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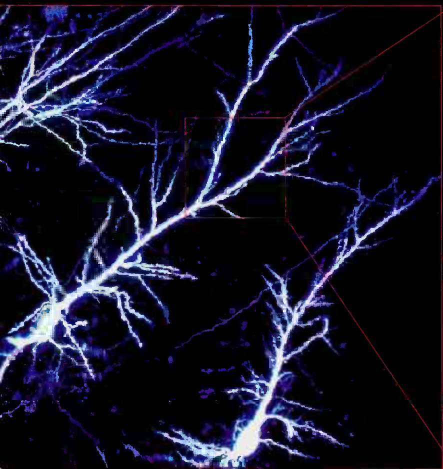

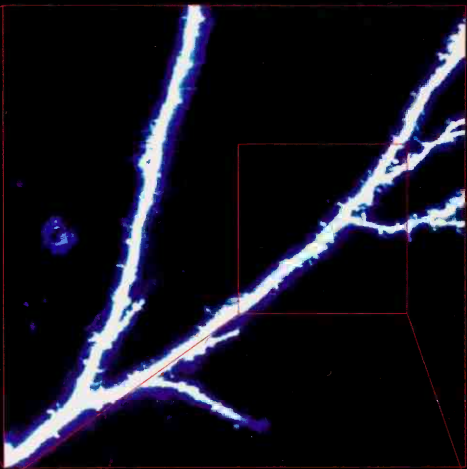

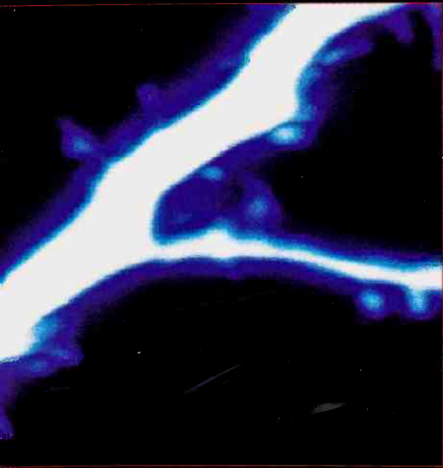

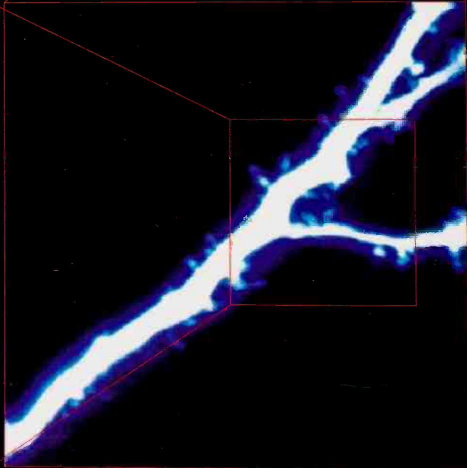

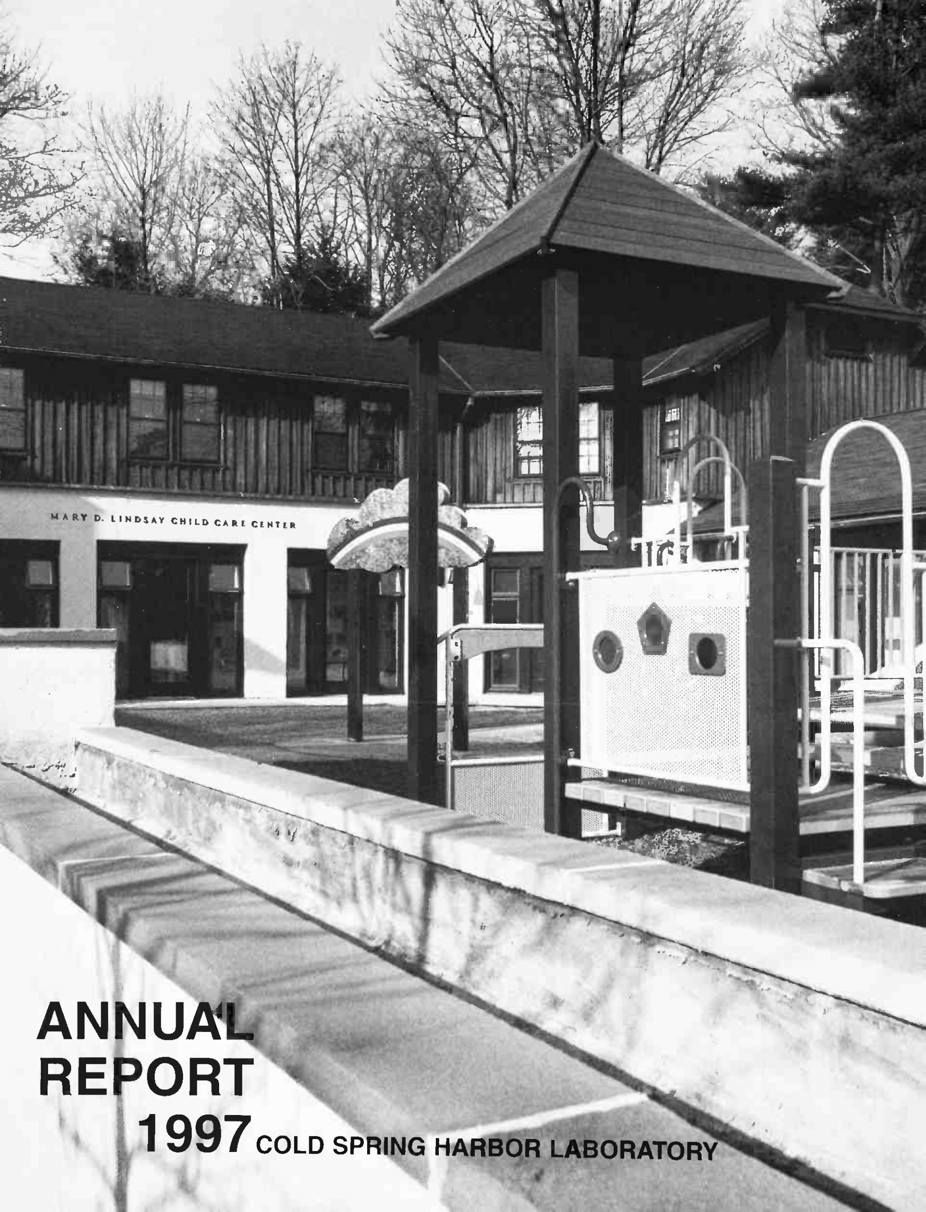
MARY D. LINDSAY CHILD CARE CENTER
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Cold Spring Harbor Laboratory
P.O. Box 100
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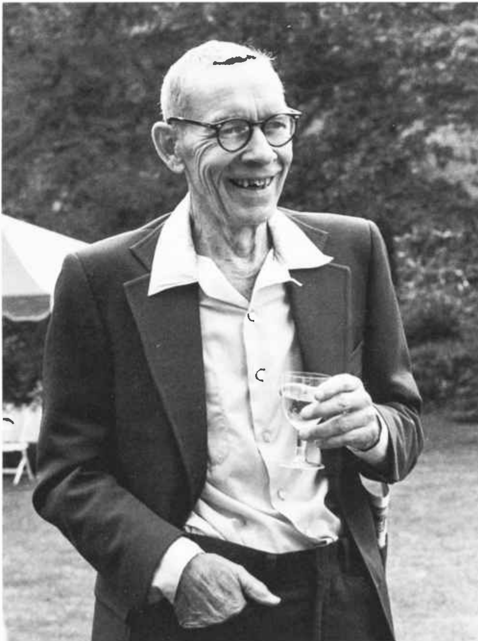


Alfred Day Hershey (1908–1997)

Most of the basic facts about the gene and how it functions were learned through studies of bacteriophages, the viruses of bacteria. Phages came into biological prominence through experiments done in wartime United States by the German physicist, Max Delbrück, and the Italian biologist, Salvador Luria. They believed that in studying how a single phage particle multiplies within a host bacterium to form many identical progeny phages, they were in effect studying naked genes in action. Soon they recruited the American chemist-turned-biologist Alfred Hershey to their way of thinking, and in 1943 the "Phage Group" was born. Of this famous trio, who were to receive in 1969 the Nobel Prize, Hershey was initially the least celebrated.

Al had no trace of Delbrück's almost evangelical charisma or of Luria's candid assertiveness and never welcomed the need to travel and expose his ideas to a wide audience. He framed his experiments to convince himself, not others, that he was on the right track. Then he could enjoy what he called *Hershey Heaven*, doing experiments that he understood would give the same answer upon repetition. Although both he and Luria had independently demonstrated that phages upon multiplying give rise to stable variants (mutants), it was Hershey, then in St. Louis, who in 1948 showed that their genetic determinants (genes) were linearly linked to each other like the genes along chromosomes of higher organisms.

His most famous experiment, however, occurred soon after he moved to the Department of Genetics of the Carnegie Institution of Washington in Cold Spring Harbor on Long Island. There, in 1952, with his assistant, Martha Chase, he showed that phage DNA, not its protein component, contains the phage genes. After a tadpole-shaped phage particle attaches to a bacterium, its DNA enters through a tiny hole while its protein coat remains outside. Key to Hershey's success was showing that viral infection is not affected by violent agitation in a kitchen blender, which removes the empty viral protein shells from the bacterial surface.

Although there was already good evidence from the 1944 announcement of Avery, MacLeod, and McCarty at the Rockefeller Institute that DNA could genetically alter the surface properties of bacteria, its broader significance was unknown. The Hershey-Chase experiment had a much stronger impact than most confirmatory announcements and made me ever more certain that finding the three-dimensional structure of DNA was biology's next most important objective. The finding of the double helix by Francis Crick and me came only 11 months after my receipt of a long Hershey letter describing his blender experiment results. Soon afterward, I brought it to Oxford to excitedly read aloud before a large April meeting on viral multiplication.

Hershey's extraordinary experimental acumen was last demonstrated through his 1965 finding that the DNA molecule (chromosome) of bacteriophage λ had 20-base-long single-stranded tails at each end. The base sequences of these tails were complementary, allowing them to find each other and circular λ DNA molecules. This was a bombshell result, because circular molecules had 2 years before been hypothesized as an intermediate in the integration of λ DNA molecules into bacterial chromosomes.

An abridged version of this essay appeared in the *New York Times Magazine* on January 4, 1998.

Key to the esprit of the phage group was its annual late summer meeting at Cold Spring Harbor. It was not for amateurs, and the intellects then exposed had no equal in biology then or even now, in the hurly burly rush of genetic manipulation of today where perhaps a quarter-million individuals think about DNA in the course of their daily lives. Logic, never emotion or commercial consideration, set the tone in those days, and it was always with keen expectation that we awaited Al's often hour-long concluding remarks. Tightly constructed, his summaries struck those of us aware of his acute taciturnity as containing more words than he might have spoken to outsiders over the course of the past year.

By 1970, the base features of λ DNA replication and functioning, both in its more conventional lytic phase and in its prophage stage, had become known, and a book was needed for their presentation to a broader biological audience. Because of his stature and honest impartiality, everyone wanted Al to be the editor and to see that the 52 different papers said what they should and no more. Through Al's ruthless cutting of unneeded verbiage, the book's length was kept in check and the final volume not painful to hold in one's hands. Working hard to make the changes that Al suggested, the young Harvard star, Mark Ptashne, noted with pleasure that Al had made no further changes in his revised manuscript's first ten pages. Then on page 11, Al wrote, "Start here." Only Al could be so direct and so admired.

This was his last scientific hurrah. Although he was only 63, he soon chose to retire. It bothered me that a mind so focused and inventive would willingly stop doing science, but he lived to his own standards, and the pursuit of new ideas was never easy. New moments of *Hershey Heaven* never lasted long, and his long summers sailing on Georgian Bay were out of place as the number of new scientists seeking gene secrets increased. In retrospect, he did not get out too soon. Recombinant DNA was but 2 years away, and soon there would have been competitors on all sides.

Retirement saw him first expanding his garden, and I could exchange words with him on my walks past his home. Later, when he became absorbed with computers, he was inside and I never thought I had something important enough for an excuse to interrupt. I now regret my lack of courage. Al always appreciated others trying to move ahead. In his last month, now 88 and much curved over by arthritis, Al drove his wife to my house for a small gathering. In asking about our daily lives at the lab, Al likely knew that this would be the last time we saw him. The time to go was at hand, and he stopped eating. No one among his friends ever expects to see another who so pushed science to that level of human endurance.

November 10, 1997

J.D. Watson

PRESIDENT'S ESSAY

I am close to completing 50 years of association with the Cold Spring Harbor Laboratory. In mid-June of 1948, I arrived here to spend the summer doing phage experiments under Salvador Luria, with whom I had just started my Ph.D. thesis research at Indiana University. It was to be an interval that set the course of my future life. Until then, my impressions of what high-level science was like had come largely from books, journal articles, and university lectures. Suddenly I had the opportunity to associate faces and voices with ideas and experiments. Not discouraged by personalities who did not always measure up to their science, I was happily finding out that high-level science could be more than long days in the lab and much mental sweat. It could also encompass a life that had room for outdoor fun and silly moments that did not always treat gently individuals who thought too well of themselves.

Dominating the intellectual esprit that summer was the German-born theoretical physicist-turned-phage biologist Max Delbrück. He, with his wife Manny, had been in Cold Spring Harbor every summer save one since 1941. Born in 1906 in Berlin, Max had moved in the circle of the great theoreticians when quantum mechanics revolutionized the world of physics. At that time, Berlin was one of the world's great centers for science, but its central role vanished as soon as Hitler took over in 1933. Coming from a distinguished Protestant academic family, Max did not have to leave, but feeling at odds with the Nazi-led university scene, he migrated to the United States in early 1937. By then he had become more interested in the gene than the atom and so used his Rockefeller Foundation fellowship to go to the California Institute of Technology to work with its strong contingent of geneticists. There he soon saw the possibility of using the viruses of bacteria (bacteriophages or phage for short) as model systems for studying the fundamental properties of genes.

It was after his move to Vanderbilt University in Nashville in early 1940 that he received an invitation from Milislav Demerec, then the Laboratory's Director, to participate in the 1941 Cold Spring Harbor Symposium. Even more important, he was asked to stay on for the remainder of the summer to do experiments in one of the unheated lab buildings assigned to summer visitors. Max extended this invitation to his new friend Salvador Luria, who had done phage experiments in Rome and Paris before fleeing ahead of advancing German troops to a boat that would take him to New York. He had restarted phage experiments at the College of Physicians & Surgeons of Columbia University when he and Max first met in late December of 1940 at an American Association for the Advancement of Science annual meeting in Philadelphia. After first talking about their phage work, Max took Salva to dinner with a friend of his from the past, the theoretician Wolfgang Pauli, legendary for both his ideas and his rudeness.

Six months later, his summer experiments with Salva were going so well that Max, who had left for Pasadena in late July to get married, brought his new bride Manny (Mary Bruce) back to Cold Spring Harbor for the remainder of the summer. In no sense was it a traditional honeymoon. But Manny was then, as throughout her life, never bound by conventions. She quickly began to share Max's enchantment with Cold Spring Harbor and eagerly looked forward to returning the following summer.

The collection of scientists that Demerec assembled that summer of '41 was extraordinary. It included the young *Drosophila* geneticists, Ed Lewis and Jim Neel, as well as the already revered Hermann J. Muller, arguably the world's best geneticist, just back in the States after nine years in Berlin, Moscow, and Edinburgh. Sewell Wright, the best population geneticist in the United States, also was there, as were four leading maize geneticists including the Cornell University-trained Marcus Rhodes and Barbara McClintock. Later that year, Barbara, personally incompatible with the University of Missouri, was appointed to the Cold Spring Harbor staff by Demerec, remaining here for just over 50 years until her death in 1992. With Bentley Glass and Alexander Hollander also in residence, this particular cohort of summer visitors represented an intellectual virtuosity never before seen in Cold Spring Harbor's 51 years of existence.

Max and Manny were to spend six years in Nashville before they returned to Pasadena where they had first met. In 1946, as soon as George Beadle moved from Stanford back to Caltech as head of its Biology Division, he asked Max to come back as a tenured professor. Accepting this invitation was easy for Max. He had greatly enjoyed his earlier stay at Caltech, and Manny's family lived nearby in an affluent San Marino home. Her father had managed copper mines in Cyprus, and most of Manny's early life had been spent in the Middle East. She had returned to the United States only when she reached college age and entered the orange-grove-surrounded Scripps Women's College in Claremont, some 20 miles to the east of Pasadena.

Although once again based in California, the Delbrücks continued to make the trek east to Cold Spring Harbor each summer, and 1948 was no exception. Max's firm yet soft way of speaking mesmerized me that summer of '48. As much as possible I tried to be near him—when he was eating in Blackford, or writing equations on the Blackford Hall Fireplace Room blackboard, or hitting tennis balls so much harder than I could, or swimming off the sand spit raft. Then, he was about to turn 42, and at 20 I was almost young enough to be his son. Others, observing our similar tall, thin shapes and my never subtle attempts to mimic Max's behavior, jokingly began to call me *son of Max*.

Manny was constantly finding novel ways to avoid boredom and relished the nonconformity of many of the personalities then in pursuit of the gene. She could not resist making fun of snobs enamored of money or possessions and took much pleasure in telling tales about friends whose character defects led them to embarrassing moments. When given the chance, she was always outdoors, never hesitating to choose a canoeing adventure to an unknown site over newspapers or magazine reports of events out of her control. I could not then imagine Max having a more suitable wife and wondered whether I would ever have such an idyllic companion for life.

That summer I relaxed in Manny's noncompetitive reflective presence, not at all embarrassed by either my excessive skinniness or my inability to easily make social remarks that put others at ease. Still necessarily more an observer than a real player in the phage group, I took pleasure in Max's letting me call him by his first name and in return soon being known to others as Jim. Although we would eagerly listen to Max's opinions, no personal penalty was incurred by not adopting his way of thinking. Instead, the scientific mood was much that of a Socratic dialogue leading toward conclusions that might someday reveal the deep nature of the gene.

With my thesis research on X-ray-inactivated phage effectively a routine extension of Luria's prior work on UV-inactivated phage, I had no need to be intellectually clever in planning the next day's experiments. Nor did they seem so immediately important as to preclude more fun activities, especially after the Lurias returned to Bloomington in early August to await the birth of their son Danny. Then Max and Manny persuaded Renato Dulbecco, whom Luria had brought over the year before from Italy to work in his Bloomington lab, to drive us up to the Marine Biological Lab at Woods Hole. There we met the German refugees Hans Gaffron and Victor Hamburger and listened to much gossip about the legendary Berlin biochemist Otto Warburg, whose ideas about photosynthesis and cancer then provoked much controversy. After our return to Cold Spring Harbor, Renato's family arrived from Italy and soon he was driving off to Bloomington. But as I was to be with Luria at a mid-September Genetics Society meeting in Washington, I stayed on in my Blackford Hall room long after virtually all of the other summer visitors were gone. Max and Manny also lingered on, and after the Blackford Hall dining room closed on Labor Day, we had meals with those Carnegie staff who lived in the big Victorian, Jones-built house on Route 25A that today we call Davenport House.

The academic year that followed at Indiana University in 1948–1949 saw me focused on chemically induced indirect effects of X-rays on phage. For days at a time, I would think I was on to something big, but then cold reality inevitably arrived. Particularly awkward were my presentations in either Bloomington or Chicago before Max or the Hungarian-born physicist Leo Szilard, the most famous of the phage course's converts to the pursuit of the gene. Leo would start interrupting me as soon as I started to speak. Later I comforted myself by hoping that he wouldn't have been so ferocious if there was nothing of importance in what I was saying. In retrospect, however, that was the sad truth, and my Ph.D. period proved primarily a time for learning until eventually, I could intellectually stand on my own feet as opposed to those of Luria. But Salva's help was always there. Seeing that I could not use the English language effectively, in April 1950, he pared my unconceptual Ph.D. thesis to 50 pages.

Manny was expecting their second child during the summer of 1949, so many of the phage group assembled in Pasadena. Like during Cold Spring Harbor summers, token

weekday experiments alternated with many blackboard discussions as well as tennis. Weekends were mapped out for camping trips, which frequently did not proceed according to plan. One of the most memorable trips occurred early in August, when Gunther Stent and Wolf Weidel, then postdocs with Max, found themselves stranded over a Saturday night below treacherous cliffs on Catalina Island. The following March Max organized a mini-Caltech meeting aimed at extending the phage world's ways of thinking to plant and animal viruses. I was then trying to finish my thesis so did not accompany Salva to Pasadena. Later Max was to make my day by asking me to help put together a syllabus on the procedures, facts, and interpretations of phage. It appeared at the end of the little book, *Viruses 1950*, that emerged from the Caltech conference.

What mattered most then were the phage facts and ideas, not the individuals who brought them forth. This was Max's biggest impact on the phage group and it allowed us to take real joy in the discoveries of others instead of being jealous of them for providing our intellectual excitement of the moment. In retrospect, Max's unchallenged scientific collectivism was helped by the still so-mysterious nature of the gene. It was not then clear what types of phage experiments could lead to a real breakthrough, and no two individuals were pursuing the same experimental approaches. Only with the 1952 Hershey-Chase experiment did the primacy of DNA became clear The discovery a year later of its double helical structure started us thinking about how genetic information is encoded by the sequences of base pairs. The ways to pin down how DNA functions in protein synthesis, however, long remained partly clouded. So for the next 15 years, we remained in the happy situation where there were more important problems to solve than scientists opting for their challenges.

By then I was on the Harvard faculty, and it was easy to propose potentially important thesis topics for my students without worrying whether someone else would be doing similar experiments. Just one of my students had his thesis work made largely irrelevant by more incisive findings elsewhere, and only once, when searching for the molecular nature of nonsense suppressors, did we consciously find ourselves racing against another lab. Happily we won that race when, in 1965, Mario Cappechi and Gary Gussin found mutant tRNAs to be the molecular entities that misread nonsense codons as sense. In retrospect, the pace of research up through the end of the 1960s was not that hurried, and summers could still be used as low-key periods for recharging our intellectual batteries. Life at Cold Spring Harbor correspondingly remained much as when I first came here, with the students taking the summer courses occasionally bringing along wives, if not families, and regulars like Rollin Hotchkiss coming each year for the occasional summer experiment in Jones lab.

After I became Director of the Laboratory in February 1968, I brought here for the coming summer some of the better of the younger scientists working on phage λ. At that time, my Harvard lab was increasingly focused on this most fascinating lysogenic phage and I saw great advantage in having my so-oriented students come in long contact with their peers from elsewhere. Max and Manny were once again regular summer visitors, with Max, at John Cairns' urging, having started in 1965 a workshop on *Phycomyces*, the mold then used as a model system for studying biological responses to light. Each summer, the Delbrücks were domiciled in the utilitarian Page Motel. Perfect for outside-oriented visitors, it easily allowed Max and Manny to assemble friends on the lawn outside for barbecues. I had just married Liz, and Max and Manny, knowing that all too many

young scientists are not people-oriented, graciously made her welcome in their home-away-from-home. The Delbrücks were to return for five more workshops between 1971 and 1976, by which time Max was 70 and saw the need for others to assume leadership of *Phycomyces* research.

During the early 1970s, improving Laboratory finances allowed us to install heating systems and modernize many of our run-down older buildings that were formerly relegated to summer activities. Cold Spring Harbor as a summer-dominated institution began to vanish as we strove to become one of the world's more exciting sites for tumor virus research. In particular, by focusing on the small DNA tumor viruses SV40 and adenovirus, we hoped to identify genes that make cells cancerous. Initially, our tumor virus lab, led by Joe Sambrook, had almost the aura of a tiny frontier mining encampment filled with upstarts waiting to take big risks. Each was hoping that their next experiment would lead to a gold strike. In so prospecting, they needed brains and brawn as well as luck. Those not of strong will and guts knew they were out of place and moved elsewhere. On Friday nights, our Blackford Hall bar had the swagger of a western saloon awaiting the inevitable gun fights that would come during our impending tumor virus meetings. Certain personalities were bound to prove more than others could bear.

These tensions, so ready to explode, were still those of the academics searching primarily for intellectual glory. The academic powers to be won were likely to be marked by family cars bearing no more cachet than a Chevrolet. No one here then thought he might soon have facts or ideas about cancer cells that would have commercial applications. Like most pure academic biologists, we looked more with disdain than envy on those chemist acquaintances whose industrial contacts let them live in homes almost suitable for bankers. So with sufficient monies coming from the War on Cancer to support our experiments, I saw no reason to taint our reputation for academic purity by unnecessarily promoting commercial activities. With no hesitation I turned down Richard Roberts' request that our Lab help start a company to commercialize the new restriction enzymes that he was isolating. For several years, he had been providing them gratis to scientist acquaintances, but now he was receiving more requests than he could handle.

The rough saloon camaraderie of tumor virology research persisted through our 1979 Viral Oncogenes Symposium. Its record-breaking 141 presentations allowed virtually everyone with a true result to speak up and be heard. Then, as the powerful new procedures of recombinant DNA became available, key objectives became much easier to reach and our cowboy era was over. Once one mastered the new gene cloning procedures, experimental success was almost foreordained. So oncogenes quickly moved from being largely hypothetical constructs to definitive DNA sequences. Correspondingly, which experiments to do next became more obvious, with the road maps to reach key objectives often available to all. Lab sizes had to grow to keep others from mining important quarries that in the past were treated as personal possessions. Summer became no different from any other time of year, and once a tumor virus meeting was over, its participants quickly departed back to their labs.

In the early 1980s, tumor virology moved beyond the concept of oncogenes to embrace the equally important concept of tumor suppressors. These were genes whose loss could also lead to the cancerous phenotype. Oncogenes became seen as the accelerators of cell growth and division, whereas tumor suppressor genes were perceived as the corresponding brakes. It was here at Cold Spring Harbor that these two means of

cancer causation first effectively came together. In our new Sambrook Lab, Ed Harlow made his seminal 1988 finding that the E1A oncogene of adenoviruses acted by neutralizing the key cellular tumor suppressor Rb protein. Through this watershed observation, we at last knew how to go after the molecular mechanisms by which the various forms of human cancer arise, either by oncogene acquisitions or by tumor suppressor gene losses. Immediately, we foresaw a rapidly escalating pace of cancer research, since the number of human oncogenes and human tumor suppressor genes was likely to be large.

In our current search for the vast multiplicity of genetic changes that underlie human cancer, the social fabric under which we work bears little relation to the cowboy days of the 1970s. Virtually all of our research objectives have human implications, and we must increasingly face the fact that our scientific competitors are now located in commercially based, as well as academic, institutions. In a real sense we should be happy, because this now means that those financial types who control big sums of money believe that soon there will be real payoffs from our cancer research. On their horizons are scores of new diagnostic tests as well as the successful utilization of cancer-gene-derived molecular targets for the generation of powerful new classes of anti-cancer drugs.

Now that commercial, as well as academic, gold lies within our potential grasp, past ways to achieve socially acceptable balances between competition and cooperation among scientists will be even harder to maintain. Our scientists will see the need not only to publish first, but also to be the first to file concomitant patent applications. In the past, scientific civility was greatly helped by individuals deciding to publish simultaneously even though one group might have been one to two months ahead in reaching the main conclusion. Unfortunately, patent law does not forgive being a few days late and the winner gets all.

For the foreseeable future, research sweat, as opposed to research fun, will thus increasingly dominate the day-to-day atmosphere of cancer research. We should not, however, moan too long for our past days of academic purity. Instead, we must accept our frenzied states as manifestations of our increasing scientific optimism that the splendid science of the past 30 years will soon lead to reduced feelings of dread when the physician's verdict is "cancer." But for us as academics to continue as vital forces in cancer research, we will necessarily have to act more and more like our more commercial compatriots. Expensive new genome technologies, for example, must not remain restricted to big pharmaceutical laboratories or well-capitalized biotech firms. Instead, they must become available to the academic community. Luckily, this is a year when federal funding for cancer research may greatly increase. If it is deployed well, academics such as ourselves can continue to be major players in turning cancer knowledge into cancer cures.

In so working to remain an important player in cancer research, we must also see to it that Cold Spring Harbor Laboratory retains its cherished role in the broader world of biology and medicine. We must remain an institution primarily focused on the generation and dissemination of ideas as opposed to their commercial exploitation. One way to so continue is to strengthen even further our advanced teaching programs. Now we offer 25 courses in contrast to just one in 1945, when Max Delbrück taught the first phage course. But to keep our courses first-rate, we must constantly improve their laboratory facilities and equipment. Now, for example, only seven years after completion of our Beckman Lab, its teaching facilities are inadequate for teaching the new nerve cell imaging tech-

niques. So we have just started construction of our new Edwin and Nancy Marks Imaging Building, with its completion anticipated as the new millennium starts.

We will always need warm and wise friends, like the Marks, who come to our aid when we need to move forward quickly. Manny Delbrück long helped us in this way. Soon after I became Director, she provided the funds that allowed us to transform the then-derelict Wawepex Laboratory into new dormitory space. In this way we were able to house the students for the new neurobiology courses to be given in newly renovated teaching space in McClintock Laboratory. Manny's help also made possible the new tennis courts on Bungtown Road on the way to the sand spit. Sadly, upon her death early this January, we have lost our last close connection to the first heroic period of phage research. Now, however, we should not weep too long for Manny but instead rejoice that she was so long a part of our lives. In the same way, we must not dwell too much on our loss of the former coziness of those Cold Spring Harbor summers that witnessed the coming of the phage world.

There will always exist those individuals who, like the early pioneers of the phage world or tumor virology, feel happiest in moving upon uncharted water. Today, the way precise information is stored in and retrieved from the human brain appears equally, if not more, mysterious than the necessarily fuzzy way I perceived the storing of genetic information during my first Cold Spring Harbor experience some 50 years ago. Small groups of young scientists, attracted by the prospect of not following their elders' paths, are bound to assemble again to meet this challenge.

As long as we remain a home for tough risk-takers, not caring too much whether or not there might be gunslingers among them, we should not worry too much about how the start of the forthcoming millennium will find us.

March 1998 **James D. Watson**

Remembering Manny

Manny and Max, 1948

Manny and Salvador Luria, 1953

Manny personified joie de vivre—the name of her game was Fun. Having relinquished personal ambition at an early stage, she was free to pursue whatever interest struck her fancy. And those interests were many, including especially the zoo of characters who passed through Max's lab and visited Caltech over the years. Manny loved stories about those people, of whom I was privileged to be one. "As tired as Seymour at the Grand Canyon" became a standard of fatigue which Manny and Max took glee in applying to novice campers whom they had introduced to the first camping trip of their lives. No such trip was complete without some kind of disaster to recount, later, with enjoyment. Herman Kalckar's broken leg, after he had deprecated the crumbly desert boulders as "Hollywood rocks," was a trophy. My confidence in rock climbing was shaken when, while dangling helplessly in space at the end of a rope held by Jean Weigle, I looked down and saw the Delbrücks slapping their sides with laughter. Practical jokes were almost de rigeur. But make no mistake: This mischief was combined with unsurpassed warmth, hospitality, generosity and concern. To know Manny and Max was to love and admire them both.

Seymour Benzer

Niccolo Visconti and Manny, 1953

Manny and Jim Watson, 1953

Delbrück (1917–1998)

From Max we learned how to do science; from Manny, how to live. As a Californian raised in the Levant, Manny had an aura of cosmopolitan glamour about her. With her quick mind, wide cultural interests, and mental rigor, on the one hand, and her casual charm, good will, and sense of humor, on the other, she was a rare combination of the best personality traits that the Old and New Worlds have to offer. She made the Delbrück home a kind of intellectual salon, where we could drop in on her and Max at almost any time, and socialize with them and with other drop-ins, warming our hearts in the enchanting ambience generated by Manny's wonderful qualities. The venue of Manny's salon was not restricted to her home. On many a weekend, we sat around a campfire somewhere in the California desert, on one of the outings that she had organized. The visits to the Delbrücks' house and the desert outings provided for us many of the most precious memories of the formative years we spent at Caltech, with Manny at their focus.

Gunther Stent

Manny and friends barbecuing in front of Page Motel, 1975

Agnes Ullmann, Manny, and Peggy Lieb
50th Anniversary of Phage Meeting, 1995

Manny lived a life that I loved. She was a person in her own right, a partner, not simply "Max's wife." Her ideas and example challenged and expanded the conventions I had been exposed to previously. It was in 1952, when I was newly married, and new to the culture of international science, that Manny and Max welcomed Dave and me into their circle, where plots and activities were hatched spontaneously and carried on with childish enthusiasm. Being part of Manny's life gave me a sense, an experience, of a life that flowed from people to culture to play to science to nature to civic life—all on the lubricants of good food, unpretentious spontaneity, and seemingly effortless immersion in the flow of life. Manny's gift for friendship was extraordinary. Although she had friends all over the world, she always remembered her particular connection with each, picking up the thread from their last time together. And at her home, friends came, friends stayed, friends left, and returned...

Anne Stadler

DIRECTOR'S REPORT

At the time of this writing, we are in the midst of celebrating several significant anniversaries for Jim Watson. It was 50 years ago that Jim, as a young graduate student working with Salvador Luria, first arrived at Cold Spring Harbor to spend the summer with that year's phage group. Twenty years later, in 1968, Jim, returned to the Laboratory as its new Director and began the remarkable revitalization of the Laboratory's facilities and research activities. By choosing to study the cancer-forming DNA tumor viruses, simian virus 40 (SV40) and adenovirus, Jim placed the Laboratory and its scientists in an excellent position to make seminal contributions to cancer research and to fundamental aspects of eukaryotic cell and molecular biology. Jim arrived here with his new bride, Liz, and together they have perfected one of the most attractive centers for research in the world, combining historic and architectural taste with an unparalleled love for the beauty and history of Cold Spring Harbor and its environs. All of us owe a great debt to both Jim and Liz, and we are happy that they continue to devote much of their time to the continued success of the Laboratory.

Science has changed profoundly in the past 30 years. The year 1997 marks the 25th anniversary of the publication by Paul Berg and his colleagues of the first joining together of DNAs from different sources; they combined SV40 DNA with either bacteriophage λ DNA or *Escherichia coli* DNA. The next year, Stanley Cohen, Herb Boyer, and their colleagues reported the first functional recombinant DNA. Twenty years ago, Fred Sanger and his colleagues reported the sequence of the complete genome of bacteriophage ϕX174 (5375 nucleotides), and they published the new chain-terminating method for sequencing DNA. Today, this technique is the basis for sequencing the entire genome of many organisms, including the human genome, which consists of about 3 billion base pairs. These accomplishments, together with the innovative use of restriction endonucleases by Kathleen Danna and Dan Nathans to map the SV40 genome in 1971, heralded a new age in biology. Soon, Cold Spring Harbor Laboratory scientists, quick to master the new science, made important advances that contributed to the emerging recombinant DNA age. Biologists were no longer limited by techniques, but only by their imagination.

One of the significant developments to come soon thereafter was the establishment of the first biotechnology companies, which used the new biology to produce contemporary pharmaceutical products and reagents more effectively. The biotech companies, with their significant resources provided by venture capital, would soon produce recombinant human growth hormone, recombinant insulin, blood cell growth factors, and other important new drugs. Company scientists realized that they had to be in the business of basic research to be the first to discover new drug targets and proteins. The initial successes of the biotech companies attracted collaborations with increasing numbers of academic researchers because their biotech colleagues often had equipment and facilities that were the envy of the academic community. Collaborations to sequence proteins and clone cDNAs that encoded important cell surface receptors and other signaling molecules became the norm. Often, the biotech companies won the race with academic laboratories to obtain key genes. But even with all its resources, the biotech industry did not surpass the quality of research in the universities and institutions like our own, for it was not always

obvious where the next important and useful discovery would emerge. With few exceptions, the great discoveries in biology during the past 20 years emerged from academic laboratories, including Cold Spring Harbor Laboratory.

The relationship between academic research and biotech research has evolved over the years, and today, a happy synergy exists that greatly benefits society as a whole. Publicly funded academic research is still governed by peer review, is published for all to see, and is focused primarily on the issues that society and individual scientists deem important. A large percentage of public funds continue to support fundamental, rather than directed, discovery. For example, many of the dramatic advances in the development of combinations of drugs against HIV relied on basic studies of many retroviruses and their interactions with the cell. Techniques and facilities for protein crystallography that ultimately played an important role in the development of the HIV pharmaceuticals were developed to understand the structure of proteins that had little if any medical or commercial value. And without recombinant DNA technology, it would have been far more difficult to develop strategies to fight the AIDS epidemic. There remains much to do in HIV research, but it is already clear that because of the vigorous support of basic academic research, scientists had the tools to deal with HIV when it surfaced. Basic research had provided an infrastructure on which to build, and although it was often frustrating that progress was not faster, the pace of dealing with the disease was relatively rapid when compared to epidemics of the past.

Biotechnology companies now play an important role in the larger biomedical research enterprise. In addition to their contributions to basic and applied research programs, they have become a very effective conduit for translating the basic research discoveries made in academic laboratories into drug discovery and, ultimately, clinical uses. In the past, basic research discoveries that might have languished for many years are now rapidly picked up by the biotech industry and incorporated into their own research programs.

Many biotech companies, in turn, seek collaborations with the much larger pharmaceutical companies that have the financial and technical resources to develop promising leads into the clinic or to the market. In a sense, the pharmaceutical industry can choose from the large number of projects put forward by the smaller biotech companies and have greater confidence that a project may go all the way to the clinic. Collaborations between the biotech and pharmaceutical companies are important because the cost of developing a new drug that will become an FDA-approved pharmaceutical is extraordinarily high, often running into the hundreds of millions of dollars. As a result of the academic–biotech–pharmaceutical collaborations, completely new approaches for the treatment of cancer are entering clinical trials at unprecedented rates, with real expectations that significant inroads to treating the disease may occur in the not too distant future.

Collaborations between academic institutions such as Cold Spring Harbor Laboratory and the biotech industry can also fulfill another need that large pharmaceutical companies may not address. Many diseases, although devastating to patients and their families, are not economically feasible for large pharmaceutical companies to pursue, principally because the market will not be large enough to justify the expense. Biotech companies, particularly the newer enterprises, are more likely to pursue these targets because they offer an opportunity for a biotech company to achieve its first independent clinical success. In some cases, where the clinical need is demonstrable but the economic incentive is absent, public funds from the National Institutes of Health and other government agencies might be money well spent. Thus, the relationship between the biotech industry and public funding might come full circle to yield clinical success.

The number of biotechnology companies continues to grow at a rapid rate, and I suspect that we are at the beginning of a significant expansion and further evolution of this industry and its relationship to academic labs. Public funding for science is increasing because every informed person realizes that extraordinary research opportunities now exist. We also know from experience that the academic community is best positioned to make the important advances that are unexpected, primarily because the goals of basic research are very different from the research conducted in industry. At the same time, however, industry cannot afford to ignore these basic discoveries and has equipped itself with the technologies to rapidly take advantage of new developments. Today, the biotech and pharmaceutical industry laboratories are much more well equipped for modern biology than are the academic laboratories; they cannot afford not to be.

The cost of modern biological research in academic laboratories is increasing with the complexity of the tasks. Unfortunately, public funding for equipping academic laboratories has not kept pace with the cost of the equipment. It is common these days to spend many hundreds of thousands of dollars on a single item of equipment for a single investigator, and federal funds are not easy to obtain in a timely and efficient manner, if they can be obtained at all. To ensure that the academic research laboratories do not fall behind the well-equipped industrial laboratories, the National Institutes of Health and the National Science Foundation should establish better mechanisms for providing infrastructure support. It would be very dangerous to create a wide gulf between the academic labs and the industrial labs because of the lack of equipment support in academia. Such a gap would eventually lead to a weakening of the process of innovative discovery that is the hallmark of academic research. One kind of funding mechanism for improved infrastructure might be to provide competitive, peer-reviewed, multi-year block grants to institutions that would then have access to these funds immediately. Although Cold Spring Harbor Laboratory spends a considerable amount of its resources obtained through fund-raising to equip its labs, we cannot keep pace with the constant demand for new equipment. This is, in part, because the equipment is more expensive, and also because the techniques in modern biology are more complex than they were 20–30 years ago.

The existing relationship between biotechnology and academia is a healthy one for society as a whole, as long as the potential conflicts of interest are declared and understood by the public, research institutions, and scientists. The vast majority of such interactions, and certainly all of those occurring at Cold Spring Harbor Laboratory, benefit without compromising the goals of publicly funded research. We have in place a very effective subcommittee of the Board of Trustees that oversees interactions with private companies and enforces strict guidelines on the nature of the collaborations.

This year saw the completion of an agreement that allows Westvaco, Novatis, and Monsanto Corporations to provide infrastructure support for our research in plant biology and to help us develop a database of gene-trap and enhancer-trap lines of *Arabidopsis*, one of our favorite plants for biological investigation. Without the support of these forward-thinking companies, we would not have the resources to pursue this research, which will ultimately benefit the entire plant biology community. This year also saw the completion of agreements with Hoffmann La-Roche Inc., OSIP (formerly Oncogene Sciences), and Helicon Corporation to study learning and memory and with Tularik Inc. to identify new cancer gene loci. These arrangements will enable our scientists to pursue their research projects and have access to resources that otherwise would not have been available.

Because future interactions between biotech and academic laboratories will be an integral part of the larger scientific picture, we have taken steps to help establish a Biotech Park on Long Island near the Laboratory. Centers of modern biological discovery such as Cold Spring Harbor Laboratory often have attracted biotech companies to locate nearby because the proximity fosters scientific interactions and aids in recruiting scientists to their companies. The Laboratory has been involved in transferring technology to many start-up biotech companies, but most of these are located in places other than Long Island. Three of these companies, however, exist nearby and we are keen to see that they remain. To facilitate this, the Laboratory sought advice and help from New York State, and we were pleased to learn recently that Governor Pataki and the State Legislature will support the establishment of a Biotech Park adjacent to the nearby State University of New York, Farmingdale campus, a short distance from the Laboratory. Although the Biotech Park will be a separate entity from Cold Spring Harbor Laboratory, we will help guide the facility to become a nationally recognized center of excellence in biotechnology. It is hoped that a nearby Biotech Park will attract many outstanding companies that will create a broader scientific environment on Long Island and at the same time benefit the area economically. John Cleary did much to guide us in our support of this important project; for this, and for many other matters on which he has provided sound counsel, we are very grateful.

Our own growing technology needs require that we provide an off-grounds facility to accommodate the increased DNA sequencing and gene-based research that is such an essential component of modern biological science. As the Laboratory expands its genome sequencing and related gene technologies, we need space that would best be provided by a large building located off campus. In addition, the success of our neurobiology research program has created new demands for mouse behavior facilities that cannot be incorporated into our existing infrastructure. As part of a solution for these urgent needs, we expect in the near future to acquire a sizable building that is located a short distance from the Laboratory, on the way to the Biotech Park. There, we will establish a state-of-the-art DNA technology center large enough to accommodate the expanded genome projects that we began 2 years ago, as well as behavior rooms that will do justice to the exciting neurobiology research.

Another development this year that should broaden the intellectual community on Long Island and take the Laboratory to new heights was the decision this year by our Board of Trustees to explore the possibilities of establishing a Graduate School of Biological Sciences. We already have a very expansive education program at the Laboratory, including the elementary and high school programs at the DNA Learning Center, a sizable contingent of graduate students from the State University of New York (SUNY) at Stony Brook, and our advanced courses and meetings program that constitute postgraduate training for scientists. We see the possibility of starting our own graduate school, while maintaining the very valuable programs and interactions we have with SUNY Stony Brook, as very exciting. It is particularly pleasing that Winship Herr is developing the new graduate school that will take Cold Spring Harbor Laboratory into a new era.

As the pace of biological discovery evolves, so too, does the Laboratory. Any research institution must adapt to the sometimes dramatic changes in modern biological research. The projects and thinking I have outlined are all essential for our research institution to remain dynamic. At the same time, we must make sure that our scientists are supported to the fullest extent possible and that the research remains of the highest possible quality, in the new academic style.

HIGHLIGHTS OF THE YEAR

Research Highlights

Cancer Genetics and Cell Division

In March, Cold Spring Harbor Laboratory (CSHL) scientists Michael Wigler and Clifford Yen with colleague Ramon Parsons, M.D., Ph.D., of the Herbert Irving Comprehensive Cancer Center and Columbia-Presbyterian Medical Center, announced the discovery of a tumor suppressor gene, which they named PTEN. The gene appears to be altered in a large percentage of brain, breast, and prostate cancers, and evidence suggests that loss of PTEN affects the way a benign tumor becomes malignant. Unlike mutations of genes such as *hMSH2* and *BRCA1*, which were found in people who have hereditary predispositions to cancer, PTEN was discovered by analyzing the more common sporadic cancers. More than 80% of all cases of cancer are sporadic, meaning that they have no obvious hereditary contribution.

M. Wigler

PTEN received its name because of its similarity to phosphatases and tensin. The similarity between PTEN and protein phosphatases, which remove phosphates from proteins, is significant because many oncogenes—genes that help to transform normal cells into cancer cells—encode tyrosine kinases, which add phosphates to proteins. Tensin is part of a complex of proteins that sits below the cell surface and controls cell shape. Thus, PTEN may also be involved in the spread of tumors, by localizing to the cell surface and removing phosphates from key signaling proteins. In a productive collaboration between the Wigler laboratory and Nicholas Tonks' laboratory at CSHL, the two groups quickly confirmed that PTEN is a phosphatase and have identified proteins with which it interacts. These studies should point to the pathway in which PTEN functions in normal cells and which is altered in tumor cells.

Representational difference analysis (RDA), an advanced genetic technology developed by Mike and Nikolai Lisitsyn, then at CSHL, played a key role in the identification of a PTEN tumor suppressor gene. RDA is a procedure used to analyze the differences between two genomes. (A genome is the entire DNA sequence of an organism.) By comparing DNA from diseased and normal cells from the same person, scientists can use RDA to identify DNA sequences that differ between the cancer cells and normal cells. In the case of PTEN, RDA was used to find unique DNA sequences present in normal tissue but missing in breast cancer. To date, the Wigler lab has located about a dozen genetic loci potentially involved in breast cancer. Each of these discoveries represents a vital step forward in the path to earlier diagnosis and improved treatment for breast cancer patients, and it illustrates the growing realization of the genetic complexity of cancer.

In 1994, to further utilize RDA in the search for cancer-related genes, the Laboratory and Mike formed Amplicon Corporation. In October 1997, the Laboratory announced the acquisition of Amplicon by biotech leader Tularik, Inc. Tularik is the largest privately held biotechnology company in the nation, and its scientists are enthusiastic about continuing collaborations with CSHL scientists while using RDA in an extensive cancer research program. Although Tularik, Inc. is located in California, the oncology division of the company will continue to operate on Long Island for at least 5 years and will continue to collaborate with CSHL scientists.

There was good news and bad news from Carol Greider's lab in 1997: The good news was the report of a line of telomerase knock-out mice. The bad news was that Carol left Cold Spring Harbor after 9 years to accept a position as Associate Professor in the Department of Molecular Biology and Genetics at Johns Hopkins University School of medicine in Baltimore, Maryland, to follow her historian husband to his new faculty position at George Washington University.

C. Greider

In October, Carol's group published a report about mice that lack telomerase, an enzyme that she discovered in 1985 and has continued to study. Telomerase is necessary for maintaining chromosome integrity. Several studies have suggested that telomerase also plays a role in cancer and cell senescence. The ends of chromosomes, called telomeres, shorten each time a cell divides. It is thought that when telomeres reach a critically short length, the cell division cycle arrests and cells enter into a senescent state after which they never divide again. Telomerase appears to sustain telomeres against this shortening.

In collaboration with Ron DePinho's lab at Albert Einstein College of Medicine, Carol's group bred a line of mice that lacked the telomerase enzyme. The results showed that mice can survive for six generations without telomerase. The studies also proved telomerase's role in chromosome stability; mice that lacked telomerase showed telomere shortening and loss of telomere function, and they eventually developed chromosomal abnormalities. After five or six generations, telomere loss in mice leads to sterility and loss of cell viability in certain highly proliferative tissues. The work confirms the suspected role for this important enzyme in cell proliferation and demonstrates that when telomeres reach a critically short length, cell and tissue viability are progressively lost. Interestingly, cells that lacked telomerase could still form tumors, demonstrating that telomerase is not essential for tumor formation in mice. As predicted, however, recent results indicate that the rate of tumor formation is lower in mice that lack telomerase. Thus, although telomerase is not essential for the development of cancer, it may play an important role in tumor formation.

Scott Lowe and David Beach made a surprising new discovery about the transformation of normal cells into cancer cells. Usually, most human cells undergo senescence,

D. Beach, S. Lowe

or permanent cell cycle arrest, after a restricted number of cell divisions. This, in effect, limits the cells' life span. Cancer occurs when cells continue dividing beyond the normal limit or fail to die when they should. In 1981, scientists, including Mike Wigler at CSHL, discovered that a gene called *ras* was involved in some human cancers. This was the first discovery of a human oncogene that was derived from a tumor. In 1983, Earl Ruley—then at CSHL, now at Vanderbilt University School of Medicine—showed that *ras* acts in concert with other oncogenes to cause cancer. It was determined that most of these cooperative oncogenes can independently extend the life span of—or even immortalize—cells. Recently, Scott and David reported the surprising observation that when the oncogenic form of *ras* is overexpressed in normal cells, it immediately induces the same sort of cell senescence that occurs during cell aging. Two other genes, p16 and p53, both extensively studied at CSHL and elsewhere, are necessary for this type of cell cycle arrest. When p53 or p16 are absent from the cell, *ras* now stimulates uncontrolled cell division, rather than cell division arrest. This research provides important information about the multistep nature of cancer and suggests that, in the right context, it may be possible to exploit oncogenes to reverse tumor cell growth.

Neuroscience

In previous reports, CSHL neurobiologist Hollis Cline described the role of the enzyme CaMKII in neuronal development. In 1997, her lab built on its previous discoveries—that expression of CaMKII is involved with neuronal maturation and with the stabilization of synapses, the intricate connections between neurons. Evidence from the Cline lab showed that CaMKII coordinates the development of the physical structure of neurons with the development of their synaptic connections. The normal, gradual increase of CaMKII expression during early brain development correlates with slowed growth rate and increased stabilization of dendrites—the branches through which neurons receive their electrochemical signals. These changes signify neuronal maturity. Holly therefore hypothesized that CaMKII, which is regulated by calcium activity, may represent an activity-dependent mediator of neuronal maturation. Her lab tested this theory by forcing the expression of CaMKII in immature neurons and obtained the exciting result that indeed CaMKII expression suppressed the development of additional, longer dendrites and prompted the stabilization of the existing branches. Thus, it promoted the maturation of neurons during brain development.

Conversely, Holly and research investigator Elly Nedivi showed that a novel protein, CPG15, increases the number of dendritic branches in neurons. Elly isolated the gene for CPG15, which belongs to a group of genes whose expression is elevated by neuronal activity. In addition to inducing neuronal branching, they found that CPG15 also controls the growth of neighboring neurons through an intercellular signaling mechanism. These studies suggest that CPG15 is capable of translating local neuronal activity into structural changes in the brain. This would represent the discovery of a new class of neuronal activity regulated growth factors.

Alcino Silva's lab continues to study the molecular and behavioral functions involved in learning and memory in mice. In 1997, Alcino tied previous results together through an experiment with collaborator Howard Eichenbaum of Boston University in which he monitored the activity of specific cells in the hippocampus in the brains of living, functioning mice.

Alcino has been studying "place cells"—specific, identifiable brain cells that fire only when an animal is in a precise place in its environment, a place that the animal's brain recognizes. These place cells are representative of the "place circuits" that are stimulated as an animal becomes acquainted with a place or area. The establishment of such a series of circuits is called spatial orientation or learning; the mouse recognizes familiar things as it travels about. Alcino tested the function of place cells in two types of mutant mice, each representing a component in learning and memory that Alcino has been studying—the αCaMKII protein and a CREB protein. Alcino created genetically modified mice that carry a point mutation in a single amino acid of the αCaMKII protein. Instead of firing at specific times like wild-type cells, place cells in animals with this αCaMKII mutation fire randomly when they are exposed to familiar and unfamiliar places, which indicates a lack of learning. Mice containing decreased levels of CREB also display this reduced learning, albeit to a lesser degree. Behavioral studies corroborate this finding, as the same mutant mice demonstrate a marked lack of spatial orientation.

In a second set of experiments, Alcino's lab successfully reversed the learning deficit in mice with the NF1 mutation characteristic of neurofibromatosis type 1. These mice have proven to be a valuable model for the study of this disease. He has confirmed a mechanism that is defective in NF1 mice and has restored the ability of mice to learn by breeding in a second mutation that counteracts the effects of the NF1 mutation. These behavioral studies may suggest targets for the search for treatments of learning deficiencies in children affected by mutations in the neurofibromatosis type 1 gene.

HIV Pathogenesis

Jacek Skowronski's studies of HIV pathogenesis have continued to produce new understanding of how the deadly virus commandeers its host cells. Jacek's laboratory has used genetic techniques to dissect the functions of the Nef protein, the product of the viral gene *nef* that is found in human and simian immunodeficiency viruses (HIV and SIV). Nef is a regulatory protein that is important for efficient viral replication and essential for the development of AIDS in humans and primates. HIVs lacking Nef do not cause AIDS, and thus inhibiting the functions of this protein is a key goal for controlling HIV pathogenesis.

J. Skowronski

Jacek has shown that Nef possesses multiple independent functions. One role is to control levels of the CD4 protein on the cell surface of $CD4^+$ T lymphocytes. CD4 is one component of the cell surface receptor for HIV. A second role is to control the levels of cell surface MHC molecules, proteins that play a key role in immune recognition of virus-infected cells. The genetic dissection of the multiple functions of Nef provides a basis for experiments directed toward understanding in detail the molecular mechanisms by which Nef affects the development of AIDS, and such experiments are currently under way in Jacek's laboratory. In addition, Jacek and his colleagues are collaborating with scientists who study SIV pathogenesis using animal models of AIDS with the goal of developing an HIV vaccine.

Cell Death during Development and Cancer Progression

In the study of programmed cell death, or apoptosis, Michael Hengartner's lab has made significant progress in identifying the genes involved and their roles. Working with post-

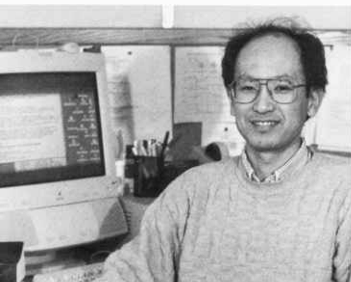
doctoral researcher Mona Spector, Michael has continued to gain new information about this process by studying the tiny worm *Caenorhabditis elegans*, an ideal model organism for genetics research. Previously, three genes were known to be essential for properly controlled programmed cell death to take place in *C. elegans*, and two of these genes were known to have counterparts, or homologs, in mammals. Michael and others had shown that CED-3 and CED-4 work in concert to kill unneeded cells, a necessary process cells during normal development, and that CED-9 suppresses this action. If the worm carries a mutation in either CED-3 or CED-4, then this necessary mechanism to rid the body of unwanted cells never takes place, resulting in excess cells in the adult worm. In contrast, if there is a mutation in CED-9, programmed cell death is not controlled and cells die at an abnormally high rate.

Recently, Michael and Mona identified a previously unknown physical interaction between the suppressor of cell death, CED-9, and the activator of cell death, CED-4. They discovered that if the interaction between the two proteins is disrupted, there is a failure to control apoptosis. These and other results suggest that CED-9 works by binding to CED-4 and regulating its activity. The new information begins to suggest how CED-3, 4, and 9 interact to control the process of programmed cell death.

Many drugs for treating cancer kill tumor cells by inducing apoptosis, or programmed cell death, but many tumor cells have mutations that produce resistance to chemotherapy. Yuri Lazebnik and Scott Lowe have been studying the process of cell death in normal and tumor cells and they reported this year that unexpectedly, extracts from drug-resistant tumor cells contain the cell death machinery and the ability to trigger this process. Interestingly, the tumor cells contain an activity that can induce programmed cell death in normal cells, a factor that Yuri and Scott call oncogene-generated activity (OGA). They are pursuing the nature of OGA with the hope that they might be able to stimulate its function in tumor cells that have become resistant to chemotherapy.

Chromosome Structure

In another vital area of our research program, Tatsuya Hirano has made significant strides in his studies of chromosome structure and function. DNA in chromosomes exists in a coiled state usually stretched out like a fully extended Slinkey (the popular children's toy) within the cell nucleus. Immediately before cell division, the DNA molecules must condense and be packaged into compact rod-shaped structures, like the Slinkey retracted into its tightly coiled shape, so that the duplicated chromosomes can be divided evenly into the daughter cells. Unlike the Slinkey, however, the packaging of DNA into chromosomes is not a spontaneous process because it requires the assistance of "packaging" proteins. Tatsuya studies the mechanisms of chromosome packaging (or condensation) via a biochemical approach using frog egg extracts and was the first to identify such a "packaging" protein, named condensin (for condensation protein).



T. Hirano

In 1997, Tatsuya and his colleagues purified and characterized condensin. They found that condensin contains several highly conserved proteins that induce, in a test tube, remarkable structural changes in DNA called positive supercoiling, which is com-

parable to the retraction and tight coiling of DNA necessary for cell division. This is the first reported evidence for how this class of proteins actually works. In addition to the condensin protein complex, Tatsuya has shown that another protein complex containing condensin-related proteins exists in cells and is involved in chromosome cohesion, the process by which the two duplicated DNA molecules remain bound to each other prior to their condensation and separation at mitosis. Further understanding of the proteins' control of higher-order chromosome structure will likely reveal more surprises.

Plant Biology

In plant genetics, the Laboratory's part of the global *Arabidopsis* Sequencing Project continues to go well. Richard McCombie's sequencing expertise and Rob Martienssen's work with the gene traps that he developed with Venkatesan Sundaresan have proved an invaluable contribution not only to the sequencing effort, but also to the determination of gene function. Rob continues to do other plant genetic research as well, and he recently made an important discovery relating to protein transport. Protein translocation is a term used to describe the process of moving proteins across cell membranes. Rob's lab identified a gene, *hcf106*, which produces a membrane protein that is vital to one of three known pathways for protein translocation in maize. Two things about this discovery were especially exciting: (1) This was the first component of the pathway to be identified and (2) the pathway in which this protein is involved was thought to be unique to higher-plant chloroplasts. However, after Rob and his lab cloned and characterized the *hcf106* gene, they found in database searches that homologous genes were present in bacteria! This interesting discovery was made possible by the worldwide bacterial genome sequencing projects and the accessibility of the data in public databases.

Bioinformatics

The Laboratory's bioinformatics program—the use of computers to analyze, store, and distribute scientific data, a kind of scientific information technology—has continued to evolve. Bioinformatics scientist Michael Zhang has been at CSHL since 1991 and studies DNA sequence pattern recognition. Recently, he was joined by three new computational biologists: Andy Neuwald, Andy Reiner, and Lincoln Stein. Andy Neuwald brings expertise in understanding the relationship between protein sequence and structure. Andy Reiner studies mechanisms for data storage and management, and Lincoln Stein is an expert in genome research and sequencing. In addition to their own research, all are providing valuable contributions to Dick McCombie's DNA sequencing efforts. Tom Marr, a member of the Laboratory's bioinformatics team since 1989, spent 1997 in transition between the Laboratory and Genomica Corporation in Boulder, Colorado. Tom is still involved in research projects here at Cold Spring Harbor, but he is now president and C.E.O. of Genomica.

The goal of the Laboratory's bioinformatics program is to develop and use computerized methods to study biology. As genomic research advances, so too does the need for efficient analysis, reliable storage, and accessibility of data. Genome scientists make up one of the most open group of research scientists today, posting programs and results on searchable Internet sites as soon as they are assembled. The bioinformatics group at the Laboratory will continue to work on original research and development projects and to collaborate with other Cold Spring Harbor scientists on a wide range of projects.

Symposium LXII: Pattern Formation during Development

One of the great scientific accomplishments of the past decade or so is the recognition that the molecular and cellular mechanisms that guide the patterning of tissues and organs during embryonic development are remarkably similar among different species. What works for flies and frogs also serves humans very well as embryos acquire their form and identity. To celebrate these marvelous discoveries, the 62nd CSH Symposium focused on pattern formation during development, with a particular emphasis on evolutionarily conserved mechanisms and molecules.

When the CSH Symposium was initiated in 1933, the length of each meeting was five summer weeks, and the length of presentations was unlimited. In 1941, director Milislav Demerec saw fit to reduce the duration of the meeting to two weeks; in 1948, he reduced it to eight days. The length of the symposium remained at a week and a day for almost half a century, but in today's fast-paced world, with many two-career families, it has become increasingly difficult for most scientists to be away from home for more than a week. After much careful consideration, the length of the Symposium was reduced to five days in 1997.

The 62nd CSH Symposium, *Pattern Formation during Development*, took place from May 28 to June 2. On Sunday evening, June 1, Sean Carroll, Professor of Molecular Biology, Genetics and Medical Genetics at the University of Wisconsin at Madison, presented the annual Dorcas Cummings lecture for meeting participants and the public. In it, Dr. Carroll presented an audiovisual short course in the development of body parts that was extremely interesting to both the lay and scientific audiences.

20 Years of Splicing

On August 23, during the *Eukaryotic mRNA Processing* meeting, the Laboratory held a special historic session and champagne toast to honor the 20th anniversary of the dis-

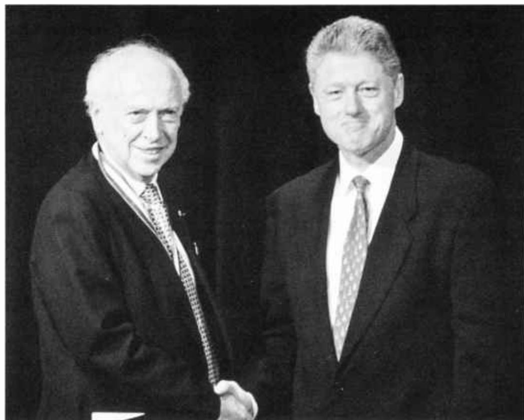
Twenty years of splicing: The Toast

covery of RNA splicing and split genes. In 1993, the Nobel Prize for Medicine or Physiology was awarded to Rich Roberts and Phil Sharp for their contributions to the discovery of split genes. The discovery—that some regions of a gene, the exons, are transcribed into messenger RNA (mRNA), whereas other regions, the introns, are spliced out—led to the creation of a new field of science, known as RNA splicing.

Phil Sharp and his colleagues in the Nobel Prize-winning work, Sue Berget and Claire Moore, were on hand for the celebration. Thoughtful reflection by James Watson familiarized the largely younger audience with the Nobel selection process, and remarks from Phil and Sue provided a candid perspective on winning a Nobel and the research that contributes to one.

National Medal of Science

On April 30, 1997, President Bill Clinton announced that Jim Watson had been awarded the National Medal of Science. The presidential honor, administered by the National Science Foundation, is this nation's highest scientific honor. The award was made in recognition of Jim's co-discovery of the structure of DNA in 1953 and for his pioneering role in the establishment of the Human Genome Project, the worldwide effort to map and sequence the human genome. Jim was appointed Associate Director for Human Genome Research of the National Institutes of Health in 1988, and in 1989 he became the first Director of the National Center for Human Genome Research, a position he held until 1992. On December 16, 1997, he traveled to Washington, DC, to accept the medal from President Clinton.



1997 National Medal of Science Award Ceremony
James D. Watson and President Clinton

Executive's Seminar meeting at Banbury

Banbury Conference Center

Executives' Seminar Weekend at Banbury Center

Our close relationship with J.P. Morgan and Co., Inc. continues and has led to their support of the annual Executives' Seminar meeting, Banbury Center's equivalent of the CSH Symposium. To be sure, the scales are different—the Banbury Center is about one-tenth the size of Grace Auditorium—but both meetings, in their different ways, are unique occasions for reviewing the most exciting and interesting biological research. This year, the topic of the Executives' Seminar was *Genetic Engineering*. The coverage was broad. Stanley Cohen (of the famous Cohen-Boyer recombinant DNA discovery) discussed the origins of his work, Jim Wells described the genetic engineering of molecules, Shirley Tilghman and Richard Michelmore discussed the genetic engineering of animals and plants, respectively, and Kay Davies led participants into the world of gene therapy. Of special note was Alan Colman's presentation on the cloning of Dolly the sheep, and on the hope of the biotechnology community for the use of cloning to produce genetically engineered proteins in animals.

Gene Therapy of Duchenne Muscular Dystrophy

An important function of Banbury Center meetings is to promote research by hosting discussions on important topics. A recent example was the meeting on *Up-regulation of Utrophin Gene Expression*. Kay Davies, of the University of Oxford, has devised a research initiative with the goal of turning on the utrophin protein to take the place of the critical protein—dystrophin—missing in patients with Duchenne muscular dystrophy. With funding from the Oxnard Foundation, we are holding a series of expert meetings to explore the ramifications of this approach, and in February, Banbury Center hosted a meeting that brought together scientists working on Duchenne muscular dystrophy, researchers studying the control of gene expression, and clinical scientists who are using a gene-reactivation strategy for treating the thalassemias. We will hold further meetings at Banbury so that the expertise and knowledge already gained in other systems can be brought to bear on this debilitating disorder.

The "Post-Genomics" World

In the past two years, a flood of complete genome sequences has been published, including those of the bacterial "workhorse," *E. coli*, and of the yeast *Saccharomyces cerevisiae*; the genome sequence of the nematode worm *C. elegans* will be completed in 1998. Knowledge of complete genome sequences will have a profound impact on the way biological research is carried out, and two Banbury Center meetings examined what is to be done in this so-called "post-genomics" world. One meeting—*Integrating Genetic, Biochemical, and Other Data*—discussed how best to make use of all the data on the functions of cells and organisms that have been acquired during the past 100 years, in light of the more recently obtained genome sequences. The goal of this meeting is to produce a "virtual cell" that can be used as a predictive tool.

Physiologists traditionally have used other techniques to study organisms on more of a systems level, and the American Physiological Society is keen to use the tools of genetics to further their research. The meeting *Genomics to Physiology and Beyond* was designed to introduce physiologists to some of the ways in which genomics and the analysis of complex genetic traits might be used to answer the kinds of questions that interest them.

Robertson Research Fund

The Robertson Research Fund has been a continuing source of support for the Laboratory since 1973. Robertson funds supported labs in each of the Laboratory's primary fields of research: cancer, neurobiology, and plant genetics. Cancer research recipients were Xiaodong Cheng, Ryuji Kobayashi, Yuri Lazebnik, Benjamin Lee, W. Richard McCombie, David Spector, Jacek Skowronski, Nick Tonks, and Rui Ming Xu. In neurobiology, the Robertson Research Fund supported Hollis Cline, Alcino Silva, Tim Tully, Jerry Yin, and Yi Zhong—all of whom have made great strides in understanding the biological basis of learning and memory. In plant research, the fund furthered the studies of Ueli Grossniklaus and Hong Ma. The Robertson Research Fund also helps to support postdoctoral researchers, graduate students, and scientific seminars.

In 1975, the Robertson family established an additional fund, designated for neuroscience, in memory of Marie H. Robertson. In 1997, allocations went to Hollis Cline for her work on neuronal growth and stabilization and to Grigori Enikolopov for his studies of the neurobiology and development of the fruit fly *Drosophila*.

Board of Trustees

Several valued trustees completed their terms in 1997. Scientific trustees Günter Blobel, M.D., Ph.D., Gerald Fink, Ph.D., and Eckhard Wimmer, Ph.D. have departed, as have individual trustees Wendy Russell and Douglas A. (Sandy) Warner III, who is taking the requisite 1-year interval after two successive 3-year terms.

At the close of the 1997 term (February 1998), John Cleary concluded his term as President of the CSHL Association and as Trustee. We are most grateful to John for his outstanding service to the Association and to the Laboratory in general and will continue to seek his valuable advice and guidance. We look forward to working with Vernon Merrill who has now assumed the position of CSHL Association President.

Wendy Russell has been named Honorary Trustee. Wendy began serving on the Board in 1984, has served four 3-year terms, and was Secretary in 1985–1987 and 1992–1997. She has served on the Development, Executive, Finance & Investment, Banbury, Building, and DNALC Committees, as well as the CSHL Association.

Wendy is a superstar in raising financial support for the Laboratory and was instrumental in starting the Corporate Advisory Board (CAB) for the DNA Learning Center. She was also a vital and wonderful part of the Laboratory's initiative to establish on-site child care. Her tireless efforts toward that end, as well as on behalf of the CSHL Association Annual Fund, are deeply appreciated.

The Laboratory's continuing success is due, in large part, to the outstanding leadership and support of the dedicated people who volunteer their time in support of an excellent cause. We offer heartfelt thanks to each of these individuals for their contributions and active participation and look forward to continuing our relationship in the future.

Our new scientific trustees, whose terms became effective in 1997, are Edward Harlow, Ph.D., of Harvard Medical School and Massachusetts General Hospital; John Kuriyan, Ph.D., a prominent X-ray crystallographer studying signal transduction and DNA replication among other things as a Howard Hughes Medical Institute Investigator and Professor at Rockefeller University; and Lorne Mendell, Ph.D, Distinguished Professor and Chairman of the Department of Neurobiology and Behavior at State University of New York (SUNY) Stony Brook and President of the American Society for Neuroscience. Ed Harlow's return to CSHL is particularly meaningful: Ed was on our scientific staff from 1982 to 1991. His demonstration here in 1989 of a relationship between the retinoblastoma (*rb*) oncogene and the E1A tumor suppressor have won him much well-deserved acclaim. Ed studied oncogenes for a decade prior to this important discovery and has had a stellar career in science since. In addition, he is also co-author with David Lane of our very successful laboratory manual *Antibodies*. Ed is currently Professor of Genetics at Harvard Medical School and the Massachusetts General Hospital and Associate Director for Science Policy at the National Cancer Institute. We are honored and grateful to have the participation of these and other scientists in charting the course for continued scientific success at Cold Spring Harbor.

In November, the Board of Trustees voted to approve the Laboratory's plan to establish a CSHL graduate program. This has allowed us to begin the application process for becoming a degree-granting institution. Our intention is to initiate a small program of approximately five Ph.D. students per year to be run in conjunction with our existing program of graduate education for students of SUNY Stony Brook. The planning and application process is being handled by Assistant Director Winship Herr, who will also be the first Dean of the graduate school.

M.J. Harris

A Friend Lost: Mary Jeanne Harris

In November, we were deeply saddened by the death of a very special friend, Mary Jeanne Harris. Mary Jeanne and her husband Henry U. Harris, Jr., have been members of the CSHL Association since 1980, and over the years, they have been extremely generous in their support of a wide variety of projects at the Laboratory. In 1982, Mary Jeanne joined the Laboratory's Board of Trustees, on which she served for six consecutive years. She served on the Building

Committee; the Robertson House Committee, through which she helped to decorate the guest accommodations at the Laboratory's Banbury Center; the DNA Learning Center (DNALC) Committee; and as Vice Chairman of the Education Committee. The Harris' support was instrumental in the establishment of the Laboratory's DNALC in 1988, and in 1991, they funded an architectural study and made the lead gift toward construction of an addition to the DNALC.

Over several terms as a CSHL Association director (1980–1988, 1991–1994, and for a short time in 1997), including a period as Vice Chairman, Mary Jeanne demonstrated a heartfelt interest in education and child care, and a deep concern for the quality of life of Laboratory scientists and their families. She organized and hosted many events designed to make the young families more comfortable in their new surroundings.

Mary Jeanne brought a unique warmth to the projects on which she worked and a determined sensibility to the goals she set. We miss her deeply.

CSHL Association

On February 2, 1997, guest speaker Philip R. Reilly, M.D., Executive Director of the Shriver Center for Mental Retardation in Waltham, Massachusetts, addressed the members of the CSHL Association at their annual meeting. His talk, entitled "Genetics, Ethics, and You," was a thought-provoking analysis of the impact of genetic information on our daily lives. He explored various issues faced by the public as advances in genetics are increasingly applied to health care, including diagnostics and prognostics. Dr. Reilly also examined the possible effects of genetic information on health insurance, life insurance, and employment.

The Association held its annual Major Donor Cocktail party on November 16 in the home of David and Jamie Deming. Association members and scientists shared a relaxed evening of wonderful food and good conversation in the Demings' warm and comfortable home.

CSHL Association Annual Meeting
J. Cleary and P. Reilly

DNA Learning Center

We were very pleased to learn that the DNALC has received a 3-year grant of $820,000 from the Josiah Macy, Jr. Foundation to create an extensive Internet site for public education about genetics. The DNALC's newly created multimedia communications group will first develop *DNA from the Beginning*, an animated "primer" to provide background information on classical genetics, molecular biology, and biochemistry. This will be followed by a *Gene Almanac*, designed to function as an animated "encyclopedia" of genetic disorders featuring detailed information on causative or predisposing genes, DNA diagnosis, and treatments. A 3-year grant of $335,000 from the Department of Energy enabled the DNALC staff to continue their tradition of nationwide teacher training. *The Science and Issues of Human DNA Polymorphisms* introduces high school biology teachers to a laboratory-based unit on human DNA polymorphisms, which provides a uniquely personal perspective on the science and ELSI aspects of the Human Genome Project. Finally, the DNALC received a 3-year grant of $600,000 from the National Science Foundation's Advanced Technological Education (ATE) Program to create and disseminate advanced technology units on genomic biology. Operating under a direct congressional mandate, the ATE program aims to ensure U.S. competitiveness in emerging technologies of the 21st century.

CSHL Press

1997 was a year of extensive change for the Press and it ended with much improvement in organizational efficiency, technical expertise, and financial performance.

The Cold Spring Harbor journals, *Genes & Development*, *Genome Research*, and *Learning & Memory* all gained ground in 1997, with record circulation, more published papers, higher impact factors, booming advertising sales, and increased visibility. There were new faces among the principal editors of all three journals, and the scope and the mix of articles in the journals broadened. Most notably, online editions of *Genes & Development* and *Genome Research* were created. These offered the entire contents of each issue via the Internet in a fully searchable format with links to other electronic resources such as Medline. Initially, these editions were made available free of charge, with the intention of providing the electronic subscription only to print subscribers until the impact of this development on the journals' circulation and revenues could be assessed. *Learning & Memory* received a generous grant from the Donaldson Charitable Trust that will support the development of its electronic edition in 1998.

A total of 18 new books were published in 1997, double the previous year's total. They included three of the most complex and colorful volumes ever to originate from the Laboratory: the remarkable laboratory manual *Cells*, by David Spector, Bob Goldman, and Leslie Leinwand; *Retroviruses,* by John Coffin, Steve Hughes, and Harold Varmus, a textbook for a community of scientists and physicians given dramatic prominence by HIV-induced disease; and *Mutants of Maize*, by Gerry Neuffer, Ed Coe, and Sue Wessler, an encyclopedic account of the extraordinary genetic diversity of corn, which was recognized as one of the three Best Books of the Year in Biology by the American Association of Publishers. These and the other new titles, such as the commanding Symposium volume on nervous system function, combined with the continued strength of classics such as *Molecular Cloning* and *Antibodies* to produce an increase of over 12% in book sales.

Major Gifts

We have been extremely fortunate over the years in the amount of support the Laboratory receives from the private sector. As federal support now constitutes less than approximately 38% of the Laboratory's budget, contributions from individuals, foundations, and businesses are ever more crucial to the Lab's survival and to the continuation of our scientific mission.

The Laboratory's neuroscience program recently received unparalleled private support. William and Marjorie Matheson, of Mill Neck, New York, and Hobe Sound, Florida, have been members and generous supporters of the CSHL Association since 1989. In 1995, they established the Matheson Fund for Neuroscience with two contributions totaling $300,000. In 1997, Bill and Marjorie added an unprecedented $2.8 million to this endowment. The fund's value at year-end was $3,537,952, after an award of $121,659 was made to support scientist Grisha Enikolopov and his work on the role of nitric oxide in development. The endowment provides a vital and enduring source of support for our neuroscience program, as its sizable principal, carefully invested, will continue to generate income that may be applied directly to research support without decreasing the balance.

William and Marjori Matheson with Liz Watson

Another very important research project that we likely could not have undertaken without private support was the *Arabidopsis* Sequencing Project. This is an organized, global effort to sequence the entire genome of a flowering plant for the first time in history. Like the Human Genome Project, the *Arabidopsis* Sequencing Project is expected to produce an entire genetic "toolbox" for plants, providing the basis for vast continued discovery as scientists explore the function and manipulation of important genes. We could not have geared up for this project and subsequently qualified for important federal grants without the support of Westvaco Corporation and of Laboratory Board Chairman David L. Luke III. Westvaco provided $290,000 for the purchase of sequencing equipment, and then subsequently and separately, Mr. Luke made two gifts totaling approximately $700,000 for additional sequencing equipment and plant research. This roughly million dollar combined investment has enabled CSHL to further secure its place in plant genetics research, a fitting step forward for the Laboratory in light of its long history of plant genetics research. George Schull's demonstration of "hybrid vigor" began CSHL's foray into plant genetics in 1908, and it yielded the sweet corn we eat today, and Barbara McClintock's 1951 description of "transposable elements" in maize, the jumping genes now studied widely in genetics, earned her the 1983 Nobel Prize in Physiology or Medicine.

For 10 years now, Edna Davenport has given generously to the Annual Fund. Her contribution of $100,000 in 1997—and each of the previous 3 years—typifies her generosity toward the Laboratory. These funds help to support young researchers who have not yet secured federal or other grant support, awards that may elude young scientists because they are often dependent on a track record in research.

During the past 8 years, Alan and Edith Seligson have fully supported 15 postdoctoral fellowships. Again in 1997, they gave a 1-year award of $35,000, this time supporting Howard Fearnhead in Yuri Lazebnik's lab. Howard has done excellent work in his studies on apoptosis, or programmed cell death, a process involved in a variety of diseases including cancer. We are most grateful for the Seligsons' continuing support of our promising young researchers.

Plans for the new Advanced Imaging Facility are well under way and we have received exceptional gifts to that end. Edwin and Nancy Marks gave $2.5 million through the Marks Family Foundation, and the W.M. Keck Foundation contributed $2 million. Our good friends George and Mary D. Lindsay gave $250,000 toward the new initiative, Burroughs Wellcome contributed $470,800 over 5 years in support of Zachary Mainen, a postdoctoral researcher working with Roberto Malinow and Karel Svoboda on the new two-photon excitation laser scanning imaging. The two-photon imaging technology around which the Advanced Imaging Facility will be built is currently being developed in Karel's temporary lab in the Beckman Neuroscience building.

The capital campaign for the Mary D. Lindsay Child Care Center continued in 1997, and was completed in excess of its $1 million goal with gifts including $50,000 from Mr. and Mrs. William R. Miller through the Miller Family Foundation. The DNALC received a most important 3-year grant of $820,000 from the Josiah Macy, Jr., Foundation to enable them to develop an extensive educational genetics website. Geri Barish and our special friends at *1 in 9: The Long Island Breast Cancer Action Coalition* presented a check for $75,000 to Mike Wigler's lab in October at their annual black tie dinner dance fund-raiser.

In other gifts, the St. Giles Foundation gave $508,000 to Mike Wigler's lab and to visiting scientist Eli Hatchwell of Southampton University, Wessex Genetics Institute in Southampton, U.K., who came here to apply representational difference analysis to his studies of human genetics. The Sidney Kimmell Foundation for Cancer Research gave $400,000 to cancer researchers Linda Van Aelst and Scott Lowe. The William and Maude Pritchard Charitable Trust gave $243,000 in unrestricted funds, which were applied to the high-powered beamline at Brookhaven National Laboratory, a vital off-site tool for our X-ray crystallographers. The Lita Annenberg Hazen Foundation awarded $200,000 to Karel Svoboda for neuroscience research, and the Pew Charitable Trust gave $200,000 to research: Recipients included Greg Hannon, Tatsuya Hirano, Yuri Lazebnik, and Yi Zhong. The Alexander and Margaret Stewart Trust gave $150,000 to support new projects in cancer research, and we are pleased that they have designated CSHL as one of the few cancer centers which they will support. The Oxnard Foundation gave $120,000 toward muscular dystrophy research; the Oliver S. and Jenny R. Donaldson Trust contributed $100,000 for sequencing equipment for the McCombie lab; and the V Foundation gave $100,000 to Linda Van Aelst for her studies on the *ras* pathway. The Helen Hay Whitney Foundation gave $87,000 to a postdoctoral fellowship for Peiqing Sun in David Beach's lab, and Pioneer Hi-Bred International, Inc., gave $70,000 toward Ueli Grossniklaus' plant research. Henry Wendt gave $56,450 for two postdoctoral fellowships in neuroscience: Frances Hannan in Yi Zhong's lab and Peter Krasnov in Grisha Enikolopov's lab. The Goldring Family Foundation gave $50,000 toward support of two postdoctoral researchers: Bill Henry in Nouria Hernandez's lab and Kaetrin Simpson in my lab. The Perkin Fund gave $50,000 to neurobiologist Alcino Silva in support of his work on learning and memory in mice.

We also received two very generous gifts of real estate. Jill Hershey of Laurel Hollow and Bob Garland of Oyster Bay have each gifted their homes to the Laboratory, with each retaining a life estate. Jill and Al Hershey have been a part of the Laboratory for many decades, and Al's death in 1997 was a loss that we shared with Jill. Bob Garland, a good friend to several of our trustees, has been a supporter of the Laboratory since 1990. We are most appreciative of these very generous planned gifts.

President's Council

The President's Council was formed four years ago in an effort to bring together a small group of individuals who have a keen interest in science and the work of CSHL. Through their annual commitment of $25,000, the members provide support for the Cold Spring Harbor Fellows program. The funding is critical in attracting top-notch young scientists fresh from their Ph.D. studies. It allows them to embark on an independent research career, rather than assisting in the laboratory of an established scientist.

A major feature of the President's Council is its annual meeting that brings together this select group of leaders from business, finance, and science to discuss the latest developments in genetics research and biotechnology. The Council's 1997 meeting, held May 16–17, commenced with lunch on Friday at Ballybung and was followed by thought-provoking lectures by Scott Lowe and Ueli Grossniklaus of CSHL. The keynote speaker, Matt Ridley, of the Evolution and Behavior Research Group, University of Newcastle, England, opened the evening session with his talk on Gender Warfare and Evolution. Saturday's highlights included lectures by Rudi Jaenisch, of the Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology; Suzanne B. Cassidy, of the Center for Human Genetics, Case Western Reserve University; and David Haig, of the Museum of Comparative Zoology, Harvard University. The mix of leaders from the business field and the scientific community evoked interesting insights as well as provocative discussions. The meeting ended on Saturday with the guests gathering once again at Ballybung for a parting luncheon. The following are Members of the President's Council:

Abraham Appel, Appel Consultants
Peter Bloom, General Atlantic Partners
James Conneen, A.T. Hudson & Co.
Michel David-Weill, Lazard Freres & Co.
Stefan Englehorn
Leo A. Guthart, ADEMCO
Charles E. Harris, Harris & Harris Group, Inc.
Walter B. Kissenger, WBK Associates
Donald A. Pels, Pelsco, Inc.
George B. Rathmann, ICOS Corporation
Hubert J. P. Schoemaker, Centocor, Inc.
James H. Simons, Renaissance Technologies Corp.
Sigi Ziering, Diagnostic Products Corporation.

Gavin Borden Visiting Fellow

On May 17, Leland Hartwell, Ph.D., now President of Fred Hutchinson Cancer Research Center, delivered the annual Gavin Borden Lecture. Dr. Hartwell discussed the role of yeast genetics in cancer research as part of the Gavin Borden Visiting Fellowship, named for the late charismatic publisher of scientific textbooks. The annual event was initiated in 1995 in honor of Gavin, who died in 1991 of salivary gland cancer, and in an effort to carry on the mission that was so dear to him: the education of graduate students. With this goal in mind, the annual lecture by an inspiring scientist is directed toward that audience, although it is open to all Laboratory staff. During their a 2-day stay at the

Leland Hartwell and Bruce Stillman

Laboratory, Gavin Borden Fellows spend time talking with the graduate students who are currently studying at the Laboratory. Discussions typically involve science, life in science and careers in science.

Major Building Projects

Over the course of 1997, we saw the completion of two very important building projects. The Mary D. Lindsay Child Care Center, which involved extensive renovation of the old De Forest Stables, was readied for its young charges in the late spring. The dedication of the building, which represented the culmination of a decade of effort to secure on-site child care for our employees, was held on Saturday, June 21. Tributes and thanks to Mary Lindsay and the Child Care Capital Campaign Committee were offered by the

Child Care Center Dedication

Laboratory's Director of Public Affairs and Development Susan Cooper, Laboratory President James Watson, and myself. The children of many lab employees, climbing on the colorful geometric playground equipment, made the event complete.

On October 19, we celebrated the completion of the Facilities Department's move to the Richards Building and facilities complex. The Richards Building resulted from the renovation of the old Kurahara house on the west side of Bungtown Road, supplemented by the construction of barns to the east and west of the building. The building was named for long-time director of Buildings and Grounds, Jack Richards, who has now assumed the more focused position of Director of Construction. His projects included major building renovations on a failing campus in the early 1970s, as well as assorted other tasks that fell under his loosely defined job, which in the early days included plowing, mowing, and distributing mail. Jack was responsible for developing a skilled and comprehensive staff of plumbers, carpenters, electricians, and others to serve the Laboratory's growing needs over the years. He also supervised the construction of Ballybung, the President's residence, together with Jim and Liz Watson in 1994. Toasts to Jack were offered by Arthur Brings, Director of Facilities; Raymond Gesteland, former Assistant Director of the Laboratory (1968–1971); contractors Arthur Herman of Herman Development Corporation and George Feraco, Sr. of Abel Grenier Inc. and Feraco Inc.; architect James Childress of Centerbrook, and me.

In 1997, we directed extensive effort toward planning of the Marks Building, which will be home to the new CSHL Advanced Imaging Facility. Centerbrook, the architectural firm recently awarded the American Institute of Architects (AIA) 1998 Architecture Firm Award, the highest honor conferred by the AIA, has been contracted by the Laboratory for this project. Lead architect Bill Grover has been involved in the planning and execution of many building projects at the Laboratory over the years, including Grace Auditorium in 1986; Beckman Neuroscience, Dolan Hall, and Hazen Tower in 1991; and the Watson's residence, Ballybung, in 1994.

The Marks Building will house teaching laboratories, which will further strengthen our educational initiative, and will expand our research initiatives through sophisticated imaging studies of the brain.

Undergraduate Research Program (URP)

In 1997, we received 375 applications—a record number—for the Undergraduate Research Program. The 23 successful candidates, 16 men and 7 women, came from six countries. The program, known as the URP program, was initiated in 1959. Many former URPs have gone on to productive careers in the biological sciences, including David Baltimore, a member of the first class, who went on to share the 1975 Nobel Prize in Physiology or Medicine. The URP program exposes students to hands-on experimental approaches to science and helps lead them to a greater understanding of the issues involved in biochemistry, genetics, and molecular and cellular biology. Participants live and work at the Laboratory for 10 weeks during the summer, so that they are exposed not only to science in the lab, but also to life as scientists.

A list of the students, their schools, mentors, and research projects may be found in the Undergraduate Research Program Section of this Annual Report. Information about the URP program and its alumni may also be accessed through the Labortory's web site at: http://www.cshl.org/URPsite/URP.html

Partners for the Future (PFF)

Each year since 1990, the Laboratory appeals to every Long Island high school science department chairman for the nomination of one student for participation in CSHL Partners for the Future program. In 1997, we were pleased to increase the number of Partners to six, each of whom spends a minimum of 10 hours per week at the Laboratory in October through March, doing original molecular biology experiments under the guidance of a scientist mentor.

The participants for the 1997–1998 school year (and their scientist mentors) are Aaron Bronfman of Syosset, Cold Spring Harbor High School (Bruce Stillman); Arti Anand of Jericho, Jericho High School (Peter Nestler); Bradley Gottfried of Plainview, Portledge School (John Connolly); Elyse Katz of East Setauket, Ward Melville High School (Hong Ma); Nancy Choi of Woodbury, Syosset High School (Michael Hengartner); and Chian Chuu of Bayville, Locust Valley High School (Michael Regulski). Arti Anand was the second Cablevision Scholar under a special Partners for the Future scholarship offered by Cablevision, the first local business to underwrite a portion of this important educational program.

Educational Outreach

The Laboratory continues to take great pride in its educational outreach programs. We remain committed to the philosophy that it is vital to provide positive scientific experiences for young people in order to pave the way to a scientifically literate, and excited, next generation. The Interschool Exchange program continues to provide tours and meetings for students and parents of local private and public schools.

In 1997, we hosted four West Side School Science Nights: "How to Teach a Mutant Fly" (Tim Tully), "Turning Genes On" (Winship Herr), "You Don't Have to Be a Chicken to Lay an Egg" (Ueli Grossniklaus), and "How to Find a Needle in a Haystack" (Peter Nestler). These programs, initiated through West Side School, are now open to all local elementary students, parents, and teachers.

For high school students, we host a lecture series called Great Moments in Science. This year's talks were about gene transcription (Nouria Hernandez), the three-dimensional structure of proteins (Leemor Joshua-Tor), and brain development (Holly Cline). Students from the East Woods School enjoyed a tour and visit with scientist Roberto Malinow. The Cold Spring Harbor High School brought their Japanese exchange students for a tour and luncheon for the third consecutive year.

The Laboratory once again participated in Project WISE—Women in Science and Engineering. Orchestrated by SUNY Stony Brook and funded by a grant from the National Science Foundation, the project involves several Long Island institutions—SUNY Stony Brook, CSHL, Brookhaven National Laboratory, and the American Association of University Women—each helping to expose bright young women to the world of science. The program involves female high school students in 9th through 12th grade, and each year the girls participate in research programs at one of the four institutions. In 10th grade, they come to CSHL for a research experience in molecular biology and genetics under the guidance of scientist mentors.

Community Outreach

Several initiatives through our Department of Development continue to go well. The Next Generation Initiative (NGI) is a series of lectures and tours designed to inspire interest in basic research in people in their 30s and 40s. The Young President's Organization (YPO) provides similar experiences for young leaders of industry and companies, and the Harbor Society is a small group of Laboratory supporters who have contributed to the Laboratory's planned giving program.

For the general public, the Laboratory holds lectures and concerts throughout the year. Jan Witkowski continues to host Lloyd Harbor Seminars at the Banbury Conference Center, and periodically, scientists who are attending scientific conferences at Banbury will deliver a public talk on their area of expertise in Grace Auditorium. In April, Mary-Claire King did just that with her talk, "Breast Cancer Update." Dr. King, professor in the Division of Medical Genetics at the University of Washington in Seattle, was responsible for locating the first breast cancer gene, *BRCA1*; she gave her lecture while participating in *The Biology of BRCA1* meeting at the Banbury Center.

In October, Dr. Svante Paablo, Professor of General Biology at University of Munich, presented a lecture entitled "DNA, Neandertals, and Us." In November, we presented the third Cold Spring Harbor Laboratory Lyme Disease Forum. Moderators of the discussion were Steven E. Schutzer, M.D., of the New Jersey Medical School Department of Medicine, and John Dunn, Ph.D., of Brookhaven National Laboratory's Department of Biology. The speakers, Patricia K. Coyle, M.D., of the University Hospital at Stony Brook Department of Neurology and Raymond Dattwyler, M.D., of the University Hospital at Stony Brook Department of Allergy and Lyme, discussed current research and clinical treatment of the disease and answered questions from the audience.

Concerts

In 1997, we hosted seven concerts, each by one or more outstanding young classical musicians. Pianists, violinists, cellists, and sopranos played to audiences of scientists,

Benjamin Loeb (piano) and Allison Charney (soprano)

Top row: Art Brings, Bruce Stillman, Dave Micklos, James Watson, David Beach, Susan Cooper
Bottom row: Cliff Sutkevich, Patricia Bird, Terri Grodzicker, Elaine Gaveglia, Barbara Terry

staff, and neighbors. These concerts began as cultural refreshment for scientists who were at the Laboratory for several days or more attending scientific conferences. The caliber of the musicians has made these concerts an attractive event for our staff and neighbors as well and we have been delighted to provide great musical performances, usually for no charge. Performances in 1997 included: April 26, Alexander Velenzon on violin and Inessa Zaretsky on piano; May 10, pianist Freddy Kempf; May 24, pianist Wendy Chen; June 13, pianist Misha Dichter; August 30, The Laurel Trio with SunghaeAnna Lim on violin, Amy Levine Tsang on cello, and Dena Levine on piano; September 6, pianist Benjamin Loeb and soprano Allison Charney; and on September 20, pianist Jon Klibonoff.

Long-term Service

A pool-side dinner at Robertson House on June 11 marked the anniversaries of several long-term employees of the Laboratory. Susan Cooper, Director of Public Affairs and Development, and Terri Grodzicker, Assistant Director of Academic Affairs, both celebrated their 25-year anniversaries. Over the years, Susan evolved from librarian, to marketing for the CSHL Press, to Director of the Library, then Director of Public Affairs, and finally took on Development as well. Her departure to become Director for Institutional Advancement at the Trudeau Institute in upstate New York (see Changes in Administrative Staff, below) left many holes to be filled at the Laboratory. Terri Grodzicker, who came to Cold Spring Harbor as a scientist in Joe Sambrook's James lab, went on to become a staff scientist and then Assistant Director of Academic Affairs and editor of *Genes & Development*.

Celebrating 15-year anniversaries were investigator David Beach, scientific secretary Patricia Bird, Director of Facilities Art Brings, typesetter Elaine Gaveglia, DNALC Director David Micklos, manager of equipment repair Clifford Sutkevich, and circulation manager Barbara Terry.

Changes in Administrative Staff

In January, Nancy Ford concluded 24 years of dedicated service to the CSHL Press. Nancy was instrumental in developing the publications program, and she worked extremely well with a wide variety of well-respected scientists to create many landmark publications in molecular biology. Her professionalism and dedication were of great value to the Press and the Laboratory for without Nancy many of the distinguished volumes produced by the Press would not have been as great as they are. Nancy's contributions to the Press will long be remembered.

Susan Cooper, Director of Public Affairs, Development, and the Library exceeded her title by a good measure in her role as confidant and friend to many, in particular Jim Watson. Her decision to accept the position of Director for Institutional Advancement at the Trudeau Institute came as a great surprise, although it is not difficult to see why Trudeau would have courted her. Susan worked double-time, with unparalleled dedication and true devotion. She arrived here in 1972 as head librarian in the Laboratory's Carnegie Library, and she grew up with the institution, later adding the roles of Director of Public Affairs and then Director of Development. Susan orchestrated and carried out the Lab's spectacular centennial celebration nearly a decade ago, as well as planning ceremonies and booklets for the Lab's many building dedications over the years. She had a remarkable rapport with our friends, neighbors, and supporters, and she helped foster the careers of many of our scientists. Susan's vitality and enthusiasm made the Laboratory an enjoyable and interesting place to work. That she has "left home" to make her mark elsewhere is saddening to many of us, but we wish her and her husband Bob the best and much success.

Nancy Ford at her retirement luncheon.

Susan Cooper in her CSHL office.

Changes in Scientific Staff

Departures

Carol Greider moved on to a position as Associate Professor with Johns Hopkins University School of Medicine, Department of Molecular Biology and Genetics, in Baltimore. Carol's work with telomeres and telomerase, the enzyme that regulates their length, earned her much scientific and popular acclaim during her years at Cold Spring Harbor. Carol came to the Laboratory in 1988 as a Cold Spring Harbor Laboratory Fellow after completing her Ph.D. with Elizabeth Blackburn at University of California Berkeley, where Carol discovered telomerase as a graduate student. In 1989, Carol was appointed to the staff of the Laboratory, eventually being appointed full investigator. The move to Maryland has worked well for Carol and her family as her husband, Nathaniel Comfort—former Science Writer at CSHL—simultaneously accepted an assistant professorship at George Washington University, in Washington DC, after completing his Ph.D. in the history of science.

X-ray crystallographer Xiaodong Cheng left for a position with Emory University in Atlanta, Georgia. Xiaodong came to us as a postdoctoral researcher in 1990 and worked for 2 years in Jim Pflugrath's lab. He became a staff member in 1992 and did much ground-breaking work with DNA methyltransferases and methylation during his time here, including the discovery of the phenomenon called "base flipping," whereby a single base turns outward from the DNA helix—in effect opening the DNA at a given point.

Erich Grotewold, who arrived here as a postdoctoral researcher in 1989, left for a position at Ohio State University, in Columbus.

Tom Marr, a member of the Laboratory's bioinformatics team since 1989, has completed the transition from CSHL to president and C.E.O. of Genomica Corporation in Boulder, Colorado.

Joe Colasanti, research investigator who came to do postdoctoral research with Venkatesan Sundaresan in 1988, has gone on to The University of California at Berkeley, Plant Gene Expression Center, as visiting assistant research geneticist.

Masafumi Tanaka, staff investigator who came as a postdoctoral researcher in 1986 and joined the staff in 1988, moved on to Tokai University School of Medicine in Japan.

Arrivals

Neurobiologist Karel Svoboda joined our scientific staff in June. Karel studied physics at Cornell University and biophysics at Harvard, where he used a technique called laser-optical tweezers to measure the force generated by individual molecular motors. More recently, at Bell Laboratories in New Jersey, he used two-photon excitation laser scanning microscopy to obtain never-before seen images of neurons in living brains. Two-photon excitation laser scanning microscopy is an emerging imaging technology; it utilizes the tremendous concentrations of light achievable with pulsed-laser light sources to "excite" fluorophores by two-photon absorption. Karel is applying his knowledge of this technique to the establishment and further development of a state-of-the-art neural imaging facility at CSHL.

David Jackson arrived in September, after doing postdoctoral research in Sarah Hake's lab at the Plant Gene Expression Center in Berkeley, California. David is a maize

geneticist; he has been using transposons—Barbara McClintock's jumping genes—to study such phenomena as development of flowering plants, including the formation of leaves from a small group of cells called the meristem. In addition, David is studying intercellular transport, the movement of proteins and other molecules from one plant cell into another.

Andy Neuwald joined us in November, from the National Institutes of Health, National Center for Biotechnology in Bethesda, Maryland. Andy is a computational biologist and is interested in the development and use of statistical and algorithmic methods to classify and model protein domains and is also working on the development of a comprehensive database in which to log the resulting data.

Promotions

Yi Zhong, a staff member with our neurobiology program since 1992, was promoted to Associate Investigator. Bill Tansey, a postdoctoral researcher in Winship Herr's lab since 1992, was appointed Assistant Investigator in 1997, and he is now combining two active areas of research, gene transcription and cell cycle control. Doug Conklin, a postdoctoral researcher in David Beach's lab since 1993, was promoted to the position of Senior Fellow. Postdoctoral researchers Neilay Dedhia from Dick McCombie's lab, Robert Lucito of Michael Wigler's lab, and Elly Nedivi of Hollis Cline's lab were each appointed Research Investigator. Graduate student John Connolly completed his Ph.D. from the Massachusetts Institute of Technology while working in Tim Tully's lab and is now doing postdoctoral research in the Tully lab.

In addition, two visiting scientists have joined our staff: Clifford Yen and Masaaka Hamaguchi, both of whom were visiting Michael Wigler's lab, have each been appointed Research Investigator.

Visiting Scientists

Nine visiting scientists wrapped up their sojourns to CSHL: Aiping Dong, visitor to Xiaodong Cheng's laboratory, has moved to a position as visiting scientist at Emory University in Atlanta, Georgia; Konstantin Galaktionov, who came from Leningrad, USSR in 1988, left David Beach's lab to accept an assistant professorship at Baylor College of Medicine in Houston Texas; and Roberta Maestro, in David Beach's lab since 1995, returned to CRO in Aviano, Italy. Eli Hatchwell, a clinician from Wessex Clinical Genetics Service in Southampton, UK, spent 6 months in Michael Wigler's lab applying RDA to his research and has since returned to the UK. Liam Dolan returned to the John Innes Center in Norwich, UK, from Rob Martienssen's lab, and Ross Bicknell concluded his visit with Ueli Grossniklaus, to return to Crop & Food Research in Christchurch, New Zealand.

Several more visiting scientists have arrived. Jiaxin An, of the China Academy of Space Technology in Beijing and Zuoping Xie of Tsinghua University, also in Beijing, have both come to do research in Yi Zhong's lab. Ming Huang of Otsuka America Pharmaceutical Inc. is visiting Michael Zhang's lab, Nobuhiro Kashige of Fukuoka University, Department of Pharmacological Science in Kukuoka, Japan, is working in Ryuji Kobayashi's lab, and Tatyana Michurina from the Institute of Developmental Biology in Moscow, Russia is visiting Grigori Enikolopov's lab.

Postdoctoral Departures

Stephen Buck, Siyuan Le, Bong-Kyeong Oh, and Michael Rudd moved with Carol Greider to Johns Hopkins University in Baltimore, MD, to continue their postdoctoral studies in her lab, and Maria Blasco, also of Carol's lab, went to a position as Staff Investigator at the Universidad Autonoma De Madrid.

Weimin Gong and Yu Liu transferred with Xiaodong Cheng to Emory University School of Medicine, Atlanta, GA, to continue their postdoctoral studies in his lab, while John Horton went with Xiaodong to Emory to become an Assistant Professor there. Margaret O'Gara and Xujia Zhang of Xiaodong's lab each moved on as well; Margaret accepted a position as scientist with Pfizer Ltd., in Sandwich, UK, and Xujia became a researcher at the Biophysics Institute, in Beijing, China.

From the Stillman lab, Gerhard Cullmann became a Project Leader at Connex, in Germany; Kim Gavin stayed on to become an Editorial Assistant for the CSHL Press; Masumi Hidaka accepted a position as Assistant Professor at the National Institute for Basic Biology in Okazaki, Japan; Masayoshi Iizuka became an Assistant Professor at the National Cancer Center in Tokyo, Japan; and Caroline Mirzayan took a position as Assistant Professor at the University of Aarhus, in Denmark.

From David Beach's lab, Kang Dai accepted a position as scientist with Chiron Company, Walnut Creek, CA; Manuel Serrano went on to become Staff Investigator with Centro Nacional De Biotechnologia in Madrid, Spain; and Amancio Carnero has moved to David's new laboratory at the Institute for Child Health in London.

From Hollis Cline's lab, James Edwards is continuing his postdoctoral studies at Virginia Commonwealth University, in Richmond, VA, and Gang-Yi Wu is doing the same at Stanford University in CA. From Hong Ma's lab, Hai Huang went on to become Professor at Shanghai Center of Life Science in China, and Hua-Ying Fan is continuing postdoctoral studies at New York University Medical Center. From Nick Tonks' lab, Salim Mamajiwalla became a patent agent with Blake, Cassels & Graydon, in Unionville, and Tony Tiganis is now a research officer with St. Vincent's Institute of Medical Research in Victoria, Australia.

From Dick McCombie's lab, Arthur Johnson became Sequencing Facility Director at North Carolina State University, Department of Forest Biology, in Raleigh, NC, while Muhammad Lodhi accepted a position as senior scientist with Sequana Therapeutics in LaJolla, CA. From the Krainer lab, Javier Caceres went on to become Group Leader at the MRC, Western General Hospital in Edinburgh, Scotland, and David Horowitz became Assistant Professor at the Uniformed Services University of the Health Sciences in Bethesda, MD.

Derek Gordon left Tom Marr's lab to continue postdoctoral studies at Rockefeller University, in New York, NY. Bing Guo went from Kim Arndt's lab to continue postdoctoral research at the Whitehead Institute in Cambridge, MA. Sui Huang left David Spector's lab to accept a position as Assistant Professor at Northwestern University Medical School in Chicago, IL, and Peter Lorenz is continuing postdoctorial research at University of Rostock, Institute of Immunology in Rostock, Germany. Stephen Brand left Mike Mathews' lab to accept a Clinical Research Scientist position with Cato Research in Durham, NC. Thillai Koothan of the Malinow lab is continuing postdoctoral research at the Mayo Clinic in Jacksonville, FL. Patricia Springer of Rob Martienssen's lab is now an Assistant Professor at the University of California, Riverside, and Mee-wa Wong of Nouria

Hernandez's lab is now at the University of Texas. Qizhi Wang finished up in Mike Mathews' lab, and Christopher Jones and Gert Bolwig left Tim Tully's lab: Christopher accepted a postdoctoral position at the University of Tennessee and Gert became an Assistant Professor at the Institute of Human Genetics in Denmark.

Graduate Student Departures

Nick Carpino went from Ryuji Kobayashi's lab to do postdoctoral research at St. Jude's Children's Hospital in Memphis, TN, and Chong Huang graduated from Winship Herr's lab and accepted a postdoctoral research position at the Molecular Neurogenetics Unit of Massachusetts General Hospital in Boston, MA.

Two graduate students made the transition to medical school and one joined the computer world, Benjamin Lee of Winship's lab is now a medical student at SUNY Stony Brook School of Medicine in NY, and Aneil Shirke of Roberto Malinow's lab is now a medical student at University of Iowa in Iowa City, IA; Jian Sheng of Yi Zhong's lab has become a student in the Computer Department at SUNY Stony Brook.

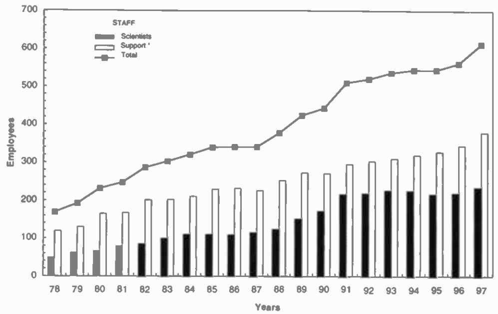
Doug Mason went to continue graduate studies with Carol Greider at Johns Hopkins School of Medicine.

A Busy Agenda

Cold Spring Harbor Laboratory has never rested on its past accomplishments. Throughout the history of the Laboratory, the introduction of new programs such as the scientific meetings in the 1930s, the laboratory courses in the 1940s, the Banbury Conference Center in the 1970s, and the DNA Learning Center in the 1980s has transformed and strengthened the institution. I am pleased that we have a vigorous research agenda and that the educational programs at the Laboratory continue to go from strength to strength. We have chosen to undertake several major initiatives this year: expansion of our research facilities, fostering a local biotechnology industry, and growth of our educational mission by developing a graduate school of biological sciences. Each of these objectives presents a great challenge, but they are all necessary to ensure that Cold Spring Harbor Laboratory remains a vital and exciting place to live and work. We have a very busy time ahead, but I am confident that we can efficiently incorporate these new programs into our existing infrastructure while maintaining a high standard of excellence and a leading role in biology and the biomedical sciences.

April 1998 **Bruce Stillman**

* Consists of Full time and Part-time Technical Support, Core Services, Publications, Meetings, Library, Public Affairs, Building and Grounds, Administrative Personnel, Banbury Center and DNA Learning Center



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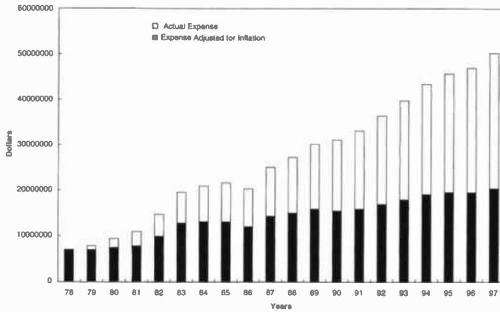
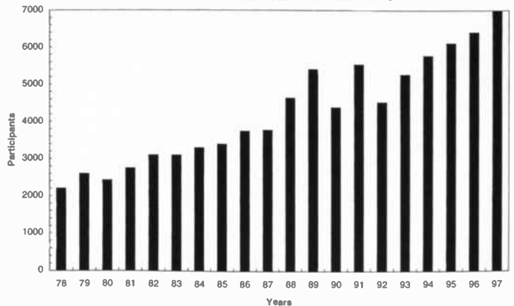


MEETINGS & COURSE PARTICIPANTS



ADMINISTRATION

As the century draws to a close, there is a growing sense among the public that biologists are making real progress in the War on Cancer, the Human Genome Project, and the great promise of biotech. The renewed public confidence in science is reflected in Congress, which now seems likely to vote substantial increases in federal funding for basic research. Here at Cold Spring Harbor Laboratory, it is exciting to know that many of our research and education programs have been at the heart of the discoveries and strategies that now seem likely to usher in a new golden age for biology.

From an administrative viewpoint, 1997 was a good year for the Laboratory. Financial results were positive with operating revenues reaching a new high of nearly $50 million. There was a surplus from operations of $317,000 after fully allowing for depreciation of $3,370,000. The operating budget for the year had been conservatively stated and was bettered by some $870,000. There was a positive cash flow from operations of $3,688,000. Sustaining a positive cash flow of this magnitude is extremely important if we are to maintain and modernize facilities, and to purchase the ever more expensive scientific equipment that has become a necessity for any world-class research center.

The better-than-anticipated financial results for the year were driven by the success of our scientists in obtaining grants, increased corporate sponsorship of our research, good attendance at meetings and courses, higher income from tech transfer and investments, and careful departmental control of costs. There were also substantial savings in electricity costs which are providing a rapid return on the major capital investment made in energy-saving programs in recent years.

The year 1997 was not without challenges, however. Early in the year, it became clear that a reorganization of the CSHL Press was needed, which required some painful reductions in staff. The result has been positive, with a much-improved ability to meet production schedules for new books. As in past years, book sales from the back list were very strong, and journal subscriptions and advertising revenues increased nicely. By year-end, the Press had published 18 important and beautifully produced new books, most notably *Mutants of Maize*, *Cells*, and *Retroviruses*, and it had achieved a small surplus before the reorganization expense.

During 1997, several relatively senior members of the scientific staff made transitions to other institutions. Such departures are a sad but sometimes unavoidable result of the efforts of other institutions to recruit from the exceptional scientific talent at the Laboratory. We have come to realize from experience that such turnover constantly refreshes our research programs with new young scientists who will make the next series of unexpected discoveries. On a practical basis, however, the departure of senior scientists also means the departure of grants that must be replaced from scarce internal Laboratory resources while newly recruited scientists begin the research that will ultimately enable them to compete effectively for federal funding. In addition, the Laboratory must pay for the expensive redesign and equipping of the new scientists' labs.

A further challenge has been the escalating cost of providing the data-processing infrastructure that in recent years has become so important to biologists. Gene sequencing, protein studies, and other new high-tech methodologies are triggering an enormous

flood of new data that must be managed and analyzed to be useful. The Laboratory has made major investments to provide a high-speed Internet connection and a campus-wide, fiber-optic local area network that is supported by a professional Information Services Department. It will be a continuing challenge to keep up with the growing demand for these services.

In 1997, The Laboratory's endowment once again achieved handsome gains as a result of good market returns and new additions. The endowment consists collectively of the Robertson Research and Cold Spring Harbor Funds and is our primary source of internal funding for research. At year end, the market value of the endowment was $144,098,000, a 12-month increase of over $14 million. The funds are invested in a balanced mix of equities, fixed-income securities, and short-term instruments. The endowment is managed by a team of investment professionals—Essex Investment Management Company, Inc., Miller Anderson & Sherrerd (a subsidiary of Morgan Stanley Dean Witter), U.S. Trust Company, and the Vanguard Prime Cap Fund. Over the years, all segments of the funds have achieved very competitive returns when compared with benchmark indices. The growth of the endowment has been enhanced by a conservative Laboratory drawdown policy of 4% per annum, based on a 3-year moving average of previous year-end market value. In very strong markets such as those of recent years, the drawdown has been held to less than targeted levels. In 1997, it was 3.7%. As a result, the endowment has more than kept up with inflation and has made it possible to expand the overall science program and react positively and quickly to opportunities created by new discoveries.

A very important component of the endowment is the Science Fund, which was established by the Board of Trustees in 1992. On December 31, 1997, it totaled $5,452,000. The fund holds a portion of royalties and all of the equity positions received by the Laboratory in return for the transfer of intellectual property to various biotech companies based in whole or in part on CSHL technology. There are now 13 such companies, 4 of which have public markets. The estimated value of these equity positions on December 31, 1997, was $3,748,000, which is believed to be very conservatively stated. The Trustees have designated the Science Fund to be used for the support of future science, and it is hoped that as some of these ventures mature, their value will become an important source of funding for the Laboratory research. In this manner, the value of intellectual property created today may be recycled in the years ahead as future support for science.

The development of a strategy to manage intellectual property at the Laboratory has been primarily the responsibility of John Maroney, Director of Technology Transfer. John and his associate Carol Dempster interact with the scientists, patent attorneys, and venture capitalists to make viable commercial ventures of the Laboratory's discoveries; the very good results of their efforts speak for themselves. The Laboratory is now thought of as a leader in the management of intellectual property and its practices are being emulated by many other research centers.

The Buildings and Grounds Department, led by Art Brings, had another very productive year marked by the dedications in June and October, respectively, of the Mary D. Lindsay Child Care Center and the Richards Building and facilities complex. A high point of the latter was the marvelous music provided by the Corinthians, led by Bill Grover of Centerbrook Architects, of Essex, Connecticut, who played a mean saxophone. Bill's

presence reminded us all of the enormous contributions that he, Jim Childress, and so many of the other Centerbrook architects and technicians have made to the style and beauty of our campus. So it was with great pleasure that we heard early this year that Centerbrook had been named the recipient of the 1998 Architecture Firm of the Year Award by the American Institute of Architects.

Other important Building and Grounds projects included the $1.7 million replacement of the complete HVAC systems in Demerec Laboratory and Bush Auditorium, which was accomplished, with the help of a cool summer, without shutting down the facilities. The lighting systems in these buildings and in Delbrück Laboratory were also upgraded, and throughout the Laboratory, all high-horsepower variable-speed drives were replaced. Savings from these renovations and previously completed energy savings programs should exceed $300,000 on an annual basis. The former Mathews' laboratory in Demerec was completely rebuilt, and in Beckman Center, a laser lab, wet lab, and office were created for Karel Svoboda from previous storage areas. In 1999, Karel will move his new photon laser imaging technology to the Edward and Nancy Marks Imaging Building, which is now under construction. The new imaging technology makes it possible for the first time to study individual neurons and circuits in the brain of living and behaving animals.

Much appreciated by the Laboratory staff are two newly constructed sand volleyball courts and a state-of-the-art fitness center. Under the very able direction of Dan Miller, the overall Laboratory grounds have never looked better. There is much new landscaping on the north campus near Ballybung, and Airslie, and around the new Lindsay Child Care Center and the Richards Building and facilities complex. Much credit should also be given to the people who provide the constant internal and external maintenance of our more than 50 buildings on the main campus, Banbury Center, the DNA Learning Center, and at Uplands Farm.

During the year, there were significant changes in the leadership of important administrative areas—development, public affairs, and library services. These were previously the sole responsibility of Susan Cooper, who has moved on to a new challenge as Director of Institutional Advancement at the Trudeau Institute in Saranac Lake, New York. As discussed earlier in the Highlights section of this report, Susan contributed greatly to a broad spectrum of Laboratory activities and she is sorely missed. Prior to her departure, it had been decided to separate the departments of Development and Public Affairs and recruit new leadership for each. After an extensive search, we were successful in attracting Rick Cosnotti, who joined us in November as Chief Development Officer. Rick has had many years of professional experience with major gifts and capital campaigns with the well-known development consulting firm of Ketcham, Inc. Most recently, he was Director of Development of the Episcopal Theological Seminary of New York. After a short stay in a Williams House apartment, Rick and his wife Shari, who is the new Curate at St. John's of Cold Spring Harbor, are now happily moved into the Galehouse residence on the spectacular waterfront between Airslie and Ballybung. As our new Chief Development Officer, Rick will have his hands full with the large and important fund-raising tasks immediately ahead.

To direct Public Affairs, we have been fortunate in attracting Deborah Barnes, a frequent visitor in the past to Laboratory meetings. Deborah has a Ph.D. in biology from Georgetown University, was a postdoc at Harvard Medical School, taught biology, wrote

for the news department of the journal *Science*, and was Editor of the *Journal of NIH Research.* She is now completing several assignments for the National Institutes of Health and will join the Laboratory on a full-time basis in July. Her primary focus at the Laboratory will be to increase the visibility of our research and education programs with the media and to make our community outreach programs even more interesting.

For the Library, the task was relatively easy. We already had the able services of Margaret Henderson as Head of Library Services, and we confidently appointed her as the new Director of Libraries. Margaret will undertake a thorough study of our future library needs, including the implications and fast-growing requirements of electronic media. With the help of a consultant, she will draw up a plan for improving the organization and usefulness of the very important archives of the Laboratory's 108-year history.

As the Laboratory continues to grow, the task of managing its finances has become more complex. Our long-term Controller, Bill Keen, can be counted on to make all surprises pleasant ones, and he deals most efficiently with the frequently changing standards for not-for-profit fund accounting. In October, Bill's assistant Kathy Didie, moved to a position at CMP Media, Inc. We are fortunate to have as her replacement Lari Russo, who joined the Laboratory in January, in time to be much involved in the year-end closing, annual audit, and 1998 budget.

The Grants Management, Human Resources, and Purchasing Departments, under the leadership of Susan Schultz, Cheryl Sinclair, and Phil Lembo, respectively, each year provide effective and very necessary support across a broad spectrum of Laboratory activities. Roberta Salant, Administrative Assistant to both John Maroney and me, also manages the often-complex task of providing for the meeting arrangements and other needs of our Board of Trustees and its many committees.

As we look forward to the years immediately ahead, there are several important new projects in various stages of development that would substantially expand the scope of the Laboratory's activities. In November, the Board of Trustees, subject to the approval of the N.Y. State Board of Regents, decided to establish a graduate degree-granting program at the Laboratory. A graduate school would complement our long-standing program with SUNY Stony Brook, which currently involves approximately 50 students. The new CSHL graduate school would be a unique accelerated program with a matriculating class of about five students of exceptional caliber each year. As such, it would require only minor refurbishment of existing facilities on our Laurel Hollow campus.

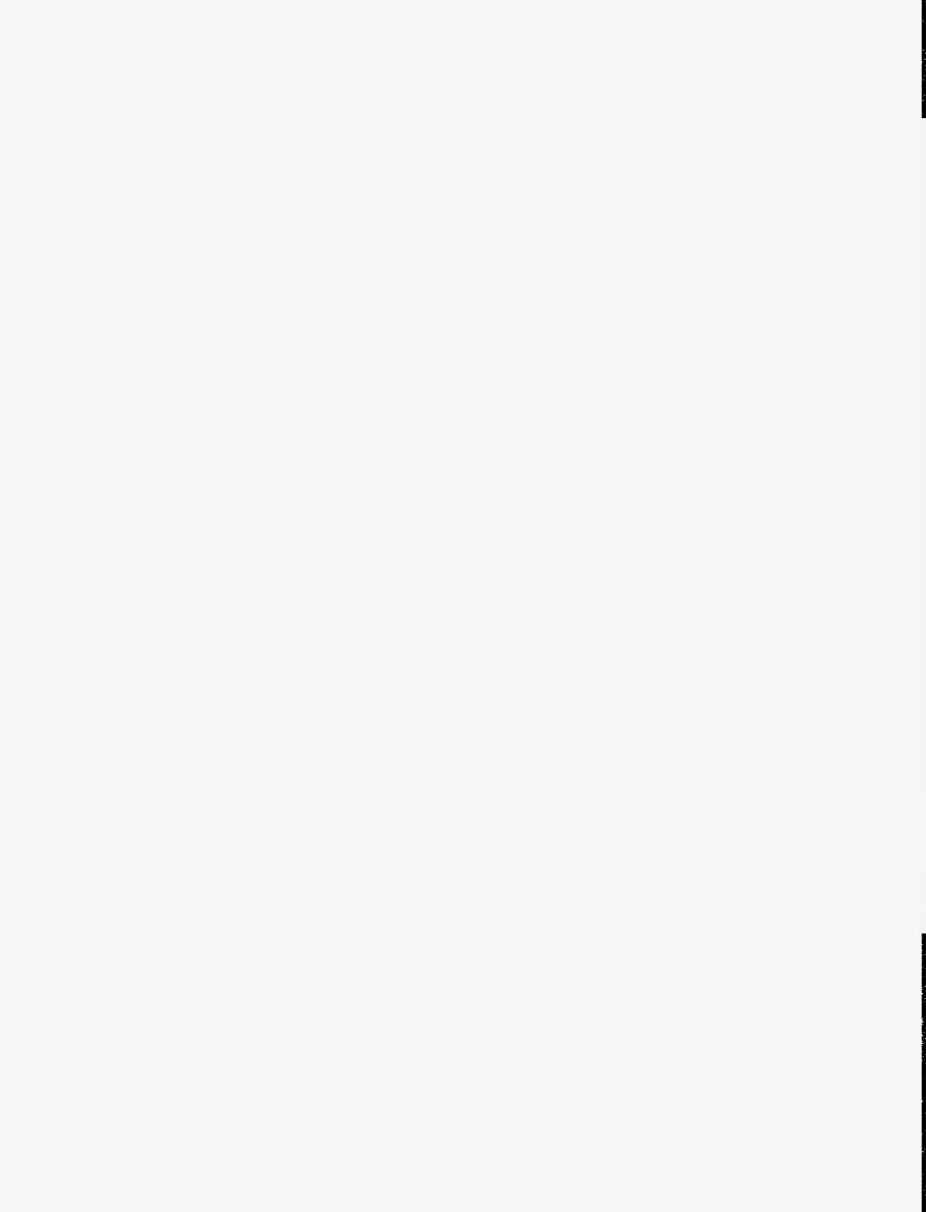
The Board of Trustees has also authorized the purchase of a 60,000-sq. ft. building located on 12 acres in Woodbury near the Long Island Expressway. This facility would be used by the Laboratory as a high-tech center for gene sequencing and DNA chip technologies and as a home for the editorial and fulfillment activities of our academic press.

In March, Governor George Pataki came to Long Island and announced his support for a CSHL-associated Biotechnology Park. The Park would be fully financed by the State of New York and located on property owned by SUNY at Farmingdale on Route 110, about 20 minutes driving time from the Laboratory. The Park will be designed to attract young companies that are commercializing new technologies from the Laboratory, as well as other companies that are located elsewhere but would value close association with the Laboratory's research and education programs. The 1998 New York State Budget, which has now passed, includes approximately $14 million for this project.

As we consider the challenges and opportunities inherent in these and other programs, we have decided to increase the depth of administrative management at the Laboratory. We are very pleased that W. Dillaway Ayres, a resident of Laurel Hollow, has agreed to join us as Associate Administrative Director. Dill is well known in the community and at the Laboratory, where he has served as a Director of the CSHL Association. He is a graduate of Princeton University and received an MBA from the Columbia University Graduate School of Business Administration. He has extensive business experience in the areas of corporate planning and finance.

All in all, it is a very exciting time at the Laboratory. Our science and education programs are first-rate, and we have both the management and financial strength to be confident of remaining a world center of biology.

G. Morgan Browne
Administrative Director



Row 1: Benjamin Boettner; Jana Steiger, Peter Nestler; Qiong Liu; Vickie Tu

Row 2: Jenny Wang; Zach Mainen; Kim Ivarson; Keiichi Shibahara

Row 3: Tracy Kuhlman; Alex Rai; Mona Spector; Shirley Pinto

Row 4: Douglas Conklin; Christine Berthier

Row 5: Marcia Belvin; Ana Losada, Keiji Kimura; Troy Messick

RESEARCH

TUMOR VIRUSES

A unifying feature of the investigations in the Tumor Viruses Section is the use of viruses to probe cell function and to understand the processes affected during cell transformation. The viruses used include adenovirus, human and bovine papillomaviruses, SV40, herpes simplex virus, and human and simian immunodeficiency viruses. Arne Stenlund and Bruce Stillman study DNA replication of papillomavirus and SV40, respectively. The Stillman laboratory complements studies of SV40 DNA replication with studies of cellular DNA replication in human and yeast cells. Winship Herr and Masafumi Tanaka, Adrian Krainer, and William Tansey study control of gene expression in human cells, in particular the control of transcription, pre-mRNA splicing, and protein stability, respectively. Yuri Lazebnik and Scott Lowe study how programmed cell death or apoptosis is regulated in normal and cancer cells, and how changes in the regulation of apoptosis influence the response of cancer cells to chemotherapy. Jacek Skowronski studies virus/host-cell interactions with human and simian immunodeficiency viruses, probing how the viral protein Nef disrupts multiple aspects of signal transduction in T cells. The synergy between the study of cellular processes and the study of how viruses affect those processes is readily apparent from a reading of the following research descriptions.

DNA SYNTHESIS

B. Stillman

S. Waga, J. Chong, G. Cullmann, V. Ellison, M. Hidaka, M. Iizuka, C. Liang, J. Mendez, C. Mirzayan, K. Shibahara, K. Simpson, A. Verreault, M. Weinreich, Y.-C. (Nancy) Du, K. Gavin, H. Yang, L. Zou, M. Waga, P. Wendel, C. Driessens

This year, our studies on the replication of DNA and chromatin assembly in eukaryotes have progressed rapidly with advances made in a number of areas. More details have emerged about the proteins that synthesize DNA at the replication fork and how the cell cycle regulator protein, p21, controls the function of the proliferating cell nuclear antigen (PCNA). We have also found that the papillomavirus transforming protein, E7, modulates the function of the p21 protein, reversing its PCNA inhibiting functions.

Our studies on chromatin assembly have focused on the role of the chromatin assembly factor, CAF-1. The year saw a major advance with the purification and characterization of CAF-1 from the budding yeast, *Saccharomyces cerevisiae*. Although CAF-1 was not essential for cell division, the inheritance of epigenetically determined transcription states of chromatin was compromised. In human cells, we demonstrated that CAF-1 exists in a complex with newly synthesized histones H3 and H4 that are modified by acetylation. Finally, we have purified the human histone H4 acetyltransferase (HAT1) and have demonstrated that it contains an essential histone-binding protein that is highly related to a histone-binding protein subunit of CAF-1.

In another important development, we have developed a chromatin-binding assay for DNA replication proteins from *S. cerevisiae* and demonstrated that the cellular initiator protein, ORC, is bound to yeast chromosomes throughout the cell cycle. In contrast, the mini-chromosome maintenance (MCM) proteins and the Cdc45 protein (Cdc45p) are associated with chromatin in a cell-cycle-regulated manner. Particularly interesting was the observation that Cdc45p loading onto chromatin required activation of the S-phase-specific cyclin-CDKs that are essential for initiation of DNA replication. Cdc45p loading also required the assembly of ORC, the MCM proteins, and Cdc6p into

a prereplication complex at origins of DNA replication. The assembly of these protein complexes normally occurs only once per cell cycle, and we have provided convincing evidence that the Cdc6 protein is the mediator of this control.

As part of our efforts to understand the mechanisms of initiation of DNA replication in mammalian cells, we have continued to isolate the human ORC proteins and to characterize the human Cdc6p. Studies this year have resulted in the cloning of cDNAs encoding human ORC4 and ORC5, and the demonstration that the human CDC6 gene is regulated by the E2F transcription factor, a finding that links initiation of DNA replication to the cell cycle regulatory machinery essential for commitment to cell division.

SV40 DNA Replication

V. Ellison, M. Waga, S. Waga, B. Stillman

This marks the 13th year that we have studied the mechanisms of DNA replication using simian virus 40 (SV40) as a model system. Three years ago, we reported the reconstitution of complete SV40 DNA replication with purified proteins, together with a fairly extensive description of the mechanisms of DNA synthesis at the replication fork. Apart from the virus-encoded T antigen, all of the proteins were from human or bovine cells. Some of the interesting mechanisms that occur at the replication fork, for synthesis of both leading (continuously synthesized) and lagging (discontinuously synthesized) strands, are the steps that require the proliferating cell nuclear antigen (PCNA) and replication factor C (RFC). This involves loading of a trimer of PCNA, the clamp that ties the DNA polymerase to the template DNA, by the RFC ATPase protein. To facilitate further mechanistic studies on this step, a better understanding of the regulation of clamp loading and the extensive protein-protein interactions between PCNA and other proteins that function at the replication fork, we have expressed recombinant human RFC in various baculovirus vectors and have purified the recombinant RFC (rRFC). The five-subunit rRFC has all of the same activities as the RFC purified from human cells, including DNA- and PCNA-activated ATPase activity, DNA binding, and an ability to support SV40 DNA replication in vitro. The large 140-kD subunit protein is essential for the PCNA-stimulated ATPase activity, although a four-subunit complex containing the RFC small subunits (40 kD, 38 kD, 37 kD, and 36 kD) and a three-subunit complex (40 kD, 37 kD, and 36 kD) have DNA-activated ATPase activity that cannot be stimulated by PCNA. The rRFC expressed from the five separate baculovirus vectors will enable us to characterize the role of RFC more extensively.

A new PCNA clamp loading assay requiring ATP, replication protein A (RPA), RFC, and a single-stranded DNA with an annealed primer was developed and used to demonstrate that RFC remains bound to the PCNA–DNA complex after it loads PCNA onto the DNA. We have shown previously that the cell cycle regulator protein p21 blocks PCNA-dependent DNA replication, and using the new DNA-binding assay, we have now shown that p21 becomes part of the PCNA–DNA complex via its interaction with PCNA. p21 remains stably bound to the PCNA and stimulates the dissociation of RFC from the DNA–protein complex. These results suggest that p21 blocks a PCNA-dependent step during either polymerase loading or subsequent polymerase activity. Since p21 binds independently to cyclin-dependent protein kinases, the new data suggest the possiblity that the p21–PCNA complex on the DNA attracts these cell cycle regulators to the DNA. These interactions are currently being tested.

In a productive collaboration with Denise Galloway and her colleagues at the Fred Hutchinson Cancer Center in Seattle, we have shown that the human papillomavirus (HPV) E7 protein can abrogate the effect of the p21 protein on PCNA replication functions. The E7 protein from the highly oncogenic HPV type 16, but not the nononcogenic HPV type 6, can block the p21-induced inhibition of PCNA stimulation of the DNA polymerase and can also block the inhibitory effect of p21 on the human cyclin-dependent protein kinases. Thus, the oncogenic form of the E7 protein targets the p21 protein, which is regulated by the tumor suppressor protein p53, by altering PCNA function in much the same way as the E7 protein blocks the function of the retinoblastoma (Rb) tumor suppressor. These results provide further biological evidence for a role for the p21–PCNA interaction in modulating DNA replication and thereby effecting genome stability.

As a result of a successful sabbatical visit to Cold Spring Harbor by Nicholas Muzyczka some years ago, we have continued to collaborate with Nick and his colleagues (University of Florida, Gainesville), who have demonstrated that adeno-associated virus (AAV) DNA replication can occur in human cell extracts in the absence of adenovirus proteins. Many of the same

human cell proteins that support SV40 DNA replication are also used by AAV to replicate the viral genome, along with the AAV-encoded Rep78 initiator protein. These cellular proteins include RPA, PCNA, RFC, and as yet unidentified proteins that are not required for SV40 DNA replication.

Replication-dependent Chromatin Assembly

K. Shibahara, A. Verreault

Concomitant with studies on the mechanism of DNA replication, we have been studying the replication-dependent assembly of chromatin, a process that requires a three-subunit protein called chromatin assembly factor-1 (CAF-1). Last year we reported identification of the CAF-1 genes from the yeast *S. cerevisiae* and the observation that the *CAF* genes are essential for the inheritance of epigenetically determined transcription states of chromatin, such as silencing of genes adjacent to telomeres. Others have extended this observation by demonstrating that CAF-1 is essential for inheritance of the silent state of mating-type genes on chromosome III in the budding yeast. Current studies are aimed at determining whether other epigenetically determined states are dependent on CAF-1 and how CAF-1 links the maintenance of these transcription states of chromatin to DNA replication.

The smallest subunit of CAF-1 in human (p48) and yeast cells (Cac3p) is a member of a family of proteins called the WD40 repeat proteins because of the presence of a repeated amino sequence motif (WD40) in the protein. Another protein from human cells, called p46, is highly related to the CAF-1 p48 subunit and is also similar to a protein in the budding yeast that is a subunit of the histone H4 acetyltransferase, HAT1. We have purified the human HAT1 enzyme and shown that it contains two subunits: one is the p46 WD40 repeat protein and another is an ortholog of the budding yeast acetyltransferase, HAT1. Like the yeast counterpart, the human HAT1 enzyme acetylates histone H4 on lysine residues located at positions 5 and 12 from the amino terminus of the protein. Characterization of the two subunits of human HAT1 shows that the activity of the acetyltransferase catalytic subunit is greatly stimulated by the p46 subunit. Both the HAT1 p46 protein and the p48 CAF-1 subunit bind tightly to an α-helix in the amino terminus of histone H4, and this binding facilitates the acetyltransferase activity. A summary of the protein complexes that contain either p46, p48, or their orthologs is shown in Figure 1.

Interestingly, the p46 or p48 interaction with the histone cannot occur if the histone is assembled into a nucleosome, suggesting that the HAT1 enzyme can only modify histone H4 when the histone amino terminus is free, such as during DNA replication or perhaps nucleosome remodeling. In this context, it is interesting that a nucleosome remodeling factor from *Drosophila* called NURF and the histone deacetylases HDAC1 and HDAC2 from human cells contain p48 or its *Drosophila* ortholog, as a subunit. We suggest that acetylation of these residues in histone H4, commonly associated with silenced transcriptional states of chromatin, can only be modified during DNA replication of locus-specific nucleosome remodeling.

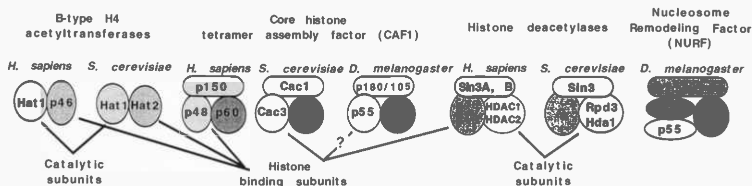


FIGURE 1 Protein complexes containing the p48/p46 proteins and their orthologs in *S. cerevisiae* and *Drosophila*. (CAF-1) Chromatin assembly factor 1; (HDA1 and HDA2) histone deacetylases; (HAT) histone acetyltransferase; (NURF) nucleosome remodeling factor.

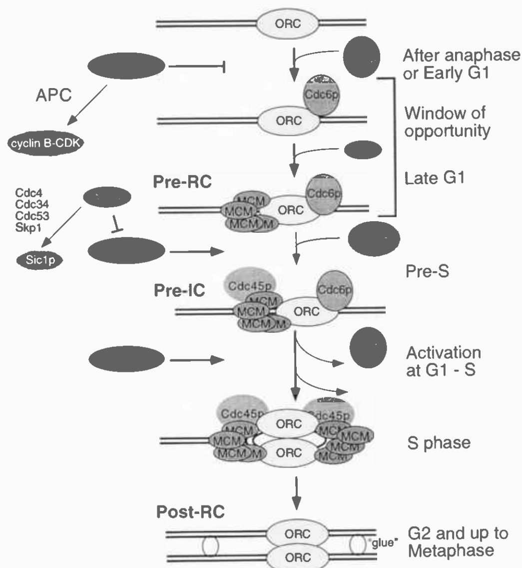


FIGURE 2 Model for the initiation of DNA replication in the yeast *S. cerevisiae*. The proteins are ORC, origin recognition complex; MCM, mini-chromosome maintenance proteins; APC, anaphase promoting complex, a ubiquitin ligase E3 that causes degradation of Clb cyclins; Cdc4, Cdc34, Cdc53, Skp1, a ubiquitin ligase E3 that causes degradation of Sic1p, a Clb-Cdc28p kinase inhibitor; Cdc45p and Cdc6p, proteins that function in initiation; Cdc7p-Dbf4p, a protein kinase required for initiation.

Initiation of Chromosomal DNA Replication in Yeast

J. Chong, Y.-C. Du, C. Liang, P. Wendel,
M. Weinreich, L. Zou

Our studies on the mechanism of initiation of chromosomal DNA replication have progressed to the stage where we can obtain the first glimpses of how the cell cycle machinery controls entry into S phase and how DNA replication occurs only once per cell division cycle. Indeed, the progress in this field by a number of laboratories, since our 1991 discovery of the origin recognition complex (ORC), the cellular initiator protein, has been dramatic. In the last year, we have fractionated yeast cells into various biochemically distinct fractions that enable us to determine whether proteins are associated with the cellular chromatin and, if so, during which stages of the cell division cycle.

The majority, if not all, of ORC, which contains six subunits, is tightly bound to yeast chromatin throughout the cell division cycle, whereas the Orc1p-related protein, Cdc6p, is only chromatin-associated during a period from exit from mitosis to late G_1. The six mini-chromosome maintenance (MCM) proteins, which are known to be required for the initiation of DNA replication, associate with the chromatin during G_1 and are gradually released during S phase. Only a subset of the available MCM proteins associate with chromatin, and a large protein complex containing

ORC, Cdc6p, and the six MCM proteins, perhaps with other proteins, forms what John Diffley has termed the pre-replication complex (pre-RC; Fig. 2).

Last year, we reported the isolation of the gene encoding Cdc45p, a protein implicated by genetic means to interact with the MCM proteins. We have now shown that Cdc45p associates with chromatin only after activation of the S-phase cyclin-dependent kinases (Clb5p-Cdc28p and Clb6p-Cdc28p) and degradation of the CDK inhibitor protein p40 Sic1p. In contrast, Cdc45p was fully loaded onto chromatin prior to the Cdc7 protein-kinase-dependent step, the last known regulatory stage prior to entry into S phase. Thus, the association of Cdc45p with chromatin defined an S-phase-specific cyclin-dependent assembly of a new complex we have called the pre-initiation complex (pre-IC).

We have also shown that the Cdc45p binds to the MCM proteins in a cell-cycle-dependent manner. The association between Cdc45p and the MCM proteins most likely occurs concomitant with the association of Cdc45p with chromatin. Indeed, we have shown that Cdc45p loading onto chromatin requires functional Cdc6p and Mcm2p. Recently, Stephen Bell and his colleagues have shown that Cdc45p associates with origins of DNA replication in late G_1. Although Cdc45p loading onto chromatin follows binding of the MCM proteins, the MCM proteins and Cdc45p appear to dissociate from chromatin by independent mechanisms. A model for some of the events that occur during the initiation of DNA replication is shown in Figure 2.

We have also characterized further the mutants in the *CDC6* gene that cause overreplication of the DNA in a single cell cycle. In the *cdc6-3* mutant cells, initiation at origins of DNA replication is deregulated and can recur in late G_2 of the cell cycle, causing accumulation of more than 2C content of DNA in a haploid cell, a lethal event. We have now shown that in the *cdc6-3* mutant cells, the MCM proteins are associated with the chromatin in late S phase and in G_2 phase of the cell cycle, stages when they are normally not chromatin-associated in wild-type cells. This argues that the pre-RC is either stable or can be formed after S phase, in the presence of the mutant Cdc6p and Clb cyclin-CDKs. These S-phase and M-phase cyclin-CDKs normally prevent formation of the pre-RC late in the cell cycle. Since Cdc6p is a known target of the cyclin-CDKs, the *cdc6-3* results suggest that Cdc6p is the key target for the control that ensures once-per-cell-cycle replication.

Initiation of Chromosomal DNA Replication in Human Cells

M. Iizuka, J. Mendez, K. Simpson, X. H. Yang

The isolation of cDNAs encoding the human Cdc6p has enabled more detailed analysis of the regulation of this key protein. In collaboration with R. Sanders Williams (University of Texas, Southwestern Medical School) and Joseph Nevins (Duke University) and their colleagues, we have shown that transcription of the human *CDC6* gene is controlled by the E2F transcription regulatory factor, itself a target of the Rb tumor suppressor protein. In unpublished studies, we have demonstrated that the amount of human Cdc6p is cell-cycle-regulated and is absent or at a low level in cells in very early G_1 phase, arguing that the timing of establishing competent origins of DNA replication is different in mammalian cells from that in yeast cells.

As part of our studies to identify proteins that are involved in the initiation of human chromosome replication, we have previously identified human Orc1p and Orc2p. During this year, in collaboration with Tim Hunt and his colleagues (Imperial Cancer Research Fund, U.K.), we have identified human and *Xenopus* Orc4p and human Orc5p and have isolated cDNAs encoding these proteins.

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MOLECULAR BIOLOGY OF PAPILLOMAVIRUSES

A. Stenlund C. Sanders G. Chen
E. Gillitzer A. Lee

The papillomaviruses infect and transform the basal epithelium in their hosts, inducing proliferation of the cells at the site of infection. The resulting tumors are in most cases benign and will usually regress after some time, but certain types of human papillomaviruses (HPVs) give rise to tumors that are prone to progress toward malignancy, especially frequently cervical carcinoma. In total, HPV DNA can now be found in biopsies from approximately 80% of all cervical carcinomas.

A key impediment to the study of papillomaviruses has been the inability to define a simple in vitro cell culture system for human papillomaviruses, largely due to the fact that these viruses normally require specialized differentiating cells that can only with great difficulty be generated in cell culture. Therefore, a bovine papillomavirus type 1 (BPV-1) has become the prototype virus for the papillomavirus group largely because a convenient cell culture system exists for this virus. In this cell culture system, viral gene expression, oncogenic transformation, and viral DNA replication can be studied. The DNA replication properties of papillomaviruses show some unique and interesting characteristics. As a part of their normal life cycle, these viruses can exist in a state of latency which is characterized by maintenance of the viral DNA as a multicopy plasmid in the infected cells. The copy number of the viral DNA appears to be tightly controlled, and the viral DNA is stably inherited under these conditions. This system therefore provides a unique opportunity to study plasmid replication in mammalian cells.

In previous years, we have reported the characterization of the papillomavirus replicon and the identification of the viral components that are required for viral DNA replication. More recently, we have directed our attention toward detailed biochemical analysis of the replication process. We are studying the biochemical properties of the virus-encoded E1 and E2 proteins that are required for viral DNA replication. We are also studying how these two proteins interact with the ori and with each other to generate initiation complexes. From these studies, we now have a relatively clear picture of the roles of the E1 and E2 proteins in replication. E1 has all the characteristics of an initiator protein, including ori recognition, DNA-dependent ATPase activity, and DNA helicase activity. E1 can also function to unwind a supercoiled plasmid that contains the ori sequence. The E2 polypeptide, whose function has remained more elusive, appears to serve largely as a specificity factor for E1. Through physical interactions with both E1 and the ori, E2 can

provide sequence specificity in the formation of the initiation complex.

Our attention has now turned toward elucidation of the precise biochemical events that precede initiation of replication at the origin of replication, i.e., binding of the initiator to the ori, the initial opening of the DNA helix, and the assembly and loading of the E1 replication helicase at the replication fork. Our studies indicate that initiation of DNA replication is an ordered multistep process involving the sequential assembly of E1 onto the ori to generate different complexes that in turn serve to recognize the ori, destabilize the double helix at the ori, and function as a replication helicase.

Interactions between the E1 and E2 Proteins

G. Chen, M. Berg

In most eukaryotic replicons that have been studied, binding sites for transcription factors constitute a part of the *cis*-acting sequences required for replication activity. In the majority of cases, including, for example, SV40, polyomavirus, and ARS elements from *Saccharomyces cerevisiae*, this auxiliary activity can be supplied by various transcriptional *trans*-activators with little apparent specificity. A similar requirement exists also for papillomavirus replicons; however, only the virus-encoded transcription factor E2 can serve as an auxiliary factor for replication. The involvement of E2 in replication of BPV extends beyond a mere requirement for E2 bound to the ori: A physical interaction with E1 is also required. This interaction can be detected as cooperative binding of the two proteins to the ori, when the respective binding sites are located in the correct position relative to each other. Because the E2 proteins are well conserved between different papillomaviruses and have a conserved overall structure, we tested E2 proteins from other papillomaviruses for interaction with BPV E1 and for replication. E2 from HPV-11 failed to interact with BPV E1 in either of these assays. This observation presented us with an opportunity to map the regions of E2 that were required for this interaction by construction of chimeric BPV/HPV-11 E2 proteins. These chimeras were tested for their ability to interact physically with BPV E1 in a biochemical assay and for the ability to support replication in vivo. The results from these experiments have revealed a more complex situation than we had anticipated. We find that multiple regions from both the amino-terminal *trans*-activation domain of E2 and the carboxy-terminal "hinge" and DNA-binding domain cooperate to generate a strong interaction with E1. The interaction between the E1 and E2 proteins appears to take place in a two-step process where an initial weak physical interaction between E1 and the DNA-binding domain of E2 allows a stronger, productive interaction to occur between E1 and the activation domain of E2. The specificity of the interaction between E1 and E2 resides in the first step, i.e., in the interaction between E1 and the DNA-binding domain of E2, whereas the activation domains of both BPV E2 and HPV-11 E2 are equally capable of interacting with E1. We have recently used site-directed mutagenesis to identify the specific residues in the E2 DNA-binding domain that are required for the interaction with E1. These studies map a small patch of residues in the DNA-binding domain of E2 that are important for the interaction with E1, verifying the specificity of this interaction and allowing us to address the importance of this protein-protein interaction in the viral life cycle.

The DNA-binding Domain of the Initiator Protein E1

G. Chen

The initiator protein E1 has a poorly characterized DNA-binding activity which serves to recognize the papillomavirus origin of DNA replication and thus determines where DNA replication initiates. We have previously established that E1 can form several different complexes with ori DNA. To understand how these forms are generated and how they function in replication, we are interested in determining the stoichiometry of binding as well as defining the sequences that constitute specific recognition sequences for E1. We have identified and isolated a minimal domain from E1 that is capable of binding specifically to the ori. This domain, which consists of the sequences between amino acids 142 and 308 in E1, can form several different complexes with the ori as judged from gel-shift analysis and can also bind cooperatively with the E2 protein. To determine the stoichiometry of binding, we performed mixing experiments using two DNA-binding domain fragments of different sizes. The results from these studies demonstrated that the E1 DNA-

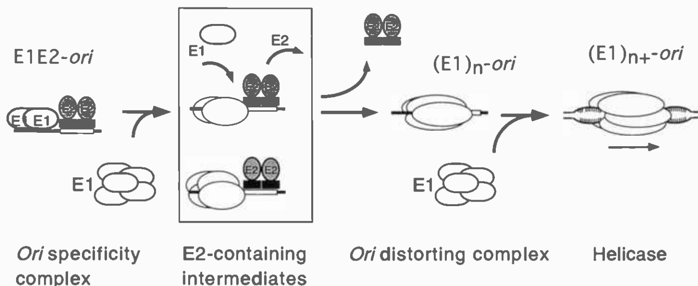

FIGURE 1 Proposed assembly pathway for initiator complexes on the BPV origin of DNA replication. The initiator E1, together with E2, forms a highly sequence specific-complex on the ori (E1E2-ori). This complex can be converted to the E1-ori complex by the addition of E1 molecules and displacement of E2, in a reaction that requires ATP hydrolysis. The E1-ori complex has a low degree of sequence specificity and displays ori distorting activity. The conversion of the E1-ori complex to the hexameric E1 helicase as shown at the right in the figure is hypothetical.

binding domain, which is monomeric in solution, can bind to the ori as one to four monomers. Interference analysis in combination with mutational analysis demonstrated that each monomer of E1 recognizes a hexameric sequence which exists in four copies in the ori sequence. The preferred form of E1 binding is as two monomers which is also the form of E1 that binds cooperatively with the E2 protein. A very striking and unexpected finding is that although the DNA-binding domains of E1 and SV40 T antigen show no homology and the recognition sequences for these two proteins are unrelated, the arrangement of the binding sites for the two proteins are similar, indicating that the positions of the binding sites might have functional significance in the biochemical activities of these proteins, possibly in the process of unwinding of the ori.

E1 Forms a Hexameric Structure That Has DNA-dependent ATPase and DNA Helicase Activities

J. Sedman

The E1 protein has been demonstrated to have both DNA-dependent ATPase activity and DNA helicase activity. All DNA-dependent DNA helicases to date have been shown to have nucleotide hydrolysis capacity. To identify the form of E1 that has ATPase activity, we assembled E1 under conditions used for ATPase assays and sedimented the material on a glycerol gradient. When the gradient was assayed for ATPase activity, interestingly, the activity was found to sediment as a discrete peak with a relative molecular mass of 400–450 kD, which is substantially larger than either a monomer of E1 or the trimeric complex of E1 that we have previously analyzed. Further molecular mass analysis of this form of E1 using gel filtration has revealed that the molecular weight is consistent with a hexameric form of E1. When isolated from a glycerol gradient, this complex has DNA helicase activity, indicating that the hexamer constitutes the active helicase. A requirement for the formation of the hexameric E1 complex is single-stranded DNA which is stably associated with the hexamer.

Assembly of Initiator Complexes

C. Sanders

An intriguing aspect of viral initiator proteins is their ability to perform several different, seemingly unrelated biochemical functions. The E1 protein is known to be a sequence-specific DNA-binding protein, but it can also distort the ori and serve as a DNA helicase. One model to explain how these activities can reside in one single polypeptide is that different oligomeric forms of the protein may have different activities. Our

previous genetic and biochemical studies have indicated that both of the two specific E1-containing complexes that we can detect on the ori are important for initiation of DNA replication. These two complexes, which we have termed the E1E2-ori complex and the E1-ori complex, differ in that E2 is present in one of the complexes and also that a larger number of E1 molecules are present in the E1-ori complex. However, the complexes are clearly related in that the E1 molecules bound in the E1E2-ori complex constitute a subset of the E1 molecules bound in the E1-ori complex. To address whether these two complexes constitute stages in an assembly pathway, we have analyzed their biochemical properties as well as their relation to each other. These studies have revealed a very interesting relationship between the two complexes, consistent with a role for both complexes in the ordered assembly of an initiator complex on the BPV ori (see Fig. 1). The E1E2-ori complex binds to the ori with very high affinity and specificity, whereas the E1-ori complex shows limited sequence specificity. By using an epitope-tagging technique to follow the fate of the E1 molecules from one particular complex, we have been able to demonstrate that the E1E2-ori complex is a preferred substrate for the formation of the E1-ori complex. In a reaction that requires hydrolysis of ATP, the E1E2-ori complex can be converted into the E1-ori complex. As a consequence of this reaction, E2 is displaced from its binding site and additional E1 molecules are added to the complex. Because the E1-ori complex formed from the E1E2-ori complex appears to be identical to the E1-ori complex formed in the absence of E2, these results identify a role for the viral transcription factor E2 as a factor required transiently and catalytically for the assembly of the initiator complex. One clear implication of these results is that a crucial function for E2 in DNA replication is to form a cooperative complex with E1, increasing the specificity and affinity of E1 binding. Thus, this pathway identifies a strategy for depositing a complex with limited sequence specificity (the E1-ori complex) onto a specific site. This is accomplished through the initial formation of the sequence-specific E1E2-ori complex which subsequently is converted to the less sequence-specific E1-ori complex.

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TRANSCRIPTIONAL REGULATION

W. Herr	D. Aufiero	C. Huang	P. Reilly
M. Tanaka	R. Babb	C. Hunt-Grubbe	W. Tansey
	R. Freiman	Y. Liu	T. Tubon
	C. Grozinger	V. Meschan	K. Wu
	C. Hinkley	J. Reader	X. Zhao

We use human viruses, in particular herpes simplex virus (HSV), as models to understand how transcription is regulated in human cells. Viruses provide simple regulatory networks in which the cellular transcriptional machinery is altered to achieve the goals of viral infection. In a cell infected by HSV, the virus can grow lytically—rapidly killing the host cell—or remain latent for many years. In the lytic cycle, HSV gene expression is initiated by a viral transcription factor called VP16, which is carried in the virion. Before activating transcription, VP16 forms a multiprotein-DNA complex—the VP16-induced complex—on viral immediate-early promoters with two cellular proteins: HCF, a protein that regulates cell proliferation, and Oct-1, a POU-homeodomain transcription factor. Once the VP16-induced complex is

assembled, VP16 initiates viral gene transcription through a potent transcriptional activation domain.

Our research is focused on three principal issues: (1) How do transcriptional regulators activate the basal transcriptional machinery? (2) How do transcription factors, as in the VP16-induced complex, modify their transcriptional activity through selective protein–protein and protein–DNA interactions? (3) What are the natural cellular roles of HCF and Oct-1, and how do they influence HSV infection?

Transcriptional Activation

C. Hinkley, W. Tansey, T. Tubon, X. Zhao

We use transcriptional activators to study how regulatory information is transferred from transcription factors to the basal transcriptional machinery assembled at the start site of transcription. In vivo, it is difficult to study how the basal transcriptional machinery responds to activators because the basal factors are highly conserved, ubiquitously expressed, and probably essential proteins. Thus, there is no direct way to study basal factors within a cellular context. To overcome these limitations, we alter the specificity of interactions between components of the basal machinery by mutagenesis, which permits the activity of mutant basal factors to be monitored in the presence of their wild-type counterparts.

Last year, we successfully assembled two altered-specificity interactions in sequence: an altered TATA box–TBP interaction developed by K. Struhl (Harvard University) and an altered TBP–TFIIB interaction that we developed. This altered TATA–TBP–TFIIB array allows us to study in human cells the structure and function of TBP, the TATA box-binding protein, and TFIIB, another general core promoter transcription factor that binds to TBP when TBP is bound to the TATA box. Using this TATA–TBP–TFIIB array, we showed that different transcriptional activation domains use the known TBP–TFIIB interaction differently: Whereas activation domains derived from viral (e.g., VP16) and some cellular (e.g., p53 and CTF) transcriptional activators depend on this interaction to activate transcription in vivo, a transcriptional activation domain derived from the cellular activator Sp1 does not rely on this TBP–TFIIB interaction. This result suggests that transcriptional activation in vivo can be achieved through differential reliance on interactions between core components of the basal machinery, a theme we have observed previously in other studies of TBP.

The sequential altered-specificity TATA–TBP–TFIIB array also allows us to probe the surfaces of TFIIB required for transcriptional activators to stimulate transcription in vivo. The amino-terminal region of TFIIB has been implicated in interactions with RNA polymerase and the basal transcription factor TFIIF and contains a zinc-binding domain. To study its role in transcriptional activation in vivo, we have analyzed the ability of a series of amino-terminal TFIIB deletions to support activation of transcription in vivo by the cellular CTF transcriptional activation domain. Results of these studies indicate that the TFIIB zinc-binding domain is important to support activation of transcription by the CTF transcriptional activation domain in vivo. We now plan to examine what activity or activities of the zinc-binding domain are important to support this activated transcription.

Modification of Transcription Factor Activity Through Selective Protein-Protein and Protein-DNA Interactions

D. Aufiero, R. Babb, C. Huang

The activity of eukaryotic transcriptional regulators can be modified by coregulators. For example, Oct-1, which is broadly expressed in human cells, acquires cell-specific promoter activation properties through association with both viral and cellular coregulators. In HSV-infected cells, Oct-1 activates HSV immediate-early (IE) gene transcription after associating with the HSV *trans*-activator VP16 and HCF on HSV IE promoters. In B cells, Oct-1 activates immunoglobulin-gene transcription after associating with the B-cell factor OCA-B on immunoglobulin-gene promoters.

Oct-1 interacts with VP16 and OCA-B through its POU domain, a bipartite structure consisting of an amino-terminal POU-specific domain joined by a flexible linker to a carboxy-terminal POU-homeodomain. In the absence of VP16 or OCA-B, Oct-1 preferentially activates transcription from small nuclear RNA (snRNA) promoters, a class of cellular promoters studied by the Hernandez laboratory here at CSHL. Previously, we have shown that VP16 association with Oct-1 alters the transcriptional activity of Oct-1 in two ways: It (1) provides a transcriptional activation domain that activates mRNA promoters, such as the viral HSV IE promoters, better than

snRNA promoters, and (2) stabilizes Oct-1 on the VP16-response elements found in the HSV IE promoters.

To study Oct-1 coregulator interactions more extensively, we have analyzed the functional properties of OCA-B. We found that OCA-B functions analogously to VP16: It contains an activation domain that preferentially activates mRNA-type promoters and it stabilizes Oct-1 binding to DNA. OCA-B, however, interacts with a different surface of the DNA-bound Oct-1 POU domain than does VP16. VP16 interacts primarily with the Oct-1 POU-homeodomain, whereas OCA-B interacts with both the POU-specific and POU-homeodomains. Indeed, the OCA-B and VP16 interactions with the Oct-1 POU domain are sufficiently different to permit OCA-B and VP16 to bind the Oct-1 POU domain simultaneously. These results emphasize the structural versatility of the Oct-1 POU domain in its interaction with coregulators.

Virus/Host Cell Interactions: The HSV VP16-induced Complex

R. Freiman, C. Grozinger, C. Hunt-Grubbe, Y. Liu, P. Reilly, K. Wu

HSV must maintain an intimate relationship with the host cell to remain latent for many years and yet retain the ability to grow lytically. Because VP16 associates with the cellular proteins HCF and Oct-1 to initiate HSV gene expression during lytic infection, we hypothesize that HCF and Oct-1 are key regulators of which mode of infection—latent or lytic—HSV enters. We are therefore interested in the natural cellular roles of HCF and Oct-1, and how these roles may influence HSV infection. We focus primarily on the cellular functions of HCF because HCF has been highly conserved during evolution and is involved in cell proliferation.

Human HCF is synthesized as a large approximately 2000-amino-acid precursor protein, which is proteolytically cleaved to generate a family of associated amino- and carboxy-terminal polypeptides. Only the amino-terminal 380 residues of HCF, however, are required to associate with VP16 and to stabilize VP16-induced complex formation. Sequence similarity to other proteins, including one of known structure, indicates that this amino-terminal region of HCF, which we refer to as the HCF_{VIC} domain, forms a structure resembling a six-bladed propeller.

In collaboration with T. Nishimoto and colleagues (Kyushu University), we have characterized a hamster cell line that has a temperature-sensitive defect in cell proliferation caused by a missense mutation in the HCF gene. This missense mutation lies within the HCF_{VIC} domain and affects the ability of VP16 to activate transcription and associate with HCF. These results indicate that VP16 targets a part of the cell machinery important for cell proliferation and suggest that VP16 mimics a cellular cofactor of HCF function that is involved in cell proliferation.

To identify a cellular factor mimicked by VP16, we asked what human proteins can associate with the HCF_{VIC} domain in a yeast two-hybrid screen. Such a two-hybrid screen revealed a single candidate: a basic leucine-zipper protein called LZIP. Consistent with VP16 mimicry of LZIP, VP16 and LZIP share the same tetrapeptide motif—$^{D}/_{E}$HXY—to associate with human HCF. Figure 1 shows how we identified this shared tetrapeptide HCF-binding motif. We individually substituted each of nine residues spanning the tetrapeptide motif in VP16 and LZIP and assayed the effect of each substitution on association with endogenous HCF in a coimmunoprecipitation assay after transient expression in human cells. Only substitution of the three conserved residues in the tetrapeptide motif (the first, second, and fourth positions) in both proteins had a severe effect on association with HCF.

The association of LZIP with HCF through this tetrapeptide HCF-binding motif has apparently been conserved during evolution because an LZIP-related protein in *Drosophila* called BBF-2 or dCREB-A also contains a related HCF-binding tetrapeptide motif. These results suggest that to control HSV infection, VP16 mimics the human basic leucine-zipper protein LZIP, which through association with HCF may control cell cycle progression.

A Genetic Analysis of Transcriptional Synergy in Yeast

M. Tanaka

Many eukaryotic promoters contain multiple binding sites for transcriptional activators, which synergize with one another to activate transcription. For this synergistic transcriptional activation to occur, the exact combinations or positions of activator-binding sites within a promoter do not appear to be generally

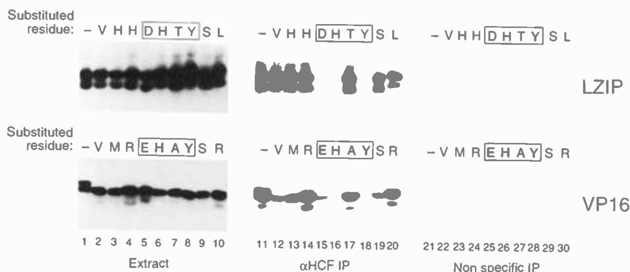

FIGURE 1 LZIP and VP16 share a small HCF-binding motif. Epitope-tagged wild-type or nine substitution mutants of LZIP (*upper panels*) and VP16 (*lower panels*) were transiently expressed in human 293 cells. The residue mutated in each mutant is indicated above each lane. In LZIP, all residues were changed to alanine, except for the threonine which was changed to asparagine. In VP16, all nonalanine residues were changed to alanine, and the alanine was changed to serine. Expression of each mutant protein is shown by direct immunoblot of the 293-cell extracts (lanes *1–10*). To assay HCF association, extracts were precipitated with an HCF antibody (lanes *11–20*) or an irrelevant antibody (lanes *21–30*). LZIP and VP16 were subsequently detected by immunoblot analysis.

important. To elucidate the mechanisms underlying this promiscuous transcriptional synergy, I isolated yeast mutants in which the activity of a promoter containing a single activator-binding site is significantly enhanced, whereas the activity of promoters containing multiple activator-binding sites is relatively unaffected. In these mutants, activator-binding sites work additively rather than synergistically, and transcriptional synergy is largely lost in these mutants.

A genetic characterization of these mutants has revealed that the mutations responsible for the loss of transcriptional synergy are recessive (i.e., cause a loss of wild-type function) and are located within either of two genes, *SIN4* or *RGR1*, both of which encode components of a subcomplex that associates with RNA polymerase II. Thus, in wild-type cells, the *SIN4*- and *RGR1*-encoded proteins Sin4p and Rgr1p have an activity that specifically represses the single-site promoter but has little effect on multiple-site promoters. These results indicate that a selective negative function of Sin4p and Rgr1p on a single-site promoter is important for producing synergistic effects in transcriptional activation.

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RNA SPLICING

A.R. Krainer J. Cáceres L. Manche E. Thomas
L. Cartegni A. Mayeda T.-L. Tseng
D. Horowitz M. Murray Q. Wu
H.-X. Liu

MECHANISMS OF CONSTITUTIVE AND ALTERNATIVE PRE-mRNA SPLICING

RNA splicing is a required step in the expression of most eukaryotic protein-coding genes. The selection and pairing of authentic splice sites within the spliceosome occur with a very high degree of fidelity, which requires that limited and dispersed sequence information present throughout introns and exons be precisely interpreted. The expression of many cellular and viral genes occurs via alternative splicing, which involves substantial flexibility in the choice of splice sites, allowing the expression of multiple protein isoforms from individual genes. The choice of alternative splice sites is commonly regulated to alter gene expression, either tissue specifically or in response to a developmental program or to extracellular signals. Both constitutive and alternative splicing mechanisms involve multiple protein components, as well as RNA components that are part of small nuclear ribonucleoprotein (snRNP) particles. Our lab has focused on the identification, purification, and molecular characterization of protein factors that are necessary for the catalysis of splicing and/or for the regulation of alternative splice-site selection.

SUBCELLULAR LOCALIZATION, NUCLEOCYTOPLASMIC SHUTTLING, AND OTHER PROPERTIES OF SR PROTEINS

The SR proteins constitute a large family of nuclear phosphoproteins required for constitutive pre-mRNA splicing. These factors also have global, concentration-dependent effects on alternative splicing regulation, and this activity is antagonized by members of the heterogeneous nuclear RNP (hnRNP) A/B family of proteins. The SR proteins have a modular structure that consists of one or two RNA-recognition motifs (RRMs) and a carboxy-terminal RS domain. J. Cáceres completed the characterization of domains required for proper localization of SR proteins within nucleoplasmic speckles. Interestingly, the RS domain was found to be necessary and sufficient for correct sublocalization of certain SR proteins, such as SRp20, but not of others, such as SF2/ASF. In the latter case, the RS domain is sufficient as a nuclear localization signal, but not for targeting to the speckles.

J. Cáceres then examined whether SR protein localization is static or dynamic, and again found unexpected differences between SR proteins. A subset of SR proteins shuttles rapidly and continuously between the nucleus and the cytoplasm, although at steady state only the nuclear population is detected by immunofluorescence. Upon inhibition of transcription, shuttling SR proteins, such as SF2/ASF, accumulate in the cytoplasm, whereas nonshuttling SR proteins, such as SC35, remain nuclear. Stronger evidence for shuttling was obtained by using interspecies heterokaryons (Fig. 1A), which showed that shuttling SR proteins are exported from human nuclei and are reimported into both human and mouse nuclei of a heterokaryon (Fig. 1B). The different shuttling properties of different SR proteins were shown to be attributable to their RS domains. However, the RS domain of SF2/ASF is not sufficient for shuttling, and RNA binding was also shown to be required.

We found that the relative accumulation of shuttling SR proteins in the nucleus and the cytoplasm can be modulated by the Clk/Sty kinase, which has been shown to phosphorylate the RS domains of SR proteins in vitro. Overexpression of catalytically active kinase resulted in cytoplasmic accumulation of shuttling SR proteins (Fig. 1C), which may account for the previously observed effects of this kinase on alternative splicing of cotransfected reporter genes. Differential modulation of hnRNP A/B and SR protein shuttling by kinases may thus serve to regulate alternative splicing in response to signal transduction pathways. The finding that some but not all SR proteins shuttle suggests that these proteins may have roles not only in nuclear pre-mRNA splicing, but also in mRNA transport, in cytoplasmic events, and/or in processes that involve communication between the nucleus and the cytoplasm.

We have continued the functional characterization of SR proteins with respect to their roles in enhancer-dependent splicing and in alternative splicing regulation. H.-X. Liu developed a randomization and iterative selection protocol based on biochemical complementation with single SR proteins, to define the types of sequences that can function as exonic splicing enhancers in the presence of each of three different SR proteins. In collaboration with M. Zhang (CSHL), short consensus sequences corresponding to enhancers specific for each SR protein were defined. These studies defined new classes of splicing enhancers, of which the well-studied purine-rich elements are only a small subset. A. Mayeda continued to define the *cis*-elements within two pre-mRNAs that splice only in the presence of particular SR proteins. This study defined two natural SR protein-specific enhancer elements. L. Cartegni has begun to study the substrate specificity of SR proteins in alternative splicing in vivo. T.-L. Tseng has been studying related proteins with RS domains in fission yeast, focusing on the genetic characterization of a protein with an amino-terminal RS domain and three RRMs.

STRUCTURE AND FUNCTION OF hnRNP A/B PROTEINS

hnRNP A/B proteins, exemplified by hnRNP A1, antagonize SR proteins to promote exon skipping and distal alternative 5′ splice site use. These proteins consist of two RRMs and a carboxy-terminal glycine-rich domain. A proteolytic fragment of hnRNP A1 lacking the carboxy-terminal domain is known as UP1. The crystal structure of human UP1 was determined by R.-M. Xu (CSHL) in collaboration with our group, and simultaneously by a team at Yale. This was the first structure with more than one RRM, which is of interest because numerous eukaryotic proteins contain multiple RRMs. The structure showed that each RRM folds independently; but unexpectedly, the two RRMs, which are antiparallel, are held together rigidly by two salt bridges. This is unlikely to be due to crystal packing forces, because the aspartic acid and arginine residues that form the salt bridges are highly conserved among the RRMs of the hnRNP A/B family of proteins. The two β-sheets—one from each RRM—form an extended RNA-binding surface. The position of the inter-RRM linker suggests a possible role in contacting bound RNA, and this is supported by its phylogenetic length and sequence conservation among the hnRNP A/B family of proteins.

A. Mayeda, in collaboration with S. Munroe (Marquette University), continued the characterization of the structure and function of hnRNP A1 with respect to its role in alternative splice-site selection, as well as in RNA binding and RNA annealing. Last year, we made the observation that although both RRMs of hnRNP A1 are required for its splicing function and are very closely related in sequence, they have distinct roles. Thus, replacement of RRM1 with a second copy of RRM2 yielded a highly active protein, whereas replacement of RRM2 by a second copy of RRM1 resulted in inactive protein. Deletion or point mutations in either RRM inactivated the protein, and swapping the positions of the two RRMs gave a protein with weak but detectable activity. Shortening of the linker that separates the two RRMs also inactivated the protein. The recent UP1 structure determination has helped us to interpret these findings and to design follow-up experiments. The symmetry of the structure and the very similar folding of the two RRMs explain in part why the RRM2 duplication is active. Regarding the salt bridges observed in the structure, they appear to be preserved in the active duplication, whereas only one of the two salt bridges is maintained in the inactive duplication and in the swap variant. We therefore repaired the missing salt bridge in the inactive variant, but this was insufficient to restore function. We are currently testing whether replacement of specific residues in the inactive RRM1 duplication variant is sufficient for gain of function. One attractive model is that RRM2 is involved in specific contacts with RNA that are essential for function, whereas RRM1 contributes to the overall free energy of binding through nonspecific contacts. Further structural characterization and mutational analysis will test this and other models.

A TRIMERIC HPRP3/HPRP4/CYCLOPHILIN COMPLEX ASSOCIATED WITH U4/U6 snRNP

D. Horowitz investigated the nature of a 55-kD protein that cross-reacts with antibodies against hPrp18. Analysis of snRNP association by Western blotting of velocity gradient fractions showed that hPrp18 is not stably associated with fast-sedimenting complexes, whereas the cross-reacting 55-kD polypeptide co-sedimented with the U4/U6 and U4/U6•U5 snRNP particles. The 55-kD protein was purified by chromatography under native conditions, using Western blotting to

follow the protein. This procedure resulted in a highly purified protein complex consisting of the 55-kD polypeptide and two additional polypeptides of 90 kD and 18 kD. This protein complex is stable even in the absence of RNA. Partial amino acid sequence for all three polypeptides was obtained by R. Kobayashi (CSHL), and this information was used to obtain complete cDNA sequences for all three proteins. Sequence analysis showed that the 55-kD and 90-kD proteins are the likely homologs of the budding yeast Prp4 and Prp3 splicing factors, respectively. The 18-kD protein is a novel cyclophilin that is similar but not identical to cyclophilin A, and we have named it USA-CyP (U snRNP-associated cyclophilin).

An alignment of Prp4 proteins from several species showed the presence of seven WD40 repeats, suggesting that hPrp4 and its homologs fold into a sevenfold β-propeller structure similar to those of the β subunits of G proteins. An alignment of Prp3 proteins did not reveal unique motifs suggestive of specific mechanisms. The presence of a cyclophilin in snRNP particles suggests possible roles for proline isomerization and/or chaperonin functions in spliceosome assembly and/or snRNP biogenesis. In particular, previous work from the Krämer, Lührmann, and Kole labs showed that the disruption of splicing upon heat shock is attributable to a defect in U4/U6•U5 snRNP particle assembly; splicing could be restored by adding a fraction containing tri-snRNP-particle-associated polypeptides. The known link between heat-shock chaperonins and cyclophilins suggests that USA-CyP may be involved in the recovery of splicing function after heat shock.

ROLE OF A TYPE 2C PROTEIN PHOSPHATASE IN PRE-mRNA SPLICING

M. Murray used fractionation of HeLa nuclear extracts and biochemical complementation to define an activity required for the first transesterification of splicing. Purification of this activity to near homogeneity yielded a 75-kD polypeptide and several fragments thereof. Microsequence analysis (in collaboration with R. Kobayashi, CSHL), followed by cDNA sequencing and sequence database comparisons, showed that this protein is a type 2C serine/threonine protein phosphatase. The phosphatase activity due to this protein precisely copurified with the splicing complementing activity. We have obtained recombinant protein and raised antibodies to it. Immunofluorescence analysis showed that this phosphatase is nuclear. Immunoprecipitation experiments demonstrated its association with spliceosomes. The recombinant protein is not active in the complementation assay, perhaps because the phosphatase is not sufficient for activity, and/or because the recombinant form lacks modifications necessary for splicing function. We are currently using the antibodies for immunodepletion experiments. Identification of specific targets for dephosphorylation by this protein within the context of splicing should yield new information on the role of phosphorylation and dephosphorylation cycles in splicing.

THE AT-AC INTRON SPLICING PATHWAY

Q. Wu has been studying the biochemistry of splicing of a small class of mammalian introns with unique 5′

FIGURE 1 Shuttling and nonshuttling SR proteins. (*A*) Diagram of interspecies heterokaryon assay of nucleocytoplasmic shuttling by transiently expressed SR proteins. The shaded nuclei indicate localization of the transiently expressed human SR protein. Cycloheximide (CHX) was added prior to fusion with polyethylene glycol (PEG) to prevent further protein synthesis from the human mRNA in the heterokaryons. (*B*) Detection of the transiently expressed SR proteins in interspecies heterokaryons. HeLa cells were transfected with expression plasmids encoding the indicated T7 epitope-tagged proteins, or hnRNP A1 as a positive control for shuttling. At 24 hr posttransfection, the cells were treated with CHX and then fused with mouse NIH-3T3 cells in the presence of PEG to form heterokaryons. The cells were further incubated for 2 hr in the presence of CHX and fixed. The localization of the transiently expressed proteins was determined by indirect immunofluorescence with anti-T7 tag monoclonal antibody and FITC-conjugated secondary antibody (*left* panels). The cells were simultaneously incubated with Hoechst 33258 dye for differential staining of human and mouse nuclei within heterokaryons (*middle* panels). The arrows indicate the mouse nuclei within human-mouse heterokaryons. Phase-contrast images of the same heterokaryons are shown (*right* panels). All of the proteins, except SC35, were exported from the human nuclei and imported into the mouse nuclei. (*C*) Effect of Clk/Sty kinase expression on SF2/ASF shuttling. HeLa cells were cotransfected with plasmids encoding either T7-tagged SF2/ASF (*top* panels) or SC35 (*bottom* panels), together with a plasmid expressing a *myc*-tagged Clk/Sty kinase. The localization of the transiently expressed SR proteins (*left* panels) was determined by indirect immunofluorescence as in panel *B*, and that of Clk/Sty (*right* panels) with rabbit anti-myc tag antibody and Texas-Red-conjugated secondary antibody. Kinase expression affects SF2/ASF nuclear export and/or reimport, causing its accumulation in the cytoplasm (*top left*). The nonshuttling SR protein SC35 remains nuclear (*bottom left*). This effect required a catalytically active form of the kinase.

and 3′ splice sites that do not conform to the consensus sequences. Whereas most introns begin with GT and end with AG, the noncanonical introns begin with AT and end with AC, and are therefore known as AT-AC introns. In addition to the AT-AC borders, most known AT-AC introns have highly conserved 5′ splice site and branch site sequence elements of seven to eight nucleotides. Intron 6 of the nucleolar P120 gene

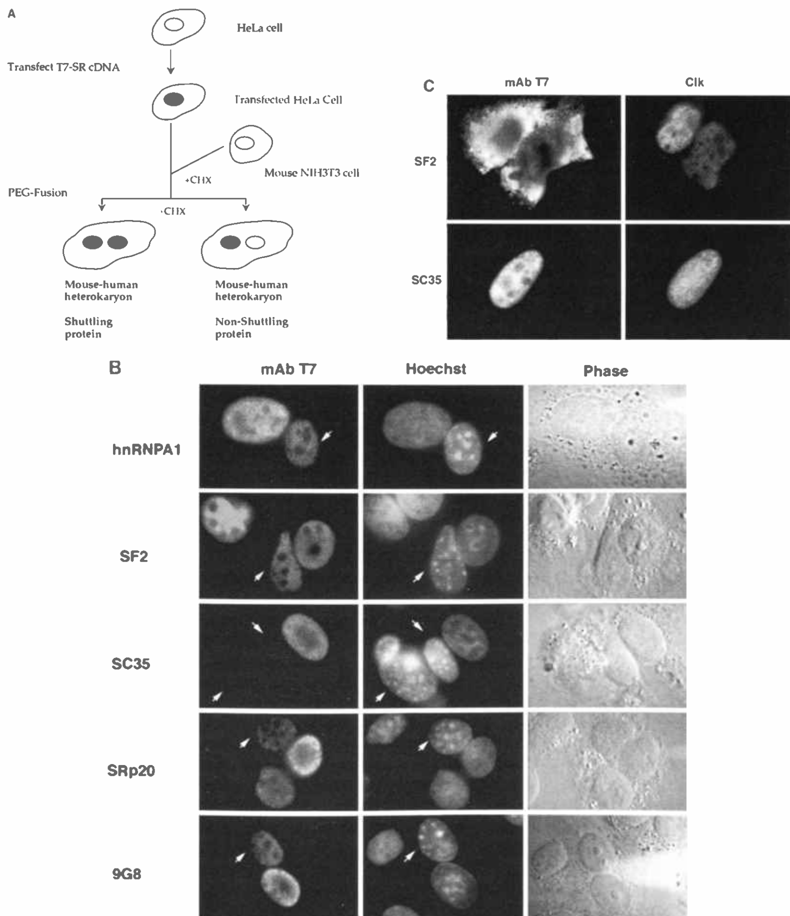

FIGURE 1 (*See facing page for legend.*)

and intron 2 of the SCN4A voltage-gated skeletal muscle sodium channel are AT-AC introns that we and other workers have recently shown to be processed via a unique splicing pathway involving several minor U snRNAs. Interestingly, intron 21 of the same SCN4A gene and the corresponding intron 25 of the SCN5A cardiac muscle sodium channel gene also have 5´-AT and AC-3´ boundaries, but they have divergent 5´ splice site and presumptive branch site sequences. We demonstrated the accurate in vitro processing of these two divergent AT-AC introns and showed that they belong to a functionally distinct subclass of AT-AC introns. Splicing of these introns does not require U12, U4atac, and U6atac snRNAs, but instead requires the major spliceosomal snRNAs U1, U2, U4, U5, and U6. Previous studies showed that G→A mutation at the first position and G→C mutation at the last position of a conventional yeast or mammalian GT-AG intron suppress each other in vivo, suggesting that the first and last bases participate in an essential non-Watson-Crick interaction. Our results show that such introns, termed AT-AC II introns, occur naturally and are spliced by a mechanism distinct from that responsible for processing of the apparently more common AT-AC I introns. Therefore, commitment to the ATAC or GTAG pathway is not specified by the boundary nucleotides, but more likely by the rest of the 5´ splice site element.

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MOLECULAR MECHANISMS OF APOPTOSIS

Y. Lazebnik A. Doseff J. Rodriguez
L. Faleiro Y. Xu
H. Fearnhead

Apoptosis is a fundamental biological process critical for the development of organisms and for maintaining tissue homeostasis. Consequently, deregulation of apoptosis contributes to diseases such as cancer and neurodegenerative disorders. Our laboratory is investigating whether the apoptotic machinery can be used to kill cancer cells. Obviously, such a killing should be selective for transformed cells, and we are trying to understand how this selectivity can be achieved.

The emerging view of apoptosis is that diverse regulatory pathways activate a conserved execution machinery. An essential component of this machinery is the caspases (formerly known as ICE-like proteases), a family of cysteine proteases. Caspases are activated at the onset of apoptosis and once active, cleave in a coordinated manner a number of proteins which results in a precisely orchestrated cell death. Preventing caspase activation prevents cell death. Reciprocally, activation of caspases results in cell death, even if this activation is achieved through means unrelated to apoptotic signaling pathways. To understand how a cancer cell can be killed, we study which caspases are involved in apoptosis, how they are activated, and how this activation leads to cell death.

ONCOGENE-DEPENDENT APOPTOSIS IN EXTRACTS FROM DRUG-RESISTANT CELLS

The failure to effectively and selectively eliminate cancer cells is a major problem of cancer therapy. Nonetheless, most mammalian cells, including those that give rise to cancer, have an intrinsic machinery whose function is to carry out cell suicide. Activation of this execution machinery results in apoptosis, a comprehensive process that can quickly eliminate large numbers of cells without triggering adverse responses such as inflammation. The efficiency of such killing prompts the examination of the apoptotic execution machinery as a potential tool for killing cancer cells.

Indeed, many anti-cancer drugs kill cells by activating the apoptotic machinery. However, this killing is inefficient as activation is indirect. The direct effect of these drugs is cell damage, such as DNA breaks or cell cycle aberrations, which then triggers signaling pathways that activate the execution machinery and eventually leads to cell death. However, a cancer cell may fail to die not because the drug does not induce cell damage but because the information about this damage fails to reach the execution machinery of apoptosis. In principle, agents that directly activate the execution machinery should bypass alterations which prevent apoptosis and kill cells that are otherwise resistant to cancer therapy. However, how to activate the apoptotic machinery in cancer cells directly and, most importantly, selectively is not clear.

During the previous year, we found that extracts from transformed but not normal cells can spontaneously activate caspases and induce apoptosis in a cell-free system. Importantly, this effect was observed even when transformed cells were highly resistant to apoptosis. We hypothesized that caspase activation is caused by an endogenous oncogene-generated activity (OGA) that is present in cells transformed with certain oncogenes. These oncogenes, which include E1A, myc, and E7, are known to induce apoptosis or sensitize cells to apoptosis-inducing factors. However, transformed cells may survive and give rise to cancer when oncogene-induced apoptosis is suppressed by overexpression of proteins with anti-apoptotic activity, such as bcl-2. Whether the pro-apoptotic activity of oncogenes is abolished when apoptosis is suppressed or whether it becomes latent was not clear.

Our hypothesis implied that this activity is latent. Using extracts from cells transformed with the adenovirus E1A oncogene, we demonstrated that our hypothesis is correct. We provided preliminary characterization of this OGA and found that it activates caspases in an ATP-dependent manner. We also found that partially purified OGA can induce caspase activation when added to extracts from untransformed cells.

During the last year, we purified OGA and identified it as APAF-1, a recently described human homolog of CED-4, a protein that is required for apo-

ptosis in *Caenorhabditis elegans*. Purified APAF-1 could induce ATP-dependent caspase activation when added to extracts from untransformed cells, which was consistent with our preliminary characterization of OGA. These results, obtained in a cell-free system, suggest that oncogene-induced apoptosis is mediated through APAF-1, although how this is achieved is not clear. The level of APAF-1 expression appears to be unchanged during transformation, suggesting that other factors or a modification of APAF-1 is involved. We are investigating these possibilities.

As mentioned earlier, the key question for us is whether the apoptotic machinery can be induced in cancer cells selectively. Our results suggest that a basis for selective activation of caspases in cancer cells may lie in the mechanisms intrinsic to carcinogenesis. Our results argue that OGA is present in transformed cells, although in a latent form, even if these cells become drug resistant. We suggest that agents that link OGA to caspases in cells would kill tumor cells otherwise resistant to conventional cancer therapy. As this killing relies on an activity generated by an oncogene, the effect of these agents should be selective for transformed cells. Our progress in identifying the molecular basis for OGA should hopefully make the testing of our model possible.

DELINEATION OF CASPASE CASCADE

Caspases are thought to be activated in a cascade. Studies in a cell-free system suggest that APAF-1 initiates caspase activation by activating caspase-9, which then cleaves other caspases. We investigated the order in which these caspases are cleaved. To do this, we generated cell extracts that are immunodepleted of one or several caspases, a technique that was called in the laboratory "the biochemical knockout." This approach allows us to determine whether a caspase is processed by another caspase or is activated autocatalytically, to identify caspases whose processing depends on the activity of the depleted caspases, and also to find the function of the depleted caspases in carrying out nuclear chances of apoptosis. Indeed, we delineated the sequence in which caspases are activated and determined the effect of caspase depletion on nuclear morphology. In a collaboration with Dr. Scott Lowe (CSHL), we found that the effects of depletion of caspase-3 in a cell-free system and in cells depleted from caspase-3 by genetic methods are similar.

LOCALIZATION OF CASPASES INVOLVED IN APOPTOSIS

Where the components of the apoptotic machinery are in a cell is one of the many mysteries of apoptosis. It is difficult without knowing where these components are to devise a viable model of cell death. Indeed, a significant part of our knowledge, as well as many assumptions, about the mechanisms of apoptosis comes from studies on cell-free systems, which are made of a homogeneous cell extract and isolated nuclei. Therefore, during the last year, we started to analyze systematically the intracellular localization of caspases.

We began with caspase-3 (cpp32) because we previously found it to be the most abundant caspase. Using a set of monoclonal antibodies that we developed, we found that caspase-3 is localized in particles. These particles, depending on cell type, are either distributed throughout the cell or are limited to the cytoplasm or the nucleus. This finding raised several questions. First, the current model of apoptosis suggests that some caspases are activated by binding to an activator complex, such as a receptor. These activated caspases then cleave the effector caspases, caspase-3 being one of them, which are assumed to be freely available for this cleavage. If caspase-3 is compartmentalized, the question is, how are the two caspases brought to the same location? The second question that we are investigating is the composition of the caspase-containing particles. One possibility is that these particles contain other known components of the apoptotic machinery.

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REGULATION OF APOPTOSIS AND SENESCENCE

S.W. Lowe A. Lin A.V. Samuelson
M.E. McCurrach M.S. Soengas
J. Polyakova R. Wallace-Brodeur

Our research is based on the premise that apoptosis, a genetically controlled form of cell death, provides a natural defense against tumor development and underlies the cytotoxicity of most current anticancer drugs. Consequently, mutations that disrupt apoptotic programs can cause tumor progression and resistance to cancer therapy. Earlier studies identified the p53 tumor suppressor as an important regulator of apoptosis and demonstrated that *p53* mutations could promote oncogenic transformation, tumor progression, and resistance to cytotoxic agents by reducing a cell's apoptotic potential. Our current research is aimed at elucidating how p53 promotes apoptosis and we are characterizing p53-independent apoptotic pathways that might function in tumor cells. We are also developing in vivo models to study apoptosis chemosensitivity in spontaneous tumors. With Y. Lazebnik here at CSHL, we are investigating how p53 and other regulators of apoptosis affect activation of the apoptotic "machinery." Finally, in a separate direction, we are investigating how cells respond to *ras* oncogenes and the consequences of this response for multistep carcinogenesis.

Modulation of Chemosensitivity

A. Samuelson, M. McCurrach, S. Lowe [in collaboration with H. Fearnhead and Y. Lazebnik, Cold Spring Harbor Laboratory; M. Mayo and A. Baldwin, University of North Carolina, Chapel Hill]

Since most anticancer agents currently in use were identified by empirical screens, the molecular mechanisms underlying drug sensitivity and resistance remain poorly understood. Work from our laboratory and elsewhere demonstrates that many anticancer agents induce apoptosis, implying that tumor cell chemosensitivity is influenced by the efficiency with which anticancer agents activate apoptotic programs. Although the precise contribution of apoptosis to tumor responsiveness remains unresolved, the fact that apoptosis is controlled by genes raises the exciting prospect that the problem of drug sensitivity and resistance will be amenable to the approaches of modern molecular biology. In principle, strategies that enhance apoptosis *specifically* in tumor cells would have widespread therapeutic potential.

Cells expressing the adenovirus early region 1A (*E1A*) oncogene provide a simple model for studying cellular processes that modulate chemosensitivity. With Y. Lazebnik, we have shown that E1A promotes apoptosis in primary cells, in part, by producing or modifying a cellular factor that enables efficient activation of the apoptotic machinery (Fearnhead et al. 1997). As a consequence, E1A-expressing cells become extremely sensitive to toxic agents, including radiation and most chemotherapeutic drugs. E1A mutations that disrupt apoptosis and chemosensitivity separate into two complementation groups, which correlate precisely with the ability of E1A to associate with either the p300/CBP or RB-related proteins (Samuelson and Lowe 1997). Furthermore, E1A mutants incapable of binding RB, p107, and p130 confer chemosensitivity to fibroblasts derived from *RB*-deficient mice, but not fibroblasts from mice lacking p107 or p130. Hence, inactivation of RB, but not p107 or p130, is required for chemosensitivity induced by E1A.

The results described above demonstrate that E1A mutants unable to bind the RB protein family *selectively* promote chemosensitivity in RB-deficient cells. Since the *RB* gene is inactivated in familial retinoblastoma and a variety of sporadic tumors (and the RB pathway is disrupted in the vast majority of cancer cells), our results identify a strategy to selectively make tumor cells more sensitive to anticancer drugs. Specifically, certain E1A mutants—or small molecules that mimic their action—should synergize with standard anticancer agents to enhance the killing of RB-defective cells. Since normal cells retain RB function, these agents are predicted to have minimal toxicity to nonmalignant cells. We are currently defining the E1A function(s) sufficient to promote chemosensitivity in RB-deficient cells and conducting "proof-of-concept" experiments to determine whether our strategy would be effective in treating human tumors.

In certain settings, oncogenic *ras* can modulate apoptosis. We participated in a study with A. Baldwin and colleagues at the University of North Carolina showing that inactivation of NF-κB function can dramatically enhance apoptosis in *ras*-expressing cells (Mayo et al. 1997). The effect appears to be independent of the p53 tumor suppressor and hence suggests an alternative strategy to manipulate apoptosis to improve cancer therapy.

p53-dependent and -independent Apoptotic Pathways

M. McCurrach, A. Samuelson, J. Polyakova, M. Soengas, S. Lowe

We continue to explore the mechanisms whereby p53 promotes apoptosis. Recently, we demonstrated that Bax (a cell death agonist that has homology with the anti-apoptotic Bcl-2 protein) can function as an effector of *p53* in chemotherapy-induced apoptosis and contributes to a *p53* pathway to suppress oncogenic transformation (McCurrach et al. 1997). In a separate direction, we have previously shown that E1A alters p53 function from promoting growth arrest to apoptosis, which is associated with the ability of E1A to increase the stability of p53 protein. Now we have shown that the E1A regions required for p53 induction and apoptosis correlate precisely with those required for E1A's oncogenic potential and that inactivation of the RB is strictly required for these effects (Samuelson et al. 1997). This argues that p53 accumulation and enhanced apoptosis is a cellular response to oncogenic "stress" rather than a direct effect of E1A on p53.

Using our simple system of fibroblasts transformed by E1A and *ras*, we have identified agents that induce apoptosis independently of p53. For example, adriamycin-induced apoptosis is p53-dependent, whereas tumor necrosis factor-α (TNF-α)-induced apoptosis is p53-independent (Lanni et al. 1997). Bax, which functions downstream from p53 in adriamycin-induced apoptosis, is dispensable for TNF-induced apoptosis. Furthermore, adriamycin synergizes with TNF-α to induce apoptosis in the absence of p53. Using MEFs deficient for specific genes and various cell death inhibitors, we are genetically characterizing these distinct pathways. We envision that this system will provide a simple model for understanding "cross-talk" between apoptotic pathways.

Caspase Function

M. Soengas, J. Polyakova, M. McCurrach [in collaboration with J. Rodriguez and Y. Lazebnik, Cold Spring Harbor Laboratory; M. Woo, R. Hakem, and T. Mak, University of Toronto Cancer Center and Amgen Institute]

Proteases related to interleukin-1β-converting enzyme, called caspases, are thought to be essential components of the apoptotic "machinery" (i.e., the molecules directly responsible for apoptotic cell death). The importance of apoptosis in cancer argues that components of this machinery may be tumor suppressors and/or drug sensitivity genes. Since proteolytic cleavage is essentially irreversible, caspase activation may represent the last regulated step in apoptosis. In this view, the endpoint of most, if not all, cytotoxic anticancer drugs is caspase activation. Consequently, most mutations that limit drug cytotoxicity act *upstream* of these proteases. If true, the caspases represent attractive drug targets.

A major goal of our laboratory is to understand how cell death regulators like p53 affect caspase activation. First, with Y. Lazebnik, we continue to identify the caspases activated during p53-dependent and -independent apoptosis in the well-defined MEF system described above. To this end, we are generating monoclonal antibodies against all known murine caspases. Second, using viral caspase inhibitors or cells deficient for specific caspases, we study the contribution of specific caspases to various apoptotic pathways.

In collaboration with T. Mak, we took a comprehensive approach to examine the role of caspase-3 (CPP32) in apoptosis using mice, embryonic stem (ES) cells, and mouse embryonic fibroblasts deficient for caspase-3 (Woo et al. 1997). *CASP3*$^{-/-}$ mice have reduced viability and, consistent with an earlier report, display defective neuronal apoptosis and neurological defects. Inactivation of caspase-3 dramatically reduces apoptosis in diverse settings, including activation-induced cell death of peripheral T cells, as well as chemotherapy-induced apoptosis of oncogenically transformed *CASP3*$^{-/-}$ MEFs. As well, the requirement for caspase-3 can be remarkably stimulus-dependent: in ES cells, caspase-3 is necessary for efficient apoptosis following UV- but not γ-irradiation. Conversely, the same stimulus can show a tissue-specific dependence on caspase-3: Hence, TNF-α treatment induces normal levels of apoptosis in *CASP3*$^{-/-}$thymocytes, but defective apoptosis in oncogenically transformed MEFs. Finally, in some settings, caspase-3 is required

for certain apoptotic events but not others: Transformed *CASP3*$^{-/-}$ MEFs undergoing cell death are incapable of chromatin condensation and DNA degradation but display other hallmarks of apoptosis. Together, these results indicate that caspase-3 is an essential component in apoptotic events that is remarkably system- and stimulus-dependent. Consequently, drugs that inhibit caspase-3 may preferentially disrupt specific forms of cell death.

In Vivo Models of Drug Sensitivity and Resistance

R. Wallace-Brodeur, M. McCurrach

Cancer is a complex phenotype that occurs only in whole animals. For this reason, we always attempt to extend observations from tissue culture studies to real tumors. To develop in vivo models for studying apoptosis and chemosensitivity, we are studying the therapeutic response of spontaneous lymphomas occurring in the *Eμ-myc* transgenic mouse. These animals constitutively express c-*myc* in the B-cell lineage and typically succumb to B-cell lymphoma within 4–6 months of age. We hypothesized that these mice would provide a useful model for studying apoptosis and cancer therapy since (1) tumor burden can be monitored externally by lymph node palpation; (2) lymphomas are detectable long before the animal dies, so animals can be treated while still healthy, (3) large numbers of tumor cells can be isolated from mice undergoing therapy, (4) tumor cells readily adapt to culture, and (5) suppression of apoptosis has been shown to accelerate lymphogenesis, suggesting that regulation of apoptosis is important in these tumors.

During the past year, we examined the response of *Eμ-myc* lymphomas to adriamycin therapy, a drug used to treat human lymphomas. As is observed in human patients with lymphoma, the vast majority (~90%) of *Eμ-myc* transgenic mice respond to therapy. Isolated tumor cells display features of apoptosis within 8 hours of therapy, including chromatin condensation, caspase activation, and PARP cleavage. In virtually every animal, however, tumors eventually return, and, moreover, treatment of animals with relapsed tumors is much less effective than in mice with primary tumors (less than 50% of the animals respond). Furthermore, in those animals that survive repeated therapy, the recurrent tumors eventually become resistant. Consequently, we feel that this model will be excellent for studying the role of apoptosis in cancer therapy, as well as the genetics of drug sensitivity and resistance.

Multistep Carcinogenesis

A. Lin, M. McCurrach, S. Lowe [in collaboration with M. Serrano and D. Beach, Cold Spring Harbor Laboratory]

Cancer is a multistep process involving a series of genetic changes that individually enhance the growth or survival of developing tumor cells. Considerable progress has been made in identifying tumor-specific mutations and how they alter normal gene function, but less is known about how these mutations interact to produce the malignant phenotype. Perhaps the simplest model of multistep carcinogenesis involves oncogenic transformation of primary cells by *ras* oncogenes. Oncogenic *ras* can transform most immortal rodent cells to a tumorigenic state. However, transformation of primary cells by *ras* requires either a cooperating oncogene (e.g., E1A) or the inactivation of tumor suppressors such as p53 or p16. Although this paradigm has been studied since the early 1980s, the biological basis for oncogene cooperation was poorly understood.

We recently showed that expression of oncogenic *ras* in primary human or rodent cells results in a permanent G_1 arrest (Serrano et al. 1997). The arrest induced by *ras* is accompanied by accumulation of p53 and p16 and is phenotypically indistinguishable from cellular senescence. Inactivation of either p53 or p16 prevents *ras*-induced arrest in rodent cells, and E1A achieves a similar effect in human cells. Recently, we have shown that *ras*-induced cell cycle arrest in primary cells proceeds through the same effector pathways responsible for *ras*-induced mitogenesis and transformation in immortal cells. Hence, these data suggest that activation of premature senescence is a cellular response to an aberrant mitogenic stimulus, rather than some novel *ras*-mediated antiproliferative pathway. Together, these observations suggest that the onset of cellular senescence does not simply reflect the accumulation of cell divisions but can be prematurely activated in response to an oncogenic stimulus. Negation of *ras*-induced senescence appears to account for the phenomenon of oncogene cooperation and may be important during multistep tumorigenesis.

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CELL SIGNALING IN HIV PATHOGENESIS

J. Skowronski S. Bronson, Y.-N. Chang, D. Desrosiers, M. Greenberg, A. Iafrate, M. Lock, N. Shohdy, T. Swigut

Our main interest is in understanding the molecular mechanisms underlying the pathogenesis of AIDS and, in particular, understanding the functional consequences of the interactions between viral proteins and the cellular regulatory machinery. The focus of our research is to understand the function of Nef, a regulatory protein of human and simian immunodeficiency viruses (HIV and SIV). Evidence from HIV-1-infected individuals and from the SIV-infected rhesus monkey model of AIDS indicates that Nef is an important determinant of viral pathogenicity. Viral loads remain low and AIDS does not develop in rhesus macaques experimentally infected with a *nef*-deleted SIV variant. Moreover, a lack of disease progression in a subset of HIV-1-infected persons is associated with deletions in *nef*. This evidence suggests that disruption of Nef function will attenuate the development of AIDS, and therefore Nef is an attractive potential target for the development of new drugs for AIDS treatment. The functions of Nef critical for the development of AIDS are not known; uncovering these functions has been a major focus of studies in our laboratory for the past several years.

Since Nef is not required for the viral life cycle under laboratory conditions, its function(s) has been difficult to study; however, Nef has been shown to have three effects on the function of cultured cells. One effect is modulating signal transduction. The other effects are down-regulating the expression of CD4 and MHC class I molecules on the cell surface. Since (1) HIV replication is modulated by the signal transduction machinery of the infected cell, (2) CD4 is a component of the receptor for HIV and SIV as well as a component of the T-cell antigen receptor, and (3) down-regulation of MHC class I surface expression in cells infected by HIV helps these cells to evade the

antiviral immune response of the host, each of these effects is likely to be important for the development of AIDS. During the last year, our effort was directed toward understanding the mechanisms mediating down-regulation of CD4 surface expression by Nef. We also initiated experiments to address the importance of the ability of Nef to down-regulate CD4 expression for the induction of AIDS by SIV in experimentally infected rhesus monkeys (in collaboration with Frank Kirchhoff, University of Erlangen, Germany). Our experiments identified putative molecular interactions of Nef with the cellular protein-sorting machinery involved in the down-regulation of surface CD4 expression and indicated that these interactions are important for immunodeficiency virus replication in the infected host. Finally, we continued our search for cellular proteins that mediate functional interactions between Nef and both the signaling and protein-sorting machineries.

NEF COLOCALIZES WITH AP-2 ADAPTOR COMPLEXES

To correlate the function of Nef with its subcellular localization, we fused Nef to the green fluorescent protein (GFP) and studied the distribution of this Nef.GFP chimera in the cell. Control experiments demonstrated that the chimeric protein was capable of down-regulating expression of CD4 on the cell surface, and therefore retained all the interactions required for this function. In transiently transfected T cells and fibroblasts, the Nef.GFP fusion protein produced a well-defined discrete pattern at the periphery of the cell and in the perinuclear region of the cell. This pattern of Nef.GFP fluorescence was similar to that observed previously for clathrin and AP-1/AP-2 adaptor protein complexes. AP-1/AP-2 adaptor protein complexes mediate the traffic of transmembrane proteins in the Golgi and at the plasma membrane via clathrin-coated membranes and vesicles. The recruitment of transmembrane proteins to clathrin coats involves recognition of sorting signals in the cytoplasmic domains of these proteins by the multisubunit adaptor protein complexes that interact directly (or indirectly) with clathrin and with cytoplasmic domains of transmembrane receptors. The observed concentration of Nef.GFP at the structures containing clathrin and adaptor complexes places Nef in physical proximity to adaptor-containing coats in the cell and imply that Nef interacts, either directly or indirectly, with a component of the coat, possibly the adaptor complex itself.

CD4 COLOCALIZES WITH NEF.GFP AND AP-2 AT THE PLASMA MEMBRANE

To address the possibility that Nef interacts with CD4 at the plasma membrane, we asked whether the NA7.GFP protein colocalizes with CD4 expressed at the cell surface. To stabilize a possibly transient association between Nef and CD4, these experiments were performed under conditions that block endocytosis. We found that in $CD4^+$ T cells expressing the Nef.GFP chimera, CD4 was redistributed into a punctate pattern at the cell margin. In contrast, in the absence of Nef, CD4 was uniformly distributed at the cell margin. The cell-surface expression of a mutant CD4 protein lacking most of the cytoplasmic domain, and unresponsive to Nef-induced down-regulation from the cell surface, was not affected by the NA7.GFP protein. The colocalization of CD4 at the cell surface with NA7.GFP, and of NA7.GFP with β-adaptin, together suggest that Nef redistributes CD4 to AP-2-containing coats at the plasma membrane. Together, this evidence supports a model whereby Nef recruits CD4 to the endocytotic machinery via AP-2 containing clathrin coats at the plasma membrane.

SCREENS TO IDENTIFY CELLULAR PROTEINS THAT MEDIATE NEF FUNCTIONS

We have initiated experiments to identify cellular proteins that mediate Nef functions, using the yeast two-hybrid system. In initial experiments, we isolated a large set of cDNA clones that specify proteins capable of binding HIV-1 Nef. Subsequently, we have been testing this pool of clones against a panel of mutant Nef proteins selectively deficient in individual functions to identify putative functionally relevant interactions. In the near future, these experiments will be further extended to SIV Nef because evidence from our previous experiments indicated that the HIV and SIV Nef proteins, although somewhat dissimilar at the amino acid level, perform the same functions, have a similar functional organization, and therefore are likely to interact with the same set of cellular proteins. We anticipate that this dual approach will greatly facilitate our search for host-cell proteins that mediate functional interactions of Nef with cell machineries.

SELECTIVE DISRUPTION OF SIVmac239 NEF FUNCTIONS

Our studies of SIVmac239 Nef indicate that it is functionally similar to HIV-1 Nef: It interferes with signal

transduction in T cells and down-regulates both CD4 and MHC class I molecules from the cell surface. Since SIV infection of rhesus macaques is currently the best animal model of human AIDS, the role of Nef in human AIDS can be inferred by studying SIVmac239 Nef in macaques. Our first goal is to identify mutant Nef proteins that are defective for only a single function. We then engineer an SIV to contain this mutant Nef and infect rhesus macaques with this mutant virus. By following the progress of the disease, and by analyzing the DNA sequence of viruses in these animals, we can make conclusions about the importance of individual Nef functions for AIDS. So far, we have identified mutations in SIVmac239 Nef that selectively disrupt CD4 down-regulation. These mutations are located in two clusters, one spanning residues 65 to 74 and the other at residues 204 and 205. We have yet to identify the residues that are critical for MHC class I down-regulation or for interfering with signal transduction.

SEPARATE FUNCTIONS OF NEF AND AIDS PATHOGENESIS

We have initiated a series of experiments that study mutant Nef proteins using SIV-infected rhesus macaques in collaboration with Dr. Frank Kirchhoff (University of Erlangen, Germany). Although experiments are still in progress which examine the importance of several conserved blocks of amino acids in Nef, we have completed two experiments. One study examined the importance of a conserved PxxP motif at positions 104–107 in SIV Nef. In HIV-1 Nef, such a PxxP motif mediates the interaction with the SH3 domain of Src-family tyrosine kinases. However, for SIV, it mediates the interaction with a p62 serine/threonine kinase. In macaques, we observed no significant reversion of mutations at this sequence prior to the development of AIDS. Therefore, the interaction of SIVmac239 Nef with the p62 serine/threonine kinase is not of critical importance for AIDS pathogenesis.

A second study examined the importance of residues in the two clusters that we had previously shown to be required for CD4 down-regulation. Three macaques were infected with a mutant SIVmac239 containing Nef with amino acid substitutions in both clusters, P73E, A74D, and D204R. Interestingly, we observed that early in infection, the mutant SIV replicated poorly. Sequence analysis showed that reversion of arginine at position 204 to the wild-type aspartic acid was rapidly selected in all three animals. Changes were also rapidly selected at position 73, although not to the wild-type proline. These changes were shown to restore the ability of Nef to down-regulate CD4 expression. These experiments indicate that these mutations at positions 73, 74, and 204 disrupted a critical function of Nef and suggest that CD4 down-regulation itself may be an important function of Nef in AIDS pathogenesis.

We are currently developing mutant Nef proteins with deletions in these regions that selectively disrupt only CD4 down-regulation. We hope that it will be more difficult for such mutations to revert in vivo and that this may allow us to more directly relate the loss of a single function (i.e., CD4 down-regulation) with lack of disease progression.

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ONCOGENE REGULATION

W.P. Tansey S.E. Salghetti

The maintenance of normal cellular growth and differentiation ultimately depends on mechanisms that regulate cell cycle progression. Two of the most important of these regulatory mechanisms are tran-

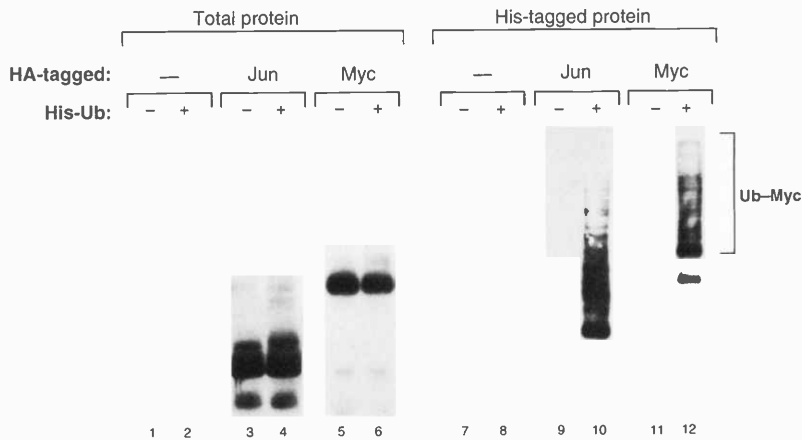

FIGURE 1 Myc is ubiquitylated in vivo. Human HeLa cells were transiently transfected with expression constructs encoding either HA-epitope-tagged Jun (lanes *3,4, 9,10*), HA-epitope-tagged Myc (lanes *5,6,11,12*), or His-tagged ubiquitin (lanes *2, 4, 6, 8,10,12*). Following transfection, 90% of the cells were lysed, and His-tagged proteins were recovered by nickel-affinity chromatography. The nickel-bound proteins (lanes *7–12*) were resolved by SDS-polyacrylamide gel electrophoresis, alongside total proteins (lanes *1–6*) from the remaining 10% of cells. Exogenously expressed Myc and Jun proteins were then detected by immunoblotting using an antibody against the HA-epitope tag. The position of Ub-Myc conjugates is indicated by the bracket.

scriptional activation and proteolytic destruction, which together control the appropriate appearance and disappearance of key cell cycle players. Work in our laboratory explores the intersection between these two processes to understand how oncogene transcription factors are regulated at the level of their own destruction.

The transcription factors controlling cell proliferation are tightly regulated at many levels, including synthesis, cellular localization, activation potential, and destruction. Indeed, many of these transcription factors, including Jun, p53, E2F, and Myc, are rapidly destroyed following their synthesis, with in vivo half-lives ranging from 20 minutes for Myc to 3 hours for E2F. The rapid and controlled turnover of these factors keeps their intracellular levels rapidly responsive to environmental factors, such as mitogenic signals and UV-irradiation, and maintains tight control over cellular proliferation, a role that is underscored by the fact that some oncogenic transcription factors escape this rapid degradation and are present in elevated levels in cancer cells. With the exception of Myc—for which little is known—these proteins are destroyed by ubiquitin-mediated proteolysis, in which their covalent attachment to the protein ubiquitin (Ub) signals their destruction by the 26S proteasome.

Ub-mediated proteolysis participates in many critical events within the cell, including signaling, DNA repair, and cell cycle progression. Protein ubiquitylation is a highly specific mutlistep process, which begins when an element within the target protein, termed a degron, is recognized by the ubiquitylation machinery. After the degron has been bound and recognized, Ub is then transferred to a lysine residue within the target protein. Repeated rounds of ubiquitylation result in a highly ubiquitylated target protein which is rapidly subject to proteasomal destruction. Because proteasomal destruction depends on prior substrate ubiquitylation, selectivity in degron recognition by the ubiquitylation machinery is central to the control of Ub-mediated proteolysis. Despite the importance of this process, however, the poor characterization of degrons and their mechanism of action have hampered our understanding of how proteins—like the oncogene transcription factors—are targeted for destruction.

Work in our laboratory is focused on uncovering the mechanism of Myc destruction. Myc is the focus of our work because (1) Myc is a prominent human oncoprotein that is aberrantly expressed in many lymphomas and lung cancers, (2) Myc activates cell cycle progression (via transcriptional activation of the

cdc25 phosphatases), and (3) the mechanism of Myc destruction has not been characterized. Indeed, although Myc has long served as a paradigm for a rapidly degraded nuclear protein, surprisingly little is known about how Myc is turned over in vivo.

We began our characterization of Myc destruction by asking if Myc, like many of its oncogene transcription factor counterparts, is destroyed by ubiquitin-mediated proteolysis. We have obtained three separate lines of evidence to support a role for the Ub-proteasome pathway in Myc turnover. First, we have shown that when a mouse cell line with a temperature-sensitive defect in an enzyme required for ubiquitin conjugation (E1) is placed at the restrictive temperature, steady-state Myc levels rise dramatically. Second, we have shown that chemical inhibitors of the proteasome also cause Myc levels to rise, both in primary human epithelial cells and in several transformed human cell lines. Finally, we have directly detected ubiquitylated forms of Myc in vivo. Figure 1 shows the results of a transient transfection experiment comparing the ubiquitylation status of exogenously expressed Myc with that of another oncoprotein transcription factor, Jun. In these experiments, approximately 1–2% of the total Myc population is trapped in a ubiquitylated state (lane 12), composed of mono-ubiquitylated Myc and a broad spectrum of higher-order Myc-Ub conjugates. The level of multi-ubiquitylation of Myc is comparable to that of Jun (compare lanes 10 and 12).

Together, these three lines of evidence demonstrate a prominent role for the Ub-proteasome pathway in directing Myc turnover. Future experiments in the laboratory will be geared toward understanding the Ub-mediated destruction of Myc in greater detail, focusing specifically on (1) identifying the Myc degron, (2) characterizing the factors responsible for targeting Myc for destruction, (3) identifying processes that regulate Myc destruction, and (4) determining whether the Myc destruction process is subverted or changed in cancer. Results of these studies should illuminate mechanisms governing an important part in the life (and death) of an important human oncoprotein.

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MOLECULAR GENETICS

This section comprises labs that study a diverse set of cellular phenomena, including signal transduction, cancer, morphogenesis, and differentiation using mainly genetic approaches in model eukaryotic systems. Michael Wigler's group identifies and analyzes the genes and loci that are mutated in human cancer and studies signal transduction in humans and yeasts, with particular emphasis on RAS, an oncogene conserved in evolution, and PTEN, a tumor suppressor that encodes a protein tyrosine phosphatase. Michael Hengartner's laboratory explores the components of the programmed cell death pathway in the nematode *Caenorhabditis elegans* and have begun to use that model genetic organism to study memory, learning, and neural cell functioning. David Beach's laboratory works on the regulation of the eukaryotic cell cycle and the abnormalities of cell cycle control that accompany oncogenic transformation. Greg Hannon's laboratory explores mechanisms of growth control in animal cells with a specific focus on the disruption of growth controls in tumor cells. Bruce Futcher's lab studies the control of cell division, with special emphasis on cyclin-dependent kinases and their substrates. The lab also works on telomeres and their relationship to aging. Rob Martienssen's laboratory is pursuing a systematic analysis of the Arabidopsis genome using gene trap and enhancer trap transposons. They are also continuing their analysis of leaf and chloroplast development, including the discovery of a chloroplast membrane protein in maize that defines a universal pathway for *sec*-independent protein translocation in bacteria and chloroplasts. Erich Grotewold's lab has made significant progress in elucidating the mechanisms by which Myb domain proteins control gene expression in plants. They have also determined that maize has more than 100 proteins containing this conserved domain, and tools to determine Myb function in plants have been developed. Hong Ma's lab identifies and studies genes important for reproductive development in *Arabidopsis*, particularly those required for normal floral organ development or controlling male meiosis and pollen development. Ueli Grossniklaus' group studies the genetic and molecular basis of plant reproduction in *Arabidopsis*, focusing on the development and function of the haploid female gametophyte and the maternal control of seed formation. David Jackson's group studies the development of the shoot apical meristem in maize and *Arabidopsis*, both model genetic organisms. They are characterizing a plant homeodomain protein that is involved in cell-to-cell communication and has the ability to traffic between plant cells.

MAMMALIAN CELL GENETICS

M. Wigler	C. Yen	D. Dong	L. Rodgers	A. Solanki
	M. Hamaguchi	J. Stolarov	M. Riggs	A. Buglino
	M. Nakamura	H. Tu	J. Troge	M. Katari
	R. Lucito	K. Chang	B. O'Conner	D. Almanzar
	L. Serina	J. West	V. Tu	J. Brodsky
	D. Esposito	Y. Han	A. Weiner	J. Douglas
	E. Hatchwell			

Our overall research objectives are to define the genetic alterations that cause human disease, especially cancer, and to determine their biochemical consequences. This effort is focused on three areas: signal transduction, in which we study the pathways of communication that become deranged in cancer cells; genomics, in which we apply and develop tools for the genetic analysis of human disease; and combinatorial chemistry, in which we are trying to develop a general methodology that will enable us to manipulate the bio-

chemical pathways involved in cancer. The ras pathway has figured prominently over the years in our studies of signal transduction, and RDA (representational difference analysis) has figured prominently in our genomic studies. RDA is a tool for genomic difference analysis developed in previous years in collaboration with Nikolai Lisitsyn (Lisitsyn et al., *Science 259:* 946 [1993]).

Studies of MAP Kinase Cascades

H. Tu, D. Dong

The MAP protein kinase cascade is a conserved motif in the signal transduction repertoire of probably all eukaryotic organisms. Our first encounter with it was in the ras-controlled sexual differentiation pathway in the yeast *Schizosaccharomyces pombe*. In that organism, ras1 acts directly on byr2, the kinase that activates byr1, itself a kinase that activates spk1, the kinase that is the homolog of the mammalian MAP/ERK family of kinases. In the control of this cascade, ras1 is joined by ste4, a leucine zipper protein that we have studied in previous years (Barr et al., *Mol. Cell. Biol. 16:* 5597 [1996]). We have demonstrated that ras1 and ste4 act independently and jointly upon the regulatory domain of byr2. We have now demonstrated the direct involvement of yet a further protein kinase, shk1, which appears to act by releasing the catalytic domain of byr2 from inhibition by its regulatory domain, thereby "opening" its conformation (Tu et al. 1997). We assess these changes in conformation by the two-hybrid system of Fields and Song (*Nature 340:* 245 [1989]). Our results suggest that ras1 exerts its influence upon byr2 through two routes: one by directly acting upon byr2 (Van Aelst et al., *Proc. Natl. Acad. Sci. 90:* 6213 [1993]), and another through a pathway involving scd1, cdc42, and shk1 (Chang et al., *Cell 79:* 131 [1994]).

byr2 is a member of the MEKK family of protein kinases, and shk1, which acts upon it, has a similar structure and belongs to another family of protein kinases, the PAK family of protein kinases. Like the MEKK kinases, these kinases also have a carboxy-terminal catalytic domain and an amino-terminal regulatory domain that contains the binding site for a member of the ras family of GTPases. We have tested whether the tools for analyzing the regulatory components of byr2 could be used for the study of the PAK family of kinases. Preliminary studies encourage us in this. The regulatory domain of shk1 binds its catalytic domain, and mutations in the regulatory region that abolish its binding to the catalytic region "open" the conformation of shk1 and genetically "activate" the kinase. Using the opening of the wild-type shk1 as a genetic screen, we have identified a novel open reading frame that may encode an upstream activator of this kinase.

PTEN, a Tumor Suppressor Encoding a Phosphoprotein Phosphatase

J. Strolarov, K. Chang, C. Yen

The use of RDA led to the identification of a region on chromosome 10 deleted in a variety of human cancers, including breast, prostate, and brain cancers. We therefore suspected that this region harbored a tumor suppressor gene. In a collaboration with Ramon Parsons of Columbia University, a gene from this region, PTEN, was identified and found to be mutated in many cancers (Li et al. 1997). In collaboration with Michael Myers and Nick Tonks at CSHL, PTEN was shown to encode a protein tyrosine phosphatase with a somewhat atypical substrate preference (Myers et al. 1997). In addition to its relation to phosphatases, the PTEN protein contains motifs also found in cytoskeletal proteins. The discovery by Parsons and collaborators that the PTEN gene is mutated in a hereditary disease predisposing the afflicted with neoplastic growths completes the demonstration that PTEN is a tumor suppressor gene (Liaw et al., *Nat. Genet. 16:* 64 [1997]; Marsh et al., *Nat. Genet. 16:* 333 [1997]).

Three lines of investigation into the biology of PTEN are being pursued. First, we have expressed the PTEN protein in cultured tumor cells that lack a functional PTEN gene. As yet we have not observed a clear phenotypic response in cell culture. Second, PTEN has a homolog in the yeast *Saccharomyces cerevisiae*, and experiments looking at the effects of deleting or overexpressing the gene in such yeast have begun. Again, as yet no phenotype is apparent. Third, we have searched for binding partners of PTEN using the two-hybrid system of Fields and Song and have found a number of binding partners, including proteins containing PDZ domains. Many PDZ domain proteins act as scaffolding proteins for proteins involved in signal transduction (Ponting et al., *BioEssay 17:* 5876 [1997]). We are attempting to explore the physiological relevance of our findings.

In a collaboration with Richard McCombie's lab at CSHL, we initiated the sequencing of the PTEN locus. Nearly 180 kbp of genomic sequence have been completed by his group.

Loci Amplified in Breast Cancer

M. Nakamura, R. Lucito, C. Yen

From the analysis of breast cancer biopsies, we have derived many probes derived from amplified loci. These loci are suspected to contain oncogenes that drive the growth and malignancy of breast cancers. Along with loci that were known previously, such as the ErbB2, c-myc, and cyclin D loci, several new loci have been observed. We are in the midst of trying to identify the genes encoded in these regions and have been concentrating on a region that lies near to but is distinct from the c-myc region.

As a first step in characterizing amplified loci, we need to perform "epicenter" mapping, a process that delineates the minimum region which is commonly amplified. To achieve this, it is necessary to search through large archives of stored breast cancer biopsies. For this purpose, we have developed a technique for "immortalizing" and amplifying the DNA of the samples for present and future analyses (Lucito et al. 1998). The technique is based on a high-complexity "representation" of tumor DNA, which entails its cleavage, ligation to adaptors, and subsequent PCR amplification. We have shown that such representations are useful for measuring gene copy number and, in collaboration with Joe Grey's laboratory (University of California, San Francisco), can be used for comparative genomic hybridization, a technique useful for the global assessment of genomic changes (Kallioniemi et al., *Genes, Chromosomes & Cancer 10:* 231 [1994]). "Quantitative" PCR can also be used to measure gene copy number in high-complexity representations (Gibson et al., *Genome Res. 6:* 995 [1996]).

Loci Deleted in Breast Cancer

C. Yen, M. Hamaguchi, R. Lucito, Y. Han, D. Esposito, M. Nakamura

Several loci have now been identified by RDA that are homozygously deleted in breast cancer. Among these are the PTEN locus and the locus encoding p16, the inhibitor of the cdc2 and cdc4 cyclin-dependent protein kinases. Ten loci remain in which we are searching for genes. This process also entails epicenter mapping, which as described above, narrows the region in which we need to search for genes. Unfortunately, the regions of homozygous deletion are quite large, often greater than a megabase upon initial characterization, and hence the process of gene searching has become the rate-limiting step in discovery. Several steps have been taken to accelerate this phase of our work. The first is the use of high-complexity representations, which has already been described. The second is the development of a solution hybridization technique, called RICH, for the cloning of cDNAs that hybridize to a given large-insert chromosomal vector such as a BAC or YAC (Hamaguchi et al. 1998).

RICH (rapid isolation of cDNAs by hybridization) is meant to complement the searching of genes by exon trapping and sequence analysis. The ends of fragments of restriction-endonuclease-cleaved cDNA libraries are modified so that when they hybridize to fragments from the insert of large cloning vectors, they can be selected and amplified and cloned. The application of this technique has been successfully tried on the c-myc locus (an example of amplification) and the PTEN locus. In a "mock" comparison with exon trapping and sequence analysis, the method fares well.

Rearrangements in Cancer and Spontaneous Genetic Disease

L. Serina, E. Hatchwell, C. Yen

We are beginning to adapt RDA to detect rearrangements in cancer. Several reasons for attempting this are, first, the present use of RDA for the discovery of tumor suppressors is biased to the discovery of large regions of homozygous deletion, and these may be "gene-poor" regions of the genome. Second, every deletion will create a rearrangement, and small deletions or large deletions do so equally. Small deletions are more likely to involve directly the rearrangement of a target gene, and hence the bridging region is more likely to be directly linked to a target gene. Although we have encountered rearranged regions with RDA as we now practice it, the method was not optimized for that task. We are now testing a variety of improved methods for the "co-representation" of tumor and nor-

mal DNAs that are designed to find differences in the mobility of restriction endonuclease fragments in the respective genomes. The same kinds of methods may find application to the discovery of the spontaneous genetic lesions that can occur in the germ line of a parent and be transmitted as de novo disease to offspring.

Combinatorial Chemistry

D. Dong

In collaboration with Peter Nestler's lab at CSHL, we have been investigating the binding capacity of a class of branched peptides, or "forcep" molecules. These have been generated as members of encoded combinatorial libraries, a method developed in collaboration with C. Still's lab at Columbia University (Ohlmeyer et al., *Proc. Natl. Acad. Sci. 90:* 10922 [1993]). As targets, we have chosen the carboxyl end of H-ras, because it is the substrate of farnesyl transferase, the first step in the essential processing of ras proteins (Gibbs, *Cell 65:* 1 [1991]) and because this end is predicted to be exposed as a peptide. Several members of this forceps library have been shown to bind specifically to the carboxy-terminal sequences of H-ras and to recognize these sequences when they are fused to other proteins. A subset of these forceps also block the farnesylation of H-ras. These results, although very preliminary at the moment, suggest that branched peptidic molecules will have the general capacity to recognize peptides and may indicate that this will be a fruitful class of molecules that can modulate biological systems.

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CAENORHABDITIS ELEGANS DEVELOPMENTAL GENETICS

M. Hengartner	S. Desnoyers	Q. Liu
	A. Gartner	S. Milstein
	T. Gumienny	G. Parvilus (URP)
	D. Hoeppner	M. Spector
	J. Keller	S. Tharin

Our laboratory uses the small nematode *Caenorhabditis elegans* as a model organism for the study of basic biological problems. Our two main areas of interest are (1) programmed cell death (apoptosis) and (2) nervous system development and function.

PROGRAMMED CELL DEATH

Programmed cell death (PCD, also known as apoptosis) is a mechanism used by multicellular organisms to eliminate cells that are not needed or are potentially dangerous. PCD plays important roles in animal development and homeostasis and occurs in a wide variety of tissues in both vertebrates and invertebrates. Proper control of PCD is crucial: Breakdown in the regulation of this process contributes to the pathogenesis of a large number of diseases, including cancer, autoimmunity, neurodegenerative diseases, ischemic stroke, and myocardial heart infarct.

The nematode *C. elegans* provides an attractive system for the study of PCD, as its development has

been extensively characterized and the animal is readily amenable to genetic and molecular manipulations. Hundreds of mutations that affect nematode cell death have been isolated over the years; these mutations identify more than 16 genes that function in the regulation and execution of apoptosis. Three genes encode key regulators of the apoptotic cell fate. Two of these (CED-3 and CED-4) are required for cell death; the third (CED-9) neutralizes CED-3 and CED-4 in cells that should live, thereby protecting them from apoptosis. Homologs of all three nematode genes have key roles in the regulation of apoptosis in mammals. CED-9 is a member of the Bcl-2 family, CED-4 is similar to the recently identified proapoptotic factor Apaf-1, and CED-3 is homologous to a family of more than ten cysteine proteases, known as the caspases.

The Central Death Machinery in *C. elegans*: The Apoptosome

M. Spector, S. Desnoyers

How does CED-9 prevent PCD? In the past year, we have reported that CED-9 interacts physically with CED-4, both in the yeast two-hybrid system and in vitro. Mutations that reduce or eliminate *ced-9* activity also disrupt its ability to bind CED-4, suggesting that this interaction is important for CED-9 function. Thus, CED-9 might control *C. elegans* cell death by binding to and regulating CED-4 activity. Interestingly, other investigators have shown that activation of CED-3 requires a physical interaction between proCED-3 and CED-4; this interaction somehow promotes autocatalytic processing of proCED-3. We suspect that in cells that should survive, CED-9 holds CED-4 in an inactive conformation, thereby preventing CED-4-mediated activation of proCED-3. The ability of CED-9, CED-4, and CED-3 to exist in a multiprotein complex has led to the "apoptosome" model of cell death regulation (Fig. 1a). Because the genetic pathway for PCD in conserved through evolution, it is tempting to speculate that a similar regulatory mechanism might exist in mammals (Fig. 1b).

We are now in the process of confirming the existence and subunit composition of the worm apoptosome, as well as the subcellular localization of its component proteins in living and apoptotic cells.

Genetic Analysis of Caspase Function in *C. elegans*

A. Gartner, D. Hoeppner [in collaboration with Yuri Lazebnik, Cold Spring Harbor Laboratory]

CED-3 is a homolog of a mammalian family of cysteine proteases known as the caspases. As is the case for CED-3, many of the caspases have been implicated in apoptosis. The ability of several of the caspases to activate other members of the family has led to the "caspase cascade" model of apoptosis, in which upstream "regulatory" caspases activate downstream "execution" caspases. Because only one caspase homolog has been described in *C. elegans*, it is not clear whether such a cascade could also be acting in the worm. We have recently identified two additional caspase homologs in the *C. elegans* genome. We are very interested in understanding the role that these genes might have in PCD (or other processes). We are using a reverse genetic approach to identify mutations in these genes. In addition, we have set up a collaboration with Yuri Lazebnik and his colleagues to study the biochemical characteristics of these two proteins.

Programmed Cell Death in the *C. elegans* Germ Line

S. Milstein, T. Gumienny, A. Gartner

How is the apoptosome regulated? The answer to this question is likely to be complex, as even a single cell might need to integrate multiple signals to determine whether it should activate the death pathway or keep on living. To address this question in *C. elegans*, we have set out to determine how specific cell types decide between life and death.

One of the cell types on which we chose to focus is the germ cell. PCD has a major role in the adult hermaphrodite germ line. Our previous studies have shown that most of the germ cells that differentiate along the oogenic pathway undergo PCD. We suspect that these deaths serve a homeostatic function and are needed to regulate the number of cells that are allowed to differentiate into oocytes.

Interestingly, we have found, in collaboration with Eric Lambie (Dartmouth University), that activation of the ras pathway is necessary for germ cells to

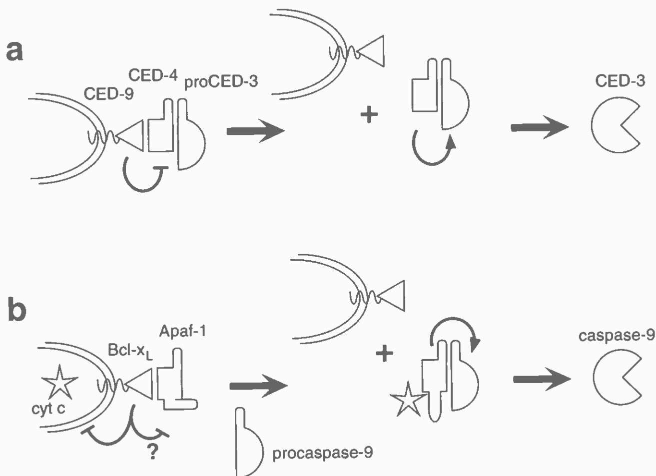

Figure 1 The apoptosome: A model for the mechanism of action of the cell death machinery. (*a*) In *C. elegans*, the cell death regulators CED-3, CED-4, and CED-9 are predicted to be stably associated in a multiprotein complex localized to the outer surface of mitochondria. This complex is expected to be present in all cells, but in an inactive state. In cells fated to die, a proapoptotic stimulus modifies the complex, possibly resulting in the dissociation of CED-3/4 from CED-9. Once freed, CED-4 allows the CED-3 proenzyme to autoactivate. The active protease then cleaves the relevant apoptotic substrates, bringing on the death of the cell. (*b*) A similar complex might exist in mammals. This complex would be expected to include a Bcl-2 family member, such as Bcl-xL, an adapter molecule such as Apaf-1, and a procaspase. For the Apaf-1/procaspase-9 complex, cytochrome c has been shown to act as an essential cofactor. (Adapted from Hengartner 1997.)

undergo PCD. We suspect that activation of the ras pathway might be required for germ cell death because it promotes progression from an early, cell-death-resistant stage to a later, cell-death-sensitive stage of meiosis. Presumably, further differentiation restores death resistance to the mature oocytes. Alternation between death-resistant and death-sensitive stages during differentiation has previously been observed, notably during B- and T-cell development. In several of these cases, a striking correlation has been observed between the sensitivity to death and levels of one or more members of the CED-9/Bcl-2 family. Whether germ cell death is regulated at the level of CED-9 is not yet clear.

To further decipher how germ cells make the decision between life and death, we have screened for mutations that specifically affect PCD in the germ line. In the last year, we have isolated more than 20 mutations that result in excessive germ cell death. We are now in the process of characterizing and classifying these mutations.

Genes Involved in the Engulfment of Dying Cells

Q. Liu, T. Gumienny

Once a cell activates the apoptotic machinery, a number of downstream "subprograms" are activated, with

the goal of rapidly breaking down the cell and removing it from the body. One important subprogram results in the generation of signals that promotes recognition and phagocytosis of the dying cell by other cells.

Six genes have previously been shown to function in this process in *C. elegans*. In the past year, we have cloned one of these six genes, *ced-6*. We found that the CED-6 protein contains a phosphotyrosine-binding (PTB) domain and a number of potential SH3-binding sites, suggesting that CED-6 might act in a signal transduction cascade activated once a cell recognizes that one of its neighbors is undergoing PCD. Consistent with this hypothesis, mosaic analysis indicates that CED-6 acts within the engulfing cells.

NERVOUS SYSTEM FUNCTION AND DEVELOPMENT

A second area under investigation in the lab is the nervous system in *C. elegans*. The small size and relative simplicity of the nematode nervous system allows us to readily address complex questions at the genetic and molecular levels.

Development of a Tetracycline-responsive System in *C. elegans*

S. Tharin, G. Parvilus

A tetracycline-responsive system for conditional gene expression has recently been developed for use in mammalian cells in culture and in transgenic mice. Because such a conditional expression/repression system would be useful for our studies of the nervous system, we have been working toward adapting this system for *C. elegans*.

In preliminary experiments, we had attempted to control gene expression in the six touch cell neurons using both a tet-transactivator and a reverse transactivator system. Unfortunately, our initial results were disappointing (some background expression, and little if any specific expression). During the last year, we have continued to experiment with different coinjection markers and stronger promoters driving the tet-transactivator in an effort to overcome these two problems.

Learning and Memory

S.Tharin [in collaboration with John Connolly, Tim Tully, Cold Spring Harbor Laboratory, and Derek van der Kooy, University of Toronto, Canada]

We are interested in analyzing the molecular basis of associative learning and memory in *C. elegans*. We are pursuing this project in collaboration with the Tully and van der Kooy laboratories. During the last year, we have continued to make progress toward the development of a satisfactory conditioning paradigm, which we will use to screen for mutants defective in acquisition, short-term memory, or long-term memory.

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EUKARYOTIC CELL CYCLE CONTROL

D. Beach	S. Allan	K. Dai	M. Serrano
	A. Carnero	K. Galaktionov	P. Sun
	J. Chen	R. Maestro	J. Wang
	D. Conklin	S. Salghetti	L. Xie

During the course of the year, Manuel Serrano accepted a staff investigator position at the Universidad Autonoma Cantoblanco in Spain, Konstantin Galaktionov became an assistant professor at Baylor College of Medicine in Houston, and Kang Dai became a scientist II at Chiron Corporation. Amancio Carnero accepted a postdoctoral fellowship at the Institute of Child Health in London; Roberta Maestro, a visiting scientist, returned to her lab at the Centro di Riferimento Oncologico Aviano in Italy; and Simone Salghetti left to have a baby and returned to work with Bill Tansey. We were joined by Pedro Otavio de Campos Lima, a postdoctoral fellow from the Karolinska Institute.

Genetic Screens to Identify Genes Involved in Cellular Immortalization and Anchorage-independent Growth in Human Cells

D. Conklin, D. Beach [in collaboration with Cathy Reznikoff, University of Wisconsin-Madison]

Cells in higher organisms are subject to genetically programmed internal constraints on proliferation. The processes of programmed cellular senescence and anchorage-dependence of growth place important restrictions on the extent and locale of cell division. What little is known of these processes is directly related to G_1 cell cycle control and tumor suppression. In an effort to identify new gene products that are involved in cellular senescence and anchorage dependence, two functional cloning screens, whose design presupposes as little about these processes as possible, have been undertaken in nearly normal human cells.

Cellular senescence was first used by Hayflick and co-workers to describe the decreased ability of human fibroblasts to divide upon continual subculture in vitro. This is now viewed as a universal property of almost all cells in vitro and, although difficult to prove, of most cells in vivo as well. Genes whose overexpression immortalizes or significantly extends the replicative life span of normal presenescent human uroepithelial cells (HUCs) are being isolated in functional screens using replication-defective retrovirus cDNA libraries. HUCs are isolated from resected ureters, which are normally discarded during kidney transplant surgeries. They are routinely cultured in vitro and predictably undergo approximately 50 population doublings before senescing. Introduction of HPV E7 results in an extended life span of two additional passages and the potential for low-frequency immortalization. Cytogenetic evidence indicates that a limited number of gene amplifications cooperate with the HPV E7 oncoproteins in the immortalization of HUCs. Retroviral cDNA libraries have been constructed using the mRNA of post-crisis immortalized HUC cells. These libraries have been introduced into presenescent HUCs, and retroviruses have been excised from immortal cell populations. At present, 42 candidate genes from approximately 15 immortal cell populations have been isolated and are being retested for authenticity.

The loss of anchorage dependence of growth is thought to be important in tumor formation and is indeed the best known cellular correlate to tumorigenicity. Genes whose overexpression or underexpression permits cells to grow in soft agarose are being isolated using retroviral sense and antisense cDNA libraries. The host strain for this screen is the human fibroblast line MSU1.1, which is immortal, yet not transformed. Retroviral cDNA libraries were constructed using the mRNA of both transformed and untransformed cells. At present, several candidate genes have been isolated and are being retested.

MDM-2 Overcomes TGF-β Growth Arrest via a p53-independent, RB/E2F-dependent Mechanism

P. Sun, D. Beach

Transforming growth factor-β (TGF-β) is a multifunctional cytokine that has important roles in suppressing tumorigenicity. In some tumors, progressive loss of

TGF-β sensitivity is associated with their aggressiveness. To identify genes that overcome cell cycle arrest by TGF-β, we performed a genetic screen in mink lung epithelial (Mv1Lu) cells, using a retroviral expression system recently developed in our lab.

A mixture of poly(A)$^+$ RNA from Swiss-3T3 and BALB-3T3 cells was chosen as the source of our cDNA library. The library was cloned into a retroviral expression vector HygroMaRXII, packaged in an ecotropic virus packaging cell line LinX E, and used to infect Mv1Lu cells, which were pseudotyped with ecotropic virus receptor. The infected cells were selected with hygromycin and then with TGF-β. Integrated provirus was then recovered from TGF-resistant cells with Cre recombinase. Three genes, MDM-2, c-Myc, and NFIX, were cloned from this screen. Among them, c-Myc was previously demonstrated to be able to overcome TGF-β arrest in mouse keratinocytes in a transient assay.

Because previously the oncogenic activity of MDM-2 was attributed to its ability to inactivate p53, and p53 was also shown to be involved in the TGF-β responses, we asked whether MDM-2 overcomes TGF-β arrest by eliminating p53. The expression of two dominant-negative alleles of p53, p53Val135 and p53175H, was unable to rescue TGF-β arrest, although they greatly reduced the p53-dependent reporter activity to almost background in a transfection assay using a p53-dependent luciferase reporter. This suggested MDM-2 probably overcomes TGF-β arrest through a p53-independent pathway in Mv1Lu cells.

The fact that MDM-2 could also bind to RB and E2F led us to investigate whether MDM-2 confers TGF-β resistance through the RB/E2F pathway. The following observations were made. (1) In wild-type Mv1Lu cells, TGF-β treatment led to dephosphorylation of RB. However, in MDM-2-expressing cells, as well as in c-Myc- and NFIX-expressing cells, RB remained hyperphosphorylated with TGF-β treatment. (2) The endogenous E2F activity in MV1Lu cells was assessed by transient transfection of a luciferase reporter gene containing three copies of E2F DNA-binding sites into wild-type cells or cells expressing MDM-2, c-Myc, or NFIX in the presence or absence of TGF-β. TGF-β treatment reduced E2F activity by twofold in wild-type cells, and expression of MDM-2, c-Myc, or NFIX abolished this reduction. (3) TGF-β treatment led to a gradual decrease in E2F-1 protein level in Mv1Lu cells. However, there was only a slight decrease in MDM-2-, c-Myc-, and NFIX-expressing cells. (4) E2F-1-overexpressing cells became TGF-resistant to a similar extent as MDM-2-expressing cells.

Our study demonstrated that in addition to inactivating p53, overexpression of MDM-2 in tumors renders cells insensitive to the tumor-suppressing function of TGF-β through a p53-independent mechanism. It is achieved by preventing TGF-β from dephosphorylating RB and down-regulating E2F expression. Since it was shown that IL-6 also leads to growth arrest by dephosphorylating RB and reducing E2F protein level, it is likely that MDM-2 also insensitizes tumor cells to other negative growth regulators, by contributing to the malignancy of tumors. This study also reveals a biological function of the p53-independent activity of MDM-2. In certain tumors, p53 inactivation and MDM-2 overexpression occur independently, which suggests that MDM-2 has p53-independent oncogenic functions. The observation that MDM2 overcomes growth arrest by TGF-β and other growth inhibitors through a p53-independent pathway may provide a potential mechanism for such functions of MDM-2.

Myc Activates Telomerase: Extension of Cellular Life Span

J. Wang, D. Beach

Telomere maintenance has been proposed as an essential prerequisite to human tumor development. The telomerase enzyme is itself a specific marker for tumor cells, but the genetic alterations that activate the enzyme during neoplastic transformation have remained a mystery. Amplification of the *myc* oncogene is prevalent in a broad spectrum of human tumors. Here, we show that *myc* induces telomerase both in normal human mammary epithelial cells (HMEC) and in normal human diploid fibroblasts. Myc increases expression of hEST2 (hTRT/TP2), the catalytic subunit of telomerase. Since hEST2 limits enzyme activity in normal cells, *myc* may control telomerase solely by regulating hEST2 levels. Activation of telomerase through hEST2 is sufficient to increase average telomere length and extend life span in normal human mammary epithelial cells. Since *myc* can also extend the life span of these cells, activation of telomerase may be one mechanism by which *myc* contributes to tumor formation.

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GROWTH CONTROL IN ANIMAL CELLS

G. Hannon P. Dong

A hallmark of tumor cells is the ability to survive and proliferate under circumstances that are incompatible with the growth and survival of normal cells. Transformation of a normal cell into a tumor cell requires myriad genetic alterations that permit escape from governance by normal growth regulatory programs and that promote unchecked proliferation. Control over the growth of normal cells comes in many forms. For example, the growth and survival of normal cells require the presence of specific extracellular factors while different factors act in a dominant manner to prevent proliferation. In transformed cells, the dependence on extracellular signals is often reduced by activation of cellular oncogenes; however, in a normal cell, oncogene activation frequently leads to programmed cell death unless additional mutations counter this response. Normal cells are also subject to a preprogrammed limit on the number of divisions that they can execute. After this limit is reached, cells enter replicative senescence, a growth arrest that is thought to be irreversible. During the past year, we have used a genetic approach to investigate multiple levels of growth control in mammalian cells. Our efforts have focused on those controls that are altered as a normal cell evolves into a tumor cell.

RESISTANCE TO GROWTH-INHIBITORY CYTOKINES: TRANSFORMING GROWTH FACTOR-β

TGF-β is a multifunctional cytokine that halts the proliferation of many normal epithelial cells. However, tumor cells often acquire resistance to the inhibitory effects of TGF-β. To investigate the mechanisms by which cancer cells resist growth inhibitory cytokines, we searched for genes that, when inappropriately expressed, bypass TGF-β-mediated growth arrest. This work, done in collaboration with Peiqing Sun and David Beach here at the Laboratory, led to the identification of three gene products: c-*myc*, NF-1X, and *mdm-2*. The c-*myc* oncogene had previously been shown to nullify TGF-β sensitivity in murine keratinocytes, so isolation of this gene in a genetic screen for TGF-β resistance validated our approach. Neither NF-1X nor mdm-2 had previously been connected with the TGF-β response.

The *mdm-2* oncogene is a regulator of the p53 tumor suppressor. Increased expression of *mdm-2* inhibits p53 function at multiple levels, including antagonism of transcriptional activation and promotion of p53 degradation. Our results therefore seemed to implicate p53 in TGF-β-mediated cell cycle arrest. To test this possibility, we used dominant-negative mutants of p53 to interfere directly with p53 function in our target cells. Surprisingly, we found that loss of p53 function did not affect the ability of TGF-β to enforce growth arrest.

We were therefore left to search for a new mechanism by which the *mdm-2* oncogene might affect cell proliferation. Our experiments and those of numerous other labs have suggested that growth arrest following treatment with TGF-β can be eliminated by inactivation of the Rb pathway. Two previous reports had suggested that *mdm-2* protein could interact in vitro and in vivo with the Rb protein itself and with a transcription factor, E2F-1, that is regulated by Rb. However, the biological consequences of these interactions were unclear. Following TGF-β treatment, the Rb protein accumulates in the hypophosphorylated, growth-inhibitory state. In addition, the abundance of E2F-1 protein is diminished, probably through posttranslational mechanisms. We found that *mdm-2* expression prevented both of these alterations. However, the possibility remained that *mdm-2* might accomplish this indirectly by effects on TGF-β signal transduction or through regulation of other cell cycle pathways.

To address more directly the biological interaction between the *mdm-2* and the Rb pathway, we asked whether *mdm-2* expression could abrogate the growth

arrest that is enforced by expression of INK4-family CDK inhibitors. INK4 proteins prevent Rb phosphorylation by inhibition of an essential Rb kinase. Rb is therefore locked in a hypophosphylated, growth inhibitory state. One member of the INK4 family, p15 INK4B, is a downstream target of the TGF-β signal transduction pathway and is a likely effector of TGF-β-mediated growth arrest. Expression of *mdm-2* substantially rescued the growth inhibition caused by either p15 INK4B or p16 INK4A or, in fact, by enforced expression of Rb itself.

Considered together, our results point to a direct connection between the *mdm-2* and the Rb pathway. Thus, *mdm-2* is a cellular oncogene that, like many viral oncoproteins, can interfere with multiple tumor suppression pathways: the p53 pathway and the Rb/p16 pathway.

RESCUE FROM ONCOGENE-INDUCED APOPTOSIS

When exposed to oncogenic insults such as increased expression of cellular oncogenes (e.g., *myc*) or introduction of viral oncoproteins (e.g., E1A), normal cells will often execute an apoptotic program that may serve to protect multicellular organisms from unwarranted proliferation. In tumors, oncogene activation is often accompanied by increased expression of anti-apoptotic genes such as *bcl2*. Thus, tumor cells not only survive oncogene activation but thrive because of it. We have used a genetic approach to identify genes that can protect cells from apoptosis provoked by a cellular oncogene, c-*myc*, that is commonly activated in human tumors.

Roberta Maestro, a visiting scientist from the C.R.O. in Aviano, Italy, introduced cDNA expression libraries into a rat fibroblast cell line that conditionally expresses the *myc* oncogene. These cells normally undergo apoptosis when *myc* is activated and growth factors are withdrawn. We therefore exploited this response to identify genes that would the prevent cell death that occurred following *myc* induction. A number of different genes were isolated by this approach. It was initially gratifying to find among protective cDNAs the *MCL-1* gene. *MCL-1* is a member of the bcl2 family, a group of antiapoptotic proteins that have been implicated in the development of a variety of human tumors. Of the remaining genes, we chose to focus our initial efforts on a transcription factor, *twist*.

The *twist* protein was originally isolated in *Drosophila* as one of the zygotic genes required for dorsoventral patterning during embryogenesis. Targeted deletion of the *twist* protein in mice is lethal, and *twist*-null embryos show massive apoptosis in the developing somites. Therefore, isolation of *twist* as an anti-apoptotic factor in a genetic screen fit well with the phenotype of *twist* disruption in vivo.

Apoptosis in response to *myc* activation depends in part on the integrity of the p53 protein. Enforced *twist* expression relieved the growth arrest caused by p53 activation in murine embyro fibroblasts, suggesting that *twist* might protect from *myc*-induced apoptosis by interfering with p53. Consistent with this notion, *twist* expression reduced the ability of p53 to activate transcription from p53-responsive elements. *twist* can interact in vitro and in vivo with a p53 coactivator, p300. Preliminary data indicate that *twist* may also affect p53 function through a direct interaction with the p53 protein.

Since *twist* can interfere with the function of a cellular tumor suppressor protein and can thus protect against oncogene-induced apoptosis, *twist* itself may contribute to tumor formation. Interestingly, patients affected by the Saethre-Chotzen syndrome due to mutations in the *twist* gene show not only developmental abnormalities, but also a tendency to develop tumors. Continuing efforts will be directed toward understanding the mechanism by which *twist* interacts genetically with cellular oncogenes and tumor suppressors and toward investigating the involvement of *twist* in tumor formation.

Myc ACTIVATES TELOMERASE: EXTENSION OF CELLULAR LIFE SPAN

Stabilization of telomeric repeats has been proposed as a prerequisite for tumorigenesis. Circumstantial support for this notion comes from the observation that telomerase is activated in a high percentage of late-stage human tumors, whereas telomerase is largely absent from somatic cells in vivo and from normal human cells in culture. As normal cells proliferate, telomeric repeats are progressively lost due to the incomplete replication of chromosome ends during each division cycle. Telomere shortening has been proposed as the mitotic clock that marks the progress of a cell toward the end of its replicative life span. According to this model, erosion of chromosome ends triggers cellular senescence. The possibility that telomere maintenance might be an essential component of the tumorigenic phenotype led us to survey known oncogenes for the ability to activate the telomerase enzyme.

In collaboration with Jenny Wang and David Beach, oncogenes were introduced into normal human

mammary epithelial cells. Of those tested, only c-*myc* showed a substantial effect on telomerase activity. In fact, expression of *myc* increased telomerase to a level similar to that seen in a number of breast carcinoma cell lines. Myc could also induce telomerase activity in normal human fibroblast cells, again to levels approximating those seen in fibroblast-derived tumor cell lines.

Recently, a number of subunits of the telomerase enzyme have been cloned. The abundance of one of these, hEST2 (the catalytic subunit), has been proposed to limit telomerase activity. In fact, expression of hEST2 alone was sufficient to activate the telomerase enzyme in a variety of cell types. In mammary epithelial cells, increased *myc* expression led to increased abundance of hEST2, providing a possible mechanism for regulation of telomerase activity by *myc*.

To probe the link between telomere length and replicative life span, *myc* and hEST2 were introduced into normal human mammary epithelial cells which were then continuously cultured. After a defined number of cell divisions (about 22 passages or 50–60 population doublings), normal cells entered replicative senescence. In contrast, cells expressing either *myc* or hEST2 have continued to proliferate. Escape from senescence was accompanied by either a stabilization or an increase in mean telomere length (for *myc* and hEST2, respectively). This suggests that the sole act of manipulating telomere length can affect cellular life span and establishes telomeres as one mechanism by which cells measure their replicative age.

Promotion of cell proliferation and oncogenic transformation by *myc* probably requires induction of a number of different target genes. As telomere maintenance may contribute to the long-term proliferative potential of tumor cells, telomerase activation may be an essential component of the ability of *myc* to facilitate tumor formation.

SUMMARY

The last 2 years were largely invested in the development of genetic approaches that can be applied to a variety of biological problems in mammalian cells. This year has seen a successful application of these technologies to three aspects of the process by which a normal cell is converted into a tumor cell. Through the study of an extracellular growth inhibitory factor, we have found that a known cellular oncogene, *mdm-2*, can interfere not only with the p53 pathway (as was previously established), but also with the Rb/p16 tumor suppressor pathway. Studies of oncogene-induced apoptosis are aimed at identifying the signals that instruct activation of the apoptotic machinery in response to inappropriate proliferative stimuli. This project has thus far yielded a connection between a developmental transcription factor and a tumor suppressor gene, p53. This link raises the possibility that the mechanisms that control apoptosis during development may overlap those that promote cell survival in tumors. Finally, our investigation of telomerase regulation has identified a connection between an oncogene that is commonly activated in human tumors and maintenance of telomeres. Although the contribution of *myc* to neoplastic transformation may require the induction of a number of target genes, activation of telomerase is likely to be a key component of the oncogenic potential of *myc*.

CELL CYCLE CONTROL IN *SACCHAROMYCES CEREVISIAE*

B. Futcher J. Donovan, N. Edgington, F. Ferrezuelo, S. Honey, M. Lessard, B. Schneider, G. Sherlock, T. Volpe, H. Wijnen, J. Zhou

Our main interest continues to be regulation of Start and mitosis in *Saccharomyces cerevisiae*. We are most interested in the tethering of division to cell growth and in the generation of the basic cell cycle oscillation. Many key cell cycle events are regulated by protein kinase complexes formed between Cdc28 and one of nine cyclins. These fall into two broad groups: the G_1 cyclins, which include Cln1, Cln2, and Cln3, that regulate Start; and the mitotic, B-type cyclins Clb1, Clb2, Clb3, and Clb4. Two other cyclins, Clb5 and Clb6, are very important for DNA replication but also have roles at Start and perhaps also in early mitosis. A second interest is yeast and human telomerase, and how telomere length relates to cell senescence.

A Network for Exit from Mitosis

J. Donovan

High Cdc28 kinase activity is required for mitosis. At the end of anaphase, when chromosomes have been separated, this Cdc28 kinase must be inactivated to allow the mitotic spindle to dissolve and to allow cytokinesis. This inactivation can be thought of as consisting of two steps: first, a signaling step, in which the completion of anaphase triggers some unknown signal, and second, an inactivation step, in which Cdc28 kinase is inactivated in two and possibly three ways. These are (1) destruction of cyclins via the APC; (2) inhibition of the kinases via the CDK inhibitor Sic1; and possibly (3) inhibitory phosphorylation of Cdc28 at Tyr-19. A network of genes is required for this signaling and inactivation pathway. The genes include *CDC5*, *CDC14*, *CDC15*, *DBF2*, *TEM1*, *SPO12*, and others. The typical loss-of-function phenotype is a large budded cell with divided nuclei and a long mitotic spindle.

We have been trying to dissect this genetic network by characterizing high-copy suppressors of the key mitotic exit mutation *dbf2-3*. We have found two such suppressors; one encodes the CDK inhibitor *SIC1* the other is a novel gene called *SDB24* (for *S*uppressor of *dbf2*).

SDB24 encodes a protein with no close homologs, but it has six potential sites for phosphorylation by Cdc28, and it has a clear coiled-coil domain, and as such it is related to structural proteins such as myosins, kinesins, and certain spindle pole body and kinetochore proteins. The transcript is cell-cycle-regulated, with a peak in S phase. The *sdb24* null mutant is viable but is synthetically lethal with *dbf2* and with *cdc23*. The synthetic lethality of *dbf2 sdb24* can be rescued by overexpression of the mitotic exit genes *CDC5*, *SIC1*, and *SPO12*, but not of *CDC15* or *TEM1*. *GAL-CLB2* and *GAL-CLB5* are lethal in an *sdb24* background. Most recently, we have found a two-hybrid interaction with a protein thought to function at the spindle pole body.

All of these results are consistent with the general idea that *SDB24* is somehow important for reducing Cdc28 activity at the end of mitosis. However, *sdb24* mutants have no obvious direct defect in cyclin proteolysis in G_1 phase, nor does Sdb24 protein seem to be a CDK inhibitor itself. Therefore, we believe that *SDB24*, and also the other mitotic exit genes, probably act to detect the end of anaphase and pass a signal to the Cdc28 inactivation machinery. This signaling machinery may reside in part at the spindle pole body; Cdc15 is at the spindle pole body, and SDB24 may be as well. In short, these genes comprise a checkpoint allowing Cdc28 inactivation and exit from mitosis.

Instability of G_1 Cyclins in G_1 Phase

B. Schneider [in collaboration with E. Patton, S. Lanker, M. Mendenhall, C. Wittenberg, M. Tyers]

There are two models explaining how the levels of G_1 cyclins rise in G_1 phase. In the first, G_1 cyclins are unstable throughout G_1, but rise in abundance because of increased *CLN* synthesis as cells approach Start. After Start, transcription is repressed and Cln synthesis drops. In the second model, G_1 cyclins are stable in G_1, accumulate partly for that reason, and then are reset to a low level after Start when Cln degradation is triggered. It has recently been suggested that the mitotic cyclins Clb1, 2, 3, and 4, which are expressed after Start, instigate turnover of G_1 cyclins. We have examined this issue in detail. We looked at G_1 cyclin half-life in G_1 phase cells obtained by elutriation and found that this half-life was 5–10 minutes, the same as the half-life seen in asynchronous cells. Furthermore, the presence or absence of the CLB1, 2, 3, and 4 genes had no influence on G_1 cyclin half-life. Therefore, we believe that G_1 cyclins are constitutively unstable.

Substrates of Cdc28, and Gene Arrays for Analysis of Cell Cycle Gene Expression

G. Sherlock

We have done a computer screen of the *S. cerevisiae* genome to find proteins with multiple clustered consensus sites for Cdc28 phosphorylation. Many of the proteins found in this screen are known to have a role in the cell cycle; in particular, many of the proteins are involved in DNA replication. We are mutating the sites in some of these proteins to see if there is a phenotype. We have knocked out all or nearly all the potential sites in Orc2, Orc6, Cdc6, Mcm3, and Cdc54. So far, all of these mutant genes are still functional if the strain is otherwise wild type. However, some of these mutant genes cause a high rate of plasmid loss (indica-

tive of inefficient initiation of replication), and when some combinations of mutants are made, severe phenotypes up to and including *ts* lethality are seen. We are now gathering further evidence that the phenotypes are due to a lack of phosphorylation, as opposed to the change in primary amino acid sequence.

The *cdc6* site mutant, which we call *cdc6**, has been particularly interesting. We have found that overexpressed wild-type *CDC6* can suppress *cdc14* and partly suppress *dbf2*. These latter two genes are involved in exit from mitosis and somehow serve to decrease levels of mitotic Cdc28 kinase after M phase. Thus, *CDC6* can be helpful in this process. However, *cdc6** is not capable of suppressing *cdc14* or *dbf2*. Furthermore, *cdc6** is synthetically lethal with *sic1*, which is known to be an inhibitor of Cdc28 kinase important for mitotic exit. One possible interpretation of these results is that *CDC6*, in addition to its role as an initiator of DNA replication, also has a role as an inhibitor of Cdc28 at the end of mitosis.

Finally, we are collaborating with the laboratories of David Botstein and Pat Brown in analysis of cell-cycle-regulated genes. These labs have arrayed all *S. cerevisiae* genes on glass slides and have done quantitative analysis of the expression of all *S. cerevisiae* genes by hybridizing cDNAs to these slides. We have done experiments using such gene arrays to find all the genes induced or repressed by the G_1 cyclin *CLN3* and the mitotic cyclin *CLB2*. Hundreds of such genes have been found and sorted into functional categories. Promoter analysis is under way. We are in the midst of analysis of changes in gene expression through the whole cycle.

Analysis of *WHI3*, a New Size Control Gene

T. Volpe

The *whi3* mutation was isolated some years ago by Rob Nash, and we are continuing its characterization. It contains an RNA-binding motif, but otherwise has little similarity to other proteins. Like other *whi* mutants, it gives a small cell phenotype. This seems to be because *whi3* mutants overexpress *CLN1* and *CLN2* G_1 cyclins. Overexpression of *WHI3* turns off *CLN1* and *CLN2* and is lethal. Presumably, then, the normal role of *WHI3* is to somehow repress *CLN1, 2* expression.

The level at which *WHI3* interferes with *CLN* expression has been unclear, however. *WHI3* does not interfere with *CLN1* or *CLN2* expression from a *GAL* promoter, so *WHI3* probably does not act directly on the *CLN1, 2* mRNAs. Because *CLN1* and *CLN2* transcription is activated by *CLN3*, we asked whether *WHI3* might inhibit *CLN3* posttranscriptionally. Recently, CLN3-galactosidase translational fusions were used to test this idea. We found that the β-galactosidase fusion was expressed at about twofold higher levels in the *whi3* mutant than in wild type. This strongly supports the idea that *WHI3* inhibits translation of *CLN3*, and this accounts for the effects on *CLN1* and *CLN2* transcription. Furthermore, we found that in a *cln3* deletion background, a *whi3* deletion has no effect (i.e., *cln3* is epistatic to *whi3*). This also suggests that *WHI3* works on *CLN3*.

Recent work from other labs suggests that *CLN3* is likely to be the target of translational controls. *WHI3* may be important for these controls. Furthermore, there are a large number of *WHI3*-like genes in yeast and human cells; our results suggest that these too may be translational regulators of specific mRNAs.

Mechanisms of Transcriptional Activation by Cyclin-Cdc28 Complexes

H. Wijnen

One effect of the Cln3-Cdc28 complex—perhaps the only effect—is to induce transcription of a large family of genes involved in the G_1/S transition. These include *CLN1*, *CLN2*, *CLB5*, *CLB6*, *RNR1*, and many genes involved in DNA synthesis and cell wall synthesis. We are trying to discover the mechanism of induction. The promoters of the *CLN3*-inducible genes all include binding sites for the Swi4 transcription factor or for its close relative Mbp1. The Swi4 and Mbp1 DNA-binding proteins each form a complex with another protein called Swi6; these two complexes are called the SBF or MBF transcription factors, respectively. We have found that *swi6* mutants are completely defective for *CLN3*-induced transcription, and this argues that Swi6 is the direct or indirect target for Cln3.

We have constructed a Gal4 (DB)-Swi6 fusion that can bind DNA via the Gal4 DNA-binding domain. This fusion places Swi6 on the DNA even when Swi4 is absent. We have found that even in the absence of Swi4, this fusion can activate transcription of a het-

erologous gene in a *CLN3*-dependent manner. This solidifies the evidence that *CLN3* works through Swi6.

However, we still do not know the mechanism of activation. The Cdc28 phosphorylation consensus sites on Swi6 are not required. We are not able to consistently detect significant amounts of Swi6 in Cln3 immunoprecipitates, so there is no evidence for a direct interaction.

Telomerase

F. Ferrezuelo, M. Lessard, J. Zhou

In the last year, other labs have identified a catalytic component of ciliate, yeast, and human telomerases. It is a reverse transcriptase called Est2. We are epitope-tagging yeast and human Est2. This will allow us to evaluate possible components of human telomerase we identified in a three-hybrid screen and will also allow direct biochemical tests of the composition of telomerase complexes. So far, we have been able to reconstitute human telomerase activity in vitro using the Est2 protein and the human telomerase RNA; we will soon see whether suspected accessory proteins affect this activity. In addition, we are continuing with the characterization of new yeast *est* mutants.

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PLANT GENETICS

R. Martienssen	D. Bush (University of Illinois, Urbana IL)	Q. Gu	R. Shen
E. Grotewold	M. Byrne	C. Kidner	J. Simorowski
	M. Curtis	M. Pizzirusso	P. Springer
	L. Dolan (John Innes Center, UK)	P.D. Rabinowicz	C. Yordan
	F. Gao	A.M. Settles	

Hcf106 Encodes an Ancient Conserved Membrane Protein Required for Sec-independent Protein Translocation

A M. Settles, D. Bush, R. Martienssen [in collaboration with K. Cline, University of Florida]

The *hcf106* mutation of maize disrupts biogenesis of the innermost (thylakoid) membrane of the chloroplast (Martienssen et al., *EMBO J. 8(6):* 1633 [1989]) and was previously shown to affect thylakoid protein uptake (Voelker and Barkan, *EMBO J. 14(16):* 3905 [1995]). This process is analogous to plasma membrane targeting in bacteria as chloroplasts are likely derived from primitive prokaryotic organelles. We have shown that the defect resides in mutant chloroplast membranes, and molecular cloning has revealed that homologs of the HCF106 membrane protein are encoded by a hypothetical bacterial gene family that includes the *Escherichia coli* gene *YigT* (Settles et al. 1997).

Several secreted proteins in *E. coli* do not depend on the protein translocation machinery encoded by the *sec* and *ffh* genes and may be substrates of *Hcf106*. A good candidate is the small subunit of hydrogenase (*hyaA*) which has a signal sequence motif found in substrates for the Hcf106 pathway in chloroplasts. In the course of generating transposon insertions in the *yigT* operon in *E. coli*, we have generated insertions in the conserved downstream ORF, *yigU*. Preliminary activity assays indicate that hydrogenase-1 is not active in the *yigU* insertion strain, consistent with a defect in hyaA membrane targeting. A second *E. coli* homolog of HCF106 is encoded by *ybeC*. Both *yigT* and *ybeC* are relatively small (300 bp) so we have chosen to knock out each ORF by gene replacement rather than transposon insertion. We will use tagged potential

substrates to monitor secretory defects in single and double knockouts. By expression of maize HCF106 in bacteria, we will determine if it directly interacts with plastid and bacterial precursor proteins. We are also using mutants in maize to investigate overlap and redundancy between sec-dependent and sec-independent translocation pathways.

The existence of a third universal pathway for protein translocation in organisms as diverse as plants and bacteria confirms the prokaryotic nature of chloroplasts and the highly conserved nature of the protein translocation process throughout evolution. Conversely, the absence of an Hcf106 or a SecA homolog from yeast suggests that mitochondrial translocation may have a different origin.

The *iojap* Gene in Maize

M. Byrne, R. Martienssen

The *iojap* mutation in maize affects chloroplast development, resulting in green and white striped homozygous mutant plants. Green sectors have normal chloroplasts while white sectors have defective plastids that fail to elaborate chloroplast membrane structures and also appear to lack ribosomes (Walbot and Coe, *Proc. Natl. Acad. Sci. 76:* 2760 [1979]). In many genetic backgrounds, heteroplastidic cells are pale green and have both classes of plastids. The plastid defect is transmitted epigenetically in that, after passage through the egg, defective plastids fail to develop into chloroplasts in the presence of the wild-type *Ij* allele (Rhoades, *Cold Spring Harbor Symp. Quant. Biol. 11:* 202 [1946]). Furthermore, reversion of the mutant allele to wild type appears to have little effect on homoplastidic white leaf tissue (unpublished results).

The *Iojap* gene has been cloned by transposon tagging (Han et al., *EMBO J. 11(11):* 4037 [1992]). Biochemical evidence suggests that the Ij protein interacts with the chloroplast 50S ribosomal subunit. A homolog has been identified in *Arabidopsis* and maps to the top arm of chromosome 3. Homologs have also been identified in a number of bacterial genomes including that of *E. coli*, where the *ij* homolog is found in an operon encoding cell division genes. We are further defining the role of *Ij* in *E. coli* via targetted mutagenesis. We have obtained soluble recombinant maize and *Arabidopsis* Ij protein. We are using these reagents to investigate interactions with ribosomal components.

Molecular Genetics of *asymmetric leaf 1* in *Arabidopsis*

M. Curtis, R. Martienssen

In plants, the genetic basis of initiation, determination, and patterning of lateral appendages such as leaves is almost entirely unknown. Leaves are initiated by the recruitment of founder cells on the flanks of the vegetative meristem. This partitioning of the meristem is reflected by the expression pattern of *KNOTTED* (*KN1*) and related homeobox genes that are down-regulated in leaf primordia. Analysis of loss-of-function mutations that affect leaf development provides useful tools to help elucidate the genetic basis of their development. One such mutant, asymmetric rosette leaves (*as1*), leads to variably lobed leaves with growth pattern changes in early leaf development characteristic of a morphological change in the proximodistal axis of the leaf. *as1* was first described by Redei and Hirono in 1965 (*Genetics 51:* 519) and is allelic to *magnifica* (Reinholz 1947 [Fiat Rep.]).

We are using our gene trap (GT) and enhancer trap (ET) system to study this mutant phenotype. We are currently examining the interaction of *as1* with several GT and ET lines that show patterns of reporter gene expression specific to the shoot apical meristem and the leaf primordia and have identified several genes whose expression pattern is altered in *as1* mutants. We are also using GT lines and ET lines with expression patterns in hydathodes, trichomes, stomata, stipules, veins, and the margins of the leaf, as both physical and molecular markers, to help determine the alteration of patterning in the mutant leaf. These mutant leaves have altered spacing of these structures relative to the leaf margin that is characteristic of a drastic change in the proximodistal axis.

We are attempting to positionally clone *as1* and have mapped the mutant allele to an interval approximately 0.3 cM north of *det2* on *Arabidopsis* chromosome 2. This map position corresponds to a contig of overlapping cosmid clones. Attempts to complement the *as1* mutation with these candidate clones are in progress.

Dorsoventral Axis Specification in Lateral Organs of *Arabidopsis*

C. Kidner, R. Martienssen

Most leaves develop as flattened blades with distinct dorsal (adaxial) and ventral (abaxial) sides. These

dorsoventral characters are thought to be specified during or soon after leaf initiation, and to involve signals from the meristem that orient the primordium relative to the main axis (I. Sussex, *Nature 167:* 651 [1951]). We have identified a number of enhancer trap and gene trap lines that have reporter gene expression confined to the abaxial side (7 lines) or adaxial side (1 line) of the immature leaf, as well as a number of lines that mark the dorsal and/or ventral boundary of the primordium. A spontaneous mutant, unrelated to transposon insertion, arose serendipitously in the Cold Spring Harbor gene trap collection. The leaves of this mutant are short and rod-like, and there is no apparent difference between the upper and lower sides. The inflorescence meristem produces short yellowish rods of tissue instead of flowers, and no lateral branches are formed. Lateral branches may also be dorsal characters as they are clonally related to the adaxial side of the leaf.

A similar mutant, *dandelion*, has been described previously (Bhatt et al., *Plant J. 9(6):* 935 [1996]). We have performed complementation tests indicating that these two mutants are allelic. However, the trans-heterozygotes produce flowers, and these flowers have the correct arrangement of severely reduced, unifacial organs. For example, they have unfused twin pistils. In wild type, the carpels fuse along the dorsoventral boundary, which is lacking in the mutant. Characterization of these two alleles of *dandelion* is proceeding by histological study and by genetic analysis. Additional mutants have been described elsewhere recently, some of which have been cloned molecularly. We are investigating whether these are related. We are considering renaming this locus *deilen ddrean* (Welsh for "prickle leaf").

FRUITFULL Encodes a MADS-box Gene Required for Cellular Differentiation during Fruit and Leaf Development

Q. Gu, R. Martienssen [in collaboration with C. Ferrandiz and M. Yanofsky, University of California, San Diego]

We have identified a mutation in *Arabidopsis* called *fruitfull* (*ful-1*), which abolishes elongation of the silique after fertilization. It arose during enhancer and gene trap mutagenesis as an F_2 plant with reduced silique (seed pod) length. The stunted silique has crowded seeds (a "full fruit"), a failure of dehiscence, and frequently ruptures prematurely. Wild-type seed pods comprise two valves joined by the replum, a strip of weakened cells that rupture to release mature seeds. *ful-1* valve cells remain small and unelongated after fertilization. Stomata, which are frequent in wild-type valves, are absent from mutant valves. *ful-1* also alters leaf shape. Wild-type cauline (stem) leaves are small, lanceolate, and lack a petiole. *ful-1* cauline leaves are broader, have an altered vascular pattern, and have a reduced number of internal cell layers.

The *ful-1* mutation is caused by the insertion of a DsE transposable enhancer trap element into the 5′-untranslated leader of the MADS-box gene *AGAMOUS-LIKE 8 (AGL8)*. GUS reporter gene expression in the enhancer trap line is observed in valve tissue, but not in the replum, the septum, or the seeds, and faithfully mimics RNA in situ hybridization data reported previously (Mandel and Yanofsky, *Plant Cell 7:* 1763 [1995]). *FRUITFULL* is also transcribed in the inflorescence meristem and in vegetative tissue such as rosette leaves. However, these tissues appear to be unaffected in the mutant. In collaboration with M. Yanofsky, a series of double mutants were made with other MADS-box genes to determine whether a redundant role might be uncovered in leaves and meristems. In most cases, no such role was discovered. However, a triple mutant combination with the closely related meristem identity genes *APETALA1* (*ap1*) and *CAULIFLOWER* (*cal*) has revealed a redundant role for *FRUITFULL* in meristem identity (C. Ferrandiz, Q. Gu, R. Martienssen, and M. Yanofsky, unpubl.). *ap1* and *cal* are thought to be responsible for cauliflower and broccoli variants within *Brassica oleraceae* (cabbages). It will be interesting to determine whether *FRUITFULL* is responsible for variations in inflorescence architecture and fruit size within the Brassicaceae and beyond.

Functional Genomics: Systematic Gene Trap and Enhancer Trap Mutagenesis in *Arabidopsis*

C. Yordan, J. Simorowski, R. Benton (URP program), R. Shen, P. Springer, R. Martienssen [in collaboration with W. Richard McCombie, and with J. Healy, G. Latter, and A. Reiner, Cold Spring Harbor Laboratory]

The *Arabidopsis* genome will be completely sequenced by the end of the year 2000. This remarkable acheivement opens up a new challenge for plant biology, i.e., to determine systematically the function of each of the 20,000 *Arabidopsis* genes, singly and in combination. We are contributing toward this goal by

systematic gene disruption using gene trap and enhancer trap transposons. As each new insertion is generated, DNA is prepared from individual plants, and sequences flanking the insertion are amplified and sequenced using TAIL PCR (Liu et al., *Plant J. 8:* 457 [1995]; Tsukegi et al., *Plant J. 10:* 479 [1996]). These sequences are then compared with genomic DNA sequence databases to map the insertion precisely within the genome. Now that the complete sequence is being determined, genome annotation can be used to identify disrupted genes, without the need for RNA-based methods, such as RACE PCR (Annual Report, 1995). Of the first few hundred lines, 17% match genomic sequence, a further 17% match an EST, and an additional 10% have some homology with an annotated gene in *Arabidopsis*. Given that 15% of the genome has been sequenced, that the gene density is 55%, and that a third of these genes are represented by ESTs, these numbers are close to those predicted for random insertion. A sophisticated relational database linking sequence to expression pattern, pedigree, and phenotype is under construction.

We are screening each transposant for lethal insertions in essential genes. Such mutations result in aborted embryos and semisterile siliques in heterozygous transposants, which are conveniently screened as they are generated (Springer et al., *Science 268:* 877 [1995]). A catalog of essential genes, comparable to those in yeast and *Drosophila*, will thus be assembled. Only a very small proportion of these genes are expected to control patterning during early development, and many of these will be required during vegetative and floral phases as well because of the reiterative nature of plant development. Reporter gene expression patterns and mosaic analysis will allow us to uncover some of these functions.

We are taking advantage of the propensity of *Ds* transposons to transpose to closely linked sites in order to saturate the region around the *prolifera* locus on chromosome 4. This is the region being sequenced by the Cold Spring Harbor/WashU/ABI *Arabidopsis* sequencing consortium. By selecting insertions from two-dimensional pools of mutagenized plants, we hope to recover insertions in most of the genes in this region.

Myb Domain Proteinsin Maize

P.D. Rabinowicz, K. Tworkowski, E. Grotewold

The regulation of gene expression is a fundamental process in all living organisms. Transcription factors are classified in structural families according to the presence of specific DNA-recognition motifs. One such family is constituted by proteins containing the Myb homologous DNA-binding domain (Myb domain), originally identified in the v-*myb* oncogene found in the virus of the avian myeloblastosis. Proteins containing the Myb DNA-binding domain have since been found in all eukaryotes in which they have been sought. Myb domains are usually formed by two or three imperfect 51- or 52-residue repeats (R_1, R_2, and R_3). Each repeat encodes three α-helices with the second and third helices forming a helix-turn-helix structure (HTH) when bound to DNA, similar to motifs found in the λ repressor and homeodomain proteins. R_2 and R_3 are usually sufficient for sequence-specific DNA binding.

According to these criteria, three Myb domain proteins have been identified to date in vertebrates, c-Myb, A-Myb, and B-Myb. c-Myb has an essential role in controlling the proliferation and differentiation of hematopoietic cells. The cellular functions of A-Myb and B-Myb are less well understood. A single c-Myb-like gene has been reported so far in *Drosophila*, and analysis of the *Saccharomyces cerevisiae* genome indicates that yeast contains less than ten Myb-like sequences. In sharp contrast, plants express a large number of Myb homologous proteins.

Plant and animal Myb domain proteins differ in several aspects. Plant Myb domains have usually two, instead of three, Myb repeats. These are most similar to the R_2 and R_3 repeats of their animal homologs. Plant Myb domains contain an extra residue inserted between the second and third helices of the R_2 repeat, and the DNA recognition helices of plant and animal Myb domains have differences that are important for their DNA-binding activities (Williams and Grotewold 1997). Little is known about the evolution of Myb domain proteins or how the Myb gene family has expanded in plants compared to animals. It is not known either whether the dramatic expansion of this family has occurred prior to or after the divergence of mono- and dicotyledons.

To answer these questions, we investigated the number and sequences of Myb domain proteins expressed in maize. We carried out RACE PCR of mRNA obtained from different parts and developmental stages of the maize plant, including young seedlings, roots, immature ears (18 days after pollination), silks, and immature tassels, using degenerate primers that recognize the conserved DNA-recognition helices of R_2 and R_3. This region encodes 41–43 residues, and we termed it Myb^{BHI} (for Myb *b*etween

DNA-*i*nteraction *h*elices). So far, we have sequenced 300 PCR fragments which correspond to 70 different Myb domains. Our data suggest that we are only at about 60% saturation of this screening, indicating that maize may express more than 130 different Myb genes. The sequences of these 70 novel Myb domains suggest that plant Myb domains are much more variable in the region between the two DNA recognition helices than previously expected, in terms of number of residues as well as amino acid composition. Our data strongly indicate that Myb^{BIH} provides a fingerprint of Myb identity, convenient to carry out studies on how this family of proteins has evolved during plant evolution.

Identification of Transposable Element Insertions in Maize Genes Encoding Myb Domain Proteins with Novel Cellular Functions

P. D. Rabinowicz, E. Grotewold

The dramatic expansion of the Myb family of regulatory proteins in plants and their cellular roles has led to the suggestion that plant Myb domain proteins are primarily involved in controlling plant-specific processes. The enlargement of the plant Myb gene family may have occurred in conjunction with the development of new cellular functions. An example of how Myb domain proteins are utilized to promote diversity in plant metabolic pathways is given by the independent regulation of branched flavonoid biosynthetic pathways by two related maize Myb domain proteins, P and C1. Whereas the C1-regulated anthocyanins are almost universally found in the plant kingdom, the P-regulated pathway is restricted to only some grasses. This suggests that the appearance of P is fairly recent, probably to supply a cellular function solely needed in some grasses. Consistent with this is the finding that P-controlled compounds accumulate in maize silks protecting corn from specific pathogens (Grotewold et al. 1998). The functions of only a few other plant Myb domain proteins are known, and these include controlling epidermal cell shape and cell fate; mediating the response to viral infection, hormones, and drought; and regulating the accumulation of phenylpropanoids.

Last year we reported the method that we developed to identify insertions of *Mutator* in gene families encoding proteins with limited sequence similarity. This method, termed SIMF (for *s*ystematic *i*nsertional *m*utagenesis of protein *f*amilies), was used to identify insertions in genes encoding Myb domain proteins. Oligonucleotides from *Mutator* ends and from conserved regions in Myb domains are used to amplify by PCR pools of genomic DNA made from from maize seedlings containing active *Mutator* elements. Currently, we use 12 macro DNA pools (6 derived from rows and 6 derived from columns) each corresponding to 10 pools, representing a population of 3600 plants grown in a 60 columns by 60 rows grid array. From a large number of possible PCR products of possible *Mutator* insertions in Myb genes, four have been confirmed to correspond to insertions in novel Myb genes. In three cases, the *Mutator* element is inserted in a conserved intron in the region encoding the Myb domain, whereas in the fourth case the intron is absent and the insertion is in the coding sequence, right between the two Myb repeats. The corresponding genes have been mapped (in collaboration with Dr. Mike McMullen, University of Missouri), and we are currently carrying out segregation analysis to determine the possible effects caused by these insertions. In addition, genomic clones for these insertions are being sequenced to determine the properties of the entire proteins.

Developing the Tools to Dissect the Myb-associated Complexes Involved in the Control of Flavonoid Biosynthesis

F. Gao [in collaboration with Arthur Kroon, Ronald Koes, and Jos Mol of the Free University, Amsterdam], M. Tanurdzic (URP Program), E. Grotewold

Anthocyanin accumulation is controlled in maize and other plants by two classes of regulatory proteins: a Myb domain-containing class (C1/Pl in maize; An2 in *Petunia*) and a basic helix-loop-helix (bHLH) domain-containing class (R/B in maize; An1/Jaf13 in *Petunia*). Anthocyanin production requires the interaction of a member of the Myb domain family and a member of the bHLH domain family. Previous studies have shown that whether or not this interaction is absolutely necessary, it is not sufficient to promote transcrip-

tion of the flavonoid biosynthetic genes, suggesting that other factors are required.

To investigate the possible nature of these other factors, biochemical and genetic approaches are being undertaken. For the biochemical strategies, monoclonal antibodies were developed against the maize C1 protein. A large number of monoclonal antibodies were obtained, which recognize the *Escherichia coli*-expressed C1 proteins with high affinity. In addition, a handful of these antibodies also recognize the maize Pl and Petunia An2 orthologous proteins. So far, however, we have not been able to detect with any of these antibodies the corresponding proteins in crude plant cellular extracts. Monoclonal antibodies against the R/B proteins are currently being generated against strategically designed peptides.

The genetic strategy consists in utilizing the yeast two-hybrid approach to identify possible partners for the known regulators. For that purpose, cDNA libraries were made from mRNA obtained from maize and *Petunia* tissues where the R/B and C1/Pl factors are fully functional. The cDNA libraries are currently being tested for interacting proteins with different parts of the cloned factors from maize and *Petunia*.

In addition, we started investigating the ability of the R, An1, and Jaf13 bHLH factors to interact. Surprisingly, the bHLH motif is not able to mediate either homo- or heterodimer formation, but the region carboxy-terminal to the bHLH motif is.

Using Reverse Genetics to Identify *Mutator* Insertions in Genes Required for Flavonoid Accumulation

Pablo Rabinowicz, M. Pizzirusso (Partner for the Future), E. Grotewold

Flavonoids are a very large class of small phenolic molecules found in all plants. Flavonoids have a number of important biological functions, including plant and flower pigmentation, sexual reproduction, protection against UV radiation, defense against attack by phytopathogens, in symbiotic interactions between plants and microbes, and in the regulation of plant hormone transport. Whereas most structural genes in the maize flavonoid biosynthetic pathway have been characterized, mutants in the genes encoding chalcone flavanone isomerase (*chi*) and flavonone 3′-hydroxylase (*f3h*) have never been described.

We used a *Mutator*-based reverse genetic approach to identify insertions in these two flavonoid biosynthetic genes. We screened 3600 plants for *Mutator* insertions and identified two transposon insertions in the 3′ regions of the *chi1* gene, and one putative insertion in the *f3h* gene. Current studies are under way to confirm these insertions, as well as to determine the phenotypic effects of mutations in these two genes for flavonoid accumulation.

Last year, we reported on the cloning of an *An11* homolog from maize (currently called MP1). In *Petunia*, *An11* encodes a protein containing WD40 repeats, and it is required for normal accumulation of anthocyanins in corollas. Using our *Mutator* stocks, we identified one insertion of Mutator in the MP1 gene, which should allow us to determine whether MP1 encodes the maize ortholog of An11.

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ARABIDOPSIS FLOWER AND POLLEN DEVELOPMENT

H. Ma J. Busch, J. Cruz, S. Dhavia, A. Debrowski, J. Ellstein, H. Fan, Y. Hu, H. Huang, E. Katz, B. Laulicht, D. Liu, C. Machare, S. Moran, P. Rubinelli, L. Shao, K. Siddiqui, J. Sylvan, S. Virani, M. Yang

We are interested in understanding the genetic control of plant reproductive development, using *Arabidopsis thaliana*. The research topics range from the control of floral meristem identity during early flower development to the regulation of meiosis and pollen development. Much of our recent effort has been devoted to the functional analysis of the floral homeotic genes *AGAMOUS* (*AG*), which regulate early flower development. AG is a member of the MADS-box gene family, which is present in all major eukaryotic kingdoms. Many other plant MADS-box genes were isolated molecularly and designated as *AGL* for *AG-L*ike.

Furthermore, we have used an enhancer trap/gene trap transposon mutagenesis method to identify new genes required for flower and pollen development. One way to isolate new genes that function during late floral organ development is to identify genes specifically expressed in floral organs. These genes are likely to be involved in cell differentiation during late flower development, and they provide molecular markers for specific cells or tissues of the reproductive organs. Another method is to screen for mutants with abnormal development; we have isolated a large number of putative mutants and have begun to characterize these mutants.

In 1997, our postdoctoral fellow Hai Huang took a position as full professor at the Shanghai Center for Life Science and the Institute for Plant Physiology of the Chinese Academy of Science. In addition, another postdoc, Hua-Ying Fan, moved back to New York City. Shao Li, a first-year student in the MCB program at SUNY Stony Brook, conducted rotation research in our lab this summer. Joshua Busch, an URP from Emory University, worked on immunodetection of the AGAMOUS protein. Several other undergraduate students, Julissa Cruz, Sumit Dhavia, and Cindy Machare from SUNY Stony Brook and Jason Sylvan from Brandeis University, participated in our projects. Kashif Siddiqui, a visiting graduate student from Adelphi University, has been working with Dehua Liu, a postdoc, to characterize new floral mutants. Sophia Virani completed her Partner for the Future program and started college at Harvard University. In the fall, Elisa Katz, our new Partner, started her project with Ming Yang, a postdoc in the lab. Furthermore, high school students Brian Laulicht, Sienna Moran, Amy Debrowski, and Joshua Ellstein joined our gene trap effort. As always, we appreciate greatly the assistance of the Uplands Farm manager Tim Mulligan and his assistant Stan Schwarz.

Characterization of Organ-specific cDNAs

P. Rubinelli, Y. Hu, S. Virani, C. Machare, H. Ma

As described in previous annual reports, we have been characterizing several cDNAs that are expressed specifically or predominantly in the anther or the pollen. A number of constructs have been made to test their functions in vivo using antisense transgenic plants. We have generated a large number of transformants carrying various constructs. Among these

plants, we have focused our effort on plants carrying constructs of ATA21 and ATA27. ATA21 is expressed specifically in the pollen grain. A fusion was generated between the pollen-specific LAT52 promoter and the ATA21 cDNA in the antisense orientation; nearly 100 T1 transformants with this construct were generated. Three of the T1 transformants showed a distorted transmission of the kanamycin resistance marker. One of these three T1 transformants showed a pollen developmental defect in about 30% of the pollen grains. These pollen grains had a collapsed appearance, failed to stain with the nonspecific cytosolic stain toluidine blue and the RNA-specific stain Azure B, and appeared translucent, indicating a lack of cytoplasm. However, these transformants with transmission distortion or pollen phenotype had apparently normal expression of the ATA21 gene as tested by Northern blot. Furthermore, one control line, a LAT52-GUS fusion, also showed the collapsed pollen phenotype, suggesting a possible promoter-associated effect on pollen development. The analysis of these transgenic lines is still in progress.

The ATA27 gene was found to be specifically expressed in the tapetum layer of the anther. To reduce its expression, a fusion of the tapetum-specific TA29 promoter and the ATA27 cDNA in the antisense orientation was constructed and introduced into *Arabidopsis* by transformation. Thirty-three transformants were generated. Among 14 transformants grown to maturity, one was sterile, and its pollen development was abnormal. This transformant had an approximately 90% reduction in ATA27 expression as tested by Northern blot. However, transmission of the transgene to the next generation was severely reduced, suggesting that both male and female fertility is affected. In addition, about 30% of the flowers of this line show ectopic stigma on both anthers and the carpal wall, accompanied by fusion of stamens to the pistil. Three out of four progeny studied so far from this transgenic line are sterile and the fourth is only partially fertile, suggesting that the phenotype is dominant with some incomplete penetrance. This segregation pattern is consistent with an antisense RNA effect. It is also possible that this phenotype may be due to a defect in an endogenous gene due to the T-DNA insertion. More study is needed to confirm that the sterility is due to the ATA27 antisense RNA.

We have also generated transformants carrying antisense constructs for ATA7, ATA18, ATA20, and ATA26 cDNAs. We are in the process of analyzing these and other transgenic plants.

Generation of New Enhancer and Gene Trap Transposants

M. Yang, A. Debrowski, J. Ellstein,
Y. Hu, S. Dhavia, J. Cruz, H. Ma

To identify new genes regulating *Arabidopsis* flower development, we have continued to generate enhancer trap/gene trap transposon insertional lines. We have performed new crosses between Ac and Ds plants; the total number of F_1 seeds now is more than 60,000. We continue to plant the F_1 seeds and harvest the F_2 families. About 11,000 F_2 families have been screened for independent new Ds insertional lines (transposants), and we have now generated about 2700 transposants. About two thirds of these lines have been screened for floral expression patterns and for visible phenotypes in the flower or in pollen development.

Characterization of a Mutant Defective in Male Meiosis

M. Yang, E. Katz, J. Sylvan, H. Ma

We have searched for male-sterile or low-fertility mutants among the enhancer/gene trap transposants. One of these mutants, from transposant ET5223, exhibits a severe defect in male meiosis. Light microscopy revealed that the mutant produced abnormal microspores with variable sizes. Examination of earlier development indicates that the mutant tetrads contain a variable number of microspores from 2 to 7 spores per tetrad. Further analysis of DNA contents using DAPI staining suggests that the amount of DNA in the abnormal spores is proportional to the size of the spores. These results suggest that the mutant is defective in male meiosis. DAPI staining of microspore mother cells undergoing meiosis uncovered an abnormal pattern of staining that strongly suggests a severe defect in separation of homologous chromosomes during meiosis I. Meiosis I differs from meiosis II or mitosis in that the homologous chromosomes pair and then separate. Normal separation of homologs is critical for proper meiosis. The mutant phenotype, therefore, suggests that it could be a useful tool to study this important process.

We have determined that mutant plants carry a cosegregating Ds insertion. In addition, we have recovered revertant sectors from mutant plants carrying an Ac element, and the revertant alleles have lost the Ds

element. We have isolated genomic sequence adjacent to the Ds, and several revertant alleles showed the restoration of wild-type sequence, indicating that they resulted from precise excisions of the Ds element. We are in the process of further characterizing this gene.

Identification and Characterization of Transposants with Floral Expression and Phenotypes

D. Liu, Y. Hu, H.-Y. Fan, B. Laulicht, K. Siddiqui, H. Ma

To identify floral genes, we have continued our analysis of the GUS reporter gene expression in transposant lines. We have analyzed about 1000 new lines for floral expressions. We found that the overall results were similar to results we had obtained previously, although specific new patterns have been recovered.

We have also performed a large-scale screening for visible developmental phenotypes in the flower. We found several mutants similar to known mutants; these presumably are alleles of known genes. We also found mutants that have novel floral phenotypes. For example, a number of mutants have abnormal floral organ sizes and/or shapes. In particular, several mutants have short filaments; some of these produce a large number of functional pollen grains, but have reduced fertility because the pollen grains are not efficiently delivered to the stigma.

We also found several new male-sterile mutants, including a mutant from transposant ET2292 with a defect in male meiosis. This mutant produced tetrads with four to six spores; therefore, the mutant phenotype is different from that of the meiotic mutant from ET5223. Pollination using normal pollen indicates that the ET2292 mutant is female-fertile. In addition, the male-sterile defect is due to a nuclear recessive mutation. We have also found several revertant sectors among mutant plants carrying an Ac element. Preliminary analysis indicates that the ET2292 mutant carries a single Ds insertion and the revertant alleles have lost this Ds element. These results strongly support the hypothesis that the mutation was caused by a Ds insertion. GUT staining experiments have not revealed any clear expression pattern, suggesting either that the gene is expressed at an extremely low level or that the GUS reporter is not expressed, possibly due to the Ds insertion being in the wrong orientation. We are currently analyzing the mutant phenotypes and the corresponding gene.

A Male-sterile Mutant Defective in Microsporogenesis

P. Rubinelli, L. Shao, H. Ma

The anther of flowering plants carries out three distinct developmental programs: cellular differentiation, microsporogenesis, and cell degeneration associated with dehiscence. Our lab has screened among *Arabidopsis* the transposant lines for male-sterile mutants. One such line, designated GT5302, segregated in its progeny male-sterile plants in a 3:1 ratio of phenotypically normal to mutant, suggesting that the phenotype is due to a single-gene nuclear recessive mutation. Genomic Southern blot hybridization of four male-sterile progeny of the GT5302 line with a fragment from the 5′ end of DsG indicated that all four plants contain a single, identical Ds element insertion in the genome. Light microscopy of sections through the mutant inflorescence revealed the following defects: incomplete dissociation of microspore tetrads, abnormal pollen wall deposition, complete degeneration of all microspores prior to pollen mitosis I, and failure of the mature anther to undergo dehiscence. In wild-type *Arabidopsis*, the stomium cell layer degenerates to allow a break in the anther wall, initiating dehiscence. Ongoing experiments will clarify if the stomium fails to degenerate in this mutant and if the mutation is transposon-tagged.

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DEVELOPMENTAL GENETICS OF PLANT REPRODUCTION

U. Grossniklaus R. Baskar A. Coluccio J. Moore
R. Bicknell M. Hoeppner Y. Redler
N. Castiaux C. Miller J.-P. Vielle-Calzada

Research in our laboratory focuses on plant reproductive biology. We are using genetic and molecular approaches to characterize the developmental control of plant reproduction, in particular the formation of the female gametes. Unlike in animals, where the meiotic products differentiate directly into gametes, the spores of plants give rise to multicellular haploid organisms that produce the gametes later in their development. Thus, the plant life cycle alternates between a diploid and a haploid phase, the sporophyte and the gametophyte. The gametophytes of flowering plants (micro- and megagametophytes) consist of a small number of cells and develop within the reproductive organs of the flower. The key events in female gametogenesis (megasporogenesis, megagametogenesis, and double fertilization) occur in ovules, which are specialized reproductive structures of sporophytic origin. The molecular and genetic bases controlling ovule development and female gametogenesis are largely unknown. The highly polar nature of the developmental events and the simple organization of ovule and megagametophyte make it an ideal system to study fundamental aspects of plant development. The goal of our research is to investigate the role of positional information, cell lineage, and cell-cell communication in plant morphogenesis and cellular differentiation.

A better understanding of the molecular mechanisms controlling megasporogenesis and megagametogenesis will not only yield important insights into fundamental concepts in plant development, but also provide tools for the manipulation of the reproductive system. In particular, we are interested in engineering components of apomixis, an asexual form of reproduction through seeds. In addition to using the tools we generate in our basic research for a biotechnological manipulation of plant reproduction, we are also using genetic strategies to isolate mutants displaying apomictic traits in maize and, in a collaborative effort with Bob Pruitt (Harvard University), in *Arabidopsis*. The introduction of apomixis into sexual crops would have revolutionary implications for plant breeding and agriculture, allowing for the immediate fixation of any desired genotype and its indefinite clonal propagation.

We would like to acknowledge the efforts of a number of high school and undergraduate students who assisted in our research. Nikhil Laud and Sean Kass, both from Roslyn High School, helped with the characterization of mutants disrupting megagametogenesis. Bill Wagner, Duke University, spent his fourth summer in our lab and helped with pollinations in the field and with molecular and cytogenetic analyses. We were sad to see Marilu Hoeppner and Nathalie Castiaux leave; both gave birth to healthy boys and moved on to new challenges. But we could welcome new members in the lab: Alison Coluccio and Yvette Redler took over the responsibilities of Marilu and Nathalie, and Ross Bicknell (Crop and Food Research, New Zealand) spent a 6-month sabbatical in our group.

Dissection of Plant Reproduction by Enhancer Detection

U. Grossniklaus, J. Moore, J.-P. Vielle-Calzada, R. Baskar, M. Hoeppner, N. Castiaux, A. Coluccio, Y. Redler

To dissect female gametogenesis in plants, we are performing a large-scale enhancer detection/gene trap screen in *Arabidopsis thaliana*, a small crucifer that has been widely adopted as a model system for plant developmental biology and genetics. Enhancer detection allows the identification of genes on the basis of their pattern of expression. We are using an enhancer/gene trap system based on the *Ac/Ds* transposon of maize that was developed here at the laboratory by R. Martienssen, V. Sundaresan, and co-workers (Sundaresan et al., *Genes Dev. 9:* 1810 [1995]). The presence of a transposon at a locus of interest greatly facilitates its subsequent molecular and genetic characterization. The transposon provides a molecular tag for the cloning of the locus and additional al-leles can be isolated by excision and local transposition. During the last few years, we generated close to 4500 lines

carrying single randomly distributed enhancer detector/gene trap transposons (transposants). We subject these lines to four independent screens: (1) To identify genes involved in early events during ovule development (axis specification and pattern formation) and megasporogenesis, young ovule primordia are characterized for expression of the *GUS* reporter gene present on the enhancer detector element. (2) Later stages of development are analyzed for *GUS* expression to isolate genes acting specifically in the megagametophyte and its constituent cell types. (3) Transposants are screened for reduced seed set and segregation ratio distortion to identify potential gametophytic mutations. (4) Screening is carried out for sporophytically required mutations that disrupt ovule morphogenesis and megasporogenesis. Using TAIL- PCR (Liu et al., *Plant J. 8:* 457 [1995]), we have isolated and sequenced flanking genomic regions of about 130 transposants of interest. On the basis of sequence homology, several genes that are likely to have important regulatory roles in female gametogenesis have been identified. The characterization of transposants at various stages of reproductive development is an ongoing long-term project in our laboratory.

Alterations of Megasporogenesis and Induction of Apomixis in *Arabidopsis*

J.-P. Vielle-Calzada, C. Miller (URP Program), U. Grossniklaus

A single cell within the ovule primordium of *Arabidopsis* differentiates into a megaspore mother cell (MMC) and undergoes meiosis to generate four haploid megaspores. Cytokinesis occurs only after the completion of meiosis II. A single megaspore gives rise to the female gametophyte, as the other three degenerate. The initiation of apomixis, or asexual reproduction through seeds, invariably takes place during megasporogenesis. Differences between sexual and apomictic reproduction may be determined by regulatory genes that act during megasporogenesis and that control events leading to the formation of unreduced female gametophytes.

During the last year, we have made significant progress in our genetic analysis of megasporogenesis. To date, we have analyzed *GUS* expression patterns in more than 1000 enhancer/gene trap lines in subsequent developmental stages, from the time when the ovule primordium forms (before MMC differentiation) to stages where the viable megaspore is differentiated. We identified 25 lines that show expression in specific regions of the developing ovule. For most of these lines (18/25) the initiation of reporter gene expression can be traced back to ovules at the onset of megasporogenesis, either before or during MMC differentiation. Specific lines show *GUS* expression restricted to either the proximal, central, or distal portion of the ovule primordium. We obtained genomic sequences for 11 lines. Several show similarity to genes that have important roles in plant and animal development. These include cell cycle regulators, leucine-rich repeat protein kinase receptors, and genes encoding proteins involved in organelle differentiation. Several others have homology with *Arabidopsis* ESTs of unknown function. One line shows *GUS* expression in a small cluster of distal cells of the young nucellus before meiosis. This region is believed to be of importance in the regulation of MMC specification. A second line showed complex *GUS* expression throughout ovule development in proximal regions, including the cells surrounding the surviving megaspore. It was found to be inserted in the β-glucanase gene cluster. β-glucanase degrades callose, which has been proposed to have an important role in megaspore selection. The absence of callose deposition around a megaspore or apomictic initial is highly correlated with this cell initiating megagametogenesis, in both sexual and apomictic species.

Using lines displaying significant *GUS* expression patterns, we plan to investigate the effects of specific alterations to megasporogenesis by misexpressing regulatory genes that could lead to the formation of unreduced megaspores, the first step in apomictic reproduction. Regulatory sequences controlling nucellar and MMC-specific expression will provide important tools to determine cellular interactions during megasporogenesis and manipulate the reproductive potential of sexual plants.

Isolation of Mutants Disrupting Megasporogenesis and Ovule Morphogenesis

J.-P. Vielle-Calzada, J. Moore, A. Coluccio, Y. Redler, U. Grossniklaus

We are conducting a genetic screen to identify transposants defective in megasporogenesis and ovule mor-

phogenesis. Transposants were screened for families that segregate sterile plants. To date, we have screened about 3193 transposants and identified 40 lines that produced sterile homozygotes; 31 candidates were analyzed in reciprocal crosses to determine whether they affect male or female reproductive development. Six were found to be exclusively female sterile. Among these, three show defects in ovule morphogenesis that are associated with a corresponding pattern of *GUS* expression, suggesting that the gene responsible for the mutation has been tagged. We identified four lines that are defective in both the male and female gametogenesis and might be associated with mutations affecting meiosis. One of the female-specific mutants forms morphologically normal ovules. Preliminary cytological analysis suggests that megasporogenesis is arrested at the onset of the haploid phase, prior to the initiation of female gametophyte formation. Most ovules contain a tetrad of meiotically derived cells, but the viable megaspore is arrested before the first mitotic division. The sequence of a TAIL-PCR fragment flanking the insertion shows homology with an *Arabidopsis* RNA helicase implicated in the regulation of the cell cycle, and with several hypothetical proteins of unknown function. The characterization of such mutants will improve our basic understanding of reproductive development in plants and will set the basis for a sustained effort in plant germ line biotechnology necessary for the transfer of apomixis into sexual crops.

Charaterization of Mutants Disrupting Female Gametogenesis

J. Moore, J.-P. Vielle Calzada, W. Gagliano, U. Grossniklaus

During megagametogenesis, the surviving megaspore divides through three consecutive mitoses to form the mature embryo sac consisting of seven cells: the egg cell, two synergids, three antipodals, and a binucleate central cell. After double fertilization of both the egg and the central cell, the ovule develops into a seed. Very few mutants affecting the gametophytic phase of the plant life cycle have been described in the literature. The isolation and characterization of novel gametophytic mutants will provide new insights into the genetic regulation of megagametophyte development and function. In a screen involving about 5000 transposants, we identifed 59 mutants that showed both reduced fertility and a distorted segregation ratio of the kanamycin resistance marker present on the *Ds* element, a characteristic typical of gametophytic mutants. We have further characterized 14 *Ds* insertion mutants that are semisterile, carry a single *Ds* element, and show a reproducibly distorted segregation pattern. By performing reciprocal crosses to wild type, we determined the efficiency of transmission through both gametophytes. Two mutants are specific to the female and ten were found to affect both sexes to varying degrees. Interestingly, two were found to specifically affect the male, indicating that the mutation disrupts a process occurring late during fertilization. It is likely that the mutations are caused by gene disruption as a consequence of the *Ds* insertion. Indeed, sequence from the genomic region flanking the element has shown that in 8 of these 14 lines, the insertion was within a sequence that has homology with genes and ESTs present in the databases. To determine unambiguously whether these mutants are molecularly tagged, we are currently attempting to isolate revertants by reintroducing *Ac* transposase.

To determine the morphological defects in these lines, we characterized the development of the female gametophyte in fixed and cleared ovules by light microscopy. For each mutant, developmentally staged ovules were compared to the equivalent stage in wild type. The mutants could be grouped into five phenotypic classes affecting all stages of megagametophyte development: (I) megagametophyte arrests before mitosis, (II) megagametophyte arrests at various points in the mitotic phase, (III) nuclei fail to migrate properly and/or cellularization is abnormal, (IV) polar nuclei fail to fuse, and (V) megagametophyte develops to maturity but the fertilization process fails. Mutants of class V can be female- or male-specific. For instance, semisterility would result if the pollen tube entered the ovule and discharged the sperm cells but fertilization was not effected. In some of the class II mutants, coordination of the mitotic cycles is lost and asynchronous divisions give rise to variable numbers of nuclei and aberrant nuclear positioning. For *hadad (hdd)*, one of the class II mutants, we performed a detailed comparison to wild type throughout megagametophyte development that gave us insight into temporal aspects of the mutant phenotype. Mutant *hdd* embryo sacs predominantly arrest after one or sometimes two mitotic cycles, but some may progress slowly through all three mitotic divisions. Although delayed, a small fraction (less than 2%) reaches maturity and can get fertilized to form seeds. We are performing similar phenotypic analyses for the other mutants that we have isolated.

Cell Specification in the Female Gametophyte

R. Baskar, J. Moore, J.-P. Vielle-Calzada, U. Grossniklaus

Although the seven cells of the megagametophyte are of clonal origin, they develop along four alternative developmental pathways. The small number of cell types in the female gametophyte makes it an ideal system to study cell specification processes in plants. We have screened more than 2000 transposants for reporter gene expression in mature ovules. Among the enhancer detector transposants, about 5% show expression in the female gametophyte. Whereas some of these show expression in all cells of the megagametophyte, others are specific to a subset of cells or to individual cell types such as the egg cell, synergids, and antipodals, suggesting that they may be involved in cell specification processes. We are particularly interested in the specification and function of the egg cell. To date, we have identified two transposants with expression in the egg, neither of which displays an obvious mutant phenotype. To confirm that the *Ds* elements were within a transcription unit expressed in the egg cell, we performed in situ hybridization with riboprobes derived from sequences flanking the insertion site. Preliminary experiments suggest that the enhancer detector expression pattern indeed reflects the expression of a gene in the immediate neighborhood of the insertion. We have isolated regions flanking the two *Ds* elements from a genomic library and are currently characterizing the promoter regions of these genes. Once identified, egg cell-specific promoters will be used for genetic cell ablation and misexpression of regulatory genes. Such experiments will allow us to investigate cellular interactions between the cells of the megagametophyte and to probe the potential of the egg cell for autonomous activation, an important component of apomictic reproduction.

MEDEA Encodes a Protein of the *Polycomb*-Group

M. Hoeppner, J.-P. Vielle Calzada, U. Grossniklaus

During the past year, we concentrated on the molecular characterization of *medea* (*mea*), a gametophytically controlled embryo lethal mutant. *MEA* regulates cell proliferation during seed development. Embryos derived from mutant eggs grow to a giant size and eventually die. The gametophytic maternal control of this growth defect is consistent with the parental conflict theory of Haig and Westoby (*Am. Nat. 134*: 147 [1989]) which explains parent-of-origin-dependent control of embryo size as the outcome of a conflict between parental genomes. We had previously shown that *mea-1* is molecularly tagged by cosegregation and reversion analyses. We used genomic fragments flanking the *Ds* element to screen a floral cDNA library and identified a single clone among 800,000 phages screened. Since the cDNA was not full length, the 5′ end was isolated by RACE PCR. Sequence analysis showed that *MEA* is similar to *Enhancer of zeste* [*E(z)*], a *Drosophila* protein best known for its involvement in the regulation of homeotic genes (Fig. 1A). The highest similarity between the two proteins (55% identity) is found at the carboxyl terminus, in the SET domain, which was named after the three founding members of the family in *Drosophila*, *Suppressor of variegation 3-9* [*Su(var)3-9*], *E(z)*, and *trithorax* (*trx*) (Fig. 1B). Although the function of the SET domain is unknown, the members of this family have been implicated in the regulation of chromatin structure, and several have been shown to associate with chromatin. *E(z)* shows characteristics of both the *trithorax*-group (*trx-G*) and *Polycomb*-group (*Pc-G*) in *Drosophila*. *Pc-G* and *trx-G* proteins play an important role in the long-term activation and repression of homeotic genes in *Drosophila*, mice, and plants. Recently, the first *Arabidopsis* member of the SET domain family, *CURLY LEAF* (*CLF*) (Fig. 1B) was shown to have a similar function in the control of floral homeotic genes (Goodrich et al., *Nature 386*: 44 [1997]).

Many of the animal members of this protein family show parent-of-origin-specific effects and are involved in the regulation of cell proliferation. For instance, the human homologs of *trx* (*All-1/Hrx*) and *E(z)* (*Enx-1*) control lymphocyte proliferation and have been implicated in leukemias and lymphomas. Thus, both aspects of SET domain protein function, the control of homeotic gene expression (*CLF*) and the regulation of cell proliferation (*MEA*), appear to be conserved across kingdoms. *MEA* shares 43% identity with *E(z)* in the CXC domain, a cysteine-rich region amino-terminal to the SET domain (Fig. 1A). The CXC domain and five additional highly conserved cysteine residues are unique to *E(z)* and its vertebrate and plant homologs. Although the function of these cysteine-rich regions is unknown, they have been shown to be required for *E(z)* function. Since *MEA* encodes a SET domain protein, it is likely that *MEA*

A

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                A        C5       N   CXC   SET
MEA
                    17%               43%   55%
E(z)
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B

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                                              Ds
                                              ▽
MEA        SDVHGWGAFT WDSLKKNEYL GEYTGELITH DEANERGRIE
CLF        ..IS.....L KN.VS.H... ........S. K..DK..K.Y
E(z)       ..IA...I.L KEGAQ...FI S..C....SQ ...DR..KVY
Enx-1      ...A...I.I K.PVQ...FI S..C..I.SQ ...DR..KVY
Su(var)    ANGS...VRA ATA.R.G.FV C..IE.I..S .......KAY
Trx        .HI..R.LYC TKDIEAG.MV I..A....RS TLTDK.E.YY

MEA        D-RIGSSYLF TLNDQ----- -LEIDARRKG NEFKFLNHSA
CLF        .-.ENC.F.. N....----- -FVL..Y... DKL..A...P
E(z)       .-KYMC.F.. N..ND----- -FVV..T... .KIR.A...I
Enx-1      .-KYMC.F.. N..ND----- -FVV..T... .KIR.A...V
Su(var)    .DNGR-T... D.DYNTAQDS EYT...ANY. .ISH.I...C
Trx        .S.GIGC.M. KID.N----- -.VV..TMR. .AAR.I..CC

MEA        RPN-----CY AKLMIVRGDQ RIGLFAERAI EEGEELFFDY CYGPEHA
CLF        E..-----.. ..VIM.A..H .V....KER. LA.....Y.. R.E.DR.
E(z)       N..-----.. ..V.M.T..H ......K... QP........ R.G.TEQ
Enx-1      N..-----.. ..V.M.N..H ......K... QT........ R.SQAD.
Su(var)    D..LAVFP.W IEHLN.ALPH LVF-.TL.P. KA....S... IRADNED
Trx        E..-----.. S.VVDIL.HK H.I...V.R. VQ....TY.. KFPF.DE
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FIGURE 1 *MEDEA* ecodes a protein of the *Polycomb*-group. (*A*) Schematic alignment of *MEA* and *E(z)* showing the relative position and amino acid identity of the SET and CXC domains, the putative nuclear localization signals (N), the five conserved cysteines (C_5) characteristic of *E(z)*, and its plant and vertebrate homologs, and a highly acidic domain (A) also found in *trx*. (*B*) Alignment of the SET domain of plant (*CLF*), *Drosophila* [*E(z)*] and human (*Enx-1*) *E(z)* homologs and the other founding members of the SET domain family, *Su(var)3-9* and *Trx*. Identities are indicated by a dot, and gaps by a dash. The *Ds* insertion site is indicated. The *MEA* SET domain shows a higher degree of similarity to the plant *E(z)* homolog *CLF* (60%) than to *E(z)* and *Enx-1* (55% and 57%, respectively).

controls gene expression during embryogenesis through the regulation of higher-order chromatin structure. Our findings suggest that similar molecular mechanisms operate in animals and plants to control cell proliferation and to mediate parent-of-origin-specific effects.

Isolation of Molecular Markers Associated with Apomixis in *Hieracium*

R. Bicknell, U. Grossniklaus

During the last several years, Ross Bicknell has developed *Hieracium*, a naturally occurring apomictic species, into a powerful model system to study apomixis. The purpose of Ross' sabbatical visit here at the laboratory was twofold: (1) The establishment of a long-term collaboration to analyze the expression of genes we identify in *Arabidopsis* in sexual and apomictic accessions of *Hieracium*, which will allow a direct comparison of gene expression during sexual and apomictic reproduction, and (2) the isolation of molecular markers associated with the locus controlling apomixis in *Hieracium*. From previous work, it appeared that apomixis was inherited as a dominant Mendelian trait. The molecular basis of the trait, however, is unknown. To develop RFLP probes linked to the principal locus, we decided to use representational difference analysis (RDA), a powerful technique developed here at the laboratory (Lisitsyn et al., *Science 259:* 946 [1993]), which allows the direct isolation of molecular differences between genomes.

Pooled segregant genomic DNA from sexual and apomictic hybrid plants would be used for RDA. In these pools, we expect all polymorphisms except for those tightly linked to apomixis to be randomized.

We did not start out very well. The first shipment of plant material was frozen in transit and the second was held up at the border. Both arrived at Cold Spring Harbor in the form of a green, smelly soup garnished with rafts of small floating labels. After several weeks of desperate nursing by Tim Mulligan and others, two segregant populations were recovered from this puree, one composed of sexual individuals and one of apomicts. Three restriction enzymes were used to fragment the genomic DNA of 11 and 15 sexual and apomictic siblings, respectively. RDA was advanced over three cycles of subtraction/amplification. Twenty- four persisting fragments were cloned that appeared to have different levels of abundance between the DNA derived from the pool of sexual plants and the DNA derived from the pool of the apomictics. These were each tested individually by Southern blotting and six were found to display reproducible differences between the pools. One of these six appears to be very promising since it has only been detected in apomictic individuals. Other tests, however, need to be conducted to confirm this preliminary result. Sadly, the 6-month period of leave ran out at this point. All the experimental material, however, is duplicated at Lincoln, New Zealand, where the work will continue.

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PLANT GENETICS

D. Jackson Z. Yuan

Research in our laboratory is aimed at understanding the control of morphogenesis in plants, using maize and *Arabidopsis* as model genetic systems. We are currently focusing on two areas: (1) intercellular trafficking of plant regulatory proteins and (2) genetic analysis of shoot meristem function. As well as adding to our basic understanding of the developmental strategies of plants, the results from these studies will aid in our understanding of cellular processes involved in the regulation of plant growth and the spread of plant pathogens.

Leaves and floral organs are initiated from shoot meristems, groups of stem cells that are responsible for the indeterminate growth characteristics of plants. Genes that define the shoot apical meristem by their expression patterns and functions have recently been identified. The maize *knotted1 (kn1)* gene and members of the *kn1* gene family from diverse species are expressed in shoot apical meristems but not in leaves; in fact, down-regulation of KN1 protein and mRNA precedes the outgrowth of a leaf primordium. *kn1* contains a homeodomain, a DNA-binding domain involved in the control of cell fate during the development of many organisms through transcriptional regulation of specific target genes.

We are studying aspects of *kn1* regulation and characterizing other mutations that affect shoot meristem function.

INTERCELLULAR TRAFFICKING OF *KNOTTED1* GENE PRODUCTS

Intercellular communication is fundamental to the development of multicellular organisms. This is especially evident in plants, where cell fate is determined largely by positional rather than lineage cues. Evidence for the importance of cell-to-cell communication throughout plant development has come from mosaic analysis, which shows that many regulatory genes in plants act non-cell-autonomously. In other words, cells that carry a mutation in a particular developmental gene can be phenotypically complemented in mosaics because they receive appropriate signal(s) from adjacent wild-type cells. The nature of these signals was previously not known, although our recent data together with data from other labs suggest that the cell-to-cell trafficking of regulatory proteins through plasmodesmata may define a novel intercellular signaling pathway.

Mosaic analysis showed that expression of *kn1* in inner cell layers of the leaf induces a signal that spreads to surrounding cells and alters their fate. To our surprise, we were able to detect the presence of KN1 protein outside of the domain of KN1 mRNA localization, suggesting that the KN1 protein itself may be the signal. We then demonstrated directly, using microinjection assays, that KN1 protein can traffic between plant cells through plasmodesmata, membrane-lined cytoplasmic channels that traverse the extracellular matrix to allow symplastic communication between cells. Functionally, plasmodesmata resemble animal gap junctions in allowing the passage of small molecules such as metabolites and small growth regulators, with an upper size limit of 1 to a few kilodaltons. Plasmodesmata were previously known to also allow the passage of macromolecules, because many viruses move through them to spread infection to adjacent cells. The virus encodes a movement protein that binds the viral genome and traffics it through the plasmodesmata. The identification of KN1 as a specific plant gene product that traffics through plasmodesmata provides an opportunity to dissect the mechanism of this novel intercellular communication pathway.

Our current research efforts are addressing the following questions: (1) What is the role of cell-to-cell trafficking of KN1 in normal development? (2) Does trafficking of KN1 protein occur in the shoot meristem, where *kn1* exerts its normal function? We are combining the use of green fluorescent protein fusions and high-resolution localization experiments with genetic analysis to elucidate the pathway of intercellular trafficking of plant proteins.

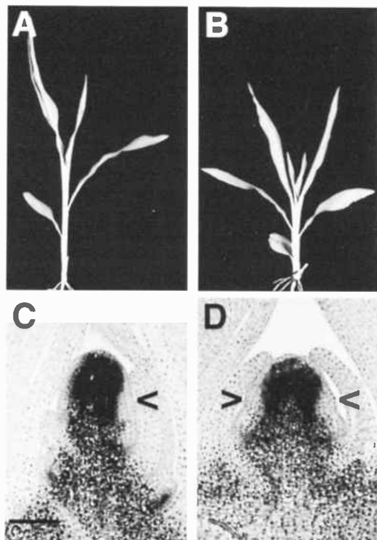

FIGURE 1 Alteration of phyllotaxy in *abph1* seedlings. Normal maize seedlings initiate leaves singly and opposite from the previous leaf (*A*). In seedlings carrying the recessive *abph1* mutation, leaves are initiated in opposite pairs (*B*). This change in phyllotaxy is reflected in changes in the expression of the *kn1* homeobox gene in the shoot apical meristem (*C*, *D*).

GENETIC ANALYSIS OF MERISTEM FUNCTION IN MAIZE

Organogenesis in plants occurs at the shoot apical meristem, a group of indeterminate stem cells that are formed during embryogenesis. Regular patterns of initiation of leaves or flowers from the meristem give rise to the familiar geometric patterns observed throughout the plant kingdom. The mechanism by which these patterns, termed phyllotaxies, are generated, remains unclear. Members of the grass family, including maize, initiate leaves singly, alternating from one side

to the other in a regular pattern. We are characterizing a new recessive maize mutant, *abphyl1*, that initiates leaves in opposite pairs rather than singly. Expression of *kn1* in the shoot apical meristem is altered in *abph1*; specifically, the prepattern of *kn1* down-regulation predicts the different patterns of leaf initiation. The *abph1* mutant phenotype is first manifest in the embryo by a larger shoot apical meristem and a larger domain of expression of *kn1*. Therefore, the *abph1* gene regulates pattern formation at an early step in maize embryogenesis, prior to visible signs of true leaf initiation. We are currently attempting to isolate the *abph1* gene using transposon tagging.

Another class of meristem mutants are those in which the balance between stem cell proliferation and primordium initiation is disturbed. Normally, the proliferation of stem cells is finely balanced with the recruitment of cells into primordia, and the meristem is maintained as an organized unit of fixed size. We are isolating and characterizing mutations that cause overproliferation and enlargement of the meristem, also known as fasciation. The normal role of these genes may be in the control of cell proliferation in the meristem, or in the control of organ initiation. We have isolated several mutations with fasciated phenotypes, one of which is from a Mutator transposon line. We have identified a transposon that cosegregates with the fasciated mutation and we are isolating flanking maize genomic sequences to test whether the gene is tagged and to isolate the fasciated gene. Isolation and molecular characterization of fasciated genes should enhance our understanding of cell proliferation in plants, and it will be interesting to determine whether the mechanisms are similar to those regulating cell proliferation in animals.

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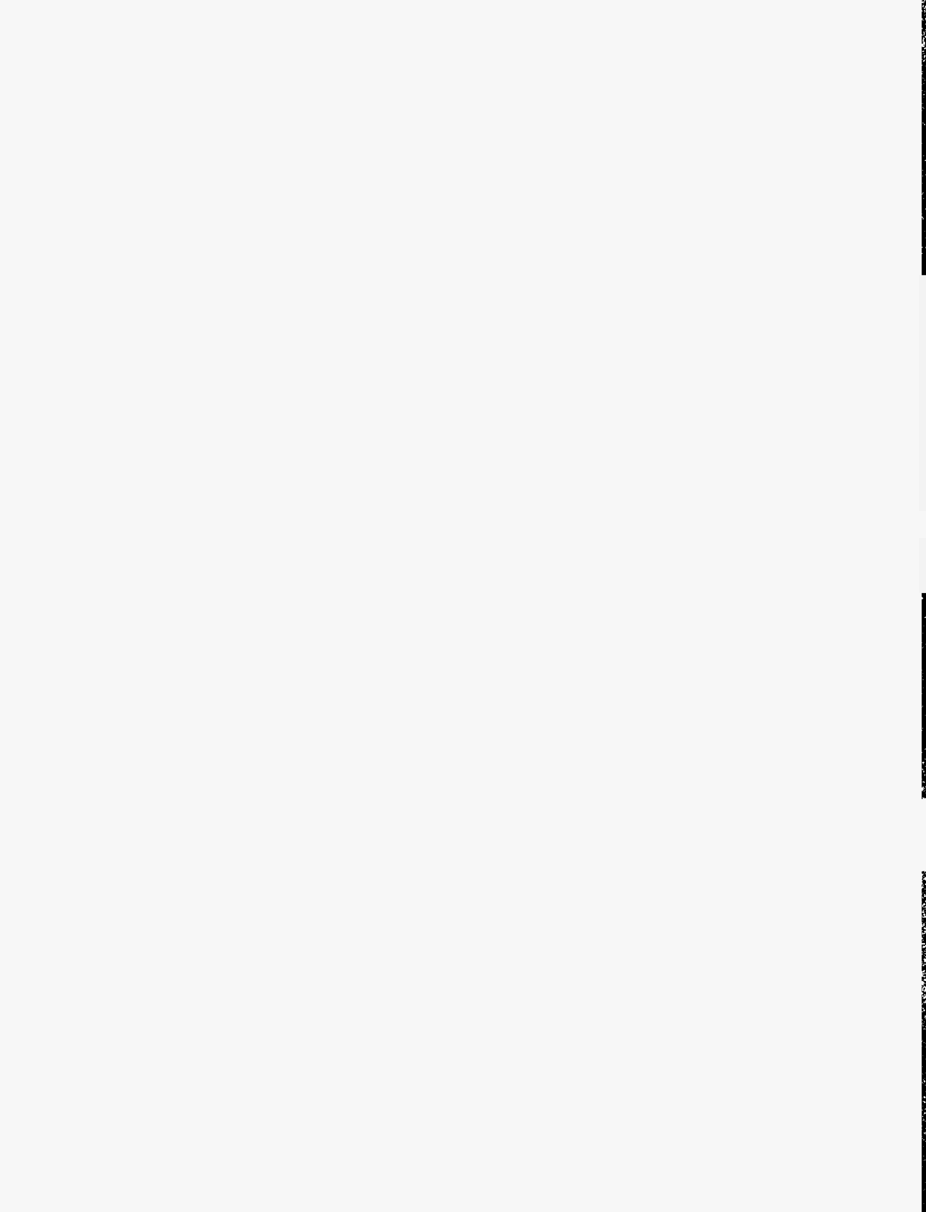

CELL BIOLOGY

This section comprises laboratories studying a diverse set of interests including signal transduction events that regulate gene expression and ultimately growth in normal and cancer cells.

- Carol Greider's laboratory continued their studies of telomerase and, together with Ron DePinho, generated knockout mice that lacked telomerase activity. In these mice, telomere function was lost after five or six generations, resulting in increased genomic instability and a progressive decline both in fertility and in viability of highly proliferative tissues. Their data suggest that although telomerase is not essential for tumor formation, its activity may contribute to tumor growth.
- David Helfman's laboratory found that activation of cellular contractility is an indispensable step in adhesion-dependent cellular signaling. Their observations suggest a mechanism whereby the changes observed in actin filaments in transformed cells could lead to aberrant cell growth by interfering with normal signaling functions.
- Nouria Hernandez and colleagues continued their studies of transcription from the human snRNA promoters and from the HIV-1 promoter. In characterizing the snRNA activating protein complex (SNAPc), they have completed isolation of cDNA clones for SNAPc subunits and reconstituted the complex from recombinant proteins. They have also been studying the ability of the HIV-1 promoter to generate short, prematurely terminated transcripts, which is dependent on a DNA element termed inducer of short transcripts (IST). A specific IST-binding factor has been purified and cloned, and its role in short transcript formation is being characterized.
- Tatsuya Hirano's laboratory uses a cell-free extract derived from *Xenopus* eggs to study the molecular mechanisms underlying mitotic chromosome condensation and segregation, with a particular emphasis on the role in these processes of two chromosomal ATPases, which are members of the SMC (Structural Maintenance of Chromosome) family.
- David Spector's group focuses on structural and functional organization of the mammalian cell nucleus. They have observed the dynamic movements of pre-mRNA splicing factors in the nuclei of living cells and have shown that these movements are dependent on ongoing RNA polymerase transcription. In addition, they have visualized directly the recruitment of splicing factors to a specific active gene in living cells using an inducible promoter system.
- Nick Tonks' laboratory continues to take a variety of approaches to study the physiological function of protein tyrosine phosphatases (PTPs), an important family of signal transducing enzymes. They have developed "substrate-trapping" mutant forms of these enzymes which form stable complexes with target substrates in a cellular context. These complexes can be isolated and the substrates identified. This approach has revealed that, contrary to popular belief, the PTPs display exquisite substrate selectivity in a cellular context and thus may be specific regulators of cellular signaling events.
- Linda Van Aelst and her colleagues focus on defining the role of the small GTPases Ras and Rac in signaling pathways that regulate cell growth. Recently, they have shown that an activated form of Rac promotes integrin-mediated T-lymphocyte adhesion. In addition, they have shown that the Rac-binding protein POR1 may be an important regulatory element in establishing specific patterns of cytoskeletal rearrangements at the plasma membrane and may play a role in peripheral membrane trafficking.

SIGNAL TRANSDUCTION

L. Van Aelst B. Boettner M. McDonough
M. Marin R. Packer

Research in our lab is focused on the study of signal transduction pathways implicated in cell growth control. In particular, one major interest of our lab is to investigate the role of Ras and Rac genes in signal transduction. The Ras and Rac genes encode low-molecular-weight guanine-nucleotide-binding proteins that function as binary molecular switches by cycling between the active GTP-bound state and the inactive GDP-bound state. These proteins have been demonstrated to have a central role in the signal transduction pathways that mediate such diverse biological phenomena as transformation, mitogenesis, metastasis transcriptional activation, and cytoskeletal organization. Our major objective is to uncover the effector pathways mediating the effects of Ras and Rac proteins. Another subject of our ongoing research is the functional characterization of $p62^{dok}$, a Ras-GAP-associated protein that is constitutively tyrosine-phosphorylated in chronic myelogenous leukemia (CML) progenitor cells.

Ras and Rac Signaling Pathways

B. Boettner, M. McDonough, R. Packer, L. Van Aelst

Functional Characterization of a Novel RAS-binding Protein, AF-6. There is abundant evidence implicating the dysregulation of the Ras proteins in the etiology of human cancers. Most importantly, cellular Ras genes have been found to be frequently activated by mutation in a wide variety of human cancers. These cancer cells exhibit morphological changes and alterations of cell adhesion. We previously identified the serine/threonine Raf kinase as a critical downstream target of Ras, required for Ras-mediated transformation in mammalian cells. However, evidence for the involvement of additional effector pathways contributing to cell transformation has been obtained. More recently, we have isolated the protein AF-6 as a novel putative Ras target. AF-6 was previously identified as an in-frame fusion partner for ALL-1 in acute lymphoblastic leukemias caused by translocation events of the t(6;11) type (AF-6 hereafter stands for All-1 fused gene on chromosome 6). ALL-1 in turn is a homolog of *Drosophila trithorax* and, in a variety of leukemias, due to chromosomal translocation, is linked to coding regions on chromosomes 4, 6, 9, or 19. However, the putative malignant function of the ALL-1/AF-6 fusion protein, as surmised for those fusions of All-1 to other loci, in lymphoblastic cells is unclear. Presently, little is known about the biochemical function of AF-6. We determined that its interaction with Ras is GTP-dependent and that the first 160 amino acids of AF-6 are sufficient for Ras binding. In addition to the amino-terminal Ras-binding domain, AF-6 contains an unc-104-like kinesin, a class V myosin homolog, a PDZ, and a proline-rich carboxy-terminal domain. A *Drosophila* homolog of AF-6, designated canoe, has been isolated, which shares a common domain organization. Canoe has been described as a downstream component in *Drosophila Notch* signaling involved in eye, bristle, and wing development and has been shown to interact genetically with the *armadillo* (*b-catenin*) gene. The products of the *Notch* and *armadillo* genes are involved in adhesive cell-to-cell communications. Furthermore, canoe is implicated in the formation of cone cells in the developing compound eye in *Drosophila*. Interestingly, the phenotypic effects of *canoe* mutations on the cone cells are dependent on the state of Ras. More recently, evidence has been obtained that AF-6 is concentrated at cell-cell contact sites of epithelial cells. One of our major aims is to assess a role for AF-6 in Ras-mediated activities, in particular, whether AF-6 may account for the functions of Ras in the regulation of cell-cell contacts. Toward this end, we are currently determining the distribution and localization of AF-6 in epithelial and endothelial cells expressing an activated mutant form of Ras, and we are assessing the effects of AF-6 WT and truncation mutants on cell-cell adhesion. In addition, we recently succeeded in isolating AF-6 interacting proteins and are in the process of further characterizing them.

Effectors of RAC1 Function. One of the first functions attributed to Rac was its involvement in the reorganization of the actin cytoskeleton induced by

growth factors and constitutively active Ras (RasV12). Later, Rac was shown to activate the transcription factors c-Jun (mediated by the c-Jun amino-terminal kinase, JNK), SRF, and the nuclear transcription factor NF-κB. More recently, Rac has been implicated in multiple aspects of cell growth control. It is well established that the GTPase Rac is an essential component of Ras-induced malignant transformation. Furthermore, evidence has been obtained that Rac has a role in cell-cell/cell-ECM adhesion (see below), invasion, and apoptosis. However, it remains a major challenge to unravel the underlying molecular mechanisms by which Rac mediates these various activities. Toward this end, we have set out to identify Rac-interacting proteins and devised a system to dissect genetically the different functions of Rac.

With regard to the identification of Rac-interacting proteins, we previously described the isolation of two Rac-interacting proteins, POR1 and POR2, and provided evidence for a role of POR1 in Rac-induced cytoskeletal rearrangements. We continued to further characterize the function of POR1. Localization studies showed that POR1, similar to Rac1, can be found both in the cytoplasm and along the surface of the plasma membrane. Our preliminary studies revealed a redistribution of POR1 from the cytoplasm to the plasma membrane in response to PDGF. Interestingly, we found more recently that POR1 interacts with the GTPase, ARF6. This study was performed in collaboration with Crislyn D'Souza-Schorey (Washington University School of Medicine, St. Louis). ARF6 is the least conserved member of the ARF family of GTPases. In addition to its role in regulating peripheral membrane trafficking, we showed that ARF6 and its activated mutant, ARF6(Q67L), elicit cytoskeletal rearrangements at the cell surface. Cytoskeletal rearrangements induced by ARF6(Q67L) could be inhibited by coexpression deletion mutants of POR1, but not with the dominant negative mutant of Rac, Rac(S17N). These findings indicate that ARF6 and Rac function on distinct signaling pathways to mediate cytoskeletal reorganization and suggest a role for POR1 as an important regulatory element in orchestrating cytoskeletal rearrangements at the cell periphery induced by ARF6 and Rac. It is possible that, depending on the nature of the extracellular stimuli, POR1 could interact with either ARF6 or Rac or both to establish highly specified patterns of cytoskeletal rearrangements at the plasma membrane. More recently, several reports suggest the possible interdependence or cross-talk between signaling pathways that regulate cytoskeletal architecture and membrane trafficking. We are assessing a role for POR1 in peripheral membrane trafficking. We are also investigating a role for POR1 in the other Rac-mediated activities.

To gain insight into the signaling pathways mediating Rac-induced biological events, we screened for Rac effector loop mutants that separate the ability of Rac to interact with different downstream effectors. In addition to the abovementioned POR proteins, a family of serine/threonine kinases known as PAKs, IQGAP, and pp70 S6 kinase were isolated as potential Rac targets. The use of Rac effector domain mutants allowed us to demonstrate that the ability of Rac to mediate transformation is not dependent on its ability to activate JNK. We expanded these studies, and in collaboration with Channing Der were able to show that SRF activation and the short-term cytoskeletal rearrangements triggered by Rac are not required for the transforming ability of Rac. Presently, the pathway(s) mediating Rac's effect on cell proliferation remains to be defined.

Several observations suggest a contribution of short-term cytoskeletal rearrangements to invasiveness and metastasis. As mentioned above, a role for Rac in invasion has been demonstrated. Adhesion, spreading, and motility are involved at various stages during invasion and metastasis and are both dependent on a functional actin cytoskeleton. We recently observed that expression of an activated mutant form of Rac, RacV12, induces increased adhesion of T cells onto fibronectin in an integrin-dependent manner and that adhesion is associated with dramatic cell spreading and integrin clustering. Cell spreading is accompanied by cytoskeletal rearrangements and requires actin polymerization. This effect was not mimicked by expression of activated mutant forms of Rho, Cdc42, Hras, or ARF6, indicating the unique role of Rac in this event. Expression of Rac did not alter the expression levels or affinity state of the integrin receptors. Moreover, our results show that the contribution of Rac to T-cell adhesion involves events following receptor occupancy, such as cell spreading and possibly integrin clustering, rather than alterations of integrin affinity or cell-surface expression. Cell spreading, which imposes a more streamlined shape to the T cells, aids in their resistance to the shear forces they are exposed to during the vascular flow in the venules. We also obtained evidence that the Rac-mediated signaling pathway leading to T-cell spreading is not due to activation of PAK, JNK, or S6 kinase. Interestingly,

the drugs wortmannin and LY294002 at concentrations of 50 nM and 100 μM, respectively, were able to inhibit the RacV12-induced cell spreading, suggesting a role of a lipid kinase in mediating Rac's effect on T-cell spreading. Since the phenotypic alterations induced by RacV12 are so distinctive, one of our long-term goals is to screen a mammalian cDNA library identifying clones that exhibit a spreading phenotype.

Functional Characterization of $p62^{dok}$

M. Marin, L. Van Aelst [in collaboration with R. Kobayashi, Cold Spring Harbor Laboratory, and B. Clarkson, P. Pandolfi, MSKI, New York]

The other research topic under investigation in the lab is the functional characterization of a constitutively tyrosine-phosphorylated, rasGAP-associated 62-kD protein (p62), which was detected within chronic myelogenous leukemia (CML) progenitor cells. To gain some understanding of the molecular mechanism underlying human CML, Clarkson and co-workers initiated a series of biochemical studies utilizing primary lin^- Ph^+ chronic-phase CML blasts. CML is characterized by the presence of the fusion protein $p210^{bcr-abl}$, which has an elevated tyrosine kinase activity relative to normal c-Abl. Their aim was to identify possible differences in the state of tyrosine phosphorylation among proteins in whole-cell lysates of primary early progenitors derived from normal or Ph^+ chronic-phase CML marrows. They observed several proteins constitutively phosphorylated on tyrosine residues in chronic-phase blasts, whereas the similar set of phosphotyrosyl-containing proteins was undetectable in normal blasts. Among them was a 62-kD phosphoprotein which they thereafter demonstrated to form an in vivo complex with p120 rasGAP. Using a human megakaryoblastic cell line expressing $p210^{bcr-abl}$ (MO7/p210), N. Carpino et al. (CSHL) purified and cloned the cDNA of p62, which they designated $p62^{dok}$. p62 encodes a novel protein that displays some interesting features: (1) A putative pleckstrin homology (PH) domain at the extreme amino terminus; (2) 15 tyrosines, of which 10 are located within a carboxy-terminal stretch spanning one-third the length of the molecule; and (3) ten PXXP motifs, the core-conserved sequence of proline-rich regions that are recognized by SH3 domains.

A RasGAP-associated 62-kD protein was also previously observed as a common target of protein-tyrosine kinases such as v-Abl, v-Src, v-Fps, v-Fms, and activated EGF receptor, and tyrosine phosphorylation of p62 appears to correlate with the transforming activity of the viral oncogenes.

Using antibodies raised against human $p62^{dok}$, we confirmed that the tyrosine-phosphorylated 62-kD protein observed in src, v-abl, and $p210^{bcr-abl}$ transformed cells is indeed $p62^{dok}$. We also confirmed that $p62^{dok}$ is rapidly tyrosine-phosphorylated after stimulation of Mo7 cells with the c-kit ligand. In addition, in fibroblasts, we observed that $p62^{dok}$ is rapidly tyrosine-phosphorylated following PDGF, insulin, and IGF-1 stimulation. With regard to $p210^{bcr-abl}$, we recently obtained evidence indicating that $p62^{dok}$ is a direct substrate of $p210^{bcr-abl}$.

In CML, evidence for constitutive Ras activation and reduced Ras-GAP activity has been found. In addition, evidence has been provided that CML progenitor cells show an altered adhesion behavior toward marrow stromal cells and some extracellular matrix proteins, in particular fibronectin, and it has been suggested that this altered adhesion may contribute to the abnormal proliferation observed in CML. In addition, c-kit ligand has been shown to increase the adhesion properties of hematopoietic progenitor cells to fibronectin. To further elucidate the function of $p62^{dok}$, we are currently measuring the catalytic activity of GAP toward ras and the GTP/GDP ratio of ras in the presence of unphosphorylated and tyrosine-phosphorylated p62 and in cells lacking $p62^{dok}$. These cells are provided by Dr. P. Pandolfi (Memorial Sloan-Kettering). We set out to identify and characterize $p62^{dok}$ interacting proteins. Furthermore, we started to determine the structural features underlying the interaction of $p62^{dok}$ with GAP and other recently identified $p62^{dok}$ interacting proteins. The information gained from these studies will provide useful tools to address the functional role of $p62^{dok}$ in the cell, in particular with an eye on its potential role in signal transduction pathways implicated in adhesion and proliferation.

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PROTEIN TYROSINE PHOSPHATASES AND THE CONTROL OF CELLULAR SIGNALING RESPONSES

N.K. Tonks A.M. Bennett, M.A. Daddario, R.L. Del Vecchio, A.J. Garton, M.J. Gutch, K. A. Ivarson, K.R. LaMontagne, M.P. Myers, S.N. Mamajiwalla, A.A. Samatar, T. Tiganis, S.H. Zhang

The phosphorylation of tyrosyl residues in proteins is a key component of the regulation of signaling pathways that control many fundamental physiological processes including cell proliferation or differentiation. Protein phosphorylation is a reversible, dynamic process in which the net level of phosphate observed in a target substrate reflects not only the activity of the kinases that phosphorylate it, but also the competing action of protein phosphatases that catalyze the dephosphorylation reaction. We study the expanding family of protein tyrosine phosphatases (PTPs) which, like the kinases, comprise both transmembrane, receptor-linked forms and nontransmembrane, cytosolic species and represent a major family of signaling enzymes. The structures of the PTPs indicate important roles in the control of key cellular functions. We are utilizing a variety of strategies to characterize the normal physiological function of several members of the PTP family. Disruption of normal patterns of tyrosine phosphorylation has been implicated as a contributor to several human diseases. Therefore, insights into the mechanisms involved in modulating PTP function may ultimately yield important information to help counter such diseases.

During the last year, Tony Tiganis and Salim Mamajiwalla completed their postdoctoral studies. Tony has returned to Australia to take a position at St. Vincent's Institute of Medical Research in Fitzroy, Victoria, and Salim has joined the Intellectual Property Group of the law firm of Blake, Cassels, and Graydon in Toronto as a Technical Advisor in Life Sciences. Martha Daddario moved to the James Lab as Building Manager, and her position in the lab as technician has been taken by Kim Ivarson.

Identification of PTP Substrates

A.J. Garton, K.R. LaMontagne, T. Tiganis

The identification of substrates of PTPs is an essential step toward a complete understanding of the physiological function of members of this enzyme family. In 1995, in collaboration with David Barford (Oxford University), we determined the crystal structure of PTP1B in a complex with a phosphotyrosyl peptide substrate. From this structure, we defined several residues in the enzyme that were important for substrate recognition and catalysis. We have characterized the function of these residues further by site-directed mutagenesis and have generated a form of PTP1B that maintains a high affinity for substrate but does not catalyze dephosphorylation effectively; i.e., we have converted an extremely active enzyme into a "substrate trap." Furthermore, the residue that is mutated to generate the substrate-trapping mutant is the invariant catalytic acid (Asp-181 in PTP1B) that is conserved in all members of the PTP family. Therefore, this has afforded us a unique approach to identification of physiological substrates of PTPs in

general. Following expression, the mutant PTP binds to its physiological substrates in the cell but, because it is unable to dephosphorylate the target efficiently, the mutant and substrate become locked in a stable, "dead-end" complex. Potential substrates can be identified by immunoblotting lysates of cells expressing the mutant PTP with antibodies to pTyr to reveal proteins whose phosphorylation state is altered as a consequence of expression of the mutant. In addition, the complex between the mutant PTP and the pTyr substrate can be isolated by immunoprecipitation, and associated proteins can be identified by immunoblotting or, on a larger scale, by primary sequence determination. We have now initiated such an approach to substrate identification with intriguing results. The major take-home message from this work is that members of the PTP family display exquisite substrate specificity in a cellular context.

TCPTP

TCPTP exists in two forms generated by alternative splicing: a 48-kD endoplasmic reticulum (ER)-associated form (TC48) and a 45-kD nuclear form (TC45). To identify TCPTP substrates, we generated substrate-trapping mutants, in which the invariant catalytic acid (D182 of TCPTP) is mutated to alanine. The TCPTP D182A substrate-trapping mutants were transiently overexpressed in COS cells, and their ability to form complexes with tyrosine-phosphorylated proteins was assessed (Fig. 1). No pTyr proteins formed complexes with wild-type TCPTP. In contrast, TC48-D182A formed a complex in the ER with pTyr-epidermal growth factor receptor (EGFR), suggesting that TC48 may regulate the state of phosphorylation of nascent EGFR that is in transit through the ER on the way to the plasma membrane. In response to EGF, TC45-D182A exited the nucleus and accumulated in the cytoplasm where it bound tyrosine-phosphorylated proteins of 50, 57, 64, and 180 kD. The 57-kD and 180-kD proteins were identified as $p52^{Shc}$ and EGFR, respectively. TC45 did not modulate EGF-induced activation of $p42^{Erk2}$ but did inhibit the EGF-induced association of $p52^{Shc}$ with Grb2, which was attributed to the ability of the PTP to recognize specifically $p52^{Shc}$ phosphorylated on Y239. These results indicate that TC45 not only recognizes selected substrates in a cellular context, but also displays preference for particular sites within substrates and thus may regulate discrete signaling events in vivo.

PTP-PEST

Previously, we had demonstrated that PTP-PEST, a ubiquitously expressed cytosolic PTP, manifested selectivity for $p130^{cas}$, a docking protein that has been implicated in assembly of multiprotein signaling complexes. We identified a high-affinity interaction between the SH3 domain of $p130^{cas}$ and a proline-rich sequence (P^{335}PPKPPR) within the carboxy-terminal segment of PTP-PEST. Mutation of Pro-337 to alanine significantly impairs the ability of PTP-PEST to recognize tyrosine-phosphorylated $p130^{cas}$ as a substrate, without qualitatively affecting the selectivity of the interaction. Thus, the highly specific nature of the interaction between PTP-PEST and $p130^{cas}$ appears to result from a combination of two distinct substrate recognition mechanisms; the catalytic domain of PTP-PEST contributes specificity to the interaction with $p130^{cas}$, whereas the SH3 domain-mediated association of $p130^{cas}$ and PTP-PEST dramatically increases the efficiency of the interaction. This represents a novel example of an emerging paradigm in tyrosine-phosphorylation-dependent signaling processes, in that substrate specificity of both protein tyrosine kinases and phosphatases is frequently influenced by the noncatalytic segments of these enzymes. This offers a versatile mechanism for restricting each enzyme to specific roles in tyrosine-phosphorylation-dependent signaling pathways.

PTP1B

The p210 bcr-abl PTK appears to be directly responsible for the initial manifestations of chronic myelogenous leukemia (CML). In contrast to the extensive characterization of this PTK and its effects on cell function, relatively little is known about the nature of the PTPs that may modulate p210 bcr-abl-induced signaling. We have demonstrated that expression of PTP1B is enhanced specifically in various cells expressing p210 bcr-abl, including a cell line derived from a patient with CML. This effect on expression of PTP1B required the kinase activity of p210 bcr-abl and occurred rapidly, concomitant with maximal activation of a temperature-sensitive mutant of the PTK. The effect is apparently specific for PTP1B because we tested several other PTPs and detected no change in their expression levels, including TCPTP, the closest relative of PTP1B. We have observed association between a substrate-trapping mutant of PTP1B (PTP1B-D181A) and p210 bcr-abl, but not v-Abl, in a

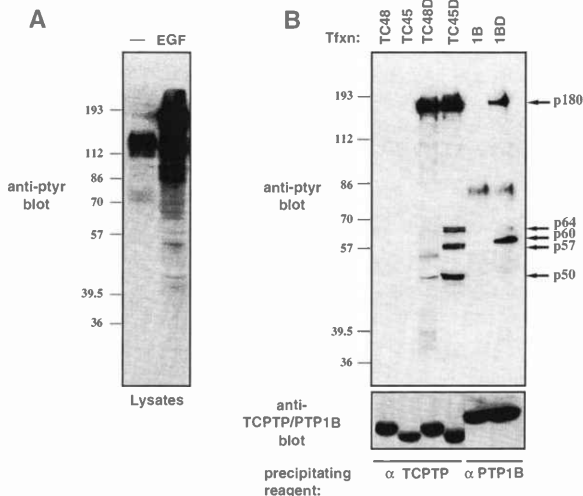

FIGURE 1 Precipitation of TCPTP and PTP1B substrates using the Asp→Ala trapping mutants. These data illustrate that PTP1B and TCPTP display substrate specificity in a cellular context, recognizing only selected pTyr proteins from the plethora of proteins phosphorylated on Tyr in response to EGF. Selectivity for substrates arises as a result of both intrinsic differences in specificity (compare the ER-localized enzymes PTP1B and TC48) and differences in localization (compare TC48 and TC45). (*A*) COS1 cells were serum-starved and stimulated with 100 ng/ml EGF for 15 min at 37ºC. Cells were lysed in 3x Laemmli sample buffer and proteins were resolved by SDS-PAGE (10%) and immunoblotted using the anti-pTyr-specific antibody G98. Molecular size markers (prestained from Sigma) are indicated on the left. (*B*) COS1 cells transiently transfected with either wild-type TC48 or TC45 or D182A mutants (TC48D, TC45D) or the PTP1B wild-type (1B) or D181A mutant (1BD) were serum-starved and stimulated with EGF (100 ng/ml) for 15 min at 37ºC. Cells were then lysed and TCPTP or PTP1B immunoprecipitates resolved by SDS-PAGE (10%) and immunoblotted with the anti-pTyr antibody G98 (*upper panel*) or with TCPTP (CF4)- or PTP1B (FG6)-specific antibodies (*lower panel*). The major pTyr-containing proteins coprecipitating with TC48D (p180), TC45D (p180, p64, p57, p50), or 1BD (p180, p60) are indicated by arrows on the right and molecular size standards are on the left. (Reproduced from Tiganis et al., *Mol. Cell. Biol. 18:* 1622 [1998].)

cellular context. Consistent with the trapping data, we observed dephosphorylation of p210 bcr-abl, but not v-Abl, by PTP1B in a cellular context and demonstrated that PTP1B inhibited binding of the adaptor protein Grb2 to p210 bcr-abl and suppressed p210 bcr-abl-induced transcriptional activation. We have now developed these studies further and demonstrated that expression of PTP1B, but not TCPTP, in p210 bcr-abl-transformed Rat1 fibroblasts inhibited the ability of these cells to grow in reduced serum, form colonies in soft agar, and form tumors in nude mice. Furthermore, we have examined the effects of PTP1B on p210 bcr-abl function in K562 cells, a Ph$^+$ CML cell line derived from a patient in blast crisis. We observed that CGP57148, a small molecule inhibitor of p210 bcr-abl (Druker et al., *Nat. Med. 2:* 561 [1996]) induced K562 cells to differentiate along the erythroid lineage. Similarly, expression of PTP1B induced erythroid differentiation of K562 cells, as measured by expression of α-globin, coincident with a decrease in the extent

of phosphorylation of tyrosyl residues in cellular proteins, including p210 bcr-abl. In contrast, expression of TCPTP did not exert a pronounced effect on the phosphorylation status of p210 bcr-abl and did not induce expression of α-globin. These results illustrate selectivity in the effects of PTP1B in a cellular context and suggest that it may function as a specific, negative regulator of p210 bcr-abl signaling in vivo.

Characterization of Mechanisms for Regulation of PTP Activity

R.L. Del Vecchio, S.N. Mamajiwalla, A.A. Samatar, T. Tiganis, S.H. Zhang

In general, the structure of PTPs can be described in terms of a conserved catalytic domain to which is fused on either the amino- or carboxy-terminal side a noncatalytic segment that serves a regulatory function and can be used to distinguish individual PTPs. It is now apparent that PTPs are regulated at multiple levels. We have been characterizing a number of protein-protein interactions involving members of the PTP family that may be important in regulating activity either directly or indirectly, through control of subcellular distribution.

TCPTP

We have examined the role of the noncatalytic, carboxy-terminal segment of TCPTP in regulating activity. Limited tryptic proteolysis of TC45 releases first a 45-kD fragment, then a 33-kD catalytic domain that displays 20–100-fold more activity toward RCML (reduced, carboxamidomethylated and maleylated lysozyme) than the full-length enzyme. Proteolytic activation occurred following cleavage of a protease-sensitive region (residues 353–387) located at the carboxyl terminus of TC45. The 33-kD catalytic domain, but not the full-length enzyme, was inhibited in a concentration-dependent manner by addition of the noncatalytic carboxy-terminal segment of TC45. A monoclonal antibody to TCPTP, CF4, which recognizes an epitope located between residues 350 and 363, was capable of fully activating TC45. These data indicate that the noncatalytic segment of TC45 contains an autoregulatory site that modulates activity via a reversible intramolecular interaction with the catalytic domain. Therefore, the carboxy-terminal noncatalytic segment of TCPTP may not only control subcellular location, but also modulate activity in response to the binding of regulatory proteins and/or posttranslational modification.

PTPH1

We pursued further our observation that the band-4.1-related PTP, PTPH1, interacts with 14-3-3β. The association is phosphorylation-dependent. Two novel motifs, RSLS^{359}VE and RVDS^{853}P, in PTPH1 were identified as major 14-3-3β-binding sites, both of which are distinct from the consensus binding motif RSxSxP recently found in Raf-1. Mutation of Ser-359 and Ser-853 to alanine significantly reduced the association between 14-3-3β and PTPH1. Association of PTPH1 and 14-3-3β was detected in several cell lines and was regulated in response to extracellular signals. These results raise the possibility that 14-3-3 may function as an adaptor molecule in the regulation of PTPH1 and may provide a link between serine/threonine and tyrosine-phosphorylation-dependent signaling pathways.

PTPμ

This year, we completed work on a paper to answer the claim made by Zondag et al. (*J. Cell Biol. 134:* 1513 [1996]) that the association we observe between PTPμ and members of the cadherin family of cell adhesion molecules results from nonspecific cross-reactivity between our anti-PTPμ antibody BK2 and cadherins. We have now characterized the association further in a number of systems and demonstrated the interaction between PTPμ and cadherins by coimmunoprecipitation using three antibodies that recognize distinct epitopes in PTPμ as well as antibodies to cadherin. In addition, we have demonstrated directly that the anti-PTPμ antibody BK2 that we used initially did not cross-react with cadherin. Our data reinforce the observation of an interaction between PTPμ and E-cadherin in vitro and in vivo, further emphasizing the potential importance of reversible tyrosine phosphorylation in regulating cadherin function. Our primary goal now is to examine the physiological function of PTPμ, concentrating on endothelial cells (ECs) as a model system.

CRYSTALLOGRAPHIC ANALYSES OF PTP STRUCTURE AND REGULATION

All of our work on crystallographic analyses of members of the PTP family is part of a long-standing col-

laboration with David Barford, formerly a Cold Spring Harbor Fellow and now a member of the faculty of the Laboratory of Molecular Biophysics, Oxford University. Recently, the crystal structure of a dimer of the membrane proximal domain of RPTPα (RPTPα D1) was described (Bilwes et al., *Nature 383:* 555 [1996]). Within this structure, the catalytic site of each subunit of the dimer is sterically blocked by the insertion of the amino-terminal helix-turn-helix segment of the dyad-related monomer. It was proposed that dimerization would lead to inhibition of catalytic activity and may provide a paradigm for the regulation of the RPTP family. We have determined the crystal structure, to 2.3 Å resolution, of RPTPμ D1 which shares 46% sequence identity with that of RPTPα D1. Although the tertiary structures of RPTPα D1 and RPTPμ D1 are very similar, with an r.m.s.d. between equivalent Cα-atoms of 1.1 Å, the quaternary structures of these two proteins are different. Neither the catalytic site nor the amino-terminal helix-turn-helix segment of RPTPμ D1 participates in protein-protein interactions. The catalytic site of RPTPμ D1 is unhindered and adopts an open conformation similar to that of the cytosolic PTP, PTP1B. This suggests that dimerization-induced modulation of RPTP activity may not be a general feature of this family of enzymes. Mechanisms by which the activity of PTPμ may be regulated, including via association with the cadherin/catenin complex, are currently being investigated.

PTPs catalyze the dephosphorylation of phosphotyrosine residues via the formation of a transient cysteinyl-phosphate intermediate. We have examined the mechanism of hydrolysis of this intermediate by generating a Gln-262 to alanine mutant of PTP1B that allows the accumulation and trapping of the intermediate within a PTP1B crystal. The structure of the intermediate at 2.5 Å resolution reveals that a conformationally flexible loop (the WPD loop) is closed over the entrance to the catalytic site, sequestering the phosphocysteine intermediate and catalytic site water molecules and preventing nonspecific phosphoryl-transfer reactions to extraneous phosphoryl-acceptors. One of the catalytic site water molecules, the likely nucleophile, forms a hydrogen bond to the putative catalytic base, Asp-181. In the wild-type enzyme, the nucleophilic water molecule would be coordinated by the side chain of Gln-262. As a result of this analysis, in combination with our previous structural data, we can now visualize each of the reaction steps of the PTP catalytic pathway.

A Genetic Analysis of PTP Function

M. Gutch

We are using the nematode *Caenorhabditis elegans* as a model system for a genetic analysis of PTP function. The experiments are being performed in collaboration with Michael Hengartner's group here at Cold Spring Harbor. An attractive feature of *C. elegans* is that many of the properties of its signaling pathways are conserved with those found in mammals. Thus, the lessons learned in *C. elegans* should be applicable to humans.

We have generated *C. elegans* strains in which two PTP genes, *ptp1* and *ptp2*, have been disrupted, producing loss-of-function mutants. PTP-1 is a receptor PTP containing three FN-III motifs in its extracellular segment and two PTP domains in the intracellular portion. We generated stable *ptp-1-GFP* transgenic worms in which green fluorescent protein (GFP) is expressed from the transcriptional regulatory sequences of *ptp-1*. GFP was expressed early in development (~100 cell stage) in a limited number of cells and was restricted to the nervous system in larval and adult hermaphrodites. We generated a Tc1 transposon insertion allele, *op147::Tc1*, in which Tc1 was inserted into the coding sequence for the membrane proximal PTP domain. This allele should generate a catalytically inactive PTP. Homozygous *op147::Tc1* hermaphrodites exhibit two incompletely penetrant phenotypes. Approximately 5% of *op147::Tc1* homozygotes are embryonic lethal and about 12.5% are morphologically abnormal. The remainder are phenotypically wild type. These incompletely penetrant phenotypes are reminiscent of a class of mutants known as vab (*v*ariable *a*bnormal). Recently, *vab-1* was cloned and found to encode a receptor PTK with similarity to the EPH family. We have generated a double mutant with a weak *vab-1* allele (*e118*), which exhibits approximately 10% embryonic lethality. Surprisingly, *vab-1(e118) ptp-1(op147::Tc1)* double mutants show greater than 90% embryonic lethality, with the remaining 10% arresting at variable stages of development prior to the generation of viable self-progeny. This enhancement of embryonic lethality appears specific to *vab-1*, since double mutants between *ptp-1(op147::Tc1)* and genes known to participate in embryonic and/or neuronal development (*vab-2, mig-2, unc-4, unc-73*) did not exhibit this

effect. We will continue to characterize the expression patterns of PTP-1, particularly from the perspective of its genetic interaction with the VAB-1 PTK.

The *ptp-2* gene encodes an SH2 domain-containing PTP. Using a Tc1 transposon targeting strategy, we isolated a recessive *ptp-2* loss-of-function allele, *op194*, which lacks the PTP domain. Homozygous *ptp-2(op194)* hermaphrodites exhibit a completely penetrant zygotic semisterile/maternal-effect lethal phenotype, characterized by the presence of abnormally large oocytes in the zygotic semisterile animals. These phenotypes indicate that PTP-2 activity is essential for proper oogenesis. Gain-of-function *let-60 ras* alleles rescued the defects associated with *ptp-2(op194)*, suggesting that LET-60 acts downstream of, or in parallel to, PTP-2 during oogenesis. Although *ptp-2* function is not required for normal vulval development, *ptp-2(op194)* altered significantly the vulval phenotypes caused by mutations in several genes of the inductive signaling pathway. The penetrance of the multivulva phenotype caused by loss-of-function mutations in *lin-15*, and gain-of-function mutations in *let-23* or *let-60 ras,* was reduced by *ptp-2(op194)*. Moreover, *ptp-2(op194)* increased the penetrance of the vulvaless phenotype conferred by a weak loss-of-function *sem-5* allele. These data position PTP-2 downstream of LET-23 in the vulval induction signaling pathway. Although PTP-2 functions to transmit a requisite signal during oogenesis, PTP-2 function during *C. elegans* vulval cell differentiation appears to be directed at regulating the overall strength of the inductive signal. Ultimately, we plan to initiate genetic screens to identify components of the signaling pathways regulated by these phosphatases.

Dual Specificity Phosphatases and Growth Control

M.P. Myers, A. Bennett

This year, a new project was initiated in the lab. In collaboration with the groups of Mike Wigler, here at Cold Spring Harbor, and Ramon Parsons, at Columbia, we have begun to characterize PTEN (*p*hosphatase and *ten*sin homolog deleted from chromosome 10), also known as MMAC1 (*m*utated in *m*ultiple *a*dvanced *c*ancers). PTEN is the product of a candidate tumor suppressor gene from human chromosome 10q23. Mutations have been observed in a variety of tumor samples including glioblastoma, advanced prostate cancer, and endometrial cancer. In addition, germ line mutations in PTEN have been identified in three related, inheritable neoplastic disorders, Cowden disease, Bannayan-Zonana syndrome, and Lhermitte-Duclos disease, which give rise to multiple benign tumors and an increased incidence of malignant cancers. When PTEN was first isolated by the Wigler and Parson labs, they noted the homology with tensin and the presence of the signature sequence motif that characterizes the PTP family. This was an exciting result because it suggested PTEN may have a defined enzymatic function—protein dephosphorylation. Furthermore, in light of the critical role that tyrosine phosphorylation is known to have in oncogenesis, it has long been suggested that PTPs would act as tumor suppressors. Nevertheless, PTEN is the first member of the PTP family to be implicated as a bona fide tumor suppressor. Obviously, characterization of the physiological function of PTEN will provide critical insights into how normal cellular signaling events are subverted in cancer. We have now shown that PTEN does possess intrinsic phosphatase activity and that it is a member of the dual specificity family of PTPs. Many point mutations in PTEN have been detected that are associated with neoplastic disease. We have demonstrated several examples in which these are detrimental to phosphatase activity, again highlighting the importance of the enzymatic function of PTEN for its ability to serve as a tumor suppressor. PTEN displays a marked preference for highly acidic substrates in vitro, suggesting that its physiological substrates also will be acidic. The search for such physiological substrates is currently a major focus of effort.

We are continuing our work on MKP-1, a highly selective phosphatase that dephosphorylates and inactivates members of the MAP kinase family of enzymes in vivo. The MAP kinases have been implicated as common and essential components of signaling pathways induced by diverse stimuli, suggesting that MKP-1 will be a crucial, central player in the control of cell function. We have been using MKP-1 as a molecular probe with which to examine physiological functions of the MAP kinases, particularly in the context of skeletal muscle differentiation. The myoblast cell line, C2C12, proliferates in high serum but differentiates into multinucleated myotubes upon mitogen deprivation. We have shown that ectopic expression of MKP-1, from a tetracycline-regulated promoter, in the presence of high serum leads to up-regulation of expression of muscle-specific genes (MyoD, myo-

genin, myosin heavy chain). Thus, expression of muscle-specific genes could be induced in vivo by inhibition of MAPK. However, myotube formation was not observed. This indicates that muscle-specific gene expression is necessary but not sufficient to commit differentiated myocytes to form myotubes and suggests a previously unexpected role for a member of the MAPK family in the late stages of skeletal muscle differentiation.

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THE CYTOSKELETON IN NORMAL AND TRANSFORMED CELLS

D.M. Helfman C. Berthier, C. Chen, J. Grossman, A.J. Rai, M. Selvakumar, A. Vaahtokari, Y.-C. Wang

Our laboratory studies the expression, structure, and function of cytoskeletal components in normal and transformed cells. We are interested in how specific actin assemblies are organized and regulated and how alterations in actin filament assembly contribute to aberrant cell growth. Actin filaments have an important role in cell movements, muscle contraction, cell division, intracellular transport, and regulation of cell

shape and adhesion. In addition, mounting evidence suggests that cellular contractility is involved in adhesion-mediated signaling. Eukaryotic cells contain three major filamentous systems involved in cytoskeletal structure: actin filaments, intermediate filaments, and microtubules. Each of these filamentous systems contains a number of different protein components, although different cell types and tissues express specific protein isoforms that comprise these structures. We are investigating the regulation and function of the cytoskeletal isoform diversity generated by alternative splicing. We are studying dynamic and stable macromolecular assemblies of distinct actin structures that are characteristic of specific cell types, for example, stress fibers, lamellipodia, filopodia, and contractile ring in fibroblasts or sarcomeres in skeletal muscle, to determine if specific isoforms are required for the assembly and regulation of specific structures. These distinct actin assemblies form by the combination of different structural components and regulatory factors. We are also interested in how the assembly of different cytoskeletal structures is regulated by extracellular signals, and how cellular contractility contributes to signaling cascades that lead to focal adhesion formation and regulation of adhesion-dependent signal transduction. Below is a description of our studies during the past year.

Regulation and Role of Cell Contractility in Adhesion-dependent Signaling

D. Helfman, C. Berthier [in collaboration with A. Bershadsky, Weizmann Institute of Science, Rehovot, Israel]

Adhesion of cells to the extracellular matrix (ECM) and neighboring cells has a critical role in various cellular processes linked to transformation, including differentiation, growth, motility, and survival. Adhesion of cells to the ECM via transmembrane receptors of the integrin family induces a rapid sequence of cytoplasmic protein events, including assembly and posttranslational modifications, e.g., formation of microfilament bundles and phosphorylation of junctional plaque proteins. The molecular components for these signaling events include cell adhesion receptors, their interaction with the cytoskeleton via specific junctional plaque proteins, and the recruitment to these sites of a number of regulatory molecules including kinases and phosphatases. Recent studies demonstrate an intimate association between the organization and expression of cytoskeletal proteins and their function in signaling in conjunction with the adhesion molecules and soluble growth factors. The loss of adhesion-dependent cell regulation is a characteristic property of transformed cells and may be attributed in part to alterations in the integrity of the cytoskeleton. These events are responsible for adhesion-induced structural alterations (formation of focal contacts and the associated actin microfilament bundles) and also affect major cellular processes including proliferation, differentiation, and cell survival. Although certain steps in this signaling cascade are well characterized, for example, tyrosine phosphorylation of focal adhesion kinase (FAK), paxillin, and $p130^{cas}$, the complete mechanism of adhesion-dependent signaling is not well understood. In addition to attachment to the appropriate ECM, the activation of adhesion-dependent signaling requires, as a rule, costimulation by soluble ligands (serum factors), whose effect is mediated by the activation of the small GTP-binding protein Rho. Rho activates cellular contractility through activation of Rho-associated kinase (Rho-kinase) that phosphorylates, and thereby activates, the regulatory light chain of myosin II (MLC) and inactivates MLC phosphatase.

To investigate the role of cell contractility in adhesion-dependent signaling, we have studied the effects of forced expression of caldesmon on the adhesion-dependent formation and tyrosine phosphorylation of focal adhesions. Caldesmon is an actin-, myosin-, tropomyosin-, and Ca^{++}-calmodulin-binding protein known to inhibit the actin-activated ATPase activity by phosphorylated myosin II and can block myosin-II-driven motility of actin filaments in vitro. Caldesmon-mediated inhibition of the actin-dependent myosin II ATPase can be released by Ca^{++}-calmodulin and possibly by some other high-affinity Ca^{++}-binding proteins. Thus, caldesmon is a potential tool for the regulation of myosin-II-driven contractility, independent of MLC phosphorylation.

We found that overexpression of nonmuscle caldesmon in cultured human fibroblasts blocks cell contractility in a Ca^{++}-sensitive manner and efficiently prevents the assembly of focal adhesions and stress fibers induced either by microtubule disruption or by expression of activated Rho. These results demonstrate that an increase in contractility is an indispensable step in these adhesion-dependent signaling processes.

Our results also suggest a physiological role for

caldesmon in the regulation of cell contractility and adhesion-dependent signaling. It has been shown in some cases that changes in fibroblast contractility may proceed without concomitant changes in the level of myosin light chain phosphorylation, implying downstream regulation. Caldesmon would appear to be a good candidate for such regulation. It is known that caldesmon can be phosphorylated by different serine/threonine kinases, including MAP kinase and $p34^{cdc2}$, and can be phosphorylated on tyrosine residues in vivo. This phosphorylation may act to integrate a variety of signaling pathways in their effect on cell contractility, which in turn can affect adhesion-dependent signaling as shown in our study.

Tropomyosin is a natural partner of caldesmon in its regulation of actomyosin-based contractility. In nonmuscle cells, the diversity of tropomyosins is very large and caldesmon specifically interacts with certain isoforms, the biological significance of which remains unclear. The spectrum of tropomyosin expression

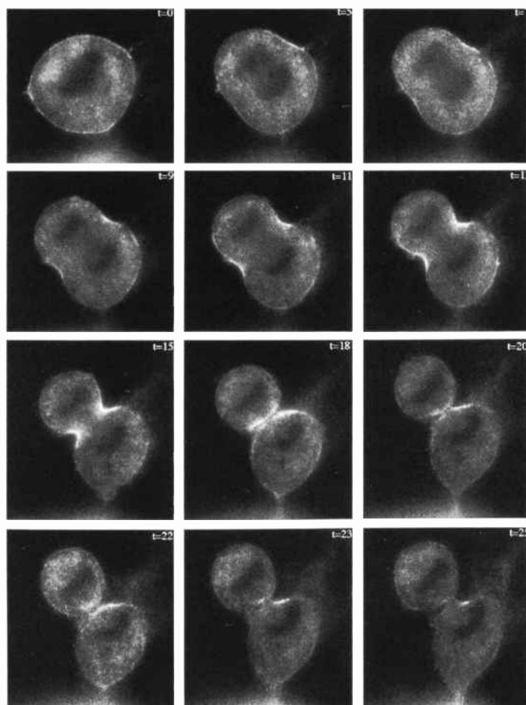

FIGURE 1 Dynamic localization of TM-5 during cell division. The localization of GFP-TM5 (NM1) was followed during cytokinesis by time-lapse video microscopy. NIH-3T3 fibroblasts stably expressing GFP-TM5 (NM1). The data show that GFP-TM5(NM1) is diffusely distributed into the cytoplasm of dividing cells before cytokinesis. During cytokinesis, GFP-TM5(NM1) progressively incorporates into the region of the contractile ring.

undergoes significant changes upon neoplastic transformation. Moreover, we and others have shown that transformation can be reversed in some cases by forced expression of particular tropomyosin isoforms. Disruption of adhesion-dependent signaling is a very characteristic feature of neoplastic transformation; such cells often display anchorage independence, growing and dividing in suspension without ECM contact. Based on our results demonstrating an ability of caldesmon to regulate adhesion-dependent signaling, we suggest that its interaction with specific tropomyosins could be important in maintaining the normal signaling function in the cell. Studies are in progress to determine how contractility leads to enhanced focal adhesion formation and what role tropomyosin and caldesmon has in the signaling cascades associated with adhesion-mediated signal transduction events.

Distribution of Tropomyosin Isoforms in Distinct and Dynamic Actin-containing Cytoskeletal Structures

C. Berthier

In nonmuscle cells, formation of actin filaments and their assembly into a variety of structures are regulated and dynamic processes that involve severing, polymerization/depolymerization cycles, and stabilization by actin-binding proteins. Several lines of biochemical evidence have suggested that nonmuscle tropomyosins (TMs) could be involved in the stabilization of actin filaments. The multiplicity of TM isoforms in one single cell (e.g., seven isoforms per fibroblast) raises the possibility that specific associations of given isoforms are required for the formation, stabilization, or regulation of specific actin-based structures in nonmuscle cells. This project was designed with regard to the following questions: (1) Do different TM isoforms achieve their function by binding to spatially distinct subsets of actin filaments, (2) how is the localization of the TMs restructured during dynamic cellular events, and (3) is the incorporation of TMs into actin filaments in vivo regulated by cooperative interactions between isoforms and is it subject to direct or indirect regulation by signaling molecules?

We have established stable cell lines of NIH-3T3 fibroblasts expressing fusion proteins of TM isoforms and green fluorescent protein (GFP). The subcellular distribution and the dynamic behavior of the GFP-tagged TMs have been investigated on living cells using fluorescence CCD-camera microscopy and time-lapse recording. Our experiments demonstrate that some differences exist between the localization of different GFP-TM fusions: TM-1, -2, and -5 appear to better incorporate into actin stress fibers than the low-molecular-weight TM-4, which is present in filopodia. Moreover, recording of cells undergoing mitosis showed an accumulation of low-molecular-weight isoforms at the contractile ring (Fig. 1). These results suggest that specific TM isoforms are involved in a different subset of actin filaments, and experiments are in progress to further characterize the dynamic localization of the different TM isoforms in various aspects of cellular motility.

Using cotransfection of tagged proteins followed by immunofluorescence, we are also currently investigating the cooperative interactions between TM isoforms and the role of caldesmon and the small GTPases in normal and transformed cells. Results strongly suggest that caldesmon, which has been shown to inhibit cellular contractility, helps the binding of TMs to actin filament bundles. We are in the process of determining if caldesmon can have a role in promoting the binding of specific isoforms to actin filaments as in vitro results suggested and if specific TM isoforms are able to regulate caldesmon function. Moreover, the role of the small GTPases in the incorporation of TM isoforms into filopodia, lamellipodia, and stress fibers is also under study. Several reports have shown that expression of TMs is altered in transformed cell lines, and we anticipate that our study will give new insights into the role of TMs in the actin cytoskeleton of cancerous cells.

Identification and Characterization of a Novel Tropomyosin-binding Protein from Nonmuscle Cells

A.J. Rai

Tropomyosin (TM) was first identified in 1948 by Bailey as a component of the actin-rich thin filament of muscle cells. Since its discovery 50 years ago, much work has been done in elucidating its function in muscle cells, which is in conjunction with the troponin complex, to serve as a calcium-sensitive regulator of actin-myosin-based contraction. However, it

was not until almost three decades later that investigators realized TM is found in virtually all eukaryotic cells and that nonmuscle cells express a multiplicity of different yet related TM isoforms. It is the functional significance of this isoform diversity that is poorly understood and still remains enigmatic.

To address this question, we have sought to identify novel TM-binding proteins from nonmuscle cells. We have conducted a two-hybrid screen with a HeLa cell library and have identified several interacting clones. One of these clones is particularly interesting in that it exhibits isoform-specific binding. The cDNA encodes a small protein of approximately 150 amino acids. We have constructed the GST fusion of this protein and have been able to demonstrate in vitro binding using Far Western blotting. Furthermore, we have been able to epitope tag this cDNA and transfect it into human fibroblasts. Antibodies to the epitope tag reveal a punctate and filamentous staining pattern. BLAST searches using the encoded protein sequence identify an EST from *Drosophila* which is 33% identical at the amino acid level. We are in the process of raising antibodies to bacterially produced recombinant protein. Isolation of these reagents will prove to be particularly useful in the further characterization and elucidation of function of this novel protein.

Regulation of Alternative Splicing of Tropomyosin RNA in Epithelial–Mesenchymal Transition

A. Vaahtokari

Several different protein products can be produced from a single gene by alternative RNA splicing. The regulation of this cellular process is poorly understood, despite its importance in the control of gene expression. TMs are a family of actin-binding proteins known to be involved in the regulation of contractility in muscle and nonmuscle cells. Because epithelial and mesenchymal cells express different splicing forms of TMs, we have begun to study the regulation of alternative splice site selection of TMs during epithelial–mesenchymal (E-M) transition. E-M transition is a crucial process in both cancer metastasis and embryonic development, e.g., during mesoderm formation and organogenesis. Recently, there has been considerable progress in discovering growth factors and other signaling molecules that regulate transition of epithelial cells into mesenchymal cells. Studies of TM isoform switching provide a model system for identifying factors that regulate E-M transition.

Human IMR-90 fibroblasts and U-2 OS osteogenic sarcoma cells transfected with the retrovirus that contains the adenovirus early region 1A (E1A) 12S oncogene are used as model systems, as it is known that E1A induces epithelialization of these cells in vitro. In addition, cells transfected with mutant forms of E1A 12S are used; one of the mutants is unable to bind retinoblastoma (Rb) protein, and one is defective in binding p300, which is a nuclear phosphoprotein homologous to CBP. The transfected cells are from Andrew Samuelson (Scott Lowe's laboratory, CSHL). Expression patterns of high-molecular-weight TMs were studied by Western blot analysis using whole-cell extracts from nontransfected IMR-90 cells and IMR-90 cells transfected with either an empty vector, 12S, or 12S mutants. In 12S-transfected cells, the expression of TM-2 and TM-4 splicing isoforms was down-regulated. The same result was observed in cells transfected with the 12S mutant that cannot bind Rb, whereas the cells transfected with the mutant form of 12S that is unable to bind p300 had the same pattern of TM expression as the nontransfected cells. Thus, p300 may be involved in the control of alternative splice site selection of TMs.

The possible role of p300 in the regulation of alternative splicing of TMs will be further studied by using p300 constructs that do not bind E1A 12S protein, and by overexpressing p300. In addition, specific inhibitors will be used to block signaling pathways (transforming growth factor-β, epidermal growth factor, hepatocyte growth factor, Wnt, E-cadherin) that are known to be involved in E-M transition, and their effects on alternative RNA splicing of TMs will be analyzed. These studies will hopefully increase our understanding of the extracellular signaling molecules and intracellular signaling pathways that are involved in the regulation of alternative splice site selection.

The Mechanism of Blocking the Use of a Skeletal Muscle Exon in Nonmuscle Cells

C. Chen

We have been using rat β-tropomyosin as a model system to study the mechanism of alternative splicing. The rat β-tropomyosin gene consists of 11 exons; two

exon pairs are alternatively spliced. Fibroblast and smooth muscle TM-1 uses exon 6 and exon 11, and skeletal muscle β-TM uses exon 7 and exon 10. We are interested in the alternative splicing of the internal exon pair, exon 6 and exon 7, which is mutually and exclusively spliced. Our previous data demonstrated that the splicing of a minigene containing exons 5–9 reflected that of the endogenous gene in HeLa cells, in which exon 6 was exclusively spliced to the common exons 5 and 8, and exon 7 was excluded. Using this substrate, we also demonstrated that at least two *cis*-acting elements were involved in the down-regulation of exon 7 splicing in nonmuscle cells. One element, which was designated as intron-regulatory element (IRE), is located in intron 6. Another element is located in the upstream region of exon 7. The ex-1 mutation, in which the element in the exon sequence is mutated, completely switches exon usage, in which the skeletal muscle exon 7 was used and the nonmuscle exon 6 excluded in nonmuscle cells in vivo.

We have further analyzed how and why the ex-1 mutation results in the use of muscle-specific exon in a nonmuscle environment. By using a pair of simple substrates that reflect the behavior of the ex-1 mutation, we have ruled out the possibilities that the effect of the ex-1 mutation is due to the creation of an enhancer element or that a secondary structure is involved. By using the UV-cross-linking assay, we found that the ex-1 mutation disrupted the binding of a putative suppressor, which we have identified as hnRNP H. The binding of hnRNP H to RNAs is specific because it can be competed out by the wild-type RNA, but not by the mutant RNAs. Work is in progress to further characterize the involvement of hnRNP H in the regulation of the rat β-tropomyosin gene alternative splicing.

Characterization of an Activity in Myogenic Cells That Stimulates Splicing of β-TM Exon 7

Y.-C. Wang

Exons 6 and 7 of the β-TM gene are mutually exclusive. During the processing of β-TM pre-mRNA, exon 7 is included in the splice product in skeletal muscle cells, whereas exon 6 is used in nonmuscle and smooth muscle cells. Previous studies suggest that muscle-specific splicing of exons 5–7 is suppressed in HeLa cells by a negative factor. To understand how skeletal muscle cells regulate exon 7 splicing, we have used mouse myogenic cells to study the *cis*-acting elements and the *trans*-acting factors involved in regulation.

Using an in vitro splicing assay comprising the 293 cell (nonmuscle) nuclear extract complemented by the mouse BC3H1 cell (muscle) nuclear extract, we have observed an activity that stimulates exon 5–7 splicing in the BC3H1 nuclear extract. This splicing activity was detected in a fraction of 25–50% ammonium sulfate precipitate followed by precipitation in 20 mM $MgCl_2$. Therefore, although we previously showed that purified HeLa SR proteins facilitated exon 5–7 splicing in BC3H1 nuclear extracts, SR proteins might not be the only factors involved in the splicing because they were in the 60–90% ammonium sulfate precipitate. To further characterize this activity, we fractionated BC3H1 nuclear extract over a CsCl density gradient and tested the fractions for the exon 5–7 splicing activity in the complementation assay with the 293 nuclear extract. An activity peak was detected coinciding with the peak of SR proteins but not U2AF, another general splicing factor essential for 3′ splice site recognition. When HeLa SR proteins were added to all fractions to relieve the limit of SR proteins, so that we could detect the presence of the other essential factor(s), the activity peak was extended three fractions toward the top of the gradient.

To identify *cis*-actin elements required for splice site selection, we have transfected the mouse BC3H1 myogenic cells and several nonmuscle cell lines, including HeLa, 293, and NIH-3T3 cells, with a chimeric minigene that contains β-TM exon 7 and portions of its flanking introns inserted between two adenoviral exons of the major late transcription unit. Although exon 7 was excluded in the splice product of this minigene in all the nonmuscle cells tested, BC3H1 cells showed detectable levels of exon 7 inclusion product. These data suggest that exon 7 and its flanking intron sequences contain the *cis*-acting elements that are, at least in part, responsible for the muscle-specific regulation of exon 7. We thus used an RNA probe containing these sequences in a UV-cross-linking assay to detect protein factors that are bound to the probe in the BC3H1 nuclear extract or its active fractions. Among the proteins detected, at least one of 70 kD showed specific cross-linking in the active fraction as well as in the whole BC3H1 nuclear extract, but not in the 293 nuclear extract. Work is in progress to further characterize these proteins and their exact roles in the regulation of exon 7 splicing in myogenic cells.

Exon Sequences and Commitment to Splicing

M. Selvakumar

Previous studies showed that the 3′ splice site of exon 6 (nonmuscle/smooth muscle exon) in β-TM pre-mRNA can be utilized only if a previous splicing event joins exons 6 and 8 together. Thus, we showed that exon sequences can contribute to splice site selection. Studies in other systems such as immunoglobulin and troponinT pre-mRNAs have identified purine-rich motifs as an important subset of exonic splicing enhancers (ESE). These ESEs are believed to act as binding sites for splicing factors such as SR proteins. Examination of sequences within exons 6 and 8 revealed the presence of two purine-rich motifs in exon 6 (6-1, 6-2) and three purine-rich motifs in exon 8 (8-1, 8-2, and 8-3) of the general consensus GARGARGAR. Substitution mutagenesis of these motifs to *T*ARG*C*R*T*AR have shown that they are required for the 3′ splice site usage of exon 6. We see that individual motifs have different effects, with 6-1, 6-2, and 8-2 being the most robust elements. Wild-type exon sequences, but not mutant exon sequences (mutated at 6-1, 6-2, and 8-2), can also specifically compete splicing of the wild-type pre-mRNA substrate. We used wild-type and mutant exon sequences as probes for UV-cross-linking in an effort to look for RNA:protein interactions. Our results have shown that SF2/ASF, a member of the SR protein family, shows much higher affinity to the wild-type exon sequences. Moreover, SF2/ASF can rescue the splicing of wild-type pre-mRNA in a competition assay. Using spliceosome assembly assays, we observed that the mutant pre-mRNA substrates are blocked at the E complex, one of the earliest steps in splicing that determine if a pre-mRNA can be committed to splicing. Thus, we have shown that there are several ESEs in exons 6 and 8 that act by binding to an SR protein, specifically SF2, thereby committing the exon to splicing. We also see that these ESEs promote not only 3′ splice site usage of exon 6, but also 5′ splice site usage (that is, in joining exons 6 and 8 together), both in vitro and in vivo.

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CELL BIOLOGY OF THE NUCLEUS

D.L. Spector	S. Huang	A. Miner
	P. Lorenz	T. Howard
	T. Misteli	J. McCann
	P. Mintz	

Studies in our laboratory are focused on the structural-functional organization of the mammalian cell nucleus. Our research program evolves around understanding the nuclear organization of factors associated with pre-mRNA splicing and the RNA substrates with which these factors interact. The microscopy core facility has been used extensively during the past year and numerous collaborations are under way with the excellent technical expertise of Tamara Howard.

The Cellular Organization of Pre-mRNA Splicing

T. Misteli, D.L. Spector

Actively transcribed genes are found throughout the nucleoplasm in mammalian cells. In contrast, essential factors required for one of the first RNA processing steps, pre-mRNA splicing, are concentrated in 20–40

distinct nuclear domains, termed speckles. The function of nuclear speckles is unknown. To investigate the behavior of nuclear speckles in vivo, we have visualized pre-mRNA splicing factors in the nuclei of living cells by fusing the autofluorescent green fluorescent protein (GFP) to pre-mRNA splicing factors. The fusion proteins are fully functional in vivo and can be detected by fluorescence microscopy in living cells. Using time-lapse fluorescence microscopy, we have demonstrated that pre-mRNA splicing factors specifically responded to transcriptional activation of single genes, by recruitment from speckles and accumulation at sites of active transcription. These observations demonstrate that transcription and pre-mRNA splicing are spatially tightly coordinated in vivo by a recruitment mechanism and suggest that the splicing factor domains are storage sites for pre-mRNA splicing factors. Other events such as preassembly of spliceosomal complexes or assembly of transcription/RNA processing complexes might also occur in these nuclear compartments.

We have investigated the molecular mechanism for this recruitment process using mutational analysis of several pre-mRNA splicing factors of the SR family of proteins. A recruitment assay was developed in which the ability of splicing factor mutants to be recruited to the stably expressed and inducible PEM gene can be monitored. We find that recruitment of splicing factors from nuclear storage/reassembly sites is likely a multistep process involving multiple protein domains. Using deletion mutants of SR proteins SF2/ASF, SRp40, SC35, and SRp20, we find that dissociation of splicing factors from nuclear speckles is mediated by the RS domain, whereas association of the protein with the target RNA involves the RNA-binding domains. Specifically, phosphorylation of the characteristic RS domain of SR proteins is required for efficient recruitment of splicing factors to transcription sites, and we suggest that phosphorylation of serine residues in the RS domain acts as a switch for the release of SR proteins from speckles. These findings indicate that protein phosphorylation coordinates transcription and pre-mRNA splicing in vivo. The data are consistent with the recent identification of kinases that specifically phosphorylate the RS domain of SR proteins and, by doing so, cause the release of splicing factors from splicing factor compartments (for a review, see Misteli and Spector 1997). Furthermore, we have previously demonstrated that a serine/threonine phosphatase I activity has the opposite effect, namely, the accumulation of splicing factors in nuclear domains. These findings allow the formulation of a working model in which the cyclic phosphorylation and dephosphorylation of SR protein splicing factors determine their nuclear localization and thus, the availability of splicing factors at sites of transcription. We are currently further investigating the molecular mechanisms of how the distribution of splicing factors is controlled in the mammalian cell nucleus by use of microscopy and molecular techniques.

Biochemical Characterization of Nuclear Splicing Factor Pools

A. Miner (Undergraduate Research Program),
T. Misteli, D.L. Spector

Fluorescence and electron microscopy studies from our lab and others have firmly established that splicing factors are distributed in at least two morphologically distinct pools in the cell nucleus: One fraction of splicing factors is in nuclear speckles and a second fraction appears diffusely distributed throughout the nucleoplasmic space. We have set out to determine the biochemical properties of these two pools and to possibly distinguish additional biochemical pools. To this end, we have developed a standardized growth and extraction procedure for cultured human fibroblasts. HeLa cells are grown in suspension and extracted with detergent and increasing concentrations of NaCl, the soluble and particulate fractions for each sample are recovered, the proteins are separated by electrophoresis, and the distribution of particular splicing factors is determined qualitatively and quantitatively by Western blotting using specific antibodies. We established that SR proteins, small nuclear ribonucleoproteins (snRNPs), and heterogeneous nuclear RNPs (hnRNPs) have distinct salt-extraction profiles, and we confirmed the presence of at least two nuclear fractions of SR proteins and snRNPs, in vivo. One fraction is soluble and most likely corresponds to the diffusely localized pool, and the second fraction is salt-resistant, representing the pool associated with nuclear speckles. In cells where transcription was inhibited by α-amanitin, a specific inhibitor of RNA polymerase II, all classes of splicing factors appeared to be more soluble, most likely due to the dissociation of splicing factors from nascent transcripts. From the observed shift, we estimated that at any given time, between 10% and 30% of total pre-mRNA splicing factors are associated with nascent transcripts. A similar increase in solubility was observed for SR protein SF2/ASF upon treatment of cells with inhibitors of protein phos-

phatases. This is consistent with the observation that SF2/ASF is heavily phosphorylated in vivo and that phosphorylation promotes its dissociation from splicing factor domains. In contrast, two less phosphorylated splicing factors, hnRNP A1 and U2-B´´, were not affected by the phosphatase treatment. These observations demonstrate that splicing factors exist in multiple, morphologically and biochemical distinct pools in the cell nucleus and that distinct classes of splicing factors have distinct biochemical properties. The developed approach will be valuable for further biochemical characterization of splicing factors in vivo.

Purification and Characterization of Interchromatin Granule Clusters

P. Mintz, D.L. Spector

Immunofluorescence studies using antibodies directed against pre-mRNA splicing factors have revealed a speckled pattern and diffuse nucleoplasmic labeling in the nucleus. It has been shown by electron microscopy that the speckled regions are composed of two structures: interchromatin granule clusters (IGCs) and perichromatin fibrils (PFs). The biochemical composition of these nuclear structures is not known. Immunocytochemical studies have shown that the IGCs are enriched with pre-mRNA splicing factors. Biochemical studies have shown that the IGCs are resistant to nuclease digestion, high salt, and detergent extractions. Although the IGCs have been shown to provide pre-mRNA splicing factors to sites of active transcription, it is unclear what other roles they may have in RNA metabolism. In addition, the architectural framework of the IGCs is not known. Therefore, it has become extremely important to isolate and biochemically characterize the IGCs in order to better understand their involvement in RNA metabolism.

We have developed a protocol to isolate the IGCs from mouse liver. Mice are treated with α-amanitin which inhibits transcription by specifically binding to the large subunit of RNA polymerase II. This drug was used in order to enrich for IGCs. Previous studies have shown that the IGCs conglomerate into a few large clusters when cultured cells or animals are treated with α-amanitin. Mouse liver nuclei were isolated using a sucrose gradient to separate the different organelles based on their density. The isolated nuclei were first treated with detergent to remove the inner and outer nuclear membrane. Next, the chromatin was digested with DNase I and extracted with high salt. After the DNase I and salt treatment, the residual nuclei contained the nuclear lamins and a few large clusters of IGCs as well as internal nuclear matrix elements. The IGCs were released from the residual nuclei by shearing through a needle. The homogenate was fractionated using a Cs_2SO_4 gradient. Samples were taken from all the stages in the protocol and examined by electron microscopy for purity and morphological characterization. The Cs_2SO_4 pellet contained mostly nuclear lamins and nuclear matrix elements, whereas the Cs_2SO_4 supernatant contained the IGCs. The purified interchromatin granules measured between 20 and 26-nm in diameter. Immunogold labeling was performed, with a monoclonal antibody that recognizes a family of SR proteins, on the final fraction (IGC fraction) to verify that it contained splicing factors. The immunogold-conjugated antibodies labeled the purified IGCs. We are currently performing biochemical analyses to identify known marker proteins in all of the fractions and to identify novel IGC-associated proteins.

Involvement of the Perinucleolar Compartment in Transcription

S. Huang, D.L. Spector [in collaboration with T.J. Deerinck and M.H. Ellisman, UCSD Microscopy and Imaging Resource]

The perinucleolar compartment (PNC) is a unique nuclear structure localized at the periphery of the nucleolus and is predominantly present in transformed cells. Several small RNAs transcribed by RNA polymerase III and two hnRNP proteins have been localized in the PNC (Getti et al., *Nucleic Acids Res. 20:* 3671 [1992]; Matera et al., *J. Cell Biol. 121:* 1181[1995]; Timchenko et al., *Nucleic Acids Res. 24:* 4407 [1996]). The PNC is a dynamic structure that dissociates at the beginning of mitosis and reforms early in G_1.

To resolve the three-dimensional organization of the PNC at high resolution, we examined PNCs in serial thin sections of optimally fixed HeLa cell nuclei by electron microscopy. The sections were not post-stained and the PNC was readily distinguishable from the nucleolus. Images acquired from the same nucleus were aligned and a three-dimensional model was reconstructed by using the programs SYNU and SynuRender (Fig. 1). The model reveals a reticulated mesh associated with a portion of the nucleolar surface. Variability in the shape, size, and association of the PNC with the nucleolus was observed. In some

cases, the PNC was predominantly positioned on the surface of the nucleolus (Fig. 1, top row), whereas in other cases, the PNC appeared to extend into the nucleolus like a plug (Fig. 1, middle row). Occasionally, the nucleolus itself extended into the PNC (Fig. 1, bottom row). The variability in the association between the PNC and the nucleolus may represent different conformations of a dynamic nuclear structure.

On the basis of the abundance of RNAs and RNA-binding proteins in the PNC, we were interested in examining whether the PNC is involved in transcription. In situ Br-UTP incorporation experiments were

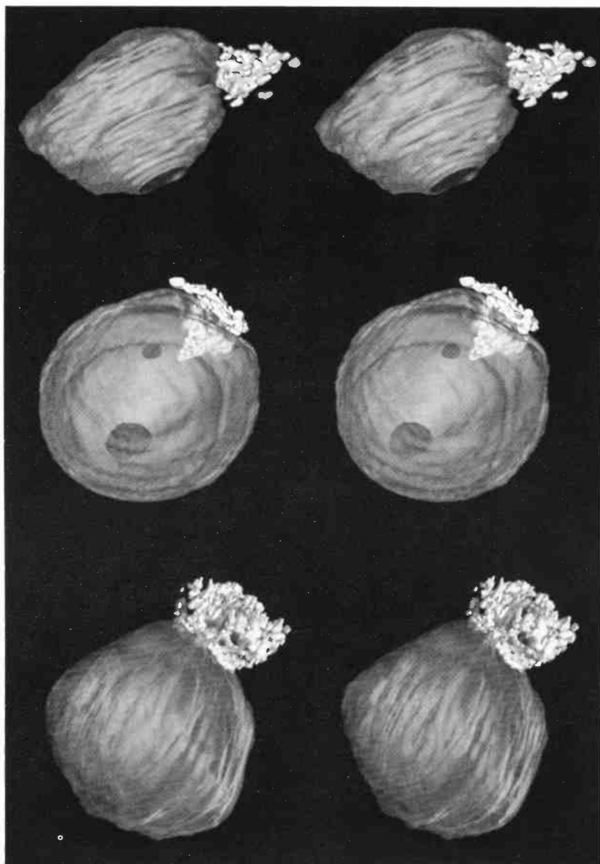

FIGURE 1 Stereopairs of three-dimensional models showing the association between the nucleolus (larger structure) and the perinucleolar compartment (smaller structure). Stereoglasses can be used to observe the three-dimensional organization of these structures.

performed to evaluate the transcriptional activity of the PNC. Simultaneous detection of transcription sites and the PNC demonstrated that the PNC coincides with sites of transcription. In fact, the Br-UTP incorporation was more prominent in the PNC than in the majority of the nucleoplasmic volume. The incorporation of Br-UTP is reduced when RNA polymerase II and/or III activity is inhibited, but it is not significantly affected when RNA polymerase I activity is inhibited. The structure of the PNC is altered upon the inhibition of RNA synthesis for 2–5 hours in cultured cells, suggesting that the integrity of the PNC is dependent on ongoing transcriptional activity. In addition, RNA polymerase II and pre-mRNA processing factors including splicing factors (snRNPs and SC35) and 3′-end processing factors (poly[A]-binding protein II), as well as poly(A)$^+$ RNA are detected in the PNC. Together, these findings support the idea that the PNC is involved in active transcription. Studies are currently under way to identify the RNAs synthesized in the PNC and the function of this nuclear compartment.

Nucleocytoplasmic Transport of Antisense Oligonucleotides

P. Lorenz, D.L. Spector [in collaboration with B.F. Baker and C.F. Bennett, ISIS Pharmaceuticals]

Antisense phosphorothioate oligonucleotides (PS-ONs) designed to specifically inhibit the expression of their target genes have recently entered clinical trials. However, it is still a matter of debate as to where within a cell the antisense activity is exerted. In an attempt to better define the cell biology of these compounds, we observed that the majority of PS-ONs localize to the cell nucleus under conditions of specific antisense activity. Their nuclear distribution consists of simple nuclear bodies set against a diffuse nucleoplasmic background (Lorenz et al., *Mol. Biol. Cell* [in press]). Although their steady-state distribution is in the nucleus, PS-ONs are not restricted to this compartment: We observed that these molecules underwent active nucleocytoplasmic transport, moving out of and into the nucleus. Thus, PS-ONs shuttle similar to such nuclear proteins as the hnRNP-A1 protein. The shuttling of PS-ONs was temperature-sensitive and energy-dependent and was abolished by addition of wheat germ agglutinin, an inhibitor of nuclear pore complex-mediated transport processes. As a test system, we used naturally occurring binucleate cells or heterokaryon fusions which were microinjected with fluorochrome-tagged PS-ONs. We were able to abolish the inhibitory effect of wheat germ agglutinin by coinjection of an excess of unlabeled PS-ONs, suggesting that we competitively inhibited the nuclear-pore-mediated active PS-ON transport. This provided evidence for a saturable, carrier-mediated transport process. An RCC1-negative mutant cell line did not export poly(A)$^+$ RNA but failed to show any influence on PS-ON shuttling. Inhibition of RNA polymerase II transcription also had no effect on shuttling. Therefore, it is not likely that mRNA export and PS-ON export are coupled. Export of a GFP-NES reporter protein was not inhibited by an excess of PS-ONs. On the other hand, a dominant-negative mutant form of the Ran protein involved in nucleocytoplasmic export, RanT24N, not only inhibited the export of GFP-NES, but also strongly decreased the nuclear export of PS-ONs. The kinetics of nucleocytoplasmic shuttling of PS-ONs were rather slow: Maximal accumulation of the oligonucleotides in the second nucleus in a binucleate cell was seen 2 hours after injection. Therefore, our observations suggest an indirect effect of the RanT24N mutant on PS-ON shuttling rather than a direct link to the Ran export pathways.

When an antisense PS-ON was injected into one nucleus of binucleate cells and a plasmid encoding the target gene into the other nucleus 2 hours later, the target gene expression was significantly reduced. This indicated that a shuttling PS-ON still had antisense activity. Taken together, our studies revealed the dynamic behavior of PS-ONs which must be taken into account when considering the sites of action of antisense oligonucleotides of this chemistry within the cell.

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INITIATION AND TERMINATION OF TRANSCRIPTION IN HUMAN snRNA GENES AND HIV-1

N. Hernandez T.L. Kuhlman, E. Ford, R.W. Henry, B. Ma, V. Mittal, D. J. Morrison, P.S. Pendergrast, L. M. Schramm, S. Sepehri, Y. Sun, M.-W. Wong

We are interested in basal mechanisms of transcription by RNA polymerases II and III, and we currently use two model systems: the human RNA polymerase II and III small nuclear RNA (snRNA) genes and the human immunodeficiency virus type 1 (HIV-1) promoter. Transcription from the human snRNA genes is unusual in several respects; in particular, the RNA polymerase II and III snRNA promoters are very similar to each other even though they are recognized by different RNA polymerases, and they are very different from the RNA polymerase II promoters of mRNA-encoding genes or the typical 5S or tRNA-type RNA polymerase III promoters.

To understand how RNA polymerase specificity is determined at snRNA promoters, we are identifying and characterizing the various factors that constitute the RNA polymerase II and III snRNA initiation complexes. The RNA polymerase II and III snRNA promoters both contain an enhancer with an octamer sequence and a proximal sequence element known as the PSE. The enhancers and PSEs can be exchanged without loss of RNA polymerase specificity. The RNA polymerase III snRNA promoters contain in addition a TATA box located around position –25, between the PSE and the transcription start site. The octamer within the enhancer recruits the broadly expressed transcription factor Oct-1, the TATA box recruits TBP, and the PSE recruits a multisubunit complex we call $SNAP_c$.

In the last year, we have obtained cDNAs for the last two subunits of $SNAP_c$, and we have been able to generate functional $SNAP_c$ from recombinant subunits. We are now pursuing two general lines of research: On the one hand, we are characterizing protein-protein and protein-DNA interactions within the RNA polymerase II and III snRNA initiation complexes. Our results so far reveal stabilizing protein-protein interactions between Oct-1 and $SNAP_c$ and, in an RNA polymerase III snRNA promoter, between $SNAP_c$ and TBP. Thus, each DNA-binding member of the RNA polymerase II and III snRNA initiation complexes stabilizes each other on the DNA. On the other hand, we want to identify the other players involved in RNA polymerase II and III snRNA gene transcription. To this end, we have pursued a collaboration with D. Reinberg and colleagues to test the role of the RNA polymerase II general transcription factors in snRNA gene transcription. We are also fractionating an in vitro transcription system to identify additional factors involved in RNA polymerase III snRNA gene transcription. These studies should help define the key protein-protein interactions that result in initiation complexes capable of recruiting specifically RNA polymerase II or RNA polymerase III.

Our interest in the HIV-1 model system is very focused and concerns the ability of the HIV-1 promoter to synthesize short, prematurely terminated, transcripts. The formation of these short transcripts is

dependent on a DNA element called the inducer of short transcripts or IST, which is located in large part downstream from the transcription start site. We do not know how the IST functions, but we imagine that it helps recruit an RNA polymerase II transcription complex that is incapable of efficient elongation, either because it is associated with a termination factor or because it lacks an elongation factor. To understand how the IST functions, we have identified, purified, and cloned a factor that binds specifically to the element. The next step is to try to demonstrate directly a role for this protein in the formation of short transcripts and to decipher its biochemical function.

Characterization of the SNAP190 Subunit of $SNAP_c$

M.-W. Wong, R.W. Henry, B. Ma, R. Kobayashi, N. Hernandez

Isolating a full-length cDNA clone encoding the largest subunit of $SNAP_c$, SNAP190, was the result of a collaboration with P. Matthias, from the Friedrich Miescher Institute in Basel, and N. Klages and M. Strubin, from the University of Geneva. We biochemically purified SNAP190 and obtained peptide sequences. From such sequence, we designed degenerate oligonucleotides, performed polymerase chain reactions (PCRs), and obtained a partial cDNA clone corresponding to a portion of the amino-terminal half of the protein. Because $SNAP_c$ binds cooperatively with Oct-1 to the DNA, we also checked whether any of the cDNAs obtained in a one-hybrid screen performed in yeast to isolate proteins interacting with octamer-bound Oct-1 might correspond to SNAP190. Indeed, two such cDNAs encoded several of the peptide sequences we had obtained from the purified protein. These cDNAs encoded the entire carboxy-terminal half of the protein. We then cloned the missing segments by a combination of PCR and library screening methods.

SNAP190 contains an unusual Myb DNA-binding domain consisting of four and a half, rather than the usual two or three, Myb repeats. The last two SNAP190 Myb repeats, Rc and Rd, bind specifically to the PSE, strongly suggesting that SNAP190 contacts the DNA within the SNAP complex. Depletion of transcription extracts with anti-SNAP190 antibodies inhibits both RNA polymerase II and III transcription of snRNA genes, and transcription can be reconstituted by the addition of purified SNAP complex. Together with previous results showing that the same is true for SNAP43, SNAP45, and SNAP50, these results suggest that the SNAP complex involved in RNA polymerase II and III transcription contains at least four common subunits.

SNAP190 could be coimmunoprecipitated with SNAP45, but not with SNAP43 or SNAP50. We had previously shown that SNAP43 and SNAP50 could be coimmunoprecipitated. Thus, at this point we were able to show interactions between SNAP43 and SNAP50, and between SNAP190 and SNAP45, but we could not assemble the two pairs of proteins into a complex. As described below, the mystery was solved with the cloning of yet another subunit of $SNAP_c$, SNAP19. SNAP190 could also interact with Oct-1 in a band shift assay, as expected from the results obtained in yeast. Thus, SNAP190 constitutes a key subunit of $SNAP_c$ that probably contacts the DNA and mediates direct protein-protein interactions with the transcriptional activator Oct-1.

Identification of the SNAP19 Subunit of $SNAP_c$

R.W. Henry, V. Mittal, B. Ma, R. Kobayashi, N. Hernandez

The observation that a complex containing SNAP43, SNAP45, SNAP50, and SNAP190 could not be assembled suggested that we might be missing an essential subunit. We have recently obtained a cDNA clone encoding SNAP19, the fifth subunit of $SNAP_c$. When all five $SNAP_c$ subunits are cotranslated, they can be coimmunoprecipitated as a complex. Indeed, a functional $SNAP_c$ can be assembled from recombinant subunits overexpressed in insect cells.

SNAP190 Domains Involved in Protein-Protein Interaction with Other Members of the SNAP Complex

B. Ma, N. Hernandez

To understand the architecture of the SNAP complex, we are defining which parts of SNAP190 interact with other members of the SNAP complex as determined by coimmunoprecipitation of in-vitro-translated proteins.

Cooperative Binding of TBP and $SNAP_c$ to the Human Basal U6 Promoter

V. Mittal, N. Hernandez

The basal U6 promoter consists of a PSE, which recruits $SNAP_c$, and a TATA box located at a fixed distance downstream from the PSE, which recruits TBP. TBP consists of two domains: A conserved carboxy-terminal domain, which performs all the functions of full-length TBP including binding to the TATA box, interacting with other basal transcription factors and activators, and directing basal RNA polymerase II transcription in vitro, and a nonconserved amino-terminal domain. The amino-terminal region contains a long run of glutamine residues and can thus be arbitrarily divided into three segments, I, II, and III, corresponding to the sequences preceding, corresponding to, and following the run of glutamine residues, respectively.

We found that $SNAP_c$ and TBP bind cooperatively to their respective sites in the U6 promoter. Thus, TBP is able to recruit $SNAP_c$ to a low-affinity PSE. Strikingly, this effect depends on the amino-terminal region of TBP, more precisely on segments I and II. In addition to its function in recruiting $SNAP_c$ to the PSE, the nonconserved amino-terminal domain of TBP performs another function: It down-regulates binding of TBP to the TATA box. Thus, full-length TBP binds poorly to the U6 TATA box, whereas the truncated protein missing the amino-terminal domain binds efficiently. This effect is dependent on segment I, and it is not limited to the U6 TATA box: It is also observed with the TATA box of the adenovirus 2 major late promoter, a typical RNA polymerase II promoter. This suggests that the amino-terminal domain of TBP negatively autoregulates the binding of TBP to DNA, perhaps to avoid binding of TBP to nonfunctional TATA boxes located outside of promoter regions.

Characterization of the Cooperative Binding of Oct-1 and $SNAP_c$ to the DNA

E. Ford, N. Hernandez

The POU domain of the transcription factor Oct-1, but not the transcription factor Pit-1, can recruit $SNAP_c$ to the PSE and activate snRNA gene transcription in vitro. We are interested in determining which subunit of $SNAP_c$ is contacted by Oct-1 POU. Our previous efforts to demonstrate protein-protein interactions between Oct-1 and SNAP43, SNAP45, and SNAP50 were unsuccessful. The identification of SNAP190 as an Oct-1 interacting protein in the yeast one-hybrid screen opened the door for the detailed analysis of the region of interaction. We are mapping and characterizing this region using a band-shift assay and, in collaboration with M. Strubin, the yeast one-hybrid assay. We expect that these studies will provide us with one of the best-characterized protein-protein interactions between an activator and the basal transcription machinery.

Characterization of Human RNA Polymerase III

S. Sepehri, N. Hernandez

We are interested in identifying additional factors required for RNA polymerase III transcription of snRNA genes, and one of the approaches we are pursuing is fractionation of transcription extracts. A problem we encountered is the lack of antibodies directed against human RNA polymerase III. To obtain such antibodies, and to characterize the human enzyme, we isolated cDNA clones encoding the largest subunit of human RNA polymerase III. The sequence revealed a polypeptide with 50% and 40% identity to the yeast and *T. brucei* homologs, respectively. The polypeptide is also 29–34% and 22–27% identical to the largest subunit of RNA polymerase II and RNA polymerase I, respectively, from various species. The availability of the cDNA allowed us to raise anti-RNA polymerase III antibodies, which we are now using to follow the enzyme during fractionation and to identify RNA polymerase-III-associated polypeptides.

Factors Required for snRNA Transcription by RNA Polymerase II

T.L. Kuhlman, N. Hernandez

The RNA polymerase II snRNA promoters require the SNAP complex for transcription, but little is known about which other factors are required. We are contin-

uing our collaboration with Dr. D. Reinberg and colleagues, who have purified all, and cloned most, of the factors required for basal transcription from the adenovirus 2 major late promoter, a typical RNA polymerase II mRNA promoter, to determine which of the general transcription factors are required for transcription from RNA polymerase II snRNA promoters.

Isolation of cDNAs Corresponding to FBI-1

D.J. Morrison, P.S. Pendergrast, R. Kobayashi, N. Hernandez

We have previously identified and purified a cellular factor whose binding to wild-type and mutant versions of the HIV-1 IST correlates with the ability of these various ISTs to direct the formation of short transcripts in vivo. We have obtained cDNAs encoding this factor, which we refer to as FBI-1, as well as anti-FBI-1 antibodies, and are characterizing its properties.

Function of FBI-1

P.S. Pendergrast, N. Hernandez

As a first step toward understanding the biochemical function of FBI-1, we are testing the effects of overexpressing the protein in transfected cells. We are also pursuing localization studies and identifying factors that interact with FBI-1.

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TELOMERASE BIOCHEMISTRY AND REGULATION

C.W. Greider C. Autexier, S. Buck, M. A. Blasco, K. Buchkovich, J. Hemish, A. Kass-Eisler, S. Le, D. Mason, B.-K. Oh, M. Rudd, S.K. Smith, H. Yang

Telomerase is a specialized reverse transcriptase that synthesizes telomere repeats onto chromosome ends. The enzyme contains an essential RNA component that provides the template for the simple telomere repeats that are synthesized. In humans, telomerase is activated in a large number of cancers, and evidence from tissue culture suggests that telomerase activation may be required for the growth of cancer cells. In the past year, we have continued the biochemical characterization of the *Tetrahymena* telomerase which we began more than 10 years ago. We characterized a large number of mutations in the RNA component and defined regions of the RNA that are essential for telomerase activity.

In addition, we have continued our analysis of a knockout mouse that lacks telomerase activity and shows telomere shortening. Finally, we have begun to explore the recombinational pathways that can contribute to telomere elongation when telomerase is absent.

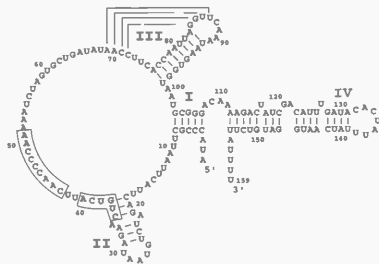

FIGURE 1 Functional dissection of the telomerase RNA. Diagram of the secondary structure of the *Tetrahymena* telomerase RNA (Romero and Blackburn, *Cell 67:* 343 [1991]). The telomerase RNA sequence is shown with functional domains, including the template and alignment regions 5′-CAACCCCAA-3′ (residues 43–51; *open* box) and the upstream conserved region 5′-(CU)GUCA-3′ (residues 35–40; shaded box), which regulates the 5′ boundary of the template.

TABLE 1 Mutational Analysis of *Tetrahymena* Telomerase RNA

Mutant RNAs[a]	Structure or sequence targeted by mutation	Specific nucleotide changes	% Telomerase activity[b]
Wild-type	none	none	100
sub4-8	stem I	CCCGC to AAACA	36 ± 18
Δ5′8 (Δstem I)	stem I	none	<10[c]
Δ5′11	stem I	none	19 ± 5
Δ5′15	stem I	none	0
sub15–16	nucleotides 15–16	CA to GU	38 ± 8
Δ5′19	stem I	none	0
Δ20–36 (Δstem II)	stem II	none	25 ± 23
Δ5′25	stem I–II	none	0
sub26–29	stem II	GUAA to CAUU	141 ± 53
Δ5′36	stem I–II	none	0
Δ37–40	conserved region (stem II)	none	13 ± 12
sub69-72 (subψ)	pseudoknot (ψ)	AACC to UUGG	53 ± 27
Δt75 (Δstem III-IV)	stem III–IV	none	<10
Δ76–99 (Δstem III)	stem III	none	64 ± 42
sub84–87 (subψ)	pseudoknot (ψ)	GGUU to CCAA	124 ± 48
sub89–90	stem III loop	AA to UU	115 ± 47
Δ103–107 (Δstem I)	stem I	none	34 ± 8
Δt111 (Δstem IV)	stem IV	none	<10
sub121–122	stem IV (GA bulge)	GA to CU	93 ± 36
sub133–136	stem IV loop	ACUA to UGAU	14 ± 11
t138	stem IV	none	<10
t146	stem IV	none	<10

[a] Nomenclature given to the mutant *Tetrahymena* telomerase RNAs: For example, Δ5′8 is a deletion of residues 1-8 from the 5′ end of the RNA. sub = substitution, t=truncation.

[b] Telomerase activity of *Tetrahymena* telomerase reconstituted with the mutant RNAs, relative to reconstitution with wild-type RNA, indicated as a percentage with standard deviation and the number of times assayed and quantitated in parentheses.

[c] For some mutants with weak activity, signal intensities are low. After substraction of background, values were often zero. However, because these mutants generated telomerase repeats, so activity was scored less than 10%.

Functional Components of the *Tetrahymena* Telomerase RNA

C. Autexier

The strong conservation of telomerase RNA secondary structure from a diverse group of ciliates suggests that the RNA secondary and tertiary structure is important for enzyme function (Fig. 1). To test what regions, besides the template, are required for in vitro telomere repeat synthesis, we made mutations in each of the four stem loop structures and a number of the other structural features such as the pseudoknot in stem III. We also tested the sequence requirements in regions of the RNA that had been predicted to bind protein based on chemical mutagenesis (Table 1). Deletion of more than 13 residues from the 5′ end of the RNA abolished telomerase activity, suggesting that this region upstream of the template has an essential function in the elongation reaction. Surprisingly, deletion of individual stems and alteration in the sequence of the loop regions did not abolish the activity of the reconstituted enzyme. All ciliate telomerase RNAs have a predicted long-range-based pairing that brings the 5′ and 3′ regions of the RNA together. Deletion of these residue or sequence alterations on both sides of the stem still allowed telomerase function. The pseudoknot formed with stem III is also a conserved feature of all ciliate RNAs and has been proposed to play a part in the translocation reaction during elongation. We found that two different mutations which abolished the base pairing required for pseudoknot formation were as active or more active than wild-type RNA in the in vitro reaction. The fact that highly conserved features of telomerase RNA appear to not be essential for the in vitro telomere synthesis reaction suggests that these regions are important for carrying out functions in vivo that are not assayed in the in vitro reaction. For example, there is evidence that the pseudoknot region may be involved in RNP assembly in vivo. These findings are particularly interesting in light of evidence from yeast and humans indicating that some proteins associated with telomerase activity are not essential for in vitro enzyme activity.

Telomerase Protein Components

J. Hemish, C. Autexier, H. Yang, D. Mason,
M. Rudd, C. Greider

To study the telomerase enzyme in vitro, we are establishing a fully recombinant telomerase enzyme. In past years, we described the isolation and characterization of two telomerase proteins components, p80 and p95. Because *Tetrahymena* has an unusual genetic code, to express recombinant proteins in *E. coli*, we needed to change the coding sequence to eliminate internal stop codons. As an alternative to multiple rounds of site-directed mutagenesis, and to ensure high-level expression, we reconstructed the two genes using synthetic DNA oligonucleotides and overlap extension polymerase chain reaction (PCR). First, the *Tetrahymena* p95 and p80 protein sequences were reverse-translated using selected codons that would allow high-level protein expression. Pairs of 100-nucleotide primers were designed to span the entire sequence of both the p95 and p80 genes. Each pair of primers overlapped by 20 bases and was used in recursive overlap extension PCR to reconstruct the entire gene. The resulting gene fragments were then sequenced and assembled together, and each gene was cloned into an expression vector for protein production.

In addition to p80 and p95, recent experiments from *Euplotes* and *Saccharomyces cerevisiae* indicated that telomerase contains a component that has homology with reverse transcriptase domains (Lingner et al., *Science 276:* 561 [1997]). Homologs to this protein, TERT, have been isolated from humans, mice, and *Schizosaccharomyces pombe*, and the high degree of sequence conservation suggested that this protein is conserved in eukaryotes. Using degenerate PCR, we have cloned a gene from *Tetrahymena* that contains protein domains which suggest that it is a TERT homolog. Expression of this protein along with the p80 and p95 will allow us to test the role of the various telomerase protein components in a fully recombinant system.

Generation of a Telomerase Null Mouse

M. Blasco [in collaboration with H.-W. Le and R.A. DePinho, Albert Einstein college of Medicine, and M.P. Hande and P. Lansdorp, University of British Columbia]

To examine the role of telomerase in normal and neoplastic growth, the telomerase RNA component (mTR) was deleted from the mouse germ line. $mTR^{-/-}$ mice lacked detectable telomerase activity, and telomeres shortened progressively with each mouse generation. The first generation of mouse that lacked telomerase was designated $mTR^{-/-}$ G_1; mating of two

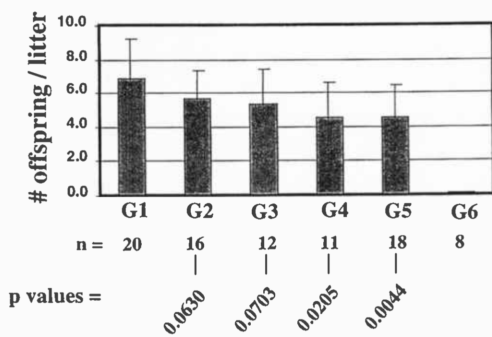

FIGURE 2 Progressive decrease in litter size with increased generations in the absence of telomerase. The number of offspring generated from crosses of each generation of cells of $mTR^{-/-}$ mice are shown. The generation of mice crossed is shown on the *x*-axis. The *p* values for statistical significance in the difference in litter size relative to wild-type mice are shown below each bar.

$mTR^{-/-}$ G_1 animals produced $mTR^{-/-}$ G_2 and additional generations were produced in a similar manner. We initially analyzed mice from G_1 through G_6 for telomere length and chromosomal abnormalities. Quantitative fluorescence in situ hybridization (Q-FISH) showed that telomeres shorten at a rate of 4.8 ± 2.4 kb per mouse generation. Metaphase analysis of $mTR^{-/-}$ cells showed chromosomal abnormalities, and aneuploidy was frequently found in later-generation mice. In late-generation animals, chromosome ends lacking detectable telomere repeats and frequent end-to-end fusions indicated that telomere function was lost. Thus, telomerase is essential for telomere length maintenance in the mouse, and loss of telomere function leads to chromosome instability as was predicted from the work of McClintock and Muller more than 50 years ago.

Telomerase Is Essential for Long Cell Term Viability

M. Blasco [in collaboration with H.-W. Lee and R. A. DePinho, Albert Einstein College of Medicine]

Early tumor formation assays using the $mTR^{-/-}$ cells indicated that telomerase is not essential in mouse cells for tumor formation. However, in vivo analysis suggests that lack of telomerase does appear to limit the growth of cells after many generations, and thus may reduce the probability of tumor formation when telomeres are very short. Phenotypic analysis of $mTR^{-/-}$ mice indicates that highly proliferative organ systems require telomerase. Although we were able to generate six generations of $mTR^{-/-}$ mice, there was a progressive decline in litter size with each generation of mouse that lacked telomerase, and, to date, no seventh generation of mouse has been produced (Fig. 2). Histology of the testes in sixth-generation $mTR^{-/-}$ mice showed a lack of germ cells, although the support tissue appeared to be normal. Strikingly, with increased generation of $mTR^{-/-}$ mouse, there was an increased number of cells in the testis that stained positive in a TUNEL assay, indicating that cells with shortened telomere are induced to undergo apoptosis.

Increased apoptosis was also seen in lymphocytes. T cells were stimulated to proliferate using a variety of stimuli and the incorporation of [^{3}H]thymidine was assayed. $mTR^{-/-}$ G_5 and G_6 mice showed a reduction in [^{3}H]thymidine incorporation, yet the cell cycle distribution of these populations determined by FACS analysis was similar to that of wild type. Staining with propidium iodide and anexin revealed that after mitogenic stimulation, cells underwent apoptosis. This suggests that the shortened telomeres in late-generation $mTR^{-/-}$ animals may signal a DNA damage pathway and induction of apoptosis. Finally, we also looked at the growth potential of bone marrow stem cells in $mTR^{-/-}$ G_5 and G_6 mice and found that there was a significant decrease in several different stem cell types compared to wild type. This suggests that the highly proliferative stem cells may be the first to be effected due to telomere shortening.

The apoptosis associated with late-generation animals in several different tissues suggests that telomerase inhibition may cause cell death when telomeres are very short. Since many human tumors have very short telomeres, telomerase inhibition may be useful in limiting the growth of tumor cells, as we proposed 7 years ago. However, the fact that cells from the $mTR^{-/-}$ G_6 mice can form some tumors suggests that in cells where telomerase is inactivated, selection of cells which have activated an alternative pathway for telomere maintenance may occur. In yeast, recombination pathways can allow telomere elongation and cell division in the absence of telomerase. Thus, understanding the alternative pathways that might be activated for in telomere maintenance in mice will be important to determine whether telomerase inhibition will be useful in cancer therapy.

Role of Recombination in Telomere Maintenance in Yeast

S. Le, C. Greider

To examine the detailed mechanism of how recombination might allow telomere maintenance, we have initiated studies in both mice and yeast. In yeast, a recombination-mediated bypass pathway has been documented which can elongate telomeres in the absence of telomerase (Lundblad and Blackburn, *Cell 73:* 347 [1993]). When the essential telomerase RNA component, *TLC1*, is deleted in yeast, telomeres shorten and most cells die. However, gene conversion mediated by *RAD52* allows telomere lengthening in some cells and these cells survive. To investigate further the role of recombination in telomere maintenance, we assayed telomere length and the ability to generate survivors in several DNA recombination mutants, including *rad50*, *rad51*, *rad52*, *rad54*, *rad57*, *xrs2*, and *mre11*. Mutations in three different genes in the *RAD52* epistasis group, *rad50*, *xrs2*, and *mre11*, resulted in significant telomere shortening. Strikingly, *rad51*, *rad52*, *rad54*, and *rad57* mutations, but not *rad50*, *xrs2*, *mre11*, increased the rate of cell death in the absence of *TLC1*. However, only the *rad52* mutants completely blocked the generation of *tlc1* survivors. Our results suggest that DNA recombination may have a role in telomere maintenance both in the presence and in the absence of telomerase in yeast.

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HIGHER-ORDER CHROMOSOME DYNAMICS

T. Hirano O. Bowes (Eton School)
M. Hirano
K. Kimura
A. Losada

Our laboratory is interested in understanding the molecular mechanisms that regulate higher-order chromosome dynamics. Our approach is a biochemical one. We use a cell-free extract derived from *Xenopus laevis* (African toad) eggs in which higher-order chromosome structures can be reconstituted in vitro in a cell-cycle-dependent manner. Using this system, we previously identified protein complexes (termed condensins) that have a central role in mitotic chromosome condensation in vitro. *Xenopus* egg extracts contain two major forms of condensins with sedimentation coefficients of 8S and 13S. The two forms share two common subunits, XCAP-C and XCAP-E, that belong to an expanding family of chromosomal ATPases, the structural maintenance of chromosome (SMC) family. Recent data from other laboratories suggest that SMC proteins are involved in a wide variety of chromosome dynamics including dosage compensation and recombinational DNA repair. Very little is known, however, about the molecular mechanisms of action of this new class of proteins. During the past year, we have purified 13S condensin from *Xenopus* egg extracts and identified a novel ATP-dependent activity that induces structural changes of DNA. We have also identified a second class of SMC protein complexes (termed cohesins) in *Xenopus* egg extracts that appear to be involved in sister chromatid cohesion. Finally, we have begun biochemical characteri-

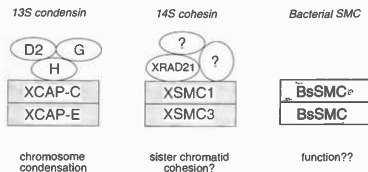

FIGURE 1 SMC protein complexes from *Xenopus laevis* and *Bacillus subtilis*. (*Left*) 13S condensin has a central role in mitotic chromosome condensation and consists of two SMC core subunits (XCAP-C and -E) and three non-SMC regulatory subunits (XCAP-D2, -G, and -H). (*Center*) 14S cohesin appears to be required for sister chromatid cohesion and contains two SMC subunits distinct from XCAP-C or XCAP-E. (*Right*) An SMC from the gram-positive bacterium *B. subtilis* forms a homodimer whose cellular function is currently unknown.

zation of a bacterial SMC protein in order to understand the "basics" of this remarkable class of proteins.

Biochemistry of Condensins

K. Kimura, T. Hirano

13S condensin is a 5-subunit protein complex consisting of two SMC (XCAP-C and -E) and three non-SMC (XCAP-D2, -G, and -H) subunits (Fig. 1). Since all SMC proteins share a nucleotide-binding motif in their amino-terminal domains, we hypothesized previously that 13S condensin displays an ATP-dependent activity that modulates higher-order chromatin structure. During the past year, we have purified 13S condensin from *Xenopus* egg mitotic extracts by immunoaffinity column chromatography and demonstrated that 13S condensin has a DNA-stimulated ATPase activity. More strikingly, 13S condensin is able to introduce positive supercoils into a closed circular DNA in the presence of topoisomerase I. The supercoiling reaction is ATP-dependent and requires ATP hydrolysis. It should be emphasized that this is a novel activity that has never been reported in the literature. We speculate that 13S condensin wraps DNA in a right-handed direction or overwinds the DNA helix by utilizing the energy of ATP hydrolysis. The energy-dependent introduction of superhelical tension of DNA might represent a key mechanism underlying the compaction of chromatin fibers during mitosis.

Cell Cycle Regulation of Condensins

K. Kimura, M. Hirano, T. Hirano [in collaboration with R. Kobayashi, Cold Spring Harbor Laboratory]

How is the function of 13S condensin regulated during the cell cycle? In the cell-free extracts, chromosomal targeting of 13S condensin is mitosis-specific and correlates with mitosis-specific hyperphosphorylation of the non-SMC "regulatory" subunits. We have found that the supercoiling activity of purified 13S condensin is mitosis-specific and phosphorylation-dependent. Unlike 13S condensin purified from mitotic extracts, the interphase form of 13S condensin exhibits little supercoiling activity. Treatment of the mitotic form with λ phosphatase abolishes its activity. Conversely, purified cdc2/cyclin B is able to phosphorylate two of the regulatory subunits of 13S condensin, XCAP-D2 and XCAP-H, and to convert the interphase inactive complex into an active one. XCAP-D2 is a frontier protein highly conserved from yeast to humans and contains a cluster of cdc2 phosphorylation sites in its carboxy-terminal domain. We are attempting to show that these sites are indeed phosphorylated by cdc2. If it turns out to be the case, our results will provide the first direct link between the major mitotic kinase and the key machinery of mitotic chromosome condensation.

Cohesins: The Second Class of SMC Protein Complexes

A. Losada, M. Hirano, T. Hirano

Eukaryotic SMC proteins can be classified into four subfamilies (SMC1 to SM4 types). XCAP-C and XCAP-E, the two core subunits of 13S condensin, correspond to the SMC4 and SMC2 types, respectively. Then what is the role of the SMC1 and SMC3 types? To address this question, we cloned cDNAs encoding the *Xenopus* SMC1- and SMC3-type proteins (termed XSMC1 and XSMC3, respectively) and prepared specific antibodies against them. During the past year, we have found that XSMC1 and XSMC3 associate with each other, forming large protein complexes with sedimentation coefficients of 9S and 14S. The 9S form is likely to be a heterodimer of XSMC1 and XSMC3, whereas the 14S form contains three additional subunits. In the cell-free extracts, the 14S

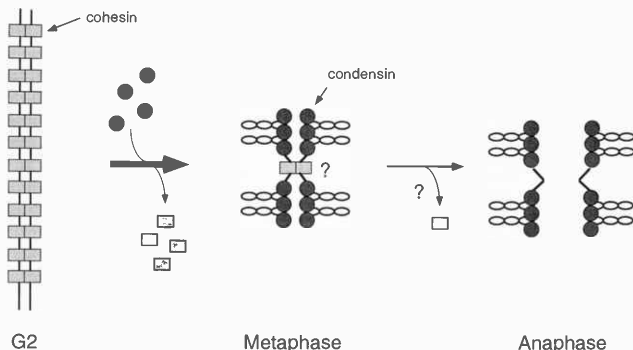

FIGURE 2 Roles of eukaryotic SMC protein complexes in higher-order chromosome dynamics. Cohesins (shown by the rectangles) mediate sister chromatid cohesion after DNA replication. At the onset of mitosis, the majority of cohesin dissociates from chromatin, leading to reorganization of cohesion. Condensins (shown by the circles) bind to chromatin and drive its condensation by changing the topological states of chromatin fibers. At the metaphase–anaphase transition, the linkage of sister chromatids is dissolved and they start to segregate. It remains to be determined how cohesins behave during this stage of the cell cycle.

form associates with interphase chromatin and dissociates from it at the onset of mitosis. Immunodepletion of the complexes from extracts produces a defect in sister chromatid cohesion, but not in chromosome condensation. Consistently, recent genetic studies in yeast from other laboratories have shown that Smc1p and Smc3p might be directly involved in sister chromatid cohesion. We therefore propose to refer to these complexes as cohesins (9S cohesin and 14S cohesin). Despite their striking similarities in structural organization, condensins and cohesins share no common subunits and have no overlapping functions. Thus, our results provide a simple biochemical picture in which the two distinct classes of SMC protein complexes, cohesins and condensins, regulate sister chromatid cohesion and chromosome condensation, respectively, thereby contributing to faithful segregation of chromosomes in eukaryotic cells (Fig. 2).

Biochemistry of a Bacterial SMC Protein

M. Hirano, T. Hirano

Recent progress in the genome sequencing projects has revealed that SMC proteins are highly conserved in the three branches of life, Bacteria, Archaea, and Eukarya. Virtually nothing is known, however, about the cellular and biochemical functions of noneukaryotic SMC proteins. Since the bacterial and archaeal genomes fully sequenced so far each contains a single SMC gene, noneukaryotic SMC proteins are likely to form homodimers. During the past year, we have cloned an SMC gene from the gram-positive bacterium *Bacillus subtilis* and expressed it in *Escherichia coli*. The recombinant SMC protein exhibits biochemical properties consistent with the homodimer model. The native SMC protein purified from *B. subtilis* is also found to be a simple homodimer containing no other subunits. We are now characterizing the biochemical activities of this "primitive" SMC and hoping to uncover the basic mechanism of action of this novel class of proteins. The recombinant SMC protein will be useful for detailed mutational analyses as well as protein crystallization. We also anticipate that this approach should provide us with fundamental insights into the evolutionary aspects of chromosome architecture and dynamics.

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STRUCTURE AND GENOMICS

Scientists in this section address a range of biological questions with a common thread of determining the correlation between structure and function in individual biomolecules and in their interactions with one another. Many of the scientists in this group work on several of the classical research areas: cancer biology, plant biology, and neurobiology. Xu Cheng (who left the Lab in the past year), Leemor-Joshua Tor, and Rui-Ming Xu use X-ray crystallography and related techniques to understand protein structure and protein–nucleic acid interactions during transcription and DNA replication. Rujyi Kobayashi and Peter Nestler use protein chemistry techniques and combinatorial chemistry, respectively, to study protein structure and protein-protein interactions. Andy Neuwald (who joined the Lab in 1997) uses computational methods to study the relationship between protein structure and function. Michael Zhang, who has been using statistical analysis of DNA sequence to develop methods to predict coding regions in genomic sequence, has expanded his efforts in order to predict regulatory signals as well. Dick McCombie's studies include determining the structure of large genomic regions using computational tools to analyze the biological function of these regions.

Two of the more experienced scientists in this group have left in the past year, Tom Marr and Xu Cheng. Despite this loss, the addition of several computational biologists in the past year and the infusion of new ideas and new technology have expanded the role this group plays in the intellectual life of the Lab, as witnessed by their many productive collaborations with other groups here at Cold Spring Harbor Laboratory.

SEQUENCE-BASED ANALYSIS OF COMPLEX GENOMES

W.R. McCombie N. Dedhia, L. Parnell, L. Gnoj, M. de la Bastide, K. Schutz, E. Huang, K. Habermann, T. Gottesman, A. Hameed, K. Jensen, N. Kaplan, M. Lodhi, N. Shohdy, A.F. Johnson, A. Hasegawa, S. Grant

The goal of genomics research is to use the latest automated techniques to determine initially the structure, but ultimately the function of complex eukaryotic genomes. We have made major advances in our ability to do this in the past year. During this time period, we have significantly enhanced our sequencing capability. This has resulted from an increase in size, from the development of new software for sample tracking and project management, and from increasing the efficiency of our laboratory operations. This increase in sequencing has been dramatic. In the years between 1992 and 1996, we placed slightly less than 200 kb of finished sequence in GenBank. From 1997 to early 1998, we completed and submitted to GenBank more than 1,000,000 bases. This increase in our capabilities has opened new possibilities for our work.

We have used this capability to advance several major projects in the past year: (1) genome sequencing of *Arabidopsis thaliana* in collaboration with Rob Martienssen here at the Laboratory and groups at the Washington University Genome Sequencing Center and Applied Biosystems; (2) cancer genetics, in which we sequenced regions of the human genome found to be deleted in human cancer (in collaboration with Mike Wigler); (3) sequencing sites of AcDs transposon insertions in the *Arabidopsis* genome in collaboration with Rob Martienssen; and (4) sequencing maize expressed sequence tags and probes used for maize mapping in collaboration with Rob Martienssen. All of these projects are linked by a common intellectual focus which is to understand the structure and/or function of large genetic regions or whole genomes. Because of the limitations of space, this report focuses only on three aspects of our lab's work in the past year: the development of new software tools to monitor and control these efforts, *Arabidopsis* genome

sequencing, and cancer genetics. The *Arabidopsis* transposon project is described in Rob Martienssen's section of this Annual Report.

ARABIDOPSIS GENOME SEQUENCING

The overall goal of this project, done as a consortium with groups led by Rick Wilson and Bob Waterston and Washington University (WUGSC) and Ellson Chen at Applied Biosystems (ABI), is to map and sequence 6.5–7 Mb of *Arabidopsis* chromosomes IV and V. *Arabidopsis* is the most important plant model organism and is extensively studied around the world. Our group's efforts are part of a larger international project to sequence the entire 100-Mb genome of this organism by the early part of the next century. The project started in October of 1996. Our plan called for sequencing and mapping to begin immediately and for sequencing to accelerate as more mapped clones became available.

MAPPING [in collaboration with R. Martienssen, M. Marra, J. McPherson, Washington University]

In the initial phase of the project, we needed to build sequence-ready bacterial artifitial chromosome (BAC) contigs in three regions of the genome: (1) the top arm of chromosome IV (YAC contig I), (2) a region from the bottom arm of chromosome IV (YAC contig II), and (3) a region from the top arm of chromosome V (YAC contig VII).

It became clear to us very early in the project that our initial mapping strategy, based on that of the European *Arabidopsis* sequencing group, was both technically very challenging and prone to error and that it had been superseded by far superior technology developed by our collaborators at the WUGSC. This latter technology enables an entire genome-wide map to be made, rather than focusing on a few areas. As a result, it was decided to use this new technology, and we have made considerable progress in mapping these areas by taking advantage of the BAC fingerprints generated by Marco Marra at Washington University. For details and data, see

http://genome.wustl.edu/gsc/arab/arabidopsis.html

These contigs have been mapped to our target sequencing regions by anchoring individual BACs with hybridization to mapped probes (cDNA, phage, SSLP, and YACs have all been used). This work was done in our lab at CSHL and at the WUGSC. These contigs have been built up to cover almost all of the regions to which we have been assigned. We have made our fingerprint data accessible in a searchable form, allowing many users to build BAC contigs using these data. The combination of fingerprinting and BAC end sequencing has proven to be enormously powerful for the community as a whole as well as for our project and should serve as an example for future large-scale genome sequencing projects.

As a result, we now have a single BAC contig from the nucleolar organizer (rDNA array) at the telomere of chromosome IV to the end of YAC CIC8B1. It spans about 20 cM, and about 2.5 Mb. This contig has been confirmed by hybridization to eight different YACs and six different genes or cDNA clones that had previously been placed on the genetic map. All 25 BACs in the minimal tiling path are currently either completed or being processed for sequencing. In addition, a 180-kb contig from the YAC CIC5B11 has been completed by Ellson Chen and colleagues at ABI. This contig spans the junction between the chromosome arm and the rDNA repeats at the telomeric end of our assigned region. It is currently being annotated by our group at CSHL and will provide the first view of such a junction from a plant genome. We have additional contigs that cover our target sequencing area. These include a contig that extends from the centromeric repeats on the long arm of chromosome IV to the region being sequenced by our European collegues.

SEQUENCING [in collaboration with R. Martienssen, R.K. Wilson, E.Y. Chen, R. Waterson]

Since our last annual report, our lab has sequenced and submitted to GenBank 731,570 base pairs of the *Arabidopsis* genome. This was composed of a total of 7 BAC clones. Our web page shows our current status at any given time and can be accessed at

www.cshl.org/arabweb/detailedsummary.htm

Our consortium has sequenced 2.03 Mb during this same time.

We are currently approaching the heterochromatic structures near the centromere. Some of our clones may be in the centromere or at least in the area of pericentromeric repeats. These have been difficult to finish due to their highly repetitive structure. We are providing help to the members of the community who study centromeres, and they are reciprocating by providing us data and valuable insights on the centromere's structure and position.

Perhaps most exciting, we have one of the first

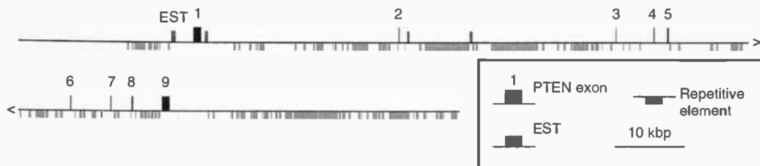

FIGURE 1 Shown is a schematic of the genomic region surrounding the PTEN gene. The region depicted here covers over 150 kbp. The PTEN gene is composed of nine exons that span over 100 kbp. Each exon is depicted by a tall dark box and identified by its corresponding number. Within this region were found four other ESTs that could potentially be incorporated into an alternately spliced version of the PTEN mRNA. Of particular note is the EST 5′ to exon 1 as this EST may represent the 5′ region of PTEN. *Alu*, LINE, LTR, and other genomic repeat elements are noted by small shaded regions beneath. The region near the cofilin-like gene has not yet been analyzed for repetitive elements.

sequence-ready contigs from a plant chromosome arm, from telomere to centromere. The functional centromere region is being defined by other investigators using complementary approaches. This will be a particularly important region to sequence. We have already sequenced the junction between the rDNA and the euchromatin, which may contain a region that controls nucleolar dominance. Several interesting cytogenetic features, including recombination hot and cold spots, chromomeres, and so on, are found in this region. The sequence data will provide a wealth of information on the organization, function, and structure of plant chromosomes. All of the clones from this area are now either sequenced or in the production queue.

In addition to sequencing this area, we have had a major effort in annotating this sequence. The WUGSC group carried out some automatic annotation on regions they sequence. Our group at CSHL carries out annotation on all of the sequences produced at ABI and CSHL, as well as adds additional annotation to the sequence from the WUGSC. The development of these Web sites is described in more detail below, in the software development section.

Even in the early phases of this project, several interesting observations have been made and a summary of some of them would be useful. In addition to structural information, of course, we have discovered more than 250 genes. They include only three genes of previously assigned genetic function (*PROLIFERA*, *LUMINIDEPENDENS*, and *GA1*). Homologs of many important genes have been identified, though, including numerous receptor kinases, a cluster of disease resistance genes, and many transcription factors and signaling components. Very interestingly, several new families of transposons have been discovered, including the MuDR autonomous transposon (Robertson's *Mutator*) that has proved to be so useful in maize. These genes are listed at

http://www.cshl.org/arabweb/GenesTransposons-table-980106.html

CANCER GENETICS SEQUENCING [In collaboration with M. Wigler, R. Lucito, M. Hamaguchi, R. Parsons, Columbia University]

Our efforts in this area have been focused on two targets. One is from chromosome 10. This region was found to contain a gene designated as PTEN (Fig. 1) that contains a tumor suppressor. We have been sequencing two BACs from this region. These clones covered the minimal deletion identified by the Wigler lab from this region. The sequencing of this region is complete. The sequence is composed of about 200 kb, which include about 108,000 bases encoding the PTEN gene and about 92,000 bases of flanking sequence. Sequence analysis indicates the possibility of other genes, in addition to PTEN, being encoded in this region. It is possible that these genes have a role in the properties of tumor cells deleted in this area.

We have also sequenced two other regions identified as being deleted in tumor cells by Rob Lucito of the Wigler lab. One of the sequences, which is completed, contains about 25,663 bases. No genes identified by computational analysis of the sequencing can be readily correlated with the malignant phenotype. In the second area, we have completed the sequence of a 140,000-bp region of the deletion area. The remainder of the deletion is composed of a clone of about 40,000 bases that is in the finishing stage of sequencing and is currently in four contigs. The sequences are being analyzed for gene content. Interestingly, the larger, 108,932-base clone contains several highly repetitive

regions. These tandem repeats are different from each other and are composed of between 16 and 70 bp and exist in 4 to 73 copies. The sequences of the unit repeats vary from each other by only a few bases. None of these repeat units are found elsewhere in the sequenced regions of the human gene.

SOFTWARE AND ANNOTATION

To make the *Arabidopsis* data available in a user-accessible form, there are two primary modes of moving through our web pages. These lead to the same information, but correspond to the two intellectual points of view from which we envision prospective users approach our data. The first approach is mapped-based and allows users to obtain information about the genes and other features found in a region of interest in the genome. The second approach provides information about genes and other elements in the sequence based on the nature of the feature itself, for instance, all kinases. In either case, the Cold Spring Harbor Laboratory Web pages now contain an extremely detailed description of what can be determined about the genes and transposons found in the BACs sequenced here at the Laboratory.

We have tried to make our annotation more closely linked to other *Arabidopsis* information, in particular, the existing genetic map. This serves two important purposes. It allows members of the community to see where their regions of interest are located relative to our sequence queue. This provides the information they need to determine whether to wait for our sequencing efforts to reach an area. These efforts should greatly facilitate positional cloning efforts being undertaken in the areas we are sequencing.

To facilitate the more global approach, we have begun an integrative function for our annotation that is also presented on our Web pages. We have assembled putatively identified genes into groups and have begun a "Master List" of all genes in our sequences. This will allow researchers to identify all members of a gene family of interest in a very rapid fashion. This list has hypertext links back to the initial, feature-rich annotation.

In other areas of informatics, we have completed the first complete version of our Web-based sample tracking system. This allows us to monitor individual projects as well as the quality of production sequencing. This system, called Kaleidaseq, tracks the flow of sequencing samples through the steps of preparing library plates, plaque-picking, preparing templates, conducting sequencing reactions, loading samples on gels, base-calling the traces, transferring the files to project directories, assembling the sequences, and conducting finishing reactions. The throughput rate at each step and the holdup at each step can be instantly viewed through the Web. The success rate of the sequenced samples can be tracked on a daily basis; the status of each project can also be tracked. Common daily operations such as base-calling of traces using base-calling engines developed by other groups and transfer to directories have been given a simple Web interface. Sample sheets for daily runs are generated through a Web interface and transferred transparently to the sequencing machine computers. A demonstration of this software can be found at

www.cshl.org/kaleidaseq

The design of the system will be described in a paper that will be in the March 1998 issue of *Genome Research* (Dedhia and McCombie 1998).

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PROTEIN CHEMISTRY

R. Kobayashi N. Carpino G. Binns
N. Kashige N. Poppito
P. Kearney K. Wanat

Research in our laboratory focused on two areas: (1) method development for protein structure analysis and (2) study of chronic myelogenous leukemia. This year, Nick Carpino, Paul Kearney, and Georgia Binns left our group and Nobuhiro Kashige joined us from Japan.

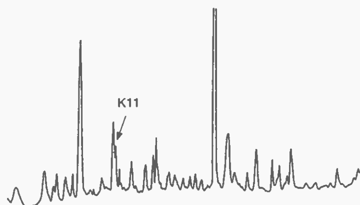
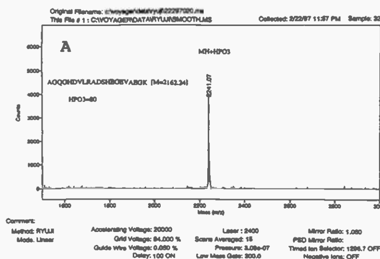


FIGURE 1 HPLC profile of Achromobacter protease I digest of p62^{dok}.

Protein Chemistry Core Facility

N. Poppito, K. Wanat, G. Binns

Our collaboration with other scientists at Cold Spring Harbor Laboratory has continued to be the major activity in our laboratory. A new instrument, a matrix-assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF-MS), showed its power in analyzing posttranslational modification of protein. With this instrument, we have been able to identify in vivo phosphorylation sites by analyzing peptide fragments separated by HPLC after protease digestion of purified protein. For example, Figure 1 shows a peptide map of p62^{dok} purified from a hematopoietic cell line expressing p210$^{bcr\text{-}abl}$, and one of the peaks, indicated as K11, was analyzed by MALDI-TOF mass spectrometry (Fig. 2A,B). In linear mode (Fig. 2A), an intact mass was observed that is 80 mass units added to the calculated molecular weight of the peptide fragment. On the other hand, in reflector mode (Fig. 2B), fragment ions are observed such as the dephosphorylated form in addition to the phosphorylated form of the peptide. This state-of-the-art instrument added to our laboratory a significant capability for structural analysis of protein to study the relationship between structure and function.



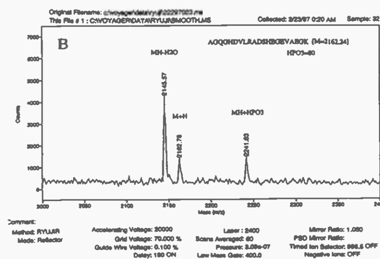


FIGURE 2 (*A*) Mass spectrometry of p62^{dok} peptide K11 in linear mode. (*B*) Mass spectrometry of p62^{dok} peptide K11 in reflector mode.

GAP-associated p62 in Chronic Myelogenous Leukemia

N. Carpino, N. Kashige

We continued to study p62^{dok} phosphorylated in chronic myelogenous leukemia (CML) in the presence of the chimeric p210$^{bcr\text{-}abl}$ protein tyrosine kinase. Hematopoietic progenitors isolated from CML patients in the chronic phase contain a constitutively tyrosine-phosphorylated protein that migrates at 62 kD by SDS-PAGE and associates with the p120^{ras} GTPase-activating protein (GAP). We are in the process of determining the phosphorylation sites of p62^{dok} to understand its role in CML.

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MACROMOLECULAR CRYSTALLOGRAPHY

X. Cheng A. Dong T. Malone
W. Gong M. O'Gara
J.R. Horton X. Zhang
Y. Liu

After almost 7 years at Cold Spring Harbor Laboratory, I moved south and took a professorship in Emory University School of Medicine and a position as a Georgia Research Alliance eminent scholar. My sense of excitement and anticipation somewhat dimmed as I looked back over the long island which had become my second home for the last 12 1/2 years. With the closure of my laboratory in the spring, this was a short year at CSHL for most of us. Tom Malone stayed on with Ruiming Xu, my former postdoctoral fellow and currently an assistant investigator at CSHL, and John Horton moved with me to Atlanta as a research assistant professor, along with Aiping Dong and Weimin Gong. Margaret O'Gara accepted a scientist position in Pfizer Central Research and migrated across the Atlantic to the United Kingdom, and Xujia Zhang accepted a position in the Institute of Biophysics in Beijing, China.

The research in my laboratory explores several different systems involved in DNA methylation and protein phosphorylation, and in eukaryotic multiprotein transcription factors. The following paragraphs summarize our current understanding on the mechanism of DNA base flipping.

MECHANISTIC LINK BETWEEN DNA METHYLTRANSFERASES AND DNA REPAIR ENZYMES BY BASE FLIPPING

Rotation of a DNA nucleotide out of the double helix and into a protein binding pocket (base flipping) was first observed in the structure of the *Hha*I DNA methyltransferase (Klimasauskas et al., *Cell 76:* 357 [1994]). There is now evidence that a variety of proteins, particularly DNA repair enzymes, use base flipping in their interactions with DNA.

Base flipping is a process by which a protein (usually an enzyme) can rotate a DNA nucleotide out of the double helix, breaking only the base-pairing hydrogen bonds, and trapping it in a protein-binding pocket. In the DNA-(cytosine C5) methyltransferase (5mC Mtase), M.*Hha*I, the catalytic side chains are situated on a typical concave surface; the target cytosine has rotated on its sugar-phosphate bonds such that it projects out of the DNA and fits nicely into the catalytic pocket of the Mtase. DNA distortion is confined essentially to this "flipped" nucleotide and its nearest neighbors on the same strand. We have suggested that flipping a base out of the DNA helix may be a widespread mechanism employed by many other proteins (Klimasauskas et al., *Cell 76:* 357 [1994]; Roberts, *Cell 82:* 9 [1995]; Cheng and Blumenthal, *Structure 4:* 639 [1996]).

Just within the last 3–4 years, base flipping has been discovered to be a common mechanism among DNA repair enzymes that function through either the base excision repair pathway (BER) or a direct reversal mechanism. The enzymes in the BER pathway that are predicted to use base flipping encompass DNA glycosylases, DNA glycosylase/abasic (AP) lyases, and possibly AP endonucleases and DNA ligase.

Overall, it appears that these enzymes utilize binding pockets for a variety of purposes that are ultimately related to recognition and/or catalysis. The concave catalytic pockets in DNA Mtases can accommodate many flipped bases, but only the target base can be methylated. Some repair enzymes have highly specific binding pockets that restrict access of nontarget bases to the binding pocket by steric interference (uracil DNA glycosylase or photolyase), whereas others achieve broad specificity by recognizing common features of the damaged bases, such as a general electron deficiency of the base to be removed (3-methyladenine DNA glycosylase II), whereas yet other enzymes simply need to get a base out of the way to achieve a catalytically competent complex (endonuclease V) (for review, see Lloyd and Cheng 1997).

Although there were subtle biochemical observations which suggested that altered base recognition was not as simple as the recognition of the damaged or mismatched bases within fully duplex DNA, no one predicted that this entire class of repair enzymes would, in some fashion, use extrahelical bases in recognition and catalysis. Clearly, the credit for this discovery rests with the cocrystal structures for DNA Mtases, M.*Hha*I (Klimasauskas et al., *Cell 76:* 357 [1994]) and M.*Hae*III (Reinisch et al., *Cell 82:* 143 [1995]), and for catalytically compromised forms of T4 endonuclease V (Vassylyev et al., *Cell 83:* 773 [1995]) and uracil DNA glycosylase (Slupphaug et al., *Nature 384:* 87 [1996]), in which nucleotide flipping has been directly observed.

The apparent universality of this mechanism has been inferred from the crystal structures of two other DNA Mtases, M.*Taq*I (Labahn et al., *Proc. Natl. Acad. Sci. 91:* 10957 [1994]) and M.*Pvu*II (Gong et al. 1997) and several other DNA glycosylases and glycosylase/AP lyases: *E. coli* endonuclease III, 3-methyladenine DNA glycosylase II or AlkA , the *E. coli* cyclobutane dimer photoreversal enzyme, photolyase and the *E. coli* hydrolytic abasic endonuclease, exonuclease III (for reviews, see Lloyd and Cheng 1997; Roberts and Cheng 1998). Although for those enzymes listed above definitive proof of the general nature of this mechanism awaits successful cocrystallization studies, the biochemical determination of active-site residues and their relative positioning within deep clefts in these enzymes strongly suggests that the DNA must conform to the structure of the protein, rather than the enzyme undergoing significant refolding.

Base flipping may be fundamental to the functioning of many DNA-binding proteins. The DNA Mtases combine base flipping with specific sequence recognition. The recognition of mismatched DNA structures by M.*Hha*I and other prokaryotic Mtases and the human DNA Mtase suggests that the evolution of these enzymes and perhaps the evolution of DNA methylation itself may have been driven by the formation of mismatches in DNA. Concerning DNA repair enzymes in the BER pathways, the use of base flipping appears to be a common component in damage recognition and catalysis. Throughout this pathway, however, the specificity of the binding pockets and purpose for which they are used varies greatly. In summary, the phenomenon of base flipping is appearing in a number of systems following its initial dis-

covery in the DNA Mtases, and many additional enzyme systems must be considered candidates to use this novel mechanism. Unfortunately, detailed mechanistic information is lacking, and it remains to be proven whether base flipping is an active process in which the protein pushes the base out of the helix, or a passive one in which the protein binds to a transiently flipped base. Further work is needed to settle the issue. Particularly intriguing is the recent finding that when M.*Hha*I binds to an abasic site, it flips the deoxyribose ring and its flanking phosphates into the same conformation as is adopted during base flipping on its normal substrate (see below). Thus, if the process is an active one, one might expect that the push will take place not on the base, but rather on the sugar-phosphate backbone.

MISMATCHED BASES (G:A, G:U, AND G:AP)

Following the discovery of base flipping by M.*Hha*I, the effects of replacing the target cytosine by mismatched bases, including adenine, guanine, thymine, and uracil, were investigated (Klimasauskas and Roberts, *Nucleic Acids Res. 23:* 293 [1995]; Yang et al., *Nucleic Acids Res. 23:* 1380 [1995]). By electrophoretic mobility shift analysis, M.*Hha*I and M.*Hpa*II were found to bind even more tightly to such mismatched substrates; the highest affinity was for a gap formed by removal of the target nucleotide and both phosphodiester linkages. Furthermore, the uracil can be enzymatically methylated and converted to thymine at low efficiencies; the binding of these Mtases at the G:U mismatch prevented its repair by uracil DNA glycosylase in vitro.

So far, three well-refined ternary structures of M.*Hha*I complexed with AdoHcy and a nonpalindromic oligonucleotide containing a G:A, G:U, or G:AP mismatch at the target base pair, respectively, have been determined (M. O'Gara, J.R. Horton, and X. Cheng, in collaboration with R.J. Roberts). The mismatched adenine, uracil, and abasic site are flipped out and located in the enzyme's active site, respectively. It seems likely that this pocket is nonspecific for the binding, but specific for methylation. In the light of the nonspecific binding pocket, the DNA Mtase may be more related to the repair enzymes such as 3-methyl-adenine DNA glycosylase II and endonuclease III, which have broad substrate specificity. On the other hand, the methylation reaction is specific in that catalysis occurs only when the flipped base is cytosine or uracil (at low efficiency), more closely resembling uracil DNA glycosylase.

4′-THIO-2′-DEOXYCYTIDINE AND DIHYDRO-5-AZA-CYTIDINE

The fact that M.*Hha*I does not show much binding specificity for the flippable base may reflect a need to leave that base unencumbered by recognition contacts. This provides an opportunity to probe the structural and chemical interactions involved in sequence-specific recognition and catalysis using nucleotide analogs incorporated into synthetic oligonucleotides in the position of the target nucleotide of M.*Hha*I. So far, two nucleotide analogs, a 4′-thionucleoside and 5,6-dihydro-5-azacytosine, have been used in our studies.

When 4′-thio-2′-deoxycytidine is incorporated as the target cytosine in the recognition sequence for M.*Hha*I, binding to the 4′-thio-modified DNA is almost identical to that of the unmodified DNA under equilibrium conditions (Kumar et al. 1997). In contrast, the methyl transfer was strongly inhibited in solution. Surprisingly, the flipped 4′-thio-2′-deoxycytidine in the crystal structure was partially methylated (Kumar et al. 1997). These results show that 4′-thio-2′-deoxycytidine does not disrupt DNA recognition, binding, or base flipping by M.*Hha*I, but they suggest instead that it interferes with a step in the methylation reaction after flipping, but prior to methyl transfer.

5,6-Dihydro-5-azacytosine (DHAC) riboside was originally synthesized to obtain a hydrolytically stable replacement for the anti-leukemic drug, 5-azacytosine riboside. DHAC contains a cytosine-like ring lacking aromatic character with an sp3-hybridized carbon (CH_2 group) at position 6 and an NH group at position 5, resembling the transition state of a dihydrocytosine intermediate in the reaction of 5mC Mtases. The structure, containing DHAC as the target, showed that DHAC is also flipped out of the DNA helix similarly to cytosine, but with no covalent bond formed between the sulfur atom of nucleophile Cys-81 and the pyrimidine C6 carbon (M. O'Gara and X. Cheng, in collaboration with J.K. Christman). This result indicates that the DHAC-containing DNA is sufficient to produce strong inhibition of the DNA Mtase and that the DHAC moiety occupies the active site of M.*Hha*I as a transition-state mimic.

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STRUCTURAL BIOLOGY

L. Joshua-Tor T. Messick D. van Aalten
P. O'Farrell D. Vaughn
J. Rosenbaum

We study the molecular basis of cell regulatory processes in terms of molecular recognition. We are using tools of structural biology and biochemistry in a combined approach to look at proteins and protein complexes associated with these processes. X-ray crystallography, our primary technique, enables us to obtain an accurate three-dimensional structure of individual proteins and the interactions in which they are involved. We use biochemistry to study properties predicted by the structure and to direct our structural studies in an iterative manner. We are also combining information from molecular biology and genetics in collaborative efforts to study their functions. Our current efforts center around two distinct themes. The first is the study of an evolutionarily conserved family of oligomeric self-compartmentalizing intracellular proteases, the bleomycin hydrolases. The second theme in the lab involves structural studies of transcription factor complexes. We are also looking at the effect of molecular motions on various biological processes.

During the past year, Paul O'Farrell and Daan van Aalten joined the group as postdoctoral fellows. Later in the year, Dan Vaughn arrived as a joint postdoctoral fellow between Tim Tully's group and ours to work on a collaborative effort which also includes Jerry Yin. Tom Malone has been promoted to the new position of crystallographic systems manager. Tom, who was previously a technician at the lab with Jim Pflugrath and Xiaodong Cheng, now runs the X-ray and computer facilities for the structural biology center.

Two important developments have greatly enhanced our technical capabilities. We acquired a new rotating anode X-ray generator for the Keck Structural Biology Center. This generator has been purchased with funds from the National Science Foundation as well as laboratory funds. Most excitingly, we have begun collecting X-ray data on our beamline X26C at the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory (BNL). This is a joint venture with SUNY Stony Brook and Brookhaven Biology and will be described in more detail by Rui-Ming Xu in this Section (Structure and Genomics).

Bleomycin Hydrolases

Resistance to antineoplastic drugs, whether intrinsic or acquired, is a central problem in treatment of human

cancers. Bleomycin hydrolase is a cysteine protease discovered because of its ability to hydrolyze the anticancer drug bleomycin and thus to limit its use in cancer therapy. The recent discovery of homologs of this hexameric 300-kD protein in yeast and bacteria implies a conserved cellular function for this protein. In humans, this protease is expressed in all tissues tested and at elevated levels in several tumors. In mice, expression is highest for newborn mice and decreases with age. These proteases belong to the group of self-compartmentalizing, or "sequestered" intracellular proteases which include the proteasome as has been realized from the crystal structure of the yeast bleomycin hydrolase, Gal6, which we have determined previously. Characteristic of these proteases, it has a ring structure with a central channel where the active sites, six in this case, can be accessed only from within the channel. Several other unique features have been revealed from this structure, including a nucleic-acid-binding activity and an unusual positioning of the completely conserved carboxyl terminus of the protein in its own active-site cleft. The yeast protein binds preferentially to single-stranded DNA or RNA, with a K_d of approximately 10 nM and to nicks in double-stranded DNA. Moreover, its expression is regulated by galactose and the Gal4 protein, and Gal6 itself acts as a repressor of galactose-regulated genes. Clearly, bleomycin hydrolase has complex cellular functions, consistent with the increasing recognition of the important role in general of intracellular proteases.

Human Bleomycin Hydrolase

P. O'Farrell, L. Joshua-Tor [in collaboration with W. Zheng, H. Whelan, and S. A. Johnston, Southwestern Medical Center]

In an effort to understand the mechanism of action of the enzyme, and with the long-term goal of designing an effective inhibitor and a cleavage-resistant bleomycin, we have determined the crystal structure of wild-type human bleomycin hydrolase (hBH) and of an active-site mutant, C73S. X-ray data were collected for the wild-type crystals at our beamline (X26C) at NSLS and mutant crystals were collected on beamline X25. Both crystallize in space group $C222_1$. Data were also collected for an additional crystal form for the mutant, R32, which diffract much better. Both structures were solved by molecular replacement using the yeast Gal6 structure as a starting model, and are currently being refined to 2.6 Å resolution for the wild type and 1.85 Å for the mutant. With these structures near completion, we have begun analysis of the structures and comparison with the yeast homolog.

The Carboxyl Terminus of Gal6, the Yeast Bleomycin Hydrolase, Dictates Carboxypeptidase, Aminopeptidase, and Peptide Ligase Activities

L. Joshua-Tor [in collaboration with W. Zheng and S.A. Johnston, Southwestern Medical Center]

As in other self-compartmentalizing proteases, the active sites of Gal6 are embedded in the channel, sequestered from the outside. A unique feature of this particular family of enzymes is that the carboxyl terminus of the protein lies in the active-site cleft, blocking the access from the exterior of the protein, as well as part of what would be the substrate-binding site. We have investigated this unusual configuration of the active site using mutational, structural, and biochemical methods. We find that Gal6 acts as a carboxypeptidase to create its active site, converting itself to an aminopeptidase. The carboxyl terminus itself is the major determinant of substrate specificity, conferring "positional" rather than sequence specificity by acting as an anchor and "molecular ruler" to position the substrate (see Fig. 1). As a consequence, deletions of the carboxyl terminus convert the protein to an endopeptidase. Interestingly, both the wild-type and the variant forms of the protein have a peptide ligase activity. Our model not only explains these diverse activities of the enzyme, but also explains its unique ability to inactivate the anticancer drug, bleomycin.

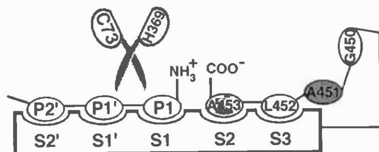

FIGURE 1 Cartoon illustration of the active site of Gal6. The carboxyl terminus of the enzyme anchors the amino terminus of a substrate and positions it for cleavage. The scissors represents the active site cysteine and histidine at the cleavage site.

Transcription Complexes

Protein-Protein Complexes Involved in Transcriptional Activation

T. Messick, L. Joshua-Tor [in collaboration with
S.A. Johnston, Southwestern Medical Center]

The interplay between various protein components in transcriptional regulatory systems and their response to induction from exterior signals is central to understanding the mechanism of transcriptional activation. We are currently screening crystallization conditions for such complexes. Initial trials have already yielded crystals of one such protein-protein complex, which are currently being refined.

Structure and Dynamics of the Myb DNA-binding Domain

D. van Aalten, L. Joshua-Tor [in collaboration with
E. Grotewold, Cold Spring Harbor Laboratory]

The Myb domain protein P is a transcriptional activator that regulates a1, a flavonoid biosynthetic gene in maize. The DNA sequences preferentially bound by P are different from the sequences bound by animal Myb proteins, and using a nuclear magnetic resonance (NMR) structure of an animal Myb domain for modeling P-Myb does not explain the binding characteristics of DNA-binding mutants of P. To understand Myb function and DNA binding at the atomic level, we are performing X-ray crystallographic and computer simulation studies of the DNA-binding domain of P from maize.

Initial purification of the Myb domain yielded insoluble protein. Various constructs were used with small amino-terminal and/or carboxy-terminal deletions to screen for optimal solubility. A suitable construct was found, giving a monodispersed protein solution, as shown by dynamic light scattering. The protein binds several oligonucleotides that contain the P-binding element. Crystallization experiments with one of these oligonucleotides yield small needlelike crystals, and optimization of these conditions is under way.

The NMR structure that is available for the Myb domain of mouse c-Myb was used to study the dynamic fluctuations that may be important for DNA binding. The NMR structure consists of the second and third Myb repeats (both are separate helix-turn-helix motifs), connected by a linker. In computer simulations the repeats were moved as rigid bodies, opening and closing the DNA-binding site. These motions pointed to a conserved proline in the middle of the linker sequence as important for controlling the amplitude of this fluctuation. A simulation of the proline-to-alanine mutant was performed, and the amplitude of the hinge-bending motions was found to be increased.

Molecular Motions Important in Binding and Signal Transduction

Structure of a Cross-linked DNA Decamer with Increased Affinity to DNA-Methylases

D. van Aalten, L. Joshua-Tor [in collaboration with
G. Verdine, Harvard University]

DNA methylases are thought to act by binding to DNA and flipping out a base, followed by the enzymatic reaction. This base-flipping has been seen in the two methylase-DNA cocrystal structures that were solved in the laboratories of Xiaodong Cheng and Richard Roberts here at Cold Spring Harbor Laboratory and in the laboratories of William Lipscomb and Gregory Verdine at Harvard University. Since it is necessary for the enzyme to flip out a base, binding should be improved when it is offered a DNA duplex with the appropriate base already flipped out. In the lab of G. Verdine, a palindromic decamer was designed, with a linker and a disulfide cross-link between a central guanine nucleotide of one strand to the same position on the other strand. The modified DNA duplex was found to bind significantly better to DNA methylase than the unmodified form. Crystals of the cross-linked DNA were grown in order to examine the structural basis for this tighter binding. The crystals diffract to 1.75 Å on the in-house RAXIS generator and to 1.65 Å on beamline X26C at NSLS. The structure was solved by molecular replacement and is currently being refined. There are two structurally very different duplexes in the asymmetric unit. One has a large bend in the middle of the helix and has the cytosine at position 6 on one strand partially flipped out; the other is straight but is stabilized by a calcium ion.

Structure and Dynamics of a Conformationally Restricting Glycine Mutant in the Human Muscle Fatty-acid-binding Protein

D. van Aalten, L. Joshua-Tor [in collaboration with
J. Veerkamp, University of Nijmegen, The Netherlands]

The muscle fatty-acid-binding protein (FABP) is a member of a large family of small hydrophobic molecule transporters. It binds fatty acids presented to it via a membrane transport system and delivers them to intracellular locations. Ligand-protein complexes of FABPs show the ligand bound deep inside the protein, with no obvious entry/exit route. Computer simulation of a FABP-ligand complex suggests large hinge-bending motions in the protein, transiently opening a tunnel through which the ligand could enter or exit. A key residue appeared to be an evolutionarily conserved glycine, which lies in the center of the hinge region and might thus control the dynamics of this tunnel opening. The Veerkamp lab has provided us with an FABP mutant in which this glycine is replaced by a conformationally more restricted serine. Two crystal forms were observed, both in space group $P2_12_12_1$. One crystal form diffracts to 2.1 Å on the in-house RAXIS and has been refined to R = 20% and R_{free} = 22%. The second form diffracts to 1.19 Å on beamline X26C at Brookhaven, and has been refined to an R = 18%, R_{free} = 19%. While the Veerkamp lab is performing kinetic measurements on the mutant, we will perform simulations to compare the hinge-bending motions to those of the wild type.

Investigation of the Photocycle of the Photoactive Yellow Protein by X-ray Crystallography and Computer Simulation

D. van Aalten, L. Joshua-Tor [in collaboration with
K. Hellingwerf and W. Crielaard, University of Amsterdam, The Netherlands]

The photoactive yellow protein is found in bacteria isolated from hypersaline, sun-bathed lakes. This photoreceptor was found to be part of a signal-transduction pathway that is responsible for the negative phototactic response of the bacterium to intense blue light. Like bacteriorhodopsin, it has a multistate photocycle in which a ligand is isomerized, followed by a conformational change in the protein, which is the first step in signal transduction. Unlike bacteriorhodopsin, which is a membrane protein, this protein is water-soluble, making it an excellent candidate to study the details of the photocycle at the atomic level. We are currently studying the structure and dynamics of three serine mutants of evolutionarily conserved glycines, which have been implicated to act as hinge-bending points in conformational changes during the photocycle.

In the Hellingwerf lab, the mutants were shown to have a slower photocycle. We have crystallized and solved the structures of the mutants. All crystallize in spacegroup $P6_5$ with approximately the same cell dimensions and diffracted on the in-house generator to 2.0 Å and on beamline X26C at Brookhaven to 1.75 Å. Refinement of two of the mutants is currently to 1.85 Å with R = 16%, R_{free} = 19% for one and R = 20% and R_{free} = 24% for the other. The photocycle occurs normally in crystals. We will perform computer simulations of the mutants, restricted by the X-ray data, to examine which areas of the protein are conformationally restricted. This might have implications for the photocycle and subsequent steps in the signal transduction pathway.

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COMBINATORIAL LIBRARIES

H.P. Nestler R. Liu
N. Batada (URP)
R. Sherlock
A. Anand (PFF)

Combinatorial "One bead–One structure" libraries have opened a new dimension for the study of interactions of small molecules with chemical and biological ligands. We are employing such encoded combinatorial libraries to identify molecules that specifically interfere with the biological function of proteins of physiological importance at specific functional sites. Hereby, we pursue an approach that outlines a new paradigm for molecular biology, as we will attempt to prevent a biological process not by inhibiting the enzyme connected with this process, but by masking the substrate with a synthetic molecule. The second aspect of our research is the identification of substrates and subsequently of inhibitors of enzymes, especially proteases. Although the identification of peptidic substrates for proteolytic enzymes can be done rather efficiently using combinatorial libraries, there is still a need for methods to efficiently identify protease substrates from protein mixtures. Finding new substrates for enzymes not only will open ways to design compounds inhibiting these enzymes, but will also help to elucidate the pathways these enzymes are involved in.

Molecular Forceps from Combinatorial Libraries That Bind RAS Proteins

R. Liu, R. Sherlock [in collaboration with D.L. Dong and M.H. Wigler, Cold Spring Harbor Laboratory]

Our initial studies have shown that simple molecules displaying two or more short peptide or peptide-like chains (which we termed "molecular forceps") can provide reasonable affinities and selectivities toward peptide substrates. We are using libraries of molecular forceps to identify molecules that are able to bind to the carboxyl terminus of RAS proteins (also termed "CaaX-box") and prevent its farnesylation. The farnesylation of RAS has been shown as a crucial step in the activation of RAS as a signal tranduction protein, and pharmaceutical trials showed that molecules that inhibit the farnesyl transferase, the farnesylating enzyme, are able to stop tumor growth. The approach we follow outlines a new paradigm for molecular biology, as we attempt to prevent a biological process not by inhibiting the enzyme connected with this process, but by masking the substrate with a synthetic molecule. To efficiently target our molecular forceps toward the carboxyl terminus of RAS, we use the isolated peptide sequence instead of the whole protein as substrate for the screening of our libraries. The molecules selected against the peptide epitope should bind RAS site-specifically at the CaaX-box. Although there is precedence that antibodies can be raised against peptide epitopes in this fashion, it is unclear whether this approach is suitable for the selection of synthetic molecules. The carboxyl terminus of RAS is a suitable test case for this novel approach, because it is conformationally not restricted and should therefore be readily accessible for the molecular forceps we intend to generate.

In our assays of a library that contained two-armed and four-armed molecular forceps with the octapeptide derived from the CaaX-box of RAS and the whole RAS protein, we observed that the molecular forceps with four peptide arms predominated as interacting molecules. In a nonexhaustive screening of this library, we identified 15 molecules binding to the above mentioned CaaX-box octapeptide and 35 molecules binding to the RAS protein. Subsequently, we resynthesized 3 molecules that bound to the peptide, three forceps that bound to the protein, and three that did not show interaction in the screening. Each of the resynthesized molecules that had been selected against the peptide binds with micromolar affinities selectively to the H-RAS protein with some specificity over other proteins such as ovalbumin or BSA, supporting our assumption that we can screen libraries with isolated peptide epitopes to identify forceps binding to the full-length protein.

Furthermore, two of the peptide binders not only bind to the RAS protein, but also slow down the farnesylation of H-RAS with IC_{50} values of 100 μM and 400 μM. None of the protein binders or nonbinding forceps prevents the farnesylation in the micromolar

range. These findings suggest that some molecular forceps (although not all of them) that were selected for binding to peptide epitopes recognize this epitope on the full-length protein, whereas forceps selected against the protein bind at other locations of the protein surface.

Beyond that, the retardation of farnesylation by the stronger acting forceps is sensitive to the peptide used as substrate for the farnesyl transferase: Whereas the farnesylation of a peptide derived from the CaaX-box of RAS is inhibited with an IC_{50} of 100 μM, the farnesylation of a peptide derived from K-RAS B is inhibited with an $IC_{50} > 1$ mM (75% farnesylation efficiency at a concentration of 1 mM) and the farnesylation of a hybrid peptide with an IC_{50} of about 500 μM. The farnesylation of a peptide derived from the carboxyl terminus of lamin B is not affected at concentrations up to 1 mM. We are currently establishing that this retardation is caused by binding of the forceps to the CaaX-box of RAS and not by coincidental inhibition of the farnesyl transferase.

Detection of His_6-tagged Peptides and Proteins on Beads

R. Liu, R. Sherlock, H.P. Nestler

Although antibodies have been employed very successfully with combinatorial libraries as screening targets or to detect tightly bound proteins, there are drawbacks to using antibodies coupled to alkaline phosphatase for detection of beads that bind proteins with low affinity. First, the extensive washing procedures required for the staining tend to elute the proteins from their ligands; second, some libraries we have tested bind to the antibody or its conjugate; and third, antibodies against certain proteins may not be readily available.

The attachment of a hexa-histidine moiety ("His-tag") to proteins that are engineered for overexpression into bacteria is now a standard means to purify these proteins by affinity chromatography over a nickel column, making use of the strong affinity of the His-tag to the nickel on the column. Beyond its ability to complex nickel ions, the His-tag also interacts with other ions of transition metals, such as copper. We could detect the interaction of copper(II) ions with a hexa-histidine sequence covalently attached to resin beads by a blue staining of these beads. However, the colorization resulting from copper(II) ions interacting with His-tagged ligands that were not covalently bound to beads was too faint to allow the unambiguous identification of beads binding these ligands.

We have developed a way to detect noncovalently bound His-tagged ligands on beads by catalytically enhancing the signal: Copper(II) ions are essential to catalyze the Benzidine Blue reaction, where the colorless substrate benzidine (biphenyl-diamine) is oxidized by hydrogen peroxide to yield a dark blue charge-transfer complex. If we perform the Benzidine Blue reaction on beads, the precipitation of the blue complex leads to a brown or violet staining of the beads. Our experiments show that the developed method is applicable to the screening of His-tagged proteins as well as peptides.

A Two-dimensional, Diagonal SDS-PAGE Technique to Screen for Protease Substrates in Protein Mixtures

H.P. Nestler, N. Batada [in collaboration with A. Doseff and Y. Lazebnik, Cold Spring Harbor Laboratory]

Proteases have emerged during the past few years as key players in the control of cell fate. Many have been identified and cloned, but to understand their function and to elucidate the pathways in which they are involved, the intracellular substrates processed by each protease must be identified. Even the intensive efforts in genome sequencing and cloning that yield new protein sequences every day do not provide a complete solution: The occurrence of a cleavage motif in a protein sequence is not sufficient for proteolytic susceptibility, since folding may prevent access of the protease to the substrate site. Indeed, many proteins in their native conformation are resistant to proteolytic digestion.

Electrophoresis of proteins in polyacrylamide gels is a standard tool for separating proteins for analytical and preparative purposes, and two-dimensional electrophoresis is widely used for the high-resolution mapping of proteins. However, two-dimensional electrophoresis has seldom been used to monitor the transformation of proteins, and in most instances isoelectric focusing is used in the first dimension and SDS-PAGE in the second dimension. We have developed a technique for identifying protease substrates in protein mixtures using two-dimensional gel electrophoresis combined with in-gel proteolysis.

A mixture of proteins, i.e., a cellular extract, is separated in a nonreducing SDS-slab gel. The proteins are renatured in the gel by eluting the SDS from the gel with a buffer appropriate for the proteolytic digestion. The equilibrated gel is then incubated with the protease of interest, and the digested proteins are separated by nondenaturing SDS-PAGE in a second dimension orthogonal to the first. Since electrophoretic development in each dimension occurs under the same conditions, undigested proteins appear in a diagonal line through the gel with the products of the proteolytic cleavage below this line. Initial attempts to achieve separation under nondenaturing conditions failed. The denaturation of the proteins with SDS during the electrophoresis proved necessary to ensure separation by molecular weight, which is crucial to the diagonal alignment of uncleaved proteins in the two-dimensional gel.

We show in our studies that we are able to successfully renature proteins after the first electrophoresis dimension. Indeed, even with highly active proteases that have a broad specificity, such as trypsin or chymotrypsin, we are able to obtain specific fragmentation patterns from the protein samples that are in agreement with reported stabilities of the respective proteins in their native state to proteolytic digestion. Using CPP-32, a highly specific protease, we only observed cleavage of Mch-2, a known substrate. The other proteins in the used sample were unaffected even over extended periods of proteolysis. Currently we are employing this technique to identify substrates of CPP-32 from cellular extracts.

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SEQUENCE-BASED PREDICTION OF PROTEIN FUNCTION

A. Neuwald

Our work addresses a source of inefficiency in protein research inasmuch as the information that researchers seek through experimentation may already be available, hidden among the mass of data present in the literature and in the sequence and structural databases. In particular, we aim to make protein functional information implicit in multiple sequence data more accessible to the research community. This is needed because important relationships are now often overlooked.

For example, some of this year's work (performed prior to coming to CHSL) led to compelling predictions concerning the structure, active-site residues, and catalytic mechanism of a fruit fly membrane protein involved in germ cell migration. Yet experimentalists studying this protein overlooked the subtle sequence relationships leading to these predictions. Other recent work has revealed (similarly overlooked) relationships relevant to our understanding of breast cancer, cardiomyopathic disorders, and spinocerebral ataxia. Thus, deciphering multiple sequence data can help clarify the roles proteins play in disease mecha-

nisms, leading to better diagnoses and treatments. To further enhance our ability to predict protein function, we are currently developing and improving methods to gather, model, and interpret multiple sequence data.

A Comprehensive Set of Protein Domains

A. Neuwald

To help develop advanced alignment models of protein domains, we are partitioning all available sequences into protein families. This allows the inclusion of as many proteins as possible in each set, which helps align the sequences and construct an accurate model of the protein family. These alignment models can be used to search a database for distant relatives, often leading to striking functional predictions.

By constructing a complete set of these alignments, we are obtaining an overview of the "protein universe" so that the idiosyncrasies of the various types of domains can be recognized, prioritized, and studied. This, in turn, is helping us to develop more highly refined alignment models. We will also use these data for comprehensive statistical and comparative analyses of protein domains. Moreover, a nearly complete set of alignments helps detect and delineate subtly conserved or interrupted domains in multiple domain proteins by first delineating more easily detected adjacent or inserted domains. Finally, by precomputing these alignments, many important relationships are being detected that would have been missed by thorough but more limited approaches.

For example, a protein that is mutated in a hereditary cardiomyopathic disorder (Barth syndrome) was found in one of the alignment sets. In this set, all of the characterized proteins are acyltransferases involved in phospholipid biosynthesis. This suggested a possible disease mechanism where the abnormalities associated with this syndrome, which is characterized by alterations in mitochondrial morphology and respiratory-chain dysfunction, may be due to alterations in membrane phospholipids. This relationship was missed during previous analyses starting with this disease-related protein because it lacks sufficient *pairwise* similarity to these acyltransferases. Nevertheless, for these alignment models to achieve better sensitivity and selectivity, they need to more accurately represent family-specific features.

Advanced Alignment Models of Protein Domains

A. Neuwald [in collaboration with J. Spouge and W.J. Wilbur at the National Center for Biotechnology Information, National Institutes of Health]

To tailor models to particular protein families, we are pursuing a strategy of stepwise refinement. This involves starting with the initial alignment models constructed during partitioning of the sequence database and then successively adding more details. First, general features are being added to the models. These include correcting for compositionally biased sequences and adding empirically derived estimates of the spacing between and gaps within conserved regions. Second, more specific features are being added, including structural constraints for families with known structures and modeling of repetitive elements within domains. Finally, hierarchical alignment models are being devised to represent commonly occurring subdomains within domains. This will help distinguish functional constraints shared by related families from those specific to a particular family.

Regarding the first model refinement step, we have recently developed statistical and algorithmic procedures to model the spacing between conserved regions in a multiple alignment. These procedures were applied in an analysis of pleckstrin homology (PH) domains, which occur in a wide range of proteins involved in intracellular signaling or as constituents of the cytoskeleton. This domain was selected for analysis because it is weakly conserved and contains numerous insertions and deletions. The modified multiple alignment model detects about 28% more PH domains than an alignment model that ignores the spacing between conserved regions and about seven times as many domains as standard pairwise analysis methods. Not surprisingly, this alignment also captures additional features of the PH domain (Fig. 1), modeling it as five conserved regions versus three previously.

Regarding the second model refinement step, we have been working recently on methods to incorporate structural constraints into alignment models. Because only structural features are conserved between the most distant members of a protein family, this approach should help maximize a model's information about distant (unknown) family members. Recent

FIGURE 1 Alignment of representative PH domains.

preliminary work confirms this notion.

Four sequences from among the top nonsignificant matches for a database search using a protein kinase alignment model were threaded through a protein kinase structure. These sequences included two non-protein kinases—an isomerase (E value = 6) and a helicase (E value = 14)—and two known viral protein kinases distantly related to other protein kinases—one from herpesvirus (E value = 3) and one from cytomegalovirus (E value = 42). A protein kinase structural core was defined using the ten conserved regions of the alignment model. Similarly, sequence threads through this core were defined by optimally aligning each sequence to the alignment model. Threading scores were based on three structural properties: solvent accessibility, secondary structure propensity, and tertiary contact potentials. The significance of these threading scores was determined using a Monte Carlo procedure for estimating small P values developed by Dr. Wilbur at the NCBI.

All of the threading score P values for these sequences were relatively small. This is as expected for nonsignificant but high-scoring sequences retrieved in a large database search using a protein kinase alignment model. However, P values for the viral protein kinases were lower by a factor of three to five orders of magnitude relative to the nonprotein kinases. Hence, these results indicate that, indeed, there is additional information to be gained by incorporating structural features.

Regarding the last model refinement step, recent preliminary work has examined the benefit of ignoring a commonly occurring subdomain within an alignment model. This preliminary work, in a crude way, mimics the effect of layering a subdomain beneath a domain model—a more sophisticated approach that we will implement in the near future. When applied to NTP-binding motifs in a model of replication factor C (RFC)-related proteins, this allowed better discrimination of this family. A database search using the RFC family alignment model detected 265 protein sequences. In contrast, a search that ignores the NTP-binding subdomain detected 68 RFC-related proteins. (Six conserved regions outside the NTP-binding motifs characterize the RFC family.) Thus, this method filters out 197 NTP-binding proteins otherwise unrelated to RFC proteins. Moreover, during construction of this alignment model, this approach was able to detect and include distantly related, unknown RFC family members that were not previously detected due to the abundance of NTP-binding proteins.

COMPUTATIONAL GENOMICS

M.Q. Zhang T. Chen (Part-time grad student) J. Feng (Part-time grad student) M. Huang (Visiting scientist) I. Ioshikhes R. Klein (URP) W. Orrick (Part-time grad student) J. Tabaska

Our research interest is to identify and to characterize genetic elements in nucleic acid sequences by computational means. As the Human Genome Project enters its large-scale sequencing phase, developing efficient computational methods for identification of genes and their control/regulatory elements has become extremely important. Knowing the organization of a gene often becomes the prerequisite for further functional studies. Our initial focus was to study statistical characters of exons and introns in protein coding regions and to develop coding-exon prediction programs by applying multivariate statistical pattern recognition techniques. More recently, we began to focus on promoters, translational start site, and polyadenylation site regions to develop new computational methods for identification of the ends of genes.

T. Chen is now a postdoctoral fellow at Harvard. W. Orrick went to Australia for a post-doc position. R. Klein is back at Harvard as a senior. Tabaska joined the lab in May as a post-doc from Stormo's lab at the University of Colorado and Ioshikhes joined the lab in July as a post-doc from Trifonov's lab at Weizmann Institute. Huang came to visit the lab in July from Otsuka America Pharmaceutical. Feng started in December as a volunteer from Queens College.

POMBE: A Fission Yeast Gene-finding Tool

T. Chen, M.Q. Zhang

Finding protein coding regions in a eukaryotic genome is very difficult due to the presence of introns. To improve our rule-based interactive computer program (INTRON.PLOT) and to meet the needs of a world-wide large-scale genomic sequencing project, we analyzed 200 genes from an important model organism—the fission yeast—and developed a new fission yeast gene-finding and exon-intron organization prediction system called POMBE. The sensitivity, the specificity, and the correlation coefficient were 98.5%, 99.9%, and 98.3%, respectively, at the nucleotide level. It is based on Linear Discriminant Analysis and is now available at

ftp://cshl.org/pub/science/ POMBE/

and at

http://c.cshl.org/gene finder/

Identification of Protein-coding Region in Human and Mouse Genomes

M.Q. Zhang

Identification of exons in vertebrates is more challenging because an average vertebrate gene consists of many short exons separated by introns that are ten or a hundred times larger. As hinted by the exon-definition model, the strategy for vertebrate gene-finding should focus on exons. We carried out comprehensive classification and statistical characterization of human exons and their flanking regions. Further analyses revealed novel correlation and constraints among different splicing features across an exon. On the basis of our findings, we have developed a new powerful exon-finding program called MZEF that uses the quadratic discriminant function for multivariate statistical pattern recognition. When compared with other leading gene-finders (such as HEXON or GRAIL2), MZEF tends to have higher specificity (less false-positive) without sacrificing sensitivity (in a test of 43 human genes, the accuracy measured by the correlation coefficient was 0.80 for GRAIL2, 0.83 for HEXON and 0.90 for MZEF). MZEF has been ranked top among all the human exon prediction programs in a recent review. It is implemented for both human and mouse gene-finding and is available at

ftp://cshl.org/pub/science/MZEF/

and at

http://www.cshl.org/gene finder/

Exploratory Study of Kohonen Network Classification of Promoters

W. Orrick, M.Q. Zhang

Classification of promoters of human genes is very useful for understanding their relationship. There are two methods: One is functional and the other is statistical. Because of the lack of functional information, we looked for a statistical technique for automated sequence classification. The Kohonen self-organized map (SOM) is a well-known unsupervised neural network learning procedure that can automatically classify high dimensional samples (such as DNA sequences) according to their degree of similarity. It has been applied to both protein and nucleotide sequence classification problems. We have implemented SOM in JAVA language on our UNIX workstation and started experimenting with human promoter sequences. Initial results showed that SOM was able to partition promoter sequences into TATA and TATA-less sectors; better visualization techniques are needed for recognizing more subtle clusters in the final Kohonen topology preserving map.

A Computational Description of the Interaction between the Transcription Factors E2F and Sp1

R. Klein, M.Q. Zhang

E2F is a eukaryotic transcription factor that has been found to regulate many genes needed for DNA synthesis. Several recent studies have indicated that E2F and Sp1 work together to regulate expression of some genes. By analyzing independent promoters containing both E2F and Sp1 sites, we found most genes had at least one E2F/Sp1 pair with interdistance less than 50 bp but larger than 10 bp. Using the weight matrices constructed from aligned E2F and Sp1 sites together with the distance rule, we have searched the eukaryotic promoter database (EPD). The scoring threshold was established by searching a control set of randomly shuffled promoter sequences. Aside from many homologs to the known genes, there are four likely candidate genes for further experimental testing for E2F/Sp1 regulation. Two are proto-oncogenes: mouse c-myb and human c-abl. The third is chicken HMG-17, which is a non-histone DNA-binding protein found in chromosomes. Its human homolog has been shown to have increased mRNA levels in S-phase. Finally, htf9-a, which was identified in the original literature search as being regulated by both Sp1 and E2F, but separated by a distance greater than 50 bp, was shown to have a potential Sp1 site closer to the E2F site. However, homologs in the EPD for both c-abl and HMG-17 did not have consensus E2F sites. Another gene, transforming growth factor β-1, which was recently shown to be E2F regulated, also showed up positive with appropriately spaced E2F and Sp1 sites in its promoter. Whether each gene is actually regulated by these two factors needs to be experimentally determined.

Database Construction and Statistical Analysis of Promoters That Contain Specified Pairs of Transcription Factors

I. Ioshikhes, J. Feng, M. Q. Zhang

We have tried to make a functional classification of promoters by requiring that each class must share some common regulatory elements. We are extending the analysis for the E2F/Sp1 pair to other transcription factors, such as Sp1/NF-Y or E2F/X, where X represents any other documented interacting factors. Databases for specific families of promoters are being built to facilitate further statistical analysis. Our goal is to use significant spatial correlation of specific transcription factor (TF) sites as a robust criterion for identification of functional promoters and for screening new genes under similar regulatory pathways. Another approach that explores the possible correlation between certain TF sites and the "nucleosomal" periodicity (Ioshikhes et al.1996) is also being pursued. Our preliminary result demonstrated some prevalence of 10.3 ± 0.2 bp periodicity in putative TF site distribution within promoter regions, which may indicate the important role of the chromatin structure in gene expression.

Identification of Human Core Promoter in Silico

M.Q. Zhang

A core-promoter, approximately from –60 to +40 bp relative to a transcriptional start site (TSS), consists of a minimal DNA element that is necessary and suffi-

cient for accurate transcription initiation in a reconstituted cell-free system. Due to the hierarchical and modular nature of the promoter architecture, we proposed a stepwise strategy: (1) given a large genomic DNA sequence of 100 kb or larger, to localize a functional promoter into a 1–2 kb region; (2) given such an extended promoter region, to further localize a TSS into a 50–100-bp (core promoter) region. Using positional dependent 5-tuple measures, a quadratic discriminant analysis method has been implemented in a new program, CorePromoter. Our experiments indicate that when given a 1–2 kb extended promoter sequence, CorePromoter will correctly localize the TSS to a 100-bp interval approximately 60% of the time. This may be compared with 37% when using a similar promoter finder, TSSG (Solovyev and Salamov 1997), which is based on a linear discriminant analysis. This program will soon become available on World Wide Web.

Computational Study of Upstream Regulatory Elements in Yeast Genes

M. Huang, M.Q. Zhang

Although the complete yeast genome is available, there are still no clues as to the function of 30% of the genes. Since the expression pattern of a gene is closely related to its function, one way to approach the function of a novel gene is to determine which regulatory system controls its transcription. From the literature, we have classified some well-characterized yeast genes into several functional groups. From published upstream regulatory elements, we are in the process of summarizing into specific rules and hope that the function of many uncharacterized genes will be predicted by these rules. By grouping functionally related genes and then aligning their 5′ regulatory region, it is also possible to detect new consensus sequences. Some of them may represent weak regulatory elements that will give us some clues to those genes that lack a strong promoter.

Computational Analysis of 5′- and 3′-untranslated Regions of mRNAs

J. Tabaska, M.Q. Zhang

The goal of this research is to find ways of recognizing the 5′- and 3′-untranslated regions (UTRs), which may then be used as markers to define the ends of genes. Since no one secondary is structure-diagnostic of all mRNAs, we need to look for general structure properties within these molecules. To this end, we have developed a structural potential score (SPS). The SPS for a base in some sequence quantitates both the number and stability of local base pairs in which the base may engage, so that bases which are liable to contribute to stable structures receive high SPSs. Analysis of a large number of mRNAs has shown that bases in the 5′- and 3′-UTRs have significantly higher SPS scores than bases in the coding regions. This is thought to reflect the fact that the coding bases are functionally constrained relative to the UTR bases, so that if there are any structures that are important for mRNA processing or translational regulation, they are most likely to occur in the UTRs. The utility of SPSs as a component of a general gene-finding algorithm is being evaluated.

mRNAs also contain signals involved in cotranscriptional and posttranscriptional processing and in translational regulation. To help us identify possible signals, we use a neural network which, when presented with a set of sequences believed to contain an unknown signal, can "learn" to mimic a protein that binds the signal (Heumann et al. 1994). We can then query the model protein to characterize the signal we were looking for. A variant of this neural network approach can also be used to generate a model that discriminates between true signals and pseudosignals. Using this approach, we have developed recognition models for the binding site of human cleavage stimulatory factor (CstF) and for some U-rich regulatory elements, such as the mRNA destabilizer signal and the cytoplasmic polyadenylation element (CPE). Models have also been developed which suggest that true AAUAAA polyadenylation signals tend to occur downstream from AAUAAA-like sequences, whereas AAUAAAs that do not direct polyadenylation appear to be hidden by upstream pyrimidine-rich sequences. Incorporation of these models into gene-finding programs is being studied, and the search for other signals by which mRNA UTRs may be recognized is ongoing.

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STRUCTURAL BIOLOGY

R.-M. Xu J. Jiang K. Witkin (URP)
M. Hayashi Y. Zhang

We use X-ray crystallography as our principal method to study protein–nucleic acid interaction in gene expressions and molecular assembly in multiprotein complexes. Our current studies focus on proteins involved in pre-mRNA splicing and eukaryotic DNA replication.

PROTEIN-RNA INTERACTIONS IN PRE-MRNA SPLICING

hnRNP A1 is one of the most abundant heterogeneous nuclear ribonucleoproteins, which has been shown to package nascent transcripts for processing, influence alternative 5′ splice site selection, catalyze the annealing of complementary RNA strands, and participate in RNA transport. RNA-protein interaction plays an essential part in these processes. We have undertaken an initiative to understand the structural basis of protein-RNA interactions by hnRNP A1, in collaboration with Adrian Krainer here at CSHL.

Last year, we determined the crystal structure of human UP1, the domain of hnRNP A1 that contains two RNA recognition motifs (RRMs). From that study, we learned that the two RRMs are antiparallel and held together in a rigid structure, enabling the two RRMs to function as a single entity in binding RNA. We are continuing to analyze the structural basis of RNA interaction by hnRNP A1. We have succeeded in obtaining co-crystals of UP1 with two short RNA oligos. Currently, we are optimizing crystallization and data collection conditions for efficient structure determination.

Additionally, hnRNP A/B proteins have been reported to bind to single-stranded d(TTAGGG)$_n$, the human telomeric DNA repeat. The human telomeric DNA repeat sequence shows striking similarity to the high-affinity RNA sequence as determined by in vitro selection, which contains one or more copies of the hexamer sequence r(UAGGGA/U). The similarity in sequence requirements for deoxy- and ribonucleotides in binding hnRNP A1 implies that perhaps the underlying structural basis of sequence-specific interactions with RNA and single-stranded DNA by hnRNP A1 is similar. We have crystallized and solved the structure of UP1 with a 12-mer oligo d(TTAGGGTTAGGG). The complex structure revealed an intriguing mode of nucleic acid binding by hnRNP A1, where one single-stranded telomeric DNA binds to the amino-terminal RRM of one protein and the carboxy-terminal RRM of another (Fig. 1). In the co-crystal structure, two consecutive purines (adenine and guanine) base-stack with two phenylalanines located in the conserved RNP motifs. Arg-92, which is located in the linker connecting the two RRMs, contacts three bases and appears to play an important part in binding the telomeric DNA. The linker between the RRMs is highly conserved both in length and in sequence among hnRNP A/B proteins. Our finding suggests that this high degree of conservation is perhaps evolutionarily selected for the unique nucleic-acid-binding property of hnRNP A/B proteins. It is worth noting that the two protein molecules binding to the same strand of DNA are related by crystallographic symmetry. This poses the question of whether the observed binding mode occurs in solution and whether it is sequence-dependent. This question is being addressed biochemically together with the Krainer laboratory.

Structural study of hnRNP A1 is a part of our thematic investigation of the RRM cooperativity in proteins that contain multiple copies of this RNA-binding module. A significant number of the RRM-containing proteins have multiple copies of RRMs; many of these multi-RRM proteins require contiguous RRMs for wild-type RNA-binding specificity. Cooperative inter-

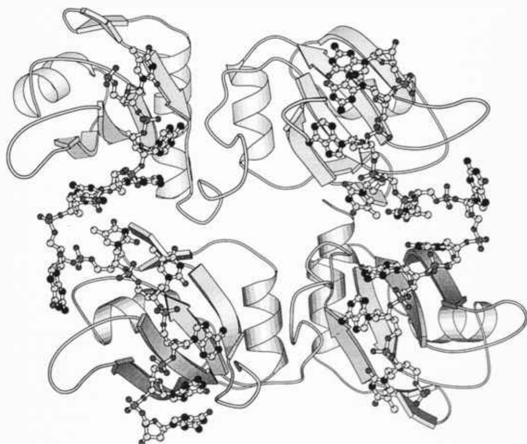

FIGURE 1 Crystal structure of UP1 in complex with human telomeric DNA repeats. Two protein molecules are represented as ribbons, and the single-stranded DNA is represented as ball and sticks.

actions between RRMs are believed to be a general feature of proteins with multiple RRMs. We are interested in understanding how multiple RRMs are spatially arranged and in what way this contributes to cooperativity in RNA binding. We have chosen U2AF65 (in collaboration with Adrian Krainer) and polypyrimidine-binding protein (PTB, in collaboration with David Helfman) for these studies. U2AF65 is the large subunit of the heterodimeric U2AF complex, which is an essential splicing factor required for U2 snRNP-branch point interaction and spliceosome formation. U2AF65 contains three RRMs and binds specifically to the polypyrimidine tract downstream from the branch point. PTB contains four copies of RRM, and it binds to the polypyrimidine tract within the intron. PTB has been shown to be involved in tissue-specific alternative splicing of tropomyosin pre-mRNAs, and that of c-*src* and the $GABA_A$ receptor $\gamma 2$ subunit. It is believed that the binding of PTB to the intronic sequence results in the repression of the splicing of nearby exons, thus leading to the tissue-specific pattern of alternative splicing. We have made some initial progress toward the structure determination of these multi-RRM proteins, and we hope that the successful determination of these structures will shed light on the common phenomenon of RRM synergy in RNA binding.

STRUCTURE AND FUNCTION OF THE SF2-ASSOCIATED PROTEIN P32

Human p32 protein, which co-purifies with splicing factor SF2, is a ubiquitous eukaryotic protein. It has been reported that p32 binds to the HIV-1 Tat and Rev proteins, as well as EBNA-1 of Epstein-Barr virus, and can potentiate *trans*-activation at the viral promoter. In addition, p32 has been reported to bind to the globular head domains of the complement component C1q and inhibits the hemolytic activity of C1q. However, the true physiological role of p32 remains unknown. It has recently been shown that p32 is localized in the mitochondrial matrix, and the disruption of the gene homologous to the human p32 in budding yeast causes growth retardation of yeast cells in glycerol medium, but not in glucose medium. This observation points to the potential role of p32 in maintaining mitochondrial oxidative phosphorylation.

We have undertaken the structural analysis of human p32 (in collaboration with Adrian Krainer), anticipating the structural information to aid in reveal-

ing the function of p32. p32 crystallizes with a $P2_1$ symmetry and cell dimensions of a = 58.63 Å, b = 56.48 Å, c = 93.83 Å, and β = 96°. We have determined the crystal structure using the seleno-methionyl multiple anomalous diffraction method, and the structure is currently being refined to 2.5 Å resolution. The crystal structure shows that p32 forms a trimeric ring. Each p32 adopts an $\alpha\beta$ structure with seven antiparallel β-strands flanked by an amino-terminal and two carboxy-terminal α-helices. The β-sheets from all three monomers form the inner surface of the ring, whereas the α-helices wrap around the ring and form the outer surface. The structure of p32 will be analyzed in detail by comparing it with the existing protein structures in the database. In the end, we hope that our structural analysis will lead to a better understanding of the cellular function of p32.

MOLECULAR ASSEMBLY IN DNA REPLICATION

The ability to replicate the entire genome is essential to all organisms. Recent progress in understanding the initiation of DNA replication in eukaryotes has demonstrated the importance of a six-subunit protein complex, the origin recognition complex (ORC). ORC is required for the recognition of the chromosomal origin of DNA replication and for the cell-cycle-dependent assembly of prereplicative complexes. Sequence-specific interaction with DNA requires ATP and involves several subunits of the ORC complex. The protein complex is quite ornate, having at least two nucleotide-binding sites in different protein subunits, and is regulated by specific DNA sequences.

Being structural biologists, we are fascinated by how large protein complexes are assembled and how individual subunits act together as a single functional unit in some cases, whereas in other cases they exhibit their individualities. ORC provides us with the opportunity to study these phenomena in the context of eukaryotic DNA replication. In collaboration with the Stillman laboratory, we have taken initial steps toward the crystallization of ORC.

UNC69 AND AXONAL OUTGROWTH IN *C. ELEGANS*

In collaboration with the Hengartner laboratory, we are carrying out the structure determination of UNC-69 protein from *Caenorhabditis elegans*. Studies from the Hengartner lab have shown that *unc-69* causes uncoordinated movement phenotype, and UNC-69 is required for proper axonal fasciculation and outgrowth. A human homolog of UNC-69 has also been identified. Interestingly, UNC-69 appears to interact with itself and forms a multimeric complex in solution. We have crystallized UNC-69 fused with a hexahistidine tag at the amino terminus. We are currently optimizing crystallization conditions to obtain bigger crystals for X-ray diffraction experiments.

BEAMLINE DEVELOPMENT AT NATIONAL SYNCHROTRON LIGHT SOURCE

We have made significant efforts in bringing the X26C beamline into operation during the last year. The X-ray crystallography operation at beamline X26C is jointly run by Cold Spring Harbor Laboratory, SUNY at Stony Brook, and the biology department at Brookhaven National Laboratory. Thanks to the work done by everyone from the CSHL X-ray crystallography groups, Malcom Capel (BNL biology), and Grace Shea (University of Chicago), X26C finally went into operation during 1997. The beamline has been heavily used by the CSHL and Stony Brook groups. Further development of the beamline will be necessary to enable full-fledged macromolecular X-ray crystallography operation. Nevertheless, the beamline has already proven to be an important asset for a productive structural biology program at Cold Spring Harbor Laboratory.

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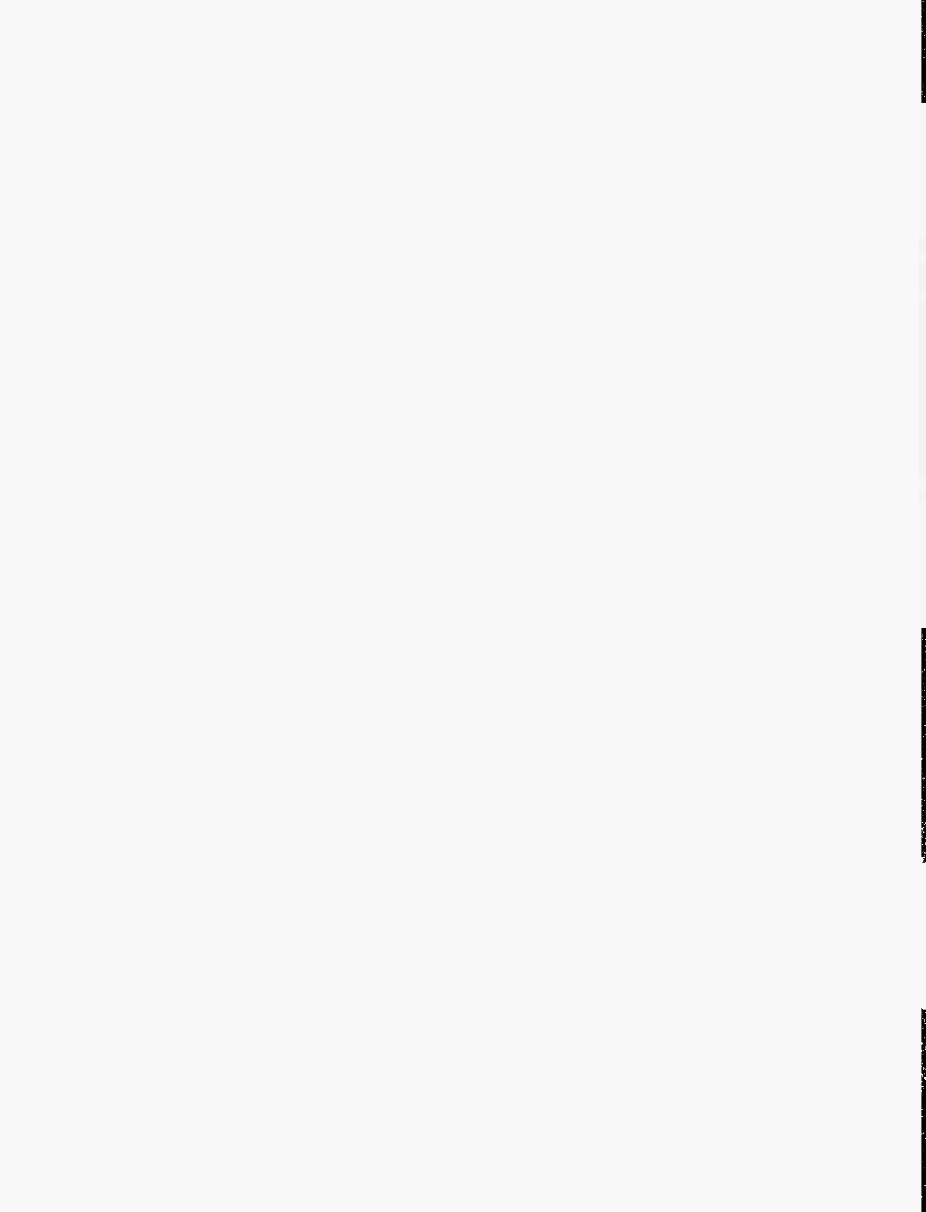

NEUROSCIENCE

Molecular biological research during the last two decades has revealed a remarkable evolutionary conservation of gene function across the animal kingdom. From cell proliferation and patterning of the embryo to the biochemistry of cell signaling, genes of similar sequence have been found to have similar biological roles. This new general law of biology holds as well for the neurosciences.

Continued neuroscience research at CSHL clearly has incorporated this new biological perspective. Single genes are manipulated in almost every experiment, thereby revealing new roles or more intricate details during neuronal development and function. These studies now often cross species boundaries. Enikolopov's group studies nitric oxide (NO) function in human and rat cultured cells and in developing flies and frogs. Malinow's group studies NMDA and AMPA receptor function in rat and mouse hippocampal slices and in the developing frog visual system. Yin's group is studying CREB function in flies and mice. Zhong's group is studying NF1 function in flies and cultured mouse cells. Cline's group is investigating candidate plasticity genes in rats and frogs. In each case, new insight is obtained from the different vantage points of each species' model system. Thus, intellectual synergy emerges from a comparison of gene function among vertebrate and invertebrate species.

As units of biological function, gene products are minute and exert their effects primarily at the cellular level. Hence, a mechanistic understanding of the roles of genes in more complex brain functions, such as neuronal networks or behavior, requires visualization of molecules in cells. To this end, Dr. Karel Svoboda has joined the neuroscience effort at CSHL. He brings with him an advanced, laser-based imaging system, which permits the study of identified molecules in living brain tissue. With this new technology, Svoboda's group has begun to visualize activity-dependent functional and structural changes in the synaptic connections among neurons—with breathtaking resolution.

MOLECULAR NEUROBIOLOGY OF LEARNING AND MEMORY IN *DROSOPHILA*

T. Tully
C. Alexander
G. Bolwig
J. Christensen
J. Connolly
J. DeZazzo
J. Dubnau
T. Gill
S. Gossweiler
M. Hannan
C. Jones
R. Jones
H. Li
J. Medalle
S. Pinto
M. Rahimzadeh
M. Regulski
M. Saitoe
J. Salvatore
P. Smith
B. Svedberg
D. Vaughn
K. Velinzon
S. Xia
A. Chu (URP)
T. Bridges (Eton College)
R. Wlodarczyk (PFF)

Two major projects have been added to our efforts to understand the biological bases of learning and memory in *Drosophila*. First, with support from the John A. Hartford Foundation, we have begun a forward genetic mutagenesis to identify new genes involved in learning and memory. With perhaps 100 genes involved in this form of learning and only 23 identified so far, our new mutagenesis promises to generate the raw material that will fuel further insight to the biological basis of learning and memory. Second, in collaboration with Helicon Therapeutics, Inc., we have begun molecular screens for genes or their products that are differentially regulated during CREB-dependent long-term memory formation. With access to state-of-the-art DNA chip technology and proteomics, this project will enhance greatly our molecular-genetic understanding of long-term memory formation in fruit flies and will allow us to "leap-frog" with such molecular information into the mammalian genome.

Molecular Search for CREB-dependent LTM Genes

T. Tully, J. Dubnau, J. Medalle, S. Gossweiler, J. Salvatore, T. Gill [in collaboration with Helicon Therapeutics, Inc., and J. Yin, Cold Spring Harbor Laboratory]

We have begun to search for genes downstream from CREB that are transcriptionally regulated during LTM formation. Parametric experiments have determined that transcriptional changes of ≥150% can be detected via five RNA extracts from adult heads. Both positive and negative controls have been established with extant genes known to be involved in cellular or behavioral plasticity. Candidate genes X and Y have been identified from a differential screen of a genomic library with whole-head RNAs isolated immediately after spaced or massed training. Via our collaboration with Helicon Therapeutics, we also have begun a differential screen with two-dimensional protein gels. To date, several candidate genes have been identified from both nuclear and cytosolic fractions.

CREB Protein Structure and Function

T. Tully, D. Vaughn [in collaboration with L. Joshua-Tor and J. Yin, Cold Spring Harbor Laboratory]

Ultimately, the modulation of long-term memory formation will involve protein::protein interactions at the molecular level. Since the expression of different isoforms of *dCREB2* is sufficient to modulate LTM formation, these CREB isoforms and their binding partners likely represent one set of interactions. Work on vertebrate CREB has identified CBP as a partner necessary for transcriptional regulation. Moreover, the CREB-binding domain has been localized to an 80-amino-acid region, and the structure of this domain—with a 25-amino-acid CREB phosphopeptide—has been solved by nuclear magnetic resonance (NMR). Both the activator and repressor isoforms of CREB contain this phosphopeptide sequence, suggesting that presentation of this peptide within the overall structural framework of a CREB isoform is what determines whether CREB functions as a transcriptional activator or blocker. To understand better this structural context, we have begun an X-ray crystal structure determination of *Drosophila* CREB.

A significant amount of soluble CREB blocker has been expressed as an amino-terminal HIS-tagged construct in *Escherichia coli*. Several milligrams of soluble blocker have been purified, and screening for crystallization conditions has begun. CREB blocker without a HIS-tag also has been purified. This will allow expression, purification, and ultimately crystallization trials with the native protein.

Identification of New Genes Involved with Associative Learning

T. Tully, C. Jones, S. Pinto, J. Christensen, G. Bolwig, T. Bridges, J. DeZazzo, K. Velinzon, M. Rahimzadeh, C. Alexander, M. Hannan, H. Li, M. Saitoe, P. Smith, B. Svedberg [in collaboration with K. Broadie, University of Utah]

We continue our molecular genetic characterizations of *latheo*, *linotte*, *nalyot*, and *golovan*. We have identified DNA sequence lesions in independently isolated mutant alleles of *latheo*, thereby confirming that we have cloned the correct transcript. Experiments continue to search for proteins that interact with the novel Lat protein. On the basis of fine-scale sequence analysis, we putatively have identified the biochemical nature of *linotte*. Combined with its preferential expression in nerve terminals of the central brain, this gene promises to open important new vistas in our understanding of the biological basis of learning. Behavioral "rescue" experiments have established that the *nalyot* mutation resides in the *Adf-1* gene, which encodes a transcription factor. Chronic expression of *hs-Adf1*$^{+}$ in *nalyot* mutants yields normal learning, whereas chronic expression of *hs-Adf1*$^{+}$ in wild-type flies yields defective learning. Interestingly, Adf1 is expressed throughout the adult brain. These and other data suggest that *nalyot* functions during

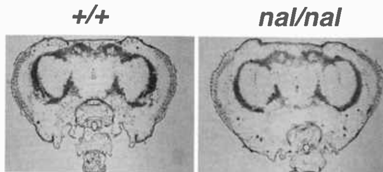

FIGURE 1 Adf1 expression in wild-type (+/+) and mutant (*nal/nal*) adult brains. Note the lower levels in mutant brain.

development to produce normal learning. We now are turning our attention to long-term memory formation in *nalyot* mutants under conditions when learning is normal. Initial results suggest an additional, functional role for *Adf1* when long-term memory is induced. Similar experiments are under way with *golovan*.

Characterization of Extant Mutants

T. Tully, C. Jones, M. Regulski, J. DeZazzo
[in collaboration with U. Heberlein, San Francisco General Hospital]

U. Heberlein's group has shown that *amnesiac* mutants are more sensitive to the anesthesia produced by chronic exposure to alcohol. Induced expression of our *hs-amn*$^{+}$ transgenes rescues this alcohol sensitivity defect in *amnesiac* mutants, thereby indicating that the neuropeptide encoded by amnesiac may be involved in modulating drug sensitivities. Experiments now are under way to determine whether the *hs-amn*$^{+}$ transgene is able to rescue the memory defect of *amnesiac* mutants.

To date, behavioral experiments have failed to demonstrate the involvement of nitric oxide (NO) in fruit fly behavioral plasticity. Wild-type flies fed inhibitors of nitric oxide synthase (NOS) show no reliable defects in olfactory learning or early memory formation thereafter. Possibly this lack of effect is due the inability of flies to ingest enough of NOS inhibitor to have effect. To address this issue genetically, we have tried, to no avail, to generate mutations in the *dNOS* gene. We also have generated various *dNOS* constructs and are screening them in cell culture to identify dominant-negative forms. Transgenic flies carrying such dominant-negative NOS constructs then will be bred ultimately for more behavioral experiments.

Functional Properties of Learning and Memory in Normal and Mutant Flies and in Worms

T. Tully, J. Connolly, R. Wlodarczyk, K. Velinzon, S. Xia, M. Saitoe [in collaboration with M. Hengartner, Cold Spring Harbor Laboratory]

In collaboration with Kendall Broadie at the University of Utah, we have characterized synaptic plasticity at the larval neuromuscular junction of *latheo* mutants. In a manner analogous to *dunce* mutants, *latheo* mutants show increased calcium cooperativity, leading to an enhancement of evoked excitatory junction currents (ejcs); spontaneous mini-ejcs, in contrast, are normal. This defect in basic synaptic transmission yields aberrant synaptic plasticity (paired-pulse facilitation, short-term facilitation, augmentation, and posttetanic potentiation).

Unlike *dunce*, however, no structural defects are observed at the neuromuscular junction. More generally, these data suggest that other learning and memory mutants will show defects in synaptic function. To this end, we have begun to look at *linotte* and *nalyot* neuromuscular junctions.

We also have begun to study plasticity in nematodes. Initial work from D. Van der Kooy's group at the University of Toronto indicates that flatworms are capable of Pavlovian learning using a task analogous to that used with fruit fly larvae. We are trying to replicate these results and then to initiate a study of memory formation thereafter, with particular emphasis on the effects of spaced and massed training. If "homology of function" exists, a mutagenesis in this species will yield new L&M genes more efficiently.

New Genetic Tools

T. Tully, J. Dubnau, R. Jones

We are progressing in our efforts to develop the next generation of genetic tools for *Drosophila*. We have nearly completed building the DNA constructs with which to generate transgenic flies carrying (1) an inducible transgene also with limited spatial expression and (2) a "gene conversion" transgene to attempt in vivo gene replacement. These constructs will be injected into fly eggs presently. Finally, molecular experiments designed to identify EMS-induced point mutations have been less successful, necessitating more extensive analysis.

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LONG-TERM MEMORY FORMATION IN *DROSOPHILA*

J. Yin M. Belvin, J. Bikoff (URP), E. Cahill, J. Daniell, E. Drier, J. Horiuchi, Z. Lin, A. Morotti, J. Nagel, M. Stebbins, M. Tello, J. Wallach, J. Waskewich, P. Wu, H. Zhou

Circadian Regulation of CRE-responsive Transcription

M. Belvin, H. Zhou, P. Wu, J. Daniell

In *Drosophila*, [CRE-TATA-luciferase] transgenic flies exhibit circadian patterns in luciferase activity, whether measured in tissue extracts or in behaving flies (see Fig. 1). There are two peaks in luciferase activity, one during the morning and the other shortly after lights out. These rhythmic transcriptional patterns are regulated through the circadian system. Flies entrained on a 12-hour light:12-hour dark cycle maintain this pattern of transcription when switched to constant darkness, and the periodicity of the pattern is affected by mutations in the clock gene *per*, being shortened in per^s, lenghthened in per^l, and destroyed in per^o mutants. High-level rhythmic transcription is dependent on a consensus CRE site (TGACGTCA), since single nucleotide changes in each of the half-sites drastically reduce activity, although the remaining low-level transcription may still show a hint of rhythmicity. We are currently determining if proteins from the dCREB2 gene regulate this pattern of transcription, and if not, which *Drosophila* CRE-binding proteins are responsible.

We are intrigued by the possibility that this pattern of rhythmic transcription may be involved in the formation of long-term memory (LTM). Since CRE-responsive transcription, mediated by the dCREB2 gene and its various protein isoforms, is critical for the *establishment* of LTM in many organisms, including flies, it is possible that the time of training (relative to the circadian clock) is important for memory formation. In addition, there is a large amount of data in various systems, at the behavioral and cellular levels, that implicate the involvement of parts of the sleep cycle in memory consolidation. It is widely believed that the physical basis for LTM is increased connectivity between the neurons that subserve the particular behavior. Since it is also likely that neuronal circuits become weaker if they are not periodically used ("use it or lose it"), systems-wide mechanisms must exist for reuse and restrengthening of circuits during the *maintenance* of LTM. We are trying to establish whether circadian-controlled, CRE-responsive transcription is the underlying molecular mechanism for this important aspect of memory consolidation.

Subcellular Localization

M. Belvin, H. Zhou, J. Bikoff, M. Tello, P. Wu

When total proteins from flies are subjected to traditional nuclear:cytoplasmic fractionation, two of the major isoforms from the dCREB2 gene appear to be largely cytoplasmic. When certain protein isoforms are overproduced in transgenic flies, they also show preferential subcellular localization, some being predominantly cytoplasmic and others more nuclear.

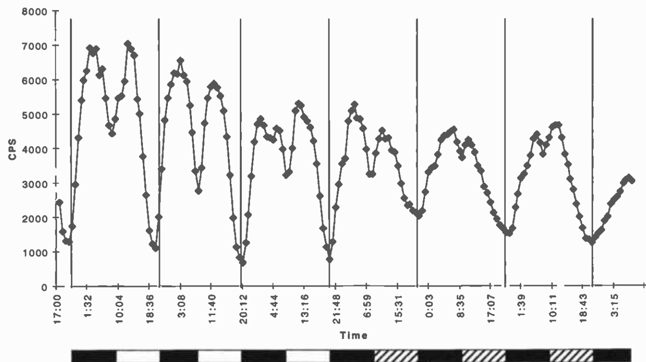

FIGURE 1 Circadian cycling of CRE-luciferase reporter.

These biochemical data are supported by immunocytochemistry, although there are clearly significant levels of nuclear staining on tissue sections.

The dCREB2-b isoform, which we previously have shown to block LTM formation completely and specifically, fractionates largely into the cytoplasm, whereas the dCREB2-a isoform is more nuclear. The dCREB2-a isoform contains three alternatively spliced exons (2, 4, and 6), which are spliced into the dCREB2-b backbone. Exon 2, the most amino-terminal of the alternatively spliced exons, was originally identified using RT-PCR analysis of head RNA. All of these protein isoforms contain extremely good matches to a consensus nuclear localization signal in their bZIP domain, suggesting that the cytoplasmic localization of some of the isoforms may reflect an active, regulated process. Mutation of eight different possible phosphorylation sites, which are conserved across CREB molecules from many species, does not affect the subcellular localization of the dCREB2-a isoform. We are currently investigating the possible phosphorylation sites, signaling pathways, and combination of exons that might control subcellular distribution of these proteins. We are also testing the dCREB2-d isoform, which only contains two of the three alternatively spliced exons, for transcriptional activity in cells, subcellular localization, and its effect on LTM formation. One of the two dominant endogenous bands that our antibodies detect probably corresponds to dCREB2-b, whereas the other band comigrates with dCREB2-d.

The Role of Phosphorylation in Regulating dCREB2 Activity

J. Horiuchi, M. Belvin, H. Zhou

Phosphorylation, notably on the well-conserved Ser-133 residue, regulates the activity of the CREB family of transcription factors. We are interested in the possible roles of the other well-conserved residues on dCREB2 transcriptional activity and LTM formation. Alanine substitution mutations have been placed on all of the known phosphorylation sites on the dCREB2-a and dCREB2-b isoforms, and transgenic flies have been made. In addition, we are analyzing some of these mutations in transiently transfected F9 cells.

We previously reported that induced overexpression of the dCREB2-a isoform enhances LTM formation, accelerating its rate of formation. Subsequent molecular analysis reveals that the transgene used in these experiments contained a sequence rearrangement generated sometime during its manipulation, prior to making the flies. This rearrangement results in premature termination of translation early in the dCREB2-a open reading frame. However, in reticulocytes, this rearranged DNA makes a truncated protein when an internal, in-frame, translation initiation site is used, making a protein of about 28 kD that contains all of the phosphorylation sites and the bZIP domain.

This 28-kD protein is also the protein that shows PKA-responsive, CRE-binding transcriptional activity in transiently transfected F9 cells. When the sequence rearrangement is repaired and expressed in reticulocyte or flies, two proteins are made, one full length (about 40 kD) and the other truncated. We are currently assaying constructs that make the shorter protein only, the longer protein only, or both, in transiently transfected cells, transgenic reporter flies, and transgenic flies for their effects on transcription and LTM formation.

Tetracycline-inducible Expression in Flies and Mice

M. Stebbins, Z. Lin

We have continued to develop our (hopefully) improved version of the tetracycline inducible system. This system consists of two separate transgenes, one carrying the *trans*-activator gene, the other the target gene of interest. We have made transgenic flies and mice carrying various versions of the *trans*-activator (forward or tet "on" in flies and mice, reverse or tet "off" in flies). In flies, the *trans*-activator is being driven ubiquitously by the actin promoter, whereas in mice it is being driven by the spatially restricted αCaM kinase II promoter, which is forebrain-specific. Two different antibodies detect a protein band of the proper size in total extracts from transgenic flies, indicating that both the forward and reverse *trans*-activators are made at detectable levels. We have made transgenic animals carrying target genes in both systems and will use luciferase and *lacZ* targets to evaluate the inducibility of the system, the amount of leakiness in the absence of the antibiotic (for the forward system), the kinetics of induction, and the spatial regions where inducible expression occurs. We have also made a number of target transgenic animals that carry genes of interest, hopefully to apply the system to the study of memory formation in both flies and mice.

Searching for Neurons Where CREB Is Activated during LTM Formation

J. Wallach, H. Zhou

We have used transgenic reporter flies and mice to try to detect CREB activation in response to behavioral training. In flies, we have used and analyzed "simple" reporters (which contain 3–15 CRE sites in front of a TATA box and *lacZ*), as well as complex reporters driven by native promoter fragments from genes that we suspect to be "downstream" target genes of CREB activation (e.g., fos, tyrosine hydroxylase, dCREB2). These reporters are being analyzed for differences in tissue staining from flies that receive spaced, massed, or no training.

In mice, we have a transgenic line that carries a 7xCRE-TATA-*lacZ* reporter. This line shows fairly high basal Xgal staining in the CA1 and CA3 fields of the hippocampus and throughout the amygdala, although the staining in the dentate gyrus region of the trisynaptic circuit of the hippocampus is very low. Kainate injection dramatically induces staining in the dentate region and increases the number and intensity of cells that stain in CA1 and CA3. We are using these mice to look for new staining after behavioral training in cued and contextual fear conditioning.

Mouse Behavior: The Development of Behavior with a Spaced Versus Massed Distinction

J. Wallach, J. Waskewich, J. Nagel [in collaboration with Tim Tully, Cold Spring Harbor Laboratory]

We are investigating behavioral parameters that might allow the identification of a mouse behavioral task which yields a memory difference after spaced versus massed training. Various parameters, such as strain differences, stimulus intensity, duration, and frequency, and behavioral tasks are being investigated.

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NEURAL BASIS OF LEARNING AND MEMORY IN *DROSOPHILA*

Y. Zhong N. Wright Y. Wang L. Luo
F. Hannan Z. Xie J. Sheng
F. Guo J. An J. Tong

Our primary interest is focused on the neural basis of learning and memory by studying *Drosophila* mutants. A number of *Drosophila* mutants have been isolated by their defects in learning, short-term memory, or long-term memory. These mutants provide a unique opportunity to gain insights into neuronal or neural network properties that are important for learning and memory. Currently, we have built our research on two model systems.

First, the larval neuromuscular junction preparation has been used because of its accessibility to electrophysiological analysis of identifiable synapses in *Drosophila*. It allows us to examine how in general synaptic transmission and neuromodulatory responses are altered in the mutants and to identify new components that act in concert or sequentially with the mutant molecules and are potentially important for learning and memory. This study has led us to pursue the biochemical and behavioral significance of the newly identified components. Second, we have developed methods of monitoring neuronal activities from living fly brains, including intracellular recordings and optic Ca^{++} imaging, in response to odors. This preparation allows us to investigate sensory information processes and neural activities more directly related to learning and memory.

K^+ Channels Mediating the PACAP Response

J. Sheng, Y. Zhong

Pituitary adenylyl cyclase-activating polypeptide (PACAP) is a vertebrate neuropeptide that can induce a 100-fold enhancement of K^+ currents in larval muscle cells (Fig. 1). This response is abolished by rut and NF1 mutants, both affecting learning and short-term memory. It has been indicated that this PACAP response is mediated by coactivation of the adenylyl cyclase/cAMP/PKA and Ras/Raf pathways. However, K^+ channel subunits mediating the PACAP response remain to be determined. We investigated a number of K^+ channel mutants. The preliminary results indicate that the PACAP response was normal in all single mutants examined, including Sh, eag, Hk, and slo, and diminished in Sh, slo double mutants, suggesting that Sh-encoded voltage-activated K^+ channels and slo-encoded Ca^{++}-activated channels may mediate the response.

Biochemical Analysis of NF1-dependent Activation of Rut-adenylyl Cyclase

J. Tong, F. Hannan, L. Luo

NF1 is a tumor suppressor gene that encodes a large protein containing a fragment homologous to the catalytic domain of ras-GAP. NF1 causes skin tumors and a variety of symptoms, including specific learning deficits, in human beings, which has been thought mainly to result from the defect of its GAP function. However, previous electrophysiological experiments have suggested that NF1 mediates the cAMP pathway in addition to inhibiting ras activity. Yet, whether and how NF1 affects cAMP synthesis remains elusive because the electrophysiological data only provide indirect evidence. Here, we analyzed how the membrane fraction of adenylyl cyclase activity is affected in NF1 mutants, but the stimulated cAMP synthesis by adding GTPrs was altered by the mutations (Fig. 2). GTPrs induced a strong increase in adenylyl cyclase activity in wild-type flies; the increase of activity was much smaller in NF1 null mutants. This suggests that there are two components of stimulated adenylyl cyclase activities: one NF1-independent and another NF1-dependent. We are currently investigating how

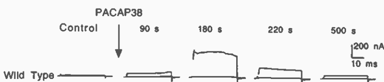

FIGURE 1 PACAP38-induced enhancement of K^+ currents in larval body-wall muscles. The arrow indicates focal application of PACAP38 (5 μM). Control current traces were recorded before application of PACAP38. The time in seconds after pressure-ejection of PACAP38 is indicated above current traces.

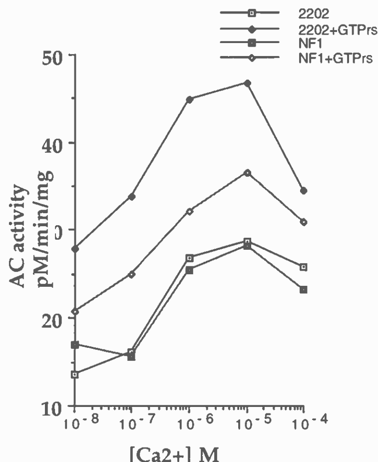

FIGURE 2 GTPrs-stimulated adenylyl cyclase activity is reduced in NF1 mutants. Adenylyl cyclase activity was assayed on the membrane fraction extracted from homogenized abdominal tissues.

mechanistically NF1 proteins affect activation of adenylyl cyclase.

Two-hybrid Screening of NF1 Targets That Mediate the NF1-dependent cAMP Pathway

F. Hannan

Because a yeast homolog of NF1, IRA, has been proposed to bind to the yeast adenylyl cyclase and Ras to mediate Ras-dependent cAMP synthesis, and because of indications from electrophysiological data that NF1 may regulate activation of rut-encoded adenylyl cyclase, we examined the possibility that the NF1 protein binds to Rut-adenylyl cyclase by the yeast two-hybrid analysis. Various combinations of fragments of the coding region of the two genes were cloned into vectors to test their interactions; none were found to give a positive result. Thus, we turn to the second scenario in which NF1 may interact with adenylyl cyclase via an intermediate. We are now setting up the yeast two-hybrid system for screening potential targets of NF1 from a fly cDNA library.

Molecular Basis of Learning Defect in *NF1* Mutants

F. Guo

The observations that NF1 patients, as well as NF1 mouse knockouts, display a learning disability have interested our group from beginning of this project. The finding that NF1 regulates activation of Rut-adenylyl cyclase in *Drosophila* provides a plausible explanation for the molecular basis of the observed learning defects because diminished activity of Rut-adenylyl cyclase in flies and mice disrupts learning and memory. Our investigation of NF1 behavior supports the hypothesis.

Greatly facilitated by the help from Tim Tully's lab, we showed that associative learning is defective (Fig. 3) in *NF1* mutants, whereas the peripheral sensory modalities related to the learning behavior are near normal. This phenotype could be rescued by expressing the normal NF1 protein. The double mutant rut;NF1 did not make the learning score any worse than that in NF1 single mutants. Moreover, the NF1 learning defects can be rescued by expressing the transgenic catalytic subunit of PKA. These results strongly suggest that NF1 is a component involved in learning processes by regulating the cAMP pathway, mainly on Rut-encoded adenylyl cyclase.

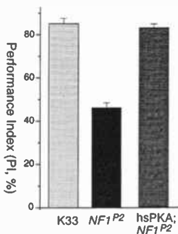

FIGURE 3 Learning defect in NF1 mutants is rescued by expression of transgenic PKA subunit. The performance index is in a scale where 1 indicates a perfect learning and 0 no learning. The learning score was obtained by using the Tully-Quinn procedure.

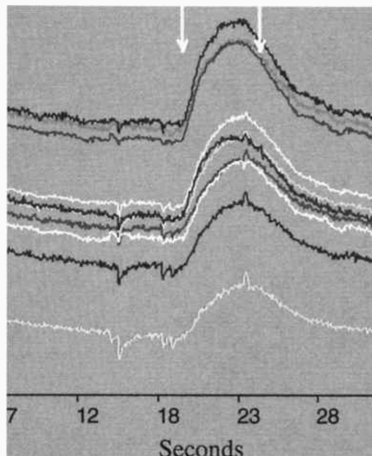

FIGURE 4 Changes in fluorescent intensity induced by odor recorded from mushroom bodies of living flies. Each trace indicates the time course of fluorescent change of different regions in the mushroom body, but recorded simultaneously. The arrows mark the period during which odor EA was puffed.

NF1 Function in Vertebrates

Z. Xie, J. An, L. Luo [in collaboration with A. Silver, Cold Spring Harbor Laboratory]

The data supporting the hypothesis that tumor suppressor NF1 mediates the cAMP pathway are all obtained from studies of *Drosophila* mutants. Whether such a mechanism also operates in vertebrates remains to be examined. Since the mouse NF1 knockouts are available and cultured NF1 mutant neurons exhibit abnormal neurotrophin-independent survival, we used cultured mouse hippocampal neurons to test this possibility. We found that puffs of neurotrophin BDNF or NT3 stimulate new neurite outgrowth from the middle of axons or dendrites. The newly formed neurites were oriented toward the gradient of the puffed neurotrophin and were at the location that was hit by the neurotrophin. This neurotrophin-dependent new neurite formation may be an important mechanism for neural plasticity. Similar neurite outgrowth was also stimulated by forskolin, a drug stimulating adenylyl cyclase activity. We are now investigating whether this neurotrophin-dependent process is altered in NF1 mutant neurons. We will also investigate whether GTPrs-stimulated adenylyl cyclase activity is affected in NF1 mouse knockouts.

Ca^{++} Imaging of Neuronal Activity of the Mushroom Body

N. Wright, Y. Wang

The mushroom body is a distinct CNS structure that is crucial for olfactory-related associative learning in insects, including flies. Since action potentials induce influx of Ca^{++}, which is required for transmitter release, we used change of Ca^{++} concentrations as an indicator of neuronal activity in the brain. A piece of cuticle above the mushroom body region was removed for loading the Ca^{++}-sensitive dye and for recordings of optic imaging. When air-borne odor was delivered to flies, changes of fluorescence intensity were observed around the mushroom body region (Fig. 4). Our current working hypothesis is that the mushroom body encodes information regarding whether a particular odor is attractive or repulsive. We will investigate how this coding of information may be modified by conditioning stimuli and how such modification may be altered in learning and memory mutants.

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ACTIVITY-DEPENDENT NEURONAL DEVELOPMENT

H. Cline R. Bari, B. Burbach, I. Cantallops, A. Demetriades (URP), J. Edwards, Z. Li, I. Miloslavskaya, E. Nedivi, I. Rajan, G-Y. Wu, D-J. Zou

Our laboratory is working toward an understanding of the cellular and molecular mechanisms that control the development of structure and function in the CNS. We perform our studies using the developing visual system of the frog *Xenopus*. We use several approaches to this system which allow us to assess and manipulate the coordinated development of neuronal morphology and synaptic transmission in the CNS. One approach is to use imaging of single retinal axons or tectal neurons in the intact anesthetized animal to observe their morphological development for up to 5 days in vivo with time-lapse confocal microscopy. A second approach is to perform functional imaging, using a calcium-sensitive fluorescent dye, to report changes in intracellular calcium in response to visual stimulus. A third approach is use a whole-brain preparation to take whole-cell patch-clamp recordings from neurons at different stages in the process of maturation. This is one of the only experimental systems that permits direct recordings of synapse formation and maturation in the intact CNS. To manipulate the genetic and protein components of the neurons, we use viral vectors to deliver genes of interest into frog neurons. We are now using this range of techniques to test the hypothesized roles of synaptic activity and activity-regulated proteins in the coordinated development of the structure and function of the CNS.

Stabilization of Dendritic Structure In Vivo by CaMKII

G.-Y. Wu, K. Bronson, I. Miloslavskaya, B. Burbach, R. Bari, H. Cline

Developing neurons must coordinate their morphological and functional development. We showed last year that CaMKII promotes the maturation of retinotectal glutamatergic synapses in *Xenopus*. Whether CaMKII activity also controls morphological maturation of optic tectal neurons was tested using in vivo time-lapse imaging of single neurons over periods of up to 5 days. We divided morphological development of tectal cells into three stages: Stage-1 neurons are newly differentiated. They undergo axonogenesis with little elaboration of the dendritic arbor. Stage-2 neurons are in a phase of rapid dendritic growth. After several days, neurons enter stage 3, a period of slower dendritic arbor growth. Short-interval observations indicate that stage-2 neurons have twice the rates of branch additions and retractions as more mature stage-3 neurons.

To examine the role of CaMKII in tectal cell morphological development, we used CaMKII antibodies to study the time course and distribution of CaMKII expression. CaMKII is expressed in a developmental gradient such that more mature neurons express intense CaMKII-immunoreactivity (CaMKII-IR), whereas younger neurons in caudal tectum have no detectable CaMKII-IR. Neurons first express detectable CaMKII-IR in their somata when they have a total dendritic branch length greater than 300 μm and are more than 3 days old, according to BrdU birthdating. This series of observations indicates that CaMKII expression in tectal neurons correlates with the transition from rapidly growing stage-2 neurons to more stable stage-3 neurons. Dendritic arbor elaboration slows with maturation, in correlation with the onset of CaMKII expression. Elevating CaMKII activity in young neurons by viral expression of constitutively active CaMKII slowed dendritic growth to a rate comparable to that of mature neurons. CaMKII overexpression stabilized dendritic structure in more mature neurons, and pharmacological CaMKII inhibition increased their dendritic growth. Thus, endogenous CaMKII activity limits dendritic growth and stabilizes dendrites and may act as an activity-dependent mediator of neuronal maturation.

These observations on neuronal morphology, taken together with the electrophysiology data, in which it appears that elevated CaMKII promotes the maturation of synapses, suggest the following type of coordinated regulation of neuronal structure and function: When NMDA receptors are active and there is calcium influx, the resultant increase in CaMKII activity has two distinct effects on the tectal cell. One

is to promote the maturation of the synaptic physiology and the other is to deter additional growth of the dendrite at the local site where CaMKII activity was elevated. As a result of the decreased branch addition and branch extension in the tectal neurons, the retinal axons retract the newly added short branch tips that have failed to establish synaptic contacts with target neurons. In our experiments, we increase CaMKII activity throughout the tectal cell, but in the normal neuron, NMDA receptor activity, calcium influx, and CaMKII activity would occur at discrete sites within the arbor corresponding to sites of converging inputs. This would result in highly localized control of synapse maturation and neuronal growth.

The Role of Glutamate Receptor Activity in the Development of Tectal Cell Dendrites In Vivo

I. Rajan, R. Bari, K. Bronson, H. Cline

The model outlined above suggests that glutamate receptor activity will also influence tectal cell development. To test this hypothesis, I. Rajan took in vivo time-lapse images of single DiI-labeled tectal neurons from normal animals and from animals treated with pharmacological blockers of the NMDA and AMPA type glutamate receptors, as well as the sodium channel blocker tetrodotoxin. Exposure to APV (100 μM), CNQX (20 μM), or TTX (1 μM) all blocked the normal development of the tectal cell dendritic arbor. These observations indicate that glutamatergic activity promotes the initial development of the dendritic arbor.

Inhibition of Protein Kinase Function In Vivo

D.-J. Zou, B. Burbach

During the last 2 years, we have generated recombinant vaccinia viruses expressing stable forms of peptide inhibitors specific for two of the major calcium-regulated protein kinases in the brain, CaMKII and PKC. We have determined the relative potency and specificity of peptide inhibitors on the activity of endogenous enzymes by doing kinase assays in tadpole brain homogenates. In the last year, D.-J. Zou has found that inhibition of endogenous CaMKII in tectal neurons increases the elaboration of the dendritic arbor in more mature tectal neurons, without significantly changing the growth rate of more simple neurons. This is consistent with our observations that simple neurons have little CaMKII and are therefore not susceptible to the effect of CaMKII inhibitor. Zou further tested the effect of inhibiting CaMKII in the postsynaptic tectal neurons on the elaboration of the presynaptic retinal axon arbors. His previous work has shown that increasing CaMKII activity in tectal neurons decreased the elaboration of retinal axons, by stabilizing their structure. In these complementary experiments, he has found that inhibition of endogenous tectal cell CaMKII causes an enhanced elaboration of retinal axons.

Candidate Plasticity Genes

E. Nedivi, I. Rajan, H. Cline, R. Bari, K. Bronson, I. Miloslavskaya, G.-Y. Wu [in collaboration with P. Worley, Johns Hopkins University]

The experiments summarized above have allowed us to test the potential function of previously identified and characterized proteins in the control of neuronal development and circuit formation. In an effort to test the function of novel genes and their protein products in developmental plasticity, we have established collaborations with scientists who have isolated activity-regulated genes through differential screening techniques. These screens were performed by E. Nedivi when she was in Y. Citri's lab at the Weismann Institute and by P. Worley's group at Johns Hopkins University. Activity-regulated genes may be induced in retinal ganglion cells or tectal neurons in response to visual activity and thereby promote the further activity-dependent development of the visual system. Experiments mentioned above by I. Rajan indicate that blocking retinotectal synaptic transmission impairs the development of tectal cell morphology. This may occur due to failure to induce activity-regulated genes. The candidate plasticity genes are regulated by neuronal activity and during development. Expression of four candidate plasticity genes in optic tectal neurons in vivo using recombinant vaccinia viral constructs results in four unique phenotypes with respect to neuronal development. Results from one such study are outlined below.

CPG15, a GPI-linked signaling molecule induced by synaptic activity, promotes dendritic growth in

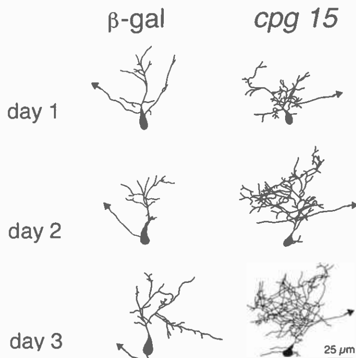

FIGURE 1 Drawings of images collected at daily intervals over 3 days of neurons from animals infected with a vaccinia virus expressing the candidate plasticity gene, *cpg15*, compared to control neurons. Neurons from cpg15-VV-infected animals grow bigger faster than normal neurons. The first image was collected about 24 hr after viral expression of *cpg15* had begun. Experiment performed by E. Nedivi and G.Y. Wu.

vivo. Using time-lapse imaging and virally mediated gene transfer, we demonstrate that CPG15 promotes dendritic arbor growth in vivo. CPG15 accelerates the growth of projection neurons, but has no effect on the growth rate of interneurons. We further show that CPG15, a GPI-linked protein, controls growth of neighboring neurons through an intercellular signaling mechanism. Thus, CPG15 is a signaling molecule capable of translating neuronal activity into structural changes. Our findings posit the existence of a new class of activity-regulated growth factors, which by virtue of being membrane bound, may permit exquisite spatial and temporal control of neuronal growth (see Fig. 1).

Calcium Imaging in Retinal Arbors

J. Edwards, I. Miloslavskaya, R. Bari, H. Cline

We have used in vivo imaging of the calcium-sensitive dye, calcium green dextran, to perform functional imaging of retinal ganglion cell axon activity in response to light stimulus to the retina. We have found that light stimulus to the eye causes changes in calcium levels in the RGC axons within the optic tectum. We tested whether the changes in calcium in the axon terminals were due to stimulation of RGCs. Intraocular application of the sodium channel blocker tetrodotoxin (TTX [10 μM]) blocked the stimulus-induced increase in axonal fluorescence, whereas lower concentrations (100 nM) had no effect. Similar intraocular application of the selective L-type voltage-sensitive calcium channel antagonist nifedipine (1 μM and 10 μM) blocked increases in fluorescence intensity in RGC axon arbors. A second series of pharmacological investigations were designed to determine the mechanism of the calcium elevation in the axon terminals within the optic tectum. Intraventricular injection of caffeine (10 mM) causes a large increase in calcium, indicating that intracellular stores contribute to the calcium signal. Presynaptic nicotinic acetylcholine receptors (nAChRs) may play a part in axon arbor development and retinal topography. Injection of nicotine (10 μM) significantly elevated RGC axonal calcium levels, whereas application of the specific nicotinic-cholinergic receptor blocker α-bungarotoxin (100 nM) blocked the stimulus-evoked rise in axonal calcium. These data suggest that light stimulus to the retina increases calcium in the axon terminal arbors through a mechanism that may include calcium-induced calcium release and could be modulated by nAChRs.

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THE BIOLOGY OF LEARNING AND MEMORY

A.J. Silva O. Carvalho, J. Coblentz, N.B. Fedorov, P.W. Frankland, S. Kida, P. Chen, Y. Elgersma, E. Friedman, K.P. Giese, J.H. Kogan

Our laboratory is studying the molecular and cellular processes that underlie learning and memory. Our approach is to study the behavior and electrophysiology of mice with specific genetic modifications. Below, are summarized our research efforts of the last year.

AUTOPHOSPHORYLATION AT T286 OF THE αMKII IS REQUIRED FOR LTP FOR PLACE CELL STABILITY AND FOR PLACE LEARNING

Long-lasting changes in synaptic strength (such as LTP) are thought to underlie learning and memory. Previous studies in our laboratory showed that the genetic deletion of the α calcium-calmodulin-kinase II (αCaMKII) impairs both LTP and learning. Autophos-phorylation at T286 allows αCaMKII to switch from a CaM-dependent to a CaM-independent state, and J. Lisman and colleagues at Brandeis have proposed that the T286 autophosphorylated (constitutively active) state of CaMKII is crucial for LTP and learning. Consistent with the model, previous results showed that both LTP induction and learning trigger a long-lasting increase in the autophosphorylated form of CaMKII. These studies, however, did not demonstrate that the autophosphorylation of CaMKII is required for either LTP or learning. To determine whether the autophosphorylation of αCaMKII at T286 is required for LTP and learning, we substituted T286 for alanine (T286A) with a novel gene-targeting procedure. The T286A mutation results in mutant mice (αCaMKII T286A) with a kinase that is unable to switch to its constitutively active state.

LTP was tested in the αCaMKIIT286A mutants both with extracellular field recordings and with whole-cell recordings (pairing protocol) in the CA1 region of hippocampal slices. This analysis revealed that NMDAR-dependent LTP is especially impaired in the mutants. In contrast, basal synaptic transmission, NMDAR function, and several forms of short-term plasticity are normal in these mutants. Our studies with the water maze also showed that the autophosphorylation of αCaMKII is required for spatial learning, a hippocampus-dependent task. During training, the mutants needed more time than wild-type mice to locate the platform, and during a transfer test given at the end of training, the wild-type mice searched selectively for the platform, whereas the mutant mice did not. In contrast, the mutants could learn a hippocampus-independent task, the visible platform version of the Morris water maze, indicating that they had the vision, motivation, and motor coordination required for learning in the water maze. Altogether, these results show that the autophosphorylation of αCaMKII at T286 is crucial for hippocampal LTP and hippocampus-dependent learning.

In the hippocampus, spatial representations are thought to be mediated by "place cells," neurons that fire selectively when an animal is in a specific location. LTP may underlie the fine-tuning and stability of firing patterns in place cells. Y. Cho, a post-doc in the laboratory, went to Howard Eichenbaum's laboratory, and there she compared the properties of place cells in two mutants with different severities of impairment in LTP and place learning: the αCaMKIIT286A mice, and mice deficient for the αΔ isoform of the cAMP-responsive element binding protein ($CREB^{\alpha\Delta-}$). Place cells from both mutants showed decreased selectivity in spatial tuning, suggesting that the spatial maps of these mutants were of "lower" resolution than in controls. In addition, consistent with the idea that stable long-lasting changes in synaptic strength underlie the stability of place maps in the hippocampus, the αCaMKIIT286A mutants with severely abnormal LTP also showed profoundly unstable place fields, the functional units that make up hippocampal place maps. Consistent with these findings, the $CREB^{\alpha\Delta-}$ mutants, which have milder LTP deficits, also have milder impairments in place field stability and in spatial learning. Together, these results indicate that LTP is important for the fine-tuning and stabilization of place cells, and these properties are key to place learning.

THE HIPPOCAMPUS IS ESSENTIAL FOR CONTEXT DISCRIMINATION, BUT NOT FOR CONTEXT RECOGNITION

Mutations can cause very specific behavioral deficits. This specificity is important because it can often suggest which brain regions should be used to search for the mechanism underlying the learning deficit of the mutants. For example, many of the mutants we have studied have been analyzed in contextual fear conditioning, a form of associative learning in which animals learn to associate an aversive event with a specific context. Previously, it has been proposed that the hippocampus is required for forming a polymodal representation of context. However, recent studies have shown apparently normal contextual fear conditioning in hippocampus-lesioned animals. This uncertainty complicates the interpretation of contextual conditioning studies of mutant mice, and therefore we decided to address this problem. Our recent studies showed how (1) the timing of hippocampal lesions and (2) the behavioral/representational demands of the task affect the requirement for the hippocampus in contextual fear conditioning. Our results showed that whereas lesions given after training abolished contextual fear conditioning, lesions given before training did not. Thus, animals lacking a functional hippocampus can be trained to recognize an aversive context, whereas lesioning the hippocampus of trained mice completely abolished their ability to recognize the aversive context in which they were trained. First, these results demonstrate that an animal's ability to recognize an aversive context can be mediated by both hippocampal and nonhippocampal neural systems; in contrast with previous reports, a functional hippocampus does not seem to be essential for contextual fear conditioning in mice. In the absence of the hippocampus, conditioned responding may be mediated by hippocampus-independent learning, such as cue/shock associations mediated by other brain regions (e.g., amygdala and neocortex). Second, our results indicate that strategies mediated by nonhippocampal systems, such as cue/shock associations, are normally suppressed by the hippocampus. If there were no suppression under normal conditions, and memory of the aversive context were processed in parallel by hippocampal and nonhippocampal systems, then lesions given after training would have the same effect as lesions given before training. It is important to note that although our studies showed that the hippocampus is not essential for contextual conditioning, they demonstrated that normally this structure is involved in contextual fear conditioning (i.e., lesions given after training abolish conditioned freezing responses).

In contrast with contextual conditioning, even hippocampal lesions given prior to training disrupted context discrimination, a novel procedure where mice are trained to discriminate between two similar chambers: one in which the animals receive a foot shock, and another in which they do not. These data dissociate hippocampal and nonhippocampal contributions to contextual fear conditioning, and they provide direct evidence that the hippocampus has an essential role in the processing of contextual stimuli. Although nonhippocampal systems can support recognition of an aversive context, they are inefficient at discriminating between two similar contexts.

These results also offer an explanation for the seemingly paradoxical results we obtained with the NMDAR/Nf1 compound heterozygous mice. These mice have hippocampus-dependent spatial learning deficits, but seemingly normal contextual conditioning. However, tests of contextual discrimination revealed a pronounced deficit in these mutant mice. The phenotype of these mutants parallels the results obtained with hippocampal lesioned mice, suggesting that the NMDAR/Nf1 mutation blocked hippocampal function. The resolution of this discrepancy demonstrates the importance of neuroanatomical lesion studies for the interpretation of experiments with mutant mice.

THE N-RAS HETEROZYGOUS MUTATION RESCUES THE LEARNING DEFICITS CAUSED BY THE NF1 HETEROZYGOUS MUTATION

Specific learning disabilities are the most common neurological complication in children with neurofibromatosis type 1 (NF1), a neurological disorder that affects 1/4000 people worldwide. The complexity of these cognitive deficits, and the difficulty of pursuing their study in patients, motivated us to study them in mice mutant for the neurofibromin gene ($Nf1^{+/-}$). Additionally, uncovering the causes for the learning deficits in Nf1 mutant mice will reveal molecular, cellular, and neuroanatomical substrates of learning and memory. We have previously shown that the $Nf1^{+/-}$ mice are a model for the learning disabilities caused by the mutation of the NF1 gene in humans. Neurofibromin, however, is a large protein that may have multiple functions. Biochemical studies showed that it is a Ras GTPase-activating protein. Additionally, recent *Drosophila* genetic studies uncovered evi-

dence suggesting that neurofibromin regulates (directly or indirectly) the activity of adenylate cyclase. To investigate whether the learning deficits of the Nf1 heterozygous mutants are caused by abnormally high Ras activity, we tested whether a decrease in N-Ras levels ameliorates/rescues the learning deficits of the Nf1 heterozygotes. Thus, we tested Nf1 heterozygotes, N-Ras heterozygotes, Nf1/N-Ras compound heterozygotes, and their wild-type littermates in the Morris water maze. Consistent with our previous results, the Nf1 mutants showed clear deficits in the spatial version of the Morris water maze. In contrast, the Nf1/N-Ras compound heterozygous mutants performed normally throughout training and testing. Just like wild-type controls, the Nf1/N-Ras mice learn the task after only 5 days of training (6 trials per day): The time required by the mice to reach the platform during training and the specificity of their searches in probe trials were indistinguishable from those of controls. Additionally, our results showed that the N-Ras heterozygous mutation does not affect performance in the Morris water maze. Similar results with other behavioral tasks confirmed that the N-Ras heterozygous mutation rescues the spatial learning impairments caused by the Nf1 mutation and suggest that the learning deficits observed in the Nf1 heterozygotes are caused by abnormally high Ras signaling. Current studies are addressing the cellular implications of these behavioral results.

DEVELOPING INDUCIBLE AND REGION-RESTRICTED KO SYSTEMS: THE CRE/LBD-ESTROGEN AND TET INDUCIBLE SYSTEMS

In the last year, we have tested the Cre/LBD-estrogen inducible system in mice. Cell culture studies showed that fusions of the LoxP/Cre site-specific recombinase with the ligand binding domain (LBD) of a human mutant estrogen receptor confer hormone-dependent regulation to the activity of that recombinase. Our laboratory derived 12 transgenic lines for a vector designed to direct the expression of Cre-LBD (from the estrogen receptor with the G525R mutation) to the hippocampus using regulatory sequences of the αCaMKII gene, a forebrain, postnatally expressed, and neuron-specific promoter highly expressed in the hippocampus. In addition, we derived mice that contain a *loxP*-flanked (floxed) *neo* gene within the αCaMKII gene. We have already tested 7 of the 12 lines generated, and so far we have not seen any deletion of the floxed gene with a variety of induction protocols. To confirm that the floxed *neo* allele can be deleted by Cre recombinase, we have deleted it in ES cells transfected with Cre. Currently, we are selecting for transgenic lines that express high levels of the fusion protein.

In addition to the LBD-Cre system, we are also testing tetracycline-regulated gene expression. This system (tet system) has become an important tool for the analysis of gene function in a variety of species. This system normally consists of two components, one encoding the *trans*-activator protein under control of a promoter (in our case we used the αCaMKII promoter), and the second component is the tet-operator minimal promoter controlling the gene of interest (in our case Cre recombinase). The tet-mice that we have generated (5 lines) were crossed with mice with two *loxP* sites flanking a *neo* gene in the αCaMKII locus. We are currently using these transgenic mice to test the efficiency of Cre-mediated deletion of the floxed allele. Preliminary results suggested inducible Cre-mediated deletion in one of the transgenic lines tested. We are currently confirming and characterizing in detail this observation.

Another system that we have tested involves transgenic mice (4 lines) that we have derived with Cre recombinase under the regulation of the αCaMKII promoter. This simpler noninducible system allows the deletion of genes only in regions of the brain that express the αCaMKII gene (forebrain neurons of the postnatal brain).

DERIVATION OF LOXP FLANKED ALLELES FOR RAF-B AND KV6.2

The Cre-mediated gene deletion systems described above work on genes/exons flanked by *loxP* sites. The Cre recombinase recognizes these sites and deletes DNA sequences between them. This past year we have generated lines of embryonic stem cells with *loxP*-flanked genes: Raf-B kinase and Kv6.2 potassium channel. The Raf-B kinase is activated by Ras function and is highly expressed in the brain. The Kv6.2 potassium channel is in axons of CA3 and dentate gyrus neurons of the hippocampal formation, and it changes the I/V kinetics of other channels.

DEVELOPMENT OF CREB-INDUCIBLE AND CREB-REPORTER TRANSGENIC MICE

To test whether the LBD system could regulate the expression of the cAMP responsive element binding

protein (CREB), our laboratory has derived three types of transgenic mice: CREB-mutant inducible mice, ICER-inducible mice, and CRE-promoter LacZ/GFP mice (CREB-function reporter mice). To derive mice in which the function of a mutant CREB is under inducible and tissue-restricted regulation, our laboratory designed a construct in which a mutant version of the CREB gene (at Ser-133) was fused to the mutant LBD described above. Cell culture studies done in M. Montminy's laboratory showed that CREB's transcriptional function can be regulated when fused with LBD-estrogen. In our laboratory, the LBD/CREB fusion protein was placed under the regulation of the forebrain αCaMKII promoter. We have tested one of the four transgenic lines generated and failed to see any evidence of CREB repression with this transgene. Constitutive expression of this transgene is known to repress CREB transcription in mice. The other three lines are currently being tested. We have also derived similar transgenic mice with ICER, a CREB repressor (two lines; others under derivation). These lines are currently being tested. To test CREB function in the inducible transgenics described, we have also created a transgenic mouse with a β-galactosidase/green fluorescence protein fusion (LacZ/GFP) under the control of a cAMP responsive element (CRE) containing promoter. For better temporal resolution of the induction events, the LacZ/GFP gene also included sequences that accelerate the degradation of its protein product.

MASSED VERSUS SPACED TRAINING EFFECTS ON PROTEIN SYNTHESIS INDEPENDENT LTP AND MEMORY

Studies from a number of laboratories, including our own, demonstrated the advantages of spaced training in the formation of long-term memory (protein-synthesis-dependent). In the last year, we discovered that memory that is not protein-synthesis-dependent is also sensitive to the effects of massed versus spaced training. Mice were trained on a contextual conditioning task with two trials separated by either 6 or 60 seconds, and they were then tested 2 hours after training. Our studies had shown that anisomycin, a protein synthesis blocker, does not impair memory 2 hours after training, but that it does block it 24 hours later. Surprisingly, we found that the animals trained with a 60-second interval between trials performed better than those trained with only a 6-second inter-trial interval. These results show that spaced training improves memory that is not protein-synthesis-dependent, demonstrating that spaced training can affect mechanisms other than transcription (e.g., synaptic mechanisms).

Previous findings from our laboratory revealed that the $CREB^{\alpha\Delta-}$ mutation caused a profound loss in long-term memory, even though short-term (30 minute) memory triggered with a single trial was unaffected, a result that we have confirmed recently. In the last year, we found that memory tested 2 hours after training (middle-term memory; anisomycin insensitive) is also affected by the CREB mutation. Interestingly, recent results reported by Kandel and colleagues suggested that besides its role as a transcription factor, CREB may also have a cytoplasmic role in protein-synthesis-independent memory formation as a modulator of cellular signaling. Thus, the CREB mutants have deficient middle-term memory, a result consistent with a cytoplasmic role for CREB in memory formation.

Previous results from our laboratory found that the long-term memory deficit of the $CREB^{\alpha\Delta-}$ mutants could be rescued by lengthening the interval between training trials. One-hour intervals triggered seemingly normal memory, whereas 1-minute intervals failed to trigger long-term memory in the mutants. These results, together with similar results in other species, indicated that CREB has a critical role in determining training schedules required for long-term memory formation. To determine whether CREB could also have a role in determining training schedules required for protein-synthesis-independent memory, we tested the impact of massed and spaced training on 2-hour memory (middle-term memory) in the $CREB^{\alpha\Delta-}$ mutants. Interestingly, spaced training (1-hour inter-trial interval) rescues the middle-term memory deficit of the mutants just as it rescued their long-term memory deficits. In contrast, massed training (1-minute inter-trial interval) does not rescue the deficits in either of these two memory phases. These data suggest that CREB is a key factor in determining the training schedules required for the formation of both middle and long-term memory.

In striking parallel with the behavioral results described above, we found that spaced tetani (10 minute) also rescue the LTP deficits measured both minutes (protein-synthesis-independent) and 5 hours (protein-synthesis-dependent) in the $CREB^{\alpha\Delta-}$ mutants. Altogether, these findings involve CREB function in two distinct memory phases, and they indicate that the hippocampal LTP deficits of the mutants underlie their hippocampus-dependent memory impairments.

THE CREM GENE IS INVOLVED IN SPATIAL LEARNING AND MEMORY

Previous results from our laboratory involved the CREB gene in learning and memory. CREB is known to form functional heterodimers with the cAMP response modulator (CREM), a closely related member of the CREB family of transcription factors. Thus, we suspected that CREM may also be involved in learning and memory. Consistent with this hypothesis, we found that the CREM null mutants have impaired spatial learning in the Morris water maze.

In the last year, we have also found that the CREM gene appears to rescue the spatial learning deficits of the $CREB^{\alpha\Delta-}$ mutants. Analysis of memory formation in the $CREB^{\alpha\Delta-}$ mutants showed that not all of the mutants are equally affected by the loss of the CREB $\alpha\Delta$ isoforms. Instead, we found that some of the $CREB^{\alpha\Delta-}$ mutants had nearly normal memory. Therefore, we examined whether up-regulation of the CREM gene could be responsible for the partial rescue of memory in these $CREB^{\alpha\Delta-}$ mutants. Immunocytochemical studies showed that all of the $CREB^{\alpha\Delta-}$ mutants showed increases in the levels of CREM, but these increases were more pronounced in the mutants with nearly normal memory. Analysis of the dentate gyrus and of CA1/CA3 regions of the hippocampus showed that the levels of CREM in these regions were directly proportional to the performance of the $CREB^{\alpha\Delta-}$ mutants in the spatial version of the water maze, a hippocampal-dependent task. RT-PCR analysis and RNase protection assays showed that both activator and repressor products of the CREM gene were up-regulated in the hippocampus of $CREB^{\alpha\Delta-}$ mutants. Thus, the loss of CREM affects spatial learning, and increases in the expression of this gene appear to compensate for the loss of the CREB $\alpha\Delta$ isoforms. Altogether, these results indicate that CREM is involved in learning and memory.

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TRANSMISSION AND PLASTICITY IN MAMMALIAN CENTRAL SYNAPSES

R. Malinow N. Dawkins M. Maletic-Savatic
J. Esteban J.-C. Poncer
Y. Hayashi S. Zaman
A. Laskey Y. Aoki (visiting medical student)
S. Shi

We address issues directed toward an understanding of learning and memory by studying the physiology of synapses. We study synaptic transmission and plasticity in rodent brain slices which are complex enough to show glimpses of emergent properties, as well as simple enough to allow hard-nosed biophysical scrutiny. To monitor and perturb the function of synapses, we use a combination of electrophysiology, microscopic imaging, and viral transfection techniques. This allows us to examine the cell biological basis for changes in electrophysiological function. It is our philosophy that synapses have key properties whose understanding will provide insight into phenomena at higher levels of complexity.

Probing Mechanisms of Plasticity with Use-dependent Synaptic Blockers

Z. Mainen [in collaboration with John Roder, University of Toronto]

The mechanisms responsible for enhanced transmission during long-term potentiation (LTP) at CA1 synapses remain elusive. Several popular models propose an increase in use of existing synaptic elements, e.g., increased probability of transmitter release or increased opening of postsynaptic receptors. To test these models directly, we have employed a GluR2 knockout mouse in which AMPA-R transmission is sensitive to use-dependent block by polyamine toxins. We developed a method that can detect increased release probability or AMPA receptor channel opening, but find no such changes during LTP. Our results suggest that previously documented enhancement of quantal size is due to increased number or conductance of AMPA receptors and that enhanced quantal content reflects activation of previously silent synapses. In general, these results argue against increased use of previously functioning structures and indicate recruitment of new functional synaptic elements during LTP. The methods developed in this study will provide tools by which to understand modulation of transmission by activity-induced and pharmacologically induced plasticity.

Delivery of Glutamate Receptors to Synapses during LTP

Y. Hayashi

One possible mechanism by which LTP can be expressed is the rapid delivery of receptors from regions not accessible by transmitter (either intracellular storage or extrasynaptic dendritic surfaces) to the synaptic surface. One method of testing this possibility is to use tissue from mice in which GluR2 is genetically deleted, and to acutely express GluR2 with Sindbis-GluR2. AMPA receptors lacking GluR2 have a clear electrophysiological phenotype: Agonist-activated currents show rectification. In mice lacking GluR2, synaptic responses show such a phenotype. We can infect neurons in hippocampal slices from GluR2$^{-/-}$ mice with a Sindbis virus that expresses GluR2-EGFP. In infected cells, we see the appearance of nonrectifying synaptic responses, indicating the appearance of GluR2-containing AMPA receptors to synapses. The amount of GluR2-containing AMPA receptors at synapses increases over the several days after infection, which provides information regarding the time course of receptor turnover. To determine if newly synthesized receptors are delivered to synapses during LTP, we can test whether LTP increases the amount of nonrectified synaptic current.

Is Phosphorylation of the GluR1 Subunit of AMPA Receptors Required to Generate LTP?

Y. Hayashi [in collaboration with Hannah Monyer and Peter Seeburg, Heidelburg]

GluR1 is a common subunit to most AMPA receptors on pyramidal neurons in CA1 hippocampus. There are several phosphorylation sites on GluR1. Ser-831 is hypothesized to be phosphorylated by endogenous CaMKII at synaptic sites. Such phosphorylation in recombinant expression systems has been shown to increase the function of AMPA receptors. We want to test the possibility that phosphorylation at this site is necessary to generate LTP. Toward this end, we have designed several experiments, in collaboration with H. Monyer and P. Seeburg. Seeburg and colleagues have generated mice in which GluR1 is deleted. We have made constructs with point mutations (or no mutations, as controls) in GluR1. These have been inserted by H. Monyer into the genome of the GluR1 KO mice. We will test if such mice (expressing only mutant GluR1) lack LTP. We can also do such experiments on acute slices by acute expression of GluR1 (introduced by recombinant viruses) in slices from the GluR1$^{-/-}$ mice.

Dendritic Trafficking, Clustering, and Synaptic Delivery of AMPA Receptors in Hippocampal Neurons

S. Shi, J. Esteban, Y. Hayashi

Using GFP-tagged subunits of AMPA receptors, we have examined the spatial, temporal, and activity-dependent characteristics of recombinant AMPA receptors expressed in hippocampal neurons. Using HEK293 cells, we first established that GluR1 with enhanced GFP tagged on the amino terminus (the expected extracellular domain) is electrophysiologically functional. Upon infection of dissociated cultured hippocampal neurons with Sindbis virus expressing GluR1-GFP, we see expression within 6 hours that increases over the next 48 hours. The distribution of receptor is initially diffuse and becomes more clustered over time. Using immunohistochemistry, we can determine the amount of receptor that is exposed to the surface (~50% at 24 hours) and the amount of clustered receptor that appears at synapses (colocalization with the synaptic marker, synapsin I; ~50% of clustered GluR1-GFP is within 1 μm of synapsin I-positive regions). With immunohistochemistry directed against GluR1, we can also determine the level of overexpression of GluR1 produced by the virus. At the cell body, the value is 10- to 30-fold; this falls monotonically to about 2-fold overexpression at sites on dendrites 100 μm from the cell body. One interesting observation is that despite the different levels of overexpression, the amount of receptor exposed at the surface is constant; indeed, it appears to be no greater in infected cells than in uninfected cells. This indicates that delivery to the surface is a saturable/regulated process that "keeps track" of how much receptor is at the surface. We are conducting similar experiments on organotypic hippocampal slices. We are currently testing whether such surface delivery is controlled by neuronal activity and/or protein kinase activity.

Silent Synapses: Electron Microscopic Analysis

J. Esteban [in collaboration with R. Wenthold and R. Petralia, National Institutes of Health]

The synapses we study (which are similar to and serve as a model for most excitatory synapses in the vertebrate brain) use glutamate as the neurotransmitter and have two types of postsynaptic glutamate-sensitive receptors: AMPA-type and NMDA-type. Roughly speaking, AMPA receptors transmit all presynaptic release events, whereas NMDA receptors require intense activity and trigger plasticity. We and other investigators have provided electrophysiological evidence that initially formed synapses contain only NMDA receptors and, through plasticity, AMPA receptors are added. There are other models, however, to explain the physiological results. To shed light on this issue, we have examined the distribution of AMPA and NMDA receptors using immunogold electron microscopic analysis. We have examined tissue from the hippocampus of rats at day 2, day 10, and adult using five antibodies against AMPA receptors and three antibodies against NMDA receptors. We find that in general, AMPA receptors increase in number during development, whereas NMDA receptors remain constant. Furthermore, the frequency of synapses with no AMPA receptor immunoreactivity is

83% at day 2, 71% at day 10, and 50% in adult. Since immunogold techniques are low-detection methods, we have used several computational methods to estimate the fraction of synapses without AMPA receptors. After compensating for low detection, the fraction of synapses lacking AMPA receptors is 75% at day 2, 51% at day 10, and 30% for adults.

Silent Synapses: Calcium Imaging Analysis

Z. Mainen [in collaboration with Karel Svoboda, Cold Spring Harbor Laboratory]

We and other investigators have observed electrophysiological responses mediated purely by action on NMDA receptors. One interpretation is that there are synapses that have only NMDA receptors (examined above). Another interpretation is that sufficient transmitter can leak out of synapses to activate only NMDA receptors on nearby synapses. The higher affinity of NMDA receptors for glutamate, compared to that of AMPA receptors, makes this scenario plausible. To examine the biophysical basis for pure NMDA responses, we are monitoring the stochastic responses of single synapses that are positioned next to each other. If there is transmitter leakage, nearby synapses should respond synchronously. If there is no leakage, synapses should respond independently.

Interactions between Short-term and Long-term Plasticity

J.-C. Poncer

If synapses are activated twice within approximately 100 msec, the second response is generally different in amplitude from the first. At some synapses, the second response is larger (paired-pulse facilitation), whereas at other synapses, the second response is smaller (paired-pulse depression). This short-term plasticity has been studied extensively at a number of synapses and likely reflects properties of the presynaptic transmitter release and recycling machinery. There have been reports that such short-term plasticity changes during LTP. Does this indicate that LTP produces changes in presynaptic function? We find that changes in short-term plasticity during LTP can be explained by recruitment of previously silent synapses. This

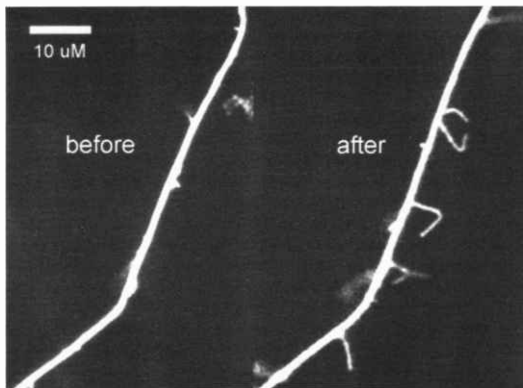

FIGURE 1 Dissociated cultured hippocampal neuron infected with GFP-Sindbis virus imaged with epifluorescence and cooled CCD camera. Before (*left*) and 20 min after (*right*) transient rise in intracellular calcium produced by application of calcium ionophore A23187. Note selective neuronal infection (underlying glia are not infected) and growth of new processes with calcium stimulus.

recruitment may occur by addition of AMPA receptors to synapses containing only NMDA receptors.

Rapid Structural Changes in Hippocampal Neurons Induced by High-Frequency Synaptic Stimulation

M. Maletic-Savatic [in collaboration with
Karel Svoboda, Cold Spring Harbor Laboratory]

A central issue regarding activity-induced synaptic plasticity is: Are there structural changes that accompany or even underlie changes in synaptic efficacy? To address this possibility, we have examined in living hippocampal slices the structure of GFP-expressing neurons with two-photon laser scanning microscopy before and after intense synaptic stimulation. We have generated a Sindbis virus that expresses enhanced GFP. When injected into hippocampal slices, this virus selectively infects neurons (~100:1 selectivity over glia) (Fig. 1). When examined 24 hours after infection, GFP appears to fill the entire cell homogeneously, including dendrites, spines, filapodia, and axonal processes. With two-photon imaging, we place a stimulating electrode close to a GFP-labeled dendritic process. After several baseline images, a high-frequency stimulus (similar to one used to induce LTP) is delivered, and several more images are obtained. Our initial observations are that there is a rapid growth of new structures, both spines and filapodia. Such growth appears to be blocked by the NMDA-receptor antagonist, DL-APV. These observations raise many interesting questions: (1) What is the relationship between the structural changes and the electrophysiological changes? (2) Are the new structures synapses? (3) Do the new structures contain NMDA receptors? AMPA receptors? On the surface or intracellularly? (4) Does the new growth require protein synthesis? New transcription? (5) Are the new structures due to growth of existing synapses contacting the dendrite, or completely new synapses? (6) Is a rise in intracellular calcium at the growing site necessary/sufficient to produce this growth? (7) What signal transduction cascades are necessary/sufficient? (8) Is dendritic exocytosis necessary to produce growth?

Electrophysiology of Transgenic Mice Carrying Mutated Presenilin 1

S. Zaman, A. Laskey [in collaboration with
Sangram Sisodia, Angele Parent, David Borchelt,
Johns Hopkins University]

A protein which when mutated is responsible for approximately 50% of familial Alzheimer's disease, presenilin-1 (PS1), was recently cloned. Little is known regarding its cellular distribution and function. We are now studying the electrophysiology of mice expressing a mutant form of PS-1 that is known to cause disease in humans. Preliminary studies indicate that various properties of synaptic transmission and plasticity in these mice show subtle abnormalities. We are defining the stimulus protocols that best show these abnormalities.

In conclusion, we are continuing to elucidate the basic mechanisms involved in central synaptic transmission. Such an understanding is necessary to derive a mechanistic flowchart of plastic processes. We also continue to probe the role of different molecules, including CaMKII and PS-1, in activity-induced and developmental synaptic plasticity.

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SIGNAL TRANSDUCTION AND DIFFERENTIATION

G. Enikolopov A. Hazan, S. John, P. Krasnov, B. Kuzin, C. Mannino, T. Michurina, J. Mignone, N. Nakaya, M. Packer, N. Peunova, V. Scheinker, Y. Stasiv

Our main interest is in signals that link cell activity with cell differentiation. In the past year, our research mostly focused on the role of nitric oxide (NO) in development. We are testing a hypothesis that NO acts as an antiproliferative agent and, ultimately, controls the shape and size of tissues and size of organs during normal development. We started several projects with different models of animal development, and our current results support the notion of NO as a critical component of the developmental program. We are now focusing on genetic and biochemical interactions of NO with systems that control cell division and cell differentiation in the developing organism.

NO and Rb Pathways Interact to Control Eye Development in *Drosophila*

B. Kuzin, G. Enikolopov [in collaboration with M. Regulski and T. Tully, Cold Spring Harbor Laboratory]

NO can reversibly suppress DNA synthesis and cell division in most cell types; however, the mechanisms of the antiproliferative action of NO in developing organisms are not known. We used a genetic approach to address this problem and tested the genetic interactions of NOS with various components of the cell cycle. We used transgenic flies that express the *Drosophila* NOS gene (*dNOS1*) under the control of the heat-shock promoter and flies that express proteins involved in the cell cycle (RBF, dE2F, dDP, dacapo, and p21) under the control of the eye-specific GMR promoter (gifts from N. Dyson and I. Hariharan). We have found that manipulations of NOS activity can enhance or suppress the effect of the RBF and E2F components. In particular, we found that (1) dNOS interacts with dRBF to suppress cell proliferation in the developing eye; (2) dNOS enhances the suppressive action of dRBF; (3) inhibition of NOS rescues the eye phenotype caused by ectopic expression of dRBF; (4) dNOS rescues the eye phenotype caused by ectopic expression of dE2F and dDP; and (5) inhibition of NOS prevents dRBF from suppressing the phenotype of dE2F/dDP transgenic flies. Our results argue that NO controls cell division in the developing eye by controlling entry in the S phase of the cell cycle. These experiments present the first example of analyzing the genetic interactions of NOS. They demonstrate that NOS cooperates with the Rb pathway to control the number of cells in the ommatidia during *Drosophila* eye development.

Molecular Mechanisms of Nitric Oxide Synthesis during *Drosophila* Development

Y. Stasiv, A. Hazan [in collaboration with M. Regulski and T. Tully, Cold Spring Harbor Laboratory]

We have recently shown that NO is involved in the control of organ size during *Drosophila* development. Now we are searching for the RNA species that might be responsible for the action of NO in the developing fly. Using RT-PCR and *Drosophila* cDNA libraries, we found a family of five alternatively spliced transcripts arising from the NOS locus. Two of them give rise to unusual new proteins, dNOS3 and dNOS4, which do not possess enzymatic activity; however, dNOS3 can inhibit dNOS1 activity in cotransfection assays. We have generated antibodies against the dNOS3-specific peptide and demonstrated that it recognizes a protein differentially expressed at various stages of *Drosophila* development. Our results argue that dNOS3 may code for a natural negative regulator of NOS activity during *Drosophila* development. Furthermore, these results also suggest that dNOS3 can be used as a dominant-negative regulator of NOS when introduced to the *Drosophila* germ line. We are currently generating transgenic flies expressing dNOS3 under heat-shock- and eye-specific promoters to test this hypotheses.

Genes Activated by NO in Neuronal Cells

N. Nakaya

Although the action of NO in cells was studied in many details, very little is known about the transcriptional targets of NO, and very few genes have been identified whose activation is dependent on NOS activity in the cell. In this project, we are searching for genes whose expression in neuronal cells is affected by NO. We expect to obtain reliable markers of NO action in the cell and to identify NO-responsive promoter elements. Using suppressive subtraction hybridization, we cloned several genes that are induced by NO in neuronal cells. We are currently searching for their control elements and using them to probe the action of NO as a transcellular messenger.

NO and *Xenopus* Brain Development

N. Peunova, C. Mannino [in collaboration with H. Cline, Cold Spring Harbor Laboratory]

To examine whether NO has an antiproliferative role during vertebrate development, we used *Xenopus laevis* as a model organism, focusing on the possible role of NO in the development of the tadpole's brain. Using cytochemical and immunochemical staining, we found that NOS is accumulated in maturing neurons, whereas the zones of active proliferation in the germinal layer remained free of NOS. This suggests that, similar to neuronal cells in culture and imaginal discs of *Drosophila*, NOS may be involved in cessation of proliferation of neuronal precursors. To test whether NOS is involved in growth arrest of precursor cells in the developing *Xenopus* brain, we blocked NO production by introducing a slow-release polymer implant impregnated with NOS inhibitors in the ventricle of the tadpole brain. BrdU labeling of the brain demonstrated a dramatic increase in the number of cells in the S phase of the cell cycle in the brains treated with inhibitors, compared to the control brains. Staining of cell nuclei with DAPI revealed a higher number of cells in the brains of experimental animals, indicating that excessive cells in the S phase successfully completed the cell cycle by mitosis. The overall size of the brain was also increased after introduction of the inhibitor. The most affected areas are the optic tectum and the area immediately adjacent to the ventricle where the impregnated matrix was implanted. Using antibodies to specific neuronal markers, we found that development of some types of neurons is grossly perturbed by excessive proliferation of precursors, whereas other types, produced at the early stages of neurogenesis, are not affected. Our results strongly argue that NOS activity is essential for the normal pattern of cell proliferation in the developing *Xenopus* brain.

Cloning of the *Xenopus* NOS Gene

V. Scheinker, Y. Stasiv

Using the information about the known NOS genes, we cloned the NOS cDNA from *Xenopus*. Its primary structure suggests that the cloned gene (*XeNOS*) is an ortholog of the Ca^{++}-dependent neuronal NOS isoform of mammals. Analysis of the gene reveals a remarkable degree of evolutionary conservation with long stretches of amino acid sequences identical to those of humans, mice, rats, and *Drosophila*. The cloned gene produces enzymatically active protein when transfected in cultured cells. Using specific antibody and RT-PCR analysis, we found that *XeNOS* is strongly expressed in tadpoles and in the frog brain.

Protein Targeting to Neuronal Cell Terminals

P. Krasnov

We have recently identified the structural elements that are necessary and sufficient for transporting the synaptotagmin molecule to the terminals of neuronal cells. Using alanine-scanning mutagenesis, we have identified two amino acids that are absolutely critical for accumulation of synaptotagmin at the terminal. These amino acids are conserved among all known synaptotagmin isoforms, suggesting that different synaptotagmins may have similar or common mechanisms of targeting. The information about the trafficking signal allowed us to isolate proteins that bind the signal. We used columns with immobilized synthetic peptides that correspond to the wild-type and mutated inactive versions of the signal to search for the interacting proteins in the extracts of neuronal PC12 cells. We found three proteins, two of which bind only the

wild-type but not the mutated peptide. We have obtained their partial sequence, and we are currently trying to identify them and to test if they are indeed responsible for the accumulation of the synaptotagmin protein at the terminals.

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NEOCORTICAL CIRCUITRY AND ITS PLASTICITY

K. Svoboda B. Burbach Z. Mainen
C. Gray P. O'Brien

The neocortex is by far the largest brain structure in mammals, and it is central to most cognitive functions. Neocortical anatomy is highly conserved between species. As its name implies, the neocortex is also the most recent arrival in evolutionary terms. Neocortical circuitry is further distinguished by its phenomenal complexity. One microliter of neocortical tissue alone contains nearly one million neurons and more than a billion synapses. A single neuron connects to a thousand other neurons, near and far. This awesome network underlies the dynamics of our brains and our perception of the world. To unravel neocortical function, we are studying how the basic hardware of this network, namely, neurons and their dendrites and synapses, functions within the intact system. To observe neuronal function, we require highly sensitive tools that can be applied to the difficult experimental situation in the intact brain. Two-photon excitation laser-scanning microscopy (TPLSM) allows us to image neuronal function and structure at micrometer-level resolutions in highly scattering tissue. Optical uncaging of neurotransmitters allows us to excite subcellular structures with great spatial and temporal control. Intracellular electrical recordings using sharp and whole-cell electrodes allow us to monitor the electrical membrane potential and synaptic currents in the soma and dendrites. As model systems, we use the rodent somatosensory cortex in vivo and in brain slices. For synaptic physiology, we use slices derived from the hippocampus.

Morphogenesis Induced by Synaptic Activity

K. Svoboda [in collaboration with M. Maletic-Savatic and R. Malinow, Cold Spring Harbor Laboratory]

How is the cortical network established and maintained? And how does it change in response to experience? The formation of precise synaptic connections between neurons during development and learning and memory in the adult brain is no doubt critical to the normal functioning of the brain. For the case of development, it is likely that activity-dependent mechanisms can induce structural reorganization, but this has not been directly demonstrated. For the case of learning and memory in the adult, it is unknown if structural changes are necessary for synaptic plasticity, despite decades of research. In previous studies, neurons in brain slices were tetanized to produce long-term potentiation (LTP) and subsequently analyzed for morphological changes. These studies have produced mixed results, we believe for two principal reasons. (1) The morphological analysis occurred in fixed tissue that made a "before-and-after" compari-

son impossible; subtle and sparse changes in synaptic structure would therefore have remained buried in the large intrinsic variability in synaptic structure. (2) Tetanically induced LTP would be expected to change only a small subset of a pyramidal neuron's 10,000 synapses; these changes would be hard to find by random sampling.

We have recently been able to circumvent these problems by directly observing morphogenesis of dendritic spines, the postsynaptic partners of synapses. To stimulate a spatially restricted set of synapses, we placed a glass electrode very close (<10 μm) to a small dendritic branch. For TPLSM imaging, CA1 pyramidal cells in cultured hippocampal slices were infected with a virus expressing green fluorescent protein (GFP), a bright fluorophore that allows excellent imaging with low photodamage. We find that synaptic activity produces rapid local sprouting of spines and fillopodia in a small region of the dendrite. These structures can be stable over hours. Synaptically induced sprouting is blocked by antagonists of the NMDA receptor. We are currently trying to determine if our observations apply primarily to the activity-dependent processes of development or if they are applicable to the adult brain.

Optical Studies of Synaptic Function at Individual Synapses

Z. Mainen [in collaboration with R. Malinow, Cold Spring Harbor Laboratory]

In contrast to the classical preparations for synaptic physiology, such as the neuromuscular junction, CNS synapses are difficult to study with electrophysiological techniques alone. Important issues regarding stochastic fluctuations and spatial specificity in synaptic transmission require direct measurements of transmission at individual synapses. For this purpose, we have begun to use high-resolution functional TPLSM imaging. Individual pyramidal neurons in hippocampal slices are filled with a calcium indicator through a whole-cell patch clamp electrode. Synaptic transmission at glutamatergic synapses is associated with postsynaptic calcium influx through NMDA receptors. We therefore use these synaptically activated calcium transients at individual dendritic spines as indicators of synaptic function. We compare these signals with measurements of synaptic currents recorded via the whole-cell electrode. This powerful approach allows us to address tenacious questions of synaptic physiology: (1) Is synaptic transmission a point-to-point or spatially distributed process? This question has profound implications for the information storage capacity of the brain. Although the canonical view is that the release of an individual vesicle activates only one dendritic spine, this view has recently come under attack. Since the release of individual vesicles is stochastic, temporal correlations in activity in close-by spines would indicate spread of transmitter to neighboring spines. We are currently looking for such correlations. (2) What is the distribution of probability of release? This question also has importance for information coding in the brain. (3) Finally, do changes in the probability of release underlie some forms of synaptic plasticity? Activity-dependent modulation of synaptic strength (such as LTP) is the most popular model for long-term information storage in the CNS. In the brain slice preparation, coincident pre- and postsynaptic stimulation can produce LTP. Although LTP has received a lot of experimental attention, the cellular mechanisms underlying LTP are highly controversial; modulation of release probability is one such candidate mechanism.

Mechanisms of Current Amplification in Neocortical Dendrites

C. Gray, K. Svoboda

Some neocortical dendrites pose an interesting problem in "neuro-engineering." In particular, the dendrites of layer 5 pyramidal cells are so long and thin (length ~1 mm; thickness ~5 μm) that it is difficult to fathom how the synapses on their distal dendrites could have an influence on the electrical behavior of the cell body and hence the cell's output. From a systems function perspective, this is an interesting problem, because feedback from higher cortical areas is mediated mostly through the distal synapses of layer 1. It has long been hypothesized that these remarkable dendrites must possess the ability to amplify synaptic currents, and a variety of detailed models of this amplification exist. Distinguishing between these models will enhance our understanding of the role and function of neocortical feedback projections and the role of dendritic electrogenesis in general. The detailed biophysical experiments required to discover

the mechanisms of current amplification in long dendrites are best performed in neocortical brain slices. Direct postsynaptic stimulation using optical uncaging of caged precursors of neurotransmitters is required for these experiments. Simultaneous whole-cell measurements of the resulting postsynaptic currents at the soma probe the coupling between dendrites and soma. To perturb current amplification, we manipulate specific dendritic voltage-sensitive conductances using pharmacological tools. The dendrites and their voltage sensitive conductances are analyzed using compartmental models constructed in NEURON.

Dendritic Excitation In Vivo

K. Svoboda

Recent experiments in brain slices have produced a wealth of data suggesting that action potential propagating from soma to dendrite is necessary for the induction of synaptic plasticity. In particular, backpropagating action potentials may be the feedback signals underlying Hebbian synaptic plasticity underlying learning and memory. With the long-term goal to bridge the gap between cellular physiology and brain function and behavior, we have begun to directly study dendritic excitation in the intact brain under sensory stimulation. We find that somatically recorded action potentials produce fast calcium transients, where the calcium concentration codes for the recent activity of the cell. Surprisingly, these transients are restricted to the proximal apical dendrite, with no influx into the tuft of layer 1. Using direct dendritic membrane potential recordings, we found that this inhomogeneous calcium accumulation is due to a failure of action potential back-propagation into these dendrites. Interestingly, these stunted back-propagating action potentials can be enhanced by subcortical modulatory systems, such as the ascending cholinergic pathway. This is important because these modulatory systems are particularly active during attention and arousal and are intimately related to learning and memory.

Cellular Basis of Neocortical Plasticity

K. Svoboda, B. Burbach

The central nervous system is distinguished by its ability to reorganize itself in response to changes in experience. These changes underlie learning and memory as well as our ability to deal with damage to our sensory periphery. Rodents are excellent model systems for the study of plasticity in the neocortex. Individual whiskers preferentially activate a small column of neurons in the primary somatosensory (barrel) cortex. Plasticity can be induced by trimming one or several whiskers with predictable effects on a cortical column and its neighbors. After whisker-trimming, the part of the cortex corresponding to the trimmed whisker will begin to preferentially respond to untrimmed whiskers. This is an example of experience-dependent receptive field plasticity and it is related to procedural learning. What happens at the level of neurons, dendrites, and synapses when the neocortex reorganizes? As a first step in determining the cellular mechanisms underlying neocortical plasticity, we are using TPLSM to image neocortical neurons over very long time scales (days) to assess their normal morphological stability. We will then perturb the sensory experience of the animal by trimming a whisker and look for morphological correlates of the resulting receptive field plasticity.

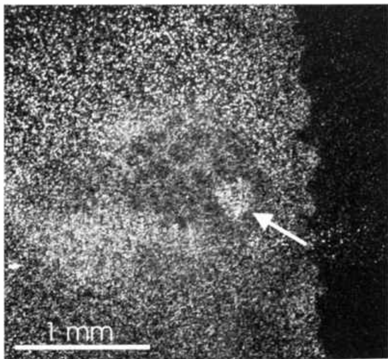

FIGURE 1 CPG15 induction in mouse barrel cortex 12 hr after whisker-trimming. In situ hybridization to a tangential section through layer 4 is shown. The barrel field appears as an approximately 1 x 1 mm^2 area of dark tiles (to the left of the arrow) All whiskers but one (D1) were trimmed, which leads to increased representation of this whisker in the barrel cortex. The cortical column corresponding to the spared whisker shows enhanced expression of CPG15 in layer 4 (light spot within the barrel field, arrow) and also in layers 2 and 3 (not shown).

Molecular Basis of Neocortical Plasticity

B. Burbach, K. Svoboda [in collaboration with E. Nedivi, Cold Spring Harbor Laboratory]

It has been known for a long time that long-term plasticity requires gene expression. The vibrissa cortex offers a good system to discover the genes that are associated with experience-dependent neocortical plasticity. Luckily, we have at our disposal several candidate plasticity genes (CPGs) that were isolated by E. Nedivi in a differential screen at the Weizmann Institute. These genes are regulated during development and by sensory activity. We are now in the process of using in situ hybridization of CPGs to rat brain sections to discover the subset of activity-dependent genes that are regulated by experience-dependent plasticity. We have already found that CPG-15 is upregulated during the early phase of receptive field plasticity (Fig. 1). In the future, we plan to explore the function of CPGs in the intact neocortical network by combining virally mediated gene transfer with TPLSM in vivo imaging of neuronal morphology and synaptic function.

Instrumentation

K. Svoboda, P. O'Brien

Much of the work we do depends on sensitive instrumentation to study neural function in intact tissue. For example, TPLSM allows imaging of neural function at submicron resolution in the intact brain, an environment that is hostile to traditional microscopies. In addition, living specimens are sensitive to photodamage. Photodamage can be minimized by maximizing fluorescence detection efficiency. Finally, suitable microscopes must be able to accommodate large specimens such as rats and assorted micromanipulators for intracellular membrane potential measurements. For this reason, we typically build the microscopes ourselves. The first microscope we have constructed is for imaging and electrophysiology in acute brain slices. We have recently completed a second microscope, based on a completely novel geometry, that is ideal for subcellular imaging in the intact neocortex. These instruments are continually being modified based on feedback gained from performing biology experiments.

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COLD SPRING HARBOR MEETINGS AND COURSES

ACADEMIC AFFAIRS

The academic program at Cold Spring Harbor now takes place during a 9-month period extending from mid-March through the first half of December. During that time, new ideas, discoveries, and technologies are communicated in a wide-ranging series of postgraduate laboratory and lecture courses, workshops, large meetings, and a summer research program for undergraduates.

The courses emphasize teaching the latest in state-of-the-art methodologies set in the context of recent scientific advances and are of relatively short duration so that senior scientists as well as junior faculty, postdoctoral fellows, and graduate students can attend. All of the courses are oversubscribed, which is a tribute to the skills and talents of the instructors as well as the timeliness of the courses. The 1997 meetings also emphasized communicating the latest results in a broad range of topics in molecular biology and neurobiology. This year, more than 6000 scientists came to the laboratory to serve as instructors, lecturers, and students in the courses as well as participants in the meetings.

This year, 25 courses were held at the laboratory, including 19 molecular genetics, genomics, structure, and neurobiology laboratory courses. These were held in the Delbrück Laboratory and the Howard Hughes Medical Institute (HHMI) teaching laboratories in the Beckman Neuroscience Center. Five neurobiology lecture courses were held at the Banbury Center. Course instructors, assistants, lecturers, and students are listed below. It is certainly a pleasure to note here the contributions of the instructors who come from universities and research institutes around the world to teach at Cold Spring Harbor. It is their creativity and skill that really make the courses work so well.

A group of scientists who have taught in the courses for several years each are retiring after this year. We are most grateful to them, even more so because we know that we can count on them to return to give advice and lectures in future years. These include Steve Smale, an instructor in the Eukaryotic Gene Expression course; Rob Last, who taught in the *Arabidopsis* Molecular Genetics course; Alison Adams, a Yeast Genetics course instructor; Fabio Rupp, who taught the course on Molecular Cloning of Neural Genes; and Arthur Konnerth, Fred Lanni, and Rafael Yuste, instructors for the course of Imaging Structure and Function in the Nervous System. Two fall session instructors are also retiring with our thanks—Dennis Burton and Roger Reeves.

The courses are supported by, and would not be possible without, a series of grants from federal and private sources. The summer molecular genetics courses have been supported for many years by grants from the National Institutes of Health (NIH) and the National Science Foundation (NSF). A grant from the National Institute of Mental Health supports several of the neurobiology courses. This year, a grant from the National Cancer Institute that supports several of the molecular biology courses was renewed, as was an NSF grant that supports the *Arabidopsis* Molecular Genetics course. A large education grant from HHMI has provided stable support for the neurobiology program and has allowed the Laboratory to begin and to expand its series of spring and fall courses. We are most pleased that an award from the Esther and Joseph A. Klingenstein Fund for the support of neurobiology courses was renewed this year. As has been the case for several years, the Grass Foundation provided funds for scholarships for students in neurobiology courses. A grant from the Department of Energy has also helped in the funding of the course in Macromolecular Crystallography. In addition, the Laboratory receives valuable support from many companies that donate supplies and lend equipment for the courses.

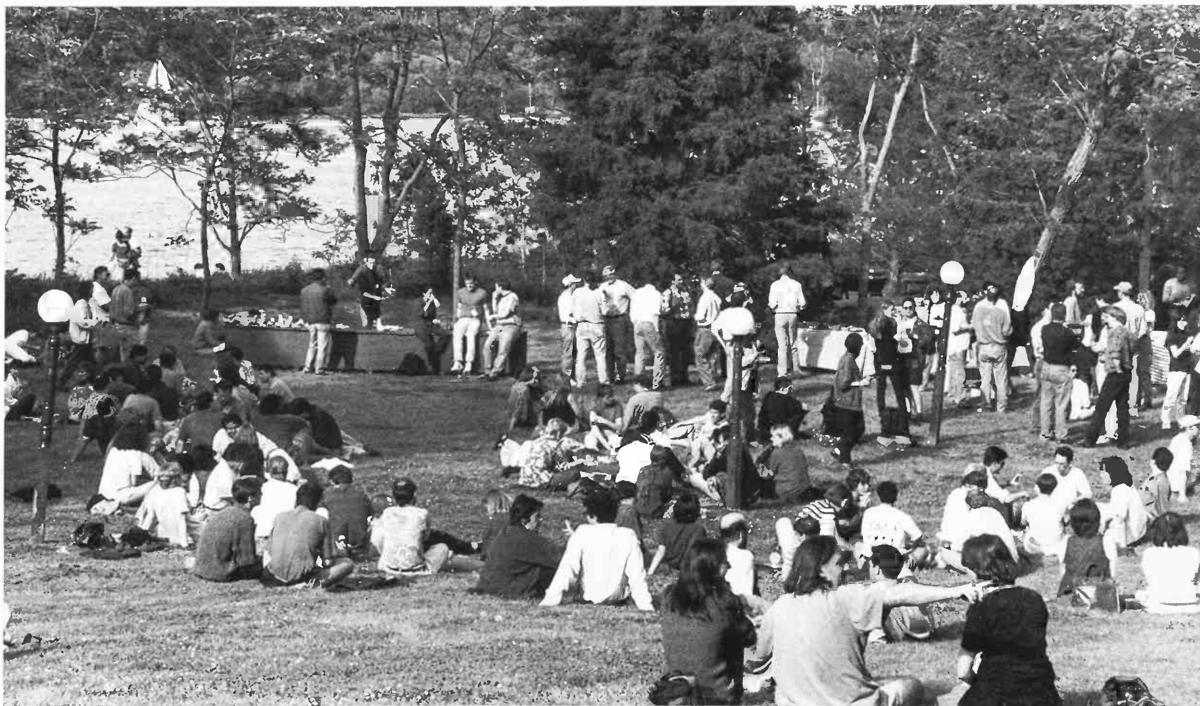
Sixteen meetings were held at the Laboratory this year, but the Symposium remains, as always, central. This year's Symposium on Pattern Formation during Development, discussed below by Bruce Stillman, brought together a broad array of developmental biologists to discuss current research. Several new meetings were held this year. These include the Biology of Proteolysis organized by Charles Craik, Susan Gottesman, Mark Hochstrasser, and Yuri Lazebnik, and Microbial Pathogenesis and Host Response organized by P.T. Magee, Stan Maloy, and Ron Taylor. A very interesting new meeting on Human Evolution was held in October, organized by Luigi Cavalli-Sforza and Jim Watson. We expect these new meetings to be held again in 2 years' time. Other

meetings, which are held in alternate years, covered topics ranging from Eukaryotic DNA Replication to Tyrosine Phosphorylation and Signaling to Neurobiology of *Drosophila*. Several conferences of long standing continue to be oversubscribed, such as the meetings on Retroviruses and Mechanisms of Eukaryotic Transcription. This year, two biotechnology conferences on Vector Targeting Strategies for Gene Therapy and The *Arabidopsis* Genome: From Sequence to Function were held outside of the regular meetings season. These biotechnology conferences, now in their second successful year, are overseen by David Stewart and will continue in future years. All organizers and contributors to the meetings are listed below. The sources of support for these meetings come from the Laboratory's Corporate Sponsor program, NIH, NSF, DOE, and various corporations and foundations. Grants helped scientists at all stages of their careers to attend the conferences.

While graduate students, postdoctoral fellows, and faculty participate in the courses and meetings, the Undergraduate Research Program (URP) provides an opportunity for college undergraduates to spend 10 weeks at the Laboratory during the summer. The program, headed by Michael Hengartner, allows students to do research in the laboratories of staff scientists.

The large numbers of courses and meetings proceed with skill and efficiency, thanks to the collaborative efforts of a large number of people at the Laboratory. The staff of the Meetings and Courses Office, headed by David Stewart, Director of Meetings and Courses, coordinates the arrangements for all of the visiting scientists. This enormous job, which seems to grow every year, is carried out not only extremely efficiently, but with patience and tact as well. The staff includes Micki McBride, the Course Registrar; Nancy Weeks, Head Concierge; Jim Koziol, Diane Tighe, Marge Stellabotte, Andrea Stephenson, Drew Mendelson, Mary Smith, and Michael Glaessgen, as well as Herb Parsons, Ed Campodonico, Bill Dickerson, and the part-time audiovisual staff. Staff from several other departments are crucial to the success of the meetings and courses, including Cliff Sutkevich and his staff who set up and maintain course equipment, and Edie Kappenberg, the course coordinator. In particular, Wendy Crowley, taking over in Grants as Educational Grants Manager after Mary Horton's departure, provides invaluable support in the preparation and management of grants in support of the meetings and courses.

Terri Grodzicker



62nd Symposium: Picnic at Airslie

62nd COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

Pattern Formation during Development

May 28–June 2 391 participants

ARRANGED BY **Bruce Stillman,** Cold Spring Harbor Laboratory

One of the great scientific accomplishments in the past decade was the recognition that the mechanisms used for patterning of tissues and organs during development are remarkably similar among species. What works for flies and frogs also serves human beings very well as embryos acquire their form and identity. To celebrate these marvelous discoveries, the 62nd Symposium focused on pattern formation during development, with a particular emphasis on the evolutionarily conserved mechanisms and molecules.

This outstanding series of meetings began in 1933, in the midst of economic depression. Each meeting lasted for 5 weeks while scientists were in residence at Cold Spring Harbor, and there were no time limits on the length of presentations or discussion! This "experiment in scientific procedure," reflecting the apparent tranquility of academic life in those days, lasted for 8 years. The second Symposium, entitled Aspects of Growth, included several papers dealing with differentiation and development.

For the 1941 Symposium, the new Director of the Laboratory, Milislav Demerec, condensed the program into 2 weeks, and his second Symposium focused on The Relation of Hormones to Development, with 17 presentations. In 1948, Demerec changed the meeting to 8 days, held in early June, and this has been the arrangement until this year. Given the dramatic changes in the pace of scientific discovery in the biological sciences, the modern methods for rapid dissemination of information, and the fact that in many families, both spouses have demanding careers, the program has been shortened to 5 days, still considered by some to be a long time for a meeting. But we must not forget the goals of this particular series, and indeed all of the meetings at Cold Spring Harbor Laboratory. The idea is not to present a series of observations from a collection of individuals, but to provide sufficient time for the experts in a field of biology to discuss the issues of the day and to determine how progress might be accomplished.

During eight days in 1954, the Symposium focused on The Mammalian Fetus: Physiological Aspects of Development. The science was descriptive, but some ideas presented were the forerunners of the modern approaches to understanding development. Salome Glueckshon-Waelsch discussed the T (Brachyury) locus and a genetic approach to understanding development that foreshadowed the powerful approaches to come. Then after a long hiatus between Symposia on this topic, Joe Sambrook organized in 1985 the 50th Symposium on the Molecular Biology of Development. The Symposium celebrated the conversion of developmental biology from a descriptive, anatomically based science to a mechanism-driven science of great interest.

In the dozen years hence, we have learned much about the exquisite mechanisms that specify development, but most importantly, the evolutionary conservation of the pathways that govern development has emerged. We are no longer bound by technology, as in the early days of these Symposia, and modern technology has taken us to new heights unimagined even 12 years ago.

The field of developmental biology is now very large, and coupled with the decision

to shorten the program, difficult decisions about the selection of speakers and topics had to be made. Brigid Hogan and Gerry Rubin gave much valuable advice and help with the scope of the meeting and potential speakers. The formal scientific program consisted of 64 oral presentations and a remarkable 233 poster presentations, and the meeting attracted more than 390 participants. Introductory talks on the first evening were given by Mario Capecci, Brigid Hogan, Roland Nusse, and Gail Martin, and the Reginald G. Harris Lecture was presented by John Gurdon. Sean Caroll presented a fascinating Dorcas Cummings Lecture on the formation and evolution of animal body patterns to both the visiting scientists and the local community.

J. Watson, M. Scott

R. Beddington, N. Rosenthal

B. Hogan, P. Beachy

G. Rubin, D. Melton, M. Levine

I. Herskowitz, T. Grodzicker, R. Losick

Essential funds to run this meeting were obtained from the National Institute of Child Health & Human Development and the National Institute of Neurological Disorders & Stroke, both branches of the National Institutes of Health. Contributions from the Corporate Sponsors provide core support for the Cold Spring Harbor meetings program and we are grateful for their continuing support: Amgen, Inc.; BASF Bioresearch Corporation; Becton Dickinson and Company; Boehringer Mannheim Corporation; Bristol-Myers Squibb Company; Chiron Corporation; Chugai Research Institute for Molecular Medicine, Inc.; Diagnostic Products Corporation; The Du Pont Merck Pharmaceutical Company; Forest Laboratories, Inc.; Genetech, Inc.; Glaxo Wellcome, Inc.; Hoechst Marion Roussel; Hoffmann-La Roche, Inc.; Johnson & Johnson; Kyowa Hakko Kogyo Co., Ltd.; Life Technologies, Inc.; Eli Lilly and Company; Merck Genome Research IInstitute; Novartis Pharma Research; Oncogene Science, Inc.; Pall Corporation; The Perkin-Elmer Corporation; Applied Biosystems Division; Pfizer Inc.; Pharmacia & Upjohn, Inc.; Research Genetics, Inc.; Schering-Plough Corporation; SmithKline Beecham Pharmaceuticals; Wyeth-Ayerst Research; and Zeneca Group plc. We also thank our Plant Corporate Associates: American Cyanamid Company; Kirin Brewery Co., Ltd.; Monsanto Company; Pioneer Hi-Bred International, Inc.; and Westvaco Corporation.

PROGRAM

Welcoming Remarks: Bruce Stillman

Introduction
Chairperson: R. Lehmann, *New York University Medical Center, New York*

Patterning and Transcription
Chairperson: M. Capecchi, *University of Utah, Salt Lake City*

Reginald G. Harris Lecture: "Long-range Signaling and a Morphogen Gradient in *Xenopus* Development"
Speaker: J. Gurdon, *University of Cambridge, United Kingdom*

Morphogens
Chairperson: B. Hogan, *Howard Hughes Medical Institute, Vanderbilt University, Nashville, Tennessee*

Signaling in Organogenesis
Chairperson: L. Zipursky, *Howard Hughes Medical Institute, University of California, Los Angeles*

Inductive Mechanisms
Chairperson: E. Robertson, *Harvard University, Cambridge Massachusetts*

Tissue Specification
Chairperson: R. Beddington, *National Institute for Medical Research, London, United Kingdom*

Neural Induction and Pathfinding
Chairperson: D. Melton, *Howard Hughes Medical Institute, Harvard University, Cambridge, Massachusetts*

Asymmetric Divisions
Chairperson: G. Rubin, *Howard Hughes Medical Institute, University of California, Berkeley*

Polarity and Position Specification
Chairperson: R. Losick, *Harvard University, Cambridge, Massachusetts*

Dorcas Cummings Lecture: "Embryos and Ancestors: The Formation and Evolution of Animal Body Patterns"
Speaker: S. Carroll, *University of Wisconsin, Madison*

Patterning Pathways
Chairperson: J. Rossant, *Samuel Lunenfeld Research Institute, Toronto, Canada*

Axis Formation
Chairperson: M. Levine, *University of California, Berkeley*

Summary: M. Scott, *Stanford University School of Medicine*

Vector Targeting Strategies for Therapeutic Gene Delivery

March 14–16 141 participants

ARRANGED BY **David T. Curiel,** M.D., University of Alabama
Wayne Marasco, M.D., Ph.D., Dana-Farber Cancer Institute

The basic premises of gene therapy are subservient to the pharmacologic goal of efficient and specific delivery of therapeutic nucleic acids to relevant somatic cells. In this regard, current generation gene transfer vectors have been suboptimal for this purpose. This is especially apparent in the context of gene therapy strategies requiring direct, in vivo, gene transfer. The basis of these shortcomings has been related to the very limited attempts to engineer vectors specifically for their delivery purposes. The technology to do this, however, has recently been developed. Specifically, methods to achieve cell-specific gene delivery via viral and nonviral vectors have been described. A second issue, however, is the definition of appropriate surface markers on target cells. In this context, the recent "high-thruput" target definition technologies based on phage panning allow the ability to define cell-specific targeting moieties. On the basis of this concept, Vector Targeting Strategies for Therapeutic Gene Delivery meeting was designed to bring together basic vectorologists endeavoring targeted vector delivery, as well as scientists working in the area of target definition. It is anticipated that the scientific linkages between these groups within the Cold Spring Harbor meeting framework will allow more rational design of vectors of utility for gene therapy purposes.

This meeting was funded in part by Canji, Inc.; the Cystic Fibrosis Foundation; Gene Medicine, Inc.; Genzyme; Glaxo Wellcome, Inc.; Intraimmune Therapies, Inc.; Microbiological Associates/MAGENTA; Prolinx, Inc.; Rhone Poulenc Rorer; Targeted Genetics Corporation; Therexsys Limited; Transgene, S.A.; Virus Research Institute; and Wyeth-Ayerst Laboratories.

PROGRAM

Introductory Lectures: D.T. Curiel, *University of Alabama, Birmingham;* W. Marasco, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Target Definition
Chairperson: H.R. Hoogenboom, *University of Mastricht, The Netherlands*

Liposomes and Molecular Conjugates
Chairperson: E. Wagner, *Vienna University Biocenter, Austria*

Targeted Retroviruses
Chairperson: W. Marasco, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Targeted Adenoviruses
Chairperson: D.T. Curiel, *University of Alabama, Birmingham*

Emerging Technologies
Chairperson: J.C. Glorioso, *University of Pittsburgh School of Medicine, Pennsylvania*

Keynote Speaker
Richard Vile, *Imperial Cancer Research Fund, United Kingdom*

V. Krasnykh, I. Dmitriev, D. Curiel

Cell and Molecular Biology of *Aplysia* and Related Invertebrates

April 16–20 77 participants

ARRANGED BY **John H. Byrne,** University of Texas–Houston Medical School
Reinhardt Jahn, Yale University School of Medicine
Leonard Kaczmarek, Yale University School of Medicine
Eric Kandel, Columbia University College of Physicians & Surgeons

This fifth international meeting continued the trend of including presentations on data gleaned from work with identifiable neurons of *Aplysia*, squid, *Lymnaea*, as well as with some crustaceans and annelids (preparations). For the neurobiologist, the major advantage to using these animals (preparations) is the large size of their somata, which allows intracellular injection of enzymes and other reagents to probe synaptic transmission and other aspects of neuronal plasticity. This feature has allowed workers to gain numerous insights into the mechanisms of exocytosis of synaptic vesicles and the modulation of membrane channels. In addition, the fact that the nerve cells of these animals are readily identifiable from preparation to preparation allows them to be used to determine the changes that occur in neuronal properties during learning and other prolonged changes in animal behavior.

This meeting was funded in part by the National Institute of Neurological Disorders and Stroke, a division of the National Institutes of Health; and the National Science Foundation. Contributions from the Corporate Sponsors and Plant Corporate Associates also provided core support for this meeting.

PROGRAM

Plenary Lecture
I. Levitan, *Brandeis University, Waltham, Massachusetts*

Cellular and Molecular Biology of Ion Channels and Synaptic Transmission I
Chairperson: R. Jahn, *Yale University, New Haven, Connecticut*

Cellular and Molecular Biology of Ion Channels and Synaptic Transmission II
Chairperson: L. Kaczmarek, *Yale University, New Haven, Connecticut*

Peptides and Small Molecule Transmitters
Chairperson: P. Lloyd, *University of Chicago, Illinois*

Neural Control of Behavior
Chairperson: J. Byrne, *University of Texas Medical School, Houston*

Tanks and Home Tanks
Chairpersons: P. Walsh, *University of Miami, Florida;* D. Gardner, *Cornell University, New York*

Rhythms: Generation and Modulation
Chairperson: I. Kupfermann, *Columbia University, New York, New York*

Learning and Memory I
Chairperson: C. Sahley, *Purdue University, West Lafayette, Indiana*

Learning and Memory II
Chairperson: C. Rankin, *University of British Columbia, Vancouver, Canada*

J.H. Byrne, T. Carew

R. Jahn, B. Garetsky

Biology of Proteolysis

April 23–27 233 participants

ARRANGED BY **Charles S. Craik,** University of California, San Francisco
Susan Gottesman, National Cancer Institute
Mark Hockstrasser, University of Chicago
Yuri Lazebnik, Cold Spring Harbor Laboratory

Proteolytic processing has emerged as a key regulatory step in a wide range of biological processes ranging from cell cycle and development to cell death. This created a need for a forum that would fertilize crosstalk between experts on these processes and experts on the proteases as enzymes. This new meeting, which will be held every other year, attracted more than 230 scientists that discussed the role of proteolysis in signal transduction, gene regulation, development, cell cycle, control of protein expression, neoplasia and cell transformation, immune control, and cell death. The reported experimental systems ranged from prokaryotes to animals and plants, whereas the interests of speakers varied from the dissection of protease structure to finding out how plants know that they are eaten by caterpillars. Despite such a diversity, the participants commented that the meeting was focused, informative, and very exciting. The crosstalk was made possible in large part by the chairpersons who presented extensive overviews at the beginning of each session. In summary, this meeting promises to be a unifying forum that will help us to understand how proteases control life and death. (For a detailed review of this meeting, see *Trends in Cell Biology 7:* 333–335 [1997].)

Contributions from the Corporate Sponsors and Plant Corporate Associates provided core support for this meeting.

PROGRAM

Signal Transduction
Chairperson: T. Maniatis, *Harvard University, Cambridge, Massachusetts*

Gene Regulation and DNA Replication
Chairperson: T.A. Baker, *Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge*

Development
Chairperson: K.V. Anderson, *Memorial Sloan Kettering Cancer Institute, New York, New York*

Neoplasia and Cell Transformation
Chairperson: P.M. Howley, *Harvard Medical School, Boston, Massachusetts*

Cell Cycle
Chairperson: J. Ruderman, *Harvard Medical School, Boston, Massachusetts*

Quality Control
Chairperson: F.U. Hartl, *Howard Hughes Medical Institute, Memorial Sloan Kettering Cancer Center, New York, New York*

Host-Pathogen Interaction and Antigen Presentation
Chairperson: H. Ploegh, *Massachusetts Institute of Technology, Cambridge*

Cell Death
Chairperson: V.M. Dixit, *University of Michigan, Ann Arbor*

F. Goldberg, C. Craik

R. Deshaies, M. Hockstrasser

Regulation of Liver Gene Expression in Health and Disease

April 30–May 4 252 participants

ARRANGED BY **Gretchen Darlington**, Baylor College of Medicine
Rebecca Taub, University of Pennsylvania School of Medicine
Ken Zaret, Brown University

This meeting featured eight sessions on topics that highlighted recent progress in the area of liver biology. This year, each session featured speakers from fields other than liver function, in order to broaden the perspective of the participants and to provide cross-fertilization of ideas within different experimental systems.

The initial session addressed the Embryonic and Neonatal Development of the Liver and presentations in this session covered the similarity of developmental mechanisms for the major organ systems in *Drosophila* and mouse. Exciting data were presented about the role of fibroblast growth factor in directing the development of the initial stages of liver organogenesis. The second session, Transcriptional Control and Chromatin Structure, featured presentations on a powerful in vitro system for examining transcription and chromatin structure. In addition, recent findings were presented on the complexity of function of several transcription factor families that have critical roles in governing gene expression in the liver. Differentiation of Other Tissues from the Gut highlighted the similarities and differences in gene regulatory events that lead to differentiation of adipose tissue, lung, pancreas, and intestine. New findings of significance to metabolism were presented that showed how alterations in adipose tissue function lead to serious complications in liver function, suggesting a new disease mechanism for fatty liver. The fifth session focused on Hormonal and Nutritional Control of liver function. The interplay between certain families of liver transcription factors, carbohydrate metabolism, and insulin regulation was presented, giving insight into the molecular mechanisms that govern metabolic homeostasis. The Liver Growth and Regeneration session contained several exciting papers that revealed new mecha-

K. Fournier, G. Darlington, R. Hanson

K. Zaret, J. Whitsett

D. Robins, K. Tullis, S. Duncan

nisms of the regenerative response. The well-studied system of liver regeneration following partial hepatectomy has only recently been dissected at the molecular level and the work presented here by several investigators has formed the basis for a new paradigm for pathways of mitotic response, showing that the control of hepatic proliferation has components that have not been identified in the cultured fibroblast systems commonly studied thus far. The role of Cytokines in Gene Expression featured talks on the signal transduction pathways for the acute phase response in the liver including the Stat factors and the IL-6 receptor. The final topic dealt with Liver Gene Therapy. This stimulating session revealed the recent advances in the use of the liver as the organ of choice in gene therapy protocols. The benefits of each method were presented leaving the audience with both insight and high expectations for future developments in this new area of medicine.

The "Liver Meeting" at Cold Spring Harbor continues to attract a large number of participants and the level of audience participation is particularly strong. Prominent investigators regard this meeting as the premier forum for presentation of their latest findings. This meeting was funded in part by the National Institute of Child Health and Human Development, the National Cancer Institute, and the National Institute of Diabetes and Digestive and Kidney Diseases, divisions of the National Institutes of Health; the Council for Tobacco Research, USA, Inc.; Dupont Merck Pharmaceutical Company; Genetic Therapy, Inc.; the March of Dimes; and Pfizer, Inc.

Contributions from the Corporate Sponsors and Plant Corporate Associates also provided core support for this meeting.

PROGRAM

Embryonic and Neonatal Development
Chairperson: K. Zaret, *Brown University, Providence, Rhode Island*

Transcriptional Control and Chromatin Structure
Chairperson: U. Schibler, *University of Geneva, Switzerland*

Differentiation of Other Tissues from the Gut
Chairperson: J. Whitsett, *Children's Hospital Medical Center, Cincinnati, Ohio*

Hormonal and Nutritional Control
Chairperson: G. Darlington, *Baylor College of Medicine, Houston, Texas*

Liver Growth and Regeneration
Chairperson: R. Taub, *University of Pennsylvania School of Medicine, Philadelphia*

Hepatocellular Carcinoma, Cirrhosis, Viral Hepatitis
Chairperson: R. Jirtle, *Duke University Medical Center, Durham, North Carolina*

Cytokines and Gene Expression in the Liver
Chairperson: G. Fey, *University of Erlangen-Nürnburg, Germany*

Liver Detoxification and Gene Therapy
Chairperson: S. Woo, *Mt. Sinai School of Medicine, New York, New York*

Tyrosine Phosphorylation and Cell Signaling

May 7–11 300 participants

ARRANGED BY **Ben Neel,** Beth Israel Hospital, Harvard Medical School
Nick Tonks, Cold Spring Harbor Laboratory

This second meeting at Cold Spring Harbor marked the 20th anniversary of the discovery of the vSrc protein tyrosine kinase. As such, it was particularly appropriate that the meeting was opened with keynote addresses by Ray Erikson and Sara Courtneidge, providing an overview of historical perspective, current progress, and future directions in the field. The format of the meeting provided a combined emphasis on the physiological roles of protein tyrosine kinases and phosphatases and how their actions are integrated to modulate signaling events in vivo. The sessions were based around physiological processes and cellular functions rather than around particular categories of enzymes, so as to try to provide biological context to the data. The program included scientists from the United States, Europe, the Far East, and the South Pacific. There were 63 speakers selected to present their data in sessions that dealt with Receptor Protein Tyrosine Kinase Signaling and Growth Control, Neuronal Signaling, Cytoskeleton and Cell Adhesion, Tyrosine Phosphorylation and the Immune System, Hematopoietic Cell Signaling Development, and Human Disease. A variety of systems were described with great progress reported in genetic and biochemical approaches to the characterization of physiological functions for tyrosine phosphorylation. The meeting continues to be successful and now alternates with a conference with the same format at The Salk Institute with the result that there is an annual meeting on "Tyrosine Phosphorylation" that alternates in venue between Cold Spring Harbor and The Salk.

The meeting was supported by grants from the National Institutes of Health, National Science Foundation, and Upstate Biotechnology Inc. with contibutions from the Corporate Sponsors and Plant Corporate Associates.

T. Hunter

PROGRAM

Keynote Speakers: Ray Erikson, *Harvard University;* Sara Courneidge, *SUGEN, Inc.*

Neuronal Signaling
Chairperson: K. Zinn, *California Institute of Technology, Pasadena*

Receptor Signaling and Growth Control I
Chairperson: T. Hunter, *The Salk Institute, La Jolla, California*

Cytoskeleton and Cell Adhesion
Chairperson: M. Streuli, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Tyrosine Phosphorylation in the Immune System
Chairperson: K. Siminovitch, *Samuel Lunenfeld Research Institute, University of Toronto, Canada*

Hematopoietic Cellular Signaling
Chairperson: A.-M. Pendergast, *Duke University, Durham, North Carolina*

Development
Chairperson: P. Soriano, *Fred Hutchinson Cancer Center, Seattle, Washington*

Human Disease
Chairperson: J. Brugge, *Ariad Pharmaceuticals, Cambridge, Massachusetts*

Receptor Signaling and Growth Control II
Chairperson: M. Roussel, *St. Jude's Children's Research Hospital, Nashville, Tennessee*

N. Tonks

Genome Mapping and Sequencing

May 14–18 461 participants

ARRANGED BY **David Bentley,** Sanger Centre
Eric Green, National Institutes of Health
Philip Hieter, Johns Hopkins University

This meeting marked the tenth annual gathering of genome scientists in this setting and, as such, the meeting was a celebration of this important milestone. Just over 460 people from around the world attended the meeting, with 238 abstracts presented describing a broad array of topics relating to the analysis of genomes from a number of different organisms.

The session topics included areas such as biological insights from multi-organismal genomics, mapping methods and technologies, DNA sequencing production and technology, functional genomics, computational genomics, and gene discovery and transcript mapping. This year's poster symposium featured progress on sequencing the human genome. Once again, projection-style, interactive computer demonstrations in Grace Auditorium effectively highlighted the critical new bioinformatics tools being developed for storing, organizing, and analyzing genomic maps and sequences. There was also a panel discussion on the genome ELSI (Ethical, Legal, and Social Implications) program, which included a timely presentation on the ethical and scientific issues relating to cloning.

The major themes of the meeting related to the exciting initiation of human genomic sequencing and the increasing number of approaches being developed for using sequence data to per-

E. Green, R. Myers, J. Witkowski, R. Gibbs

D. Bently

M. Boguski, L. Goodman

P. Hieter, D. Porteous

form important biologic studies. Specific presentations reported major achievements in the sequencing of microbial genomes, the development of new sequencing technologies, and the use of microarrays for performing genome analysis. As a major pioneer of the Human Genome Project and initiator of the annual Cold Spring Harbor meeting, it was only fitting that the keynote speaker at the tenth annual meeting be Dr. James D. Watson. His talk was nothing short of spectacular, focusing on the ethics of genome research and the history of eugenics. With the anticipated achievements in the next decade, future Genome Mapping and Sequencing meetings should continue to serve as the premiere showcase for the Human Genome Project and genome research.

This meeting was funded in part by the National Human Genome Research Institute, a division of the National Institutes of Health. Contributions from the Corporate Sponsors and Plant Corporate Associates also provided core support for this meeting.

PROGRAM

Multi-Organismal Genomics: Biological Insights
Chairpersons: D. Porteous, *MRC Human Genetics Unit, Edinburgh, United Kingdom;* R. Reeves, *Johns Hopkins University, Baltimore, Maryland*

Mapping Methods and Technologies
Chairpersons: C. Cantor, *Boston University, Massachusetts;* S. Foote, *Walter and Eliza Hall Institute, Victoria, Australia*

Computer Demonstrations I
Chairperson: M. Boguski, *National Institutes of Health, Bethesda, Maryland*

DNA Sequencing: Production and Technology
Chairpersons: E. Mardis, *Washington University, St. Louis, Missouri;* A. Rosenthal, *Institute of Molecular Biotechnology, Jena, Germany*

Human Genome Sequencing Poster Symposium
Chairpersons: D. Bentley, *Sanger Centre, Cambridge, United Kingdom;* E. Green, *National Institutes of Health, Bethesda, Maryland*

Computer Demonstrations II
Chairperson: M.P. Reeve, *Whitehead Institute/MIT Center for Genome Research, Cambridge, Massachusetts*

ELSI Panel Discussion
Moderator: E. Meslin, *National Institutes of Health, Bethesda, Maryland*

Functional Genomics
Chairpersons: P. Brown, *Stanford University, California*; P. Hieter, *Johns Hopkins University, Baltimore, Maryland*

Computational Genomics
Chairpersons: R. McCombie, *Cold Spring Harbor Laboratory, New York;* R. Myers, *Stanford University, California*

Computer Demonstrations III
Chairperson: T. Matise, *Rockefeller University, New York, New York*

Keynote Speaker
James Watson, *Cold Spring Harbor Laboratory*

Gene Discovery and Transcript Mapping
Chairpersons: R. Roberts, *New England Biolabs, Beverly, Massachusetts;* R. Waterston, *Washington University, St. Louis, Missouri*

Genomics: The Next Step

May 18–19 145 participants

ARRANGED BY **Mary Ann Gray,** Dillon, Read & Co. Inc.
David Stewart, Cold Spring Harbor Laboratory

This second annual Dillon Read genomics conference sponsored jointly by the Laboratory and the New York investment bank Dillon, Read & Co. Inc. (now SBC Warburg Dillon Read) brought leaders in the investment and financial communities to Cold Spring Harbor to learn from current leading practitioners and companies about the present state of knowledge and technology in genomics research. Participation in the meeting was by invitation only from both East and West coast financial communities and also from abroad, and included senior executives of biotechnology, pharmaceutical, and venture capital companies. The meeting was relatively informal in style, and all the participants, scientists as well as executives, found the meeting to be both intellectually stimulating and very enjoyable. Eminent keynote speakers including Jim Watson, Ham O. Smith, Rich Roberts, and Jan Witkowski were able to give a broad overall view of certain aspects of the field to a predominantly lay though informed audi-

ence. The keynote talks set the scene for discussions of particular technologies given by senior researchers from leading biotech companies (both established and start-up) who explained the science underlying their technology platforms in the context of the commercial environment. Presentations were made by 30 biotechnology companies including Acacia Biosciences, Algene Biotechnologies Corporation, AlphaGene, Inc., Ariad Pharmaceuticals, Aurora Biosciences Corporation, Cadus Pharmaceutical Corporation, Darwin Molecular Corporation, deCODE Genetics, Digital Gene Technologies, Inc., Exelixis Pharmaceuticals, Gene Logic, Inc, Gene/Networks, Genetics Institute, GeneTrace Systems Inc., Genome Therapeutics Corporation, Genzyme Molecular Oncology, Human Genome Sciences, Incyte Pharmaceuticals, Millenium Pharmaceuticals, MitoKor, Myriad Genetics, Inc., Nanogen Inc., Neurocrine Biosciences Inc., Novalon Pharmaceutical Corporation, Ontogeny Inc., Progenitor Inc., SEQ Ltd, Sequana Therapeutics, Variagenics Inc., and Visible Genetics Inc. A roundtable discussion on "Building a Business in Drug Discovery" (moderator Karen Bernstein) was of considerable interest to the audience, and Kevin Kinsella's post-banquet multimedia presentation once again provided an amusing personal and anecdotal history of the genome biotechnology industry.

This meeting was sponsored by Dillon, Read & Co. Inc., New York.

PROGRAM

Introduction to Genomics: J. Witkowski, *Cold Spring Harbor Laboratory*

Keynote Speaker: K.J. Kinsella, *Sequana Therapeutics*

Welcome: J.D. Watson, *Cold Spring Harbor Laboratory*

Keynote Speaker: H.O. Smith, *Johns Hopkins University School of Medicine*

Corporate Presentations I

Progenitor
Myriad Genetics
Sequana Therapeutics
Exelixis Pharmaceuticals

Roundtable: Building a Business in Drug Discovery
Chairperson: K. Bernstein, *BioCentury Publications, Inc.*

Corporate Presentations II
Genome Therapeutics
Incyte Pharmaceuticals
Mitokor
Ariad Pharmaceuticals

Keynote Speaker: R.J. Roberts, *New England Biologicals*

Corporate Rapid Fire Session
Acacia
AlphaGene
deCODE
Digital Gene
Gene Logic
GeneTrace Systems
Neurocrine Biosciences
Novalon
SEQ
Variagenics

Corporate Presentations III
Human Genome Sciences
Genzyme Molecular Oncology
Nanogen
Visible Genetics

Corporate Presentations IV
Millennium Pharmaceuticals
Genetics Institute
Darwin Molecular, Chiroscience Group
Aurora Biosciences
gene/Networks
Ontogeny
Algene Biotechnologies
Cadus Pharmaceutical

A. Walton, R. Whitsfield

D. Stewart, R. Roberts

Retroviruses

May 20–25 437 participants

ARRANGED BY **Stephen H. Hughes,** National Cancer Institute
John M. Coffin, Tufts University School of Medicine

Although there were exciting developments in a number of areas of retrovirus research, assembly, reverse transcription, integration, expression, HIV accessory proteins, and fish retroviruses, the greatest progress was made in understanding the envelope glycoproteins and their interactions with host-cell receptors. We saw, for the first time, the structure of the critical elements of an SU protein. The structure is both aesthetically pleasing and informative. The segments of SU involved in receptor recognition are positioned on the surface of the protein, and a plausible model of a trimeric SU was presented. We learned more about the HIV-1 coreceptors and the role these proteins have in defining the tropism of particular strains of HIV-1. Despite the fact that in nature the envelope glycoprotein is always on the virus and the receptor always on the cell, there is nothing magical about this arrangement. If by experimental manipulation the receptor is expressed on the virus and the envelope glycoprotein on the surface of the cell, efficient infection can still occur. Happily, these experiments answer important questions and, at the same time, provide us with new problems to solve. Contributions from the Corporate Sponsors and Plant Corporate Associates provided core support for this meeting.

PROGRAM

Entry
Chairpersons: L. Albritton, *University of Tennessee, Memphis;* M. Eiden, *National Institutes of Health, Bethesda, Maryland*

Reverse Transcription
Chairpersons: R. Bambara, *University of Rochester, New York;* W.-S. Hu, *West Virginia University, Morgantown*

Integration
Chairperson: A. Engelman, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Assembly
Chairpersons: W. Sundquist, *University of Utah, Salt Lake City;* H.-G. Kraüsslich, *German Cancer Research Center, Heidelberg*

Vectors/Evoluton
Chairpersons: J.A. Anderson, *West Virginia University, Morgantown;* B. Preston, *University of Utah, Salt Lake City*

Accessory Proteins
Chairperson: J. Elder, *Scripps Clinic and Research Foundation, La Jolla, California*

HIV Pathogenesis
Chairpersons: R. Swanstrom, *University of North Carolina, Chapel Hill;* L. Ratner, *Washington University, St. Louis, Missouri*

Expression
Chairpersons: M.-L. Hammarskjöld, *University of Virginia, Charlottesville;* M. Malim, *Howard Hughes Medical Institute, University of Pennsylvania, Philadelphia*

TAT I/II
Chairperson: A. Rabson, *University of Medicine and Dentistry of New Jersey, Piscataway*

Non-HIV Pathogenesis I/II
Chairperson: D. Derse, *National Cancer Institute, Bethesda, Maryland*

Special Topics
Chairpersons: H. Fan, *University of California, Irvine;* J. Stoye, *National Institute for Medical Research, London, United Kingdom*

J. Coffin, A.-M. Skalka, J. Lew

P. Jolicocur, J. Wills

Yeast Cell Biology

AUGUST 12–17 472 participants

ARRANGED BY **Trisha Davis,** University of Washington
Mark Rose, Princeton University
Tom Stevens, University of Oregon

T. Davis

This conference was the sixth bi-annual international meeting devoted to major aspects of cell biology in yeast. It is unique in that the study of all major areas of cell biology are integrated in a single simple eukaryotic organism, *Saccharomyces cerevisiae*.

A common interest in one organism allowed for extensive cross-fertilization of ideas and methodologies. Important insights were further gained by studies in the fission yeast *Schizosaccharomyces pombe* as well as other yeasts. The completion of the sequencing of the *Saccharomyces* genome had a profound impact on the meeting, greatly facilitating the identification of genes identified by their effects on critical cellular processes. One major area of interest included the functions of the actin cytoskeleton particularly with respect to cell polarity and organelle movement. A second area that was extensively described concerned the roles of microtubules and associated proteins and organizing structures in chromosome and nuclear movement. Third was targeting and sorting of proteins in the secretory, endocytotic, and nuclear localization pathways, and integration of these processes was discussed in various sessions devoted to mating pathways, the cell cycle, and protein transport and degradation. Additional sessions focused on organelle biogenesis and genome analysis, and signaling, ions, and cell wall. It was a very exciting meeting with 368 abstracts, 114 talks, and 254 posters.

Contributions from the Corporate Sponsors and Plant Corporate Associates provided core support for this meeting.

PROGRAM

Mating Pathways
Chairperson: K. Blumer, *Washington University School of Medicine, St. Louis, Missouri*

Cell Cycle and Microtubule Organization
Chairperson: B. Andrews, *University of Toronto, Canada*

Secretion and Endocytosis
Chairperson: N. Segev, *University of Chicago, Illinois*

Microtubule Organization
Chairperson: D. Botstein, *Stanford University School of Medicine, California*

Organelle Biogenesis and Genomic Analysis
Chairperson: A. Hopper, *Hershey Medical Center, Pennsylvania*

Nuclear Transport, ER Protein Degradation, and Folding
Chairperson: J. Rine, *University of California, Berkeley*

Actin Cytoskeleton and Cell Polarity
Chairperson: J. Pringle, *University of North Carolina, Chapel Hill*

Cell Polarity and Development
Chairperson: L. Pon, *Columbia University, New York, New York*

Signaling, Ions, and Cell Wall
Chairperson: J. Thorner, *University of California, Berkeley*

Golgi Function and Protein Sorting
Chairperson: S. Michaelis, *Johns Hopkins School of Medicine, Baltimore, Maryland*

D. Botstein, T. Stevens, F. Sherman

T. Fox, J. Shaw

Eukaryotic mRNA Processing

August 20–24 272 participants

ARRANGED BY **Adrian R. Krainer,** Cold Spring Harbor Laboratory
James Manley, Columbia University
Timothy W. Nilsen, Case Western Reserve University

This first meeting focused on the mechanisms and regulation of pre-mRNA splicing and 3′-end formation in yeast and multicellular eukaryotes. The format of this meeting was modeled after the highly successful RNA processing conferences that were held annually or every other year at Cold Spring Harbor from 1982 to 1995. The major difference is in size and scope, in that the current meeting, which will be held every other year, deals exclusively with eukaryotes and with the processing of messenger RNA.

A major area of discussion at this meeting was in the characterization of SR proteins, which are involved in multiple aspects of constitutive and alternative splicing, as well as in the recognition of splicing enhancer elements. Many presentations focused on other mammalian, invertebrate, or yeast splicing factors, including U2AF, SF1, Prp proteins, as well as several RNA helicase motif proteins, or on further characterization of hnRNP proteins and individual polypeptides from snRNP particles. RNA-RNA interactions among the spliceosomal snRNAs and critical sites on pre-mRNA were described in increasing detail.

Related studies were presented in the context of *trans*-splicing in nematodes and trypanosomes, as well as in the context of the recently identified ATAC *cis*-splicing pathway for a minor class of introns present in several genes; this splicing reaction involves a distinct set of U snRNAs. Recent progress in characterizing a novel mRNA splicing reaction involving the yeast Hac1 transcription factor pre-mRNA was presented; this reaction does not involve the major or minor spliceosomes but rather tRNA ligase. It now appears that Ire1p—a transmembrane kinase involved in the unfolded protein response signaling pathway—also serves as the splicing endonuclease to cleave the Hac1 unusual intron in Hac1. Interestingly, this peculiar splicing pathway appears to be conserved in mammalian cells.

Other presentations at the meeting dealt with 3′-end processing mechanisms and regulation, mRNA editing, mRNA turnover, cellular and viral RNA transport, and localization of mRNA and RNA processing factors in yeast and metazoans. Genetic, biochemical, and cell biological approaches were brought to bear on each of these problems. Finally, several presentations dealt with the integration of mRNA processing reactions—including capping, splicing, 3′-end formation, and mRNA turnover—with each other and with transcription, translation, and the cell cycle.

1997 marked the 20th anniversary of the discovery of split genes in adenovirus. Reports of this remarkable discovery were given at the 1977 XLII Cold Spring Harbor Annual Symposium by teams from Cold Spring Harbor Laboratory and MIT. To commemorate this important occasion, copies of the 1977 abstracts, Symposium articles, and papers, as well as pictures of Symposium participants, were on exhibit after the Saturday afternoon session. Cold Spring Harbor Laboratory President James Watson talked about the XLII and other annual symposia, followed by remarks from two meeting participants and co-authors of one of the landmark 1977 papers: Nobel Laureate Philip Sharp from MIT and Susan Berget, now at the Baylor College of Medicine. This was followed by a champagne toast.

This meeting was funded in part by the National Cancer Institute and the National Institute of Child Health and Human Development divisions of the National Institutes of Health, by the National Science Foundation, and by Promega Corporation. Contributions from the Corporate Sponsors and Plant Corporate Associates also provided core support for this meeting.

The Laboratory would in addition like to thank the RNA Society for its support of this meeting.

S. Berget, J. Alwine, J. Manley

B. Blencowe, P. Sharp, A. Beyer

PROGRAM

Splicing Regulation
Chairperson: T. Maniatis, *Harvard University, Cambridge, Massachusetts*

Mechanisms and Regulation of Polyadenylation
Chairperson: N. Proudfoot, *Sir William Dunn School of Pathology, Oxford, United Kingdom*

Splicing Mechanisms
Chairpersons: M. Konarska, *Rockefeller University, New York, New York;* A. Newman, *MRC Laboratory of Molecular Biology, Cambridge, United Kingdom*

Localization and Transport
Chairperson: R. Lührmann, *Philipps-Universität Marburg, Germany*

snRNPs and Splicing Factors
Chairperson: S. Berget, *Baylor College of Medicine, Houston, Texas*

SR Proteins and Phosphorylation
Chairperson: P. Sharp, *Massachusetts Institute of Technology, Cambridge*

In Celebration of Twenty Years of Splicing
Remarks by James D. Watson, *Cold Spring Harbor Laboratory*

mRNA: Beyond Splicing and 3′ Ends
Chairpersons: L. Maquat, *Roswell Park Cancer Institute, Buffalo, New York;* J. Richter, *Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts*

Mechanisms of Eukaryotic Transcription

August 27–31 424 participants

ARRANGED BY **Nouria Hernandez,** Cold Spring Harbor Laboratory
Robert Kingston, Massachusetts General Hospital
Keith Yamamoto, University of California, San Francisco

This was the fifth biennial meeting devoted to mechanisms of transcriptional regulation in eukaryotes. The conference focused on the structure and function of the basal transcription apparatus involved in transcription initiation and elongation, on activation and repression mechanisms, on mechanisms of chromatin remodeling, and on the role of chromatin and chromatin remodeling for the regulation of transcription initiation and elongation. The conference was attended by scientists from around the world who study transcription in systems as diverse as yeast, plants, and invertebrate and vertebrate animals. Important advances in our understanding of transcriptional regulation were presented, including the characterization of protein-protein and protein-DNA interactions in transcription initiation complexes, the identification of new factors involved in the regulation of transcription elongation, and the characterization of the interplay between chromatin remodeling factors and transcription regulation. The meeting was summarized by Arnold J. Berk.

R. Kingston, R. Treisman

A. Berk, G. Gill

M. Carey

R. Roeder, W. Slumph

This meeting was funded in part by the National Cancer Institute and National Institute of Child Health and Human Development, both branches of the National Institutes of Health; the National Science Foundation; Ariad Pharmaceuticals Inc.; and Tularik, Inc. Contributions from the Corporate Sponsors and Plant Corporate Associates also provided core support for this meeting.

PROGRAM

Basal Machinery
Chairperson: W. Herr, *Cold Spring Harbor Laboratory*

Basal Machinery/Elongation
Chairperson: C. Prives, *Columbia University, New York*

Activation Mechanisms
Chairperson: C. Abate-Shen, *University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway*

Activation Mechanisms/Chromatin Remodeling
Chairperson: D. Allis, *University of Rochester, New York*

Chromatin: Acetylation, Deacetylation, and Elongation
Chairperson: S.M. Gasser, *ISREC, Epalinges, Switzerland*

Modifying Activator Function
Chairperson: E.N. Olson, *University of Texas Southwestern Medical Center, Dallas*

Repression and Activation
Chairperson: M. Carey, *University of California, Los Angeles*

Transcription Factor Function In Vivo
Chairperson: L. Guarente, *Massachusetts Institute of Technology, Cambridge*

Eukaryotic DNA Replication

September 3–7 395 participants

ARRANGED BY **Thomas Kelly,** Johns Hopkins University School of Medicine
Bruce Stillman, Cold Spring Harbor Laboratory

T. Kelly

This was the sixth biannual meeting on eukaryotic DNA replication held at Cold Spring Harbor. Studies of eukaryotic DNA replication are advancing rapidly and advancing on many fronts, and this meeting is now established as the most important in the field. A record number of investigators participated in the ten scientific sessions and there were nearly 300 platform and poster presentations. Thus, interest in the mechanisms and regulation of DNA replication in eukaryotic cells remains extremely high and the meeting is playing a key part in fostering the exchange of new ideas and experimental approaches.

A major goal of work in the field is to define the mechanisms of initiation of eukaryotic DNA replication at replication origins. Several laboratories reported important advances in identifying the proteins that assemble at cellular origins of replication prior to initiation and in defining the critical protein-protein and protein-DNA interactions required for assembly. The nature of the replication switch that links initiation of DNA synthesis to the eukaryotic cell cycle was the subject of a number of genetic and biochemical studies. This work highlighted the central role of cyclin-dependent kinases in triggering initiation and in preventing re-replication prior to mitosis. The enzymology of the eukaryotic replication fork also remains a major focus of study for many laboratories. Work presented at the meeting provided new perspectives on the mechanisms of action of purified DNA polymerases and accessory proteins, as well as insight into the functional interactions that contribute to the efficiency and fidelity of DNA chain elongation. Finally, several groups reported exciting progress in defining and characterizing the protein components of yeast and human telomerase, the enzyme responsible for maintaining the integrity of the ends of chromosomes. The initial characterization of a strain of mice lacking telomerase activity was also discussed.

Essential funding for the meeting was provided by in part by the National Cancer Institute, a division of the National Institutes of Health, and the National Science Foundation. Contributions from the Corporate Sponsors and Plant Corporate Associates also provided core support for this meeting.

PROGRAM

Initiation of Replication
Chairperson: J. Diffley, *Imperial Cancer Research Fund, South Mimms, United Kingdom*

Control of Initiation
Chairperson: E. Fanning, *Vanderbilt University, Nashville, Tennessee*

Chromosome Replication
Chairperson: K. Downey, *University of Miami, Florida*

DNA Unwinding?
Chairperson: B. Brewer, *University of Washington, Seattle*

Replication Fork Accessory Proteins
Chairperson: T. Wang, *Stanford University School of Medicine, California*

Polymerases and Mitochondria
Chairperson: M. DePamphilis, *NICHD, National Institutes of Health, Bethesda, Maryland*

Telomeres and Telomerase
Chairperson: P. Traktman, *Cornell University Medical College, New York, New York*

Virus Chromosome Replication
Chairperson: A. Stenlund, *Cold Spring Harbor Laboratory*

B. Stillman, S. Bell, M. Botchan

L. Harrington, C. Greider

Microbial Pathogenesis and Host Response

September 10–14 305 participants

ARRANGED BY **P.T. Magee,** University of Minnesota
Stanley Maloy, University of Illinois
Ronald Taylor, Dartmouth Medical School

Understanding microbial pathogenesis demands a detailed knowledge of the host response as well as the pathogen itself, and requires an interdisciplinary approach, integrating the fields of microbiology, eukaryotic cell biology, and immunology. This first Cold Spring Harbor meeting was planned to facilitate such interactions, and the meeting attracted more than 300 international scientists who approach the study of bacterial and fungal pathogens from a broad range of perspectives.

Each session focused on recent insights into a specific mechanism of pathogenesis. The first session dealt with bacterial pathogens that produce toxins which cause the primary disease symptoms. A major highlight of this session was a talk by John Mekalanos on how a bacterial virus can convert nonvirulent strains of *Vibrio cholerae* into a highly virulent strain. Another session dealt with *Shigella*, *Listeria,* and *Legionella*, three bacterial pathogens that evade the host immune system by growing inside eukaryotic cells. A highlight of this session was a talk by William Dietrich on the creative combination of classical mouse genetics and genomics to identify genes involved in susceptibility to *Legionella* infections. A session dealt with the infamous bacterial pathogens *Salmonella* and *Yersinia*, which invade a host and cause serious systemic disease, whereas another session dealt with another growing threat, opportunistic gram-positive pathogens such as *Staphylococcus*, *Streptococcus,* and *Bacteriodes*. These pathogens are often resistant to multiple antibiotics and are a common cause of life-threatening, hospital-acquired infections, and several talks highlighted new approaches for developing novel antibiotic targets by identifying genes required for virulence in a eukaryotic host. Another session dealt with *Mycobacterium*, the causative agent of tuberculosis. Two sessions focused on the fungal pathogens *Candida*, an opportunistic yeast pathogen that causes serious illness in immunocompromised hosts, and *Histoplasma* and *Coccidiodes*, two fungal pathogens that cause widespread diseases but are less well studied, and in which William Goldman demonstrated how the clever development of new molecular genetic tools can facilitate the dissection of the mechanism of pathogenesis in an organism that was previously intractable. One session dealt with how the recent developments in genomics have provided powerful new approaches to study microbial pathogenesis, with highlights being talks by Christopher Gray, on how genome sequences can be used to rapidly determine the structure and function of proteins (proteomics), and Daniel Shoemaker, on the use of high-density arrays (DNA chips) to rapidly identify genes that are turned on or off under specific conditions. Another session dealt with novel model systems for dissecting host-pathogen interactions and examined the innovative uses of worms, goldfish, and plants to identify virulence genes that also play a role in human pathogenesis. Finally, Abigail Salyers gave an introspective talk on future challenges facing the field of microbial pathogenesis. Based upon the support and enthusiasm of the participants, the meeting was a resounding success. Thus, a second Microbial Pathogenesis and Host Response meeting will be held in Fall of 1999.

This meeting was funded in part by the National Institute of Allergy and Infectious Diseases, a division of the National Institutes of Health; and the United States Department of the Army. Contributions from the Corporate Sponsors and Plant Corporate Associates also provided core support for this meeting.

S. Maloy

P. Sansonetti, H. Shuman

J. Scott, J.-F. Tomb, P. Model

PROGRAM

Toxigenic Pathogens: *Vibrio Cholerae* and Toxinogenic *E. coli*
Chairperson: R. Taylor, *Dartmouth Medical School, Hanover, New Hampshire*
Keynote Speaker: J. Mekalanos, *Harvard Medical School, Boston*

Intracellular Pathogens: *Shigella, Listeria*, and *Legionella*
Chairperson: P. Sansonetti, *Institut Pasteur, Paris, France*
Keynote Speaker: P. Cossart, *Institut Pasteur, Paris, France*

Disseminating Pathogens: *Salmonella* and *Yersinia*
Chairpersons: V. Miller, *Washington University School of Medicine, St. Louis, Missouri*; J. Slauch, *University of Illinois, Urbana*
Keynote Speaker: R. Isberg, *Tufts University School of Medicine, Boston, Massachusetts*

Opportunistic Gram-positive Pathogens: *Staphylococcus*
Chairperson: M. Schmid, *Microcide Pharmaceutics, Inc., Mountain View, California*
Keynote Speaker: A. Tomasz, *Rockefeller University, New York, New York*

Opportunistic Bacterial Pathogens II: *Streptococcus* and *Bacteriodes*
Chairperson: S. Maloy, *University of Illinois, Urbana*

Chronic Bacterial Pathogens: *Mycobacterium*
Chairperson: I. Behlau, *Tufts University School of Medicine, Boston, Massachusetts*
Keynote Speaker: W. Jacobs, *Albert Einstein College of Medicine, Bronx, New York*

Genomics
Chairperson: J.-F. Tomb, *The Institute for Genomic Research, Rockville, Maryland*

Opportunistic Yeast Pathogens: *Candida*
Chairperson: P.T. Magee, *University of Minnesota, St. Paul*
Keynote Speaker: G. Fink, *Whitehead Institute/MIT, Cambridge, Massachusetts*

Other Fungal Pathogens: *Histoplasma* and *Coccidiodes*
Chairperson: B. Keath, *St. Louis University, Missouri*
Keynote Speaker: W. Goldman, *Washington University School of Medicine, St. Louis, Missouri*

Novel Model Systems for Dissecting Host-Pathogen Interactions
Chairperson: S. Maloy, *University of Illinois, Urbana*

Programmed Cell Death

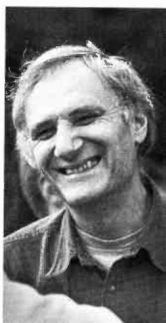
September 17–21 450 participants

ARRANGED BY **Stanley J. Korsmeyer,** Washington University School of Medicine
Eileen White, Rutgers University
H. Robert Horvitz, Massachusetts Institute of Technology

The field of programmed cell death has greatly expanded since the first Cold Spring Harbor meeting on the topic 2 years ago. With the auditorium filled to capacity, the meeting proceeded with a consistent stream of new and engaging information on the mechanisms to regulate cell

death, and components of the cell death machinery. The scope of the talks and posters spanned yeast, nematodes, *Drosophila*, viruses, mice, and humans. The approaches were genetic, developmental, disease-related, and biochemical. Highlights of the meeting were numerous and included identification of genes encoding *Drosophila* caspases and their requirement for development, and the discovery of direct protein complex formation between Ced-3, Ced-4, and Ced-9 gene products of *C. elegans*. Mammalian Bcl-xL and FLICE can replace Ced-9 and Ced-3, respectively, in the Ced-4 protein complex, suggesting that control of caspase activation by members of the Bcl-2 family may be mediated by direct binding to Ced-4. Ion channel activity associated with Bcl-2, Bax, and Bcl-xL in artificial bilayers was reported, in support of the structural similarity of Bcl-xL with the pore-forming domain of diphtheria toxin. The role of mitochondria in the cell death process and caspase activation was also reported. Bax localizes to mitochondria, and in the presence of a death stimulus, cytochrome c is released causing Ced-4/Apaf-1-dependent caspase activation. The protein kinase Akt, which is a component of survival factor signal transduction pathways, was reported to phosphorylate Bad and thereby prevent inhibition of Bcl-2 by Bad. Induction of apoptosis by deregulated c-Myc was found to be mediated by the Fas pathway since blocking that pathway with dominant-negative FADD prevents apoptosis. The specificity of caspase cleavage sites has been elucidated suggesting a hierarchy of activation and a grouping of activities. Caspase cleavage of Bcl-2 and Bcl-xL was found to completely reverse their function to that of pro-apoptotic proteins, suggesting the existence of an amplification step in the apoptotic process. Signal transduction pathways from the Fas and TNF receptors to induce cell death are now well understood. The identification of decoy receptors for Fas ligand-related molecules was reported as a novel mechanism to regulate cell death. The identification of new p53 target genes and their role in cell cycle arrest and apoptosis was reported. The induction of Fas ligand expression in melanoma and other tumors was established as a possible mechanism for tumor cells to evade immune surveillance. Finally, viral and cellular proteins were identified that encode direct antagonizers of the Fas and TNF pathways through interaction with adaptor molecules and caspases. These and numerous other discoveries, with lively discussions thereafter, produced a meeting of extraordinary intellectual interest which was well received in this very fast-paced field.

This meeting was funded in part by the National Institute of Aging, and the National Institute of Child Health and Human Development, both branches of the National Institutes of Health. Contributions from the Corporate Sponsors and Plant Corporate Associates also provided core support for this meeting.


M. Roth

S. Nagata, P. Krammer

M.-C. Hsu, E. White

PROGRAM

Opening Address
A. Wyllie, *CRC Laboratories*

Invertebrate Development
Chairpersons: M. Hengartner, *Cold Spring Harbor Laboratory*; K. White, *Massachusetts General Hospital, Charlestown*

BCL-2 Family
Chairpersons: C. Thompson, *University of Chicago, Illinois*; G. Nuñez, *University of Michigan Medical School, Ann Arbor*

Caspases
Chairpersons: N.A. Thornberry, *Merck Research Laboratories, Rahway, New Jersey*; W.W. Wong, *BASF Bioresearch Corporation*

Special Lecture
S. Nagata, *Osaka University Medical School, Japan*

Vertebrate Development
Chairpersons: J. Yuan, *Massachusetts General Hospital, Boston*; S. Cory, *Walter and Eliza Hall Institute of Medical Research, Parkville, Australia*

Signal Transduction
Chairpersons: D. Goeddel, *Tularik, Inc.*; V.M. Dixit, *University of Michigan, Ann Arbor*

Oncogenesis
Chairpersons: M. Oren, *Weizmann Institute of Science, Rehovot, Israel*; D. Hanahan, *University of California, San Francisco*

Viral Control of Apoptosis
Chairpersons: L. Miller, *University of Georgia, Athens*; J.M. Hardwick, *Johns Hopkins School of Medicine, Baltimore, Maryland*

Immunology/Neurobiology
Chairpersons: P. Krammer, *German Cancer Research Center, Heidelberg*; B.A. Barres, *Stanford University School of Medicine, California*

Neurobiology of *Drosophila*

September 24–28 314 participants

ARRANGED BY **Chris Doe,** University of Illinois
Linda Hall, State University of New York, Buffalo

The primary goal of this year's meeting on *Drosophila* neurobiology was to provide a forum for exchange of ideas, techniques, and new discoveries within the field of *Drosophila* neurobiology. The meeting consisted of platform and poster presentations by 314 junior and established investigators. The platform sessions were arranged to reflect the exciting advances that have been made in understanding the molecular mechanisms underlying neuronal-glial specification, cell-cell signaling, axon guidance, synaptic cell biology, receptor and ion channel function, sensory systems, and complex behavior. The work presented included genetic, cell biological, molecular, neurophysiological, and behavioral approaches to address questions that spanned from nervous system development to nervous system function. The highlights of the meeting included presentations of exciting new developments in several areas, including neuronal-glial determination, cell-cell signaling, axon guidance, sensory systems, biological rhythms, learning and memory, plasticity, and synaptic cell biology. Also noteworthy were reports of cloning and characterization of several novel genes encoding molecules of importance to nervous system development and function, new behavioral paradigms, and a new way to visualize nervous system function in the living animal. The setting of the meeting provided for ample opportunities for informal discussions. The high quality of presentations and the novel findings in many areas made it amply clear that this format, which is inclusive of all aspects of neurobiology, is extremely useful to young and seasoned scientists because it provides an ideal opportunity to cover all the different aspects of *Drosophila* neuroscience.

This meeting was funded in part by the National Institute of Neurological Disorders and Stroke, a branch of the National Institutes of Health, and the National Science Foundation. Contributions from the Corporate Sponsors and Plant Corporate Associates also provided core support for this meeting.

P. Kolodzig, S. Kunes

D. Ebert, L. Hall

PROGRAM

Neuronal-Glial Specification
Chairperson: S. Datta, *Texas A&M University, College Station*

Cell-Cell Signaling
Chairperson: S. Kunes, *Harvard University, Cambridge, Massachusetts*

Axon Guidance
Chairperson: H. Keshishian, *Yale University, New Haven, Connecticut*

Synapse Formation and Function
Chairperson: M. Bate, *University of Cambridge, United Kingdom*

Neurotransmitters, Receptors, Ion Channels
Chairperson: L. Iverson, *Beckman Research Institute of the City of Hope, Duarte, California*

Sensory Systems
Chairperson: J. Fischer, *University of Texas, Austin*

Behavior
Chairperson: R. Greenspan, *New York University, New York*

Human Evolution

October 4–8 199 participants

ARRANGED BY **Luca Cavalli-Sforza,** Genetics Department, Stanford University
James D. Watson, Cold Spring Harbor Laboratory

This meeting originated from a suggestion by Jim Watson and was organized in collaboration with him. Approximately 200 participants, mostly molecular geneticists, physical anthropologists, paleoanthropologists, primatologists, and linguists attended. The meeting was most successful, judging from reactions of participants and reviews. The subject had been rarely touched upon in previous symposia; there were previously three Cold Spring Harbor meetings on human genetics or related subjects in which attention was also paid to human evolution, and they took place in 1957, 1964, and 1985. The meeting will most probably be repeated in 1999. The focus was largely on molecular evolution at the DNA level, with much attention on the new techniques of detection of single nucleotide polymorphisms, comparison of entire sequences, studies on mitochondrial DNA and Y chromosomes, their relative merits and differences, and applications to human fossils, in particular the recent results on the original Neanderthal remains showing that it is not on the direct evolutionary line to modern humans. A definite majority favored the recent African origin of modern humans, but there were supporters of the multiregional hypothesis. Members interested in the development of the Human Genome Diversity Project had three meetings in which programs were discussed. At the time of the meeting, the report of the National Research Council Workshop on the usefulness and feasibility of the study of Human Genome Diversity was not yet published, but it appeared shortly thereafter, finally removing the major political obstacles that had been generated largely by antiscience organizations.

This meeting was largely supported by the Oliver Grace Professorship Fund with additional funding provided by the John D. and Catherine T. MacArthur Foundation. Contributions from the Corporate Sponsors and Plant Corporate Associates also provided core support for this meeting.

Participants watching a video poster

S. Pääbo

PROGRAM

Genetic Markers for Human Molecular Evolution
Chairperson: K.K. Kidd, *Yale University School of Medicine, New Haven, Connecticut*

Molecular Genetics of Living Populations
Chairperson: K. Weiss, *Pennsylvania State University, University Park*

Genes and Language
Chairperson: L.L. Cavalli-Sforza, *Stanford University, California*

Ethics and the HGDP
Chairperson: L.L. Cavalli-Sforza, *Stanford University, California*

Special Lecture
S. Pääbo, *University of Munich*

Paleoanthropology and Human Evolution
Chairperson: R. Klein, *Stanford University, California*

Primate Behavior and the Reconstruction of Human Social Evolution
Chairperson: F.B.M. de Waal, *Emory University, Atlanta, Georgia*

Mitochondrial DNA Studies
Chairperson: M.-C. King, *University of Washington, Seattle*

Y-chromosome Variation
Chairperson: S. Pääbo, *University of Munich, Germany*

Methods for Microsatellite and Other Studies
Chairpersons: M. Feldman, *Stanford University, California;* M. Slatkin, *University of California, Berkeley*

Isolated Populations and Disease
Chairperson: N. Risch, *Stanford University, California*

HLA and Population Genetics
Chairperson: W.F. Bodmer, *Imperial Cancer Research Fund, Oxford, United Kingdom*

R. Cooke, M.-C. King

The *Arabidopsis* Genome: From Sequence to Function

December 11–14 133 participants

ARRANGED BY **Rob Martienssen,** Cold Spring Harbor Laboratory
Michael Bevan, John Innes Center, United Kingdom

The sequence of the *Arabidopsis* genome is likely to be completely, or nearly completely, determined by the year 2000. This meeting served as the first of a series of annual biotechnology symposia aimed at bringing together those groups sequencing and interpreting the sequence and those groups attempting to determine the biological function of genes and other chromosomal sequences. Sessions on sequencing progress and annotation were followed by a session on positional cloning of genes identifed by mutation. This session outlined progress in cloning a number of genes using novel PCR-based markers, as well as progress in identifying centromeric regions by recombinational analysis. Sessions devoted to insertional mutagenesis using transfer DNA and transposons gave a comprehensive overview of insertion banks and their utility in gene disruption. It was clear that sufficient material had already been generated in many laboratories for site-selected mutagenesis by screening pools of plants via the PCR. Systematic characterization of gene expression via EST sequencing, enhancer and gene trapping, and biochip technology presented an impressive array of tools for analyzing gene expression at high resolution throughout the genome. A final session on bioinformatics highlighted novel means to catalog, assemble, interpret, and disseminate this vast flood of new data. The remarkable conservation of genes and gene clusters emerging from comparative studies of plant genomes was an eye-opener to many. The need to examine gene function and expression simultaneously in a systematic way was emphasized in a large number of the presentations. Perhaps an unexpected outcome of the meeting was the surprising degree of duplication and redundancy and the relatively low level of lethality associated with disrupted *Arabidopsis* genes, compared with animal and fungal studies. It is clear that imaginative approaches are going to be required to take maximum advantage of this exciting new era in plant biology.

This meeting was funded in part by DEKALB Genetics Corporation; Diatech, Ltd.; Keygene (The Netherlands); and Monsanto Company. Contributions from the Corporate Sponsors and Plant Corporate Associates also provided core support for this meeting.

J. Mundy, M. Bevan, P. Rouze

R. Flavell, D. Jackson, R. Martienssen

PROGRAM

Progress in Genome Sequencing
Chairperson: F. Ausubel, *Massachusetts General Hospital, Boston*

Advances in Positional Cloning
Chairperson: J. Jones, *Sainsbury Laboratory, John Innes Centre, Norwich, United Kingdom*

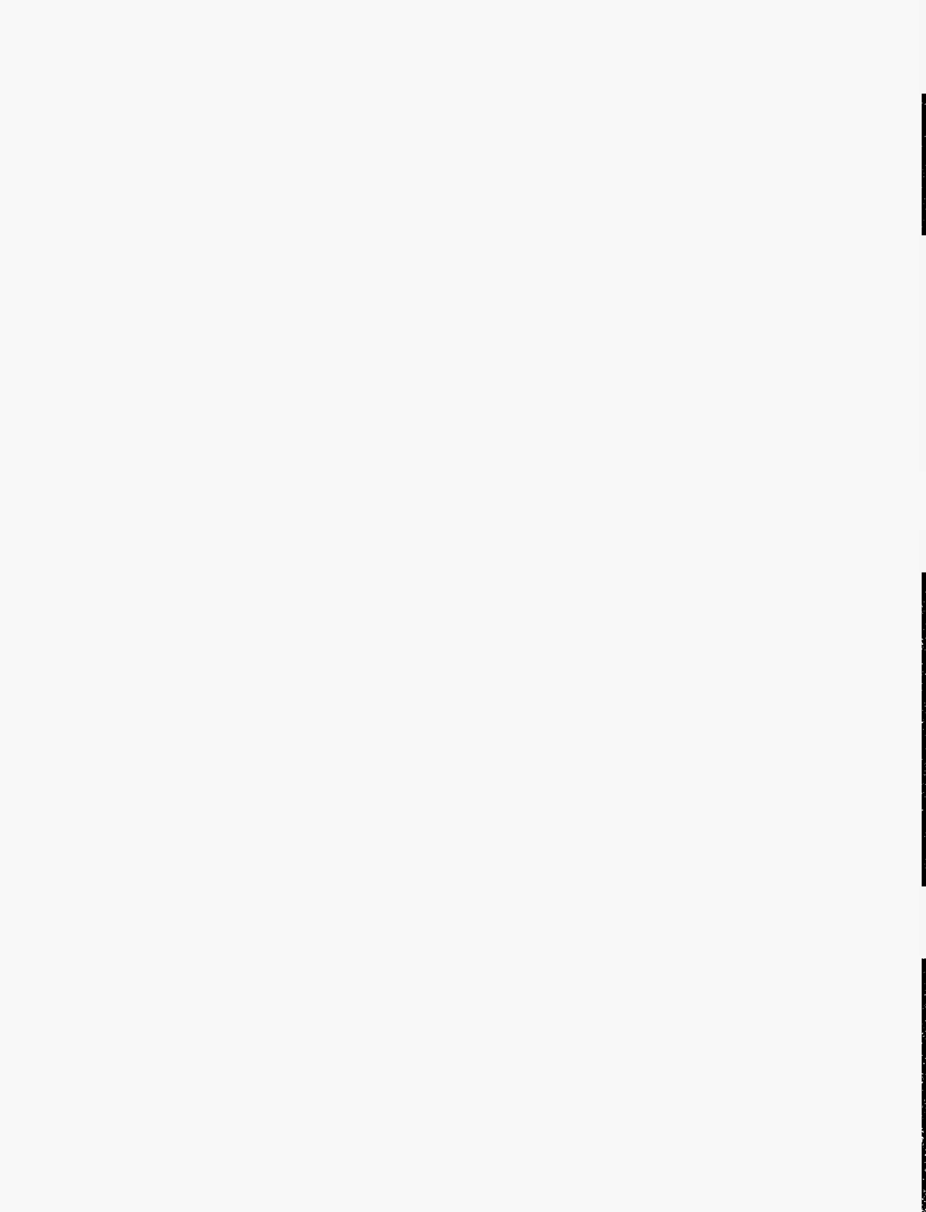
Annotation Workshop
Moderator: S. Rounsley, *The Institute for Genomic Research, Rockville, Maryland*

T-DNA Mutagenesis and Antisense Strategies
Chairperson: N. Fedoroff, *Pennsylvania State University, University Park*

Transposon Mutagenesis
Chairperson: R. Martienssen, *Cold Spring Harbor Laboratory*

Novel Approaches to Gene Expression
Chairperson: M. Bevan, *John Innes Centre, Norwich, United Kingdom*

Informatics and Databases
Chairperson: N. Federspiel, *Stanford University DNA Sequencing and Technology Center, Palo Alto, California*



POSTGRADUATE COURSES

The summer program of Postgraduate Courses at Cold Spring Harbor Laboratory is aimed at meeting the special need for training in interdisciplinary subjects that are either so new or so specialized that they are not adequately treated by universities. Our aim is to provide intensive study in the most recent developments and techniques in these subjects and to prepare students to enter directly into research in a particular area. To ensure up-to-date coverage of current research work, we bring together a workshop staff from many laboratories around the world and supplement this staff with a series of seminar speakers.

Advanced Genome Sequence Analysis

March 14–27

INSTRUCTORS

Chen, Ellson, Ph.D., Applied Biosystems Division of Perkin Elmer Corporation
Gibbs, Richard, Ph.D., Baylor College of Medicine
McCombie, Richard W., Ph.D., Cold Spring Harbor Laboratory
Wilson, Richard, Ph.D., Washington University School of Medicine

CO-INSTRUCTORS

Mardis, Elaine, Washington University School of Medicine
Muzny, Donna, Baylor College of Medicine
Zuo, Lin, Sequana Therapeutics

ASSISTANTS

Anari, Ali, Baylor College of Medicine
Chen, Chun-Nan, Applied Biosystems Division of Perkin Elmer Corporation
De La Bastide, Melissa, Cold Spring Harbor Laboratory
Dedhia, Neilay, Cold Spring Harbor Laboratory
Evans, Cheryl, Genetics Institute
Fitzgerald, Michael, Massachusetts General Hospital
Gnoj, Lidia, Cold Spring Harbor Laboratory
Greco, Tracie, Washington University School of Medicine
Johnson, Doug, Washington University School of Medicine
Kaplan, Nancy, Cold Spring Harbor Laboratory
Nahn, Michael, Washington University School of Medicine
Parnell, Larry, Cold Spring Harbor Laboratory
Schutz, Kristin, Cold Spring Harbor Laboratory
Touchman, Jeffrey, National Institutes of Health, National Human Genome Research Institute

The purpose of the course was to teach students the range of techniques and project management skill required to carry out a large-scale sequencing project. The course provided a 2-week, extensive laboratory experience in which the students work as a team to carry out a large-scale sequencing project.

Recent advances in the automation of DNA sequencing have opened new possibilities for the analysis of complex genomes at the DNA sequence level. This 2-week course provided intensive training in this rapidly evolving field. The course emphasized techniques and strategies for using automated sequences to sequence large, contiguous genomic regions. Students carried out all

of the steps in the sequencing process from preparing cosmid DNA to computer analysis of the finished sequence. Topics included subclone library generation, large-scale template purification, sequencing reactions, gel analysis on automated sequencers, sequence assembly, gap filling, and conflict resolution. Students worked in groups to sequence a large region of DNA. For instance, in the 1997 course, a 143-kb human BAC clone containing two chemokine receptor genes was sequenced. Through this process, they were trained in crucial project and data management techniques. A series of lecturers discussed their applications of these techniques as well as alternate strategies for high-speed automated DNA sequencing.

PARTICIPANTS

DeSilva, U., B.S., National Institutes of Health, Bethesda, Maryland
Diaz-Perez, S., B.S., Ph.D., University of California, Los Angeles
Doggett, N., B.A., Ph.D., Los Alamos National Laboratory, New Mexico
Dragan, Y., B.A., Ph.D., University of Wisconsin, Madison
Garcia, D., B.S., M.S., University of Texas Health Science Center, San Antonio
Giacalone, J., B.A., Ph.D., New York University
Pae, A., B.S., Millennium Pharmaceutical, Inc., Massachusetts
Porcel, B.M., B.S., Ph.D., Uppsala University, Sweden
Powell, E., B.A., M.P.A., Ph.D., Tuskegee University, Alabama
Sagripanti, J.-L., B.S., Ph.D., Food & Drug Administration, Rockville, Maryland
See, L.H., B.S., M.S., Defence Medical Research Institute, Singapore
Solinsky, K., B.S., Case Western Reserve University, Cleveland, Ohio
Tang, M., B.S., Ph.D., University of California, Berkeley
Watanabe, M., B.S, Ph.D., Chiba University, Chiba, Japan
Yu, Y., B.S., M.S., Clemson University, South Carolina
Zhou, X., M.S., Millennium Pharmaceutical, Inc., Cambridge, Massachusetts

SEMINARS

Bevan, M., John Innes Centre, Norwich, United Kingdom. The European *Arabidopsis* genome initiative.
Chee, M., Affymetrix. Santa Clara, California. New technologies for DNA sequencing.
Fraser, C., Institute for Genomic Research, Rockville, Maryland. Microbial genome sequencing at TIGR.
Green, E., National Institutes of Health, Bethesda, Maryland. Physical mapping of human chromosomes.
Mathies, R., University of California, Berkeley. New developments In fluorescent sequencing.
McPherson, J., Washington University School of Medicine, St. Louis, Missouri. Developing sequence-ready maps of the human genome.
Roe, B., University of Oklahoma, Norman. Experience from sequencing more than 1 megabase of human genomic DNA.
Schwartz, D., New York University Medical Center. Optical mapping of large-molecular-weight DNA molecules.
Watson, J., Cold Spring Harbor Laboratory. History of the human genome project.

Early Development of *Xenopus laevis*

April 8–17

INSTRUCTORS

Krieg, Paul, Ph.D., University of Texas, Austin
Moody, Sally, Ph.D., George Washington University Medical Center

ASSISTANTS

Kenyon, Kristy, George Washington University Medical Center
Kroll, Kristin, Harvard Medical School
Zorn, Aaron, University of Cambridge/Wellcome CRC Institute

This course provided extensive laboratory exposure to the biology, manipulation, and use of embryos from the frog, *Xenopus laevis*. The course was suited both for investigators who had no experience with *Xenopus* and for those who had worked with *Xenopus* and wished to learn new techniques. All students had current training in molecular biology and some knowledge of developmental biology. The course consisted of intensive laboratory sessions, supplemented by daily lectures and demonstrations from experts in both experimental and molecular embryology. Areas covered included (1) care of adults and embryo isolation; (2) stages of embryonic development and anatomy; (3) whole-mount in situ hybridization and immunocytochemistry; (4) microinjection of eggs and oocytes, including fluorescent lineage tracers, DNA constructs, mRNA, and antisense oligonucleotides; (5) micromanipulation of embryos, including explant and transplantation assays; and (6) use of cell cycle extracts, including preparation of transgenic embryos.

PARTICIPANTS

Brittingham, J., B.S., Ph.D., University of Iowa, Iowa City
Coumailleau, P., B.S., Ph.D., Karolinska Institute, Stockholm, Sweden
Furutani-Seiki, M., M.D., Ph.D., Max Planck Institute für Entwicklungsbiologie, Tübingen, Germany
Hoskins, S., B.S., Ph.D., City College of the City of New York
Lee, Y.J., B.S., Ph.D., Harvard University/Dana Farber Cancer Institute, Boston
Liu, X., B.S., Ph.D., Harvard Medical School/Center for Blood Research, Boston
Lupo, G., B.S., University of Pisa, Chezzano, Italy
Matsuo-Takasaki, M., B.S., Ph.D., National Institutes of Health, Bethesda, Maryland
Nick, T., B.A., B.S., Ph.D., University of Colorado Health Sciences Center, Denver
Pandur, P., B.S., University of Ulm, Germany
Peunova, N., M.S., Ph.D., Cold Spring Harbor Laboratory
Popsueva, A., M.S., Engelhardt Institute of Molecular Biology RAS, Moscow, Russia
Rettig, J., B.S., Ph.D., Max Planck Institute for Biophysical Chemistry, Goettingen, Germany
Sible, J., B.S., Ph.D., University of Colorado School of Medicine/HHMI, Denver
Sokac, A.M., B.S., University of Wisconsin, Madison
Samanas, S., B.S., University of Minnesota, Minneapolis

SEMINARS

Cho, K., University of California, Irvine. Molecular mechanisms of activin and BMP signaling during *Xenopus* embryogenesis.
Christian, J., Oregon Health Sciences University, Portland. Regulation of BMP signaling during vertebrate development.
Grainger, R., University of Virginia, Charlottesville. Lens induction, and genetics as a strategy.
Harland, R., University of California, Berkeley. Expression cloning of developmental genes.
Heasman, J., University of Minnesota School of Medicine, Minneapolis. Adhesion molecules and antisense oligonucleotide inhibition of function.
Keller, R., University of Virginia, Charlottesville. *Xenopus* gastrulation.
Kessler, D., University of Pennsylvania School of Medicine, Philadelphia. Regulation of vertebrate cell fate by maternal inducers.
Sive, H., Massachusetts Institute of Technology, Cambridge. Early events in *Xenopus* anteroposterior patterning: Approaches using subtractive cloning and inducible gene expression.
Slack, J., University of Bath, United Kingdom. The role of fibroblast growth factors in *Xenopus* development.

Cloning and Analysis of Large DNA Molecules

April 8–21

INSTRUCTORS

Abderrahim, Hadi, Ph.D., Stanford University
Riethman, Harold, Ph.D., The Wistar Institute
Wing, Rod, Ph.D., Clemson University

CO-INSTRUCTOR

Dewar, Ken, University of Pennsylvania

ASSISTANTS

Choi, Sangdun, Clemson University
Frisch, David, Clemson University

This course covered the theory and practice of manipulating, cloning, and mapping large segments of DNA. The course focused on the hands-on construction of bacterial artificial chromosome (BAC) clone libraries and the use of several types of large-insert clone libraries (YAC, BAC, PAC) and genomic DNA for the analysis of large chromosome regions in the physical mapping and contig-building phases of positional cloning projects. Lectures and laboratory work included BAC clone library preparation, preparative and analytical pulsed-field gel electrophoresis (PFGE), techniques

for the isolation and manipulation of high-molecular-weight DNA from a variety of sources (including plants and animals), and an introduction to relevant bacterial and yeast genetics. BAC- and YAC-based positional cloning methods included library screening, contig assembly, long-range restriction and RecA-assisted restriction endonuclease (RARE) cleavage mapping, and recovery/analysis of large-insert clone end-fragments. Lectures by outside speakers on topics of current interest supplemented the laboratory work.

PARTICIPANTS

Basinski, M., M.S., Lilly Research Laboratories, Indianapolis, Indiana
Berghmans, S., D.V.M., University of Liege, Belgium
Champagne, G., B.S., M.S., DNA LandMarks, Inc., Quebec, Canada
Di Girolamo, M., B.S., Consorzio Mario Negri Sud, S. Maria Imbaro, Italy
Ebeling, S., B.S., Ph.D., The Netherlands Red Cross, Amsterdam
Goff, S., B.A., Ph.D., Novartis Corporation, Research Triangle Park, North Carolina
Hornum, L., M.S., Ph.D., Hagedorn Research Institute, Gentofte, Denmark
Jeon, J.-T., B.S., Ph.D., Swedish University of Agricultural Sciences, Uppsala, Sweden
Lidmer, A.-S., M.S., Astra Hassle AB, Molndal, Sweden
Lodhi, M., M.S., Ph.D., Cold Spring Harbor Laboratory
Morshed, S.A., M.D., Ph.D., Yale University, New Haven
Parkin, I., B.S., M.S., Ph.D., John Innes Center, Norwich, United Kingdom
Podlutsky, A., B.S., Karolinska Institute, Huddinge, Sweden
Reddy, A., B.S., M.S., Ph.D., University of Michigan, Ann Arbor
Rogel-Gaillard, C., B.S., Ph.D., Institut National de la Recherche Agronomique, Jouy en Josas, France
Zhang, Y.H., B.S., M.S., Ph.D., University of Massachusetts, Boston

SEMINARS

Birren, B., Whitehead Institute, Cambridge, Massachusetts. Large library screening physical mapping.
Cheung, V., University of Pennsylvania/Children's Hospital of Philadelphia. Gene chip technology.
Doggett, N., Los Alamos National Laboratory, New Mexico. Integrated maps of human chromosome 16.
Ganal, M., Institute for Plant Genetics and Crop Plant Research, Gatersleben, Germany. Plant genome analysis.
Gnirke, A., Mercator Genetics, Inc., Menlo Park, California. RARE cleavage.
Larionov, V., National Institutes of Health, Research Triangle Park, North Carolina. TAR cloning.
McCombie, W.R., Cold Spring Harbor Laboratory. Large-scale sequencing.
Reeves, R., Johns Hopkins University School of Medicine, Baltimore. Positional cloning strategies.

Protein Purification and Characterization

April 8–21

INSTRUCTORS

Burgess, Richard, Ph.D., University of Wisconsin, Madison
Courey, Albert, Ph.D., University of California, Los Angeles
Lin, Sue-Hwa, Ph.D., University of Texas/MD Anderson Cancer Center
Mische, Sheenah, Ph.D, Rockefeller University

ASSISTANTS

Bai, Yijian, University of California, Los Angeles
Earley, Karen, University of Texas/MD Anderson Cancer Center
Gharahdaghi, Farzin, Rockefeller University
Pietz, Brad, University of Wisconsin, Madison
Thompson, Nancy, University of Wisconsin, Madison
Valentine, Scott, University of California, Los Angeles
Weinberg, Catherine, Rockefeller University

This course was intended for scientists who were not familiar with techniques of protein isolation and characterization. It was a rigorous program that included laboratory work all day and a lecture with discussion session every evening. Each student became familiar with each of the major techniques in protein purification by actually performing four separate isolations including (1) a regulatory protein from muscle tissue; (2) a sequence-specific, DNA-binding protein; (3) a recombinant protein overexpressed in *E. coli*; and (4) a membrane-bound receptor. A variety of bulk fractionation, electrophoretic, and chromatographic techniques were employed including precipitation by salts, pH, and ionic polymers; ion-exchange, gel-filtration, hydrophobic interaction, and reverse-phase chromatography; lectin affinity, ligand affinity, oligonucleotide affinity, and immunoaffinity chromatography; polyacrylamide gel electrophoresis and electroblotting; and high-performance liquid chromatography. Procedures were presented for solubilizing proteins from inclusion bodies and refolding them into active monomeric forms. Methods of protein characterization were utilized including immunological and biochemical assays, peptide mapping, amino acid analysis, protein sequencing, and mass spectrometry. Emphasis was placed on strategies of protein purification and characterization rather than on automated instrumental analysis. Guest lecturers discussed protein structure, modification of proteins, methodologies for protein purification and characterization, and applications of protein biochemistry to cell and molecular biology.

PARTICIPANTS

Belien, A., B.S., University of Zurich, Switzerland
Bertaux, F., B.S., M.S., Ph.D., Guy's Hospital, London, United Kingdom
Bordo, D., B.S. Ph.D., Advanced Biotechnology Center, Genoa, Italy
Chalfie, M., B.A., Ph.D., Columbia University, New York
Delgado, A., B.S., M.S., Ph.D., University of California, Los Angeles
Drabkin, H., B.A., M.D., University of Colorado Health Science Center, Denver
Dumont, E., B.A., M.A., Ph.D., Northeastern Ohio University, Rootstown
Flower, A., B.S., Ph.D., University of North Dakota School of Medicine, Grand Forks
Gershenson, A., B.A., Ph.D., California Institute of Technology, Pasadena
Gwynn, B., B.S., M.S., The Jackson Laboratory, Bar Harbor, Maine
Ingham, C., B.S., Ph.D., Nottingham University, United Kingdom
Koshland, D., B.A., Ph.D., Carnegie Institute of Washington, Baltimore, Maryland
Li, D.W.-C., B.S., Ph.D., Columbia University, New York
Little, E., B.S., University of Arizona, Tucson
Roll, U., B.S., M.S., Ph.D., University of California, San Francisco
Schuyler, S., B.A., Harvard Medical School/Dana Farber Cancer Center, Boston

SEMINARS

Baker, T., Massachusetts Institute of Technology, Cambridge. Assembly, organization, and remodeling of the recombination machinery.
Courey, A., University of California, Los Angeles. Transcriptional regulation of dorsal/ventral pattern formation in *Drosophila*.
Greenblatt, J., University of Toronto, Canada. Multiprotein complexes involved in transcriptional initiation and elongation.
Greider, C., Cold Spring Harbor Laboratory. Telomerase: Biology and biochemistry.
Guidotti, G., Harvard University, Cambridge. Are insulin receptors dimers?
Lin, S.-H., University of Texas/MD Anderson Cancer Center, Houston. Role of cell adhesion molecule (C-CAM) in prostate cancer.
Marshak, D., Osiris Therapeutics, Baltimore, Maryland. Mesenchymal stem cells and associated glycoproteins.
Mische, S., Rockefeller University, New York. Microanalytical protein preparation and characterization.
Raines, R., University of Wisconsin, Madison. Protein folding and disulfide bond formation
Stillman, B., Cold Spring Harbor Laboratory. Biochemical approach to understanding replication of the eukaryotic cell genome.

Developmental Neurobiology

June 4–17

INSTRUCTORS

Bargmann, Cornelia, Ph.D., University of San Francisco
Burden, Steven, Ph.D., New York University Medical Center
Cline, Holly, Ph.D., Cold Spring Harbor Laboratory

The aim of this lecture course was to discuss principles and recent advances in developmental neurobiology. Major topics considered included determination, proliferation, and differentiation of neural cells; trophic interactions in neural development; gradients and compartments; guidance of axons to targets; and the formation of synapses. These topics were considered within the context of the development of both invertebrate and vertebrate neural systems. Prospective students had a background in neurobiology or molecular biology.

PARTICIPANTS

Alcamo, E., B.S., Massachusetts Institute of Technology, Cambridge

Bradke, F., B.S., European Molecular Biology Laboratory, Heidelberg, Germany

Brosamle, C., M.A., University of Zurich, Switzerland

Durand, B., M.S., Ph.D., University College London, United Kingdom

Gary, S., B.S., Ph.D., Yale University, New Haven

Hines, P., B.A., Ph.D., *Science Magazine*, Washington, D.C.

Hummel, T., B.S., Universitat Munster, Germany

Lothian, C., B.S., M.D., Karolinska Institute, Stockholm, Sweden

Monaghan, A.-P., B.A., Ph.D., German Cancer Research Center, Heidelberg, Germany

Moore, A., B.A., Medical Research Council, Edinburgh, United Kingdom

Murgia, M., B.S., Ph.D., University of Padova, Italy

Ren, P., B.S., Ph.D., University of Missouri, Columbia

Schaefer, A., B.S., Washington University School of Medicine, St. Louis, Missouri

Shiraishi, Y., M.S., Tokyo University, Japan

Sigal, C., B.S., Ph.D., National Institutes of Health, Bethesda, Maryland

Smithers, L., B.S., Imperial Cancer Research Fund, London, United Kingdom

Solomin, L., M.D., Karolinska Institute, Stockholm, Sweden

Van der Sar, A., M.S., The Netherlands Institute for Developmental Biology, Utrecht

Wang, S., B.S., M.D., Ph.D., Stanford University, Palo Alto,California

SEMINARS

Bargmann, C., University of California, San Francisco. The cell biology of neuronal development/Mechanisms of controlling axon guidance in *C. elegans*.

Barres, B., Stanford University School of Medicine, California. Myelination in the central nervous system.

Bonhoeffer, T., Max Planck Institute, Munich, Germany. Role of activity and neurotrophins in the development of the visual cortex.

Hemmati Brivanlou, A., Rockefeller University, New York. Control of ectoderm and mesoderm patterning during *Xenopus* development.

Burden, S., New York University Medical Center, New York. Neuromuscular synapse formation.

Cline, H., Cold Spring Harbor Laboratory. Regulation of presynaptic differentiation.

Eisen, J., University of Oregon, Eugene. Motor neuron development in Zebrafish.

Fox, K., University of Wales, Cardiff, United Kingdom. Activity-dependent development in the barrel cortex.

Goodman, C., University of California, Berkeley. Pathfinding in the central and peripheral nervous systems of *Drosophila*.

Hatten, M.B., Rockefeller University, New York. Neuronal migration during cortical development.

Jan, Y.N., University of California, San Francisco. Control of cell fate decisions during neural differentiation in *Drosophila*.

Jessell, T. Columbia University College of Physicians & Surgeons, New York. Patterning in the developing neural tube.

Jin, Y., University of California, Santa Cruz. Control of neural fate and differentiation in *C. elegans*.

Kinter, C., The Salk Institute, San Diego, California. Neural induction and neurogenesis in *Xenopus*.

Lemke, G., The Salk Institute, San Diego, California. Regulation of Schwann cell differentiation.

Lichtman, J., Washington University, St. Louis, Missouri. Editing of synaptic connections.

O'Leary, D., The Salk Institute, San Diego, California. Pathfinding in the developing visual system.

Raff, M., University College London, United Kingdom. Control of cell number.

Reichardt, L., University of California, San Francisco. Regulation of neural differentiation by adhesion molecules and neurotrophins.

Zipursky, L., University of California, Los Angeles. Signaling during development of the *Drosophila* eye.

Advanced Bacterial Genetics

June 6–26

INSTRUCTORS

Bassler, Bonnie, Ph.D., Princeton University
Manoil, Colin, Ph.D., University of Washington
Slauch, James, Ph.D., University of Illinois

ASSISTANTS

Callahan, Jennifer, University of Washington
Freeman, Jeremy, Princeton University
Ho, Theresa, University of Illinois

The laboratory course presented logic and methods used in the genetic dissection of complex biological processes in bacteria. The methods presented included mutagenesis using transposons, mutator strains, and chemical and physical mutagens; mapping mutations using genetic and physical techniques; generation and analysis of gene fusions; molecular cloning; polymerase chain reaction; Southern blot analysis; epitope insertion mutagenesis; and site-directed mutagenesis. A key component of the course was the use of sophisticated genetic methods in the analysis of pathogenic and "undomesticated" bacteria. Invited lecturers described the use of genetic approaches to study biological processes in a variety of bacteria.

PARTICIPANTS

Blum, J., B.S., M.D., Ph.D., Harvard Medical School, Boston, Massachusetts
De Wulf, P., M.S., Ph.D., Harvard Medical School, Boston, Massachusetts
Durrant, L., M.S., Ph.D., Campinas, State University, Sao Paulo, Brazil
Ershova, G., M.S., Ph.D., Institute of Molecular Genetics, Moscow, Russia
Gizatullin, F., M.S., Ph.D., Massachusetts Institute of Technology, Cambridge
Handfield, M., B.S., M.S., Laval University, Quebec, Canada
McKay, D., B.S., Ph.D., Stanford University, California
Morfeldt, E., B.S., Ph.D., Karolinska Institute, Stockholm, Sweden

Moxley, R., D.V.M., Ph.D., University of Nebraska, Lincoln
Pace, J., B.S., M.S., Ph.D., Antex Biologics, Inc., Gaithersburg, Maryland
Rech, S., B.S., M.A., Ph.D., University of California, Los Angeles
Sanders, G., B.A., Ph.D., Massachusetts Institute of Technology, Cambridge
Schauder, S., B.S., University of Bern, Switzerland
Schuler, D., B.S., Ph.D., Iowa State University, Ames
Sibley, M., B.A., Ph.D., New England Biolabs, Beverly, Massachusetts
Wu, G., B.S., Ph.D., University of Sheffield, United Kingdom

SEMINARS

Gross, C., University of California, San Francisco. Sensing stress in the periplasm.
Kleckner, N., Harvard University, Boston. Mechanisms and regulation of Tn*10* transposition.
Isberg, R., Tufts University, Boston. Intracellular trafficking in *L. pneumophila*.
Mekalanos, J., Harvard University Medical School, Boston. Molecular genetics analysis of *V. cholerae* pathogenesis.
Ohman, D., University of Tennessee, Memphis. Genetic analysis of protease processing and secretion in *P. aeruginosa*.
Rudd, K., University of Miami, Florida. Whole genome sequence: Ultimate bacterial genetics.
Silhavy, T., Princeton University, New Jersey. Parallel pathways perceive periplasmic problems.
Wright, A., Tufts University, Boston. Visualizing chromosome behavior in *E. coli*.

Integrated Approaches to Ion Channel Biology

June 6–26

INSTRUCTORS

Caldwell, John, Ph.D., University of Colorado
Levinson, Rock, Ph.D., University of Colorado
Maue, Robert, Ph.D., Dartmouth Medical School

ASSISTANTS

Koszowski, Adam, University of Colorado
Shah, Ruta, Dartmouth Medical School
Spooner, Edward, Dartmouth Medical School

This intensive laboratory/lecture course introduced students to the multidisciplinary use of molecular biological, biochemical, immunological, and electrophysiological approaches to the study of ion channels. The laboratory focused on the cellular regulation of channel expression and function. Hands-on exercises included characterization of regulatory elements that control transcription of channel genes, identification of ion channel isoform transcripts and proteins, visualization of channel distributions, and the biophysical analysis of channel isoform function in excitable cells and tissues and in exogenous expression systems. Specific techniques employed included PCR, Western blot, transient transfection and expression, immunocytochemistry, and patch-clamp analysis of ionic currents. Lectures covered the techniques employed while providing broad exposure to current issues surrounding ion channel mechanisms and cellular expression. This course was intended for advanced students with specific plans to apply the techniques taught to a defined problem, and students were encouraged to bring their preparation to the course for preliminary studies.

Lectures were given by Dave Dawson, Dan Goldman, Bill Green, Leslie Henderson, John Kusiak, Gail Mandel, Hannah Monyer, Lorna Role, Peter Sargent, Terry Snutch, and Jim Trimmer.

PARTICIPANTS

Bertaso, F., B.S., Kings College School of Medicine, London, United Kingdom
Charlier, C., D.V.M., University of Utah, Salt Lake City
Didier, M., B.S., Ph.D., Universite Laval, Quebec, Canada
Escayg, A., B.S., Ph.D., University of Michigan, Ann Arbor
Ferreira, G., M.S., M.D., Rush Presbyterian St. Luke's Medical College, Chicago, Illinois
Groot Kormelink, P., B.S., University of Leuven, Belgium
MacDonell, K., B.S., M.S., Ph.D., University of Calgary, Alberta, Canada
O'Connell, D., B.S., Ph.D., University College Dublin, United Kingdom
Sampo, B., B.S., M.S., INSERM U 464, Marseille, France
Santi, C., M.D., University of Mexico, Mexico DF, Mexico
Thompson, R., B.S., McMaster University, Ontario, Canada
Verbitsky, M., B.S., National Council of Science and Technology Research, Buenos Aires, Argentina

SEMINARS

Bargmann, C., University of California, San Fancisco. Genetic approaches to the study of ion channels and behavior in *C. elegans*.
Barres, B., Stanford University, California. Induction of sodium channel clustering by oligodendrocytes.
Caldwell, J., University of Colorado Health Sciences Center, Denver. Sodium channel six: Developmental expression and distribution in the nervous system.
Froehner, S., University of North Carolina, Chapel Hill. Regulation of ion channel distribution.
Gardner, P., University of Texas Health Science Center, San Antonio. Brawn and brain and nicotinic acetylcholine receptor gene expression.
Henderson, L., Dartmouth Medical School, Hanover, New Hampshire. Sex and GABAA receptors: The Ego and Id of neurotransmission.
Levinson, R., University of Colorado Health Sciences Center, Denver. Sodium channel expression and distribution in the peripheral nervous system.
Malinow, R., Cold Spring Harbor Laboratory. Synaptic transmission and plasticity in hippocampal slices.
Mandel, G., State University of New York, Stony Brook. Silencing the type II Na channel gene: A model for neuron-specific gene regulation.
Maue, R., Dartmouth Medical School, Hanover, New Hampshire. Structural and functional analysis of epitope-tagged Na channel B1 subunits/Mechanisms underlying Na channel induction by neurotrophic factors.
O'Dowd, D., University of California, Irvine. Development of electrical excitability and synaptic connections in the mammalian CNS.
Salkoff, L., Washington University, St. Louis, Missouri. The impact of the *C. elegans* genome sequencing project on potassium channel biology.
Sargent, P., University of California, San Francisco. Structural and functional diversity of nicotinic receptor families in the brain.
Snutch, T., University of British Columbia, Vancouver, Canada. Modulation of calcium channel activity by protein kinases and G-proteins.
Zweifach, A., University of Colorado Health Sciences Center, Denver. Analysis of CRAC channel regulation in lymphocytes.

Molecular Embryology of the Mouse

June 6–26

INSTRUCTORS

Koopman, Peter, Ph.D., University of Queensland, Australia
Magnuson, Terry, Ph.D., Case Western Reserve University

CO-INSTRUCTORS

Nagy, Andras, Ph.D., Samuel Lunenfeld Research Institute, Canada
Tam, Patrick, Ph.D., Children's Medical Research Institute, Australia

ASSISTANTS

Bowles, Jo, University of Queensland, Australia
Schumacher, Armin, Case Western Reserve University

This course was designed for biologists interested in applying their expertise to the study of mouse embryonic development. Laboratory components provided an intensive introduction into the technical aspects of working with and analyzing mouse embryos, and lecture components provided the conceptual basis for current research. Procedures described included isolation and culture of pre- and postimplantation embryos; oviduct and uterine transfer; formation of aggregation chimeras; isolation of germ layers in gastrulation-state embryos, establishment, culture and genetic manipulation of embryonic stem cell lines; in situ hybridization to whole mounts of embryos; LacZ staining; skeletal preparation, electrofusion, microinjection of DNA into pronuclei; and microinjection of embryonic stem cells into blastocysts.

Last year's speakers were: R. Behringer, A. Bradley, M. Bronner-Fraser, A. Efstratiadis, R. Hammer, P. Koopman, T. Jessell, A. Joyner, R. Lovell-Badge, J. Mann, T. Magnuson, A. McMahon,

A. Nagy, V. Papaioannou, L. Parada, E. Rinchik, E. Robertson, J. Rossant, D. Solter, P. Soriano, P. Tam, and D. Wilkinson.

PARTICIPANTS

Aris De La Fuente, Maria Del Carmen, B.S., Centro de Investigaciones Biologicas, Madrid, Spain
Blair, H., B.S., Medical Research Council, Oxon, United Kingdom
Blank, V., B.S., Ph.D., Harvard Medical School, Boston
Brown, N., B.A., Ph.D., University of Michigan, Ann Arbor
Chang, A., B.S., Ph.D., Children's Medical Research Institute, New South Wales, Australia
Holcik, M., B.S., Ph.D., University of Ottawa, Ontario, Canada
Jensen, J., B.S., M.S., Hagedorn Research Institute, Gentofte, Denmark
Kettunen, P., D.D.S., University of Helsinki, Finland
Kielman, M., B.S., Ph.D., Leiden University, The Netherlands
Klarman, L., B.A., Ph.D., Harvard University, Boston
Schulz, H., B.S., Ph.D., San Raffaele Scientific Institute, Milan, Italy
Stover, P., B.S., Ph.D., Cornell University, Ithaca, New York
Suh, H., B.S., M.S., Ph.D., Ajou University School of Medicine, Kyeongi-do, South Korea
Wallis, J., B.A., Ph.D., University of Edinburgh, United Kingdom

SEMINARS

Behringer, R., University of Texas/MD Anderson Cancer Center, Houston. Organization of pattern during mouse embryogenesis/Transgenic animals in biomedical research/Transgenic mouse video.
Bowles, J., University of Queensland, Brisbane, Australia. Structural and regulatory analysis in Sry in mice.
Bradley, A., Baylor College of Medicine, Houston, Texas. Introduction to ES cells/Screening the genome for tumor suppressor genes.
Costantini, F., Columbia University College of Physicians & Surgeons, New York. The role of the *Fused* gene in vertebrate embryogenesis and signal transduction.
Efstratiadis, A., Columbia University, New York. Genetics of growth: Developmental roles of insulin-like growth factors.
Hogan, B., Vanderbilt University Medical School, Nashville, Tennessee. Functional analysis of *forkhead/winged* helix genes in skeletal and brain development.
Jaenisch, R., Whitehead Institute/Massachusetts Institute of Technology, Cambridge. Methylation, imprinting, and X inactivation.
Jenkins, N., National Cancer Institute, Frederick, Maryland. The role of unconventional myosins in development and disease.
Joyner, A., New York University, New York. Genetic control of mid/hindbrain and limb patterning.
Koopman, P., University of Queensland, Brisbane, Australia. Strategies for molecular genetic analysis of mouse development/*Sry* and *Sox* genes: Multiple roles in mammalian development.
Krumlauf, R., Medical Research Council, United Kingdom. Events patterning the hindbain and cranial neural crest.
Lovell-Badge, R., Medical Research Council, United Kingdom. Sex determination and the role of Dax1/*Sox* genes and neural fate.
Magnuson, T., Case Western Reserve University, Cleveland, Ohio. The role of eed during mouse gastrulation.
Mann, J., Beckman Research Institute, Duarte, California. Allele-specific expression of imprinted genes and its role in development.
Martin, G., University of California, San Francisco. Limb bud induction, outgrowth, and patterning/The use of DNA recombinases to control gene expression in mice.
McLaren, A., Wellcome/CRC Institute, Cambridge, Massachusetts. Origin and development of germ cells.
Nagy, A., Mount Sinai Hospital/Samuel Lunenfeld Research Institute, Ontario, Canada. Applications of gene knockout technology in mice.
Papaioannou, G., Columbia University College of Physicians & Surgeons, New York. Chimeras in experimental embryology/T-box genes and development.
Rinchik, G., Sarah Lawrence College, Bronxville, New York. Recovery of radiation- and chemical-induced germline mutations in the mouse.
Robertson, L., Harvard University, Cambridge. Roles of TGF-β proteins in axis formation and organogenesis.
Rossant, J., Mount Sinai Hospital/Samuel Lunenfeld Research Institute, Ontario, Canada. Preimplantation development, implantation, and extraembryonic structures/Patterning the developing body axis.
Schumacher, A. Case Western Reserve University, Cleveland, Ohio. Genetic control of axial patterning in the mouse.
Skarnes, B., University of Edinburgh, United Kingdom. Gene trapping and functional analysis of the mammalian genome.
Solter, D., Max Planck Institute, Freiburg, Germany. Imprinting and control of gene expression during preimplantation mouse development.
Soriano, P., Fred Hutchinson Cancer Research Center, Seattle, Washington. Insertional mutagenesis screens and tyrosine kinase signaling during mouse development.
Tam, P., Children's Medical Research Institute, Wenworthville, Australia. Postimplantation development/Analyzing X-chromosome activity with a *lacZ* transgene.
Wilkinson, D., Medical Research Council, United Kingdom. In situ hybridization: Methods and research applications/Segmental restrictions in vertebrate development.

Structure, Function, and Development of the Visual System

June 19–July 1

INSTRUCTORS

Bonhoeffer, Tobias, Ph.D., Max Planck Institute
Fitzpatrick, David, Ph.D., Duke University

This lecture/discussion course explored the functional organization and development of the visual system as revealed by the use of a variety of anatomical, physiological, and behavioral methods. It was designed for graduate students and more advanced researchers who wished to gain a basic understanding of the biological basis for vision and to share in the excitement of the latest developments in this field. Topics included phototransduction and neural processing in the retina; functional architecture of striate cortex; cellular basis of cortical receptive field properties; the anatomy, physiology, and perceptual significance of parallel pathways; functional parcellation of extrastriate cortex; sensory-motor integration in the superior colliculus; human functional imaging; and role of patterned neuronal activity in the development of central visual pathways. Past lecturers included B. Chapman, D. Dacey, C. Gilbert, C. Gray, D. Hubel, L. Katz, K. Martin, J. Maunsell, T. Movshon, K. Nakayama, C. Shatz, A. Thompson, D. Van Essen, and R. Wong.

PARTICIPANTS

Bar, M., B.S., M.S., University of Southern California, Los Angeles
Crowley, J., B.A., Duke University, Durham, North Carolina
Dantzker, J., B.A., The Salk Institute, La Jolla, California
Demains, R., B.S., O.D., M.S., McGill University, Montreal, Canada
Gepshtein, S., M.S., University of Virginia, Charlottesville
Herzog, A., B.A., Ph.D., The Salk Institute, La Jolla, California
Hooper, J., B.A., Ph.D., Eastman Kodak, New Haven, Connecticut
Huang, J., B.S., M.S., Ph.D., Massachusetts Institute of Technology, Cambridge
Huber, K., B.S., Ph.D., Brown University, Providence, Rhode Island
Hung, C., B.S., Yale University, New Haven
Mrsic-Flogel, T., B.A., Oxford University, United Kingdom
Nace, K., B.A., University of California, Davis
Nase, G., B.S., Max Planck Institute for Brain Surgery, Frankfurt, Germany
Sharon, D., B.S., M.S., Weizmann Institute, Rehovot, Israel
Song, H., B.S., M.A., University of California, San Diego
Starsinic, S., B.S., University of California, Davis
Tavazoie, S., B.A., Harvard Medical School, Boston
Tong, P., B.S., M.D., Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland
Trachtenberg, J., B.A., Ph.D., University of California, San Francisco
Wainwright, M., B.M., Harvard University, Cambridge
Wu, S.-M., B.S., Ph.D., Georgetown University Medical Center, Washington, D.C.

SEMINARS

Bonhoeffer, T., Max Planck Institute, Munich, Germany. Intrinsic circuits and functional architecture/Development of central visual pathways.
Dacey, D., University of Washington, Seattle. Phototransduction, retinal circuitry.
Ferster, D., Northwestern University, Evanston, Illinois. Connectivity between retina, LGN, and striate cortex.
Fitzpatrick, D., Duke University, Durham, North Carolina. Intrinsic circuits and functional architecture.
Hendry, S., Johns Hopkins University, Baltimore, Maryland. Molecular aspects of visual system organization.
Hockfield, S., Yale University, New Haven. Molecular aspects of visual system organization.
Hubel, D., Harvard Medical School, Boston. Fundamentals of visual cortical processing.
Katz, L., Duke University Medical Center, Durham, North Carolina. Development of central visual pathways.
Konig, P., The Neuroscience Institute, La Jolla, California. Synchronization and oscillations.
Logothetis, N., Max Planck Institute, Germany. Studies of the human visual system.
Kevan, M., University of Zurich, Switzerland. Connectivity between retina, LGN, and striate cortex.
Maunsell, J., Baylor College of Medicine, Houston, Texas. Higher cortical processing and parallel pathways.
Newsome, B., Stanford University School of Medicine, California. Higher cortical processing and parallel pathways.
Reid, C., Harvard Medical School, Boston. Connectivity between retina, LGN, and striate cortex.
Sereno, M., University of California, San Diego. Studies of the human visual system.
Wong, R., Washington University School of Medicine, St. Louis, Missouri. Development of central visual pathway.

Arabidopsis Molecular Genetics

June 30–July 20

INSTRUCTORS

Glazebrook, Jane, Ph.D., University of Maryland
Lam, Eric, Ph.D., Rutgers University/Ag Biotech Center
Last, Robert, Ph.D., Boyce Thompson Institute/Cornell University

ASSISTANTS

Jirage, Dayadevi, University of Maryland
Meisel, Lee, Rutgers University
Saracco, Scott, Boyce Thompson Institute/Cornell University

This course provided an intensive overview of topics in plant growth and development, focusing on molecular genetic approaches to understanding plant biology. It emphasized recent results from *Arabidopsis thaliana* and other model plants and provided an introduction to current methods used in *Arabidopsis* research. The course also demonstrated the use of microbial systems in plant research, including *Agrobacterium rhizobium*, *E. coli*, and *S. cerevisiae*. It was designed for scientists with experience in molecular techniques or in plant biology who wish to work with *Arabidopsis*. The course consisted of a vigorous lecture series, a hands-on laboratory, and informal discussions. Speakers provided both an in-depth discussion of their own work and a review of their specialty.

Discussions of important topics in plant research were presented by the instructors and by invited speakers. These seminars reviewed plant anatomy; plant development (including development of flowers, roots, meristems, embryos, and the epidermis); perception of light and photomorphogenesis; responses to pathogens and to other environmental stresses; synthesis and function of secondary metabolites and hormones; nitrogen assimilation; unique aspects of plant cell biology (including the plant cytoskeleton, cell wall, and chloroplasts); biotechnological improvement of plants; the importance of transposons and *Agrobacterium* for manipulating plant genomes and current approaches to genome analysis.

The laboratory sessions provided an introduction to important techniques currently used in *Arabidopsis* research. These included studies of *Arabidopsis* development, mutant analysis, studies of epidermal features, in situ detection of RNA, histochemical staining and immunolabeling of proteins, transformation with *Agrobacterium*, transient gene expression in protoplasts, expression of plant proteins in microorganisms, detection and analysis of plant pathogens, and techniques commonly used in genetic and physical mapping.

PARTICIPANTS

Adhami, F., B.S., Universitat für Bodenkultur, Vienna, Austria
Azumi, Y., B.S., Ph.D., Kanagawa University, Hiratsuka, Japan
Barker, B.M., B.A., University of Montana, Missoula
Bougourd, S., B.S., Ph.D., University of York, North Yorkshire, United Kingdom
Bustos, M., B.S., Ph.D., University of Maryland, Baltimore
Kahn, K., B.S., Ph.D., University of Missouri, Kansas City
Kargul, J., B.S., M.S., University of Warwick, Coventry, United Kingdom
Mande, K., B.S., National University of Singapore, Singapore
Mouradov, A., Ph.D., ForBio Research, Brisbane, Australia
Panda, S., B.S., M.S., Scripps Research Institute, La Jolla, California
Raman, R., B.S., M.S., Scripps Research Institute, La Jolla, California
Ramsay, N., B.S., University of Warwick, Coventry, United Kingdom
Sathyanarayanan, P, B.S., Washington State University, Seattle
Stranger, B., B.A., University of Montana, Missoula
Sattler, S., B.S., University of Minnesota, St. Paul
Weaver, A., B.S., Purdue University, West Lafayette, Indiana

SEMINARS

Arntzen, C., Boyce Thompson Institute/Cornell University, Ithaca, New York. Genetic engineering in plants.
Banks, J., Purdue University, West Lafayette, Indiana. Ferns.
Briggs, W., Carnegie Institute of Washington, Stanford, California. Light perception.
Chapple, C., Purdue University, West Lafayette, Indiana. Secondary metabolism.
Cook, D., Texas A&M University, College Station. Rhizobium.
Curtis, M., Cold Spring Harbor Laboratory. Enhancer traps in *Arabidopsis*.
Drews, G., University of Utah, Salt Lake City. Flower development.
Glazebrook, J., University of Maryland, College Park. Plant defense responses/Map-based cloning strategies.
Guerinot, M.L., Dartmouth College, Hanover, New Hampshire. Ion channels and transport.
Hobbie, L., Adelphi University, Garden City, New York. Hormones.
Irish, V., Yale University, New Haven. Plant anatomy, plant development.
Katagiri, F., Massachusetts General Hospital, Boston. Plant resistance genes.
Lam, E., Rutgers University/Ag Biotech Center, New Brunswick, New Jersey. Programmed cell death.

Larkin, J., Louisiana State University, Baton Rouge. Trichomes.
Last, R., Boyce Thompson Institute/Cornell University, Ithaca, New York. Amino acid biosynthesis/Plant stress responses.
Lemieux, B., Stanford University, Palo Alto, California. The *Arabidopsis* genome project.
Ma, H., Cold Spring Harbor Laboratory. Molecular analysis of plant development.
McClung, R., Dartmouth College, Hanover, New Hampshire. Circadian rhythms.
Preuss, D., University of Chicago, Illinois. Male and female gametophytes and fertilization.
Reed, J., University of North Carolina, Chapel Hill. Photomorphogenesis.
Richards, E., Washington University, St. Louis, Missouri. Genome organization and epigenetics.
Rose, A., University of California, Davis. Cloning genes by complementation in microbes.
Rounsley, S., The Institute for Genomic Research, Rockville, Maryland. *Arabidopsis* resources AtDB.
Shirley, B., Virginia Tech, Blackburg. Protein-protein interactions.

Molecular Cloning of Neural Genes

June 30–July 20

INSTRUCTORS

Darnell, Robert, Ph.D., Rockefeller University
Dulac, Catherine, Ph.D., Harvard University
Rupp, Fabio, Ph.D., Johns Hopkins University
Serafini, Tito, Ph.D., University of California, Berkeley

CO-INSTRUCTOR

Lai, Cary, Ph.D., Scripps Research Institute

ASSISTANTS

Herrada, Gilles, Harvard University
Jensen, Kirk, Rockefeller University
Lander, Cynthia, Yale University
Okano, James, Rockefeller University
Yang, Yolanda, Rockefeller University

This intensive laboratory and lecture course was intended to provide neuroscientists at all levels with an introduction to modern molecular neurobiology. The course consisted of daily laboratory exercises, detailed discussions on the practice of molecular biology and modern approaches to cloning neural genes, and a series of evening research seminars by invited speakers. This lecture series emphasized the ways in which molecular techniques studied in the laboratory have been successfully applied to the study of neural genes.

The laboratory portion of the course included a module on basic gene cloning and a module on more advanced methods in cloning and expression of neural genes. Gene cloning techniques included isolation and characterization of poly(A)$^+$ RNA; synthesis of cDNA libraries; standard screening and expression screening of cDNA libraries; analysis of cDNA clones, including phagemid rescue, restriction analysis, Southern blotting, ligations, transformation, electroporation, and subcloning; PCR analysis including oligonucleotide design, synthesis, and purification, and an exploration of advanced cloning techniques including single-cell library synthesis and PCR-based subtraction cloning. Gene expression studies included Northern blot analysis, RT-PCR analysis, in vitro transcription, in situ hybridization, and mammalian cell transfection.

PARTICIPANTS

Bi, G., B.S., Ph.D., University of California, San Diego
Bruses, J., M.D., Ph.D., Case Western Reserve University, Cleveland
Cesare, P., B.S., M.S., King's College London, United Kingdom
Degtiar, V., M.S., Ph.D., Stanford University, Stanford, California
Haas, K., B.S., Ph.D., Albert Einstein College of Medicine, Bronx, New York
Halfter, W., B.S., Ph.D., University of Pittsburgh, Pennsylvania
Hoke, K., B.S., Stanford University, Stanford, California
Hong, W., B.S., George Washington University, Washington, D.C.
Kelic, S., B.S., Ph.D., Albert Einstein College of Medicine, Bronx, New York
Nagan, N., M.S., Ph.D., Boston University School of Medicine, Massachusetts
Schacher, S., B.S., Ph.D., Columbia University College of Physicians & Surgeons, New York
Schmid, A., B.S., Ph.D., University of Illinois, Urbana
Schulz, D., B.S., M.S., University of Illinois, Urbana
Tole, S., B.S., Ph.D., University of Chicago, Illinois
Trainer, P., B.S., Ph.D., National Institute of Medical Research, London, United Kingdom
Wizenmann, A., B.S., Ph.D., UMDS at Guy's & St. Thomas's Hospital, London, United Kingdom

SEMINARS

Darnell, R.B., Rockefeller University, New York. Using autoimmune neurologic disease antisera to clone neuron-specific genes.
Dulac, C., Harvard University, Boston. Molecular biology of pheromone perception in mammals.
Edwards, R., University of California, San Francisco. Neurotransmitter packaging and the synaptic vesicle cycle: From bioenergetics to synaptic transmission.
Friedman, J.M., Rockefeller University, New York. Thin mice do it with leptin: Cloning hypothalamic hormones.
Hemmati-Brivanlou, A., Rockefeller University. New York. In vivo systems for expression cloning in vertebrates.
Lavery, D., Glaxo Wellcome Research & Development, Zurich, Switzerland. Isolation of novel circadian target genes by a new subtractive cloning method.
Mombaerts, P., Rockefeller University, New York. Targeting olfaction.
Orr, H.T., University of Minnesota, Minneapolis. So, you have a neural gene now what? From gene to function.
Rupp, F., Johns Hopkins School of Medicine, Baltimore, Maryland. Molecular analysis of motor neurons: It isn't how big it is, it's how you screen it.
Reed, R., Johns Hopkins School of Medicine, Baltimore, Maryland. Regulating gene expression in neurons. How the nose knows and why the worm turns.
Serafini, T., University of California, Berkeley. In vitro analysis of axon guidance and targeting.
Walsh, C., Beth Israel Hospital/Harvard Medical School, Boston. Genetic control of neuronal migration to the cerebral cortex.
Yancopoulos, G., Regeneron Pharmaceutical Inc., Tarrytown, New York. Expression cloning growth factors and receptors in the nervous system.

Neurobiology of *Drosophila*

June 30–July 20

INSTRUCTORS

Dickinson, Michael, Ph.D., University of California, Berkeley
Patel, Nipam, Ph.D., University of Chicago
Taylor, Barbara, Ph.D., Oregon State University, Corvallis

ASSISTANTS

Lear, Bridget, University of Chicago
Sane, Sanjay, University of California, Berkeley

This laboratory/lecture course was intended for researchers at all levels who wish to use *Drosophila* as an experimental system for studying behavior, physiology, and development. Daily seminars introduced students to a variety of research topics drawn from recent experimental contributions in the field of *Drosophila* neurobiology. The lectures and laboratories focused on both the development of the nervous system and its role in controlling larval and adult behaviors. Guest lecturers brought original preparations for viewing and discussion and direct laboratory exercises and demonstrations in their area of interest. The course provided students with hands-on experience using a variety of experimental preparations that are used in the investigation of current neurobiological questions, including embryo in situ hybridization, labeling of identified neurons, electrophysiological recording from nerves and muscles, and the analysis of larval and adult behavior. Collectively, the course provided a comprehensive and practical introduction to modern experimental methods for studying the *Drosophila* nervous system. The specific topics from this year's course included neurogenesis, axon pathfinding, synaptogenesis, membrane excitability, learning and memory, courtship, walking, flight, and crawling behaviors.

PARTICIPANTS

Afshar, K., B.S., Ph.D., University of Wisconsin, Madison
Barron, A., B.A., Cambridge University, United Kingdom
Bergmann, A., B.S., Ph.D., Massachusetts Institute of Technology, Cambridge
De Sousa, D., B.S., McMaster University, Ontario, Canada
Koh, Y.H., B.S., M.S., University of Massachusetts, Amherst
Korey, C., B.S., Harvard University Medical School, Boston
McClung, C., B.S., University of Virginia, Charlottesville
Prichard, M., B.A., University of Hawaii, Manoa, Honolulu
Prokopenko, S., B.S., Baylor College of Medicine, Houston, Texas
Straube, A., B.S., Ph.D., University of Freiburg, Germany
Vijayraghavan, S., B.S., Tata Institute of Fundamental Research, Bangalore, India
Zhu, J.-c., B.S., State University of New York, Stony Brook

SEMINARS

Atwood, H., University of Toronto, Canada. Larval neuromuscular physiology.
Bossing, T., Wellcome/CRC Institute, Cambridge, United Kingdom. Neuroblast lineages and CNS midline formation.
Dickinson, M., University of California, Berkeley. Physiology and mechanisms of flight.
Gaul, U., Rockefeller University, New York. Visual system development.
Hall, J., Brandeis University, Waltham, Massachusetts. Behavioral mutants and genetics: An overview.
Heberlein, U., University of California, San Francisco. Genetics of alcohol and drug sensitivity.
Meinterzhagen, I. and Sun, X.J., Dalhousie University, Nova Scotia, Canada. Synaptic plasticity and the adult visual system.
Murphey, R., University of Massachusetts, Amherst. Sensory systems and adult behaviors.
O'Dowd, D., University of California, Irvine. Ion channels and neuronal diversity.
Patel, N., University of Chicago, Illinois. Embryogenesis and early pattern formation.
Restifo, L., University of Arizona, Tucson. Metamorphosis and adult development.
Sink, H., University of California, Berkeley. Axonal pathfinding.
Skeath, J., Washington University School of Medicine, St. Louis, Missouri. Early neurogenesis.
Sokolowski, M., York University, Ontario, Canada. Larval behaviors.
Strauss, R., Max Planck Institute für Biologie Kybernetik, Tübingen, Germany. Central processing centers and walking behavior.
Taylor, B., Oregon State University, Corvallis. Genetics of sex-specific behaviors.
Tully, T., Cold Spring Harbor Laboratory. Learning and memory.
Zhong, Y., Cold Spring Harbor Laboratory. Peptide neurotransmitters and plasticity.

Brain Mapping

July 8–14

INSTRUCTORS

Mazziotta, John, M.D., Ph.D., University of California, Los Angeles
Toga, Arthur, Ph.D., University of California, Los Angeles

The aim of this lecture and practical course was to describe the rapidly evolving developments in brain mapping that have been applied to the problem of mapping the structure and function of the brain. The goal of brain mapping is to understand cerebral anatomy and physiology in health and disease and to evaluate neurological, neurosurgical, and psychiatric disease states. This course described new methods as well as the application of traditional techniques to the study of brain structure and function. Methodologies discussed included magnetic resonance imaging (including functional, spectroscopic, and angiographic approaches), positron emission tomography, electrophysiological techniques, optical intrinsic signal imaging, digital approaches to conventional postmortem neuroanatomical investigations, data analysis, statistical analysis, statistical approaches, visualization, and stereotaxy. The course was not designed to simply describe methods, but rather to discuss how brain mapping strategies can be employed in combination with biological models for

understanding the structure and function of the brain. Findings relevant to the function of the visual, motor, language, memory, and cognitive brain systems, as well as diseases that adversely affect them, were discussed. Specific hypotheses and experimental designs were developed by the students for remote execution, as an actual experiment. In addition, a field trip to a local imaging laboratory was arranged. Invited speakers included world leaders in each of the respective brain mapping subspecialties.

PARTICIPANTS

Breen, E., B.A., M.A., Indiana University School of Medicine, Indianapolis
Elizondo, I., B.A., National Institutes of Health, Bethesda, Maryland
Goldbloom, L., B.S., Albert Einstein College of Medicine, Bronx, New York
Hoffman, E., B.A., M.S., National Institutes of Health, Bethesda, Maryland
Jiang, Y., B.S., Ph.D., National Institutes of Health, Bethesda, Maryland
Kupers, R., B,S., Ph.D., Karolinska Institute, Huddinge, Sweden
Lang, D., B.S., University of British Columbia, Vancouver, Canada
Pankhaniya, R., B.S., M.D., University of California, San Francisco
Rey-Hipolito, C., B.S., National Institute of Mental Health, Bethesda, Maryland
Salat, D., B.A., Oregon Health Sciences University, Portland
Schmid, A.M., B.S., London University, United Kingdom
Tzelepi, A., B.S., State University of New York, Brooklyn
Vaina, L., M.S., Ph.D., Boston University, Massachusetts

SEMINARS

Belliveau, J., Massachusetts General Hospital, Charlestown. Image integration: Visual system.
Cohen, M., University of California, Los Angeles. MRI.
Fletcher, P., Wellcome Institute of Neurology, London, United Kingdom. Language and memory.
Goldman-Rakic, P., Yale University, New Haven. Cerebral cortex: Cyto- and chemoarchitecture.
Haxby, J., National Institutes of Health, Bethesda, Maryland. Object recognition: Visual system.
Mazziotta, J., University of California, Los Angeles. PET/SPECT.
Pascual-Leone, A., Harvard University Medical School, Boston. Transcranial magnetic stimulation (epilepsy and motor system).
Petersen, S., Washington University, St. Louis, Missouri. Experimental design.
Simpson, G., Albert Einstein College of Medicine, New York. EEG/MEG/ERP (visual system).
Toga, A., University of California, Los Angeles. Optical, morphological analyses.
Weinberger, D., National Institute of Mental Health, Washington, D.C. Mental illness.
Woolsey, T., Washington University School of Medicine, St. Louis, Missouri. Intravascular dyes.
Zeffero, T., Sensor Systems, Inc., Sterling, Virginia. Data analysis.

Biology of Memory: From Molecules to Behavior

July 18–31

INSTRUCTORS

Byrne, Jack, Ph.D., University of Texas, Houston Medical School
Eichenbaum, Howard, Ph.D., Boston University
Pearson, Keir, Ph.D., University of Alberta, Canada
Squire, Larry, Ph.D., University of California, San Diego

This lecture course provided an introduction to cell, molecular, and systems approaches to learning and memory. It was suited for graduate students in molecular biology, neurobiology, and psychology as well as research workers who are interested in an introduction to this field. The course covered topics ranging from behavioral considerations of learning and memory to gene regulation in the nervous system. The lectures provided an intensive coverage of six selected areas: (1) an introduction to modern behavioral studies of learning and memory; (2) an overview of the cell biology of neuronal plasticity and second messenger systems; (3) the regulation of gene expression; (4) cellular and molecular mechanisms of simple forms of learning and memory in invertebrates and vertebrates; (5) cellular and molecular mechanisms of long-term potentiation and depression in various regions of the mammalian brain; and (6) systems approaches to learning in vertebrates and humans.

PARTICIPANTS

Ikema, M., B.S., M.S., Ph.D., Massachusetts Institute of Technology, Cambridge
Barria-Roman, A., B.S., Oregon Health Sciences University, Portland
Brodie, C., B.A., B.S., University of Minnesota, Minneapolis
Candy, S., B.S., Imperial College School of Medicine at St. Mary's, United Kingdom
Celikel, T., B.S., Bogazici University, Istanbul, Turkey
Chan, K.-H., B.A., Purdue University, West Lafayette, Indiana
Jimenez de Asua, L., M.D., Ph.D., Fundacion Campomar, Buenos Aires, Argentina
Joiner, M.-L., B.S., M.S., Brandeis University, Waltham, Massachusetts
Kellendonk, C., B.S., German Cancer Research Center, Heidelberg, Germany
Kirchhoff, B., B.A., Boston University, Massachusetts
Korzus, E., B.S., M.S., Ph.D., University of California, San Diego
Lechner, H., B.A., University of Texas, Houston
Lipton, P., B.A., Boston University, Massachusetts
Olmstead, M., B.S., M.S., Ph.D., University of Cambridge, United Kingdom
Panchal, P., B.S., M.S., Tata Institute, Maharashtra, India
Pike, F., B.A., University of Oxford, United Kingdom
Steele, P., B.S., University of Texas, Houston
Swensson, P., B.S., Lund University, Sweden
Teng, E., B.S., University of California, San Diego

SEMINARS

Ball, G., Johns Hopkins University, Baltimore, Maryland. Ethological approaches to learning.

Byrne, J., University of Texas, Houston. Introduction to the cellular study of learning/Overview of membranes and synaptic transmission.

Eichenbaum, H., Boston University, Massachusetts. Role of LTP and LTD in learning/Memory systems in rodents.

Greenough, W., University of Illinois, Urbana. Morphological correlates of learning and experience.

Gilbert, C., Rockefeller University, New York. Dynamics of adult visual cortex.

Holland, P., Duke University, Durham, North Carolina. Introduction to learning theory I/Introduction to learning theory II.

Kandel, E., Columbia University, New York. Nonassociative learning in *Aplysia* I/Nonassociative learning in *Aplysia*/ Long-term potentiation II.

LeDoux, J., New York University, New York. Modulation of memory.

Levitan, I., Brandies University, Waltham, Massachusetts. Structure, function, and modulation of ion channels.

Lo, D., Duke University Medical Center, Durham, North Carolina. Growth factors.

Malenka, R., University of California, San Francisco. Long-term potentiation I/Long-term depression.

Pearson, K., University of Alberta, Edmonton, Canada. Mechanisms of motor learning.

Pfaffinger, P., Baylor College of Medicine, Houston, Texas. Cloning of genes important to learning.

Posner, M., University of Oregon, Eugene. Neuropsychology of cognition.

Schulman, H., Stanford University School of Medicine, California. Overview of second-messenger systems and their role in learning and memory I/Overview of second-messenger systems and their role in learning and memory II.

Silva, A., Cold Spring Harbor Laboratory. Gene-knockout approaches to study learning.

Squire, L., University of California, San Diego. Memory systems in non-human primates/Human memory and disorders of memory.

Steinmetz, J., Indiana University, Bloomington. Classical conditioning of the nictitating membrane response.

Tully, T., Cold Spring Harbor Laboratory. Genetic approaches to study associative learning in *Drosophila*.

Eukaryotic Gene Expression

July 23–August 12

INSTRUCTORS

Carey, Michael, Ph.D., University of California, Los Angeles
Gill, Grace, Ph.D., Harvard Medical School
Gilmour, David, Ph.D., Pennsylvania State University
Smale, Stephen, Ph.D., University of California, Los Angeles

ASSISTANTS

Ellwood, Katherine, University of California, Los Angeles
Soccio, Ray, Harvard Medical School
Le, Trinh, University of California, Los Angeles

The rapid cloning of eukaryotic promoters and regulatory factors has led to a dramatic increase in studies of gene regulation. This course was designed for students, postdocs, and professors who have recently ventured into this dynamic area of contemporary biology. The course focused on state-of-the-art strategies and techniques that are employed to study gene expression, with a special emphasis on transcriptional regulation. Regulation of transcription by nuclear proteins was examined by in vitro transcription in cell-free extracts and by transfection of mammalian tissue culture cells. Analytical techniques for measuring gene expression included primer extension ies of protein-DNA interactions. Transcription factors were expressed in a heterologous system such as *E. coli* or baculovirus and purified by affinity chromatography. A detailed characterization of the DNA-binding properties of the recombinant factors was carried out using mobility shift, DNase I footprinting, and methylation interference assays. During the past few years, the gene

regulation field has begun to emphasize the importance of in vivo approaches to studying protein-DNA interactions. Students were therefore instructed in the most advanced in vivo footprinting and UV cross-linking methodologies. Students also learned strategies and techniques for executing a structure-function study of a regulatory factor, including an introduction to site-directed mutagenesis, basic computer analyses, and the two-hybrid approach in *S. cerevisiae* for studying protein-protein interactions. Experience with basic recombinant DNA techniques was a prerequisite for admission to this course. Lectures by the instructors covered the current status of the gene expression field, theoretical aspects of the methodology, and broader issues regarding strategies for investigating the regulation of gene expression in eukaryotes. Guest lecturers discussed contemporary problems in eukaryotic molecular biology and technical approaches to their solution.

PARTICIPANTS

Antonnsson, C., B.S., Karolinska Institute, Stockholm, Sweden
De Luca, P., B.S., University "Federico II" of Naples, Italy
Englund, M., M.S., Sahlgrenska University Hospital, Goteborg, Sweden
Ernst, J., B.S., Yale University, New Haven
Hastings, M., B.A., Marquette University, Milwaukee, Wisconsin
Holmberg, C., M.S., Turku Center For Biotechnology, Turku, Finland
Huh, S., B.A., Yale University, New Haven
Joutel, A., M.S., M.D., Ph.D., INSERM U 25, Paris, France
Kapatos, G., B.S., Ph.D., Wayne State University, Detroit, Michigan
Martin, M., B.S., M.S., M.D., University of California, Los Angeles
Minchin, S., B.S., Ph.D., University of Birmingham, U.K.
Morgan, K., B.S., Ph.D., Nottingham University, U.K.
Mourelatos, Z., M.D., University of Pennsylvania, Philadelphia
Warnmark, A., M.S., Karolinska Institute, Huddinge, Sweden
Yoon, C., B.A., Harvard Medical School, Cambridge
Yu, C., B.A., Ph.D., University of California, Irvine

SEMINARS

Abate-Shen, C., UMDNJ/ Robert Wood Johnson Medical School, Piscataway, New Jersey. Homeoprotein function in development and oncogenesis.
Burley, S., Rockefeller University, New York. X-ray crystallographic studies of eukaryotic transcription factors.
Carey, M., University of California, Los Angeles. Biochemical mechanism of gene activation.

Fink, G., Whitehead Institute, Cambridge, Massachusetts. The latest and greatest in yeast.
Gill, G., Harvard Medical School, Boston. A transcriptional repressor that antagonizes E1A-mediated activation.
Gilmour, D., Pennsylvania State University, University Park. In vivo and in vitro analysis of the hsp70 heat shock gene promoter from *Drosophila*.
Goldstein, J., University of Texas, Dallas. The SREBP pathway: Regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor.
Goodrich, J., University of Colorado, Boulder. RNA polymerase II transcriptional regulation.
Grosschedl, R., University of California, San Francisco. Role of MARs in the regulation of gene expression and chromatin accessibility.
Jones, K., The Salk Institute, La Jolla, California. Regulation of HIV-1 transcription: From chromatin to Tat.
Kingston, R., Massachusetts General Hospital, Boston. Moving through chromatin.Lis, J., Cornell University, Ithaca. Multiple mechanisms of eukaryotic transcription regulation: Different schemes for different genes.
Maniatis, T., Harvard University, Boston. Mechanism of NF-κB activation.
Sen, R., Brandeis University, Waltham, Massachusetts. Factors regulating Ig gene expression.
Smale, S., University of California, Los Angeles. Regulation of IL-12 expression in antigen-presenting cells.
Orkin, S., Harvard Medical School, Boston. Transcriptional control of blood cell development.
Pugh, F., Pennsylvania State University, University Park. Kinetic steps in transcription complex assembly.
Roeder, R., Rockefeller University, New York.The role of general and gene-specific cofactors in transcriptional regulation.
Stillman, B., Cold Spring Harbor Laboratory. Cell cycle control of yeast chromosomal DNA replication.
Struhl, K., Harvard Medical School, Boston. Molecular mechanisms of yeast transcriptional regulation.
Young, R., Massachusetts Institute of Technology, Cambridge. New insights into eukaryotic gene regulation.
Zaret, K., Brown University, Providence, Rhode Island. Specifying tissues and gene expression programs in development.

Imaging Structure and Function in the Nervous System

July 23–August 12

INSTRUCTORS

Konnerth, Arthur, M.D., Ph.D., University of Saarland, Germany
Lanni, Fred, Ph.D., Carnegie-Mellon University
Yuste, Rafael, Ph.D., Columbia University

ASSISTANTS

Eilers, Jens, University of Saarland, Germany
Smetters, Diana, Columbia University
Tsiola, Areti, Columbia University
Vanni, Steve, Carnegie-Mellon University

Advances in light microscopy and digital image processing and the development of a variety of powerful fluorescent probes present expanding opportunities for visualizing and measuring the structure and function of neurons, synapses, and networks in the brain. This intensive laboratory and lecture course provided participants with the theoretical and practical tools to utilize these emerging technologies. The primary emphasis of the course was on light microscopy, including fluorescence, differential interference and phase contrast, confocal scanning, and 2-photon scanning, as well as the use of different types of electronic cameras and the application of digital processing to enhance and analyze microscope images. Students learned the principles of light microscopy, the use of calcium-sensitive probes (e.g., Fura-2), voltage-sensitive dyes, photo-activated ("caged") compounds, exocytosis tracers, whole-cell patch-clamp methods, single-cell microinjection of fluorescent indicators, and other methods to explore neuronal function. They used a variety of neural systems, including living animals, brain slices (e.g., hippocampus, cerebellum, and neocortex), and cultured cells. Applicants had a strong background in the neurosciences or in cell biology.

PARTICIPANTS

Batchler, A., B.S., Ph.D., University College London, U.K.
Cambridge, S., B.S., Ph.D., Carnegie-Mellon University, Pittsburgh, Pennsylvania
Castro-Alamancos., M., B.S. M.S., Ph.D., Brown University, Providence, Rhode Island
Dill, T., B.A., M.S., Ph.D., College of Notre Dame of Maryland, Baltimore
Episkopou, V., B.S., Ph.D., Medical Research Council, London, United Kingdom
Esteban, J., B.S., Ph.D., Cold Spring Harbor Laboratory
Goedecke, I., B.S., Ph.D., Max Planck Institute, Munich, Germany
Myhr, K., B.S., Ph.D., Washington University School of Medicine, St. Louis, Missouri
Naegerl, V., B.S., University of California, Los Angeles
Patzke, H., B.S., Max Planck Institute, Frankfurt, Germany
Takahashi, M., L.I.B., B.S., Ph.D., Duke University Medical Center, Durham, North Carolina
Zhou, Q., B.S., M.S., State University of New York, Stony Brook

SEMINARS

Augustine, G., Duke University, Durham, North Carolina. Using calcium indicators.
Betz, B., University of Colorado, Denver. FM 1-43 imaging.
Chalfie, M., Columbia University, New York. GFP.
Christenson, M., Princeton Instruments, Princeton, New Jersey. Cameras.
Cline, H., Cold Spring Harbor Laboratory. In vivo observations of neuronal form and function.
Cohen, L., Yale University, New Haven. Voltage-sensitive dyes.
Denk, W., Lucent Technologies Bell Labs, Murray Hill, New Jersey. Two-photon microscopy.
Helmchen, F., Max Planck Institute, Heidelberg, Germany. Calcium dynamics-1.
Katz, L., Duke University Medical Center, Durham, North Carolina. Photostimulation.
Kay, S., Scripps Research Foundation, La Jolla, California. Bioluminescence.
Konnerth, A., University of Saaland, Sarr, Germany. Calcium dynamics-2/Imaging calcium in Purkinje cells.
Lanni, F., Carnegie Mellon University, Pittsburgh, Pennsylvania. Basic microscopy.
Lichtman, J., Washington University, St. Louis, Missouri. Confocal microscopy.
O'Malley, D., State University of New York, Stony Brook. Imaging zebrafish in vivo.
Svoboda, K., Cold Spring Harbor Laboratory. Imaging calcium in vivo.
Tsien, R., University of California, San Diego. Design of calcium indicators/GFP and voltage-sensitive dyes.
Wong, R., Washington University School of Medicine, St. Louis, Missouri. Imaging retinal waves.
Yuste, R., Columbia University, New York. Imaging calcium in spines.
Zucker, B., University of California, Berkeley. Uncaging calcium.

Yeast Genetics

July 23–August 12

INSTRUCTORS

Adams, Alison, Ph.D., University of Arizona
Gottschling, Daniel, Ph.D., Fred Hutchinson Cancer Research Center
Stearns, Tim, Ph.D., Stanford University

ASSISTANTS

Dubois, Michelle, Fred Hutchinson Cancer Research Center
Feierbach, Becket, Stanford University
Kandl, Kimberly, University of Arizona

The major laboratory techniques used in the genetic analysis of yeast were studied, including the isolation and characterization of mutants, tetrad analysis, complementation, and mitotic recombination. Micromanipulations used in tetrad analysis were carried out by all students. Molecular genetic techniques, including yeast transformation, gene replacement, analysis of gene fusions, and generation of mutations by transposon mutagenesis, were studied. Indirect immunofluorescence experiments were performed to identify the nucleus, microtubules, and other cellular components. Lectures on fundamental aspects of yeast genetics were presented along with seminars given by outside speakers on topics of current interest.

PARTICIPANTS

Barros, M.H., B.S., M.S., University of Sao Paolo, Brazil
Bognar, A., B.D., Ph.D., University of Toronto, Ontario, Canada
Brown, B.J., B.S., M.D., Ph.D., University of Michigan, Ann Arbor
Greger, I., B.S., M.S., University of Oxford, United Kingdom
Hammar, M., M.S., Ph.D., Karolinska Institute, Stockholm, Sweden
Janssen, M., M.S., Utrecht University, The Netherlands
Jin, Q.-W., B.S., M.S., University of Vienna, Austria
Kalaitzis, P., B.S., Ph.D., University of Maryland, College Park
Kok, R., B.S., Ph.D., Gist-Brocades, Delft, The Netherlands
Lin, H., B.S., M.S., St. John's University, Jamaica, New York
Mayer, A., B.S., Ph.D., Max Planck Institute, Tübingen, Germany
Nemoto, Y., M.D., Ph.D., Yale University, New Haven
Schmekel, K., B.S., M.D., Stockholm University, Sweden
Swire-Clark, G., B.S., Clemson University, South Carolina
Then, A.B.S., University of Heidelberg, Germany
Tsukiyama, T., B.S., M.S., D.V.M., Ph.D., National Institutes of Health, Bethesda, Maryland

SEMINARS

Boeke, J., Johns Hopkins University, Baltimore, Maryland. Transposition in yeast.
Dawson, D., Tufts University, Boston, Massachusetts. Meiosis in yeast.
De Shaies, R., California Institute of Technology, Pasadena. Role of proteolysis in triggering G_1-S transition in yeast.
Elion, E., Harvard University, Boston. Signal transduction in the yeast mating pathway.
Fink, G., Massachusetts Institute of Technology, Cambridge. The latest and greatest in yeast.
Fox, T., Cornell University, Ithaca. Mitochondrial function in yeast.
Kaiser, C., Massachusetts Institute of Technology, Cambridge. The yeast secretory pathway.
Lindquist, S., University of Chicago, Illinois. Heat shock proteins in yeast.
Michaelis, S., Johns Hopkins University, Baltimore, Maryland. Mating factor synthesis in yeast.
Petes, T., University of North Carolina, Chapel Hill. Recombination and repair.
Rose, M., Princeton University, New Jersey. Nuclear fusion.
Sanders, S., Massachusetts Institute of Technology, Cambridge. Cell polarity in yeast.
Stillman, B., Cold Spring Harbor Laboratory. Cell cycle control of yeast chromosomal DNA replication.
Struhl, K., Harvard Medical School, Boston. Molecular mechanisms of yeast transcriptional regulation.
Young, R., Whitehead Institute for Biomedical Resesarch, Cambridge, Massachusetts. New insights into eukaryotic gene regulation.

Neurobiology of Human Neurological Disease: Mechanisms of Neurodegeneration

August 4–10

INSTRUCTORS

Gandy, Sam, M.D., Ph.D., Cornell University
Sisodia, Sangram, Ph.D., Johns Hopkins University Medical School

How and why do neurons die in specific acute or chronic human neurological disorders? What are the molecular and biochemical manifestations of specific genetic lesions in neurodegenerative disorders? Do different pathological neuronal death phenotypes share common mechanisms? What practical treatments can be contemplated? This lecture course explored possible answers to these important questions. Recent advances in neurogenetics and in molecular and cell biology have begun to shed light on the mechanisms that underlie nervous system injury in disease states such as Alzheimer's disease, amyotrophic lateral sclerosis, prion diseases, Huntington's disease, epilepsy, and stroke. Taking advantage of small class size and extensive discussion, invited faculty lecturers examined critical issues in their areas of expertise. Overview was provided and course participants did not need to be familiar with neurological diseases. The course focused principally on the specific hypotheses and approaches driving current research. Emphasis was placed on the highly dynamic interface between basic and clinical investigation, including the interdependence of clinical research and disease model development, and the value of disease research in understanding the function of the normal nervous system.

PARTICIPANTS

Bertuzzi, S., B.S., Ph.D., The Salk Institute, La Jolla, California
Coulliard-Despres, S., B.S., McGill University, Quebec, Canada
Debburman, S., B.A., Ph.D., University of Chicago, Illinois
Gschwentner, C., B.S., M.S., University of Vienna, Austria
Heinonen, O., M.S., Ph.D., National Public Health Institute, Helsinki, Finland
Hofmann, S., B.A., M.D., Ph.D., University of Texas Southwestern Medical Center, Dallas
Holder, J., B.A., University of Texas Southwestern Medical Center, Dallas
Karpuj, M., B.S., M.S., Weizmann Institute of Science, Rehovot, Israel
Lindblad, K., M.S., Karolinska Hospital, Stockholm, Sweden
Meyer, T., M.D., University of Ulm, Germany
Perez, M., B.S., University of Pennsylvania, Philadelphia
Perry, E., B.A., National Institutes of Health, Bethesda, Maryland
Peters, M., B.S., Ph.D., University of North Carolina, Chapel Hill
Pinter, J., B.A., M.D., University of California, San Francisco
Pong, K., B.S., Amgen, Inc., Thousand Oaks, California
Ray, J., B.S., M.S., Washington University Medical School, St. Louis, Missouri
Sanicola, M., B.S., Ph.D., Biogen, Inc., Cambridge, Massachusetts
Servadio, A., B.S., Ph.D., Telethon Institute of Genetics & Medicine, Milan, Italy
Shamraj, O., B.S., Neurogen Corporation, Branford, Connecticut
Takahashi, R., M.D., Ph.D., The Burham Institute, La Jolla, California
Tasaki, Y., B.S., Ph.D., Yamanouchi Pharmaceutical Co., Ibaraki, Japan

SEMINARS

Bredesen, D., La Jolla Cancer Research Foundation, California. What is the relationship between developmental and degenerative neural cell death?
Chao, M., Cornell University Medical College, New York. Receptor signaling mechanisms for cell survival and death.
Collinge, J., St. Mary's Hospital Medical School, London, United Kingdom. Human and veterinary prion diseases.
Edwards, R., University of California, San Francisco. The pathogenesis of Parkinson's disease.
Gandy, S., Cornell University Medical College, New York. Vesicle biology of the Alzheimer amyloid precursor and the presenilins.
Griffin, J., Johns Hopkins University Medical College, Baltimore, Maryland. Molecular pathology of peripheral neurological disorders.
Lipton, S., Harvard Medical School, Boston. Pathways to neuronal injury in AIDS dementia and stroke: Apoptosis, necrosis, excitotoxins, and free radicals/Potential treatment of AIDS dementia and stroke: NMDA channel blockers and NO-related species.
McNamara, J., Duke University Medical Center, Durham, North Carolina. Epilepsies and neurodegeneration.
Mobley, B., University of California, San Francisco. Neurotrophic factors and neurological disease.
Posner, J., Memorial Sloan-Kettering Cancer Center, New York. Paraneoplastic syndromes.
Price, D., Johns Hopkins University School of Medicine, Baltimore, Maryland. Motor neuron disease.
Relkin, N., Cornell University Medical College, New York. Clinical case study of Alzheimer's disease.
Ross, C., Johns Hopkins University Medical College, Baltimore, Maryland. Huntington's disease and other triplet repeat disorders.
Rothstein, J., Johns Hopkins University School of Medicine, Baltimore, Maryland. Excitotoxicity.
Sisodia, S., Johns Hopkins University School of Medicine, Baltimore, Maryland. Molecular neuropathology of Alzheimer's disease.
Young, A., Massachusetts General Hospital, Boston. Huntington's disease and other triplet repeat disorders.

Positional Cloning: Contig to Candidate Gene

October 14–27

INSTRUCTORS

Reeves, Roger, Ph.D., Johns Hopkins University School of Medicine
Silverman, Gary, Ph.D., Harvard Medical School
Spencer, Forest, Ph.D., Johns Hopkins University School of Medicine

CO-INSTRUCTORS

Church, Deanna, Ph.D., Mount Sinai Hospital/Samuel Lunenfeld Research Institute
Parimoo, Satish, Ph.D., Johnson & Johnson

ASSISTANTS

Bartuski, Alison, Harvard Medical School
Wiltshire, Tim, Johns Hopkins University School of Medicine

This lab-based course was aimed at investigators using genetic and physical mapping and functional assays to isolate a gene of interest. It was designed to cover those procedures used from the time a gene is localized genetically to the point where candidate genes are isolated and characterized for mutations in a positional cloning project. Methods for physical mapping included contig construction using YACs and BACs/PACs; analysis and characterization of large-insert clones by STS-content mapping, fingerprinting, and pulsed-field gel electrophoresis; creation of nested deletion derivatives of YACs by fragmentation at repeat sequences or exons; and end sequence rescue. Several procedures were used to manipulate sequences in YACs by homologous recombination to introduce mammalian selectable markers or to engineer specific mutations into target sequences. YAC DNA was prepared for transfer into cells in tissue culture or into the mouse germ line by lipofection, spheroplast fusion, and pronuclear injection. Gene identification from large cloned segments was accomplished by laboratory exercises in cDNA selection and exon trapping. Computer-based sessions on accessing data from the World Wide Web and the use of a variety of databases in gene searches were covered in class and by invited instructors. The practical component of the course was supplemented with lectures by invited speakers who are prominent researchers utilizing many of the procedures taught in the course. All participants presented their own research interests to serve as the basis for structured discussions of how to apply current technologies to their specific research projects.

PARTICIPANTS

Cicila, G., B.S., Ph.D., Medical College of Ohio, Toledo
De Gregorio, L., B.S., Istituto Nazionale Tumori, Milan, Italy
Furukawa, K., M.D., Ph.D., University of Kentucky, Lexington
Heresbach, D., M.D., Ph.D., North Shore Hospital, Manhasset, New York
Lee, H.-G., B.S., Ph.D., Memorial Sloan-Kettering Institute, New York
Maiwald, R., M.D., University of California, San Francisco
McKenzie, J., B.S., M.S., Peter MacCallum Cancer Institute, Victoria, Australia
McMahon, F., B.A., M.D., Johns Hopkins University, Baltimore, Maryland
Mestroni, L., B.S., M.S., M.D., International Center for Genetic Engineering & Biotechnology, Trieste, Italy
Mills, D., B.S., M.S., Ph.D., Brown University/Rhode Island Hospital, Providence
Nicholson, S., B.S., Imperial College School of Medicine at St. Mary's, London, United Kingdom
Passos-Bueno, M.R., M.D., University of Sao Paulo, Brazil
Robert, M., B.S., M.S., Ph.D., John Innes Centre, Norwich, United Kingdom
Romero, G., B.S., Ph.D., Philippine Rice Research Institute, Munoz, Philippines
Sharp, J., B.S., Ph.D., University College London Medical School, United Kingdom
White, R., B.S., Ph.D., The Children's Mercy Hospital, Kansas City, Kansas

SEMINARS

Baxevanis, A., National Institutes of Health, Bethesda, Maryland. Informatics in positional cloning.
Birren, B., Whitehead Institute, Cambridge, Massachusetts. Physical mapping: BACs to the basics.
Church, D., University of Toronto, Ontario. Physical and transcription mapping in the Cri du chat region.
Green, E., National Institutes of Health, Bethesda, Maryland. Clone-based physical mapping.
Parimoo, S., Johnson & Johnson, Skilman, New Jersey. Positional cloning of genes controlling hair follicle growth.
Reeves, R., Johns Hopkins University, Baltimore, Maryland. Mapping the unmappable and cloning what's not there.
Roe, B., University of Oklahoma, Norman. Genome sequencing: How, why, wherefore?
Silverman, G., Harvard Medical School, Cambridge. SCCA1 and SCCA2: A tale of two serpins.
Spencer, F., Johns Hopkins University, Baltimore, Maryland. A tale of two topics: Chromosomes controlled and cross-referenced.
Trask, B., University of Washington, Seattle. Cytogenetics in genome analysis.

Macromolecular Crystallography

October 14–27

INSTRUCTORS

Furey, William, Ph.D., V.A. Medical Center, Pennsylvania
Gilliland, Gary, Ph.D., National Institute of Standards & Technologies, Maryland Texas
McPherson, Alexander, Ph.D., University of California, Riverside
Pflugrath, James, Ph.D., Molecular Structure Corporation, The Woodlands

ASSISTANTS

Vasquez, Gregory, National Institute of Standards & Technologies, Maryland

Crystallography and X-ray diffraction yield a wealth of structural information unobtainable through other methods. This *intensive* laboratory/computational course focused on the major techniques used to determine the three-dimensional structures of macromolecules. It was designed for sci-

entists with a working knowledge of protein structure and function, but who were new to macromolecular crystallography. Topics that were covered included crystallization (proteins, nucleic acids, and complexes), crystal characterization, X-ray sources and optics, crystal freezing, data collection, data reduction, anomalous dispersion, multiple isomorphous replacement, phase determination, solvent flattening, molecular replacement and averaging, electron density interpretation, structure refinement, molecular graphics, molecular dynamics, and multidimensional NMR. Participants learned through extensive hands-on experiments where they crystallized and determined a protein structure, along with lectures and informal discussions on the theory behind the techniques.

PARTICIPANTS

Agianian, B., B.S., European Molecular Biology Laboratory, Heidelberg, Germany
Bouvier, M., B.S., Ph.D., University of Connecticut, Storrs
Downing, A.K., B.S., Ph.D., University of Oxford, United Kingdom
Eklof-Spink, K., B.A., Stanford University, Stanford, California
Gaiser, O., M.S., Max Delbruck Center, Berlin, Germany
Gampe, R., B.S., M.S., Glaxo Wellcome, Research Triangle Park, North Carolina
Kappler, J., B.A., Ph.D., Howard Hughes Medical Institute, Denver, Colorado
Li, J., B.S., University of Virginia, Charlottesville
Locher, K., M.S., University of Basel, Switzerland
McGrath, W., B.S., Ph.D., Brookhaven National Laboratory, Upton, New York
Mellman, D., B.S., Vanderbilt University, Nashville, Tennessee
Nguyen, D., B.A., Johns Hopkins University, Baltimore, Maryland
Nieves, R., B.S., University of Puerto Rico, San Juan
Sertchook, R., B.S., M.S., Ph.D., Hebrew University, Jerusalem, Israel
Thoma, N., B.A., University of Cambridge, United Kingdom
Wiencek, J., B.S., Ph.D., University of Iowa, Iowa City

SEMINARS

Brunger, A., Yale University, New Haven. Maximum advances in refinement: Torsion-angle, simulated-annealing, cross validation, maximum likelihood.
Clore, M., National Institutes of Health, Bethesda, Maryland. NMR structure determination of proteins and protein complexes beyond 20 kD.
Davies, D., National Institutes of Health, Bethesda, Maryland. Structure determinations of two enzymes that utilize cofactors.
Fitzgerald, P., Merck Research Laboratories, Rahway, New Jersey. Crystallographic studies in structure-based drug design.
Fleming, P., Yale University, New Haven. Xenon: Binding to proteins and use as a heavy atom derivative.
Gilliland, G., National Institute of Standards & Technology, Gaithersburg, Maryland. Crystal structure of recombinant tetradeca-(3-fluorotyrosyl)- glutathione *S*-transferase.
Hendrickson, W., Columbia University, New York. Structural biology of immune signaling through CD4 and Lck.
Joshua-Tor, L., Cold Spring Harbor Laboratory. The carboxyl terminus of Gal6/bleomycin hydrolase acts as a molecular ruler for proteolysis.
Sussman, J., Brookhaven National Laboratory, Upton, New York. Weizmann Institute of Science, Rehovot, Israel. Acetylcholinesterase in 3D: New mysteries revealed from the crystal structure.
Sweet, R., Brookhaven National Laboratory, Upton, New York. Laue diffraction and other methods as a tool for study of dynamic effects in protein crystals.
Tronrud, D., University of Oregon, Eugene. Strange density.
Xu, R., Cold Spring Harbor Laboratory. Crystal structure of the RNA-binding domain of human hnRNP A1.

Advanced In Situ Hybridization and Immunocytochemistry

October 14–27

INSTRUCTORS

Murray, John, Ph.D., University of Pennsylvania
Ochs, Robert, Ph.D., Scripps Research Institute
Ried, Thomas, Ph.D., National Institutes of Health
Schrock, Evelin, Ph.D., National Institutes of Health
Spector, David, Ph.D., Cold Spring Harbor Laboratory

ASSISTANTS

Howard, Tamara, Cold Spring Harbor Laboratory
Quinlin, Margot, University of Pennsylvania

This course focused on specialized techniques in microscopy related to localizing DNA and RNA sequences and proteins in cells for microscopic examination. The course emphasized the use of the latest equipment and techniques in epifluorescence microscopy, confocal laser scanning microscopy, electron microscopy, and digital image processing. The aim of the course was to provide state-of-the-art technology and scientific expertise in the use of microscopic applications to address basic questions in genome organization and cellular and molecular biology. The course was designed for the molecular biologist who was in need of microscopic approaches and for the cell biologist who was not familiar with the practical application of the advanced techniques presented. Among the methods presented were the preparation of tagged nucleic acid probes, fixation methods, detection of multiple DNA sequences in single nuclei or chromosome spreads, comparative genomic hybridization, use of a variety of reporter molecules and nonantibody fluorescent tags, indirect antibody labeling and detection of multiple proteins in a single cell. In addition, this year, a new section of the course emphasized the cellular localization of RNA. In each method, several experimental protocols were presented allowing the students to assess the relative merits of each and to relate them to their own research. Students were encouraged to bring nucleic acid or antibody probes to the course which could be used in addition to those provided by the instructors. The laboratory portion of the course was supplemented by invited lecturers who gave up-to-the-minute reports on current research using the techniques being presented in the course.

PARTICIPANTS

Ahmad, S., D.V.M., M.S., Ph.D., University of California, Davis
Averboukh, L., B.S., M.S., Ph.D., Ergo Science Corporation, Charlestown, Massachusetts
Bell-Matesoi, D., M.D., Baylor Research Institute, Dallas, Texas
Brink, A., B.S., University Hospital Vrije Universiteit, Amsterdam, The Netherlands
Connolly, B., B.A., Ph.D., Merck Research Laboratories, West Point, Pennsylvania
Hay-Schmidt, A., M.S., Ph.D., Royal Veterinary & Agricultural University, Frederiksberg, Denmark
Inada, M., B.S., University of California, San Francisco
Katz, J., B.A., M.D., University of Pennsylvania, Philadelphia
Laub, F., B.S., Mount Sinai School of Medicine, New York
Levenson, C., B.A., Ph.D., Florida State University, Tallahassee
Linney, E., B.S., M.S., Ph.D., Duke University Medical Center, Durham, North Carolina
Lu, J., B.S., Ph.D., AMC Cancer Research Center, Denver, Colorado
Maltzman, T., B.S., Ph.D., University of Colorado Health Sciences Center, Denver
Pramatarova, A., B.S., M.S., McGill University, Montreal General Hospital, Quebec, Canada
Radu, C., B.S., M.D., University of Texas Southwestern Medical Center, Dallas
Skopicki, H., B.A., Ph.D., Columbia University College of Physicians & Surgeons, New York

SEMINARS

Deerinck, T., University of California, San Diego. Electron microscopy as a tool in cell and molecular biology.
Garini, Y., Spectral Diagnostics, Carlesbad, California. Spectral karyotyping.
Herman, B., University of North Carolina, Chapel Hill. Fluorescence microscopy.
Huang, S., Northwestern University Medical School, Chicago, Illinois. Localization of RNA.
Murray, J., University of Pennsylvania, Philadelphia. Basic introduction to light microscopy and video microscopy/Principles of confocal microscopy and deconvolution techniques.
Piston, D., Vanderbilt University, Nashville, Tennessee. Localization of proteins in living cells using GFP.
Ried, T., National Institutes of Health, Bethesda, Maryland. Comparative genomic hybridization to detect amplifications and/or deletions in human cancers.
Salmon, T., University of North Carolina, Chapel Hill. High-resolution multi-mode digital microscopy of microtubules and mitosis.
Singer, R., Albert Einstein College of Medicine, Bronx, New York. Cytoplasmic organization of mRNA.
Spector, D., Cold Spring Harbor Laboratory. Immunocytochemistry.
Waggoner, A., Amersham Life Sciences, Pittsburgh, Pennsylvania. Development of fluorochromes and filters for fluorescence microscopy.

Mouse Behavorial Analysis

November 4–17

INSTRUCTORS

Silva, Alcino, Ph.D., Cold Spring Harbor Laboratory
Gallagher, Michela, Ph.D., Johns Hopkins University

ASSISTANTS

Han, Jung-Soo, Johns Hopkins University
Kogan, Jeff, Cold Spring Harbor Laboratory
Paylor, Richard, National Institutes of Mental Health
Smith, Dani, University of North Carolina
Wolfer, David, University of Zurich, Switzerland

This course provided a theoretical and experimental introduction to behavioral analysis in the mouse, with a focus on learning and memory. It was especially designed for geneticists,

lar biologists, pharmacologists, and electrophysiologists with a need for a hands-on introduction to behavioral analysis of the mouse. Additionally, the course covered the principles of using mutant mice in behavioral studies, as well as the issues involved in integrating behavioral, neuroanatomical, neurophysiological, and molecular findings. Among the methods that were presented were the water maze, cued and contextual fear conditioning, natural/ethologically relevant learning, open field behavior, the rotor-rod, and other activity tests. In addition, there were demonstrations of several aspects of in vitro electrophysiology (fields and whole-cell recordings of synaptic plasticity).

The speakers in the course included Howard Eichenbaum, Boston University; Larry Squire, University of California, San Diego; Peter Holland, Duke University; Mathew L Shapiro, McGill University; Roberto Malinow, Cold Spring Harbor Laboratory; Richard Thompson, University of Southern California; Peter R. Rapp, Mt Sinai; Joseph Takahashi, Northwestern University; Randal Reed, Johns Hopkins University School of Medicine; Michael Fanselow, University of California, Los Angeles; P. Read Montague, Baylor College of Medicine.

PARTICIPANTS

Brandon, E., B.S., Ph.D., The Salk Institute, La Jolla, California
Brown, G., B.S., Ph.D., Mount Sinai School of Medicine, New York
Chen, J.-F., M.S., M.D., Ph.D., Harvard Medical School, Charlestown
Hagan, J., B.S., Ph.D., SmithKline Beecham Pharmaceuticals, Essex, United Kingdom
Hasan, M., B.A., Ph.D., Massachusetts Institute of Technology, Cambridge
Isles, A., B.S., University of Cambridge, United Kingdom
Lau, D., B.A., New York University Medical Center, New York
Lin, Z., B.S. Ph.D., Cold Spring Harbor Laboratory
Magnusson, K., B.S., D.V.M., Ph.D., Colorado State University, Fort Collins
Pittenger, C., B.S., M.S., Columbia University College of Physicians & Surgeons, New York
Xu, B., B.S., Ph.D., University of California, San Francisco
Xu, W., B.S., Ph.D., Baylor College of Medicine, Houston, Texas

SEMINARS

Eichenbaum, H., Boston University, Massachusetts. Real life and laboratory learning in humans and animals/Brain systems/Recognition memory.

Fanselow, M., University of California, Los Angeles. Fear conditioning.

Fedorov, N., Cold Spring Harbor Laboratory. Measuring EPSCS and EPSPS in hippocampal slices.

Frankland, P., Cold Spring Harbor Laboratory. The involvement of the hippocampus on contex conditioning and contex discrimination: Neuroanatomical and genetic evidence.

Gallager, M., Johns Hopkins University, Baltimore, Maryland. The impact of aging on cognitive function: Rodent studies/Comparative approaches in the development of animal models.

Giese, K., Cold Spring Harbor Laboratory. The involvement of the aCaMKII in the plasticity of cells, circuits, and behavior.

Holland, P., Duke University, Durham, North Carolina. Appetitive conditioning/Brain systems that mediate Pavlovian conditioning.

Kogan, J., Cold Spring Harbor Laboratory. Memory studies in CREB mutant mice.

Malinow, R., Cold Spring Harbor Laboratory. Mechanisms of hippocampal synaptic plasticity.

Montague, P.R., Baylor College of Medicine, Houston, Texas. The value of modeling brain function.

Nelson, R., Johns Hopkins University, Baltimore, Maryland. Using converging methods to study nitric oxide synthase in mice: Studies of aggression.

Rapp, P., Mt. Sinai School of Medicine, New York. Advanced stereological techniques for quantitative neuroanatomy.

Shapiro, M., McGill University, Montreal, Canada. Assessing the performance of neural systems: Memory and long-term potentiation/Assessing the performance of neural stems: Recording neuron activity in behaving animals.

Silva, A., Cold Spring Harbor Laboratory. How to control genetic background.

Takahashi, J., Northwestern University, Evanston, Illinois. Genetics of circadian rhythms.

Thompson, R., University of California, Los Angeles. Mutant mice in the analysis of cerebellar-dependent learning.

Phage Display of Combinatorial Antibody Libraries

November 4–17

INSTRUCTORS

Barbas, Carlos, Ph.D., Scripps Research Institute
Burton, Dennis, Ph.D., Scripps Research Institute
Silverman, Gregg, M.D., University of California, San Diego

CO-INSTRUCTOR

Siegel, Donald, M.D., Ph.D., University of Pennsylvania Medical Center

ASSISTANTS

Cary, Steven, University of California, San Diego
Fuller, Roberta, Scripps Research Institute

Recent advances in the generation and selection of antibodies from combinatorial libraries allow the rapid production of antibodies from immune and nonimmune sources. This library/lecture course focused on the construction of combinatorial antibody libraries expressed on the surface of phage and selection of desired antibodies from the library. Students learned the theoretical and practical aspects of constructing combinatorial libraries from immune and nonimmune sources as well as the construction of synthetic antibody libraries. Antibodies were selected from the library by panning. Production, purification, and characterization of Fab fragments expressed in *E. coli* were also covered. The lecture series presented by a number of invited speakers focused on PCR of immunoglobulin genes, the biology of filamentous phage and the utility of surface expression libraries, expression of antibodies in *E. coli* and mammalian cells, antibody structure and function, catalytic antibodies, the whole biology of antibody activity, and recent results on the use of antibodies in therapy.

PARTICIPANTS

Chung, J., M.D. Ph.D., Dongguk University College of Medicine, Kyung-Ju, Korea
Flavell, D., B.S., Ph.D., University of South Hampton, Hampshire, United Kingdom
Frank, H.-G., M.D. Ph.D., University of Aachen, Germany
Gomez Roig, A., B.S., MERCK Farma y Quimica, S.A., Barcelona, Spain
Greenfield, E., B.S., Ph.D., Dana Farber Cancer Institute, Boston, Massachusetts
Hocking, D., B.S., CSL Ltd., Victoria, Australia
Kaushik, A., B.S., M.S., Ph.D., University of Guelph, Ontario, Canada
Mantis, N., B.A., Ph.D., Harvard Medical School, Boston
Nord, K., M.S., Ph.D., Royal Institute of Technology, Stockholm, Sweden
Schenk, J., B.S., Max Delbruck Center for Molecular Medicine, Berlin, Germany
Selisko, B., B.S., Ph.D., National Autonomous University of Mexico, Morelos
Tayapiwatana, C., B.S., M.S., Chiang Mai University, Chiang Mai, Thailand
Wilkins, J., B.S., M.S., Ph.D., University of Manitoba, Canada
Xing, P.X., M.D., Ph.D., University of Melbourne, Victoria, Australia
Zvi, A., M.S., Ph.D., Weizmann Institute of Science, Rehovot, Israel

SEMINARS

Barbas, C., The Scripps Research Institute, La Jolla, California. Phage display of antibodies and zinc finger protein.
Kelsoe, G., University of Maryland School of Medicine, Baltimore. B-cell development: Phenotype, anatomy location, and diversification of the antibody gene repertoire.
Lowman, H., Genetech, Inc., S. San Francisco, California. Phage display of antibodies and other proteins.
Model, P., Rockefeller University, New York. Phage biology.
Nestler, P., Cold Spring Harbor Laboratory. Encoded combinatorial libraries: Chemistry and beyond?
Pasqualini, R., La Jolla Cancer Research Institute, California. In vivo panning.
Sanz, I., University of Texas Health Science Center, San Antonio. Generation and features of antibody diversity.
Scanlan, T., University of California, San Francisco, San Francisco. Catalytic antibodies.
Scott, J., Simon Fraser University, Burnaby, British Columbia, Canada. Phage display of peptides.
Siegel, D.L., University of Pennsylvania Medical Center, Philadelphia. Cell surface selection of combinatorial Fab libraries.
Silverman, G. University of California, San Diego. Repertoire cloning of SLE autoantibodies.
Wilson, I., The Scripps Research Institute, La Jolla, California. Structural biology of immune recognition.

Molecular and Cell Biology of *S. pombe* and Other Yeasts

November 4–17

INSTRUCTORS

Chappell, Thomas, Ph.D., University College London, United Kingdom
Young, Paul, Ph.D., Queens University, Ontario, Canada

ASSISTANTS

Karagiannis, James, Queens University, Ontario, Canada
Gachet, Yannick, University College London, United Kingdom
Gregg, James, Oregon Graduate Institute of Science & Technology, Portland
Smith, Jennifer, University of Alberta, Canada

Fission yeast is often chosen as a model system for studies of cell and molecular biology. This course was designed to introduce the basic genetic, molecular, and cell biological techniques necessary for an investigator to adapt this system to their own laboratory/project. Topics included formal genetics and tetrad dissection, cytology including fluorescent and immunological techniques, transformation and gene replacement, plasmid recovery, and cell fractionation. Many of the experiments exploited mutant strains affected in the cell cycle or cytoskeleton. Against this background, the course introduced several other yeast systems of industrial or pathological interest. The *Pichia* system was used for protein expression and *Yarrowia* for cell fractionation studies. In addition to the laboratory component, invited speakers addressed various aspects of the biology of these systems.

PARTICIPANTS

Akoulitchev, A., B.S., M.S., Ph.D., New Jersey University of Medicine & Dentistry, Piscataway, New Jersey
Ayoub, N., B.S., M.S., Hebrew University, Jerusalem, Israel
Blad, S., M.S., Institute for Molecular Cellular Biology, Amsterdam, The Netherlands
Dove, S., B.S., Ph.D., University of Birmingham, United Kingdom
Gauss, P., B.S., Ph.D., Western State College, Gunnison, Colorado
Guevara-Lara, F., B.S., M.S., Unidad Irapuato, Irapuato, Mexico
Hanninen, A.-L., M.S., Ph.D., University of Helsinki, Finland
Hastings, C., B.S., M.S., Pioneer Hi-Bred International Inc., Johnston, Iowa
Kuznetsov, N., M.D., Ph.D., Marie Curie Research Institute, Surrey, United Kingdom
Lechelt-Kunze, C., B.S., Bayer AG, Leverkusen, Germany
Novoa, I., B.S., New York University Medical Center, New York
Raponi, M., B.S., University of South Wales, New South Wales, Australia
Sterner, J., B.S., Eli Lilly & Company, Indianapolis, Indiana

SEMINARS

Chang, F., Columbia University, New York. Polarity in fission yeast.

Chappell, T., University College London, United Kingdom. Functional and genetics studies of Golgi enzymes in *S. pombe*.

Cregg, J., Orgeon State Institute of Science & Technology, Portland. *Pichia* expression.

Leatherwood, J., State University of New York, Stony Brook Regulation of the cdc2 kinase.

McLeod, M., State University of New York, Brooklyn. Regulation of meiosis in fission yeast.

Smith, J., University of Alberta, Canada. Peroxisomes in *Yarrowia*.

Young, P., Queens University, Ontario, Canada. Sodium transport in *S. pombe*.

Computational Genomics

November 6–11

INSTRUCTORS

Pearson, William, Ph.D., University of Virginia, Charlottesville
Smith, Randall, Ph.D., SmithKline Beecham Pharmaceuticals

The course presented a comprehensive overview of the theory and practice of computational methods for gene identification and characterization from DNA sequence data. The course focused on approaches to extracting the maximum amount of information from protein and DNA sequence similarity through sequence database searches, statistical analysis, and multiple sequence alignment. Additional topics included gene recognition (exon/intron prediction), identifying signals in unaligned sequences, and integration of genetic and sequence information in biological databases. The course combined lectures with hands-on exercises; students were encouraged to pose challenging sequence analysis problems using their own data. This year, the course made extensive use of local World Wide Web pages to present problem sets and computing tools to solve them. The course was taught using Unix servers and X-windows terminals; participants were expected to be comfortable using the Unix operating system, the GCG sequence analysis package, and a Unix text editor (programming knowledge was not required). The course was designed for biologists seeking advanced training in biological sequence analysis, for computer core directors and staff for molecular biology or genetics resources, and for scientists in other disciplines, such as computer science, who wish to survey current research problems in biological sequence analysis.

PARTICIPANTS

Bailey, W., B.A., B.S., Ph.D., Merck & Co., Inc., West Point, Pennsylvania
Brown, S., B.S., Ph.D., New York University Medical Center, New York
Cai, L., B.S., Ph.D., Children's Hospital, Boston, Massachusetts
Cao, W., B.S. Ph.D., Cornell University Medical College, New York
Duchateau-Nguyen, G., M.S., Ph.D., Universite Paris Sud, Orsay, France
Gao, G.-J., B.S., Ph.D., University of Louisville, Kentucky
Gatewood, B., B.S., Ph.D., Johns Hopkins University, Baltimore, Maryland
Greene, J., B.S., Ph.D., Human Genome Sciences, Inc., Rockville, Maryland
Hatzimanikatis, V., B.S., Ph.D., Dupont Company, Wilmington, Delaware
Hinkle, G., B.A., Ph.D., University of Massachusetts, No. Dartmouth
Karnovsky, A., M.S., Ph.D., University of Colorado, Boulder
Leal, S., Ph.D., Rockefeller University, New York
Sanchez, R., B.S., Rockefeller University, New York
Schlink, G., B.A., Ph.D., Missouri Southern State College, Joplin
Schmid, K., M.S., Ph.D., University of Munich, Munchen, Germany
Simon, R., B.S., D.Sc., National Cancer Institute, Bethesda, Maryland
Taylor, R., B.S., M.S., National Institutes of Health, Bethesda, Maryland
Williams, S., B.A., Ph.D., Meharry Medical College, Nashville, Tennessee
Yang, U.-C., B.S., Ph.D., National Yang-Ming University, Taiwan, China
Zhu (Joe), X., B.S., Ph.D., SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania

SEMINARS

Eddy, S., Washington University School of Medicine, St. Louis, Missouri. Blocks, motifs, domains, and other protein databases/Multiple sequence comparison with hidden Markov models.
Gish, W., Washington University, St. Louis, Missouri. Statistics of sequence similarity scores/Searching databases with BLAST and friends/NCBI tools for sequence analysis.
Overbeek, R., Argonne National Laboratory, Argonne, Illinois. Genomic databases and genome informatics/ Beyond gene sequences: Recognizing biological function
Pearson, W., University of Virginia, Charlottesville. Protein evolution-biology/Practical issues in protein sequence searching.
Retief, J., University of Virginia, Charlottesville. Introduction to computer lab/GCG.
Smith, R., SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania. Introduction to multiple sequence comparison.
Stormo, G., University of Colorado, Boulder. Identifying sites in unaligned sequences/New approaches to discovering features in biological sequences.

The Laboratory would like to acknowledge the generosity of the following companies who loaned equipment and reagents to the various courses:

ALA Scientific Instruments, Inc.
Amersham Corporation
Amresco
Atto Instruments
Axon Instruments
Bachem Biosciences
Beckman-BREA
Becton-Dickinson Labware
Bio-101
Bio-Rad
Boehringer Mannheim Corporation
Burleigh Instruments, Inc.
Carl Zeiss, Inc.
CBS Scientific
Chroma Technology Corporation
Codonics Inc.
Corning Costar Corporation
Diagnostic Instruments, Inc.
Drummond Scientific Company
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Life Technologies, Inc.
MatTek Corporation
Medical Systems Corporation
Micro Video Instruments
MJ Research, Inc.
Modulation Optics, Inc.
Molecular Dynamics
Molecular Probes
Nalge Labware
Narishige International USA, Inc.
NEN Life Science Products
New England Biolabs
Nikon
Olympus America Inc.
Operon Technologies Inc.
Optronics Engineering
Owl Scientific, Inc.
Packard Instruments
Perkin-Elmer
PerSeptive Biosystems
Pfanstiehl Laboratories, Inc.
Pharmacia Biotech, Inc.
Pierce
Princeton Separations
Promega Corporation
Qiagen Inc.
Robbins Scientific Corporation
Savant Instruments
Sherwood-Davis & Geck
Shimadzu
Sooner Scientific, Inc.
Sorenson
Stratagene
Sutter Instruments
Techne, Inc.
Technical Manufacturing Corp.
TMC
Tropix
Vector Laboratories
Vorhies Technologies, Inc.
VWR Scientific Products
Wallac, Inc.

SEMINARS

Invited Speaker Program

Each year, Cold Spring Harbor Laboratory invites speakers from outside the institution to present their latest findings on a weekly basis. These seminars keep the CSHL staff current on the latest developments and broaden their perspectives. Graduate students and postdoctoral fellows meet with the seminar speakers for lunch immediately after the seminar, allowing an opportunity for the exchange of ideas in an informal setting.

1997

January

Michael Greenberg, Harvard Medical School. Neurotrophin and neurotransmitter regulation of gene expression and neuronal adaptive responses. (Host: Holly Cline)

Richard Young, Whitehead Institute. Positive and negative factors in gene expression. (Host: Winship Herr)

Walter Mangel, Brookhaven National Laboratory. Cofactors (peptide and viral DNA) and structure (1.6 Å resolution) of the adenovirus proteinase. (Host: Xiaodong Cheng)

Jonathan Stamler, Duke University. Hemaglobin: A microcosm of NO biology. (Host: Grisha Enikolopov)

February

Rudolf Jaenisch, Whitehead Institute. X-chromosome inactivation. (Host: Terri Grodzicker)

Keith Burridge, University of North Carolina. RHO and focal adhesions: Signaling and assembly. (Host: Nick Tonks)

Daniel Littman, Skirball Institute of Biomolecular Medicine. New York University Medical Center. Chemokine receptors: Roles in HIV entry and pathogenesis. (Host: Jacek Skowronski)

March

Richard Assoian, University of Miami School of Medicine. Cyclins and CDK inhibitors regulated by growth factors and the extracellular matrix. (Host: Nick Tonks)

Richard Firtel, University of California, San Diego. Regulatory pathways controlling morphogenesis and cell fate decisions in *Dictyostelium*. (Host: Hong Ma)

James Ihle, St. Jude's Children's Research Hospital, Memphis. Signaling by the cytokine receptor superfamily. (Host: Nick Tonks)

Aravinda Chakravarti, Case Western Reserve University. Genetic dissection of a complex disease: Hirschprung disease. (Host: Tom Marr)

April

Ed Harlow, Massachusetts General Hospital. Closing the G_1 gap. (Host: Lavina Faleiro)

Lorena Beese, Duke University Medical Center. DNA replication in a crystal: A high-resolution snapshot of a polymerase in action. (Host: Leemor Joshua-Tor)

Donald Ganem, University of California, San Francisco. Human herpesvirus 8 and the biology of Kaposi's sarcoma. (Host: Nouria Hernandez)

Douglas Koshland, Carnegie Institute of Washington. The five Cs of mitotic chromosomes: Cohesion, condensation, centromeres, and cell cycle. (Host: David Spector)

October

Jeff Dangl, University of North Carolina, Chapel Hill. Arabidopsis loci controlling cell death and disease resistance. (Host: Rob Martienssen)

Walter Gehring, Biocenter, University of Basel. Morphogenesis and evolution of eyes. (Host: Ueli Grossniklaus)

Joan Steitz, Yale University. The cell nucleolus: An RNA machine. (Host: Nouria Hernandez)

November

Larry Abbott, Brandeis University. Functional roles of synaptic depression in cortical processing. (Host: Rui-Ming Xu)

Judah Folkman, Children's Hospital, Boston. New directions in angiogenesis research. (Host: Scott Lowe)

Leonard Guarente, Massachusetts Institute of Technology. A molecular cause of aging. (Host: Bruce Stillman)

December

Michael Caudy, Cornell University Medical College. Control of neuronal cell fate decisions and neuronal cell signaling by helix-loop-helix transcription factors in *Drosophila* and mammals. (Host: Tim Tully)

William Weis. Stanford University. Structural analysis of the Armadillo repeat region of β-catenin and its interactions with cadherins. (Host: Leemor Joshua-Tor)

Carol Prives, Columbia University. Regulation of the p53 tumor suppressor protein. (Host: Terri Grodzicker)

In-House Seminar Program

Cold Spring Harbor In-House Seminars were initiated to provide a semiformal avenue for communication between the various research groups at the Laboratory. They are particularly useful for research personnel who have recently joined the Laboratory. The seminars also afford a necessary opportunity for the graduate students and postgraduate staff to develop their skills in organizing, presenting, and defending their research.

1997

January

Vivek Mittal (Hernandez Lab): Transcriptions a SNAP: Communication between regulatory factors bound to an unusual RNA polIII promoter.
Chun Liang (Stillman Lab): Control of DNA replication in the budding yeast *S. cerevisiae*.
Nick Carpino: Chronic myelogenous leukemia and p62.
Ken LaMontagne (Tonks Lab): PTPs, PTKs, and chronic myelogenous leukemia.

February

Gregory Hannon: Toward genetics in animal cell.
Clifford Yen (Wigler Lab): Genomic analysis of breast tumors by RDA.
Yi Zhong: The Ras-NF1 complex regulated adenylyl cyclase activity and its role in learning and memory.
Elly Nedivi (Visiting Scientist; Cline Lab): Differential cloning of genes responsive to neuronal activity.

March

John Iafrate (Skowronski Lab): Functional analysis of the HIV *nef* gene.
Cyril Sanders (Stenlund Lab): Assembling the BPV-1 replication initiation complex.
Richard Freiman (Herr Lab): Virus/host cell interactions: Cell cycle and transcriptional regulation by the VP16-associated protein HCF.
Dan Bush (Sabbatical Visitor; Martienssen Lab): Life without a heart: Understanding plant sugar and amino acid transporters in the context of resource allocation and multicellular growth.

April

Tatsuya Hirano: Protein machines that build up chromosomes.
Michael Hengartner: Death and the hermaphrodite: The hows and whys of programmed cell death in *C. elegans*.

Octrober

Viola Ellison (Stillman Lab): Characteriation of replication factor C: A component of the eukaryotic DNA replication machinery.
Howard Fearnhead (Lazebnik Lab): Is the enemy of my enemy my friend? (and how to find him).
David Helfman: What I did on my sabbatical: Stable and dynamic cytoskeletal assemblies.
Leemor Joshua-Tor: The carboxyl terminus of Gal6/Bleomycin hydrolase: A molecularler in proteolysis.

November

Michael Murray (Krainer Lab): Role of a type-2C Ser/Thr protein phosphatase in pre-mRNA splicing.
Zack Mainen (Malinow Lab): Receptor recruitment during LTP in GluR2 knockout mice.

December

Jim DeZazzo (Tully Lab): Molecular genetics of the Drosophila learning mutant nalyot.
Brandt Schneider (Futcher Lab): Divide or bust: Size matters and the cell cycle.

UNDERGRADUATE RESEARCH

Program Director: Michael Hengartner

Program Assistant: Jane Reader

An important aspect of the summer program at the Laboratory is the participation of college undergraduate students in active research projects under the supervision of full-time laboratory staff members. The program was initiated in 1959. Since that year, 485 students have participated in the course, and many have gone on to productive careers in biological science.

The objectives of the program are to provide (1) a greater understanding of the fundamental principles of biology; (2) an increased awareness of experimental approaches to science; (3) a deeper understanding of the major issues in the fields of biochemistry, genetics, and molecular and cellular biology; and (4) a personal acquaintance with research, research workers, and centers for study.

The following students, selected from more than 370 applicants, took part in the program.

Nizar Batada, Carleton University
Advisor: **Peter Nestler**
Sponsor: Frederica von Stade Fund
Screening and detection of substrates of apoptotic protease, CPP32.

Richard Benton, University of Cambridge
Advisor: **Robert Martienssen**
Sponsor: Emanuel Ax
Use of gene-trap and enhancer-trap systems to determine pattern formation in the vegetative development of *Arabidopsis thaliana.*

Scott A. Berkowitz, Yale University
Advisor: **Yuri Lazebnik**
Sponsor: Burroughs Wellcome Foundation
Searching for substrates of apoptotic proteases.

Jay Bikoff, Brown University
Advisor: **Jerry Yin**
Sponsor: Burroughs Wellcome Foundation
Regulation of the subcellular localization of the *d*CREB2 transcription factor.

Joshua Busch, Emory University
Advisor: **Hong Ma**
Sponsor: Burroughs Wellcome Foundation
Immunological analysis of AGAMOUS.

Alice Chu, Drew University
Advisors: **Shirley Pinto and Tim Tully**
Sponsor: National Science Foundation
Alternate cDNA copies of *Latheo*, a gene implicated in associative learning in *Drosophila*.

Andreas Demetriades, University College London
Advisor: **Hollis Cline**
Sponsors: Libby Internship; Jephson Educational Trust
Analysis of aberrant axon trajectories in homer-expressing neurons.

Daniel Desrosiers, Saint Anselm College
Advisor: **Jacek Skowronski**
Sponsor: Garfield Fund
Deletion analysis of HIV-1 Nef

Yanfei Feng, Peking University
Advisors: **Frances Hannan and Yi Zhong**
Sponsors: Glass Foundation; Jephson Educational Trust
Yeast two-hybrid system screen for interactors of *Drosophila* NF1 and *Rutabaga* adenylyl cyclase.

Andrew Fry, University of Glasgow, Scotland
Advisor: **Linda Van Aelst**
Sponsor: Shakespeare fund
Rac small GTPase and exchange factor TIAM1: An investigation of T-cell adhesion.

Christina Grozinger, McGill University
Advisor: **Winship Herr**
Sponsor: Olney Foundation
Determination and characterization of the DNA-binding site of the transcription factor LZIP.

Alberto Hazan, Harvard University
Advisor: **Grigori Enikolopov**
Sponsor: National Science Foundation
Transcription initiation in *Drosophila* nitric oxide synthase.

Kirstin Knox, Swarthmore College
Advisor: **Scott Lowe**
Sponsor: National Science Foundation
A genetic analysis of Ras-induced cell cycle arrest.

Robert J. Klein, Harvard University
Advisor: **Michael Zhang**
Sponsor: Burroughs Wellcome Fund
A computational description of the interaction between the transcription factors E2F and Sp1.

Carson Miller, The College of Wooster
Advisor: **Ueli Grossniklaus**
Sponsor: The Natioπnal Science Foundation
Molecular and genetic analysis of an enhancer detector line affecting megagametophyte development in *Arabidopsis*.

Andrew Miner, Duke University
Advisors: **David Spector and Tom Misteli**
Sponsor: Burroughs Wellcome Foundation
Biochemical characterization of pre-mRNA splicing factor pools in vivo.

Geralda Parvilus, Tuskegee University
Advisor: **Michael Hengartner**
Sponsor: National Science Foundation
Temporal control of gene expression in the nervous system of the nematode, *C. elegans*.

Nikos Reppas, Balliol College, Oxford University
Advisor: **Bruce Stillman**
Sponsor: Bliss Internship
The interaction of DNA polymerase α–primase with the origin recognition complex (ORC) in *Saccharomyces cerevisiae*.

Joel Stern, Columbia University
Advisor: **Alcino Silva**
Sponsor: Burroughs Welcome Fund
The N-Ras heterozygous mutation rescues the spatial learning deficits caused by the NF1 heterozygous mutation.

Milos Tanurdzic, University of Novi Sad
Advisor: **Erich Grotewold**
Sponsor: Read Fellowship
Identification of additional factors interacting with regulators of flavonoid biosynthesis.

Elizabeth Thomas, The Evergreen State College
Advisor: **Adrian Krainer**
Sponsor: National Science Foundation
Characterization of p54, a putative splicing factor.

Hung Tran, Columbia University
Advisor: **Michael Wigler**
Sponsor: National Science Foundation
Characterization of the binding partners of the tumor suppressor gene pTEN.

Keren Witkin, Wellesley College
Advisor: **Rui-Ming Xu**
Sponsor: National Science Foundation
Purification and preliminary crystallization studies of UNC-69.

NATURE STUDY PROGRAM

The Nature Study Program gives elementary and secondary school students the opportunity to acquire a greater knowledge and understanding of their environment. Through a series of specialized field courses, younger students can engage in introductory programs such as Nature Bugs, Nature Detectives, and Nature Discovery, and older students can enroll in more advanced programs such as Marine Biology and Nature Photography.

During the summer of 1997, a total of 383 students participated in 28 courses within the program. The classes were held outdoors, weather permitting, at the West Side School. The Laboratory has equipped and maintains classroom and laboratory facilities as well as a darkroom at West Side School. This facility is used as a base for the students' exploration of the local environment. Field classes are held on Laboratory grounds, St. John's Preserve, Fire Island National Seashore, Caumsett State Park, the Cold Spring Harbor Fish Hatchery and Aquarium, as well as other local preserves and sanctuaries.

In addition to the three, 2-week sessions, the Adventure Education course meets on two Fridays for trips. The students go on a three-masted schooner to navigate and explore the waters of Long Island Sound and on a 12-mile canoe trip on the Nissequogue River.

PROGRAM DIRECTOR

William M. Payoski, M.A., Adjunct Professor, Nassau Community College

REGISTRAR

Amy Stiso, Cold Spring Harbor Laboratory

INSTRUCTORS

Alison Forte, B.S., Marine Science, University of Rhode Island
Donna Pandaliano, M.S., Science Teacher, Valley Stream School District
Linda Payoski, M.S., Science Teacher, Uniondale School District
Marjorie Pizza, M.S., Science Teacher, Locust Valley School District
Brian Withers, Fine Arts Teacher, New York City School System

COURSES

Nature Bugs
Nature Detectives
Nature Discovery
Ecology Explorers
Frogs, Flippers, and Fins
Pebble Pups
Freshwater Life
Seashore Life
Bird Study
Marine Biology
Flora, Fauna, and Fun with watercolors
Nature Photography
Adventure Education

BANBURY CENTER

BANBURY CENTER DIRECTOR'S REPORT

Banbury Center had a very busy 1997, with no fewer than 18 meetings in the course of the year. In addition, the Center was used on a further 18 occasions for Laboratory meetings and by local nonprofit organizations. The meetings program was as eclectic as usual, covering fundamental research and topics of special interest in biotechnology as well as scientific education.

Participants

More than 570 participants came to the scientific meetings. The majority were of course drawn from institutions in the United States, but the fact that 15% came from other countires underscores the international character of biomedical research and the high esteem in which Banbury Center meetings are held. Most foreign visitors came from the United Kingdom (29), followed by France (15) and Germany (9). The majority of participants from the United States came from the Northeast, with New York, Massachusetts, and Maryland accounting for 26% of our visitors. The second largest grouping came from California (12%). The Corporate Sponsors sent 42 scientists, and 45 of the invited participants came from other companies.

Meetings on Genetics

Banbury Center has had a long-standing interest in genetics, originally dealing with the mutagenic effects of environmental hazards. But, more recently, a sizable proportion of the Banbury Center year has been devoted to human genetics, reflecting, in part, the success of the Human Genome Project. Meeting topics have dealt with technical matters such as cloning genes or have been devoted to specific disorders. We had examples of both meetings in 1997.

Duchenne muscular dystrophy (DMD) has the distinction of being one of the first genetic disorders on which recombinant DNA techniques made a significant impact. The DMD gene was cloned in 1986, and DNA-based diagnosis has played an important part in the lives of many thousands of afflicted families. Unfortunately, progress has been slow in developing treatments based on this molecular knowledge. The *Up-regulation of Utrophin Gene Expression* meeting examined a new approach based on the finding that there is a fetal form of the protein defective in DMD. This fetal gene is largely turned off in adults, but it may be possible to make it active again. Experiments in mice have shown that this protein can replace defective dystrophin, and the hope and expectation are that utrophin will be able to do the same in young males with DMD. We brought human geneticists working on this approach together with scientists interested in expression of muscle proteins and researchers who have been using this strategy successfully in treating thalassemias.

We held also the third in a series of meetings on neurofibromatosis (NF). Banbury Center has played a part in the NF story since the first meeting was held in 1990—an opportune moment for the gene was cloned later that same year. Our 1997 meeting, *The Pathogenesis of NF1 and NF2: Therapeutic Strategies*, had the goal of examining the special clinical features of NF1 and NF2 in the light of what is known of molecular changes in the genes. For example, cognitive dysfunction is a common feature of these disorders but its basis is poorly understood. Here at Cold Spring Harbor Laboratory, the groups of Alcino Silva and Yi Zhong are making important contributions using NF1 mutant mice. In addition, there was much discussion of how knowledge of the biochemical pathways involved in NF1 and NF2 might be exploited in devising therapies.

One of the attractions of doing scientific research is when unexpected connections are made

Robertson house provides housing accommodations at Banbury Center.

in what had appeared to be unrelated fields. Such a connection had been made in research on Parkinson's Disease in the summer of 1997 when it was found that Parkinson's Disease in one family was associated with a mutation in the gene for a protein called synuclein. This same protein is known to be involved in birds learning and remembering their songs! So here is a highly suggestive link between a protein and memory in two very different organisms, and our meeting, *Genetics of Parkinson's Disease,* held in the fall, examined this new development. Although it appears that this mutation is rare, it is hoped that the synuclein connection will provide clues as to what goes wrong in this devastating disorder.

Research in Cancer

There were three meetings on cancer-related topics. The first of the year was *The Biology of p53 and Its Implications for Diagnosis and Therapy*. p53 was originally thought to be a gene involved in making cancers, but further research showed that it suppresses cancer by being a key player in controlling cell division. This workshop first reviewed what is currently known of the biology of p53—how it exerts its effects in the cell and its interactions with other proteins. The workshop then turned to the practical applications of our knowledge of p53, in particular the fact that different mutations in p53 may lead to tumors with different properties. Thus, determining the type of p53 mutation in a patient's tumor may have important implications for both diagnosis and treatment.

The Biology of BRCA1 meeting had a similar purpose. The clonings of the *BRCA1* gene in 1996 and of *BRCA2* in 1997 were significant advances in research on breast cancer. However, as in so many cases where disease genes have been cloned, the next steps have been problematic. The meeting reviewed the spectrum of mutations so far found in the *BRCA1* and *BRCA2* genes, as well as the mutations found in other tumor suppressor genes in breast cancer. The participants then turned to the vexing question of the biological functions of the BRCA1 protein,

an area of considerable controversy, but an understanding of which is essential for a rational approach to devising therapies.

For many years, it has been hoped that an immunological attack on cancer would be possible. Cancer cells are different from the cells of the body and have different molecules on their surface, molecules that the immune system might be expected to use to identify cancer cells to be destroyed. Perhaps because overoptimistic hopes were not fulfilled, research on tumor immunology has not been among the leading areas of cancer research. Nevertheless, very good research continues in this area—research that deserves to be encouraged. The Alexander and Margaret Stewart Trust has as its goal the development of less toxic therapies than those in current use, and immunological therapies could provide these. The meeting *Immunological Attacks on Cancer* brought together scientists working on a very broad range of topics; many of these researchers had not met before for any extended period of time.

Genomics

Three meetings dealt with genetic studies at the level of complete genomes. The first—*Finding Genes: Computational Analysis of DNA Sequences*—discussed highly technical issues relating to what is becoming a critical problem. DNA sequence is being produced at an ever-increasing rate, and computer analysis of the sequence is essential for trying to identify genes in the hundreds of millions of bases. However, there is still considerable discussion as to which of the present methods are best and what new developments are needed to improve the accuracy and efficiency of the strategies and computer algorithms.

But all the computer-based gene detection in the world will count for very little unless experimental biologists make use of the information for understanding how organisms function. This is the so-called "post-genomics" world where genomics data will be used to design experiments.

An important intermediate step is to make use of all the biochemical, physiological, and cell biology research that has been performed throughout this century. An amazing wealth of data exists that must be assembled into a more coherent whole, using genetics as a framework. The first steps are already being taken using the detailed knowledge of, for example, metabolic pathways in bacteria. These are not trivial tasks and *Integrating Genetic, Biochemical, and Other Data in the Post-Genomics Era* examined the problems involved. Not least of these is devising rational and consistent classifications of current knowledge. This is complicated further by the very different forms data take in different fields.

One discipline that is likely to benefit is physiology, where the knowledge of genes and their interactions should provide new insights into physiological processes. The American Physiological Society funded a meeting at Banbury Center called *Genomics to Physiology and Beyond: How Do We Get There?* Participants included geneticists who are producing genomic data, scientists already using such data to examine interesting biological processes, and physiologists who will be using genome data increasingly in planning their research.

Cell and Developmental Biology

Cells synthesize many tens of thousands of molecules, but for many of these—in particular, proteins—synthesis is not the end of the story. There follows a series of changes in which various groups, for example, sugars in glycosylation, are added to the proteins. These modifications are essential for proper functioning of the proteins and pose problems for companies making human proteins in bacteria that cannot make the necessary modifications. The *Posttranslational Modifications* meeting was not restricted to proteins but examined a wide variety of changes that cellular molecules undergo following synthesis.

A key element in the life of a cell is its interactions with its environment: the detection of molecules in that environment, the conveyance of that information to the interior of the cell, and finally

the development of an appropriate response. Signal transduction is of special interest to those looking for therapeutic molecules to modify cell behavior. *Signal Transduction in Endothelial Cells* examined the molecules, their receptors, and the pathways that are activated in this important group of cells. A final discussion reviewed various models and mechanisms by which endothelial cells integrate the many positive and negative signals that they receive.

Neuregulins and Neuregulin Receptors examined a particularly important group of molecules involved in signal transduction. This family of proteins is large and their receptors are diverse, and thus they are involved in many systems and not just, as their name implies, in neurons. They have a role, for example, in signaling at synapses and the neuromuscular junction, in interactions with glial cells, and in signaling in sensory systems.

Development was the theme of what was, perhaps, the most "basic" and intriguing of the year's meetings. Animals are generally symmetrical during their early development and then develop asymmetries, that is to say, handedness becomes apparent. *Handedness and Symmetry in Development* brought to Banbury Center scientists whose expertise went from studies of molecular asymmetry, through embryology, to social studies of handedness in human beings. There was extensive and speculative discussion of the genetics, mechanisms, and evolutionary implications of this symmetry breaking during development.

Plant Molecular Biology

Banbury Center's plant meeting for 1997 dealt with *Molecular and Genetic Approaches to Transport in Plants*, a topic of considerable economic and social importance. The geographical distribution of plants is limited not only by climate, but also by the nature of the soil. The presence of metals such as aluminum and heavy metals and the nitrogen and phosphate content of the soil restrict the areas in which plants can be grown. Recombinant DNA techniques have led to the isolation of genes for the transporters that take up these elements and compounds. The practical consequences of these studies are of great potential significance for increasing the range of habitats in which a crop plant can be grown. We have been trying to promote plant molecular biology and genetics meetings, and the enthusiasm of both the participants and the members of the Laboratory's Plant Corporate Associates Program for this meeting was very gratifying.

Lyme Disease

Lyme Disease is of special interest in our region and Banbury Center has held a series of meetings on the topic since 1991. Research on Lyme Disease has changed dramatically since that meeting, becoming increasingly molecular in its basis. This was reflected in the 1997 meeting, entitled *Molecular Immunobiology of Lyme Disease*, which covered the latest findings on topics such as antigen expression, immune response, the importance of mixed infections, and progress in developing a vaccine. We can expect the nature of Lyme Disease research to change even more dramatically in the coming years, now that the complete genome sequence of the spirochaete has been determined. I expect Banbury Center to continue its role of promoting this research through further workshops.

Neuroscience

Banbury Center continues to hold meetings in the field of neuroscience, especially in topics related to learning and memory, research areas of special interest to the Laboratory. The John A. Hartford Foundation is interested in problems of learning and memory in human aging and has made a substantial grant to the Laboratory for exploring how our research on learning and memory in *Drosophila* and mice relates to human beings. Banbury Center held a discussion workshop on *Human Cognition and How It Fails* as part of this program. The subjects covered were extremely diverse, ranging from the development of learning and memory in infants through hippocampal functioning and Alzheimer's Disease to models of memory formation.

Science Education for Nonscientists

We were very pleased to join with the Federal Judicial Center in holding the second seminar on *The Art of Judging: Perspectives of Science*. Some 30 Federal and State judges came to Banbury Center to hear presentations on a wide variety of topics, ranging from the history of biology through human genetics to viruses and plagues. A highlight of the meeting was a talk by Leon Lederman, Nobel Laureate for Physics, on the very first moments after the Big Bang.

The J.P. Morgan/Cold Spring Harbor Laboratory Executives' Seminar

This was the 12th in this unique series of weekend seminars, intended to introduce areas of novel biological research to the senior executives of pharmaceutical and biotechnology companies and financial institutions. Each seminar has seven scientists as speakers, chosen both for the excellence of their research and for their ability to communicate with others. This year's seminar was on *Genetic Engineering*—a particularly appropriate subject, it being 25 years since Stan Cohen and Herb Boyer published their classic paper on cloning. We were very fortunate to have Stan Cohen as the opening speaker. In addition, we moved to the latest form of cloning, when Alan Colman of PPL Therapeutics told us about Dolly the Sheep. And in-between we fitted genetic engineering of molecules, plants, and human beings.

Other Meetings

The Banbury Center is a facility of Cold Spring Harbor Laboratory and is used by scientists for small discussion meetings on the Laboratory's research. For example, the DNA Tumor Virus group used the Center for the annual review of their research. We also make the Center available on a necessarily limited number of occasions for local nonprofit groups, including the Lloyd Harbor Village Conservation Board and Cold Spring Harbor High School, and for Board meetings of Holiday House and Huntington Hospital. The Lloyd Harbor Village Seminar series continued successfully.

Funding

With each passing year, Banbury Center's debt to the Corporate Sponsors continues to grow. The Program supports one third of Banbury Center meetings and provides us with the opportunity to have meetings that are both important for their relevance to biotechnology and pharmaceutical research and at the forefront of "basic" research. This year, the Corporate Sponsor Program funded the following meetings: *Signal Transduction in Endothelial Cells; Finding Genes: Computational Analysis of DNA Sequences; Integrating Genetic, Biochemical, and Other Data in the Post-Genomics Era; Neuregulins and Neuregulin Receptors; Handedness and Symmetry in Development; and Molecular and Genetic Approaches to Transport in Plants.*

Funding from other companies is playing an increasing role in our activities. This year, for example, Applied Microbiology, Inc. supported *Posttranslational Modifications*; OncorMed, Inc. funded *The Biology of p53 and Its Implications for Diagnosis and Therapy*; and J.P. Morgan again provided us with the means to do the Executives' Seminar *Genetic Engineering*.

We are particularly pleased that Foundations continue to find that Banbury Center meetings make important contributions to their efforts. In 1997, four Foundations came here: the Oxnard Foundation (*Up-regulation of Utrophin Gene Expression*); The Alexander and Margaret Stewart Trust (*Immunological Attacks on Cancer*); The John A. Hartford Foundation (*Human Cognition and How It Fails*); and the National Neurofibromatosis Foundation (*The Pathogenesis of NF1 and NF2: Therapeutic Strategies*). The American Physiological Society was the primary supporter of the *Genomics to Physiology and Beyond: How Do We Get There?*, and a contribution was made to the meeting by the Burroughs-Wellcome Fund.

Federal funding is important, although the long lead times needed to make grant applications

conflict with Banbury Center's goal of holding meetings on current and controversial research. Nevertheless, the National Cancer Institute was very helpful in contributing to both *The Biology of* BRCA1 and *Integrating Genetic, Biochemical, and Other Data in the Post-Genomics Era*, while the National Human Genome Research Institute and the National Institute for Neurological Disorders and Stroke funded the meeting on the *Genetics of Parkinson's Disease*. Two Institutes—the Centers for Disease Control and Prevention and the Federal Drug Administration—were the primary funding agencies for *Molecular Immunobiology of Lyme Disease*. Federal funding, through the Federal Judicial Center, covered the costs of *The Art of Judging: Perspectives of Science*.

Companies were also generous in providing supplemental funding, all of which contributed significantly to the Center's program. These companies included Cambridge Neuroscience, Inc. (*Neuregulins and Neuregulin Receptors*); CIBA-GEIGY, Ltd. (*Genomics to Physiology and Beyond: How Do We Get There?*); Amgen, Inc. (*The Biology of BRCA1*); and Fort Dodge Animal Health; Glaxo-Wellcome, Inc. and Pasteur Merieux Connaught (*Molecular Immunobiology of Lyme Disease*).

Acknowledgments

That Banbury Center is able to hold 18 meetings in a single year is a great tribute to the Center's staff—Bea Toliver and Ellie Sidorenko—and Katya Davey at Robertson House. Chris McEvoy and Andy Sauer continue to make the Center a beautiful place for our participants. Our schedule also places a burden on Housekeeping and Facilities, especially when we have meetings back-to-back with those in the main Cold Spring Harbor Laboratory meetings program. I thank all these people and the scientists at the Laboratory who continue to support our activities.

Jan Witkowski

Banbury Conference Center

Up-regulation of Utrophin Gene Expression

February 7–9

FUNDED BY
Oxnard Foundation

ARRANGED BY
K.E. Davies, University of Oxford, United Kingdom
D. Weatherall, University of Oxford, United Kingdom

SESSION 1: DMD and Utrophin
Chairperson: K.E. Davies, University of Oxford, United Kingdom

K.E. Davies, University of Oxford, United Kingdom: Background.
M. Grady, Washington University School of Medicine, St. Louis, Missouri: Double knockouts.
J.M. Tinsley, University of Oxford, United Kingdom: Transgenic utrophin *mdx* mice.
Discussion: Implications of this research.

SESSION 2: Defining Promoters in Muscle Genes
Chairperson: P.W.J. Rigby, National Institute for Medical Research, London, United Kingdom

B. Jasmin, University of Ottawa, Ontario, Canada: The utrophin promoter.
S.D. Hauschka, University of Washington, Seattle: Muscle genes.
P.W.J. Rigby, National Institute for Medical Research, London, United Kingdom: Muscle genes.
Discussion: What do we know about the properties of muscle promoters?

SESSION 3: Up-regulation Therapy
Chairperson: D. Weatherall, University of Oxford, United Kingdom

V.L. Funanage, Alfred I. DuPont Institute, Wilmington, Delaware: Up-regulation of utrophin: Effects of hemin.
D. Weatherall, University of Oxford, United Kingdom: The hemoglobinopathies: Background.
G.D. Ginder, University of Minnesota Medical School, Minneapolis: Embryonic globin gene activation in adult erythroid cells.
D. Weatherall, University of Oxford, United Kingdom: Clinical trials update.
Discussion: What can up-regulation of the hemoglobinopathies tell us?

SESSION 4: Strategies for Targeting Promoters
Chairperson: A.M. Bruskin, Oncogene Science, Inc., Uniondale, New York

C. Passananti, University of Rome, Italy: Targeting zipper motifs.
A.M. Bruskin, Oncogene Science, Inc., Uniondale, New York: Oncogene Science general strategies.
P. Baeuerle, Tularik, Inc., South San Francisco, California: Targeting gene transcription.
K. Giese, Chiron Corporation, Emeryville, California: Positive selection system to screen for modulators of transcription.
Discussion: Strategies to find new compounds.

SESSION 5: Other Possible Consequences of Up-regulation of Utrophin
Chairperson: L.M. Kunkel, Howard Hughes Medical Institute, The Children's Hospital, Boston, Massachusetts

B. Jasmin, University of Ottawa, Ontario, Canada: The neuromuscular junction and transcriptional control-1.
K.P. Campbell, Howard Hughes Medical Institute, University of Iowa College of Medicine, Iowa City: Reconstitution of the complex at the membrane.
J.S. Chamberlain, University of Michigan Medical School, Ann Arbor: Toxicity of overexpression; levels of expression needed; timing of delivery.

SESSION 6: Screening for Compounds Which May Up-regulate Utrophin
Chairperson: G.D. Ginder, University of Minnesota Medical School, Minneapolis

J. Haley, Oncogene Science, Inc., Uniondale, New York: Oncogene Science assay.
G. Karpati, Montreal Neurological Institute & Hospital, Canada: Studies of utrophin in culture.
Discussion: The best assay in muscle.

Summary and Discussion: Optimizing Strategies: Screening and Testing
Chairperson: S.D. Hauschka, University of Washington, Seattle

S. Hauschka

Genomics to Physiology and Beyond: How Do We Get There?

February 23–26

FUNDED BY
The American Physiological Society, with additional support from the Burroughs Wellcome Fund and CIBA-GEIGY, Limited

ARRANGED BY
A.W. Cowley, Medical College of Wisconsin, Milwaukee
H.J. Jacob, Medical College of Wisconsin, Milwaukee

SESSION 1: Looking for Genes Involved in Interesting Processes
Chairperson: A.W. Cowley, Medical College of Wisconsin, Milwaukee

A.W. Cowley, Medical College of Wisconsin, Milwaukee, and J.D. Watson, Cold Spring Harbor Laboratory: Opening remarks—Goals and challenges.
D.R. Cox, Stanford University School of Medicine, California: The human genome.
M.J. Welsh, Howard Hughes Medical Institute, University of Iowa College of Medicine, Iowa City: Cystic fibrosis/CFTR.
P. Corvol, INSERM, Paris, France: Predisposing genes to human essential hypertension.
L. Cardon, Sequana Therapeutics, Inc., La Jolla, California: Positional cloning of genes involved in asthma.
G. Duyk, Millennium Pharmaceuticals, Inc., Cambridge, Massachusetts: Analysis of complex traits in a post-genome scan world: Real examples—Real solutions.
C.T. Caskey, The Merck Genomics Research Institute, Inc., West Point, Pennsylvania: ESTs.

SESSION 2: Comparative Genomics as a Key to Human Physiology
Chairperson: D.R. Cox, Stanford University School of Medicine, California

H.J. Jacob, Medical College of Wisconsin, Milwaukee: Is man a rat? Gene hunting for complex phenotypes.
J.E. Womack, Texas A&M University, College Station: From the human genome to animal physiology.
M.C. Fishman, Massachusetts General Hospital-East, Charlestown: Zebra fish as a model vertebrate.
M.R. Riley, Marine Biological Laboratory, Woods Hole, Massachusetts: Progress report on the sequences of *E. coli* and other prokaryotes.
Breakout Groups: How to Find Genes of Interest to Physiologists?

SESSION 3: Determining Gene Function
Chairperson: M.J. Welsh, Howard Hughes Medical Institute, University of Iowa College of Medicine, Iowa City

O. Smithies, University of North Carolina, Chapel Hill: Knockout mice for elucidating gene function.
J.S. Takahashi, Northwestern University, Evanston, Illinois: Using forward genetics in the mouse to study the function of genes.
K.R. Chien, University of California, San Diego: Molecular

analysis of complex cardiovascular phenotypes in genetically engineered mice.

R. Roberts, Baylor College of Medicine and Methodist Hospital, Houston, Texas: Structure/function analysis of the β-MHC gene and protein.

G.J. Nabel, Howard Hughes Medical Institute, University of Michigan, Ann Arbor: Gene transfer in animal models and human disease.

Discussion: Problems of Using Transgenic Techniques for Physiological Studies
Chairperson: C.T. Caskey, The Merck Genomics Research Institute, Inc., West Point, Pennsylvania

SESSION 4: Going Beyond Genomics
Chairperson: C.T. Caskey, The Merck Genomics Research Institute, Inc., West Point, Pennsylvania

A.R. Rut, SmithKline Beecham, King of Prussia, Pennsylvania: Genomics and physiology.

M. Riley, Marine Biological Laboratory, Woods Hole, Massachusetts: Integrating *E. coli* genomics and metabolic pathways.

J.B. Bassingthwaighte, University of Washington, Seattle: Formulating the cardiome project: A beginning for the physiome project?

K.G. Hofbauer, Ciba-Geigy Ltd., Basel, Switzerland: A rational approach to target identification, concept validation, and patient selection.

General Discussion
Chairpersons: J.B. Bassingthwaighte, University of Washington, Seattle; **V.J. Dzau,** Brigham & Women's Hospital, Boston, Massachusetts

SESSION 5: Factors Affecting Implementation of Gene-based Approaches in Physiology
Chairperson: A.W. Cowley, Medical College of Wisconsin, Milwaukee

A.W. Cowley, Medical College of Wisconsin, Milwaukee: Introductory remarks.

C.T. Caskey, The Merck Genomics Research Institute, Inc., West Point, Pennsylvania: Industry.

D.R. Cox, Stanford University School of Medicine, California: Genetics.

T. Unger, University of Kiel, Germany: Physiology.

V.J. Dzau, Brigham & Women's Hospital, Boston, Massachusetts: Clinical.

S.C. Mockrin, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland: NIH.

Discussion: How to Encourage Exploring Approaches in Physiology
Chairperson: J.A. Schafer, University of Alabama at Birmingham

C.T. Caskey, J. Witkowski, A. Cowley, D. Cox

The Biology of p53 and Its Implications for Diagnosis and Therapy

March 5–8

FUNDED BY
OncorMed, Inc.

ARRANGED BY
A.J. Levine, Princeton University, New Jersey
S. Lowe, Cold Spring Harbor Laboratory

SESSION 1: Basic Biology of p53—What are the aspects of p53 biology that may be relevant to the clinic?
Chairperson: S. Lowe, Cold Spring Harbor Laboratory

D.P. Lane, Dundee University, Scotland, United Kingdom: Regulating p53 function through amino- and carboxy-terminal domains.
T. Waldman, The Johns Hopkins Oncology Center, Baltimore, Maryland: p21 checkpoint function and sensitivity to anticancer agents.
J. Windle, University of Texas Health Science Center at San Antonio: Effect of p53 on tumor properties and response to chemotherapy in a transgenic mouse model.
A.J. Levine, Princeton University, New Jersey: The functions of the MDM2 oncoprotein.
S.P. Linke, The Salk Institute for Biological Studies, San Diego, California: p53-dependent cell cycle effects of γ-radiation and nucleotide antimetabolites.

SESSION 2: Complexities of p53 Biology—How might the complexities of p53 biology confound simple analysis of clinical data? Are the biological consequences of p53 mutation dependent on the type of p53 mutation? Tissue type?
Chairperson: A.J. Levine, Princeton University, New Jersey

P.A. Hall, Ninewells Hospital and Medical School, Dundee, Scotland, United Kingdom: The heterogeneity of the p53 response in mice.
T.D. Tlsty, University of California, San Francisco: Loss of genomic integrity in preneoplastic cells.
C. Prives, Columbia University, New York, New York: Impact of p53 status on drug treatment of cells.
M. Oren, The Weizmann Institute of Science, Rehovot, Israel: Anti-apoptotic gain of function of mutant p53.
S. Lowe, Cold Spring Harbor Laboratory: Activation of p53 by oncogenes.

SESSION 3: Determining p53 Mutational Status—What are the current strategies for identifying p53 mutations? Their limitations? Can we assess pathways rather than genes?
Chairperson: C. Cordon-Cardo, Memorial Sloan-Kettering Cancer Center, New York, New York

M. Bywater, Cytometrics, Inc., Philadelphia, Pennsylvania.
B. Neri, Third Wave Technologies, Inc., Madison, Wisconsin: The detection of p53 mutations using cleavase fragment-length polymorphism (CFLP).
T. Soussi, Institut Curie, Paris, France: Serological analysis of p53 alterations in human cancer.
D. Mack, Affymetrix, Inc., Santa Clara, California: Profiling of cancer gene expression patterns and genotypic analysis using high-density oligonucleotide arrays.
R. Iggo, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland: Analysis of p53 tumor suppressor gene function in yeast.

C. Prives, R. Iggo

SESSION 4: p53 Mutations and Clinical Parameters—What are the clinical implications of p53 mutations for cancer patients? How does this relate to p53 biology? Should patients be tested for p53 mutations?
Chairperson: P.A. Hall, Ninewells Hospital and Medical School, Dundee, Scotland, United Kingdom

R. Cote, University of Southern California School of Medicine, Los Angeles: p53 and bladder cancer: Tumor progression, response to therapy, and analytical methods.
C. Cordon-Cardo, Memorial Sloan-Kettering Cancer Center, New York, New York: p53 as a biological determinant in human solid tumors.
E. Newcomb, New York University Medical Center, New York: p53 mutations and survival: Our experience with lymphoid, brain, and ovarian cancers.
R. Elledge, University of Texas Health Science Center at San Antonio: Prognostic and predictive value of p53 in breast cancer.
J.S. Kovach, City of Hope National Medical Center, Duarte, California: Null and missense p53 mutations in primary breast cancers are associated with adverse prognosis.

SESSION 5: Using p53 for Therapeutic Gain—How can p53, or knowledge of p53 status, be used to improve cancer management? What are the potential problems? What is needed from basic research to overcome these problems?
Chairperson: D. Lane, Dundee University, Scotland, United Kingdom

D. Kirn, Onyx Pharmaceuticals, Richmond, California: The use of an E1B-55 kD gene-deleted adenovirus for the treatment of p53-deficient tumors.
P. O'Connor, National Cancer Institute, Bethesda, Maryland: Can molecular characterization aid drug discovery? A test case with the p53 pathway in the NCI Anticancer Drug Screen.
M. Rolfe, Mitotix Inc., Cambridge, Massachusetts: Small-molecule inhibitors of p53 ubiquitination.
M.I. Sherman, PharmaGenics, Inc., Allendale, New Jersey: Strategic approaches to restoring lost p53 function.
D.P. Carbone, Vanderbilt University Cancer Center, Nashville, Tennessee: Oncogene-targeted cellular immunotherapy.
M. Harper, Introgen Therapeutics, Inc., Houston, Texas: Strategies for p53 gene therapy in cancer: Molecular, biological, and clinical endpoints.

Posttranslational Modifications

March 9–12

FUNDED BY
Applied Microbiology, Inc.

ARRANGED BY
S.L. Mowshowitz, Applied Microbiology, Inc., Tarrytown, New York
R. Pollack, Columbia University, New York, New York

SESSION 1
Chairperson: S.L. Mowshowitz, Applied Microbiology Inc., Terrytown, New York

H. Freeze, The Burham Institute, La Jolla, California: Mannose and *N*-glycosylation: A minority report.
B. Stillman, Cold Spring Harbor Laboratory: Histone acetylation in chromatin assembly.
R.G.W. Anderson, University of Texas Southwestern Medical Center, Dallas: Tyrosine kinase signal transduction from caveolae.

SESSION 2
Chairperson: B. Stillman, Cold Spring Harbor Laboratory

E.R. Stadtman, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland: Reactive oxygen-mediated modification of proteins.
W.I. Sundquist, University of Utah School of Medicine, Salt Lake City: Proteolytic processing and cyclophilin a binding by the HIV-1 capsid protein.
A.J. Franzusoff, University of Colorado Health Sciences Center, Denver: Intracellular trafficking pathways and HIV infectivity.

F. Perlin, R. Morimoto

SESSION 3
Chairperson: S.L. Mowshowitz, Applied Microbiology, Inc., Tarrytown, New York

F.B. Perler, New England BioLabs, Inc., Beverly, Massachusetts: Protein splicing.
M.-Q. Xu, New England BioLabs, Inc., Beverly, Massachusetts: Mechanism of protein splicing and its applications.
S.B. Prusiner, University of California, San Francisco: Conformational templating in the formation of the scrapie prion protein.
D.J. Selkoe, Brigham and Women's Hospital/Harvard Medical School, Boston, Massachusetts: Posttranslational processing of the β-amyloid precursor protein and the genesis of Alzheimer's disease.
M. Gasson, Institute of Food Research, Norwich, United Kingdom.
J.N. Hansen, University of Maryland, College Park: Formation, properties, and biological roles of the unusual residues in nisin and subtilin.
R.I. Morimoto, Northwestern University, Evanston, Illinois: Molecular chaperones in protein folding and protein degradation.
T. Muir, Rockefeller University, New York, New York: Protein synthesis via chemical ligation: New tools for probing protein structure and function.

NEUREGULINS AND NEUREGULIN RECEPTORS

March 16–19

FUNDED BY
Cold Spring Harbor Laboratory Corporate Sponsor Program, with additional support from Cambridge NeuroScience, Inc.

ARRANGED BY
M.A. Marchionni, Cambridge NeuroScience, Inc., Massachusetts
G. Lemke, The Salk Institute for Biological Studies, San Diego, California

SESSION 1: Neuregulin Receptors and Signaling
Chairperson: G. Plowman, Sugen, Redwood City, California

Y. Yarden, National Institute of Child Health & Human Development, Bethesda, Maryland: Signal diversification by neuregulin receptors.
K.L. Carraway, Beth Israel Hospital, Boston, Massachusetts: A ring-finger-containing protein that binds and clusters ErbB receptors.
M.X. Sliwkowski, Genentech, Inc., South San Francisco, California: Structure-function relationships of neuregulin with its receptors.
D.F. Stern, Yale University School of Medicine, New Haven, Connecticut: Hormonal regulation of the erbB receptor family network.
D.L. Falls, Emory University, Atlanta, Georgia: Functions of the neuregulin cytoplasmic domain.

SESSION 2: Genetics of Neuregulins and Neuregulin Receptors
Chairperson: C. Ibanez, Karolinska Institute, Stockholm, Sweden

C. Birchmeier, Max-Delbrueck-Centrum, Berlin, Germany: Genetic analysis of neuregulin and its receptors.
S.L. Erikson, Genentech, Inc., South San Francisco, California: ErbB3 is essential for normal cardiac and cerebellar development: A comparison with NRG- and ErbB2-deficient mice.
K.-F. Lee, The Salk Institute, La Jolla, California: Role of neuregulin receptor erbB2 in mammalian development.
M. Gassmann, The Salk Institute, La Jolla, California: Neural phenotypes in ErbB4 neuregulin receptor mutant embryos.
G. Lemke, The Salk Institute for Biological Studies, San Diego, California: Neuregulin ablation by ribozymes.

SESSION 3: Neuregulins in Neuron/Glial Interactions
Chairperson: S. Scherer, University of Pennsylvania Medical Center, Philadelphia

N. Ratner, University of Cincinnati College of Medicine, Ohio: Modulation of Schwann cell proliferation through alterations in Ras signaling and neuregulin production.
C.D. Stiles, Dana-Farber Cancer Institute, Boston, Massachusetts: Activation of erbB2 during Wallerian degeneration of sciatic nerve.
K.R. Jessen, University College London, United Kingdom: Neuregulin signaling in early Schwann cell development.
J. Grinspan, Children's Hospital of Philadelphia, Pennsylvania: Neuregulin as a survival factor for Schwann cells in development peripheral nerve.
A. Mudge, University College London, United Kingdom: Role of neuregulin in Schwann cell development.
J. Salzer, New York University Medical Center, New York: Role of the neuregulins in the axonal-glial interactions of myelination.
R.H. Miller, Case Western Reserve University, Cleveland, Ohio: Role of neuregulins in the induction of oligodendrocyte precursors.
C.S. Raine, Albert Einstein College of Medicine, Bronx, New York: Effect of neuregulins on autoimmune demyelination.
E. Anton, Yale University School of Medicine, New Haven, Connecticut: GGf/neuregulin is a mediator of reciprocal interactions between migrating neurons and radial glia in the developing cerebral cortex.
G. Corfas, Harvard Medical School, Boston, Massachusetts: Neuregulins in the developing cerebellum.

SESSION 4: Neuregulin Signaling at Synapses
Chairperson: A. Goodearl, Millennium Pharmaceuticals, Inc., Cambridge, Massachusetts

W. Thompson, University of Texas, Austin: Neuregulins and Schwann cells at the neuromuscular junction.
G.D. Fischbach, Harvard Medical School, Boston, Massachusetts: Neuregulins at the neuromuscular junction.
S. Burden, New York University Medical Center, New York: NRG-mediated signaling at neuromuscular synapses.
X. Yang, New York University Medical Center, New York: Regulation of neuronal nAChR expression by neuregulins.

SESSION 5: Neuregulin Signaling in Sensory Systems and New Ligands
Chairperson: C. Birchmeier, Max-Delbrueck-Centrum, Berlin, Germany

J.T. Corwin, University of Virginia, Charlottesville: Neuregulins in the sensory epithelia of the ear.
H. Chang, Stanford University School of Medicine, California: Ligands for ErbB family receptors encoded by a newly characterized neuregulin-like gene.
C. Lai, The Scripps Research Institute, La Jolla, California: ErbB receptor expression in the nervous system and neuregulin-2, a novel neuregulin-like molecule.
M.A. Marchionni, Cambridge NeuroScience, Inc., Massachusetts: Perspectives and summary.

G. Lemke, S. Scherer

Finding Genes: Computational Analysis of DNA Sequences

March 23–26

FUNDED BY
Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY
R. Gibbs, Baylor College of Medicine, Houston, Texas
P. Green, University of Washington, Seattle
J.-M. Claverie, CNRS, Marseille, France

SESSION 1: Overview

R.H. Waterston, Washington University, St. Louis, Missouri: Challenges in genome sequencing interpretation.
J.-M. Claverie, CNRS, Marseille, France: Introductory talk on practical methods for gene identification current concepts and current problems.

SESSION 2: Computational Analysis

S. Karlin, Stanford University, California: Computational biases in eukaryotic and prokaryotic genome sequences.
M. Borodovsky, Georgia Institute of Technology, Atlanta: Statistical determinants of protein-coding regions in DNA sequence.
M. Adams, The Institute for Genomic Research, Rockville, Maryland: Use of constraints, consensus, and contradiction in merging gene-prediction methods.
G.D. Stormo, University of Colorado, Boulder: New kinds of information for DNA parsing.
A. Krogh, Technical University of Denmark, Lyngby: Hidden Markov models for gene finding.
M. Gelfand, University of Southern California, Los Angeles: Las Vegas algorithms for gene recognition.

SESSION 3: Signatures in DNA

S. Audic, CNRS, Marseille, France: Promoter detection in mammalian DNA.
J.W. Fickett, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania: Eukaryotic promoter recognition: Where do we stand?
M.Q. Zhang, Cold Spring Harbor Laboratory: What do we know about core promoters?
A. Smit, University of Washington, Seattle: Improving database searches and gene prediction by identifying repetitive and low-complexity DNA.
L. Hillier, Washington University, St. Louis, Missouri: Use and interpretation of EST hits in gene prediction.
E.-C. Uberbacher, Oak Ridge National Laboratory, Tennessee: Large-scale gene modeling with pattern recognition and ESTs.
S. Banfi, Telethon Institute of Genetics & Medicine, Milan, Italy: DRES: *Drosophila*-related expressed sequences.

SESSION 4: Comparisons of Computational and Experimental Gene Finding

A. Ansari-Lari, Baylor College of Medicine, Houston, Texas: Large-scale sequencing in human chromosome 12p13: Experimental and computational gene structure determination.
B.A. Roe, University of Oklahoma, Norman: Human and bacterial genomic DNA sequence annotation.
E.M. Rubin, University of California, Berkeley: Computational and biological results from the analysis of ~700 kb of genomic sequence from a megabase region at human 5q31.
Discussion: Testing computational models: Designing a benchmark.

SESSION 5: Gene Finding and Databases

W. Gish, Washington University, St. Louis, Missouri: Analysis of human genomic sequence data at the GSC.
R. Durbin, Sanger Centre, Cambridge, United Kingdom: Managing gene annotation for genomic sequencing projects.
R.A. Manning, ApoCom Inc., Oak Ridge, Tennessee: Discovery tools for genomics.
D. Haussler, University of California, Santa Cruz: Design of the Genie Genefinder.
V. Solovyev, Amgen Inc., Thousand Oaks, California: Sequence analysis by WWW: From gene finding to structure prediction.
Summary: What Next?

The Biology of *BRCA1*

April 2–4

FUNDED BY
National Cancer Institute, with additional support from Amgen Inc.

ARRANGED BY
M.-C. King, University of Washington, Seattle
D. Livingston, Dana-Farber Cancer Institute, Cambridge, Massachusetts

SESSION 1: Mutations in Breast Cancer
Chairperson: M.-C. King, University of Washington, Seattle

Opening discussion: Identifying the Key Questions.
Chairperson: E. Harlow, Massachusetts General Hospital Cancer Center, Charlestown

A. Borg, University Hospital, Lund, Sweden: Biological and genetic features of *BRCA1* and *BRCA2* tumors.
D. Haber, Massachusetts General Hospital Cancer Center, Charlestown: Mutational analysis of *BRCA1*, *BRCA2*, and ataxia telangiectasia in early onset breast cancer.
Discussion: Mutations in Breast Cancer.
A. Efstratiadis, Columbia University, New York, New York: Phenotypes of *BRCA1*, *BRCA2*, *BRCA1/BRCA2*, *BRCA1*/p53, and *BRCA2*/p53 nullizygous mouse embryos.
T. Mak, Ontario Cancer Institute, Toronto, Canada: *BRCA1* and *BRCA2* are required for embryonic cell proliferation.

SESSION 2: Biology of BRCA1
Chairperson: D. Livingston, Dana-Farber Cancer Institute, Cambridge, Massachusetts

L.A. Chodosh, University of Pennsylvania School of Medicine, Philadelphia: Role of *BRCA1* in mammary epithelial growth and differentiation.
P. Polakis, Onyx Pharmaceuticals, Richmond, California: Response of the *BRCA1* protein in cellular stress.
E. Solomon, UMDS-Guy's Hospital, London, United Kingdom: Transcriptional regulation of *BRCA1*.
Discussion: Biology of *BRCA1*.

SESSION 3: BRCA1-Protein Interactions and Tumor Suppression I
Chairperson: D. Livingston, Dana-Farber Cancer Institute, Cambridge, Massachusetts

R. Baer, University of Texas Southwestern Medical Center, Dallas: Proteins that associate with the *BRCA1* gene product.
F.J. Rauscher, The Wistar Institute, Philadelphia, Pennsylvania: BAP-1, a novel enzyme that binds to the RING finger of the *BRCA1* gene product and exhibits properties of a tumor suppressor.

Coffee break

SESSION 4: BRCA1-Protein Interactions and Tumor Suppression II
Chairperson: T. Mak, Ontario Cancer Institute, Toronto, Canada

C. Wilson, University of California, Los Angeles, School of Medicine: Expression and subcellular localization of *BRCA1* and *BRCA1Δ11b*.
D. Livingston, Dana-Farber Cancer Institute, Cambridge, Massachusetts: Functional analysis of the *BRCA1* gene product.
J. Feunteun, Institut Gustave Roussy, Villejuif, France: Role of *BRCA1* in tumor suppression.
R. Jensen, Vanderbilt University School of Medicine, Nashville, Tennessee: Mechanisms of tumor suppression by *BRCA1*.
M.-C. King, University of Washington, Seattle: Possible genetic mechanisms underlying the biology of *BRCA1* in sporadic tumors.

SESSION 5: BRCA1-Protein Interactions and Tumor Suppression III
Chairperson: E. Harlow, Massachusetts General Hospital Cancer Center, Charlestown

J. Holt, Vanderbilt University School of Medicine, Nashville, Tennessee: Tumor suppression by *BRCA1*.
S. Tavtigian, Myriad Genetics, Inc., Salt Lake City, Utah: Predisposing mutations in *BRCA1:* Drawing connections between protein-protein interactions and cancer risk.

SESSION 6: Discussion: What Don't We Know? Where Next?
Chairperson: E. Harlow, Massachusetts General Hospital Cancer Center, Charlestown

Integrating Genetic, Biochemical, and Other Data in the Postgenomics Era

April 6–9

FUNDED BY
Cold Spring Harbor Laboratory Corporate Sponsor Program, with additional support from the National Cancer Institute

ARRANGED BY
M. Ashburner, University of Cambridge, United Kingdom
E. Harlow, Massachusetts General Hospital Cancer Center, Charlestown
P. Karp, SRI International, Menlo Park, California

SESSION 1
Chairperson: C. Sander, European Bioinformatics Institute, Cambridge, United Kingdom

E. Harlow, Massachusetts General Hospital Cancer Center, Charlestown: Opening remarks.
S. Letovsky, Johns Hopkins University School of Medicine, Baltimore, Maryland: Representation of gene function in GDB.
M. Ashburner, University of Cambridge, United Kingdom: On the representation of gene function in genetic databases.
J.T. Eppig, The Jackson Laboratory, Bar Harbor, Maine: De-fined vocabularies and functional/phenotypic classifications.
N. Maltsev, Argonne National Laboratory, Illinois: Representation of function in the PUMA/WIT systems.

M. Kanehisa

SESSION 2
Chairperson: M. Ashburner, University of Cambridge, United Kingdom

C. Sander, The European Bioinformatics Institute, Cambridge, United Kingdom: Functional genome comparison.
A. Danchin, Institut Pasteur, Paris, France: Integration of methodological knowledge and genetic knowledge.
P. Slonimski, Centre National de la Recherche Scientifique, Gif-Sur-Yvette, France: Population genetics of genes within genomes.
S. Lewis, University of California, Berkeley: Issues in data management for large-scale genomic analysis.
O. White, The Institute for Genomic Research, Rockville, Maryland: Bacterial sequence annotation in high throughput systems.
M. Fonstein, University of Chicago, Illinois: The Rhodobacter capsulatus genome project.

SESSION 3
Chairperson: A. Danchin, Institut Pasteur, Paris, France

M.L. Mavrovouniotis, Northwestern University, Evanston, Illinois: Metabolic pathways.
P. Karp, SRI International, Menlo Park, California: A pathway ontology is the key to computing with pathways.
R. Overbeek, Argonne National Laboratory, Illinois: WIT: A system to support metabolic reconstruction.
E. Selkov, Argonne National Laboratory, Illinois: Genome reconstruction: Methodology, status, and outlook.
M. Kanehisa, Kyoto University, Japan: From gene catalogs to functional catalogs: The KEGG project.

SESSION 4
Chairperson: P. Karp, SRI International, Menlo Park, California

A. Zollner, Max-Planck-Institute for Biochemistry, Martinsried, Germany: Functional categories of proteins (yeast genome).
C. Ouzounis, The European Bioinformatics Institute, Cambridge, United Kingdom: Representing and classifying protein function.
M. Riley, Marine Biological Laboratory, Woods Hole, Massachusetts: Functions of almost all *E. coli* gene products.

SESSION 5
Chairperson: L.T. Williams, Chiron Corporation, Emeryville, California

H. McAdams, Infernus, Stanford, California: What do we have to know to model developmental decision points in the cell?
J. Reinitz, Mount Sinai Medical School, New York, New York: Circuitry from gene expression: Computational analysis of segment determination in *Drosophila*.
S. Shaw, National Cancer Institute, National Institutes of Health, Bethesda, Maryland: Strategies for efficiently harnessing the expertise of biologists in cumulative information assembly: The PROW experiment.
J. Wagg, The Rockefeller University, New York, New York: Computational tools for bridging the gap between molecular and integrative biology.

The Pathogenesis of NF1 and NF2: Therapeutic Strategies

July 14–17

FUNDED BY
The National Neurofibromatosis Foundation and The Wilson Foundation

ARRANGED BY
E. Casper, Memorial Sloan-Kettering Cancer Center, New York, New York
J. Gusella, Massachusetts General Hospital, Boston
D. Gutmann, Washington University School of Medicine, St. Louis, Missouri
B. Korf, Children's Hospital, Boston, Massachusetts
K. North, Royal Alexandra Hospital for Children, Parramatta, Australia
A. Rubenstein, Mt. Sinai School of Medicine, New York, New York

SESSION 1: Cognitive Function in NF1
Chairperson: K. North, Royal Alexandra Hospital for Children, Parramatta, Australia

K. North, Royal Alexandra Hospital for Children, Parramatta, Australia: Overview of cognitive deficit in NF1.
P. Frankland, Cold Spring Harbor Laboratory: Molecular and cellular mechanisms underlying the learning impairments of NF1 mutant mice.
A. Bernards, Massachusetts General Hospital Cancer Center, Charlestown: Genetic analysis of NF1 function in *Drosophila*.
Y. Zhong, Cold Spring Harbor Laboratory: Molecular basis of learning deficits in NF1 mutants.

SESSION 2: Optic Glioma

Chairperson: D.H. Gutmann, Washington University School of Medicine, St. Louis, Missouri

D.H. Gutmann, Washington University School of Medicine, St. Louis, Missouri: Overview.

R. Listernick, Children's Memorial Hospital, Chicago, Illinois: Clinical overview and natural history of pathway optic glioma.

C.D. James, Mayo Foundation, Rochester, Minnesota: Molecular genetics of astrocytomas.

A.J. Wong, Kimmel Cancer Institute, Philadelphia, Pennsylvania: Signal transduction in astrocytes and astrocytomas.

D.H. Gutmann, Washington University School of Medicine, St. Louis, Missouri: Neurofibromin as a negative growth regulator for astrocytes.

R.J. Packer, Children's National Medical Center, Washington, D.C.: Chemotherapy and investigational drugs for optic pathway gliomas.

SESSION 3: Neurofibroma

Chairperson: B.R. Korf, Children's Hospital, Boston, Massachusetts

B.R. Korf, Children's Hospital, Boston, Massachusetts: Overview of neurofibromas in NF1.

D. Viskochil, University of Utah, Salt Lake City: Molecular genetics of neurofibroma.

J.B. Gibbs, Merck & Company, West Point, Pennsylvania: Farnesyl transferase inhibitors.

F. Lieberman, Mt. Sinai Medical Center, New York, New York: Differentiation induction strategies for neuroectodermal tumors.

N. Ratner, University of Cincinnati College of Medicine, Ohio: Experimental systems.

T. Jacks, Massachusetts Institute of Technology, Cambridge: Mouse model.

J.A. Epstein, University of Pennsylvania, Philadelphia: Cardiovascular defects in NF1-deficient mice.

L.F. Parada, University of Texas Southwestern Medical Center, Dallas: Neurotrophin-independent survival of neurons in the NF (–/–) mouse.

SESSION 4: Malignancy in NF1

Chairperson: E.S. Casper, Memorial Sloan-Kettering Cancer Center, Denville, New Jersey

E.S. Casper, Memorial Sloan-Kettering Cancer Center, Denville, New Jersey: Overview of malignancy in NF1.

A.I. Neugut, Columbia-Presbyterian Medical Center, New York, New York: A critique of the association between neurofibromatosis and sarcomas.

J. Woodruff, Memorial Sloan-Kettering Cancer Center, New York, New York: Pathology of malignant peripheral nerve sheath tumors.

LH. Baker, University of Michigan Cancer Center, Ann Arbor: The relationship of cafe au lait spots and sarcomas other than neurofibrosarcoma.

K.M. Shannon, University of California, San Francisco: Biologic and therapeutic studies in a murine model of NF1-associated leukemia.

SESSION 5: NF2

Chairperson: J.F. Gusella, Massachusetts General Hospital, Charlestown

J.F. Gusella, Massachusetts General Hospital, Charlestown: Overview.

V. Ramesh, Massachusetts General Hospital, Charlestown: Cell biology of NF2 protein merlin.

R.G. Fehon, Duke University, Durham, North Carolina: Structure/function analysis of *Drosophila* merlin.

G. Thomas, Foundation Jean Dausset/CEPH, Paris, France: Toward a mouse model for NF2.

M. MacCollin, Massachusetts General Hospital, Charlestown: Phenotype suppression using aminoglycoside antibiotics: A potential treatment for NF2.

Coffee break

The Art of Judging: Perspectives of Science

October 14–17

FUNDED BY
The Federal Judicial Center, Judiciary Leadership Development Council, and Cold Spring Harbor Laboratory

ARRANGED BY
J.A. Apple, The Federal Judicial Center, Washington, D.C.
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory: Cold Spring Harbor Laboratory and its place in science.

SESSION 2

A.N.H. Creager, Princeton University, New Jersey: From Darwin to Dolly: Developments in the biological sciences in the 20th century.
D. Wilkinson, Princeton University, New Jersey: Life in an inhospitable universe.

SESSION 3

M.D. Lemonick, *Time Magazine,* New York, New York: An exploration of life.
J.A. Deddens, University of Cincinnati, Ohio: Statistics and probability in science.

SESSION 4

D. Wilkinson, Princeton University, New Jersey: Discussion on new concepts of the universe.

SESSION 5

M. Gallo, Robert Wood Johnson Medical School, Piscataway, New Jersey: Toxicology, the environment, and risk assessment.
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Human genetics: A look at the past.

SESSION 6

J.A.Witkowski, Banbury Center, Cold Spring Harbor Laboratory: DNA and the Human Genome Project.

SESSION 7

R.M. Henig, Takoma Park, Maryland: Viruses and plagues: The menace of the 21st century?
P. Reilly, Shriver Center of Mental Retardation, Waltham, Massachusetts: Social implications of genetic research.

SESSION 8

L. Lederman, Fermi Laboratory, Chicago, Illinois: Great issues in science; the challenge of the 21st century.

R. Henig, J. Apple, J. Witkowski, D. Boggs

Immunological Attacks on Cancer

October 19–22

FUNDED BY
The Alexander and Margaret Stewart Trust

ARRANGED BY
B. Stillman, Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: MHC and Antigen Processing

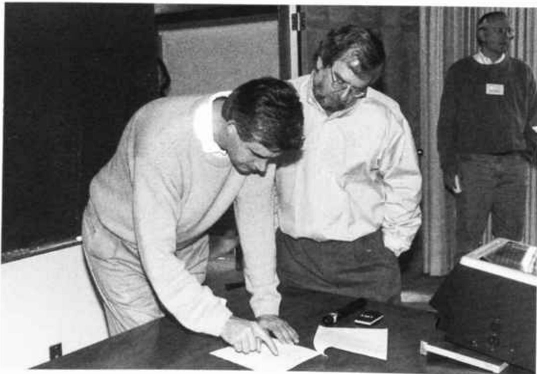
D.M. Pardoll, Johns Hopkins Oncology Center, Baltimore, Maryland: Harnessing the cryptic universe of endogenous antitumor T cells.
P.J. Lehner, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut: A critical role for tapasin in the assembly of MHC class I molecules.
L.L. Lanier, DNAX Research Institute, Palo Alto, California: Inhibitory receptors for MHC class I regulate immune responses.
P.K. Srivastava, University of Connecticut School of Medicine, Farmington: The case for uniqueness and pan-valency.
R. Glas, Harvard Medical School, Boston, Massachusetts: A nonproteasomal pathway for the generation of MHC class I ligands in mouse tumor cells.
General Discussion: MHC and Antigen Processing

SESSION 2: Tumor Antigens

V.H. Engelhard, University of Virginia, Charlottesville: Isolation and characterization of class-I-associated tumor antigen peptides.
W. Zhou, The Johns Hopkins Oncology Center, Baltimore, Maryland: Identifying tumor markers by looking at gene expression.
S. Ladisch, Children's Research Institute, Washington, D.C.: Shedding and immunosuppression by tumor ganglioside.

SESSION 3: Immune Regulation: Activation vs. Tolerance

H. Levitsky, Johns Hopkins University School of Medicine, Baltimore, Maryland: Peripheral tolerance to tumor antigens.
G. Trinchieri, Wistar Institute, Philadelphia, Pennsylvania: Interactions of innate and adaptive immunity in cancer immunotherapy.
R.N. DuBois, Vanderbilt University Medical Center, Nashville, Tennessee: Colorectal carcinogenesis and prevention: Underlying mechanisms. Immunomodulation by prostaglandins.
T.F. Gajewski, University of Chicago, Illinois: The role of costimulation, cytokines, and Th1/Th2 differentiation in antitumor immunity in vivo, and application for tumor antigen immunization protocols in patients with melanoma.
J. Allison, University of California, Berkeley: The yin and yan of T-cell activation.



B. Stillman, J. Allison

SESSION 4: Immune Regulation: Activation vs. Tolerance II

C. Lieping, Mayo Clinic, Rochester, Minnesota: The role of T-cell costimulators in the induction of tumor immunity against tumor antigens.
O.J. Finn, University of Pittsburgh School of Medicine, Pennsylvania: The importance of tumor-specific helper T cells and examples of tolerance at the helper T-cell level in case of tumor antigens that are also autoantigens.
Y. Hahn, University of Virginia Health Sciences Center, Charlottesville: Mechanism of immune evasion by tumorigenic hepatitis C virus.
E. Gilboa, Duke University Medical Center, Durham, North Carolina: Tumor RNA transfected dendritic cell vaccine: Preclinical and clinical studies.
D.P. Carbone, Vanderbilt University Cancer Center, Nashville, Tennessee: Mechanisms of dendritic cell dysfunction in cancer patients.

SESSION 5: Vaccines

M.T. Lotze, University of Pittsburgh School of Medicine, Philadelphia: Dendritic cells enhance effector mechanisms in the immune response to cancer.
W.M. Kast, Loyola University of Chicago, Illinois: Vaccine development against HPV-induced cervical cancer.
H. Kaufman, Albert Einstein College of Medicine, Bronx, New York: Recombinant viral vaccines and adjuvants for immunotherapy of human cancer.
R. Newman, IDEC Pharmaceuticals Corporation, San Diego, California: Monoclonal antibodies: Evolution to mainstream therapeutics.

J.P. Morgan & Co. Incorporated/Cold Spring Harbor Laboratory Executive Conference on Genetic Engineering

October 24–26

ARRANGED BY
J.D. Watson, Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

S. Cohen, Stanford University, California: Genetic engineering.

SESSION 2

J. Wells, Genentech, Inc., South San Francisco, California: From bigger molecules to smaller ones.
S. Tilghman, Howard Hughes Medical Institute, Princeton University, New Jersey: Exploring gene function in mice.
A. Colman, PPL Therapeutics plc, Edinburgh, Scotland, United Kingdom: A tale of three sheep, Tracy, Dolly & Polly: Implications for the biomedical uses of transgenic livestock.

SESSION 3

S. Lowe, Cold Spring Harbor Laboratory: Oncogenes, tumor suppressor genes, and chemosensitivity.

SESSION 4

K. Davies, University of Oxford, United Kingdom: Gene therapy–genetic engineering in human beings.
R. Michelmore, University of California, Davis: The second green revolution.
S. Fodor, Affymetrix, Inc., Santa Clara, California: Genes, chips, and the human genome.

P. Ringose, J. Witkowski, A. Colman, J. Watson

Molecular Immunobiology of Lyme Disease

November 2–5

FUNDED BY
Centers for Disease Control and Prevention, Food and Drug Administration, Fort Dodge Animal Health, Glaxo Wellcome, Inc., MedImmune, Inc., Pasteur Merieux Connaught, and SmithKline Beecham Pharmaceuticals

ARRANGED BY
J.J. Dunn, Brookhaven National Laboratory, Upton, New York
S.E. Schutzer, UMDNJ-New Jersey Medical School, Newark

SESSION 1: Antigen Expression
Chairperson: M.T. Philipp, Tulane Regional Primate Research Center, Covington, Louisiana

E. Fikrig, Yale University School of Medicine, New Haven, Connecticut: Arthropod- and host-specific *Borrelia* gene expression.
A. deSilva, Yale University School of Medicine, New Haven, Connecticut: *Borrelia* gene expression and transmission from ticks.
T.G. Schwan, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, Montana: Common themes in *Borrelia* proteins expressed in ticks.
S.E. Schutzer, UMDNJ-New Jersey Medical School, Newark: In vivo expression of *Borrelia* antigens in cerebrospinal fluid.
S.W. Barthold, University of California, Davis: Biologically relevant antibody responses to *B. burgdorferi* antigens expressed in vivo.

SESSION 2: Diagnostics
Chairperson: B.J. Luft, State University of New York at Stony Brook

. Ticehurst, ODE/CDRH/FDA, Rockville, Maryland: FDA Public Health Advisory on assays for antibodies to *B. burgdorferi:* Background/advice (2-step testing)/responses/future.
M.E. Schriefer, DVBID, NCID, CDC, Ft. Collins, Colorado: Diagnostic serology: Recommendations, performance, future.
P.K. Coyle, State University of New York at Stony Brook: Markers for neurological involvement.
A.E. Levin, Immunetics, Inc., Cambridge, Massachusetts: Total automation of the two-step testing procedure for Lyme antibodies: From esoteric procedure to routine clinical lab test.
R.J. Dattwyler, State University of New York at Stony Brook

SESSION 3: Genetics
Chairperson: J.J. Dunn, Brookhaven National Laboratory, Upton, New York

A.G. Barbour, University of California, Irvine: Analysis of expression of OspA in bacterial and eukaryotic cells.
C.L. Lawson, Brookhaven National Laboratory, Upton, New York: Structural analysis of *B. burgdorferi* outer surface proteins A and B.
J. Radolf, University of Texas Southwestern Medical Center, Dallas: Model systems for studying differential expression of *B. burgdorferi* antigens.
P.A. Rosa, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, Montana: Gene activation by allelic exchange in *B. burgdorferi.*
S. Bergstrom, Umea University, Sweden: OspH, a novel chromosomally encoded outer-surface-exposed lipoprotein of Lyme disease *Borrelia.*

J. Dunn, P. Coyle

SESSION 4: Immune Response

Chairperson: S.E. Schutzer, UMDNJ-New Jersey Medical School, Newark

A.C. Steere, New England Medical Center, Boston, Massachusetts: Phase III SmithKline Beecham Vaccine Trial.

J.J. Weis, University of Utah School of Medicine, Salt Lake City: The role of CD14 in signaling mediated by outer membrane lipoprotiens of B. *burgdorferi.*

L.K. Bockenstedt, Yale University School of Medicine, New Haven, Connecticut: T-cell regulation of murine Lyme carditis.

M.T. Philipp, Tulane Regional Primate Research Center, Covington, Louisiana: Is *B. burgdorferi* able to modulate the inflammation it elicits?

J.L. Benach, State University of New York at Stony Brook: Utilization of host proteases by *Borrelia.*

R.H. Jacobson, Cornell University, Ithaca, New York: Canine cytokine responses to *B. burgdorferi.*

SESSION 5: Vaccines

Chairperson: W. Golde, State University of New York at Stony Brook

R.C. Huebner, Pasteur Merieux Connaught, Swiftwater, Pennsylvania.

M. Hanson, MedImmune, Inc., Gaithersburg, Maryland: B. burgdorferi decorin binding protein A (DbpA) as a second generation vaccine candidate.

J.N. Miller, University of California School of Medicine, Los Angeles.

W. Zhong, Max-Planck-Institute fur Immunbiologie, Freiburg, Germany: New strategies to vaccinate against Lyme disease.

SESSION 6: General Discussion

Chairperson: W. Golde, State University of New York at Stony Brook

SESSION 7: Mixed Infections

Chairperson: J.N. Miller, University of California School of Medicine, Los Angeles

E. Hofmeister, Mayo Clinic, Rochester, Minnesota: Naturally occurring coinfections in mammalian hosts.

E.M. Bosler, State University of New York at Stony Brook: Coinfection in ticks and mammal hosts.

W. Golde, State University of New York at Stony Brook: Multiple cases of human coinfection with three different pathogens, *Babesia*, *Ehrlichia*, and *Borrelia*, transmitted by *Ixodes scapularis.*

G.P. Wormser, Westchester County Medical Center, Valhalla, New York: HGE and Lyme disease coinfections.

SESSION 8: Open Discussion

Chairperson: A.G. Barbour, University of California, Irvine

B.J.B. Johnson, Centers for Disease Control, Fort Collins, Colorado

A.R. Marques, LCI/NIAID, Bethesda, Maryland

J. Soreth, U.S. Food and Drug Administration, Rockville, Maryland

Signal Transduction in Endothelial Cells

November 9–12

FUNDED BY

Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY

D. Hanahan, University of California, San Francisco

F. McCormick, University of California, San Francisco

W. Risau, Max-Planck-Institut, Bad Nauheim, Germany

Introductory Remarks: D. Hanahan, University of California, San Francisco

SESSION 1: The Big Picture: Endothelial Cell States and Their Regulators
Chairperson: J.M. Folkman, Children's Hospital, Boston, Massachusetts

W. Risau, Max-Planck-Institut, Bad Nauheim, Germany: Overview of vasculogenesis, angiogenesis, and morphogenesis of endothelial cells for nonspecialists.

G.D. Yancopoulos, Regeneron Pharmaceuticals, Inc., Tarrytown, New York: Introduction to the major angiogenesis activators and their receptors (including FGFs, VEGFs, HGF, IL-8, TGF-α, B61/Lerck1, and the angiopoeitins).

L. Holmgren, Karolinska Institute, Stockholm, Sweden: Introduction to the major negative regulators of angiogenesis (including interferon, PF4, AGM1470, 16-kD prolactin, angiostatin, and endostatin).

SESSION 2: Signal Transduction: Major Pathways, Latest Concepts
Chairperson: F. McCormick, University of California, San Francisco

M. Whitman, Harvard Medical School, Boston, Massachusetts: TGF-β signals.

N.C. Reich, State University of New York, Stony Brook: IFN signaling and overview of the jak/stat circuits.

N. Tonks, Cold Spring Harbor Laboratory: Regulation by phosphatases.

SESSION 3: Cell-Matrix and Cell-Cell Interactions
Chairperson: R.A. Weinberg, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

D.A. Cheresh, The Scripps Research Institute, La Jolla, California: Integrins controlling angiogenesis.

R.O. Hynes, Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge: Angiogenesis and vasculogenesis in α-v-null mice.

E. Dejana, Instituto Mario Negri, Milan, Italy: Cadherins controlling endothelial cell states.

SESSION 4: Endothelial Receptors
Chairperson: J. Rossant, Samuel Lunenfeld Research Institute, Toronto, Canada

G.D. Yancopoulos, Regeneron Pharmaceuticals, Inc., Tarrytown, New York: Inhibitory signals imparted by angiopoeitins.

C. Betsholtz, Goteborg University, Sweden: PDGF/PDGF-R control of pericytes.

W.A. Frazier, Washington University School of Medicine, St. Louis, Missouri: TSP-1 and its receptors.

J. Liu, Massachusetts Institute of Technology, Cambridge: The AGM1470 receptor.

R. Hynes, B. Stillman, R. Weinberg

SESSION 5: Signaling Events in Endothelial Cells
Chairperson: W. Risau, Max-Planck-Institut, Bad Nauheim, Germany

E.F. Wagner, Research Institute of Molecular Pathology, Vienna, Austria: src family kinases.
A. Zimmer, National Institute of Mental Health, National Human Genome Research Institute, Bethesda, Maryland: rafB signals in endothelial cells.
D. Falb, Millennium Pharmaceuticals Inc., Cambridge, Massachusetts: Novel smads in endothelial cells.
R.I. Weiner, University of California, San Francisco: Signals from the 16-kD prolactin receptor.
D. Linzer, Northwestern University, Evanston, Illinois: Placental hormones sending angiogenic signals.

SESSION 6: General Discussion on the Integration of Positive and Negative Signals in Endothelial Cells
Chairperson: D. Hanahan, University of California, San Francisco

M. Krasnow, Stanford University School of Medicine, California: Genetic studies of signaling during branching morphogenesis in the *Drosophila* tracheal network.
M.C. Fishman, Massachusetts General Hospital-East, Charlestown: Genetic screens for vascular patterning mutants in zebrafish.
Discussion: Mechanisms by which endothelial cells might integrate so many positive and negative signals; models and wild speculation.

Handedness and Symmetry in Development

November 16–19

FUNDED BY
Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY
C. Tabin, Harvard Medical School, Boston, Massachusetts
L. Wolpert, University College London, United Kingdom
H.J. Yost, University of Utah, Salt Lake City

SESSION 1: Theories of Development of Asymmetry
Chairperson: H.J. Yost, University of Utah, Salt Lake City

L. Wolpert, University College London, United Kingdom: Models for the generation of left/right asymmetry.
J. Frankel, University of Iowa, Iowa City: Hereditary handedness in cell surface structural patterns of ciliates.
D.R. McClay, Duke University, Durham, North Carolina: Specification of symmetry in the sea urchin embryo.

SESSION 2: Human Behavioral Handedness I
Chairperson: H.J. Yost, University of Utah, Salt Lake City

I.C. McManus, University College London, United Kingdom: Human handedness: Genetics, neurobiology, and evolution.
A.J.S. Klar, NCI-Frederick Cancer Research & Development Center, Maryland: Tests of genetics and cultural models for human handedness.

SESSION 3: Human Behavioral Handedness II
Chairperson: C. Tabin, Harvard Medical School, Boston, Massachusetts

M.C. Corballis, University of Auckland, New Zealand: Genetics and evolution of human handedness and cerebral asymmetry.

J. Buon, C. Wolpert, J. Watson

SESSION 4: Heart Asymmetry
Chairperson: C. Tabin, Harvard Medical School, Boston, Massachusettss

M.C. Fishman, Massachusetts General Hospital-East, Charlestown: Genetic dissection of asymmetry in zebrafish.
R.P. Harvey, Royal Melbourne Hospital, Victoria, Australia: Intrinsic control of heart asymmetry.
E.N. Olson, University of Texas Southwestern Medical Center, Dallas: Regulation of heart handedness by hand genes.

SESSION 5: Signals
Chairperson: L. Wolpert, University College London, United Kingdom

H.J. Yost, University of Utah, Salt Lake City: The left-right coordinator and the initiation of the left-right axis.
M. Mercola, Harvard Medical School, Boston, Massachusetts: Role of Wnt-responsive signals.
C.V.E. Wright, Vanderbilt University School of Medicine, Nashville, Tennessee: Xnr-1 and L-R determination in *Xenopus*.
J. Cooke, National Institute for Medical Research, London, United Kingdom: The lateralized expression component of the cSnR gene and its position in a putative cascade of L-R information.
C. Tabin, Harvard Medical School, Boston, Massachusetts: Transfer of left-right asymmetric positional information to and from Hnesen's node in the chick embryo.
M. Levin, Harvard Medical School, Boston, Massachusetts: Gap junctions and L-R asymmetry.

SESSION 6: Genetics I
Chairperson: E.J. Robertson, Harvard University, Cambridge, Massachusetts

W.B. Wood, University of Colorado, Boulder: A gene affecting initial establishment of handedness in *C. elegans* embryos.
H. Hamada, Osaka University, Japan: The roles and transcriptional regulation of lefty genes.
M. Brueckner, Yale University School of Medicine, New Haven, Connecticut: L-R dynein: An axonemal dynein involved in L-R pattern formation in the mouse.
S.S. Potter, University of Cincinnati College of Medicine, Ohio: Dyneins and L-R asymmetry in mice.
M.R. Kuehn, National Cancer Institute, Bethesda, Maryland: Beyond *iv* and *inv:* Phenotypic and molecular analysis of four additional mouse mutants with abnormal left/right development.

SESSION 7: Genetics II
Chairperson: J. Burn, University of Newcastle, Newcastle upon Tyne, United Kingdom

B.M. Casey, Baylor College of Medicine, Houston, Texas: Genetic aspects of *situs inversus* and other human L-R axis malformations.
P.A. Overbeek, Baylor College of Medicine, Houston, Texas: YAC gene cure of the *inv situs inversus* mutation.
M. Penman Splitt, University of Newcastle, Newcastle upon Tyne, United Kingdom: Human malformations associated with disturbance of L-R asymmetry.

General Discussion
Chairperson: L. Wolpert, University College London, United Kingdom

Genetics of Parkinson's Disease

December 2–5

FUNDED BY
National Institute of Neurological Disorders & Stroke and National Human Genome Research Institute

ARRANGED BY
Z.W. Hall, National Institute of Neurological Disorders & Stroke, Bethesda, Maryland
R.L. Nussbaum, National Human Genome Research Institute, Bethesda, Maryland

SESSION 1: Synaptic Function and Biology of Synuclein I
Chairperson: Z.W. Hall, National Institute of Neurological Disorders & Stroke, Bethesda, Maryland

Z.W. Hall, National Institute of Neurological Disorders & Stroke, Bethesda, Maryland: Introduction.
T.C. Sudhof, University of Texas Southwestern Medical Center, Dallas: Synaptic proteins, protein phosphorylation, and the genetic analysis of synaptic functions.
P. DeCamilli, Yale University School of Medicine, New Haven, Connecticut: Molecular mechanism in synaptic vesicle endocytosis.
D.F. Clayton, University of Illinois at Urbana-Champaign: Constraints on evolution and expression of synucleins: Functional implications.
K. Nakaya, Showa University, Tokyo, Japan: The role of phosphoneuroprotein 14 (β-synuclein) in neuronal formation and function.
J. George, University of Illinois at Urbana-Champaign: Lipid-dependent changes in α-synuclein structure.

SESSION 2: Pathophysiology
Chairperson: M. Goedert, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

M. Goedert, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom: Introduction.
M.G. Spillantini, University of Cambridge, United Kingdom: α-synuclein in lewy bodies.
V.M.-Y. Lee, University of Pennsylvania, Philadelphia: α-synuclein in lewy bodies in Parkinson's Disease and dementia with lewy bodies.
A.L. Goldberg, Harvard Medical School, Boston, Massachusetts: Functions of the ubiquitin proteasome pathway in mammalian tissues in normal and diseased states.

SESSION 3: Genetics I
Chairperson: R.L. Nussbaum, National Human Genome Research Institute, Bethesda, Maryland

R.L. Nussbaum, National Human Genome Research Institute, Bethesda, Maryland: Genetic studies of Parkinson's disease, an overview.
M.H. Polymeropoulos, National Center for Human Genome Research, Bethesda, Maryland: Alpha synuclein and autosomal dominant Parkinson's disease.
Z.K. Wszolek, University of Nebraska Medical Center, Omaha: Clinical assessment of Parkinson's disease and parkinsonian-plus syndromes, genealogical investigations, and longitudinal observations.
T. Gasser, University of Munich, Germany: Genetic linkage and association studies Parkinson's disease: Evaluation of candidate genes.
D.J. Selkoe, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts: Multiple genotypes produce a common phenotype in Alzheimer's disease.

SESSION 4: Genetics II
Chairperson: R.L. Nussbaum, National Human Genome Research Institute, Bethesda, Maryland

W. Scott, Duke University Medical Center, Durham, North Carolina: Unraveling the complex etiology of Parkinson's disease: Alzheimer's disease as a model.
R.H. Myers, Boston University School of Medicine, Massachusetts: Contrasting the genetic component in Parkinson's disease with that of Alzheimer's disease.
L. Ozelius, Massachusetts General Hospital, Charlestown: Identification of a gene for early-onset dystonia.

SESSION 5: Models
Chairperson: D.L. Price, The Johns Hopkins University School of Medicine, Baltimore, Maryland

J.Q. Trojanowski, University of Pennsylvania, Philadelphia: NFH-LacZ transgenic mouse with lewy body-like inclusions show increased behavioral deficits following brain trauma.
D.L. Price, The Johns Hopkins University School of Medicine, Baltimore, Maryland: Transgenic models of neurodegenerative disease.
A.Y. Chiu, City of Hope Medical Center, Duarte, California: Motor neurons are more vulnerable to injury in a transgenic mouse model of familial amyotrophic lateral sclerosis.
P.H. St. George-Hyslop, University of Toronto, Ontario, Canada: Genetic models of human neurodegenerative disease.

Human Cognition and How It Fails

December 7–10

FUNDED BY
The John A. Hartford Foundation

ARRANGED BY
A. Baddeley, University of Bristol, United Kingdom
D.L. Price, Johns Hopkins University School of Medicine, Baltimore, Maryland
T. Tully, Cold Spring Harbor Laboratory

SESSION 1: Dynamics of Memory Formation
Chairperson: Y. Dudai, Weizmann Institute of Science, Rehovot, Israel

A. Baddeley, University of Bristol, United Kingdom: Working memory deficits in normal aging and Alzheimer's disease.
C.K. Rovee-Collier, Rutgers University, Piscataway, New Jersey: Dissociations in infant memory.
K. Thoroughman, Johns Hopkins University, Baltimore, Maryland: Human motor memory processes.
Y. Dudai, Weizmann Institute of Science, Rehovot, Israel: Dynamics of memory formation: The unique case of taste.
D.B. Willingham, University of Virginia, Charlottesville: A neuropsychological theory of motor skill learning.
T.J. Carew, Yale University, New Haven, Connecticut: Temporally and mechanistically distinct phases of constitutive PKA activity in *Aplysia* sensory neurons.

SESSION 2: Structure of Memory Formation
Chairperson: J.P. Aggleton, University of Wales, Cardiff, United Kingdom

L.R. Squire, Veterans Administration Medical Center, San Diego, California: Memory and the hippocampal formation.
M. Mishkin, National Institute of Mental Health, Bethesda, Maryland: Hierarchical organization of cognitive memory.
J.P. Aggleton, University of Wales, Cardiff, United Kingdom: Dissociating aspects of event memory.
P.S. Goldman-Rakic, Yale University School of Medicine, New Haven, Connecticut: Domain-specific and receptor-specific aspects of working memory.
A.P. Shimamura, University of California, Berkeley: Role of the prefrontal cortex in human memory and cognition.

SESSION 3: Disruption/Enhancement of Memory Formation
Chairperson: E.R. Kandel, Columbia University, New York, New York

H.P. Davis, University of Colorado, Colorado Springs: Changes in declarative memory, nondeclarative memory, and frontal lobe functioning across the life span.
P.W. Landfield, University of Kentucky College of Medicine, Lexington: Neurobiology of memory impairment with aging: Implications for organization of memory.
Y. Stern, Sergievsky Center, Columbia University, New York: Understanding individual differences in memory performance in normal aging and Alzheimer's disease: Reserve and compensation.
A.R. Mayes, University of Sheffield, United Kingdom: The specific effects on memory of hippocampal lesions and the amnesia syndrome.
T. Tully, Cold Spring Harbor Laboratory: Genes, memory, and rest.
E.R. Kandel, Columbia University, New York, New York: Memory suppressor genes in the hippocampus.

D. Price, K. Hsiao, R. Mayeux

SESSION 4: Memory Dysfunction
Chairperson: Tim Tully, Cold Spring Harbor Laboratory

A. Silva, Cold Spring Harbor Laboratory: Gene targeting: A tool to unravel mechanisms of learning and memory.
D.L. Price, Johns Hopkins University School of Medicine, Baltimore, Maryland: Animal models of aging and Alzheimer's disease.
R. Mayeux, Columbia University, New York, New York: Genetic epidemiology of Alzheimer's disease and related disorders.
J. Gabrieli, Stanford University, California: Roles for prefrontal cortex in episodic memory.
M.S. Albert, Massachusetts General Hospital, Charlestown: The boundary between aging and Alzheimer's disease.

SESSION 5: Memory Models
Chairperson: J.H. Byrne, University of Texas Medical School at Houston

K. Hsiao, University of Minnesota, Minneapolis: Behavioral deficits and electrophysiological abnormalities in transgenic mice overexpressing the Alzheimer amyloid precursor protein.
A. Sailer, The Salk Institute for Biological Studies, La Jolla, California: Functional analysis of kainate receptors using gene targeting.
J.H. Byrne, University of Texas Medical School at Houston: Insights into the neural mechanisms of operant conditioning: The neglected form of associative learning.
S. Dehaene, INSERM U-334, Orsay, France: A neuronal model for the role of prefrontal cortex in evaluation and planning.

SESSION 6: Closing Discussion: What are the critical next experiments for memory research?
Moderator: T.J. Carew, Yale University, New Haven, Connecticut

Molecular and Genetic Approaches to Transport in Plants

December 14–17

FUNDED BY
Cold Spring Harbor Laboratory Corporate Sponsor Program

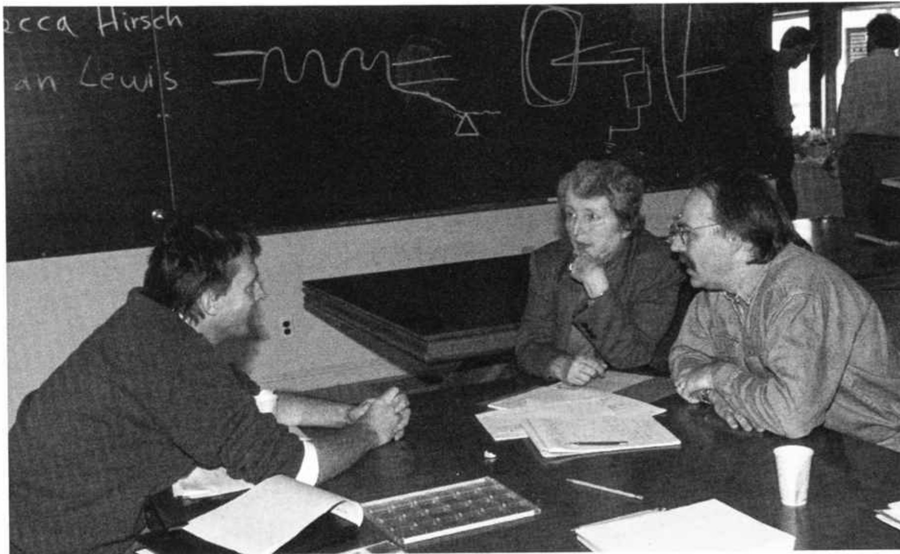
ARRANGED BY
J.I. Schroeder, University of California, San Diego, La Jolla
M.R. Sussman, University of Wisconsin, Madison

SESSION 1: Nitrogen Nutrition in Plants
Chairperson: A. Goffeau, University of Louvain, Belgium

W.-B. Frommer, Universität Tübingen, Germany: Molecular biology of nitrogen uptate.
N. Crawford, University of California, San Diego, La Jolla: Recent advances in nitrate uptake.
S.D. Tyerman, Flinders University of South Australia, Adelaide: Ammonium exchange across symbiotic membrane and aluminum-activated anion channel.
D.P.S. Verma, Ohio State University, Columbus: Regulation of nitrogen assimilation in root nodules.
A.D.M. Glass, University of British Columbia, Vancouver, Canada: Nitrogen absorption by plant roots: Regulation of fluxes.
G. Coruzzi, New York University, New York: *Arabidopsis* mutants define rate-limiting steps in nitrogen assimilation into N-transport amino acids.

SESSION 2: Proton Pumps
Chairperson: J.I. Schroeder, University of California, San Diego, La Jolla

M.R. Sussman, University of Wisconsin, Madison: Proton pumps and K^+ channel knockouts.
A. Goffeau, University of Louvain, Belgium: Functional expression of plant membrane proteins in yeast.
C. Slayman, Yale University School of Medicine, New Haven, Connecticut: H^+ ATPases.



D. Hilgemann, C. Slayman, D. Sanders

SESSION 3: K^+ Nutrition and Na^+ Stress I
Chairperson: R.F. Gaber, Northwestern University, Evanston, Illinois

H.J. Bohnert, University of Arizona, Tucson: Potassium transport and water uptake in relation to plant salinity tolerance.
E.J. Kim, University of California, San Diego, La Jolla: Characterization of novel potassium transporters in plants.
D. Sanders, University of York, United Kingdom: Monovalent cation transport: K^+ nutrition and salinity tolerance.

SESSION 4: K^+ Nutrition and Na^+ Stress II
Chairperson: C. Slayman, Yale University School of Medicine, New Haven, Connecticut

D. Hilgemann, University of Texas Southwestern Medical School, Dallas: Study of membrane transports in giant membrane patches.
J.-K. Zhu, University of Arizona, Tucson: Mutational analysis of salt tolerance and osmotic signal transduction in *Arabidopsis thaliana.*
E.P. Spalding, University of Wisconsin, Madison: Electrophysiological analysis of K^+ channel knockouts.

SESSION 5: Sucrose, Glucose: Sensing and Transport I
Chairperson: G. Coruzzi, New York University, New York

R.F. Gaber, Northwestern University, Evanston, Illinois: Glucose sensing and signaling in budding yeast.
J.-Y. Sheen, Massachusetts General Hospital, Boston: Sugar sensing and signaling in plants.
W. Tanner, University of Regensburg, Germany: Structure-function analysis of hexose/H^+-symporters.

SESSION 6: Sucrose, Glucose: Sensing and Transport II
Chairperson: W.-B. Frommer, Universität Tübingen, Germany

D.R. Bush, University of Illinois, Urbana: Sucrose and amino acid transporters: Recent advances in defining their structure and regulation.

SESSION 7: Micronutrients and Heavy Metal Transport
Chairperson: J.A.C. Smith, University of Oxford, United Kingdom

D.W. Ow, USDA-Plant Gene Expression Center, Albany, California: Heavy metal transport and sequestration.
Stephan Clemens, University of California, San Diego: A plant Ca^{++} and heavy metal transporter.
M.L. Guerinot, Dartmouth College, Hanover, New Hampshire: Characterization of a new family of metal transport proteins.

SESSION 8: Whole-plant Metal Transport and Tolerance
Chairperson: M.L. Guerinot, Dartmouth College, Hanover, New Hampshire

J.A.C. Smith, University of Oxford, United Kingdom: Metal transport and accumulation.

L. Herrera-Estrella, Centro de Investigaciones y Estudios Avanzados, Guanajuato, Mexico: Genetically engineered tolerance to aluminum toxicity.

J. Harper, The Scripps Research Institute, La Jolla, California: Cadmium and calcium pumps.

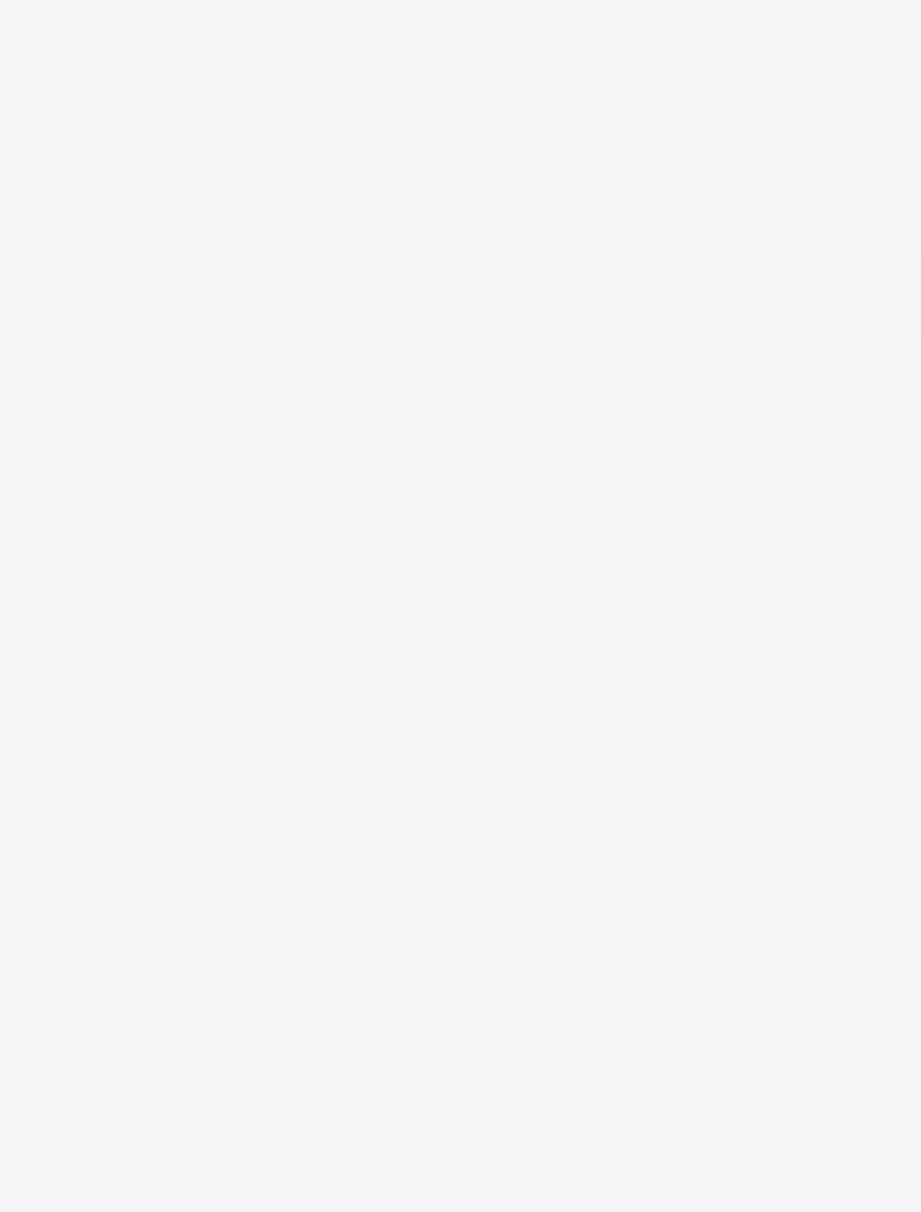
SESSION 9: Phosphate and Root-Soil Interactions
Chairperson: A.D.M. Glass, University of British Columbia, Vancouver, Canada

M. Harrison, The Samuel Roberts Noble Foundation, Inc., Ardmore, Oklahoma: Two phosphate transporters from *M. truncatula* roots: Regulation of expression in response to phosphate and to colonization by arbuscular mycorrhizal fungi.

D. Shibata, Mitsui Plant Biotechnology Research Institute, Ibaraki, Japan: Overexpression of an *Arabidopsis thaliana* high-affinity phosphate transporter gene in tobacco-cultured cells enhances cell growth under phosphate-limited conditions.

P. Doerner, The Salk Institute for Biological Studies, La Jolla, California: Cell division control and nutrient availability.

DNA LEARNING CENTER



DNA LEARNING CENTER

David A. Micklos
Judy Cumella Korabik

Mark V. Bloom
Scott Bronson
Michael Greenberg
Joan Alexander

Malissa Hewitt
Patricia Harrison
Andrew Morotti

John A. Kruper
Susan Lauter
Shirley Chan

As John Houseman once stated in a Smith-Barney ad, "We make our money the hard way, we *earn* it." In our case, this means living primarily on the "soft" money provided by gifts and grants. In this lifestyle, we come to value benefactors who appreciate one's work before it is well known and who provide funding without the usual requirements for detailed reporting. Invariably, the people who practice such "agape" giving become friends and mentors.

This summer, we mourned the death of one such special friend—Mary Jeanne Harris. She was that rare woman one meets in life who can be considered in the same league as one's own mother. She was organized and knew how to get things done. She had a certain can-do sensibility, which likely came from a traditional Midwest upbringing. When something was not getting done, she would jump in and do it herself.

Mary Jeanne and her husband Henry were the DNA Learning Center's (DNALC's) first benefactors, and, in a real sense, we owe our careers to them. In the fall of 1987, Mary Jeanne was on the trustee committee that was involved with the Laboratory's new training program for high school teachers. We were all fretting over taking an initial 6 months' lease of the old elementary school building in Cold Spring Harbor to use as a base of operations for our educational programs. Of course, we had no money for such an undertaking and were not even sure in our hearts that it would really work. Everyone knew the risk. Still, we needed $40,000 to move into the building. The weekend after this had been discussed in committee, Mary Jeanne phoned Dave Micklos from a golf outing in Texas to say that she and Henry would donate the first $10,000 toward the initial lease. The DNA Learning Center opened the following year.

Dave Micklos and Mary Jeanne Harris (left), listen to Dr. James Watson at the DNA Learning Center's dedication ceremony in September 1988.

On another occasion in 1989, Dave was struggling to find a little quiet time to finish up a textbook he had been working on for several years with our colleague Greg Freyer. In the fall, they were under fairly strict publication deadlines and just could not get the writing done in New York. Mary Jeanne suggested a "mini-sabbatical" at Henry's family farm on Squam Lake in New Hampshire. Of course, they agreed immediately and were captivated to find themselves on the "Golden Pond" of movie fame. Between morning and afternoon writing sessions, there was ample time for jogging amid the splendid fall foliage, antiquing, and listening to otherworldly calls of the resident loons. It was an incredibly productive week, during which the bulk of eight chapters were drafted. *DNA Science* was published the following year.

In 1991, Mary Jeanne and Henry funded an architectural study and made the lead gift toward construction of an addition to the DNA Learning Center. That project has taken longer to percolate, but prospects now are very good for construction to begin in 1998. It makes us terribly sad to think that Mary

Jeanne will not see the fruition of this project, which will further secure our unique place in American science education. Her death has made us feel more vulnerable in many ways, and we worry that it may be a very long time, indeed, before we find another friend of her stature.

Preparing for an Enlarged Facility

In May, we learned that the Dolan Family Foundation had provided a $1 million grant in support of a 6000 square-foot *BioMedia* Addition to the DNALC. The Dolan support adds to the lead grant of $50,000 from the Harris Trust and $400,000 in tax-exempt funding from the Suffolk County Industrial Development Corporation. Conceptual design was completed, with plans to begin construction late in 1998. We are currently seeking an additional $1.5 million to complete construction and to purchase laboratory, computer, and video equipment.

BioMedia expresses our goal to explore the creative use of computer and telecommunications technology in modern biology education. The focal points on the main level will be an octagonal multimedia computer laboratory, and a new exhibit gallery containing a *Visible DNA Sequencing Laboratory*. The *BioMedia* Addition, and reconstruction of existing space, will create a suite of three teaching laboratories and a student research laboratory, which will allow students to move effortlessly between hands-on science and multimedia computer experiences. A lunchroom and rest rooms are practical elements needed to deal with our ever-increasing number of visitors. The upper level will feature a video studio/production suite and multimedia conference room. Staff offices will be located on the upper and lower levels, according to function.

The facilities in the *BioMedia* Addition will allow us to capitalize on $1.75 million in program grants received this year (described in following sections), which will make the DNALC one of the largest Internet providers of multimedia learning materials for biology education. By pairing high-level computation with video production, we will be able to explore the "convergence" of CD/ROM, video, broadcast, and cable media. Equipment for videoconferencing will provide leverage for the DNALC's unique instructional resources, including the Laboratory's unparalleled pool of resident and visiting scientists. Distance learning will allow us to provide seminars, laboratory training, and follow-up support to many more schools than we can reach by traditional instructional methods. A streaming audio/video server will allow us to incorporate videoconference materials into our Internet site, along with content from commercial broadcast and cable sources.

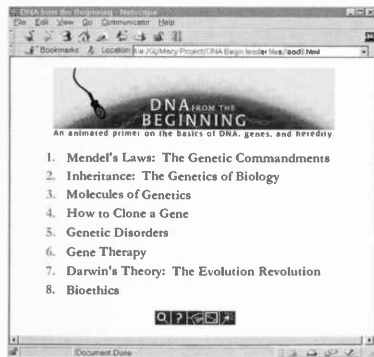
In addition to allowing us to capitalize on new opportunities, the *BioMedia* Addition offers the only solution to very serious space limitations in our existing facility. Although lab instruction was increased by over 30% in the 1997–1998 academic year, there is no possibility for any further increase in the existing building. The DNALC staff has grown from 9 positions in 1996 to 15 positions in 1998–a 67% increase. The current staff offices, located in the basement, have become so cramped that it is more and more difficult to maintain a high level of morale and productivity. We do not have reasonable workspaces for several additional staff we are currently recruiting to fulfill contractual obligations on new grants.

The *BioMedia* expansion is in line with the current effort to develop commercial biotechnology on Long Island. Since its inception in 1988, the DNALC has helped develop the science-literate workforce and informed constituency

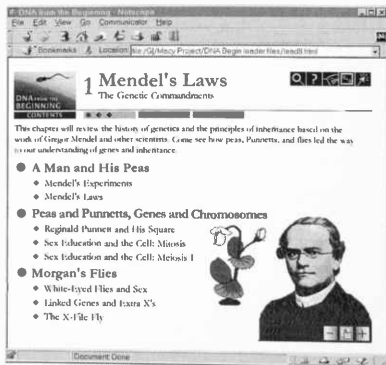
needed to support a biotechnology industry. Because of the on-site and outreach programs of the DNALC, Long Island ranks alongside the San Francisco Bay area as the world leader in advanced biotechnology education. The DNALC remains the nation's largest single provider of biotechnology lab instruction at the precollege level. The DNALC and teaching faculty it has trained provide lab experiences to thousands of local students each year. Students introduced to biotechnology in area schools are now pursuing advanced degrees in science, doing basic biological research, and assuming roles as opinion leaders in nonscience fields.

Creating the First On-line Genetics "Text"

In November, we received a 3-year grant of $820,000 from the Josiah Macy, Jr. Foundation to develop an extensive Internet site for genetics education. The first element to be developed will be *DNA From the Beginning*, an animated "primer" to provide background information on classical genetics, DNA and protein structure, the genetic code and protein synthesis, DNA mutations and polymorphisms, and gene mapping and cloning. The primer will make extensive use of multimedia animation, which can enliven unseen molecular events and involve young people accustomed to video games. This component will be the world's first on-line, animated genetics "text." The second element to be developed, *Gene Almanac*, will be an animated "encyclopedia" of genetic



The *DNA From the Beginning* web site will be tested with visiting students before it is available on-line.



disorders. The object will be to provide detailed information on diseases for which causative or predisposing genes have been identified, including current status of DNA diagnosis and treatments. Reviewed links will direct interested users to research news, patient support groups, clinical trials, and gene/protein sequences.

The Macy project was envisioned by CSHL President James Watson, who for three decades has sought mechanisms to disseminate information on genetics. Several of his works revolutionized science publishing: *The Double Helix* (1968) was the first popular account of the process of scientific discovery, as well as a critical and commercial success. *Molecular Biology of the Gene* (1965) was the first college text in molecular genetics. *Molecular Biology of the Cell* (1983) was the first "super-text" unifying cell and molecular biology. During Dr. Watson's tenure, the CSHL Press has evolved into a major book and journal publisher. Now, Dr. Watson sees the Internet as the next important channel through which to broadly encourage genetic literacy.

It is especially appropriate that the Macy Foundation should support this project. Lead support from the Macy Foundation in 1987 provided core staff funding needed to expand laboratory programs to utilize the new DNALC facility, which opened in 1988. Now, a decade later, Macy funding will catalyze a new program to effectively utilize facilities in the *BioMedia* Addition. Initial Macy funding allowed us to demonstrate new laboratory methods in science education; the next generation of funding will allow us to lead development of the Internet as an effective tool in science education.

Creating a Multimedia Communications Group

For several years, we have been involved in the applications of computer technology in biology education. A key collaboration with John Kruper, at the University of Chicago, allowed us to incorporate custom computer elements into our program to popularize the educational use of human DNA fingerprinting. With funding from the Howard Hughes Medical Institute, we worked with John's computation group to develop an educational thermal cycler and Internet-accessible database for *Alu* insertion polymorphisms. The receipt of large-scale support from the Macy Foundation provided the impetus to create the multimedia communications group we have dreamed about for several years. Thus, we were overjoyed when John arrived in November to head the new *BioMedia* Group.

John has been a close collaborator since 1987, when he spent several summers here as a graduate intern and based part of his doctoral thesis on our longitudinal survey of *Vector* Workshop participants. We first tried to recruit John in 1991, but our offer was bettered by the University of Chicago, where he stayed until 1996 as Director of Academic Computing for the Biological Sciences Division. While at Chicago, he oversaw the development of a purpose-built computer center, a server system for faculty and students, and over $4 million in grant-funded projects. As Director of Educational Computing at Allegheny College (1996–1997), he wrote and began to implement a strategic plan for a campus-wide shift to modern distributed computing.

Also arriving in November was Shirley Chan, who will be the content expert and primary author of *DNA From the Beginning* and *Gene Almanac*. Shirley had just received her doctorate from the Department of Molecular and Medical Genetics at the University of Toronto, where she worked in the laboratory of Joseph Culotti. Her thesis research on the cloning of the *unc-40* gene in

C. elegans was published in the journal *Cell*. Despite this early success in research, Shirley desired a position that combined science with her avocation of creative writing. The fact that she developed a demonstration Internet site on genetics education was evidence to us of her intent. Appropriately enough, we located Shirley through an advertisement on the Internet.

This nucleus team is rounded out by Creative Director Susan Lauter, who has been with the DNALC since its inception. Over the years, Susan has become a world-class computer animator and scientific illustrator. Sue joined the Laboratory in 1985 as an undergraduate photography intern in the public affairs and development office. She joined the full-time staff after receiving her bachelor's degree in fine arts from The Cooper Union. Sue is among the first generation of computer designers, now working almost exclusively in digital media. She computer-rendered over 200 pieces of artwork for the textbooks *DNA Science* (1990) and *Laboratory DNA Science* (1995) and has developed numerous interactive computer tutorials. She led development of the DNALC's Internet site and the early deployment of on-line *Shockwave* animations in 1996.

Moving Biology Education into the Genome Era

During the past several years, we have worked to develop an accurate analog of human genome research that allows students to learn about science in the same way as do modern scientists. We advocate that students experimentally determine their own DNA polymorphisms ("fingerprints"), then use Internet computer resources to analyze and share results. This approach received considerable support in 1997.

At the beginning of the year, we received a 3-year grant of $335,000 from the Department of Energy (DOE) for a nationwide training program, *The Science and Issues of Human DNA Polymorphisms*. This 3-day workshop introduces high school biology faculty to a laboratory-based unit on human DNA polymorphisms, which provides a uniquely personal perspective on the science and Ethical, Legal and Social Implications (ELSI) of the Human Genome Project. By targeting motivated biology faculty who currently perform student laboratories with viral and bacterial DNA, this program offers a cost-effective means to bring high school biology education up to the minute with genomic biology.

Between October and December, we instructed the first of 12 workshops nationwide at Mt. Sinai School of Medicine (New York), Boston University School of Medicine (Massachusetts), and Cañada College (California). Each workshop mixed theoretical, laboratory, and computer work with practical and ethical implications. Program participants learned simplified lab techniques for amplifying two types of chromosomal polymorphisms—an *Alu* insertion and a VNTR (D1S80). These polymorphisms illustrate the use of DNA variations in disease diagnosis, forensic biology, and identity testing and provide a starting point for discussion of the uses and potential abuses of genetic technology.

At mid-year, we received a $600,000 grant from the National Science Foundation (NSF) for a 3-year program to develop and disseminate advanced technology units on genomic biology. This project is funded by the Advanced Technological Education (ATE) Program, which focuses on improving technical education in high schools and 2-year colleges. Operating under a direct congressional mandate, the ATE program aims to ensure U.S. competitiveness in emerging technologies of the 21st century.

The ATE Project made a strong start with a workshop held at Cold Spring Harbor on June 23–28 to bring together DNALC staff with staff from project partners at the Center for Occupational Research and Development (CORD, of Waco, Texas) and members of a national Editorial Advisory Board:

Lesli Adler, Thomas S. Wooton High School, Rockville, MD
Clint Brown, Thomas S. Wooton High School, Rockville, MD
Ginny Brown, Winston Churchill High School, Potomac, MD
DeeDee Glassett, Merced College, Merced, CA
John LaPolla, Stuyvesant High School, New York, NY
Robert McKown, James Madison University, Harrisonburg, VA
Jean McLain, Wilson Technological Center, Dix Hills, NY
KumKum Prabhakar, Nassau Community College, Garden City, NY
Judy Price, Montgomery County Public Schools, MD
Gary Sarinsky, Kingsborough Community College, Brooklyn, NY
Janet Shagam, TVI Community College, Albuquerque, NM
Jerry Watkins, Central Islip High School, Central Islip, NY

The project team reviewed prototype lab materials to be included in the technology that represents a continuum of modern methods of gene identification and analysis. The units stress fundamental themes of evolutionary similarity and individual variation in genetic information. Comparative genomics and evolutionary biology are illustrated by computer manipulations of gene databases, including on-line projects using student-generated data, as well as data from DNA/protein databases. The high school unit focuses on length polymorphisms in human DNA and human population genetics, and articulates with CORD's nationally used curriculum *Applications in Biology/Chemistry* (*ABC*). The college unit analyzes human sequence polymorphisms in mitochondrial DNA, as well as polymorphisms in plants. One experiment examines how CSHL researchers use the *Ds* transposon (discovered by Barbara McClintock) to "tag" genes in the model plant *Arabidopsis*. Another shows the molecular basis of Mendel's wrinkled seed trait in peas. Following a year of development and testing, the *Genomic Biology* units will be introduced to 216 faculty at training institutes held at eight sites nationwide.

The project will be guided by a national mail survey that will reach a purposive sample of 12,000 high school biology teachers—equal to about one fourth of all such faculty in the U.S. This survey should give us the first careful measure of the number of high school students who participate in hands-on DNA labs. These students are the logical targets for *Genomic Biology* units.

Improving PCR for the Classroom

With support from DOE and NSF, we made significant biochemical modifications that will make it much easier for teachers to bring human PCR into high school and college classrooms.

We optimized the DNA extraction protocol from hair follicles, thus removing the potential objection to using cheek scrapings or saliva as a cell source for human DNA typing. For several years, we have advocated isolation of DNA from buccal cells obtained by saline mouthwash and Chelex extraction as the most reproducible method for isolating human template DNA for PCR amplification. Other educational groups advocate the use of buccal cells obtained by cheek scraping. Although noninvasive, both methods have the disadvantage of being perceived as "body fluids" potentially harboring pathogens; some school districts prohibit the use of buccal cells in student experiments. Overall, hair preps are about 10% less effective than mouthwash. Many people believe

the root to be a good source of DNA, but we have found that reproducible DNA recovery is, in fact, most highly correlated with the presence of a sheath. This structure is a bundle of several thousand squamous cells, which surround the hair shaft above (and usually separate from) the root. Brief incubation with proteinase K, followed by boiling with Chelex, disrupts the sheath membrane, releasing small groups of squamous cells that are readily lysed by boiling. Both sheath cells *and* hair shaft have mitochondria, making hair an ideal source of DNA for sequencing the mitochondrial control region.

We optimized PCR reaction conditions for Pharmacia "Ready-to-Go-Beads," which incorporate *Taq* polymerase in a dried bead. Each bead comes in its own reaction tube and is stable at room temperature. Use of Ready-to-Go Beads simplifies set up of PCRs, ensures reagent consistency, and reduces contamination. Primers and DNA template (from buccal or hair cells) are simply added to the beads. As another time saver, we incorporate the loading dye cresol red into the primer mix. This has no effect on PCR amplification and saves the step of adding loading dye prior to loading samples in an agarose gel.

We have synthesized new primers that amplify *Alu* alleles of approximately 500 bp and 800 bp, as compared to the 100- and 400-bp alleles we have amplified in the past. These larger alleles stain reproducibly with methylene blue (as well as with ethidium bromide). The larger alleles can be resolved in a lower percentage agarose gel (1.2% vs. 2.0%), reducing cost and staining time.

Our several-year effort to develop and disseminate an inexpensive DNA thermal cycler culminated in February with receipt of a license from PE Applied Biosystems to distribute the *Biogenerator* as an "authorized thermal cycler for PCR." This is, to our knowledge, the first thermal cycler specifically licensed for educational use. Although the *Biogenerator* is clearly a "Rube Goldberg" apparatus, it gives results comparable to commercial machines, and its open design allows students to better understand the process of thermal cycling. In anticipation of scaling up to the first production run of 100 machines, in October, we invested in a custom-printed circuit board for the key analog-digital controller. By year's end, we had sold 43 *Biogenerators*, through the DNALC Internet site and to distributor Carolina Biological Supply Company.

Sequencing Mitochondrial DNA

In collaboration with staff at the CSHL *Arabidopsis* Genome Sequencing Center, run by Dick McCombie, we developed reliable methods for generating mitchondrial (mt) DNA sequences from cheek and hair cells. Sequencing the variable "control region" proved an immense hit at a June workshop for members of the advisory board of our NSF Advanced Technological Education program. This technology proved readily reproducible at teacher workshops presented by Dave Micklos in Great Britain during the month of September. Two-day workshops were conducted at the University of Edinburgh, the University of Newcastle, and the Sanger Center at Hinxton Hall. Dave also gave a special lecture, "Cloning the DNA Classroom" at the London headquarters of the Wellcome Trust, which was the primary sponsor of the "tour." The enthusiastic response to mtDNA sequencing abroad encouraged us to immediately introduce this new technology at teacher-training workshops conducted under our DOE grant.

John Kruper speaks to Editorial Advisory Board at June workshop.

Although several educational groups have programs in student DNA sequencing, these focus on hand-sequencing of unknown sequences. Conversely, our program focuses on the student's own DNA sequence, devoped from colorimetric sequencing, as an entree to modern bioinformatics.

In this example, the on-line sequence analysis tutorial was used to compare modern human mitochondrial DNA sequence with Neanderthal DNA.

We advocate a system where an initial PCR amplification and dye labeling reactions are done in class (a several-hour commitment). Then, the ready-to-sequence DNAs are sent to regional centers for sequencing. Under our new NSF program, we are developing a model *Sequencing Service*, which will develop student sequences and post the results via Internet.

Experience gained in generating mtDNA sequence for more than 125 teachers at the Wellcome Trust and DOE workshops has demonstrated the feasibility of the *Sequencing Service*. We can obtain clean sequence from about 80% of buccal and hair samples; we are testing reagent modifications that can potentially put us within our projected total cost of $3.00 per sequence. Although we are currently using an Applied Biosystems 377 sequencer in the *Arabidopsis* Genome Sequencing Center, our objective is to obtain a sequencer for the DNALC site. We envision a functioning, mini-sequencing lab—enclosed by picture windows—as a permanent exhibit in the new gallery of the *BioMedia* Addition.

In the fall, we published our first sequence analysis tutorial on DNALC's web site (http://darwin.cshl.org/SequenceAnalysisExercise/index1.html). This exercise is designed to allow students and teachers to use their own mtDNA sequences to explore on-line genome resources, to test theories of human evolution, and to "solve" cases in forensic biology. In the exercise, site users choose a sequence from a database of mtDNA sequence submitted by teachers and students. The chosen sequence is then used as a query in a BLAST search to identify similar sequences in the Genome Project's primary repository, *Genbank*. After determining that the chosen sequence is from the control region of mtDNA, students then perform a CLUSTAL analysis to localize this area within the entire mitochondrial genome (16,569 bp).

Students then apply these bioinformatics tools to perform three comparisons that use mitochondrial sequence variations as a "molecular clock" to determine whether Neanderthal hominids were our direct ancestors:

- How similar are the mtDNA sequences of different modern humans?
- How similar are modern human sequences to Neanderthal sequences?
- How similar are modern human sequences to chimpanzee sequences?

The tutorial uses current *Frames* and *JavaScript* technologies to present an inquiry-based exercise that "wraps" difficult to use genome resources within an easy to use contextual guide. The sequence analysis tutorial quickly became the second most popular destination on the DNALC's Internet site, demonstrating the success of this novel approach.

Local Programs Expand Greatly

As shown in the table below, student and teacher participation in DNALC programs topped 27,000 in 1997, an increase of nearly 5,000 over 1996. Most importantly, lab instruction for local students increased by 31%. Virtually all of this growth came from the middle school program, *Genetics as a Model for Whole Learning (GMWL).* The majority of the increase came from in-school instruction, which can be scheduled independent of lab use at the DNALC. Additional lab field trips to the DNALC were made possible by redeveloping the computer laboratory into a genetics laboratory for middle school students. The low lab tables are well scaled for younger children, and the lab was further equipped with a projection microscope. The existence of a dedicated middle school laboratory allowed us to schedule "block" visits during which a single district completes all its lab field trips over several consecutive days. It also freed up the *Bio2000* Laboratory for 30 additional high school classes per year. Despite this significant increase in capacity, demand for labs was so keen that all available lab dates for the 1997–1998 academic year were essentially filled in May. Thus, any further increase in on-site instruction will have to await new laboratory space in the *BioMedia* Addition.

The *GMWL* program expanded to reach 8440 students from 40 elementary and middle schools from 13 school districts in New York City, Nassau County, and Suffolk County (see following table). This was a 72% increase over 1996 participation. Thirty-eight percent of program participants were minority students: 25% Black, 6% Hispanic, 7% Asian/Pacific Islander, and 1% Native American.

GMWL offers each school district a unique program based on its individual needs. The program encompasses three components: teacher training, in-

	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	Total
Student labs (on-site)	2,031	3,753	3,758	4,248	4,624	3,422	3,961	4,682	6,088	7,105	**43,672**
Student labs (off-site)				291	435	1,305	1,434	2,328	5,045	7,665	**18,503**
Teacher labs	58	278	270	234	270	254	302	379	302	392	**2,739**
Student workshops	32	13	24	176	234	351	361	503	437	402	**2,533**
Teacher workshops	496	285	314	333	441	249	171	110	183	245	**2,827**
Lab subtotal	2,617	4,329	4,366	5,282	6,004	5,581	6,235	7,993	12,023	15,809	**70,239**
Student lectures	553	449	660	600	1,000	734	575	520	575	407	**6,073**
Exhibit/LI discovery	3,231	2,547	2,964	1,480	848	6,416	9,943	10,366	10,122	11,150	**59,067**
Total	**6,401**	**7,325**	**7,990**	**7,362**	**7,852**	**12,731**	**16,753**	**18,879**	**22,720**	**27,366**	**135,379**

school instruction, and field trips to the DNALC. Typically, DNALC staff work with district science coordinators to create a sequential program that targets at least two grade levels. Prior to in-school instruction, a teacher training session is held to expose classroom teachers to the various labs their students will perform. A DNALC middle school instructor visits each class several times to perform a core of hands-on labs designed to increase genetic literacy while enhancing critical and creative thinking. This core experience is then supplemented with follow-up activities that classroom teachers design with the assistance of DNALC staff. Most schools include a field trip to the DNALC as the culmination of the *GMWL* unit. This field trip includes an instructional tour of the *Story of a Gene* Exhibit and a hands-on laboratory.

Tricia Harrison instructs a DNA extraction experiment to middle-school students in the former *BioMedia* Computer Lab.

Making Inroads into New York City

Our ongoing studies of instructional innovation have documented that about 175,000 hands-on DNA experiments are performed annually in American high schools. However, this has been largely a suburban movement, effectively excluding the vast majority of students in urban settings. Likewise, younger children in city schools are much less likely to receive the benefits of hands-on learning stipulated by the *National Science Education Standards*. Sadly, this is true of the New York City schools in our own backyard.

Within the last year, however, we have forged important partnerships that will provide opportunities in modern genetics education to large numbers of minority and disadvantaged students in New York City public schools. A collaboration with Community School District 29 in southeast Queens is a model for the large-scale implementation of the *GMWL* program, and a collaboration with the Gateway to Higher Education Program has provided a means to reach large numbers of high school students.

Located immediately to the north of Kennedy Airport, District 29 serves 25,000 students in grades K–8, of whom 95% are minorities, 67% are eligible for lunch assistance, and 31% have limited English proficiency. By working closely with Superintendent Celestine Miller, Director of Funded Programs Ellen Schlesinger, and Supervisor of Science Diane Ehrlich, we have developed a program for *systemic change* that maximizes involvement at all levels in the district. Parents and school board members participated in three workshops, giving them a chance to do some of the same activities done by the students.

Faculty participated in 25 hours of after-school and Saturday workshops designed to increase their command of multidisciplinary lab instruction. Administrators, school board members, and faculty also participated in a recognition dinner at Cold Spring Harbor Laboratory.

In 1997, we doubled the number of District 29 students involved in the *GMWL* program. DNALC staff presented 29 days of in-school instruction, reaching 90 sixth grade classes. Students performed four labs in school: constructing cell models, observing cells under compound microscopes, constructing DNA models, and extracting DNA from bacteria. A fifth lab—observing fruit fly mutations under stereo microscopes—was conducted during field trips to the DNALC. We further expanded the program by piloting a follow-up chemistry program for seventh graders. The chemistry lab sequence includes experiments on molecular modeling, enzyme structure and function, practical enzymology, and bioreactors.

Mort Slater, director of the Gateway to Higher Education Program. Photo by Janis Lewin.

Mort Slater, of Mount Sinai School of Medicine, has been our guide into city high schools. Mort runs the Gateway to Higher Education Program, a 4-year program that prepares minority and low-income students to graduate from high school and to make a smooth transition into university science programs. The program involves 2000 students in seven high schools representing all five boroughs of New York City. In the past year, we have begun to effectively merge the DNALC's content expertise in hands-on lab instruction with Gateway's expertise in school change.

Tangible evidence of this unique instruction/infrastructure partnership was the opening in the spring of a dedicated DNA laboratory in Brooklyn Technical High School. Mort found about $20,000 to purchase research-quality electrophoresis and cell culture equipment, refrigerator/freezer, autoclave, and ice maker. With Mort's deft guidance, the project miraculously sidestepped the notoriously slow New York City bureaucracy and was finished in several months. Support for the project was as surprising as it was heartwarming: Learning-disabled students fabricated lab benches from discarded lunchroom tables; custodians arranged to paint the room in noninstitutional colors; and central administration approved the installation of heavy-duty air conditioners so that the lab can effectively be used for summer courses.

The Brooklyn Tech laboratory found immediate use in the spring semester, serving 306 students in Advanced Placement (AP) biology and genetics classes. During the summer, the laboratory was the site of two workshops sponsored by the Greenwall Foundation, which were attended by 40 teachers from throughout New York City. Two student workshops drew 33 students, of whom 82% were minorities. In the fall, the DNA Laboratory was a key stop in a tour of Gateway schools by Luther Williams, head of the Directorate for Education and Human Resources at the National Science Foundation.

Gateway High School	NYC Borough	Enrollment	Poverty level*	Demographics (%)				
				Asian	Hispanic	White	Black	Other
Stuyvesant	Manhattan	3100	31%	47	3.9	44.8	4.7	–
Jamaica	Queens	2600	35%	17	14	–	60	9
John F. Kennedy	Bronx	5000	73%	5	71	5	17	1
Port Richmond	Staten Island	2000	22%	5	16	58	21	–
Brooklyn Tech	Brooklyn	4500	30%	32	14	16	37	–

*Poverty levels determined from Lunch Eligible Program (LEP) statistics.

Acclaim for the lab was so great that the Board of Education approved $175,000 to equip additional DNA laboratories in each of the five boroughs. Each of the DNA laboratories will also be wired for Internet communication, so these schools will be able to fully participate in both the experimental and on-line aspects of our genomic biology curriculum. When fully operational, with integrated experimental and computation, the DNA laboratories in New York City will have no peer in illustrating the modern synthesis of science and technology. As shown by the demographics for five participating schools, the DNA laboratories will serve populations that are truly representative of urban America (see table above).

Getting Out Word on Marfan Syndrome

As a complement to the *GMWL* program, we took on a key collaboration with the National Marfan Foundation (NMF). Our task was to develop a teacher's guide to complement *How Do Your Genes Fit?*, an award-winning video on Marfan syndrome for middle-school students. To expand the video's usefulness, we used Marfan syndrome as a means to introduce general concepts of cell biology, DNA structure/function, and genetics. The teacher's guide incorporates three laboratories from the *GMWL* curriculum: baggie cell model of a fibroblast, DNA extraction, and Punnett square statistics.

The guide is currently undergoing final editing and review by NMF staff and is scheduled for publication in the near future. The guide will be distributed nationally along with 700 free copies of the video, with additional copies provided at cost. The first major distribution will take place at the annual meeting of the National Science Teachers' Association, to be held in April in Las Vegas.

We believe that this project provides a particularly important outlet for our curriculum expertise. Perhaps more than any other genetic disorder, it is critical to get the word out about Marfan syndrome to young people and their parents. Only within the last several years have physicians and geneticists realized that there are a large number of related disorders caused by defects in the Marfan gene, which produces the structural protein fibrillin. Outward physical effects tend to be subtle, so all of these disorders are difficult to diagnose. Experts believe that tens of thousands of young people have undiagnosed Marfan syndrome or related fibrillin disorders and need to be evaluated by a physician.

All fibrillin disorders weaken connective tissue, which provides strength and resiliency to muscle and bone. The weakening of the wall of the aorta, the major artery carrying blood from the heart, is the only life-threatening risk of undiagnosed Marfan syndrome. Over time, the connective tissue in the aorta weakens and the high pressure of blood pumped out of the heart causes the aorta wall to bubble out and burst—an aneurysm. There have been several publicized cases of apparently healthy athletes who died of aneurysms during exercise, and who later proved to have undiagnosed Marfan syndrome. We feel that our guide can help raise awareness of this "ticking time bomb" and encourage the early diagnosis of the disorder. With early intervention, blood pressure can be regulated to minimize damage to the aorta, and affected children can limit strenuous exercise.

Corporate Advisory Board

The Corporate Advisory Board (CAB) provides a crucial link to local businesses that serve the same population base as the DNALC. Represented on the

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Vice Chairman

Gary E. Frashier, OSI Pharmaceuticals, Inc.

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Raymond A. Walters, Ph.D., CSH Central School District

board are companies of all kinds—from family-owned to multinational and from biotechnology to banking. A major goal of the board is to raise awareness of the DNALC among opinion leaders on Long Island and to involve others in our work.

To support this goal, two hands-on workshops were held at the DNALC for CAB members, family, and friends. All of the 48 participants produced their own DNA fingerprints, illustrating the uses of DNA typing in court cases. To show appreciation for the CAB's achievements, CSHL Director Bruce Stillman hosted a dinner party at Airslie for CAB members and other DNALC donors on May 4. The evening began on the main campus with a briefing by Mike Wigler on breast cancer and continued with lab tours by Jerry Yin and Dick McCombie.

Under the leadership of Jack Leahy, the CAB raised $181,361 in support of the DNALC, a 13% increase over 1996. Key to this huge success was the fouth annual golf tournament, held on June 18 at Piping Rock Club. Under the chairmanship of Horst Saalbach, the tournament drew 156 players and netted $97,714. J.P. Morgan was the major corporate sponsor; other corporate sponsors were Cablevision, Chase Manhattan Bank, Festo Corporation, Luitpold Pharmaceuticals, and Price Waterhouse.

Staff and Interns

Malissa Hewitt took over the management of the rapidly growing middle school programs after the departure of Jane Conigliaro and Diane Jedlicka in March. We are much indebted to Malissa for rising to the task under difficult circumstances and ensuring the continued quality of the *GMWL* program. Her complex position involves liaision with superintendents, principals, and faculty at 13 different school districts, and coordinating in-school and on-site instruction, conducting teacher training, and managing four other staff members. Patricia Harrison joined the middle school staff as a laboratory instructor in January

New staff to the DNALC in 1997 (top left to lower right): John Kruper, Shirley Chan, Patricia Harrison, and Scott Bronson.

and ably assisted Malissa in managing the *GMWL* program. Tricia has a bachelor's degree in elementary education from the University of Scranton and is currently a Masters candidate in special education and reading at Long Island University. In the fall, middle school instructor Andrew Morotti began to divide his time between teaching at the DNALC and working in a half-time position as a lab tech with CSHL Scientist Jerry Yin.

Scott Bronson joined the full-time DNALC staff as laboratory instructor, and in the summer assumed responsibility for managing the teaching and prep labs, as well as the student intern program. Scott brings to the staff expertise in current research methods in molecular biology. He formerly worked for 3 years as a technician in the laboratory of CSHL scientist Jacek Skowronski, where he investigated the role of the *nef* protein in HIV pathogenesis. Scott holds bachelor's degrees in molecular and marine biology, and he is currently pursuing a master's degree in science education.

The laboratory and computational staff was ably assisted by a number of veteran interns: Trevor Carlson (Central Islip High School), Gerry DeGregoris (Chaminade High School), Karin Glaizer (Portledge School), Mera Goldman (Walt Whitman High School), Hana Mizuno (Cold Spring Harbor High School), Rachael Neumann (Syosset High School), Trevor Sammis (Huntington High School), and Jermel Watkins (New York Institute of Technology). Stacey Trotter (Cornell University) and Salley Ann Gibney (Johns Hopkins University) returned during the summer to assist with the student and faculty workshops. In August, we bid farewell to Dan Gibson (Cold Spring Harbor High School) who began his freshman year at Lehigh University. Newcomers Michele Hollander (Jericho High School) and Jennifer Kirschenbaum (Jericho High School) joined the staff in fall.

Amy Cross, a valuable and popular member of our administrative team, left her position as program assistant in August to teach first- through third-grade science at Friends Academy. Her sunny disposition and office support have been greatly missed. Staff Associate Jerry Watkins, of Central Islip High School, once again dedicated several weeks of his summer break to instructing student workshops hosted at the American Museum of Natural History in Manhattan. Joining him as lab assistants were his sons Jermel (a veteran DNALC lab intern) and Justin.

Workshops, Meetings, and Collaborations

January 7	Laboratory for *Women in Science and Engineering Program,* SUNY Stony Brook, DNALC
January 9	Presentation to McClintock Book Club, Hofstra University, Hempstead, New York
January 10	Community School District 29 Meeting, Rosedale, New York
January 14	Site visit by Betty Faber, Liberty Science Center, Jersey City, New Jersey
January 16	*Science for the 21st Century,* Seminar given at Chicago Academy of Science, Illinois
January 30–31	Workshop, Brooklyn Technical High School, Brooklyn, New York
February 6–7	National Science Foundation Grant Review, Washington, D.C.
February 11	Laboratory for *Women in Science and Engineering Program,* SUNY Stony Brook, DNALC
February 16–17	American Association for the Advancement of Science Meeting, Seattle, Washington
February 22	Laboratory for Corporate Advisory Board, DNALC
February 28	Site visit by Alan Thaw, Brooklyn Technical High School, Brooklyn, New York
	Site visit by Andrea Thompson, *Newsday*
March 4–5	Howard Hughes Medical Institute Grant Review, Bethesda, Maryland
March 11	Site visit by Jinx Perullo and John LaPolla, Stuyvesant High School, New York, New York
March 12	Site visit by Bonnie Kaiser, Rockefeller University, New York, New York
March 13	Laboratory for parents of students in Community School District 29, Queens, New York
March 28	Site visit by Monica Volkmann, Miles Gordon, and Nancy Hechinger, American Museum of Natural History, New York, New York
April 4	Site visit by Rob Kelly, Computer Associates, Islandia, New York
April 4–6	National Science Foundation Workshop, *Human Genome Diversity–Student Allele Database,* University of Chicago, Illinois
April 8	*Great Moments in DNA Science* Honors Students Seminar, CSHL
April 11–12	National Science Foundation Follow-up Workshop, University of Alaska, Fairbanks
April 14	*Applications of Recombinant DNA Seminar*, Westpoint, New York
April 15	*Great Moments in DNA Science* Honors Students Seminar, CSHL
April 18–20	National Science Foundation Workshop, *Human Genome Diversity–Student Allele Database,* Foothill College, Los Altos Hills, California
April 23	National Institute of Social Sciences Meeting, Harvard Club, New York, New York
April 29	*Great Moments in DNA Science* Honors Students Seminar, CSHL
May 1	Site visit by Diane Ehrlich and Ellen Schlesinger, Community School District 29, Queens, New York
May 9	Site visit by June Osborn, Macy Foundation, New York, New York, and David Luke, Chairman of CSHL Board of Trustees
May 9–11	National Science Foundation Workshop, *Human Genome Diversity–Student Allele Database,* Washington University, St. Louis, Missouri
May 20	Brinkmann Instruments Meeting, Westbury, New York
May 29	*Creating Futures,* Seminar at C.W. Post/Long Island University, New York
June 16	Site visit by Cablevision, filming Oceanside #8 Middle School
June 23–28	Editorial Advisory Board Workshop, DNALC
June 30–July 3	*World of Enzymes* Workshop, DNALC
July 7–11	*Fun With DNA* Workshop, IS59, Queens, New York
	DNA Science Workshop, DNALC
	DNA Science Minority Teacher Training Workshop, Brooklyn Technical High School, New York
July 7–16	*Advanced DNA Science* Minority Workshop, Central Islip, New York
July 10–14	Center for Image Processing Meeting, Rochester, Minnesota
July 14–18	*Fun With DNA* Minority Teacher Training Workshop, Brooklyn Technical High School, New York
	Introduction to Computer Design Workshop, DNALC
July 14–22	*Advanced DNA Science* Workshop, DNALC
July 19	Site visit by Phil Talbot, Skyline High School, Salt Lake City, Utah
July 21–25	*Fun With DNA* Minority Workshop, IS 59, Queens, New York
	DNA Science Minority Workshop, Brooklyn Technical High School, New York
July 28–August 1	*Fun With DNA* Workshop, DNALC
August 4–8	*DNA Science* Workshop, DNALC
	DNA Science Minority Workshop, Brooklyn Technical High School, Brooklyn, New York
	DNA Science Minority Workshop, American Museum of Natural History, New York, New York
August 11–15	*Fun With DNA* Workshop, DNALC
	DNA Science Minority Workshop, American Museum of Natural History, New York, New York
August 14–22	*Advanced DNA Science* Workshop, Delbruck Laboratory, CSHL

August 18–22	*Fun With DNA*, Portledge School, Locust Valley, New York
	DNA Science Workshop, DNALC
	DNA Science Minority Workshop, American Museum of Natural History, New York, New York
	Introduction to Computer Design, DNALC
August 21	Site visit by Mark Schoofs, *Village Voice*
August 25–29	*Fun With DNA* Workshop, DNALC
	DNA Science Workshop, Delbruck Laboratory, CSHL
September 10–11	The Wellcome Trust Workshop, *Science and Issues of Human DNA Polymorphisms*, University of Edinburgh, Scotland
September 12–13	The Wellcome Trust Workshop, *Science and Issues of Human DNA Polymorphisms*, Newcastle-On-Tyne, Great Britain
September 16	Site visit by Lalita Khosla, Metro News
September 17	*Cloning the DNA Classroom*, Seminar presented at The Wellcome Trust, London, Great Britain
September 19–20	The Wellcome Trust Workshop, *Science and Issues of Human DNA Polymorphisms*, Cambridge, Great Britain
October 9	Site visit by Dr. Mort Slater, Gateway to Highway Education
October 14	*Cold Spring Harbor Laboratory and It's Place in Science*, seminar presented at Banbury Center, CSHL
October 18	Laboratory for Corporate Advisory Board, DNALC
October 24–26	Department of Energy Workshop, *The Science & Issues of Human DNA Polymorphisms*, Mt. Sinai School of Medicine, New York, New York
November 8–10	Department of Energy Workshop, *The Science & Issues of Human DNA Polymorphisms*, CityLab, Boston University School of Medicine, Massachusetts
November 11–13	Department of Energy Meeting, Santa Fe, New Mexico
November 14–16	Department of Energy Workshop, *The Science & Issues of Human DNA Polymorphisms*, Canada College, Redwood City, California
November 21–23	National Science Foundation Advanced Technological Education Meeting, Washington, D.C.
December 5–7	Department of Energy Workshop, *The Science & Issues of Human DNA Polymorphisms*, Morehouse College, Atlanta, Georgia
December 11	National Institutes of Health ELSI Grant Review
December 18	Site visit by Rockefeller University, New York

Sites of Major Faculty Workshops 1985–1997

Key: High School **College** *Middle School*

ALABAMA	University of Alabama, Tuscaloosa	1987–1990
ALASKA	University of Alaska, Fairbanks	1996
ARIZONA	Tuba City High School	1988
ARKANSAS	Henderson State University, Arkadelphia	1992
CALIFORNIA	**Foothill College, Los Altos Hills**	**1997**
	University of California, Davis	1986
	San Francisco State University	**1991**
	University of California, Northridge	**1993**
	Canada College, Redwood City	1997
COLORADO	Colorado College, Colorado Springs	1994
	United States Air Force Academy, Colorado Springs	**1995**
CONNECTICUT	Choate Rosemary Hall, Wallingford	1987
DISTRICT OF COLUMBIA	**Howard University**	**1992, 1996**
FLORIDA	North Miami Beach Senior High School	1991
	University of Western Florida, Pensacola	1991
	Armwood Senior High School, Tampa	1991
GEORGIA	Fernbank Science Center, Atlanta	1989
	Morehouse College, Atlanta	**1991, 1996**
	Morehouse College, Atlanta	1997
HAWAII	Kamehameha Secondary School, Honolulu	1990
ILLINOIS	Argonne National Laboratory	1986, 1987
	University of Chicago	**1992**
	University of Chicago	**1997**

INDIANA	Butler University, Indianapolis	1987
IDAHO	University of Idaho, Moscow	1994
IOWA	Drake University, Des Moines	1987
KANSAS	University of Kansas, Lawrence	1995
KENTUCKY	Murray State University	1988
	University of Kentucky, Lexington	1992
	Western Kentucky University, Bowling Green	1992
LOUISIANA	Jefferson Parish Public Schools, Harvey	1990
	John McDonogh High School, New Orleans	1993
MAINE	Bates College, Lewiston	1995
MARYLAND	Annapolis Senior High School	1989
	Frederick Cancer Research Center, Frederick	1995
	McDonogh School, Baltimore	1988
	Montgomery County Public Schools	1990–1992
	St. John's College, Annapolis	*1991*
MASSACHUSETTS	Beverly High School	1986
	CityLab, Boston University School of Medicine	1997
	Dover-Sherborn High School, Dover	1989
	Randolph High School	1988
	Winsor School, Boston	1987
	Boston University	**1994, 1996**
MICHIGAN	Athens High School, Troy	1989
MISSISSIPPI	Mississippi School for Math & Science, Columbus	1990, 1991
MISSOURI	Washington University, St. Louis	1989
	Washington University, St. Louis	**1997**
NEW HAMPSHIRE	St. Paul's School, Concord	1986, 1987
NEVADA	University of Nevada, Reno	1992
NEW YORK	Albany High School	1987
	Bronx High School of Science	1987
	Columbia University, New York	**1993**
	Cold Spring Harbor High School	1985, 1987
	DeWitt Middle School, Ithaca	*1991, 1993*
	DNA Learning Center	1988–1995
	DNA Learning Center 1990, 1992, 1995	
	DNA Learning Center	*1990–1992*
	Fostertown School, Newburgh	*1991*
	Huntington High School	1986
	Irvington High School	1986
	Junior High School 263, Brooklyn	*1991*
	Lindenhurst Junior High School	*1991*
	Mt. Sinai School of Medicine, New York	1997
	Orchard Park Junior High School	*1991*
	Plainview-Old Bethpage Middle School	*1991*
	State University of New York, Purchase	1989
	State University of New York, Stony Brook	1987–1990
	Titusville Middle School, Poughkeepsie	*1991, 1993*
	Wheatley School, Old Westbury	1985
	US Military Academy, West Point	**1996**
NORTH CAROLINA	North Carolina School of Science, Durham	1987
OHIO	Case Western Reserve University, Cleveland	1990
	Cleveland Clinic	1987
	North Westerville High School	1990
OKLAHOMA	School of Science and Mathematics, Oklahoma City	1994
PENNSYLVANIA	Duquesne University, Pittsburgh	1988
	Germantown Academy	1988
SOUTH CAROLINA	Medical University of South Carolina, Charleston	1988
	University of South Carolina, Columbia	1988
TEXAS	J.J. Pearce High School, Richardson	1990
	Langham Creek High School, Houston	1991
	Taft High School, San Antonio	1991
	Trinity University, San Antonio	**1994**
UTAH	University of Utah, Salt Lake City	1993

VERMONT	University of Vermont, Burlington	1989
VIRGINIA	Eastern Mennonite University, Harrisonburg	1996
	James Madison University, Harrisonburg	1997
	Jefferson School of Science, Alexandria	1987
	Mathematics and Science Center, Richmond	1990
WASHINGTON	**University of Washington, Seattle**	**1993**
WEST VIRGINIA	Bethany College	1989
WISCONSIN	Marquette University, Milwaukee	1986, 1987
	University of Wisconsin, Madison	1988, 1989
WYOMING	University of Wyoming, Laramie	1991
AUSTRALIA	Walter and Eliza Hall Institute and University of Melbourne	1996
CANADA	Red River Community College, Winnipeg, Manitoba	1989
ITALY	International Institute of Genetics and Biophysics, Naples	1996
PANAMA	**University of Panama, Panama City**	**1994**
PUERTO RICO	University of Puerto Rico, Mayaguez	1992
	University of Puerto Rico, Mayaguez	**1992**
	University of Puerto Rico, Rio Piedras	**1993**
	University of Puerto Rico, Rio Piedras	1994
RUSSIA	Shemyakin Institute of Bioorganic Chemistry, Moscow	1991
SWEDEN	Kristineberg Marine Research Station, Fiskebackskil	1995

COLD SPRING HARBOR
LABORATORY PRESS

1997 PUBLICATIONS

Laboratory Manuals

Fly Pushing: The Theory and Practice of Drosophila *Genetics*
Ralph J. Greenspan

Analyzing DNA: A Laboratory Manual
Genome Analysis, Volume 1
Bruce Birren, Eric D. Green, Sue Klapholz, Richard M. Myers, and Jane Roskams (eds.)

Discovering Neurons: The Experimental Basis of Neuroscience
Carol Ann Paul, Barbara Beltz, and Joanne Berger-Sweeney

Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual, 1997 Edition
Alison Adams, Daniel E. Gottschling, Chris A. Kaiser, and Tim Stearns

Cells: A Laboratory Manual
David L. Spector, Robert Goldman, and Leslie Leinwand (eds.)

CSHL Monograph Series

Oxidative Stress and the Molecular Biology of Antioxidant Defenses
John G. Scandalios (ed.)

C. elegans *II*
Donald L. Riddle, Thomas Blumenthal, Barbara J. Meyer, and James R. Priess (eds.)

Cell Cycle and Cell Biology
The Molecular and Cellular Biology of the Yeast Saccharomyces, *Volume 3*
John R. Pringle, James R. Broach, and Elizabeth W. Jones (eds.)

RNA Structure and Function
Robert W. Simons and Marianne Grumberg-Manago (eds.)

General Books

Vaccines 97: Molecular Approaches to the Control of Infectious Diseases
Fred Brown, Dennis Burton, Peter Doherty, John Mekalanos, and Erling Norrby (eds.)

Mutants of Maize
M. Gerald Neuffer, Edward H. Coe, and Susan R. Wessler

Function and Dysfunction in the Nervous System
Cold Spring Harbor Symposia on Quantitative Biology LXI

Correcting the Blueprint of Life: An Historical Account of the Discovery of DNA Repair Mechanisms
Errol C. Friedberg

Toward the 21st Century: Incorporating Genetics into Primary Health Care
Nancy Touchette, Neil A. Holtzman, Jessica G. Davis, and Suzanne Feetham

Retroviruses
John M. Coffin, Stephen H. Hughes, and Harold E. Varmus (eds.)

Murderous Science: Elimination by Scientific Selection of Jews, Gypsies, and Others in Germany, 1933-1945
Benno Müller-Hill

Cancer Surveys Series

Vol. 29: *Checkpoint Controls and Cancer*
M.B. Kastan (ed.)

Vol. 30: *Lymphoma*
A.C. Wotherspoon (ed.)

Journals

Genes & Development (Volume 11, 24 issues)
T. Grodzicker and N. Hastie (eds.)

Genome Research (Volume 7, 12 issues)
L. Goodman, M. Boguski, A. Chakravarti, R. Gibbs, E. Green, and R. Myers (eds.)

Learning & Memory (Volume 4, 6 issues)
J.H. Byrne (ed.)

Slides

The Art of Retroviruses
A Companion Slide Set from *Retroviruses*
J.M. Coffin, S.H. Hughes, and H.E. Varmus (eds.)

Other

CSHL Annual Report 1996

Banbury Center Annual Report 1996

Administration and Financial Annual Report 1996

COLD SPRING HARBOR LABORATORY PRESS

For Cold Spring Harbor Laboratory Press, 1997 was a year of extensive change. Significant improvement was made in organizational efficiency, technical competence, and financial performance. A two-stage administrative restructuring reduced staff costs and produced a more coordinated, responsive organization. Production methods were radically overhauled to permit greater flexibility in design and illustration, and output in varying formats. Books were printed with faster schedules, more competitive pricing, and higher quality. Eighteen new books were published, twice the 1996 total, and several were exceptional, even by the customary high standards of our program. Journal subscriptions and advertising pages increased and two titles were published on-line for the first time. Revenues increased by 9% to $5.24 million and produced a satisfactory operating excess.

Book Program

The book program maintained its strategic focus on techniques manuals and monographs, with more modest objectives in the history of science. The complete list of 1997 titles is printed opposite, but space permits specific mention of only some of the 18 books published.

Among the laboratory manuals published this year were *Genome Analysis: Analyzing DNA*, the first of a series on genomics edited by Eric D. Green, Bruce Birren, Sue Klapholz, Richard M. Myers, and Philip Hieter, the updated *Methods in Yeast Genetics* by Alison Adams, Dan Gottschling, Chris Kaiser, and Tim Stearns, and most notably *Cells*, edited by David Spector, Robert Goldman, and Leslie Leinwand. This impressive three-volume manual is beautifully illustrated with outstanding color photomicrographs. It was a complex project involving more than 130 contributions, and determined administration and vigorous editing were needed to achieve the necessary completeness and coherence. Since its release at the American Society for Cell Biology meeting, the book has been greeted with sustained enthusiasm. It may well rival our classic publications *Molecular Cloning* and *Antibodies*, collections of techniques that, even a decade after publication, are regarded as essential to their fields.

Two manuals were published with clearly educational goals. Ralph Greenspan's *Fly Pushing* responded to the long-felt need for a simple introduction to the theory and practice of *Drosophila* genetics. *Discovering Neurons*, by Carol Ann Paul, Barbara Beltz, and Joanne Berger-Sweeney, is a unique collection of techniques for college teachers introducing juniors and seniors to practical neurobiology.

A very warm response has greeted *Retroviruses*, the new monograph edited by John Coffin, Stephen Hughes, and Harold Varmus. A generous review in *Nature* hailed it as "the new testament" for investigators in the field. A descendant of two celebrated books on tumor viruses published by the Laboratory in the 1970s, this handsome volume has the look, feel, and intention of a textbook, with fine illustrations that are also available in slide form. As elders of the retrovirus research community, the editors proved able to coax particularly thoughtful contributions from distinguished investigators in this field and then boldly reshaped the material to fit their ambitious vision of the book. Their aim was not only to assist professional virologists, but also to convince physicians treating AIDS and its consequences that the biology of HIV needs to be understood in the context of its retroviral cousins. They have created an important and lasting contribution to the literature.

Additions to the Cold Spring Harbor Monograph series included C. elegans *II*, edited by Don Riddle, Thomas Blumenthal, Barbara Meyer, and James Priess, the core reference work for the expanding community of scientists studying this nematode. Our trilogy on the biology of the

yeast *Saccharomyces* was completed with the long-awaited appearance of the volume *Cell Cycle* and *Cell Biology*, edited by John Pringle, James Broach, and Elizabeth Jones.

Our growing history list was graced by an expanded edition of *Murderous Science,* Benno Müller-Hill's compelling account of eugenics in Nazi Germany, and *Correcting the Blueprint of Life,* Errol Friedberg's widely praised history of the study of DNA repair.

We were also delighted to publish after several years' preparation the lavishly illustrated reference work *Mutants of Maize* by Gerald Neuffer, Edward Coe, and Susan Wessler. Generous grant support for the production of this book was gratefully received from corn seed companies, especially Pioneer Hi-Bred. The collective effort of authors, developmental editors, and supporters was rewarded when the book was honored by the American Association of Publishers as one of the Books of the Year in biology.

Journal Publishing

All three journals, *Genes & Development*, *Genome Research*, and *Learning & Memory,* were offered more and better manuscripts for publication, and the number of pages published by each rose substantially. Content diversified with the addition of short papers and regular reviews to *Genes & Development* and a commentary section *Inside/Outlook to Genome Research*. Editorial administration was strengthened with the appointment of Laurie Goodman as Editor of *Genome Research* and Managing Editor of *Learning & Memory*, and Kim Gavin as Assistant Editor of *Genes & Development*.

In September, Nicholas Hastie, European Editor of *Genes & Development* since 1990, was succeeded by the distinguished developmental biologist Davor Solter, from the Max-Planck Institute of Immunobiology in Freiburg, Germany. Nick deserves our thanks and gratitude for his dedicated service to the journal through a period of mounting responsibilities as Director of the MRC Human Genetics Unit in Edinburgh.

The measured influence of our journals continued to grow in 1997, an important matter as libraries worldwide strive to rationalize the use of shrinking resources for print publications. The impact factor of *Genes & Development*, calculated by the Institute for Scientific Information, increased to 18.81 in 1997, maintaining its ranking in the top ten of all primary research journals. Editor Terri Grodzicker, her colleagues in the European editorial office, and the board of advisors continue to place an insistent demand on quality, even as the journal has broadened into new areas of molecular and cell biology.

With the March 1 issue, the journal celebrated its tenth anniversary. These days, many journal launches occur by binary fission, as newcomers bud from established entities. Ten years ago, *Genes & Development* was a bold initiative from a publisher new to journals, in partnership with a society rooted in population genetics, not molecular biology. All those who shared the vision of what was possible a decade ago, and have contributed to the journal's current success, deserve a feeling of quiet satisfaction with the outcome.

Genome Research acquired an impact factor for the first time, based solely on citations to the first four issues of the journal in 1995. The editorial team of Laurie Goodman and scientists Mark Boguski, Aravinda Chakravarti, Richard Gibbs, Eric Green, and Richard Myers is intent on raising the quality of the journal and expanding its scope beyond mapping and sequencing into biology.

Learning & Memory, with the widely respected neurobiologist Jack Byrne now well established as editor, continued to advance in all respects and was accepted by a number of important bibliographic resources, including the prominent *Current Contents Life Sciences* database.

In September, the full text of issues of *Genes & Development* and *Genome Research* was published on the World Wide Web, in collaboration with the HighWire Press, a division of Stanford University Library. Access was free to all until the end of 1997, when it became part of the 1998 subscription package.

The value of these on-line editions to users is high. A keyword-based search permits recovery

of all articles on a given subject from the journal database. In addition, links can be made from papers in our journals to other articles, either via Medline abstracts or to the full text if they are available on-line. However, among the many misunderstood issues in the exciting new world of on-line publishing is the cost of its development. The economics of this publishing model are certainly different from those of print publication, but when done properly, on-line publishing is not inexpensive, and, like many publishers, we found it necessary to pass on some of the additional cost to subscribers.

Meanwhile, the print circulation of both *Genes & Development* and *Genome Research* rose by a gratifying 6% in 1997, notable in an era of generally flat or falling journal sales. The subscription base of *Learning & Memory* changed more modestly but will be boosted in the future by a regular production schedule made possible by increased manuscript submission. Successful special issues were published on mushroom bodies and gene knockouts. With the help of Laboratory Trustee William Murray, a generous grant from the Oliver S. and Jennie R. Donaldson Charitable Trust was obtained to support the growth of this journal and will likely be used to fund the journal's on-line publication in 1998.

Advertising sales for the two larger journals increased by a remarkable 48% over the previous year, in response to more vigorous, focused, and creative sales activities.

Marketing and Distribution

Marketing activities in 1997 relied on the trusted strategies of direct mail, meeting exhibits, and journal advertising but also for the first time incorporated some on-line advertising. The direct mail program centered on a widely distributed catalog entitled *Genes, Cells, and Proteins,* a fall *New Book Titles* newsletter, and a variety of title-specific brochures. These were attractively redesigned and produced on the Mac/Quark page composition platform, with consequent benefits in speed of production, cost savings, and flexibility.

The Press had exhibits at the seven major scientific society meetings this year and at several smaller, more topic-focused conferences. These appearances introduce the Press to young scientists and to potential authors and others with ideas to share. The thriving Cold Spring Harbor meetings and courses offer similar promotional opportunities to display our books and journals in the campus bookstore. Many positive reviews in major journals, a consistently updated Press website, and electronic advertisements on other websites also helped increase the visibility of our publications.

Our own website—at 3 years old, now ancient in web terms—has a growing role in our marketing activities. The on-line catalog is definitive since our complete print catalog was discontinued, and it is linked with detailed announcements of new books and journal issues, sample chapters from forthcoming titles, and the opportunity to purchase books electronically. On-line journal access has increased traffic at the site and book sales have steadily increased. The next step is a major revision of the site to improve its design and ease of use and to introduce new technologies to fulfill its potential as a dynamic sales tool.

Web-based information and the opportunity to communicate by E-mail is particularly appreciated by customers outside the United States, who represent a high percentage of on-line purchasers and inquirers. However, we maintain valuable relationships with agents who represent us in several international markets, and the Frankfurt Book Fair this year was an important opportunity to confer with them and scout for candidates to open up new regions.

In the Fulfillment Department, events were dominated by the decision to decommission our 10-year old minicomputer-based sales management system and convert to a new client-server platform running Windows NT software and a new proprietary package for order fulfillment. Installation of the new system, begun in October for first quarter 1998 completion, will offer unprecedented operational power and flexibility together with major improvements in the analysis of sales, costs, and customers.

New Projects

During the year, 11 major new publishing projects were placed under contract, and at year's end, more than 20 others were in discussion. In the second half of the year, responsibility for acquisition of new projects was diversified in order to strengthen this vital component of the publishing process. The new acquisition team brought in Judy Cuddihy and Kaaren Janssen, both experienced editors widely acquainted with science and scientists. Their ideas, contacts, and energy will be invaluable in building our program to a new level of productivity.

Staff Changes

The year's reorganization of the publishing staff resulted in the formation of a Production Department charged with print and electronic production of books and journals, and a Book Editorial Development Department responsible for the timely extraction and appropriate preparation of publishable manuscripts from authors. New scheduling, transition, and reporting procedures were instituted to coordinate the work of these two vital units and ensure that accurate, up-to-date information on progress reaches all other parts of the publishing enterprise.

The staff members of the Press as of December 1997 are listed elsewhere in this volume. Our thanks are extended to each one of them for their commitment, dedication, and participation in this challenging year of change. It is a pleasure to note that those directly responsible for the creation of our publications are now appropriately acknowledged on the mastheads of journals and the title verso pages of books.

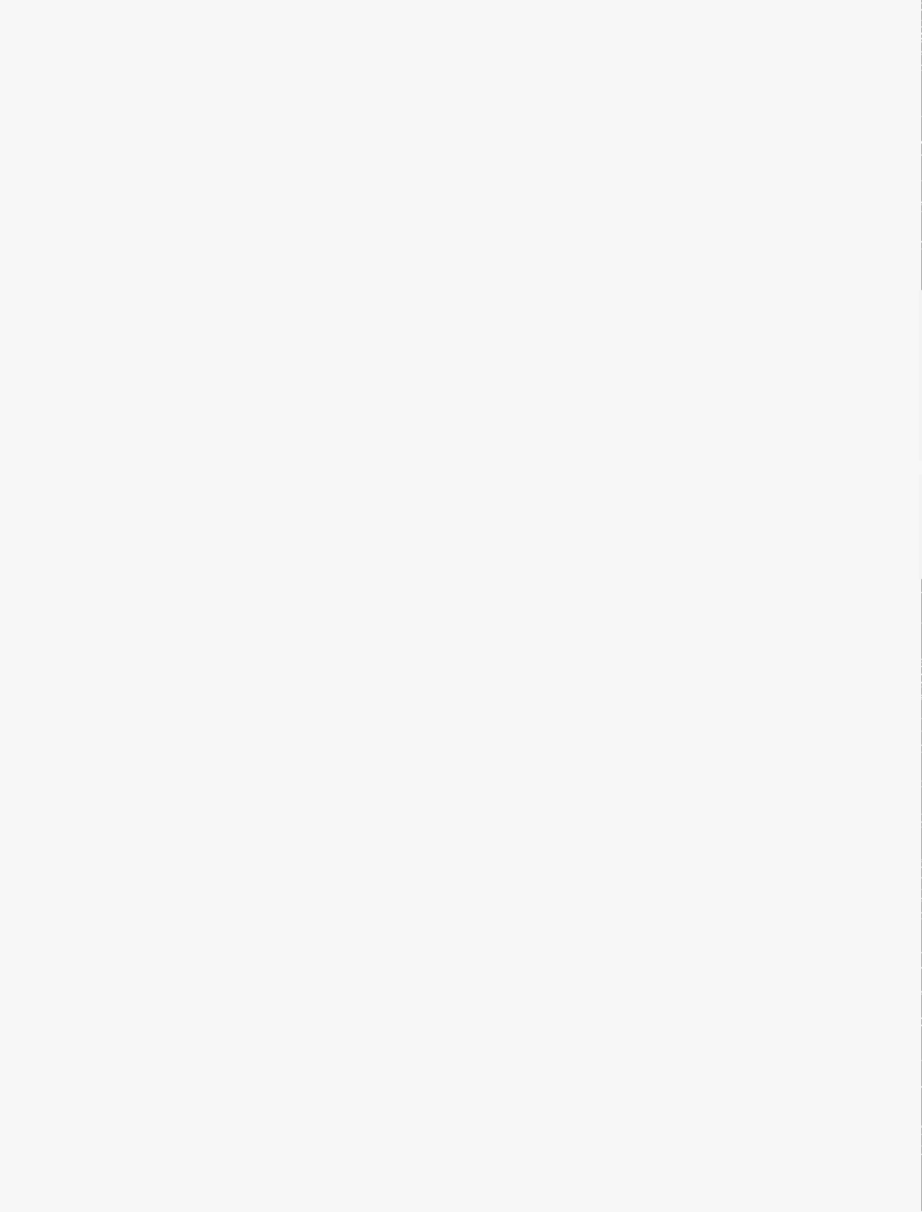
Special mention must be made of the members of the new management team: Denise Weiss, whose appointment this year as Production Manager for books and journals was the catalyst for much technological innovation and staff education; Jan Argentine, who as Editorial Development Manager maintained the coordination of book publishing; Nancy Hodson, our Business Manager who carried forward our exhausting computer conversion; Ingrid Benirschke, Marketing Manager, and Guy Keyes, Fulfillment Manager, whose dedication to their responsibilities was essential to the implementation of change; Laurie Goodman, a talented editor with energy and flair that will serve the entire publishing program well; and Marcie Ebenstein, our newly appointed Journal Advertising Manager, whose skills are tapping unexploited revenue potential from this source.

We welcomed several other new members of staff: Chris Bianco, Production Editor; Cynthia Weidman, Marketing Assistant; Peggy Calicchia, Editorial Secretary; and Joanne Thomas, Fulfillment Assistant. We bade farewell to Nancy Ford, Managing Editor of the Press, whose retirement was noted in last year's Annual Report, at a January luncheon honoring her 24 years' service to the Laboratory and her key role in establishing the publishing program. Another crowded luncheon, in August, marked Barbara Terry's retirement after 15 years of devoted service to our book and journal purchasers in the Fulfillment Department. Doris Lawrence, Editorial Secretary, also left us, for a college career. And we celebrated with Judy Cuddihy her transition from the position of Managing Editor, in which she had played a pivotal role in journal publishing since 1987, to a new life high in the mountains of New Mexico where, thanks to the marvels of telecommuting, she continues to be invaluable in the development of several important forthcoming books and the acquisition of future projects.

This is an extraordinary time in the communications industry. Corporate consolidation has reduced the number of publishers in science to a handful of powerful commercial companies and a small but influential group of learned societies. Cold Spring Harbor Laboratory Press is unusual in this environment—tiny in financial terms compared with our commercial colleagues, lacking the societies' security of member dollars, but respected for the distinction of our book list and the excellence of our journals. Our mission is to contribute to the Laboratory's economy, not drain from it, a distinction that became clear in interactions with other participants in the HighWire Press on-line publishing initiative.

The rush to on-line publication has been swift but the consequences are still uncertain. So far, only a minority of journal subscribers are making use of the electronic subscription, but this may reflect technical unreadiness among libraries and subscription agents rather than readers' lack of interest. It seems highly likely that, soon, all journals will have to be on-line, to be noticed at all. And what about books? Is there still a role for the first-class technical monograph in science, or is it only really useful as a database with links to other on-line resources? Like most publishers, we are watching this evolving marketplace with enormous fascination and experimenting to see what is technically and financially feasible. But as means of distribution and access change, one constant remains: the value of quality. In this respect, the goal of publishing at Cold Spring Harbor is what it has always been: to provide the best, in a medium of choice. In 1997, much was done to secure that position.

John R. Inglis



FINANCE

FINANCIAL STATEMENTS

CONSOLIDATED STATEMENT OF FINANCIAL POSITION
December 31, 1997
With comparative amounts as of December 31, 1996

	1997	1996
Assets:		
Cash and cash equivalents	$ 16,542,395	12,959,798
Investments	140,573,553	123,682,720
Accounts receivable:		
Publications	829,653	489,932
Other	158,235	191,162
Grants receivable	3,038,893	2,251,645
Contributions receivable	1,707,605	3,518,165
Publications inventory	1,991,707	1,426,118
Prepaid expenses and other assets	1,377,264	1,308,461
Investment in employee residences	2,269,345	2,370,943
Land, buildings, and equipment:		
Land and land improvements	9,980,071	9,037,008
Buildings	66,519,640	60,467,671
Furniture, fixtures, and equipment	4,950,130	4,310,386
Laboratory equipment	12,655,257	11,721,056
Library books and periodicals	365,630	365,630
Construction in progress	626,728	1,103,850
	95,097,456	87,005,601
Less accumulated depreciation and amortization	(30,500,090)	(27,354,072)
Land, buildings, and equipment, net	64,597,366	59,651,529
Total assets	$ 233,086,016	207,850,473
Liabilities and Net Assets:		
Liabilities:		
Accounts payable and accrued expenses	$ 2,599,761	1,470,007
Notes payable	645,839	864,961
Bonds payable	30,000,000	30,000,000
Deferred revenue	5,554,037	5,715,510
Total liabilities	38,799,637	38,050,478
Net assets:		
Unrestricted		
General operating	5,760,936	6,030,181
Designated by Board for:		
Research programs	1,150,000	1,150,000
Capital expenditures	3,709,145	5,753,947
Endowment	70,713,010	63,334,506
Net investment in plant	33,951,527	28,786,568
Total unrestricted	115,284,618	105,055,202
Temporarily restricted	4,723,413	1,269,123
Permanently restricted	74,278,348	63,475,670
Total net assets	194,286,379	169,799,995
Total liabilities and net assets	$ 233,086,016	207,850,473

CONSOLIDATED STATEMENT OF ACTIVITIES
Year Ended December 31, 1997
With comparative totals for the year ended December 31, 1996

	Unrestricted	*Temporarily Restricted*	*Permanently Restricted*	*1997 Total*	*1996 Total*
Revenue, gains, and other support:					
Public support (contributions and nongovernment grant awards)	$ 9,012,034	4,663,412	335,498	14,010,944	14,355,769
Government grant awards	15,392,415	–	–	15,392,415	13,756,042
Indirect cost allowances	10,000,564	–	–	10,000,564	9,817,300
Other revenue:					
Program fees	2,117,904	–	–	2,117,904	1,948,793
Rental income	389,658	–	–	389,658	443,934
Publications sales	5,237,814	–	–	5,237,814	4,804,744
Dining services	2,172,196	–	–	2,172,196	2,080,803
Rooms and apartments	1,712,294	–	–	1,712,294	1,639,324
Royalty and licensing fees	679,082	–	–	679,082	464,652
Net appreciation in fair value of investments	6,716,633	–	10,467,180	17,183,813	9,146,939
Investment income (interest and dividends)	5,677,213	–	–	5,677,213	6,258,338
Miscellaneous	167,324	–	–	167,324	109,733
Total other revenue	24,870,118	–	10,467,180	35,337,298	26,897,260
Net assets released from restrictions:					
Expiration of time restrictions	1,209,122	(1,209,122)	–	–	–
Total revenue, gains, and other support	60,484,253	3,454,290	10,802,678	74,741,221	64,826,371
Expenses:					
Research	27,715,449	–	–	27,715,449	25,258,285
Summer programs (meetings and courses)	6,848,437	–	–	6,848,437	7,022,511
Publications	5,400,006	–	–	5,400,006	5,104,457
Banbury Center conferences	953,562	–	–	953,562	795,244
DNA Learning Center programs	627,763	–	–	627,763	604,605
General and administrative	6,091,915	–	–	6,091,915	5,694,590
Dining services	2,617,705	–	–	2,617,705	2,487,117
Total expenses	50,254,837	–	–	50,254,837	46,966,809
Increase in net assets before cumulative effect of change in accounting principle	10,229,416	3,454,290	10,802,678	24,486,384	17,859,562
Cumulative effect of change in accounting for investments	–	–	–	–	12,671,355
Increase in net assets	10,229,416	3,454,290	10,802,678	24,486,384	30,530,917
Net assets at beginning of year	105,055,202	1,269,123	63,475,670	169,799,995	139,269,078
Net assets at end of year	$ 115,284,618	4,723,413	74,278,348	194,286,379	169,799,995

CONSOLIDATED STATEMENT OF CASH FLOWS
Year ended December 31, 1997
With comparative amounts for the year ended December 31, 1996

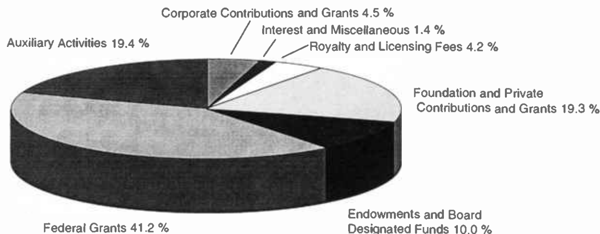
	1997	1996
Cash flows from operating activities:		
Increase in net assets	$ 24,486,384	30,530,917
Adjustments to reconcile increase in net assets to net cash provided by operating activities:		
Cumulative effect of change in accounting principle	–	(12,671,355)
Depreciation and amortization	3,370,584	2,988,112
Net appreciation in fair value of investments	(17,183,813)	(9,146,939)
Contributions restricted for long-term investment	(4,998,910)	(4,716,321)
Changes in assets and liabilities		
(Increase) decrease in accounts receivables	(306,794)	278,633
(Increase) decrease in grants receivable	(787,248)	777,102
Decrease (increase) in contributions receivable	1,810,560	(3,018,165)
Increase in publications inventory	(565,589)	(31,398)
(Increase) decrease in prepaid expenses and other assets	(68,803)	769,773
Increase in accounts payable and accrued expenses	1,129,754	520,928
(Decrease) increase in deferred revenue	(161,473)	363,841
Net cash provided by operating activities	6,724,652	6,645,128
Cash flows from investing activities:		
Capital expenditures	(8,316,421)	(4,628,242)
Proceeds from sales and maturities of investments	100,940,803	162,766,609
Purchases of investments	(100,647,823)	(169,651,705)
Net change in investments in employee residences	101,598	(545,302)
Net cash used in investing activities	(7,921,843)	(12,058,640)
Cash flows from financing activities:		
Permanently restricted contributions	335,498	3,537,198
Contributions restricted for investment in land, buildings, and equipment	4,663,412	1,179,123
Repayment of notes payable	(570,122)	(352,559)
Issuance of notes payable	351,000	550,000
Net cash provided by financing activities	4,779,788	4,913,762
Net increase (decrease) in cash and cash equivalents	3,582,597	(499,750)
Cash and cash equivalents at beginning of year	12,959,798	13,459,548
Cash and cash equivalents at end of year	$ 16,542,395	12,959,798
Supplemental disclosures:		
Interest paid	$ 1,239,341	1,277,376

COMPARATIVE OPERATING HISTORY 1993–1997
(Dollars in Thousands)

	1993	1994	1995	1996	1997
Income:					
Main Lab:					
Grants and contracts	$ 18,136	19,293	19,653	20,879	22,743
Indirect cost reimbursement	8,383	8,460	8,881	9,704	9,910
Other	6,049	6,808	7,461	7,859	8,472
CSHL Press	4,319	4,390	5,119	4,805	5,238
Banbury Center	1,281	1,569	1,732	1,214	1,495
DNA Learning Center	796	824	954	754	875
Total income	38,964	41,344	43,800	45,215	48,733
Expenses:					
Main Lab:					
Research and training	18,136	19,293	19,653	20,879	22,743
Operation and maintenance of plant	4,777	5,141	5,266	5,446	5,274
General and administrative	2,785	2,909	3,329	3,438	3,625
Other	4,385	4,847	4,959	5,367	5,759
CSHL Press	4,134	4,309	5,079	5,032	5,320
Banbury Center	1,226	1,498	1,643	1,225	1,437
DNA Learning Center	768	798	958	781	887
Total expenses, excluding depreciation	36,211	38,795	40,887	42,168	45,045
Excess before depreciation and release of designated funds	2,753	2,549	2,913	3,047	3,688
Depreciation	(2,522)	(2,668)	(2,821)	(2,988)	(3,371)
Release of funds designated (1)	–	200	–	–	–
Net operating excess	$ 231	81	92	59	317

The above amounts are presented on a combined basis for all funds for which Cold Spring Harbor Laboratory prepares operating budgets.
(1) Funds designated to underwrite future direct and indirect expenses of the neuroscience and other research programs.

COLD SPRING HARBOR LABORATORY
SOURCES OF REVENUE
YEAR ENDED DECEMBER 31, 1997



FINANCIAL SUPPORT OF THE LABORATORY

Cold Spring Harbor Laboratory, Banbury Center, and the DNA Learning Center receive a substantial portion of their funding through grants from the Federal Government and through grants, capital gifts, and annual contributions from private foundations, corporations, and individuals. The following section summarizes funding that occurred during 1997.

GRANTS January 1, 1997–December 31, 1997

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1997 Funding*⁺
FEDERAL GRANTS			
NATIONAL INSTITUTES OF HEALTH			
Program Projects	Dr. Herr	1/92–12/01	$ 3,941,478
	Dr. Marr	6/95–5/98	958,072
	Dr. Stillman	8/90–7/00	2,701,460
	Dr. Tully	5/96–4/99	698,729
Research Support	Dr. Arndt/Dr. Tonks	1/95–12/98	135,200
	Dr. Beach	5/94–2/99	315,935
	Dr. Beach	8/95–5/00	264,618
	Dr. Cline	12/95–11/98	281,273
	Dr. Enikolopov	9/94–8/98	220,491
	Dr. Futcher	4/93–3/01	266,020
	Dr. Futcher	1/91–12/99	168,445
	Dr. Greider	8/91–7/01	285,689
	Dr. Greider	12/94–11/97	96,899
	Dr. Helfman	4/94–3/98	247,050
	Dr. Helfman	8/93–5/98	309,655
	Dr. Hengartner	5/95–4/00	233,236
	Dr. Hernandez	7/87–6/00	147,108
	Dr. Herr	8/96–7/00	206,305
	Dr. Hirano	5/96–4/00	206,660
	Dr. Joshua-Tor	8/96–7/99	225,062
	Dr. Krainer	7/94–6/98	349,086
	Dr. Malinow	5/95–4/00	265,474
	Dr. Malinow	4/95–2/98	266,785
	Dr. Nedivi	7/97–6/02	108,241 *
	Dr. Skowronski	12/93–11/97	296,587
	Dr. Spector	4/95–3/99	327,844
	Dr. Silva	7/95–6/00	260,301
	Dr. Stillman	7/91–5/00	502,212
	Dr. Tonks	8/91–3/01	476,957
	Dr. Tonks	5/97–4/01	277,933 *
	Dr. Tully	4/94–3/02	382,106
	Dr. Tully	8/96–6/00	277,309
	Dr. Tully	5/97–4/99	84,500 *
	Dr. Van Aelst	12/97–11/01	348,875 *
	Dr. Wigler	7/95–4/99	1,937,881
	Dr. Xu	12/97–11/02	325,184 *
	Dr. Yin	9/96–8/99	264,784
	Dr. Zhang	9/97–8/00	372,815 *
	Dr. Zhong	2/96–1/00	240,754

*New Grants Awarded in 1997
⁺Includes Direct and Indirect Cost

Grantor	Program/Principal Investigator	Duration of Grant	1997 Funding+
Fellowships	Dr. Dickinson	11/97–6/00	14,806 *
	Dr. Dubnau	7/96–6/99	24,420
	Dr.Huang	8/97–7/02	69,762 *
	Dr. Kogan	1/97–12/98	23,700 *
	Dr. Le	2/96–1/99	16,143
	Dr. Yang	9/97–8/98	25,420 *
Training Support	Training in Cancer Cell Biology and Tumor Virology	7/94–2/99	171,686
Course Support	Advanced Bacterial Genetics	5/93–4/98	60,852
	Cancer Research Center Workshops	4/92–3/98	260,570
	Neurobiology Short-term Training	5/82–4/01	143,585
	CSHL Analysis of Large DNA Molecules	4/91–3/01	52,727
	Computational Genomics for Molecular Biologists	1991–1998	40,478
	Advanced In Situ Hybridization and Immunocytochemistry	1992–1998	62,845
	Automated Genome Sequencing	4/95–3/98	73,962
	Molecular Biology and Development of *Xenopus laevis*	4/96–3/99	10,000
Meeting Support	Genome Mapping and Sequencing	4/90–3/99	32,354
	Tyrosine Phosphorylation and Cell Signaling	4/97–3/98	5,000 *
	Cell and Molecular Biology of *Aplysia*	4/97–3/98	10,000 *
	Regulation of Liver Gene Expression in Health and Disease	5/97–4/98	14,000 *
	62nd Symposium: Pattern Formation during Development	6/97–5/98	8,000 *
	Eukaryotic DNA Replication	7/97–6/98	5,000 *
	Programmed Cell Death	7/97–6/98	19,273 *
	Microbial Pathogenesis and Host Defense	8/97–7/98	5,000 *
	Mechanisms of Eukaryotic Transcription	7/97–7/98	7,000 *
	Neurobiology of *Drosophila*	7/97–6/98	15,000 *
	Eukaryotic mRNA Processing	8/97–8/98	6,000 *
NATIONAL SCIENCE FOUNDATION			
Cooperative Agreement	Drs. Martienssen/McCombie	9/96–8/99	1,430,000
Research Support	Dr. Cline	9/96–8/99	100,000
	Dr. Grossniklaus	8/97–7/00	120,000 *
	Dr. Grotewold	2/97–4/98	5,000 *
	Dr. Grotewold	9/97–9/00	90,000 *
	Drs. Martienssen/McCombie	8/97–7/98	228,000 *
	Dr. Peunova	9/95–8/98	101,125
Training Support	Undergraduate Research Program	6/91–5/98	50,000
Course Support	*Arabidopsis* Molecular Genetics	6/94–5/00	60,000
	Early Development of *Xenopus Laevis*	9/96–8/98	14,682
Meeting Support	Tyrosine Phosphorylation and Cell Signaling	3/97–2/98	6,000 *
	Cell and Molecular Biology of *Aplysia* and Related Invertebrates	3/97–2/98	11,017 *
	Mechanism of Eukaryotic Transcription	7/97–6.98	3,000 *
	Eukaryotic DNA Replication	7/97–6/98	3,000 *

*New Grants Awarded in 1997
+Includes Direct and Indirect Cost

Grantor	Program/Principal Investigator	Duration of Grant	1997 Funding+
	Eukaryotic mRNA Processing	8/97–7/98	3,000 *
	Neurobiology of *Drosophila*	8/97–7/98	13,795 *
Equipment	Multiple Column Peptide Synthesizer for Combinatorial Chemistry	9/97–8/98	85,000 *
	A Rotating Anode X-ray Generator for Biological Macro-Molecular Crystallography	3/97–2/98	84,140 *
DEPARTMENT OF ENERGY			
Research Support	Dr. Martienssen	8/91–2/98	96,000
Course Support	Macromolecular Crystallography	9/93–12/97	15,000 *
UNITED STATES DEPARTMENT OF AGRICULTURE			
Research Support	Drs. Ma/McCombie	9/96–8/98	48,574
	Drs. McCombie/Martienssen	6/95–6/98	78,654
	Drs. McCombie/Martienssen	9/97–8/00	123,186 *
	Dr. Martienssen	9/97–8/00	67,888 *
UNITED STATES DEPARTMENT OF ARMY			
Research Support	Dr. Futcher	6/94–6/99	218,997
	Dr. Futcher	7/97–6/00	97,180 *
	Dr. Hannon	9/96–8/00	150,000
	Dr. Hengartner	7/97–6/00	95,490 *
	Dr. Wigler	7/94–8/99	200,000
Fellowship Support	Dr. Liu	12/96–11/99	41,783
	J. Polyakova	7/97 6/00	20,000 *
	Dr. Schneider	8/97–7/00	41,000 *
	Dr. Sherlock	7/97–6/00	41,000 *
Meeting Support	Microbial Pathogenesis and Host Defense	9/97–8/98	4,500 *
NONFEDERAL GRANTS			
Research Support			
American Cancer Society	Dr. Wigler, Professorship	1986–2012	50,000
	Dr. Wigler, Supply Allowance	1986–1998	10,000
Amplicon Corporation	Dr. Wigler	6/94–10/97	192,736
Arnold and Mabel Beckman Foundation	Dr. Joshua-Tor	7/96–6/98	100,000
Calbiochem-Novabiochem Foundation	Dr. Krainer	2/96–1/98	40,000
Council for Tobacco Research	Dr. Futcher	1/97–12/99	65,000 *
	Dr. Skowronski	7/97–6/00	75,000 *
	Dr. Tonks	1/95–12/97	93,000
Charles A. Dana Foundation	Dr. Marr	1/96–6/97	36,000
Oliver S. and Jennie R. Donaldson Charitable Trust	Dr. Hengartner	7/96–6/98	62,500

* New Grants Awarded in 1997
+Includes Direct and Indirect Cost

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1997 Funding+*
Irving A. Hansen Memorial Foundation	Dr. Tonks	8/95–7/98	10,000
Lita Annenberg Hazen Foundation	Dr. Svoboda	12/96–11/97	200,000
The John A. Hartford Foundation	Dr. Tully	1/97–12/99	269,082 *
Helicon Therapeutics	Drs. Tully/Yin	9/97–8/00	975,000 *
Helen Hoffritz Foundation	Dr. Cline	12/96–11/98	25,000
ISIS Pharmaceuticals	Dr. Spector	6/93–6/97	15,000 *
Esther A. and Joseph Klingenstein Fund, Inc.	Dr. Enikolopov	7/94–6/98	40,000
Charles Henry Leach II Foundation	Dr. Enikolopov	1/97–12/97	25,000 *
1 in 9: L.I. Breast Cancer Action Coalition	Dr. Wigler	1997	146,565 *
Long Beach Breast Cancer Coalition	Dr. Wigler	1997	1,000 *
G. Harold and Leila Mathers Charitable Foundation	Dr. Malinow	1/95–12/97	278,975
The McKnight Endowment Fund for Neuroscience	Dr. Silva	7/95–6/98	50,000
	Dr. Yin	7/96–6/99	50,000
Mellam Family Foundation	Dr. Tonks	12/96–11/00	50,000
Merck Genome Research Institute	Dr. Zhang	11/97–10/99	100,000 *
NIH/Sloan Kettering Subcontract	Dr. Wigler	9/95–8/98	330,498
	Drs. Tonks/Van Aelst	8/97–5/01	434,681 *
NIH/Nanoprobes, Inc Subcontracts	Dr. Spector	9/96–8/98	31,508
	Dr. Spector	9/96–8/98	31,444
	Dr. Spector	9/97–2/98	12,727 *
NIH/University of Pennsylvania Subcontract	Dr. Yin	10/97–9/01	102,760 *
N.A.T.O.	Dr. Nestler	1/96–12/98	7,049 *
Otsuka American Pharmaceutical, Inc.	Dr. Zhang	6/97–6/98	30,000 *
Perkin Fund	Dr. Silva	6/97–5/99	25,000 *
Pew Charitable Trust	Dr. Hannon	7/97–6/01	50,000 *
	Dr. Hirano	7/96–6/00	50,000
	Dr. Lazebnik	7/95–6/99	50,000
	Dr. Zhong	7/94–6/98	50,000
Pioneer Hi-Bred International	Dr. Grossniklaus	10/97–9/99	35,000 *
Lauri Strauss Leukemia Foundation, Felix Schnyder Memorial Fund	Dr. Tonks	12/97–11/98	15,000 *
Tularik, Inc.	Dr. Wigler	10/97–10/03	604,402 *
Volkswagen Foundation	Dr. Silva	7/96–6/99	26,652
Equipment Support			
The Louis Berkowitz Family Foundation, Inc.	Equipment	12/97–11/98	26,000 *
E.S. Moore Foundation	Equipment	1997	10,000 *
Fellowships			
Rita Allen Foundation	Dr. Hengartner	9/94–8/99	30,000
American Cancer Society	Dr. Ellison	7/95–6/98	28,000
	Dr. Kass-Eisler	11/96–10/99	26,000
American Heart Association	Dr. Berthier	7/96–6/98	30,000
Argentinian Research Council Fellowship	Dr. Rabinowicz	2/96–1/98	18,900

*New Grants Awarded in 1997
+Includes Direct and Indirect Cost

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1997 Funding*+
Burroughs Wellcome Fund	Dr. Mainen	9/97–8/99	55,000 *
Cancer Research Fund of the Damon Runyon–Walter Winchell Foundation	Dr. Liang	9/94–8/97	34,000
CSHL Association	Fellowships	4/95–9/98	165,152
Demerec Kaufmann Hollaender Fellowship	Dr. Grossniklaus	7/97–6/98	5,574 *
European Molecular Biology Organization	Dr. Chong	3/97–10/97	16,753 *
	Dr. Gartner	6/97–3/99	29,097 *
Fundacion Ramon Areces	Dr. Mendez	10/96–9/97	18,000
Joseph G. Goldring Foundation	Fellowship	1997	50,000 *
Human Frontier Science Program	Dr. Cartegni	10/97–9/99	36,400 *
	Dr. Chong	11/97–10/99	5,900 *
	Dr. Curtis	6/97–6/99	38,700 *
	Dr. Mendez	10/97–9/99	8,850 *
	Dr. Poncer	4/97–3/99	28,550 *
	Dr. Soengas	6/97–5/99	35,400 *
Japan Society for Promotion of Science	Dr. Shibahara	4/97–3/99	40,000 *
Sidney Kimmel Foundation	Dr. Lowe	7/97–6/99	100,000 *
	Dr. Van Aelst	7/97–6/99	100,000 *
New Zealand Crop and Research	Dr. Bicknell	1/97–6/97	9,000 *
New Zealand Foundation for Research Science and Technology	Dr. Packer	12/97–11/99	38,554 *
Max Kade Foundation, Inc.	Dr. Gartner	2/97–1/98	10,100 *
Leukemia Society of America, Inc.	Dr. Blasco	7/96–3/97	7,830
	Dr. Kimura	7/97–6/00	36,700 *
	Dr. Tansey	7/96–6/99	33,480
	Dr. Weinreich	7/97–6/00	36,700 *
Ministerio de Educacion y Cultura, Spain	Dr. Losado	10/96–9/98	18,000 *
Neurofibromatosis Foundation	Dr. Hannan	6/97–6/98	35,000 *
Andrew Seligson Memorial Fellowship	Dr. Fearnhead	1/97–12/98	35,000 *
The V Foundation	Dr. Van Aelst	8/97–7/99	50,000 *
Helen Hay Whitney Foundation	Dr. Sun	1/97–12/99	27,000 *
The Wellcome Trust	Dr. Connolly	6/97–5/98	7,850 *
	Dr. Sanders	1/96–12/97	11,700
	Dr. Zaman	8/96–7/98	20,253
Henry Wendt	Fellowship Support	1997	50,000 *

Training Support

Grantor	Program/Principal Investigator	Duration of Grant	1997 Funding+
Emanuel Ax Fund	CSHL Summer Undergraduate Program	1997	5,385 *
Burroughs Wellcome Fund	CSHL Summer Undergraduate Program	1997	43,984 *
Cornelius N. Bliss Memorial Fund	CSHL Summer Undergraduate Program	1997	8,000 *
Dorcas Cummings	CSHL Summer Undergraduate Program	1997	5,440 *
Garfield Fellowship	CSHL Summer Undergraduate Program	1997	7,550 *
H. Bentley Glass	CSHL Summer Undergraduate Program	1997	2,692 *
Howard Hughes Medical Institute	Graduate Student Support	1994–1997	20,000
Jephson Educational	CSHL Summer Undergraduate Program	1997	7,500 *
Libby Fellowship	CSHL Summer Undergraduate Program	1997	3,771 *
Olney Fellowship	CSHL Summer Undergraduate Program	1997	5,531 *
Joan R. Read Fund	CSHL Summer Undergraduate Program	1997	4,342 *
W.M. Shakespeare Fellowship	CSHL Summer Undergraduate Program	1997	5,131 *
Von Stade Fellowship	CSHL Summer Undergraduate Program	1997	5,119 *

*New Grants Awarded in 1997
+Includes Direct and Indirect Cost

Grantor	Program/Principal Investigator	Duration of Grant	1997 Funding+
Course Support			
Athena Neurosciences, Inc.	Neurological Diseases Course	1/97–12/97	500 *
Grass Foundation	Scholarships	5/94–4/98	15,000
Howard Hughes Medical Institute	Advanced Neurobiology Courses	1991–1999	275,000
Esther A. and Joseph Klingenstein Fund, Inc.	Advanced Neurobiology Courses	5/94–4/98	60,000
Meeting Support			
Ariad Pharmaceuticals, Inc.	Mechanisms of Eukaryotic Transcription	1997	500 *
Canji, Inc.	Vector Targeting Strategies for Therapeutic - Gene Delivery	1997	1,000 *
Council for Tobacco Research	Regulation of Liver Gene Expression in Health Disease	1997	1,500 *
Cystic Fibrosis Foundation	Vector Targeting Strategies for Therapeutic Gene Delivery	1997	5,000 *
DEKALB Genetics Corporation	The *Arabidopsis* Genome: From Sequence to Function	1997	1,000 *
Dupont Merck Pharmaceutical	Regulation of Liver Gene Expression in Health Disease	1997	2,000 *
GenMedicine, Inc.	Vector Targeting Strategies for Therapeutic Gene Delivery	1997	2,000 *
Genzyme	Vector Targeting Strategies for Therapeutic Gene Delivery	1997	2,500 *
Glaxo Wellcome Inc.	Vector Targeting Strategies for Therapeutic Gene Delivery	1997	500 *
Invitrogen Corporation	Mechanisms of Eukaryotic Transcription	1997	1,000 *
Intraimmune Therapies, Inc.	Vector Targeting Strategies for Therapeutic Gene Delivery	1997	1,000 *
JIC Innovation	The *Arabidopsis* Genome: From Sequence to Function	1997	1,563 *
Key Gene N.V.	The *Arabidopsis* Genome: From Sequence to Function	1997	1,500 *
March of Dimes	Regulation of Liver Gene Expression in Health Disease	1997	5,000 *
Microbiological Associates, Inc.	Vector Targeting Strategies for Therapeutic Gene Delivery	1997	1,000 *
Monsanto Company	The *Arabidopsis* Genome: From Sequence to Function	1997	5,000 *
E.I. DuPont De Nemours and Company	Differential Display and Related Techniques for Gene Discovery	1997	2,000 *
Novartis Crop Protection, Inc.	The *Arabidopsis* Genome: From Sequence to Function	1997	1,000 *
Pfizer, Inc.	Regulation of Liver Gene Expression in Health Disease	1997	1,000 *
Prolinx, Inc.	Vector Targeting Strategies for Therapeutic Gene Delivery	1997	1,000 *
Stanford University/Morrison Institute	Human Evolution	1997	9,135 *
Targeted Genetics Corp.	Vector Targeting Strategies for Therapeutic Gene Delivery	1997	2,000*
Therexsys Limited	Vector Targeting Strategies for Therapeutic Gene Delivery	1997	5,000 *
Tularik Inc.	Mechanisms of Eukaryotic Transcription	1997	1,000 *
Upstate Biotechnology, Inc.	Tyrosine Phosphorylation	1997	1,000 *
Virus Research Institute, Inc.	Vector Targeting Strategies for Therapeutic Gene Delivery	1997	2,000 *
Wyeth-Ayerst Laboratorie	Vector Targeting Strategies for Therapeutic Gene Delivery	1997	3,000 *

*New Grants Awarded in 1997
+Includes Direct and Indirect Cost

BANBURY CENTER

Grantor	Program/Principal Investigator	Duration of Grant	1997 Funding+
FEDERAL SUPPORT			
CDC Centers for Disease Control and Prevention	Lyme Disease Workshop: In Vivo Expression and Recognition of Antigenes of *Borelia burgdorferi*	1997	10,000 *
FDA Food and Drug Administration	Molecular Immunobiology of Lyme Disease Workshop	1997	15,000 *
The Federal Judicial Center Judiciary Leadership Developmental Council	The Art of Judging Workshop	1997	18,122 *
NINDS National Institute of Neurological Disorders and Stroke	Genetics of Parkinson's Disease	1997	27,849 *
NONFEDERAL SUPPORT			
Meeting Support			
Albert B. Sabin Vaccine Foundation, Inc.	Case Studies in Vaccine Development	1996	27,243
Albert B. Sabin Vaccine Foundation, Inc.	Sabin HIV	1996	14,987
Alexander and Margaret Stewart Trust	Immunological Attacks on Cancer	1997	28,760 *
American Physiological Society	Genomics to Physiology and Beyond	1997	34,146 *
Amgen, Inc.	Breast Cancer	1997	5,000 *
Applied Microbiology, Inc. (AMBI)	Posttranslational Modifications	1997	17,007 *
Cambridge Neuroscience, Inc.	Neuregulins and Neuregulin Receptors	1997	3,000 *
Fort Dodge Animal Hospital	Lyme Disease	1997	1,000 *
Glaxo Wellcome Inc.	Lyme Disease	1997	5,000 *
John A. Hartford Foundation	Human Cognition	1997	33,000 *
MedImmune, Inc.	Lyme Disease	1997	2,000 *
The National Neurofibromatosis Foundation, Inc.	Neurofibromatosis	1997	30,189 *
OncorMed, Inc.	p53	1997	28,278 *
Pasteur Merieux Connaught	Lyme Disease	1997	5,000 *
SmithKline Beecham Pharmaceuticals	Lyme Disease	1997	5,000 *
The Wilson Foundation	Neurofibromatosis	1997	6,813 *
SPECIAL PROJECT SUPPORT			
Oxnard Foundation	Utrophin Project	11/96–10/99	40,000

ADDITIONAL SUPPORT FOR THE UTROPHIN PROJECT

Barbara Bancroft
Bernard F. and Alva B. Gimbel
Mr. and Mrs. William T. Lai
Mr. and Mrs. Paul G. Pennoyer, Jr.
Martha P. Thomas
Mr. and Mrs. Andrew M. Blum
Mr. and Mrs. Thomas S. Gimbel
Valerie Louthan Designs, Inc.
Gertrude Colletti Perucca
Kevin and Gloria Daigh
Anne D. Glenn
OBX, Inc.
Robin and Enrique Senior Philanthropic Fund of the Jewish Communal Fund

*New Grants Awarded in 1997
+Includes Direct and Indirect Cost

Grantor	Program/Principal Investigator	Duration of Grant	1997 Funding+
FEDERAL GRANTS			
NATIONAL SCIENCE FOUNDATION	A Two-part Program to Develop and Support a Nationwide Corps of Human and Molecular Genetics Resource Teachers at the Secondary Level, David Micklos	4/93–6/97	10,435
	A Novel Mechanism for Introducing Human Genome Research in Freshman Biology Classes, Mark Bloom	4/95–4/98	89,556
	A Partnership to Develop Advanced Technology Units on Genomic Biology	8/97–7/00	49,954 *
DEPARTMENT OF ENERGY	The Science and Issues of Human DNA Polymorphisms: An ELSI Training Program for High School Biology Teachers	1/97–1/00	67,755 *
NONFEDERAL GRANTS			
Genentech, Inc	Story of a Gene Exhibit	4/95–4/97	722
Hearst Foundation	Genetics as a Model for Whole Learning	1/97–12/97	50,000 *
Howard Hughes Medical Institute	Precollege Science Education Initiative for Biomedical Research Institutions	7/94–8/99	35,738
Josiah Macy, Jr. Foundation	Gene Almanac	10/97–9/00	57,555 *

The following schools each awarded a grant for the *Genetics as a Model for Whole Learning Program:*

School	Amount
China Town School District 1	5,040
Community School District 29	26,700
East Meadow Union Free School District	2,450
Elwood Union Free School District	3,425
Green Vale School	2,375
Half Hollow Hills Central School District	2,750
Jericho Union Free School District	4,700
Lawrence Union Free School District	10,000
Locust Valley Central School District	7,160
Plainview-Old Bethpage Central School District	1,175
South Huntington Union Free School District	10,400
Syosset Central School District	14,700

The following schools each awarded a grant for *Curriculum Study* of 1,100:

Commack Union Free School District
East Meadow Union Free School District
Elwood Union Free School District
Garden City Union Free School District
Great Neck Union Free School District
Half Hollow Hills Central School District
Harborfields Central School District
Herricks Union Free School District
Island Trees Union Free School District
Jericho Union Free School District
Lawrence Union Free School District
Locust Valley Central School District
Massapequa Union Free School District
North Shore Central School District
Oyster Bay-East Norwich Central School District
Plainedge Union Free School District
Plainview-Old Bethpage Central School District
Portledge School
Port Washington Union Free School District
Ramaz School
Roslyn Union Free School District
Sachem Central School District
West Hempstead Union Free School District

of 1,500:
Long Beach School District

of 2,000:
East Woods School
Friends Academy
Green Vale School
Oceanside Union Free School District

*New Grants Awarded in 1997
+Includes Direct and Indirect Cost

We wish to express the genuine gratitude of the trustees, administration, and most especially the scientists of Cold Spring Harbor Laboratory for the generous financial support of those persons, corporations, and foundations whose names appear on the following pages of the annual report.

Richard L. Cosnotti, Chief Development Officer

Cold Spring Harbor Laboratory is a nonprofit research and educational institution chartered by the University of the State of New York. Less than half (44.1%) of our annual support is derived from Federal grants and contracts, and thus we rely heavily on support from the private sector: foundations, corporations, and individuals. Contributions from the private sector are tax exempt under the provisions of Section 501(c)(3) of the Internal Revenue Code. In addition, the Laboratory is designated a "public charity" and therefore is enabled to receive funds resulting from the termination of "private foundations."

METHODS OF CONTRIBUTING TO COLD SPRING HARBOR LABORATORY

Gifts of money can be made directly to Cold Spring Harbor Laboratory.

Securities: You can generally deduct the full amount of the gift on your income tax return, and, of course, you need pay no capital gains tax on the stock's appreciation.

We recommend any of the following methods:

(1) Have your broker sell the stock and remit the proceeds to Cold Spring Harbor Laboratory.

(2) Deliver the stock certificates to your broker with instructions to open an account for Cold Spring Harbor Laboratory and hold the securities in that account pending instructions from Cold Spring Harbor Laboratory.

(3) Send the *unendorsed* stock certificates directly to the Laboratory: Comptroller, Cold Spring Harbor Laboratory, One Bungtown Road, Post Office Box 100, Cold Spring Harbor, New York 11724. In a separate envelope, send an *executed* stock power.

Pooled Income Funds: Combine gifts from a number of donors in a pool for attractive investment and tax purposes.

Appreciated real estate or personal property: Sizable tax benefits can result from such donations; the Laboratory can use some in its program and can sell others.

Life insurance and charitable remainder trusts can be structured to suit the donor's specific desires as to extent, timing, and tax needs.

Bequests: Most wills probably need to be updated. Designating Cold Spring Harbor Laboratory as beneficiary ensures that a bequest will be utilized as specified.

Conversion of private foundation to "public" status on termination: This may be done by creating a separate fund within Cold Spring Harbor Laboratory whereby the assets of the private foundation are accounted for as a separate fund and used for the purposes specified by the donor. Alternatively, the private foundation can be established as a supporting organization of Cold Spring Harbor Laboratory.

For additional information, please contact the Chief Development Officer, Cold Spring Harbor Laboratory, One Bungtown Road, Post Office Box 100, Cold Spring Harbor, NY 11724, or call 516-367-8840.

CAPITAL AND PROGRAM CONTRIBUTIONS

January 1, 1997–December 31, 1997

Contributions of $5,000 and above, exclusive of Annual Fund

In 1997, the Laboratory received significant support in the form of capital and program contributions from individuals, foundations, and corporations.

Rita Allen Foundation, Inc.
Arrow Electronics
Banbury Fund
Louis Berkowitz Family Foundation, Inc.
Burroughs Wellcome Fund
Carnegie Institution of Washington
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1 in 9: The Long Island Breast Cancer Action Coalition
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Alexander and Margaret Stewart Trust
Laurie S. Strauss Leukemia Foundation
The V Foundation
Henry Wendt
Helen Hay Whitney Foundation

Total **$ 10,063,021**

CHILD CARE CENTER CAPITAL CAMPAIGN

January 1, 1997–December 31, 1997

Contributions of 1,000 and above, exclusive of Annual Fund

The child care center capital campaign is on-going. This list reflects only gifts received in 1997.

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R. H. Cushman
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The Tilles Family
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Mr. and Mrs. Colton P. Wagner
Mrs. Henry G. Walter, Jr.
Mr. and Mrs. H. G. Wellington in memory of Mrs. J. Harvey Humpstone
Dr. and Mrs. Michael Wigler
Zebra Media Productions

Total **$ 245,628**

ANNUAL CONTRIBUTIONS

Corporate Sponsor Program

The Cold Spring Harbor Laboratory Corporate Sponsor Program continues to make a very substantial contribution to the meetings programs in Grace Auditorium on the main campus and at Banbury Center. This funding enables us to bring specially invited speakers to the meetings, to help underwrite the expenses of young scientists attending meetings, and to ensure that the meetings at Banbury Center are on cutting-edge research. More than 6000 scientists attended meetings at Cold Spring Harbor Laboratory and Banbury Center in 1997. Full details of these meetings are found elsewhere in this Annual Report.

The members of the Program receive special privileges in acknowledgment of their contributions. We waive all on-site fees for eight representatives of each company at our meetings. Three of these scientists may attend meetings at Banbury Center, where attendance is otherwise only by invitation of the organizers. Corporate Sponsors receive gratis copies of Cold Spring Harbor Laboratory Press publications including the journals *Genes & Development*, *Learning & Memory*, and *Genome Research*. Special meetings held in Grace Auditorium in the Spring and Fall are also available to Corporate Sponsor Program members.

In addition, we acknowledge our Sponsors in all relevant publications, including the books of abstracts given to every participant. The names of the sponsoring companies are listed on the poster describing the meetings. This is mailed to approximately 17,000 scientists throughout the world. There were 36 members of the Program in 1997, including virtually all of the pharmaceutical companies and major players in biotechnology. This number is a tribute both to the scientific acumen and generosity of the member companies and to the quality of the Program. However, continuing mergers in the pharmaceutical and biotechnology worlds are reducing the pool of potential members, and each year it becomes ever more important that members renew. The following companies participated in the Program in 1997:

COLD SPRING HARBOR LABORATORY CORPORATE SPONSORS

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American Cyanamid Company
Kirin Brewery Co., Ltd.
Monsanto Company
Pioneer Hi-Bred International, Inc.
Westvaco Corporation

Total $ 792,000

DNALC Corporate Advisory Board Annual Fund

An important objective of the Corporate Advisory Board of the DNA Learning Center is to provide a sustainable level of annual funding for the Learning Center's programs and to increase visibility in the local business community. As a means of reaching this objective, the Board conducts an Annual Fund and an Annual Golf Tournament with proceeds benefiting the DNA Learning Center.

Chairman: John J. Leahy, Grant Thornton, LLP
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Ms. Elaine Ronnenburger
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Total $83,647

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The Country Printer
Tony's Fish & Seafood Corp.
Uniflex

Beverage Donations
Arizona Beverage
The Lion Brewery

Gift Bags
Badge Agency, Inc.
The Hartford

Total	**$ 97,714**

Total DNA Learning Center Annual Fund $ 181,361

Cold Spring Harbor Laboratory Association (CSHLA)

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Mrs. James M. Large, Vice President
Mrs. Robert G. Merrill, Secretary/Treasurer

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President's Report

It was a year of continued growth in the support activities of the Association, aimed at furthering the mission of Cold Spring Harbor Laboratory. The activities of the members were many and varied, manifesting a broad range of interests in serving this community.

On February 2, 1997 our year commenced with the annual meeting of the CSHL Association. The guest speaker was Philip R. Reilly, M.D., who spoke to us about "Genetics, Ethics, and You." The lecture was thought-provoking, leaving most of us with thoughts about various issues that face us as the science of genetics increasingly affects our daily lives. Dr. Reilly discussed topics including the future of genetic testing and the impact of genetic information on health and life insurance and employment. The lecture raised important questions that must be addressed in this increasingly scientific world.

Five directors stepped down after having served the Association generously and unselfishly. They are Joyce Green, Laurie Genovese, Dr. Alan M. Kisner, Robert L. Marcus, and Edward F. McCann II. At our February meeting, new directors were elected. They are W. Dillaway Ayres, Jr., Mary Jeanne Harris, Gilbert R. Ott, Jr., and Cynthia Stebbins. The Association was deeply saddened by the passing of Mary Jeanne Harris on August 19, 1997. Mary Jeanne served the Laboratory in many capacities including Trustee of the Laboratory, Director of the Association for a number of terms, and as a volunteer in helping young scientists adjust upon arrival at the Laboratory. Her unselfish enthusiasm, support, and contributions will be truly missed.

On June 1, Dr. Sean Carroll of the University of Wisconsin addressed the Association and friends at the annual CSHLA Dorcas Cummings Memorial Lecture during the CSHL Symposium. Dr. Carroll's talk was entitled "Embryos and Ancestors: The formation and evolution of animal body patterns." His in-depth discussion of the similarities in development between various species was a concept that few of us had previously considered. Following the lecture, dinner parties were hosted by members of the Association for scientists and friends of the Laboratory. The hosts and hostesses entertained diverse groups of neighbors, visiting scientists, and the Lab's own scientists on a lovely evening. The Association wishes to express its special thanks to each:

Mr. and Mrs. W. Dillaway Ayres, Jr.
Mr. and Mrs. Henry Babcock, Jr.
Ms. Mary Lenore Blair
Mr. and Mrs. Robert Walton
Mr. and Mrs. G. Morgan Browne
Mr. and Mrs. Harry Charlston
Mr. and Mrs. David Clark
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Mr. and Mrs. John Grace
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Mr. and Mrs. Robert O'Connor
Mr. and Mrs. John Phelan
Mr. and Mrs. Whitney Pidot
Mr. and Mrs. Douglas Soref
Mr. and Mrs. John Stevenson
Mr. and Mrs. Daniel Tenney

In the fall, the Association commenced its major annual giving campaign. As a result of the tireless efforts of many of our directors and members of the Association, in excess of $654,000 was raised from 682 members.

CSHLA Volunteer Reception
Franny Elder, John Cleary, Duncan Elder

West Side School Science Night
Winship Herr speaks to students and parents.

The directors are pleased with this outcome as it represents an increase in the number of contributors and surpasses our goal. Additionally, it is the intention of the directors to continue to develop new friends and enhance the level of contributions in furtherance of the effort to support the Laboratory's mission. The funds raised in the annual giving campaign are earmarked for the support of young scientists who are, for the most part, recent graduates of doctoral programs throughout the world. Their arrival at the Laboratory campus is an exciting time for them, as indeed it is for the Laboratory.

As alluded to above, the activities of the Association and its members cover a diverse range of interests and activities that include not only support of the Lab, but also dissemination of information to the public. On April 2, Dr. Mary-Claire King, Professor in the Division of Medical Genetics at the University of Washington, delivered a public lecture entitled "Breast Cancer Update." Dr. King was responsible for identifying *BRCA1*, the first known breast cancer gene, and she is well known for her outspoken advocacy of increased research on breast cancer.

Another timely and important topic to many of us is Lyme disease. Long Island has traditionally been an area in which Lyme disease is a constant threat to the health and well being of its residents. On November 2, a panel discussion, "Lyme Disease: Research and Clinical Update," was held in Grace Auditorium. The evening was moderated by Steven E. Schutzer, M.D. and John Dunne, Ph.D. and addressed the status of current research and the prognosis for conquering the disease. The speakers were Patricia K. Coyle, M.D. and Raymond Dattwyler, M.D.

In another area of interest, Barbara Conolly, a Long Island naturalist and resident of the community, gave a tour of the Laboratory on May 18 to individuals interested in flora and fauna. Her program, "Bird and Flower Walk," highlighted much of the natural beauty located on the Laboratory grounds and was both well attended and well received with great interest and enthusiasm from the participants.

On the music front, an outstanding concert, open to all members of the Association, was held on June 13. The performance featured world renowned pianist Misha Dichter. This concert was like many that the Laboratory supports each year usually free of charge and open to the public.

In addition to the above-mentioned activities, the Laboratory, with the participation of Association members, conducted several school tours, permitting students to observe Laboratory scientists at work. This exposure is vital to enhancing children's understanding of the manner in which science is done at the Laboratory and other scientific institutions. The intention is to interest children in science at a young age in the hope of cultivating a continuing interest in science.

Another program warranting special attention is the Next Generation Initiative (NGI). This effort was conceived and implemented by younger members of the community and its activities have

been well received. This year's NGI lecture was held on January 10, 1998 in Grace Auditorium. Dr. Bruce Stillman, Director of Cold Spring Harbor Laboratory, introduced Dr. Nicholas Tonks, a principal investigator at the Lab, who spoke on "Drug Targets for Cancer and Diabetes." Dr. Tonks has discovered an entirely new class of enzymes which have been implicated in cancer, diabetes, and other diseases. He is now striving to determine the function of these enzymes. Many thanks go to Lola and John Grace, who hosted a dinner party for all attendees following the lecture. Special thanks also go to the members of the committee who organized the evening, including Tony Kemper, Mary Alice Kolodner, Calvert Saunders, Cathy Soref, Laura Swiggett, and of course, Lola and John Grace.

I am stepping down as president of the Association, and at this time I wish to state that it has been a particular privilege and pleasure for me to serve the Association and the Laboratory. I know that the Association and the Laboratory will continue to grow and prosper, and contribute to the furtherance of science in the years to come. I would like at this time to thank Laurie Nesi, Gerry Olin, Holly Brooks, and Maureen Augusciak, who have retired as Directors this year. Each of them, in many different and varying ways, gave unstintingly of their time and effort in furthering the work of the Association and the Laboratory. The Association is very grateful to them for their support. Special thanks also go to Jean Houghton who served as our Staff Director and also to the other members of the Laboratory's Development Office who supported the day-to-day activities of the Association.

February 1998 **John P. Cleary**

Members of the Cold Spring Harbor Laboratory Association

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Carol Greider+
David Helfman
Nouria Hernandez*
Adrian Krainer
Roberto Malinow
Robert Martienssen
David Spector
Nicholas Tonks
Timothy Tully
Michael Wigler

Associate Investigators

Xiaodong Cheng+
Hollis Cline
Grigori Enikolopov
Bruce Futcher
Ryuji Kobayashi
Hong Ma
Richard McCombie
Alcino Silva
Jacek Skowronski
Arne Stenlund
Yi Zhong

Assistant Investigators

Ulrich Grossniklaus
Erich Grotewold
Gregory Hannon
Michael Hengartner
Tatsuva Hirano
David Jackson
Leemor Joshua-Tor
Yuri Lazebnik
Scott Lowe
Peter H. Nestler
Andrew Neuwald
Karel Svoboda
William Tansey
Linda Van Aelst
Rui Ming Xu
Chi-Ping (Jerry) Yin
Michael Zhang

Research Investigators

Joseph Colasanti+
Neilay Dedhia
Masaaki Hamaguchi
Robert Lucito
Akila Mayeda
Elly Nedivi
Natalia Peunova
Andrew Reiner
Shou Waga
Clifford Yen

Adjunct Investigator

Tom Marr

Adjunct Associate Investigator

Scott Powers

Adjunct Assistant Investigator

Craig Ogata

Visiting Scientists

Jiaxin An
Eli Hatchwell
Ming Huang
Nobuhiro Kashige
Marjana Maletic-Savatic
Tatyana Michurina
Mariko Nakamura
Vladimir Scheinker
Zuoping Xie

Senior Fellow

Douglas Conklin

Postdoctoral Fellows

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Anton Bennett
Christine Berthier
Benjamin Bottner
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Isabel Cantallops
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Shaohui Zhang

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Degui (Charlie) Chen
Grace Chen
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Faculty

Xiaodong Cheng
Joseph Colesanti
Carol Greider

Visiting Scientists

Ross Bicknell
Daniel Bush**
Liam Dolan**
Ai-Ping Dong
Konstantin Galactionov
Satoshi Kida
Boris Kuzin
Roberta Maestro

Postdoctoral Fellows

Maria Blasco
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Stephen Brand
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Javier Cáceres
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Qizhi Wang
Gang Yi Wu
Xujia Zhang

Graduate Students

Nicholas Carpino
Chong (Chris) Huang
Benjamin Lee
Jian Sheng
Aneil Shirke

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