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Book Reviews

Chromosome Puffs

The Science Times Book of Genetics
Edited by Nicholas Wade
New York: The Lyons Press (1999). 256 pp.
$25.00

Readers of *Cell* do not rely upon the *New York Times* Science Times section to keep up with the latest discoveries in biology, but apparently other people do. Every Tuesday, the *Times* publishes a section on science and health. Most weeks, the coverage features new contributions to biomedical research, and especially modern genetics, the origins of disease, gene therapy, genomics, and development. The weekly, prominent commitment of that much space in our most nearly national newspaper makes the Science Times the most important print source, and probably the most respected source, of news about biology. It is not coincidence that basic biomedical science and its applications now receive this level of attention. The public is aware of biological research more than ever before, because biomedicine is moving closer to what people want from it, and in some instances to what they fear from it. Biologists in turn have a serious stake in a well-informed public in order to sustain support, to retain biomedical research's privileged budgetary status among discretionary programs, and to assuage those fears.

This collection presents 43 articles about modern genetics that appeared in the Science Times over the last several years, representing the work of four writers. Their subjects range broadly: modern approaches to the natural history of our species, molecular approaches to classical questions in development, new approaches to human diseases and aging. The subjects of most articles are well chosen to present exciting science at the forefront of what is understood. Practicing biologists may be impressed that the authors chose such complex subjects, and it is interesting to see how well new research results travel from scientific presentation to popular presentation. More important than those considerations, the book is to be taken seriously because it is shows how a significant segment of the public—consumers, taxpayers, patients—gets its contemporary education in biology.

The results are uneven. Some of the articles are lucid and crisp analyses of the importance of the science, presenting results in both contemporary and historical contexts that illuminate the questions and potential answers. They discuss openly the scientific and public controversies that accompany the new findings, and appropriately foresee controversies to come. In other articles, however, the science is sacrificed to simplicity and hype, selling the prospects of the work in a way that risks creation of false expectations. Worse, some articles ignore the standards of both science and journalism, and, in fact, do damage.

In the best articles, generally those dealing with basic research, the combination of depth and accessibility is impressive. One on Hox genes introduces the notion of a conserved body plan among species, illustrating the point with the fauna of Star Trek, and continues with an explanation of the relationships between Hox gene arrangements and Hox gene activities. Quotations from scientists involved in the research, and a terrific graphic, explain the singularity and compelling nature of this relationship. The article's treatment of a novel model for coordination of Hox gene function makes clear that it is only theory, as yet unsupported by evidence, while managing to demonstrate its power in clear and effective language.

Most of the articles feature multiple quotes from scientists who participated in the work, or who know it. In some instances, the scientist is objective and understated, portraying honest uncertainty in a fashion that supports the credibility of the scientific enterprise. A model of candor is the comment from a leading investigator in the olfaction field: "It's really not clear what is going on yet." In other cases, the quotes show enthusiasm and inject a human element into research. That's understandable, but often the authors have somehow elicited and then selected for publication some unfairly silly and unhelpful remarks. A surprisingly large fraction of quotes from scientists begin with the word "Wow." And it isn't clear whether calling a particularly striking result (the characterization of the *eyeless* gene) "Frankensteinian science at its best" will make the work more or less attractive to *Times* readers.

Inevitably, these articles deal not only with science but also with the expectations of what science can offer. For those discoveries that have potential medical applications, the weight of those expectations increases. The worst thing these articles can do for the reader and for science is to create unrealistic hopes for imminent cures to fatal diseases. In some instances, that risk is mitigated by balance within the story. For example, in the course of a highly optimistic description of a gene discovery "that offers a new way of preventing many cases of colon cancer," the author also offers Francis Collins's caution against overstating the risk associated with these particular mutations.

But too often, the stories appear without the caveats. The presentation of a possible therapy for glioblastoma is particularly troublesome. It describes a single surviving patient in a gene therapy protocol, unusual but not entirely unprecedented. The investigator cautiously makes little claim of efficacy, but the article notes that the sponsoring company has proceeded with tests in a small group of patients. That was eight months before the article was written, and more than three years before this book was published. In the case of the article, there should have been a follow-up story; and when the article was reprinted in the book, there should have been a footnote. There are people waiting for this news. In several other stories, the other side, the side of caution, is just missing. "[I]t is probably only a matter of time before

some of these defects can be corrected," says the Introduction, referring specifically to Huntington's disease and hereditary forms of cancer but presaging several articles in the book. That's very likely true, of course, but how much time? With what obstacles?

Some of the articles, to their credit, note the way our society and economy affect science, and vice versa. For example, the dilemma presented by the development of a genetic test for a condition that has no cure is raised in appropriate contexts. However, the influence of the biotechnology and pharmaceutical industries and their profit motives on the direction and pace of research is treated only occasionally. One article deals with the promises and risks of gene therapy. In it, N.I.H. Director Harold Varmus is quoted, noting soberly that companies motivated to raise money for their ventures are tempted to claim that they have work in progress. He worries that such pressure harms the entire gene therapy enterprise. Juxtaposed with Varmus's remarks are the opinions of both industrial and academic scientists involved in the work who take a more pragmatic view, arguing that there's enough information in hand to justify attempts at gene therapy now. The value of other articles dealing with experimental approaches to disease would have been enhanced had the authors similarly integrated these two perspectives.

Some of the articles lose their way entirely. A 1997 piece, which someone chose to title "Can the Common Cold Cure Cancer?" focuses on an adenovirus engineered to grow in cancer cells but not in normal cells. The article explicitly, and unfortunately, recalls the "war on cancer" declared by Richard Nixon. Potentially promising results from a completed phase 1 trial are reported—so, remarkably, are results from a phase 2 trial in progress "which seem[s] to be working ... although [the researcher] declines to give the success rate." Publishing results from an unfinished trial would be irresponsible, in any venue. Even if the journalist were not otherwise sensitive to this point, the fact that the researcher declined to give the success rate might have alerted him. A few paragraphs later, readers get a second chance to see what the author may have missed and the problem he has unwittingly contributed to. Another scientist is quoted as observing that the first patients in clinical trials always do best, but that luckily Wall Street hasn't figured that out yet. All together, it amounts to a sad, cynical example of how ill-considered journalism can injure the delicate interrelationships among biology's aspirations, patients' hopes, and the participation of private enterprise.

The articles that work best for both scientists and the public are those that describe a new result in a way that makes its importance clear, convey its intrinsic excitement, and connect it to legitimate public concerns. When these articles go most wrong, it's not because they fail to understand the science or to appreciate its significance. Rather, it's because there has been a suspension of standards—of critical thinking, even of reporting. As the national model for science journalism, it's important that these articles be held to the highest principles of rigor, thoughtfulness, honesty, and responsibility. It is possible to write consistently about this subject, appropriately recognizing the interests of scientists, consumers, and business but without pandering; as an example, consult the weekly Science and Technology section of *The Economist*.

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The Cell: The Development of an Idea

The Birth of the Cell
By Henry Harris
New Haven, CT: Yale University Press (1999).
288 pp. $30.00

The concept of the cell is central to modern biology. Indeed the story of the evolution of the cell is the history of the origin of life. Many of us have a rather poorly formed concept of the history of our field from reading of E. B. Wilson's famous book *The Cell in Development and Heredity*, which was a summary of the field in the early 20th century rather than a historical development. In his recent book *The Birth of the Cell*, Henry Harris has summarized his 50 years of reading of the primary literature into a learned and judicious history of the evolution of the concept of the cell from the development of the microscope early in the 17th century to the theory of the cellular determinants of heredity, which was developed during the early part of the present century. In the course of his book, Harris restores the reputation of a number of scientists whose work was essential for developing the concept of the cell. This is a book by a biologist, for biologists, and is a notable example of how much scientists can contribute to the history of their own subject if they are prepared to treat developments in the complexity of the real historical circumstances.

The conventional view of the development of the study of cells is that it was driven by advances in optical microscopy. Although optical developments were clearly essential for increasing the resolution of observation, Harris makes the interesting point that scientists were looking for discrete and common structure in living matter because the scientific community had absorbed the atomist principles. Atomism was developed by the Greeks and proposed that all matter was made up of elementary particles. Although atomist principles were not completely lost after the end of classical civilization, they were given much greater prominence by the work of Gassendi in the 17th century. Harris describes in detail two centuries of observation, starting from 1650, that began to elucidate the nature of these elementary particles and gradually developed the concept of the cell as the basic building block of living matter.

In the course of his discussion, Harris shows that one problem in the description of the history of the cell has been the extent of national biases among biologists when discussing the origins of their work. In particular

the rise of Prussian nationalism after the defeat at Jena is reflected in the German treatment of French rivals, and vice versa. British historians in turn have been prone to exaggerate the achievements of Robert Hooke, who contributed so much experimental work during the early years of the Royal Society of London. However, it is clear from Harris's book that by the 19th century, the driving force behind the development of the cell theory was from scientists operating in the German sphere of influence. Harris provides an extensive selection of quotations in the original language, a perusal of which shows that a knowledge of German is essential for understanding the primary literature of this history.

Harris begins his discussions with Galileo, a committed atomist, who by 1614 had developed a microscope that he used to examine the cuticle of the fly. By 1660, Hooke had begun development of compound microscope with which he saw microscopic cavities in cork, probably the cell walls, and coined the term cellulae—possibly from the latin *cella*, meaning room or cubicle. Six years after the appearance of Hooke's *Micrographia* in 1665, manuscripts from Nehemiah Grew and Marcello Malpighi laid the basis for the modern understanding of the microanatomy of plants, while Malpighi also established a systematic methodology for the study of animal tissue. By the early 18th century and the work of van Leeuwenhoek, it was clear that plant tissue was composed of microcavities but their nature was unclear. During the 18th century there was a great deal of microscopic study and much discussion about the basic components of tissue. By the end of the 18th century, most botanists agreed with the idea that all plants were composed of cells. During the early 19th century the common properties of plant cells were investigated using greatly improved microscopes, and Prof. Harris gives a knowledgeable and balanced discussion of the many contributions, as well as of the controversies in estimating these contributions. In particular he threads his way through the research of the 17th and 18th centuries with remarkable clarity through what is a complex and many-sided subject. He lists (p. 38) four topics still under consideration at the beginning of the 19th century: (1) How are new cells formed? (2) Do cells communicate with each other, and if so how? (3) What do cells contain? (4) Are all tissues comprised of modified cells?

The study of these issues appears to mimic to some extent the recent studies on the molecular basis of cell physiology and development in the second half of the 20th century. Just as investigators studying the molecular basis of development did not initially equate the development of a fruit fly with that of a mouse, so the investigators of the 18th and early 19th century did not equate the cells of plants with those of animals: the unicellular organisms first seen by Leeuwenhoek or the red blood corpuscles studied by Swammerdam, Leeuwenhoek, and Malpighi, the first animal cells to be visualized.

The clear establishment of the separate cellular nucleus, and also of the basic similarity of structure of animal and plant cells, developed after about 1825 and was the work of a number of people. One of the major figures was Jan Evangelist Purkyne, a Czech nationalist who wrote largely in Czech, though educated in the main Germanic tradition. He is another of Prof. Harris's candidates for the role of victim of historical injustice, though how far this was due merely to his nationalism and how far it was because few could read his articles remains unclear. His institute in Breslau has been called "the cradle of histology."

In the gradual development during the 1830s and 1840s of the understanding of the ubiquity of cell propagation by binary fission, and of the complexity of the cell, Prof. Harris urges that the role of Müller, Schleiden, and Schwann has been greatly exaggerated, not only at the expense of Purkyne "who argued that all solid animal tissues were composed essentially of cells and fibres, and who referred specifically, to the homology between animal and plant cells" (p.93), but also to the detriment of the later work of Franz Unger, who worked in Graz and Vienna, and of Ferdinand Cohn who worked in Breslau all his academic life. During the same period "The demonstration that the egg was itself a cell and that it begat daughter cells by binary fission marked a decisive step in the growth of what later became the science of genetics" (p. 127).

Harris writes "If there was one individual who, above any other, was responsible for bringing order into the confusion that shrouded the origin of animal cells, it was Robert Remak. It was not acknowledged in his lifetime . . . nor is it adequately recognized even now" (p. 128). Reading quotations from Remak, it is hard to disagree with Harris. As in the case of Purkyne, the reasons were partly nationalistic: Remak was born in Posen and was an orthodox Jew. "By 1852 Remak had come to the view that the only form of cell multiplication to be found in the animal body was multiplication by means of binary fission", which he set out in a paper entitled "Ueber extracellulare Entstehung thierischer Zellen and über Vermehrung derselben durch Theilung (On the Extracellular Formation of Animal Cells and Their Multiplication by Division)" (p. 130). However, "the rapid and widespread acceptance of Remak's ideas was not due to the meticulous observations of Robert Remak, but to the propagandist skill of Rudolf Virchow (p. 135)," and particularly to the success of his celebrated book *Die Cellularpathologie*.

During the third quarter of the 19th century there was a gradual development of understanding of the division of the cell nucleus, and also of the indispensability of the cell membrane. Then "With the work of Balbiani and van Benenden we move away from debate about the mechanism of nuclear division to a precise delineation of chromosomes and what they do during division of the cell. . . . By 1876, Balbiani's observations had reached an altogether different level of precision. Balbiani saw essentially all the stages of mitosis and noted that, when the cell divided, the nucleus dissolved into a collection of 'bâtonnets étroits' (narrow little rods)" (p. 153). Moreover, it was clear the "bâtonnets" were not identical. "The principal impetus for the transition from purely descriptive to analytical chromosome cytology was, however, the accumulation of data concerning the fertilization and early development of the egg." The properties of chromosomes and their role in heredity were beginning to be elucidated, and early in the present century they were related to Mendel's heredity factors. After this time the problems being considered in the biology of the cell bear a remarkable family resemblance

to the problems occupying cellular biologists today, even though modern knowledge and technical resources are incomparably greater.

Prof. Harris has written a clear, erudite, and balanced history of the development of knowledge of the cell up to the beginning of the 20th century that will appeal to all biologists with an interest in the origins of their subject.

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Why Mutton Doesn't Taste Like Lamb

Molecular Biology of Aging
Edited by Vilhelm A. Bohr, Brian F. C. Clark, and Tinna Stevnsner
Copenhagen: Munksgaard (1999). 404 pp.

A Means to an End: The Biological Basis of Aging and Death
By William R. Clark
New York: Oxford University Press (1999). 234 pp. $27.50

Time of Our Lives
By Tom Kirkwood
London: Weidenfeld and Nicholson (1999). 277 pp. $27.50

Writing a book on aging is as intimidating as trying to cover "the how and why of sickness." Asking "how we age?" is almost as difficult as asking "how do we die?," insofar as it covers a vast array of tissues, physiological processes, and underlying biological mechanisms. Three recent publications provide contrasting examples of how to deal with this topic in book form.

Vilhelm Bohr et al. bring together nearly 30 papers presented at the 1998 Alfred Benzon Symposium on the *Molecular Biology of Aging*, covering such areas as genetic and population studies, replicative senescence, biomarkers, and protein changes. This is a typical "conference proceedings," with papers ranging from general overviews to more specialized technical works. In essence a bound collection of journal-style papers, a particular attraction is that each section finishes with an edited transcript of a general discussion session conducted by the symposia participants. Despite nominally being a symposium on aging, several papers have wider appeal, including several useful reviews on DNA repair. Similarly, of seven papers devoted to replicative senescence, two are reviews on telomeres and telomerase (predominantly in cancer) and telomerase-mediated immortalization. The book focuses more on molecular biology than on aging, but this does mean that it will interest a wider readership. There are bigger and better reviews in the literature—this is no *Annual Review*—and although the "bound papers" format does not provide for much cohesion within the book, it would be a useful addition to an institutional library.

In *A Means to an End*, William Clark illustrates the opposite approach, that of using a single author to cover the entire field. The potential hazards are obvious, since no single author could ever hope to have direct experience in all the topics one would need to cover. Clark's thesis seems to be that all aging comes down to senescence at the cellular level, and that ultimately all "cellular senescence"—be it in yeast, paramecium, neurons, or dividing fibroblasts—will reveal the same underlying cause. Although such a reductionist approach would certainly make the field easier to deal with, so it must be said would discovering the fountain of youth. There is little experimental support and even less by way of evolutionary rationale in favor of a single "cause" of aging (Kirkwood and Kowald, 1997); anyone arguing that *Podospora anserina* aging must be a model for replicative senescence in human cells (or vice versa) is not helping either species. Clark's book is confusing, especially his definition of cellular senescence, which is unfortunate given that the importance of cellular senescence is the main thrust of his argument. It also contains large chunks of basic cell biology that provide little relevant background and would simultaneously stun a lay audience and bore an academic reader.

Successful books are usually written when a single author sticks to what he knows best. One such example is Tom Kirkwood's *Time of Our Lives*, which covers the evolutionary aspect of aging. The use of a single author makes for cohesion and interrelatedness between chapters. In contrast to Michael Rose's rather academic *Evolutionary Biology of Aging* (1991), full of data and equations, *Time of Our Lives* is competing with Steven Austad's *Why We Age* (1997) for the wider audience. Both focus on two big questions for the lay audience: why we age, and what (if anything) we can do about it.

The evolutionary perspective provides a much-needed unifying thread in a field often marred by less than edifying arguments between overenthusiastic supporters of the various "causes" of aging, be they mitochondrial dysfunction, altered proteins, telomere-related cell senescence, or whatever. The tenor of these exchanges is often that if a mechanism cannot explain all aging everywhere then it must be irrelevant to any aging, anywhere. Understanding why aging has evolved provides a powerful aid in understanding how aging mechanisms might work, and many causes of aging proposed in the past are now recognized to be about as valid as the medieval idea that maggots originated from filth. Evolutionary arguments impose a simple test for any "cause": if there is no plausible evolutionary route whereby it could have arisen, then it should be discounted.

The consensus among evolutionary biologists is that aging is not an actively selected process; aging was not selected "for" any reason, such as altruistic benefits to the species as a whole, but rather it is a nonselected "by-product" of selection for maximal reproductive success in natural populations. This can be formalized in

the concept of antagonistic pleiotropy, which recognizes that in any age-structured population with progressively fewer individuals alive at older ages (even if simply due to accidental death) the force of natural selection will dwindle with age. Thus, any mutation that confers improved reproductive success in the early years, even if associated with deleterious effects in later life, will be selected for.

Kirkwood's "disposable soma" theory (Kirkwood, 1977) can be thought of as something of a "worked example" of this rather abstract population genetics concept. Central to this theory is the concept of evolutionary trade-offs between somatic repair and reproduction. The plight of the short-tailed field vole (described by one of my colleagues as "every predator's favorite junk food") illustrates this principle. In the wild, enthusiastic predation ensures that an enormously high percentage of the members of this species soon end their lives with a crunch and a single terrified squeak. Those that escape such "predation events" still face major problems with thermoregulation. Thus, the best evolutionary strategy for a vole is to channel the bulk of its resources (that is, energy from food) into reproduction rather than somatic tissue maintenance. After all, there is no point in having a body that will last for a hundred years if your chances of seeing the year out are slim. When taken out of the wild and into the safer environment of captivity the consequences of this life-history strategy are what we call aging, a fact anyone who has ever had a pet rodent will appreciate. Bats provide a different example of a life-history strategy. Despite being smaller than a pet rodent, some species can live for over thirty years in the wild. This shows us that there is no intrinsic biomechanical or developmental biological reason why a small furry animal cannot be designed to have a 30-year life span. Why voles and bats have evolved different life histories becomes clearer when one considers the protection from predation that flight confers (and probably the thermostable environment of a cave). One challenge for the future is to understand the basis of this vole–bat difference in mechanistic terms.

Problems in gerontological texts often start when authors begin to address which mechanisms cause aging, probably because we really do not yet know the answer. Clark, for example, appears unwilling to acknowledge aging mechanisms that might function at a level of complexity greater than individual cells in isolation, and he largely ignores age-related changes in tissue structure such as those caused by "wear-and-tear." This is a valid causal mechanism of aging for many organ systems for which there is no adult mechanism of replacement, such as the teeth of herbivores or the ragged wings of aged *Drosophila*. Wear-and-tear fits well with evolutionary considerations, which argue that such repair systems would only evolve if there is selective pressure to do so. As proof, there are numerous special cases where this has indeed occurred, such as continuous tooth replacement in many species of shark.

Another example illustrates the power of evolutionary thought when applied to a potential mechanism of aging. The limited division capacity of human cells in culture (replicative senescence), when coupled with analogous in vivo cell division during life, has been argued to lead to the progressive accumulation of senescent cells whose altered phenotype might contribute to age-related tissue degenerations (Campisi, 1996). However, this phenomenon did not evolve to cause aging, but rather as a barrier to tumorigenesis (Wynford-Thomas, 1999). The accumulation of multiple genetic mutations during tumor development, with intervening periods of clonal expansion to provide a sufficiently large number of target cells for the next mutation event, requires extensive cell division. Thus, any barrier to unlimited cell division will be a barrier to tumor formation. In evolutionary terms, this developmental program provides for reproductive success, since there is nothing more guaranteed to prevent reproduction than being dead before sexual maturity. However, this requirement also brings with it the potential for detrimental effects later in life.

Although the intellectual rigor provided by evolutionary considerations are a current strength in the aging field, they can only provide a framework within which to ask questions about the validity of potential mechanisms and are powerless to suggest specific causes. The literature on causation is strong on observational data but weak on direct interventional tests. The phenotype of the telomerase knockout mouse (Rudolph et al., 1999) has already provided the "proof of principle" that experimentally imposing a reduced cell division capacity can produce a phenotype with many features of premature aging. Similarly, the observation that replicative senescence in many human cell types can be prevented by forced expression of telomerase (Bodnar et al., 1998) provides an obvious next test: in a species possessing telomere-dependent senescence, does its removal (via forced expression of telomerase in animo) reduce or remove any aspect of the aging of that animal in any tissue? Interventional tests similarly can be devised for other mechanisms, such as asking whether increased aberrant protein production (such as via the use of misincorporating tRNA genes, making what might be termed a transgenic "error mouse") causes a premature aging phenotype.

Until recently it was probably fair to say that the aging field lacked depth but had breadth to spare. Getting to grips with the primary literature is now impossibly time consuming, so a useful research tool for investigators who wish to understand human aging without having to undergo it remains Ed Masoro's multiauthored *Handbook of Physiology, Section 11: Aging* (1995). Very heavily referenced, at nearly 700 pages it provides an excellent guide to the primary biomedical literature regarding human senescence. Similarly, Caleb Finch's *Longevity, Senescence, and the Genome* (Chicago, 1990) is an awe-inspiring magnum opus and remains the ultimate resource for anyone interested in aging across the animal kingdom.

Against these classic publications, how do the newer works compare? Clark's *A Means to an End* in particular does not fare well. Although a valiant attempt to cover such a massive field, there are no major new insights or perspectives into the aging process, and most of the topics are covered with greater detail and quality elsewhere. Kirkwood's *Time of Our Lives*, as would be expected from a leader in the evolutionary field, is excellent on this topic. There are moments of unintentional humor, such as the anecdotal vision of a nude Tom Kirkwood prostrate in the bath where "it suddenly

dawned on me why aging occurs" (p. 63), a scene from the history of ideas over which a blind (or even a small flannel) could have been decently drawn. The book only really falters when it strays away from evolutionary matters, with the bathwater clearly cold by the time his thoughts turn to cancer biology and cell senescence. However, of the various books aimed at the wider audience, Austad's *Why We Age* (1997) is the one I would personally choose to give to a lay person or a graduate student new to the field. Despite an unfortunate hagiographic tendency, it is full of wonderful anecdotes and examples, and superbly conveys the intellectual excitement that fires many to work in this area.

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Metazoan Larvae: When, How, and Why

The Origin and Evolution of Larval Forms
Edited by Brian K. Hall and Marvalee H. Wake
San Diego, CA: Academic Press (1999). 425 pp. $79.95

During the second half of the 19th century, Carl Gegenbaur and Ernst Haeckel began research programs in Jena, Germany in comparative morphology, hoping to reconstruct the phylogenetic history of metazoans and to discern the processes that underlie their evolution. Gegenbaur focused on adult anatomy while Haeckel focused on development, having formulated his biogenetic law that ontogeny recapitulates phylogeny. Initially both men believed that their programs were complementary, but they eventually reached very different conclusions regarding the evolution of the forelimbs of vertebrates based on adult and embryonic morphological data, respectively. Their disagreement regarding whether comparison of adult structures or comparison of developmental sequences would allow the accurate reconstruction of phylogenies was taken up by their respective students in one of the most raucous and vicious debates in the history of biology. By the time the debate ended, most biologists were skeptical that either type of data would allow the reconstruction of phylogenies.

Now, after almost a century, research programs in developmental and evolutionary biology are undergoing a rapprochement spurred by the development of new methods of comparison (cladistic phylogenetics) and insights into the molecular basis of development. Not surprisingly, larvae are again playing an important role, as biologists explore Walter Garstang's axiom (Zoo. J. Linn. Soc. London *35*, 81–101, 1922) that ontogeny does not recapitulate phylogeny but creates it. In spite of the important roles that larvae have played in the origin and subsequent evolution of metazoans, it is amazing that biologists still can not agree on a definition of what larvae are, when they arose, how they evolve, and the nature of their role(s) in complex life histories. All of these concerns are explored in *The Origin and Evolution of Larval Forms*. It is fitting that this new work—which is almost certain to become as important as Gavin DeBeer's *Embryos and Ancestors*—should bear the same title as a presidential address delivered by Garstang in 1928 to the British Association for the Advancement of Science. The editors have wisely organized the book into three sections that basically address three main questions: what are larvae and when did they arise?; what mechanisms underlie larval development and evolution?; and what roles do larvae play? In addition to these three main sections, a short introduction outlines the major questions that are addressed, and an epilogue provides an excellent summary of the book, as well as suggesting future directions for research on larval origins and evolution.

The difficulty of defining what constitutes a "larva" is exemplified by the staggering diversity in developmental stages and sequences that occurs among marine invertebrates. Carole Hickman notes in her chapter that it is possible to generate structural, ecological, and morphogenetic regulatory definitions of a larva. She concludes that a structural definition best facilitates analysis of larval development and evolution: "a structural state or series of states that occurs between the onset of the divergent morphogenesis following embryonic development and metamorphosis to the adult body plan." While invertebrate larvae have usually been grouped into a small number of "types," she notes that no classification scheme even comes close to recognizing the immense structural diversity that characterize marine invertebrate larvae and that many of the larval "types" recognized actually represent grades of evolution that have been reached independently a number of times (homoplasy). In spite of this diversity and rampant homoplasy, the larvae of many invertebrate groups do share a sizable number of shared derived characters, which suggests that indirect development, involving one or more larval stages that go on to metamorphose to produce an adult, arose very early in metazoan phylogeny. The discovery

of Lower Cambrian phosphatized eggs containing metazoan embryos (Bengtson and Zhao, Science *277*, 1645–1648, 1997) has, however, been interpreted as supporting direct development (i.e., a developmental sequence that does not involve larva or metamorphosis). In contrast, Davidson et al. (Science *270*, 1319–1325, 1995) have speculated that the earliest metazoans consisted of microscopic multicellular organisms whose organization was comparable to that of modern metazoan larvae. In their view, the origin of set-aside cells or an imaginal rudiment in these organisms represented a key evolutionary innovation that was necessary for the generation of a new adult phenotype. As Hickman notes in her chapter, the Late Precambrian fossil record is potentially the final arbiter of the nature of the earliest metazoans and whether they were direct or indirect developers.

Although the nature of the earliest metazoans is unknown, James Hanken concludes in his contribution that free-living aquatic larvae represent an ancient feature of amphibians and that variations in reproductive modes and life histories have allowed modern amphibians to adapt to a wide range of environments. In spite of the importance of the role played by larval adaptations among modern amphibians and the wide use of a few species as developmental models, Hanken notes that the embryonic derivation of either larval or adult features is poorly known. Jacqueline Webb points to a similar situation concerning fish larvae, whose even greater diversity of larval adaptations promises to provide considerable insight into the origin of morphological novelty.

Many biologists have suggested that heterochrony—i.e., changes in the relative timing of events in the development of an ancestor and its descendant—is the single most important, if not the sole, mechanism responsible for changes in life histories. Michael Hart and Gregory Wray, however, emphasize the importance of distinguishing between heterochrony as a pattern and heterochrony as a process. They and several other contributors suggest that many larval changes reflect a heterochronic pattern but that this fact provides no insight into the mechanisms of larval origin and evolution. They conclude that mutation, recombination, drift, migration, and selection are the mechanisms responsible for larval evolution as they are for other phenotypic changes in all organisms.

Christopher Rose also examines the role of larvae in the evolution of modern amphibians and convincingly demonstrates that most structural variation is generated by changes that affect the timing and duration of larval development, which is primarily under the control of thyroid hormone. Hormones also play a major role in the development of insects, but, as Frederik Nijhout demonstrates, these hormones (ecdysteriods and juvenile hormones) only function in an all-or-none fashion. Because there are multiple short "windows" during which specific tissues will react to these hormones, however, there are myriad possibilities for changing life histories and body parts independently, generating a vast range of organismal diversity.

Historically, comparative studies of cell lineages within and between closely related species have provided some of the most important insights into how embryos and larvae develop and how ontogenies change over time. While many interspecific cell lineage fates are conserved, some have changed dramatically. Until now, there has been little information regarding the nature of the genetic changes associated with cell lineage changes. Rudolf Raff provides considerable insight into this phenomenon by examining changes in cell lineage fate in one species of indirectly developing sea urchin and in one species that has evolved a derived pattern of direct development. In this particular example, many and perhaps most genes are conserved, but their pattern of expression has changed greatly. The example also suggests, as Raff notes, that many features, such as indirect development, have not been conserved due to developmental constraint but because selection may act to maintain a suite of features necessary to produce a feeding larva.

Although echinoderm development utilizes cell lineages to rapidly assemble a feeding larva, insect development is characterized by dissociation of cell lineage from cell patterning. Insect embryos are essentially a cellular syncytium in which groups of nuclei obtain patterning information via domains of gene expression whose positions are determined by molecular gradients within the egg. Lisa Nagy and Miodrag Grbic suggest that groups of cells under the control of a gene network (gene expression domains) can be viewed as an analog for the traditional concept of cell lineage. Because marker genes have been identified for many gene expression domains whose developmental fate is well established, it should be possible to compare the activity of these gene networks between species to see how cell fates are established and how these networks evolve. They explore the utility of this strategy by identifying patterns in insect life histories and attempting to show how new morphologies (larval stages) can emerge by the shuffling of old gene networks.

The final section (larval function, morphology, physiology, and ecology) is surprisingly brief, but is a clear indication of how little is known about larvae as functioning animals. Laurie Sanderson and Sarah Kupferberg lay the groundwork for assessing the feeding behavior of larval fishes but are clearly frustrated by the paucity of functional studies, a critical first step in identifying changes in behavior and their underlying mechanisms as part of a phylogenetic analysis.

In a final provocative chapter, Erick Greene clarifies the types of phenotypic variation and explores how this variation is maintained over time and its role in speciation and evolution. Greene and the other contributors to this volume make a strong argument that broadly based comparative studies that integrate developmental, ecological, genetic, and functional morphological data are our best hope of understanding how ontogenies change over time. This integrative and organismal approach forms the core of the emerging field of evolutionary developmental biology which is well served by this volume.

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The First Four Billion Years

The Molecular Origins of Life: Assembling Pieces of the Puzzle
Edited by André Brack
Cambridge: Cambridge University Press (1998). 417 pp. $85.00 (hard), $34.95 (paper)

The Touchstone of Life: Molecular Information, Cell Communication, and the Foundations of Life
By Werner R. Loewenstein
Oxford: Oxford University Press, (1999). 366 pp. $30.00

The Origins of Life: From the Birth of Life to the Origin of Language
By John Maynard Smith and Eörs Szathmáry
Oxford University Press (1999). 189 pp. $25.00

These are three very different books. The *Molecular Origins of Life*, edited by André Brack, is a straightforward collection of essays intended for a strictly academic audience. Brack rounds up many of the usual (expert) suspects to summarize what we know, or would like to know, about the earliest history of life on earth. There are chapters devoted to each of the key topics: the early atmosphere (maybe reducing, maybe not); prebiotic synthesis (conceivably terrestrial, but more likely extraterrestrial due to bombardment by comets and micrometeorites); early metabolism (surface-catalyzed iron-sulfur chemistry and thioesters might be important, synthesis of membrane lipids is still problematic, the precursor of RNA-based genetic systems—if indeed there was one—is still unknown); discrepancies between the paleobiological record and phylogenetic deductions (paleobiology will always be the "court of last resort" because it generates hard evidence and hard numbers); and last, but not least, the search for extraterrestrial life (this is the ultimate control experiment—why did life evolve on earth but not on Titan, Saturn's largest satellite, or Mars where conditions may have been almost right?).

The *Molecular Origins of Life* is not full of surprises, but it is a good reference book for those seeking down-to-earth up-to-date facts and theories without a trace of popular hype. As a disinterested molecular biologist, and an avocational contrarian, I was especially amused to read in the chapter by Stanley Miller that deep sea thermal vents—often portrayed as the cauldrons of life—are so hot that any useful organic compounds would almost instantly be destroyed by the heat ("submarine vents do not synthesize organic compounds, they decompose them").

The *Origins of Life* by John Maynard Smith and Eörs Szathmáry is a superb read—expansive in scope, rich in ideas, light in touch, admirably brief, and equally appealing to lay readers and hard-nosed professionals. The subtitle "From the birth of life to the origin of language" provides ample warning that the authors are either brave or foolhardy, wise or simple. Happily, the authors are brave, wise, and also gifted teachers. Once every few pages, I had to put the book down and let the full implications of a new idea sink in. One of my favorite new ideas emerged in the discussion of the Just-So-Stories we tell ourselves to explain how the urkaryote got its organelles. One problem with the usual endosymbiont hypothesis is that "before a symbiont producing ATP would be of any use to its host, some means of transporting ATP across the symbiont membrane would be needed" (p. 77). Smith and Szathmáry go on to explain that no such difficulty arises with the proposal of Martin and Müller (1998) that the original eubacterial endosymbiont generated H_2 and CO_2 which could be metabolized by an archaeal host. The obvious virtues of this scheme are that gaseous nutrients would freely diffuse from endosymbiont to host, finessing the need for a transport system, and an archaeal urkaryotic host would explain why eukaryotic information-processing machinery looks more archaeal than eubacterial.

This critique of the conventional endosymbiont hypothesis illustrates one of the virtues of the book. Whether the authors are right or wrong, it is almost always fun to watch them thinking and wondering out loud, venturing hypotheses instead of pontificating, and trying to make sense out of this amazing universe we live in ("It is pleasing when peculiar and otherwise baffling facts such as these make sense in terms of a theory that was developed in ignorance of them" [p. 92]). The joy of thinking is particularly evident in the wonderful chapters on "The origin of sex" (geneticists have yet to agree on why sex is good for us), "The origin of many-celled organisms" ("multicellular organisms never have single-celled descendants" because there is "no way back"), and "The origin of language" ("It is especially lucky that eyes of intermediate levels of complexity still exist in animals; reconstruction of the evolutionary history of the eye is an easy task compared with that of the 'language organ': language not only does not fossilize, but there are no living intermediate forms either." [p. 154]). On the other hand, it must be said that a few chapters—for example, the ten brief pages entitled "From the RNA world to the modern world"—are not strong, but it is the scope and tone of this book, not the bare facts, that recommend it.

The Touchstone of Life: Molecular Information, Cell Communication, and the Foundations of Life by Werner R. Loewenstein is a very ambitious book, but the beauty of the central insight—the fundamental equivalence of Shannon's definition of information and Boltzmann's definition of entropy—is marred by purple prose, long-windedness, and a fatal compulsion to develop a Theory of Everything. Ironically, the delightfully slim volume by Smith and Szathmáry, also under review, is a deliberate popularization of a much longer take-no-prisoners academic treatise by the same authors (*The Major Transitions in Evolution*, 1995). My recommendation is that Loewenstein take a cue from Smith and Szathmáry, and rewrite his ponderous, almost unreadable tome as a brief inspirational volume—perhaps entitled *Information Flow in Living Systems*—patterned in style and spirit on Schroedinger's *What is Life?* The barely elaborated central insight would be more powerful if room were left for each reader to apply the new viewpoint to his or her own favorite biological problems. Instead, Loewenstein

attempts to have the very last word on every possible topic. I started to count pages. Less can be more.

The book is hard to summarize. I will begin with a gripe, move on to some praise, and conclude with another gripe. The first gripe concerns the writing. In contrast to the unremarkable academic prose in *The Molecular Origins of Life*, or the simple transparent prose in *The Origins of Life*, *The Touchstone of Life* is written in a strange amalgam of perfect colloquial English and Old High German. Some of the more distracting hybrids were:

"An old clung-to notion thus was stung to the quick" (p. 43).

"If we look through a higher lens, we see where the shoe pinches the toe" (p. 98).

"What about errors? Their lack hits one still more in the eye than the lack of ambiguity" (p. 145).

Although I am as willing as the next fellow to wade through thick swamps of prose to discover the dark secrets of the intellectual jungle, I often found myself in over my head:

"It is that that (sic) one may hope one day to find the stationary states which are giving the slip to the present unifying physics theories, the states which may allow us to understand whatever it is that underlies the very nature of matter—all matter, including the biological one. Alas, it may take a while until that unifying sermon will be preached on the streets of Gotham" (p. 333).

Overreaching for lively prose can also have impolitic consequences. For example, Loewenstein introduces a metaphorical character called "Lady Evolution" (evolution may be female, but she is certainly not a lady) and entitles a subsection of the book "The Mistress We Can Live Without" (the mistress, it turns out, is none other than Teleology personified). As Auden said of one of his early poems, "It would have been bad enough if I had ever held this wicked doctrine, but that I should have stated it simply because it sounded to me rhetorically effective is quite inexcusable" (Foreword to the *Collected Shorter Poems, 1927–1957*).

Now for the praise. Loewenstein argues that we should try to resee all living systems and indeed the entire biosphere not as the conventional flow of thermodynamic (Gibbsian) free energy, but as the flow of information. And by information Loewenstein decidedly does not mean anything as pedestrian as the mere genetic information that flows from DNA to RNA to protein. Instead, Loewenstein begins by pointing out that Shannon's classic definition of information $I = \Sigma\ p_i \log_2 p_i$ (where p_i refers to the number of subsets of the system) and Boltzmann's definition of entropy $S = -k \Sigma\ p_i \ln p_i$ (where k is Boltzmann's constant in calories/°C) differ only by a scaling factor and a sign. Thus I and S are flipsides of one reality: information is a measure of order (which living systems accumulate and transmit), entropy a measure of disorder (the price living systems pay for staying alive), and both I and S can be quantified by describing the distribution of the components of the system over the states available to it. The big question is, How far can you take the notion of information in biology before the notion starts taking you?

Loewenstein's thesis echoes the criticism of Watson and Crick (quintessential molecular biologists) made by Barry Commoner (ever the integrative biologist) in the pages of *Nature* more than 30 years ago (Commoner, 1968). In response to widespread claims that DNA was the "master molecule" of life, containing all the information required to build another cell, Commoner countered with ancient wisdom, going back to Virchow, that only preexisting cells give rise to new cells. DNA means nothing without a preexisting cell to interpret and replicate it. Admittedly, molecular biologists at that time tended to view cells as the grand result of an elaborate self-assembly process, not unlike assembly of bacteriophage particles. In this sense, the existence of genetically encoded macromolecular parts was nearly tantamount to assembling a living whole. Commoner defended a more inclusive definition of genetic inheritance, embracing cellular architecture and the intracellular flow of molecules as equally legitimate, albeit nondigital, forms of genetic information. In a nutshell, DNA replicates but cells do too, and just as it takes DNA to make more DNA, so it takes a cell to make another cell. Q.E.D. Cells must contain other forms of information besides DNA.

Although much of the quantifiable information in cells may at first appear to be static (especially cell architecture as glimpsed through the microscope) almost all of this information, except the actual sequence of DNA, corresponds to continual motion: macromolecules moving every which way, small molecules establishing gradients, ions flowing, G proteins undergoing conformational changes in response to GTP hydrolysis, transmembrane receptors changing oligomerization state in response to ligand binding, etc. Similarly, subcellular structures are constantly rearranging, dissolving, and reassembling. One has only to think of vesicle traffic between lamellae in the Golgi stack, cell movement in response to cytokines, or assembly and disassembly of the mitotic apparatus. Loewenstein rightly argues that all of these states of the living system are information-rich; moving between states rearranges or creates information while consuming energy and generating entropy.

Three major areas where an informational rather than a Gibbsian view of life may be particularly apt are transcriptional regulation, signal transduction, and cell cycle progression. Combinatorial control of transcription, cross talk between signaling pathways, and cell cycle checkpoints are often described in the literature—borrowing the language of computer science—as integrative (perhaps integrated!) circuitry, able to combine many diverse inputs into a single practical output. This is biological computing at its best, and Loewenstein argues that macromolecular switches (G proteins for example) and microelectronic switches (transistors on the motherboard) are fundamentally similar. In each case, the switch exists in two (or more) states; switching back and forth between states consumes energy and generates entropy, while transmitting or creating information.

My second gripe is more serious. Although the two states of a G protein may formally resemble a transistor in action, the nature of the information storage, and the detailed thermodynamics of the two states, are profoundly different: one switch is a macromolecule composed of 3,000 covalently attached atoms, the other is a microscopic sandwich with a handful of electrons moving through a doped silicon wafer. Similarly, a wave of depolarization traveling down an axon may be formally analogous to electrons flowing through a copper

wire, but the physical nature of these two processes is so different that the analogy does not readily suggest new biological experiments or new theories of neural function. Apparently, there is little in biology that cannot be formally viewed in terms of the acquisition, maintenance, replication, transmission, and degradation of information. This allows Loewenstein to bend much of biology to his purpose, exploiting the information metaphor as a rhetorical device to construct popular accounts of many interesting tales in modern biology. The metaphor is often invigorating, but it does not seem to be intellectually or experimentally useful in the long run. As Smith and Szathmáry put it in *The Origins of Life*, "The point of analogies of this kind is not that they are true but that they suggest questions to ask, and predictions to test" (p. 106). Loewenstein's insistence on viewing all of nature as the flow of information ultimately fragments biology instead of unifying it. The natural world cannot be fully appreciated through a kaleidoscope.

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Origin of Life and RNA; Achievements and Disillusions

The RNA World, Second Edition
Edited by Raymond F. Gesteland, Thomas R. Cech, and John F. Atkins
Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press (1999). 709 pp. $129.00

How to produce a book of superb scientific quality for a broad audience such as the readers of *Cell*? Here is the recipe: First, base the project in a publishing company (fostered by a scientist who in Japan would be declared as a living national monument) with an excellent record of timely books on really hot topics within the biological sciences. Second, select as the general unifying theme a central unresolved question of biology. Third, have a team of editors who are enthusiastic and work at the forefront of this area. As a result of this combination the editing should be easy; the editors should have no difficulty in convincing the researchers most competent in their areas to contribute chapters, and the remaining editing effort should boil down to bringing the reviews into an optimal sequence and to introduce some cross-referencing between the articles.

This is at least the impression of a reader who has read all of the 24 contributions arranged under the three slightly forced and enigmatic section headings "The origins of RNA and RNA at the origin," "How to build a functional RNA" and "Transition to the RNP world." Since the editors have refrained from eliminating redundancies, all chapters can be read independently as self-contained entities.

As a warning it must be mentioned that the comprehensive reading of the book is quite time-consuming even if one scans relatively rapidly through the impressive tables and the very informative figures, some of which are arranged into an appendix. The book contains a wealth of information in a condensed form and thus, for some years to come, will constitute a gold mine of bibliographic value even for those specializing in the various areas that are covered. Due to the independence of the chapters as mentioned above, a comprehensive reading is not required. Nevertheless, I suspect that the temptation for an integral reading and thus the probability of a considerable expenditure of time is great for anybody interested in the mysteries about the origin of life and molecular evolution, be it for personal curiosity or for the classroom. (Many of the illustrations will be invaluable for teaching purposes.) Although many of the reviews, and in my opinion those that are most rewarding and novel, are only rather loosely connected with these mysteries, the enthusiasm of most contributors in treating their subjects is so great and most of the subjects considered are so central to the current understanding of the properties of RNA that it appears rather difficult for anybody not to be drawn into the reading of chapters even with purely structural and biochemical contents, such as "The interactions that shape RNA structure," "The role of metal ions in RNA biochemistry," or "The RNA folding problem."

How does this second edition compare to its predecessor? Although the first edition dates back only six years, and the single new editor to join the crew is Thomas Cech, the second edition is essentially a new book. This is mainly due to the fact that during this time so many new insights into the properties of RNA have accumulated. The first volume was produced with great enthusiasm related to the then relatively recent discovery of ribozymes; it emphasized speculations regarding the origin of life in an RNA world as defined by Walter Gilbert in the mid-1980s. In contrast, the present book, while retaining much of the enthusiasm, emphasizes the many remaining and newly emerging difficulties to understand the origin of life and deals mainly with the RNA world of today as revealed by the hard work of specialists dissecting the physical and chemical properties of the myriad of different extant RNAs, either occurring naturally or produced by selection using sophisticated in vitro Darwinian evolution experiments. Probably most contributors to the book will agree with me that the accumulated wealth of new data, although showing a surprising variety of previously unknown properties of RNA, also define its chemical limitations in comparison to peptides and thus render the formation of even the simplest replicating entity based exclusively on RNA extremely unlikely. As it happens almost invariably, more knowledge provides some answers but results in more new questions. Was there a more readily arising replicating entity before RNA, as has been speculated by many,

or did life start from a complicated mixture, a sort of RNP world containing RNA and peptides together, and possibly other small polymers as well? One lesson (of many) seems to emerge from the book: it does not help to postulate additional reactive side chains in the nucleotides of the first replicating polymers or to suggest that these polymers arose from chemically less demanding nucleotides. In other words, any entity endowed with genetic capacity (i.e., the ability to transmit information to descendants) must be simple and requires additional inorganic and organic components supplementing catalytic properties to allow the replicating polymer to come into being.

It is impossible to properly appreciate, within the limits of a book review, a volume of such wide-ranging scope that deals with so many seemingly loosely interconnected subjects. To give an idea of its content it is probably best to relate some of the 24 chapter titles, in addition to those given above: (1) Before RNA and after: geophysical and geochemical constrains on molecular evolution; (4) Probing RNA structure, function, and history by comparative analysis; (9) Introns and the RNA world; (13) Building a catalytic site using only RNA; (17) RNA recognition by proteins; (19) The growing world of small nuclear ribonucleoproteins; (22) RNA editing—an evolutionary perspective; (24) Dynamics of the genetic code.

Are there negative aspects to be mentioned? If one properly sets out to find a hair in the soup, one could note that in a few chapters the authors rather excessively refer to their own previous papers, which can be annoying despite the fact that the ideas previously proposed are intellectually very stimulating. An example of this can be found in the chapter reporting the so-called genomic tag hypothesis; incidentally, this is the only chapter which, in only marginally different form, was contained already in the first edition. Nevertheless, since the hypothesis is still interesting, it is appropriate to include the chapter again.

Other remnants from the first edition, reprinted without any changes, are the foreword by Francis Crick and the prologue by James Watson. The prologue remains interesting as it reports on the birth and the very early days of molecular genetics and provides a vivid picture of the scientific and personal endeavours of the first players within the new discipline. The foreword might be found instructive inasmuch as it essentially consists of a description of the chapters found in the first edition; thus, this foreword might indicate to young scientists (who may not have had a chance to read the first edition) how much has happened during the last six years in the laboratories dedicated to unravelling the wonderful properties of RNA. All in all, a great book!

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Chromatin Structure and Function

Chromatin: Structure and Function, Third Edition
By Alan Wolffe
San Diego, CA: Academic Press (1998). 447 pp. $79.95 (hardcover), $39.95 (paper)

Chromatin structure and function have grabbed the headlines this past year. Essential new roles for the four core histones—which are among the most abundant proteins in the eukaryotic nucleus and have been intensively studied for decades—are being reported weekly in this and other high profile journals. Remarkably, the role of the "linker histone" H1—which is also among the most abundant proteins in the eukaryotic nucleus—is growing increasingly *less* clear at a similar rate. Past assertions that chromatin structure was irrelevant to the problem of gene regulation, or that chromatin provided a uniformly repressive (and therefore insignificant) effect, overnight seem to have become quaint relics—or perhaps wishful thinking, now regrettably obsolete: it will be much harder to understand gene action at the molecular level when the "substrate" is chromatin, with its hierarchies of ill-defined and intractable structures, rather than naked DNA. Recent advances have come from many directions; perhaps foremost among these are the increasingly numerous discoveries from biochemical and genetic studies that reveal that particular gene activator (or coactivator) and repressor (or corepressor) proteins actually function (somehow) through chromatin. These discoveries raise big new questions that are certain to fuel even bigger advances in the future. This is a rich field.

As investigators discover that the regulatory factors they have been studying actually act on or through chromatin they will also discover that there is a tremendous literature on chromatin structure and function that has suddenly become relevant to their studies. These investigators, among others, will be grateful for the publication of the updated new edition of Alan Wolffe's book, *Chromatin: Structure and Function*. The first edition of this book appeared in 1992, with ~170 pages of text and ~600–700 references, and immediately proved useful to researchers in the field. The latest edition covers the same broad topics—chromatin structure, chromatin and nuclear assembly, and how nuclear processes occur in chromatin—but doubles the length of text and more than doubles the number of references to give a more comprehensive and, especially, up-to-date treatment that does justice to the remarkable progress made in this field.

This book may best be appreciated as an integrated collection of in-depth review articles comparable in scope, for example, to 15–20 *Annual Reviews*–style articles, that together address many of the most important discoveries and key ideas for future research. It does an excellent job summarizing and explaining what many investigators currently think. It is appropriately critical as useful review articles should be: for example, Wolffe frequently (and appropriately) notes when a widely cited experiment may have an alternative interpretation or may be susceptible to artifact. The book also does an

excellent job of highlighting specific unresolved questions. One can literally pick a page at random and be almost certain to find mention of a specific question that is worthy of future study. Other valuable aspects of the book include its clear focus on important biological questions and Wolffe's astonishing command of the literature. These aspects make the book particularly valuable for several distinct groups of readers. In addition to new investigators wishing to be brought quickly up to speed, students will benefit from the book's focus on the broader biological questions that we seek to address, and established investigators in the chromatin field will be grateful for the extensive and up-to-date references—which help remind us why we currently think in certain ways or believe certain ideas and will likely remind us also of important facts that we had forgotten.

What remains missing in this field is a unified theoretical understanding, for example, along the lines of Ptashne's *A Genetic Switch* (Cell Press and Blackwell Scientific Publications, 1986). Perhaps the problem of eukaryotic gene regulation is so complicated that any real understanding will of necessity incorporate thousands of disparate facts. Or perhaps there are simple unifying truths that we have not yet managed to recognize.

Most likely we are still missing critical pieces of the puzzle. In addition to the biological questions highlighted in this new book, there remain numerous fundamental mechanistic questions that seem likely to be at the heart of the problem of gene action but that remain largely unexplored. One such example is the question, Is gene regulation under equilibrium or kinetic control? Progression through the cell cycle and through development implies an arrow of time; hence, regulation must in one sense be under kinetic control. But our present understanding of gene regulation in prokaryotic and bacteriophage systems such as the *lac* genes of *E. coli* and the life cycle of phage λ is largely one of equilibrium. Phenomena such as the response to addition of an inducer of the *lac* operon or to induction of lysogenic phage λ are understood as representing transitions from one distinct set of chemical equilibria to another in which there may be changed affinities, changed concentrations of key molecules, and changes in which states are accessible to the system and thus able to participate in regulatory decisions. The situations both before and after the transition are understood in terms of their distinct chemical equilibria—with no regard to the particular sequence of events taking the system between states, such as whether a repressor or a polymerase happens to bind first. That is, the regulatory apparatus is presumed to be in dynamic physical equilibrium, with microscopic chemical events (e.g., binding and release of a repressor molecule) occurring rapidly in comparison to the timescales of regulatory decisions. In this context, can it be that regulatory decisions during eukaryotic cell differentiation are established by a "winner take all" outcome of a race between binding of transcription factors and nucleosome formation—as many investigators evidently imagine? Or should developmental outcomes instead be understood as arising from the changing balances of changing chemical equilibria? Are regulatory complexes long-lived, so that they simply do not participate in dynamic chemical equilibria? This is certainly possible, but would represent a substantial paradigm shift from our understanding of regulation in the better-understood prokaryotic systems.

There are many other big but underappreciated questions at the heart of gene action. For example, if one wishes to understand the level of occupancy that would be achieved by a gene regulatory protein (at equilibrium), one needs to know, among other things, the concentration of *free* regulatory protein that is available to bind to specific sites. Free concentrations are reduced by the ability of DNA-binding proteins to bind (albeit more weakly) to nonspecific sites, which are numerous: they could potentially start at every base pair in the genome. However, many or most of these potential nonspecific sites may be inaccessible. At present, we do not know, even to within several orders of magnitude, what is the effective concentration of nonspecific sites, hence we cannot estimate what levels of regulatory site occupancy are to be expected. To understand regulatory site occupancy one also needs to know, once a regulatory complex is established, whether the histones do, or do not, continue to compete with regulatory protein binding—and, if they do compete, is it as free subunits (perhaps dimers and tetramers) or as intact octamer? At present we are not even certain whether the histone octamer (or pieces of it) remain bound. In yet another level of complexity, recent data suggest that some or many gene regulatory proteins act at levels of chromatin structure above that of the individual nucleosome. Repression and activation may reflect a combination of actions at many levels of chromatin structure, each possibly having only modest quantitative significance but together yielding a large dynamic range of regulatory response. At present we lack even the most basic information about these levels of chromatin structure and dynamics that will be needed in order to understand the action of such regulatory proteins. For example, while it is clear that DNA target sites internal to nucleosomes remain accessible to exogenous proteins, we do not know whether this is also true for target sites in nucleosomes packed within condensed chromatin domains.

These are just a small sampling of the many big unanswered and underappreciated mechanistic questions that lie at the heart of the molecular basis of gene action. As we move forward in the future, uncovering more and more specific facts, it will also be important to consider these broader mechanistic questions, and to strive to unify the thousands of relevant but disparate facts into a real understanding. Perhaps the chief significance of this new book is that it directly raises some of these larger questions and paints a picture in which others come sharply into focus. This book and its subsequent editions will be useful for the foreseeable future.

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Protein: A Reading Diet for Experimentalists

Protein: A Comprehensive Treatise, Volume 2
Edited by Geoffrey Allen
Stamford, CT: JAI Press (1999). 305 pp. $128.00

Perhaps you have cloned a new gene. However, you know little about analysis of proteins, but would like to find out quickly the characteristics of the gene product. You want to be directed to only the most straightforward and informative methods, without having to sort through jargon, excessive detail, or techniques of limited utility. If so, *Protein*, a series of review books edited by Geoffrey Allen, may be an essential supplement to your reading diet.

Volume 2, reviewed here, describes physical methods and is comprised of seven chapters, written by experts in their respective fields. The topics include hydrodynamic measurements of size, stability and folding studies, covalent modification, electrostatics properties and calculations, analysis of bound metal ions, and x-ray crystallography. The articles are short, to-the-point, and highly accessible. State-of-the art techniques are presented. References to the primary literature are emphasized and include time-tested classics and the best-accepted, recently published methods and applications. Descriptions of underlying theory are for the most part simultaneously simple and rigorous. In short, this is an excellent review book, for principal investigators and students alike. The volume could be used effectively as an auxiliary text in a graduate biophysics course.

The state of oligomerization of a protein is often one of the first pieces of physical data we need. Stephen Harding contributed a treatise on size and shape measurements of proteins. Molecular weight determinations using gel filtration and light scattering are described. The body of the treatise concerns hydrodynamic measurements of molecular mass and shape. It is stated clearly what problems can be approached with each technique. The relationship between observed and derived quantities, and the assumptions made in data analysis can be quickly grasped from the presentation.

Chemical modification of protein side chains is also an essential tool. The chapter included on this topic by Gary Means and colleagues is particularly good. The uses to which the modifications can be put in identification of active site residues and labeling for structural and dynamic studies is made clear through numerous references to the primary literature. The descriptions are succinct, and the chapter offers useful information on the reaction conditions to be used for each reagent and what side reactions can occur. Cross-reactivities are collated in a comprehensive reference table.

Of course, you will want to determine the three dimensional structure of your protein. The volume includes a short treatise on modern protein crystallography by A. Achari and D. K. Stammers. Described are the greatly improved methods we now have for structure determination using selenomethione derivatives and synchrotron radiation. By comparison with volumes 276 and 277 of *Methods in Enzymology*, this chapter provides a very short but up-to-date summary of the experimental methods and treatment of data. *Volume 1* of this series treats the topics of covalent and secondary structure of proteins in detail, as well as current methods of deriving structural information from multiple sequence alignments.

It is also often important to determine if metals are bound to our proteins, either as catalytic cofactors or structural metals. A wonderful description of ion-binding sites in proteins, by Jenny Glusker, is included in this volume. You will quickly gain an understanding of which amino acid side chains bind to which metals, what the observed geometries are and why. The most important known binding sites for the cations of magnesium, calcium, and zinc, and the anions sulfate and phosphate are clearly illustrated in schematic and stereochemical representations. This article should be a great resource to relate observations on a new ion-binding site to the existing literature. How to spot anions and cations in crystallographic electron density maps, and how to predict binding sites from clusters of charged residues in cavities in the protein structure is also presented.

A chapter by Norma Allewell and colleagues describes calculations and experimental methods relevant for the determination of electrostatic properties of proteins. The theoretical calculations range from simple estimation of pKa's from amino acid sequence, and the popular DelPhi program for calculations of the electrostatic field, to contributions of electrostatics to protein stability and ligand binding. Among the experimental approaches described are the use of NMR to carry out single-site pH titration curves, and the use of electrostatic properties in protein purification.

For those with a deeper interest in protein folding dynamics, a chapter by Alan Cooper addresses stability measurements. The relevant thermodynamic equations are treated simply but completely. The use of differential scanning calorimetry is described. You can learn the origin of the line shape, and how to extract the folding enthapies, entropies, and free energies from the data. The effects of disulfides, ligands, pH, denaturants, and osmolytes on folding stability are also discussed.

The review by Franz Schmid discusses two-state folding reactions. Several classic folding behaviors, their experimental observation, and the interpretation of data are described. The mathematical handling of denaturation data derived from fluorescence measurements or NMR is also described. Some of the most important modern work in protein folding is summarized. In particular, the Fersht laboratory's mutagenic analysis of folding intermediates and its interpretation with respect to secondary structure folding reaction intermediates are covered in some detail. Also, recent data on stopped flow measurements of fast-folding reactions are described. An especially good section describes how the heat capacities of the folding rate can reveal how well folded the structure is in the transition state.

In summary, many techniques that have been developed over the last several decades can now be incorporated into studies of new proteins, by laboratories interested more in the system than the technique. This volume is directed to that audience.

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Calcium—From Preconception to Postmortem

Calcium as a Cellular Regulator
Edited by Ernesto Carafoli and Claude Klee
New York: Oxford University Press (1999).
642 pp. $150.00

Everyone who is interested in cellular function knows that Ca^{2+} is, at the very least, one of the (and some would say *the*) key regulators of what happens inside a cell. Indeed, some years ago Jim Putney used to incorporate a joke slide into his lectures in which he showed a histogram with "importance" as its ordinate, and with a column labeled "Ca^{2+}" being much taller than the other column, labeled "everything else." Perhaps the most interesting chapter in this book is the first one, by Bob Williams, which explains *why* this is so. It was inevitable—once life began to evolve in the soup from which it emerged, "decisions" on the use of phosphate as a form of energy currency were almost unavoidable and were made final by its resultant incorporation into the backbone of nucleic acids. And once you have phosphate in a nonacidic compartment, there is no option—the Ca^{2+} has to be removed, or the incipient life comes to a precipitate end, quite literally.

It was this elimination of Ca^{2+} from the cytoplasm, allied with some other unique aspects of its chemistry (which Williams also outlines), that led to it becoming such a force in cellular regulation. Most cells are surrounded by an aqueous solution of about 2 mM Ca^{2+}; and they cannot make Ca^{2+} or break it down, but can only put it into, or remove it from, different places. The myriad of ways in which cells do this, and the consequences of this movement of Ca^{2+}, are what this book is about. It deals entirely with eukaryotic cells, and their evolution probably coincided with or caused a further reduction in resting free intracellular Ca^{2+} from above 1 μM to around 100 nM, and also the appearance of a variety of intracellular compartments into which Ca^{2+} could be sequestered. Of these compartments (stores), the most important in actually regulating cytosolic Ca^{2+} is the endoplasmic/sarcoplasmic reticulum (probably the vacuole in higher plants).

Intracellular Ca^{2+} stores are a truly remarkable invention. Not only have they the advantage of being a way of "spreading" or localizing precisely a Ca^{2+} signal, but they also have the huge asset that they are, by their very nature, finite. Ca^{2+} is as deadly as it is useful—for example, too much Ca^{2+} gated into the cell is probably the main trigger of neuronal death that follows a stroke. David Clapham (Nature *375*, 634–635, 1995) once drew a rather nice analogy of a cell and its relationship with Ca^{2+} as resembling being inside an enormous tent during a heavy rainstorm. If the tent has not been well pitched, so it does not have a taut roof, a huge great pool of water will accumulate in a "bulge" of canvas. If you stand under that bulge you would be very well advised not to touch the canvas or, even worse, stab it with a knife. The cell is in a similar position vis-à-vis Ca^{2+}—there is a 10,000-fold higher concentration outside than inside. So, to extend Clapham's analogy, how do you get a drink of water if you are in this badly-pitched tent, without getting wet? The sanest solution is to have buckets of water distributed strategically around the tent's interior, so that you can get a drink when you want it, yet if you happen to knock a bucket over, you won't drown. The buckets are the intracellular Ca^{2+} stores. And to stretch the analogy one more time, the ideal way of getting water out of those buckets when and where you need it, is to send someone on a mission around the tent, telling him or her to open taps in the buckets wherever they are. That someone is inositol 1,4,5-trisphosphate (IP_3), and the evolution of IP_3 as a freely diffusible second messenger that can release Ca^{2+} anywhere the cell desires (simply by having the IP_3 receptors and Ca^{2+} stores in the right location) can be seen in this context as the ultimate refinement of an already extraordinarily sophisticated way of regulating events in the cell.

The other advantage of Ca^{2+} stores is that they can exhibit Ca^{2+}-induced Ca^{2+} release. This is a process whereby a small rise in Ca^{2+} is amplified by triggering release from the stores, and this enables cells to overcome the problem of the very limited diffusion of Ca^{2+} in the cytosol by generating Ca^{2+} waves (the poor diffusion stems from numerous Ca^{2+}-binding sites and sequestering mechanisms). These waves in turn gave cells the opportunity to refine and extend frequency-modulated (FM) Ca^{2+} signals. If you listen to your radio on FM and compare it with AM, especially at night, the much clearer reception on FM should convince you just how superior is FM—electronic engineers have rediscovered what evolution invented a long time ago. Ca^{2+} oscillations probably first evolved in excitable tissues as rapid bursts of cell membrane potential that resulted in pulses of Ca^{2+} entry through voltage-gated channels in the plasma membrane. (The speed with which Ca^{2+} can bind to and dissociate from specific recognition sites is another one of its crucial characteristics discussed by Bob Williams). Repetitive Ca^{2+} waves spreading through and between cells due to intracellular Ca^{2+} mobilization from stores is the way in which nonexcitable cells can generate Ca^{2+} oscillations, and these pulsatile Ca^{2+} waves are one of the most remarkable and exciting discoveries of the last decade.

All these aspects of Ca^{2+} are covered in the book under review here. In particular, the "reading" of FM Ca^{2+} signals by Ca^{2+}-controlled protein kinases is another crucial part of our understanding of how Ca^{2+} functions as a second messenger, which is particularly well covered in the chapter on CaM Kinases by Shulman and Braun. The list of proteins that recognize Ca^{2+} with high affinity and specificity is now enormous, and about a third of the book is devoted to them—the exact details of how an "EF hand" (the key to most Ca^{2+} recognition) actually binds Ca^{2+} is the subject of an entire chapter

by Slupsky and Dykes. Organelles themselves can of course also be major "targets" for Ca^{2+} signals, and the ways in which, for example, nuclei or mitochondria respond to Ca^{2+} signals and thus modulate their specialized functions (naturally, these organelles have their own intrinsic Ca^{2+} control mechanisms to ensure that they *can* respond) forms the basis for the thoughtful chapters by Stephen Bolsover and Dick Denton and their colleagues. It is also useful to find several chapters devoted to specific phyla (e.g., plants, yeasts, and fungi) or processes (e.g., apoptosis).

It is something of a truism to say that Ca^{2+} influences every part of an organism's life—"A life and death signal" as Berridge, Bootman, and Lipp called it (Nature, *395*, 645–648, 1998). It plays, for example, a major role in meiosis (which, from an individual's viewpoint is "prelife"), in the explosion in the egg that follows fertilization by sperm, in the patterns of development that govern the formation of the organism, in almost everything that organism does (need one go further than the central role of Ca^{2+} channels, intracellular stores, and Ca^{2+} target proteins in the functions of the brain and skeletal muscle?), and finally in the processes of apoptosis and necrosis that signal our end. If we consider extracellular events, on which this book only impinges occasionally (see below), Ca^{2+} also is crucial for the most long-lived (*pace* peat bogs) of our remains—our skeletons—and even, in the words of Tom Lehrer, of the "occasional pieces of skin" that accompany them; a "preconception to postmortem" signal is more like it!

So how well does the present book succeed in covering what in one way or another seems to be half of biology? It claims (probably correctly) to be the first to cover the whole Ca^{2+} story. Of course such a claim must fall short, and some parts are covered better than others. Anyone who actually works on a particular aspect of Ca^{2+} may well find that their bit is rather superficially covered, but that is inevitable—entire books have been written about topics covered by only a part of a chapter here, and inconsistent and patchy coverage is also inevitable (for example, compare the comprehensive and excellent coverage of calmodulin structure and action with the mere five pages about ryanodine receptors). Also, although the editors have confined the coverage mostly to intracellular regulation (with a few exceptions, such as an excellent chapter on the extracellular γ-carboxy-glutamate containing proteins, the most prominent being those in the blood clotting cascade), there is another whole field of Ca^{2+} research out there on the regulation of extracellular Ca^{2+} by vitamin D, parathyroid hormone, et al. (covered to some extent by Brown and coauthors) and its use in forming bones, teeth, and shells. But this is a book mostly about intracellular Ca^{2+}, and all the aspects of Ca^{2+} regulation and function I have touched on above, and many more besides, get an airing, mostly in well-written chapters. The editors are to be congratulated for very largely succeeding in an almost outrageously ambitious concept. Even the most ardent calciophile will find something to educate, amuse, or enlighten them in this volume because of its broad scope. Moreover, even in such a fast-moving field of research this book will stand for a considerable time as an excellent starting point for the uninitiated toward an appreciation of just what Ca^{2+} means to us all.

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A Plethora of Trees in the Cytoplasm: But Where's the Forest?

Subcellular Biochemistry—Volume 31: Intermediate Filaments
Edited by Harald Herrmann and J. Robin Harris
New York: Plenum Press (1998). 622 pp. $149.50

The cytoplasm of most vertebrate cells is structured by an array of protein polymers comprised of actin filaments, microtubules, and intermediate filaments. First identified as a primary component of muscle, the intrinsic polarity of the 4 nm actin polymer and an ever-growing series of myosins that use ATP to power movement along those filaments have long implicated actin as a key component in contraction, cell migration, and intracellular movement. So too for microtubules, whose organized arrays of 25 nm filaments provide the tracks for intracellular transport mediated by the kinesin and cytoplasmic dynein motor families.

Not so for the final filament class. Named not for its own apparent functional properties, but rather for the 8–10 nm filament diameter that is intermediate between that of actin filaments and microtubules, these polymers have long been seen as a static set of structural elements that, somehow, provide structural integrity to the cytoplasm. Feeding this view, the filaments are generally easy to isolate because they are so resistant, at least in vitro, to disassembly.

Using molecular genetics in mice and men, the last decade has demonstrated that intermediate filaments do provide flexible structural support, which in the case of skin cells and muscle cells can be essential to cell integrity and/or organization. Of course, it is not individual filaments that do this; rather, it is the array of intermediate filaments cross-linked (weakly and reversibly) to each other, to actin filaments and microtubules, or to components of the plasma membrane, that provides three-dimensional structural support to the cytoplasm. This was especially highlighted by discovery of the family of intermediate filament-associated proteins, including the 550 kDa plectin and the newest identified member BPAG1n/dystonin, both of which can form cross-bridges between intermediate filaments and actin and/or microtubules (T.M. Svitkina et al., J. Cell Biol. *135*, 991–1007, 1996; Y. Yang et al., Cell *86*, 655–665, 1996). Such linker elements have been shown to be essential components for cytoplasmic organization, as demonstrated initially in the case of plectin by human and mouse genetics and for BPAG1n/dystonin by genetics in mice. They are also likely to represent only the tip of the iceberg of such components (as a simple homology search of the current mouse and human gene libraries reveals).

To this observer, the last decade of discovery has thus provided a most satisfying view of the principles of cytoplasmic organization and the role that intermediate filaments play. But although the 20 chapters in this new volume *Intermediate Filaments* (edited by Herrmann and Harris) recount various aspects of the search for the properties of the family of intermediate filaments, they do not emphasize the central discoveries nor highlight the questions now at the forefront. Rather, as in many such multiauthored compendia, those wanting a thorough introduction to intermediate filament biology will have more than a little difficulty in finding the forest amid what is, by any measure, a plethora of trees. The 610 pages of heavily referenced, detailed accounts of specific aspects of intermediate filament biology (or methods to assess those properties) can serve as a refresher for those in the field, but the novice will very likely be lost in the wilderness.

To be sure, some of the key findings of the last decade are here: intermediate filaments really do play important and, in some contexts, essential roles. This is especially true for the keratins, the intermediate filament proteins of skin, where 85% of the dry mass of the fully differentiated layers is comprised of keratin bundles. Use of molecular genetics in mice and discovery of mutations in several keratin genes as the primary cause of human skin blistering diseases has provided unequivocal evidence for an essential structural role for these intermediate filaments. Overlapping chapters by Magin and Coulombe provide a summary of these discoveries. Magin focuses on the efforts in mice, providing a readable and comprehensive catalog; Coulombe adds a succinct and useful table summarizing the human keratin mutations and their associated diseases, with a subsequent focus on those keratins implicated in recovery from wounding.

Perhaps the most notable contribution is the chapter by Janmey and colleagues, which provides a very readable introduction to the biophysics of how protein polymers with different properties can combine to provide resistance to structural deformation. If you're one of those who can feel your eyes glazing over when you hear the seminar speaker say "rheology," this chapter can give you both a feel for why this is of interest in a cellular context and a readable introduction to the underlying physics. The chapter by Capetanaki can be recommended to provide a fine overview of the essential nature of the muscle intermediate filament subunit desmin in alignment of myofibrils (tethering adjacent Z bands) and in linking those fibrils to the overlying plasma membrane (an accompanying figure was particularly instructive in providing a digestible overview).

But these highlights not withstanding, three prime discoveries go almost unmentioned. First, the properties of intermediate filament–associated proteins such as plectin and BPAG1n/dystonin (and the likelihood of many others) in cross-bridging between filaments and other cytoplasmic or membrane-bound components get very short shrift. Second, the extreme stability of the assembled filaments has long suggested intermediate filaments to be hyperstable, something akin to petrified trees in a cytoplasmic forest. I remember back 20 years ago when microtubules were thought to be pretty static, too, only to learn (unequivocally) in the mid-1980s that microtubules were growing and shrinking at astounding rates. Efforts using fluorescently tagged subunits (particularly from Bob Goldman's group at Northwestern; K.L. Vikstrom et al., J. Cell Biol. *118*, 121–129, 1992) have demonstrated that intermediate filaments can be remarkably dynamic in vivo. But there is no consideration in this volume that assembly may be a dynamic process, nor of methods with which such problems can be approached.

Lastly, conditions long used for intermediate filament assembly in vitro have required highly nonphysiological conditions (e.g., dialysis starting from two extremes—8 M urea or extraordinarily low ionic strength). Those efforts and their use to identify the assembly intermediates and final filament lattice are presented with clarity, but what of the in vivo pathway? It will likely seem all too obvious to those outside the intermediate filament field that the in vitro efforts are probably telling us very little about the in vivo assembly pathway, except that we're missing some key concept or component(s). Neither this possibility nor thoughts on how to extend the effort in an in vivo direction are included.

So for whom can *Intermediate Filaments* be recommended? Not the novice wanting to quickly catch up on intermediate filament biology. Absent a unifying thread (and overview) to tie it into a whole, the heavily referenced chapters will best serve as relatively thorough summaries useful primarily to those already in the field, even if important overlying questions have been left unaddressed. At $149.50, I expect that few will feel the need to have it on their shelves.

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And Still It Moves (E Pur Si Muove)

Cell Behaviour: Control and Mechanism of Motility
Edited by J. M. Lackie, G. A. Dunn, and G. E. Jones
Princeton, NJ: Princeton University Press (1999). 346 pp. $75.00

Directed motion is one of the properties we most closely associate with living organisms. Biologists study cell motility because it is a widespread behavior, critical in biological processes ranging from embryonic development through wound-healing and immune system function. Its study demands a multidisciplinary approach, requiring that the investigator appreciate molecular structure and function, biochemical kinetics, mechanical forces, and the mathematics of self-organization of complex systems. But finally, most scientists enter this field because cell motility is endlessly fascinating to watch.

Several books have presented a coherent and unified account of the diverse processes that contribute to cell motility. One of the best is *Cell Movement and Cell Behaviour*, written by John M. Lackie in 1986 (Allen and Unwin); another excellent and more recent example is

Cell Movements by Dennis Bray (1992, Garland Publishing). This volume, proceedings of the 4th Abercrombie Conference on Cell Behaviour held in the fall of 1997, includes the voices of many authors and inevitably presents a fragmented and sometimes contradictory picture. This is more descriptive of the current state of the motility field than is the unified approach of the single-author volumes, and indeed is more descriptive of cell motility itself. Cell motility, like cell motility research, is a dynamic process that requires opposing tensions for function and fidelity. The organizers of the conference (and editors of the volume), Lackie, Graham Dunn, and Gareth Jones, have deftly balanced multiple viewpoints ranging from single-molecule characterization to tissue-level phenomenology.

Motility here is narrowly defined as migration of whole eukaryotic cells over solid substrates, separate from swimming and intracellular transport. The back-cover blurb claims that, "This book describes the latest molecular and genetic advances in the study of...the movement of cells," but this statement is misleading. The greatest strength of the book is a clear editorial vision that molecular and genetic approaches are limited in their ability to explain the process of cell motility, that the success of the reductionist paradigm has been in setting the stage for "...embarking on the more formidable task of understanding how the functioning of these components is integrated and controlled (Preface, p. ix)." Solving this complex problem of cell motility will demand that cell biologists develop and refine new ways of thinking that incorporate the specificity of the molecular approach with an appreciation for whole-cell behavior and aspects of mechanical and systems engineering.

From Molecules to Movement. The 1990s has been a tremendously successful decade in the study of the molecular basis of cell motility. Ten years ago, a central problem in this field was understanding the enormous differences in kinetic behavior between purified actin in a test tube and actin filaments inside living cells. In the meantime, classes of proteins have been identified that account for most of the disparities (Carlier, Curr. Opin. Cell Biol. *10*, 45–51, 1998; Sun et al., Curr. Opin. Cell Biol. *7*, 102–110, 1995). Master regulators of actin organization have been identified in the small GTPases of the rho subfamily (Hall, Science *279*, 509–514, 1998). Advances in the fields of integrin- and cadherin-mediated adhesion have filled in most of the obvious molecular gaps in understanding how the actin cytoskeleton interacts with the world outside the cell, and how signals are relayed back and forth (Schoenwaelder and Burridge, Curr. Opin. Cell Biol. *11*, 274–286, 1999). While many details remain to be worked out, I believe it is fair to say that we have now identified most of the major classes of molecules directly involved in cell motility, a remarkable and satisfying state of affairs.

However, identifying the proteins that participate in cell motility has not taught us how cells move. The central problem now lies in understanding how the biochemical activities of these many factors are mechanically integrated over distances thousands of times the size of individual proteins to generate the emergent properties of whole-cell motility. Due to partially overlapping functions of numerous cytoskeletal components, this problem has proved difficult to address by classical approaches. In *Dictyostelium*, a haploid cellular slime mold strikingly similar to a mammalian neutrophil in its motile and chemotactic behavior, numerous actin-associated proteins and myosins have been knocked out one by one, generally with extremely subtle cell motility phenotypes. Substantial effects on motility appear only in double or triple mutants. This bane of "so-called redundancy" (Günther Gerisch's term, p.7) has forced an enormously useful rethinking of the meaning of individual protein function in a robust, multifunctional distributed network.

Complex processes most successfully elucidated by the genetic approach, such as pattern formation in developing embryos, are sensitive to single protein changes because they are governed by simple linear or branched pathways. Cell motility is an entirely different sort of biological self-organization. It must be robust, since the same basic motile apparatus is used in nearly all vertebrate cell types. But at the same time, motility must be exquisitely sensitive to variations in local environmental conditions to enable accurate pathfinding. Robustness and sensitivity can be combined in a distributed network where signal information is passed through multiple, highly branched, and partially overlapping pathways. The multiplicity of members in every class of actin-associated proteins appears to represent not multiplicity of distinct functions, but rather the number of individual nodes in the distributed network.

Sorting out the nodes is a complex task. Some researchers are finding ways to maximize the information gained from mutant studies. Bulk measurements of cell speeds such as wound-healing or filter transmigration miss many types of informative variation. A better approach is to carefully measure multiple aspects of motility for a large number of individual wild-type and mutant cells behaving under a wide variety of conditions, using semiautomated high-throughput analysis systems. Because there is a large amount of cell-to-cell, day-to-day, and subclone-to-subclone variation in motile behavior, comparison of multiple movement parameters in two groups of cells is quite likely to yield significant quantitative differences even if the two groups are genetically identical. Thus, sophisticated statistical techniques must be used to smooth out the enormous noise of random variation and properly differentiate real and accidental differences (M. Peckham et al., p. 281–299).

A completely different approach, not being used to any extent, would be to analyze the information lurking in cell-to-cell variability. Rather than thinking of this variability as noise to be eliminated from the system, we should embrace it as a rich source of data. Making mutants is just one form of perturbation analysis, where a single component in a complex network is perturbed in a well-defined way, but other types of perturbations can also be analyzed. Fluctuation analysis of the behavior of wild-type cells might enable us to understand the importance of environmental and stochastic molecular perturbations as well as genetic ones. Cell biologists will need to become systems engineers.

Oh, Behave! The complexity of the system in question is best appreciated by just watching cells move, and descriptive studies of cell behavior in different environments still yield surprises. For experimental simplicity and reproducibility, most work on eukaryotic cell motility

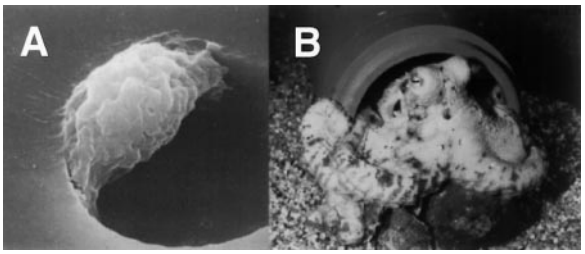

Figure 1. Cell Behavior and Animal Behavior

(A) Macrophage lurking at the opening of a 5 μm–diameter pit on a silica surface (from A. Curtis and C. Wilkinson, p. 19). In populations of macrophages cultured on a surface with microscopic pits, essentially every cell will crawl inside a pit. This complex cellular behavior is reminiscent of the well-characterized tendency of cephalopods to crawl inside of pots and jars.

(B) *Octopus vulgaris* lurking at the opening of a piece of PVC pipe (from J. W. Forsythe, http://www.nrcc.utmb.edu/images/img_cat.html).

has focused on individual cells crawling across flat solid substrates such as glass coverslips. The drawback is that very few cells in nature ever crawl across a plane of glass. Planar substrates, when encountered, tend to be irregular. Recent improvements in nanofabrication technology have enabled precise and reproducible observations of the behavior of cells encountering topographical features on otherwise flat substrates (A. Curtis and C. Wilkinson, p. 15–26). Virtually every fibroblast or macrophage cultured on a patterned substrate will align itself along a ridge or groove, or will crawl into a circular pit (Figure 1). It is not yet clear how cells sense and respond to the shape of features in their environments, though it presumably involves both adhesion and stretch receptors. Local mechanical information must be relayed to the rest of the cytoplasm for large-scale physical organization and to the nucleus for regulation of gene transcription. These pathways are likely to be as important in determining a cell's transcriptional status as the detection of soluble signals and growth factors; cells are, after all, physical entities as well as biochemical ones.

Most crawling cells in vertebrates actually move through three-dimensional environments. They influence the structure of the extracellular matrix as they move through it and are influenced by it, a two-way communication that is a major feature of tissue self-organization (R. T. Tranquillo, p. 27–42). In cell motility, the biomechanics of the third dimension are largely an unexplored frontier of enormous biological relevance to understanding cell behavior in vivo.

Cell behavior studies can be complemented by studies of protein behavior within cells. In the past few years, the most eye-opening advance in subcellular observation has been the use of fusions to GFP. Previous technologies made real-time observation feasible only for proteins that could be purified, covalently modified with a fluorescent compound, and microinjected; GFP has enabled behavioral descriptions of rare, fussy, and membrane-bound proteins in the wilds of their natural cellular habitat. In many cases, this has helped to redefine cellular morphological events (e.g., chemotaxis, phagocytosis) in molecular terms by identifying the order of recruitment of proteins to a newly forming structure (Gerisch et al., p. 1–14). It has also defined new types of cellular behaviors, protein reorganizations that may not have an immediately detectable morphological consequence (Merrifield et al., Nat. Cell Biol. *1*, 72–74, 1999).

Feeling Tense? One area where behavioral and molecular studies are coming together successfully is in understanding how tension and traction forces are generated during cell locomotion. In order to move across a solid substrate, a cell must be able to adhere tightly but transiently to the substrate, and generate traction force at the sites of attachment to pull itself forward. Advances in techniques for measuring force on a substrate (Oliver et al., J. Cell Biol. *145*, 589–604, 1999) combined with a variety of careful observations are allowing us to begin to understand how molecules cooperate in this integrated function. Assembly of adhesion structures is regulated by tension, both tension generated within the cell by contractile proteins and outside of the cell by the compressibility of the substrate. Intriguingly, it has been shown that essentially all motile cells generate much more force on their substrates than is necessary for their propulsion (Elson et al., 299–314). It seems likely that much of this force in vivo is used to remodel the environment, enabling self-organization of tissues.

For a cell to maintain its shape, contractile forces must be balanced by load-bearing structural elements. Since microtubules are the stiffest filaments in the cell, and microtubule depolymerization increases cell contractility, microtubule resistance to compression might be thought to balance acto-myosin contraction. However, given the known force required to buckle a single microtubule, there are a thousand-fold too few microtubules in the cell to perform this function (Elbaum et al., p. 147–172). If microtubules are not bearing compressive load, why does their depolymerization trigger cell contraction? Like adhesion complexes, they must playing a signaling role as well as a mechanical one. Recent work suggests that dynamic microtubules regulate actin cytoskeletal dynamics through the same rho family GTPases used to transduce extracellular signals (Ren et al., EMBO J. *18*, 578–585, 1999; Waterman-Storer et al., Nat. Cell Biol. *1*, 45–50, 1999).

Now that identification of the molecules involved in cell motility is approaching completion, we are in the position of really being able to address complex and interesting cell biological questions; how molecular function is integrated over such a large spatial scale to generate cell polarity and directional movement, how biochemical and mechanical molecular functions cooperate, and how the interaction of all these individual inanimate molecules gives rise to the emergent and dramatically lively behavior of whole cells. We can look forward to many more volumes in the *Cell Behaviour* series, and I hope they continue to maintain the uniformly high quality of the present one.

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Of Mice and Urchins: Toward a Holistic Approach to Developmental Biology

Cell Lineage and Fate Determination
Edited by Sally A. Moody
San Diego, CA: Academic Press (1998). 644 pp. $149.95

The field of developmental biology has made major gains in the last couple of decades, with fundamental insights gleaned from a variety of organisms and approaches. It has been especially striking to learn that the same general signaling and transcriptional mechanisms are used in organisms that are morphologically and phylogenetically distant, illustrating the fact that evolution works with a fairly limited set of tools to produce creatures that are stunningly different in design.

A major problem that affects this field is that most investigators only have the time to focus on their own organism, and often have difficulty appreciating the important issues in other species. Moreover, the development and morphology of different creatures vary in major ways that are hard to remember, and the terminology specific to each system typically represents a major difficulty.

Cell Lineage and Fate Determination is therefore a welcome step in the direction of breaking down the barriers between organisms. It succeeds in some aspects, and yet is less successful in other ways that represent an important lesson for developmental biologists trying to bridge the gaps between different systems. To begin with the positive, Dr. Moody has organized a series of essays on the development of nine model organisms. The book also includes five chapters devoted to the determination of specific tissues such as endoderm and muscle, which take a pan-species view of the formation of a specific tissue. These chapters are the most intriguing part of the book, as they demonstrate how results from different developmental systems integrate to provide fundamental insights into specific questions of cell type specification. To her great credit, Dr. Moody has not only included the major systems such as *Drosophila* and mouse, but has recruited essays in systems such as ascidians and the leech. While the weight given to particular organisms is arguably imbalanced, the inclusivity of this book is very welcome.

But one must also ask, what is the point of such a book? When approaching another species, one really wants to understand how the system develops, where the current research stands in relatively general terms (mostly at the levels of themes and models), and what major questions remain. This book attempts to deal with the problem by including a short introductory chapter at the start of each section on a specific organism. These chapters, written by an expert in each area, provide a very good introduction and overview of the field. These chapters are really the most important part of a book like this, and it would have been better if they were longer and more comprehensive. In contrast, the detailed essays could have been much shorter and possibly more plentiful, focusing on a specific point of interest brought out in the introductory chapter. Thus, the reader could gain a broad overview of the important issues in each field and then focus on specific areas of personal interest to obtain more details. As it is, the essays are often redundant with the introduction, since they were written before the introductory chapter, and cover too many different points.

There are several journals that publish review articles of the type found in *Cell Lineage and Fate Determination*. Reviews in these journals have the benefit of being published in a more timely fashion than the essays in this book, which will be almost a year old by the time this review is out. Thus, the principal advantage of a collection such as this is that it could help scientists at all levels learn about systems other than the ones they work on. And here lies a major problem with this book. While detailed explanations of gene expression patterns or cell lineage are of endless fascination when they apply to one's own system of research, they become distinctly less interesting and far more difficult to comprehend when one wants to understand the key questions in another organism.

A second issue is that the essays are heavy on text and therefore hard to follow if one's knowledge of the development of that organism is relatively naive. The book proclaims its use of color figures, but many of these figures are primary data that generally are not very useful for people who don't know the system well enough to interpret the results. Some of the authors clearly understood this issue and provided a fair number of explanatory and model figures, but I would argue that essays such as these should be far more figure oriented, a task which is now much easier with the advent of excellent computer drawing programs. As one example, Satoh provides a beautiful picture that shows the fate of blastomeres in the ascidian embryo from the 4-cell stage to the tadpole that readily allows any developmental biologist to understand how this relatively unfamiliar organism specifies cell fate. If the limitation is that it is too expensive to print many figures, especially in color, then perhaps a project such as this book might be better suited to a CD-ROM format.

Finally, one of the most interesting aspects of recent developmental biology is the interconnection of findings in different systems. While some authors do make the occasional attempt to mention other systems (and the final five chapters are a very deliberate attempt to work across species), one would really like to have pointers that connect between the different chapters. For example, Wnt signaling is described in essays on sea urchin, *C. elegans*, *Drosophila*, frog, zebrafish, and mouse, but it would not be readily apparent to the reader of one essay that there are some interesting other essays to read without looking up keywords in the index. Even then, using the index one would not make the urchin connection without knowing the details of the Wnt intracellular signaling cascade.

Most of the essays in this book are well written and will be very useful to people starting work in a new organism, or for researchers who would like to broaden their perspective in their field of interest or a related field that they know fairly well. Most developmental biologists will find at least several articles in this book that are well worth reading, though at $150 it is the type of book one would rather borrow than buy. But it does represent a very worthwhile attempt at breaking down the barriers

between different model systems, which is becoming ever more necessary as increased information accumulates. We can hope for future books of this type that include a larger number of more general essays, are more pictorially based, and work to connect the results found in different systems.

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Developmental Anatomy: The Beauty of the Wee Sleekit Beastie

The Anatomical Basis of Mouse Development
By Matthew Kaufman and Jonathan Bard
San Diego, CA: Academic Press (1999). 291 pp. $99.95

The anatomy of animals and their embryos reveals the intrinsic beauty of ontogeny and the evolution of body form. However, many consider the study of anatomy, even developmental anatomy, a dead, descriptive science of little interest to current biologists. In the poem by Robert Burns about the best laid schemes of mice and men, the central character, a mouse, is described as a wee, sleekit, cow'rin', tim'rous, beastie because it is in a panic as its orderly home has suddenly been disrupted by the farmer's plough. As we quickly approach a postgenomics era of mammalian biological research, it is the mouse that is disturbing the apparently complete subject of anatomy. Currently, there are only old and incomplete text resources available that describe mouse developmental anatomy (*The Mouse* by R. Rugh, 1968; *The House Mouse* by K. Theiler, 1972). Thus, the new *Anatomical Basis of Mouse Development* by Kaufman and Bard is a welcome addition to the textual resources available to laboratories studying the mouse.

The laboratory mouse is currently the primary mammalian species used in experimental biomedical research to understand human biology. Because of the mouse's direct relevance to human development and disease, we are witnessing an explosion of efforts to understand the genetic basis of mouse development and its physiology. Realizing this, the National Institutes of Health recently convened a meeting to set priorities for mouse genomics and genetic resources to facilitate this goal (http://www.nih.gov/welcome/director/reports/mgenome.htm).

This small eutherian mammal, *Mus musculus*, has been associated indirectly and directly with human culture over the millennia. The initial fascination with genetic variation in mice began with the mouse fanciers who for personal pleasure maintained stocks of variant mice with overt phenotypes such as attractive coat colors or neurological quirks. Mouse research efforts became formalized earlier this century with the development of genetically homogenous strains of inbred mice for cancer research. The subsequent pioneering efforts in embryo collection, transfer, and preimplantation in vitro culture led to experimental embryological manipulations to generate mouse chimeras. The study of teratocarcinomas and embryonal carcinoma (EC) cell lines that can differentiate into many diverse tissue types sparked the idea that tissue culture cell lines could incorporate into developing mouse embryos and possibly contribute to the germline. The successful incorporation of wild-type and mutant EC cells into mice certainly set the groundwork for today's popular embryonic stem (ES) cells and their genetic manipulation.

The development of the atomic bomb led to the initiation of studies in mice to examine the health effects of radiation. Genetic screens for radiation-induced mutations in the mouse resulted in new insights for gene function. It also became clear that chemicals could be exploited to mutagenize the mouse genome. Transgenic animals were first developed in the mouse, providing the means to study the activity of introduced genes in the context of the developing animal. Most recently we were amazed to learn that Cumulina is but one of many Hawaiian mouse clones derived from a variety of differentiated somatic nuclei. It is inevitable that animal cloning and genetic manipulation will soon be combined for novel studies of gene function.

Whereas homologous recombination (gene targeting) in mouse ES cells is a unique genetic tool to create precise genetic alterations, it is labor intensive and will probably only be a supportive methodology in a genome-wide screen of gene function. It now seems more apparent that random mutagenesis will be the primary tool to unlock the secrets of the mammalian genome through phenotypic analysis of the animal. These genetic screens are bolstered by the knowledge that the sequence of the mouse genome will be completed within the next few years. Thus, the challenge for mammalian geneticists and developmental biologists in the next few decades will be to understand the functional roles of genes in development and how these genes are organized into pathways.

At the moment, mouse geneticists are capable of manipulating the mouse genome in almost any way conceivable, from point mutations to centimorgan alterations to chromosome ablations. Thus, it may not be surprising that these genetic tools have led to the generation of a tremendous number of novel mouse mutants that are revealing important biological insights. Many of these mutants have been generated by laboratories with extensive expertise in mouse genetics, reproductive physiology, and embryology. However, perhaps just as many mouse mutants have now been generated by institutional core facilities and then transferred to principal investigators for phenotypic analysis. This may be the first time that biochemists, molecular biologists, and cell biologist find themselves dealing with the demands of breeding animals let alone trying to determine the primary defects that cause complex mutant phenotypes. The *Anatomical Basis of Mouse Development* by Kaufman and Bard will certainly become a standard resource for both experts and novices analyzing mouse phenotypes.

The *Anatomical Basis of Mouse Development* contains, as one might expect, a compendium of detailed descriptions of developmental anatomy with illustrative figures and diagrams. There are a myriad of anatomical

terms and more indexes than one might really have wanted. In addition, there are numerous useful wire diagrams showing the relationships between the "parents" and "children" of a particular structure. Furthermore, there are many references to human developmental anatomy to provide links with clinical conditions. I doubt that even the most diligent individual will ever read this book from cover to cover. It is more likely that a specific question will arise that will lead one to refer to a particular section of this book.

The developmental anatomy covered in this book is first rate and provides exceptional detail about many important organ systems. The depth of information will provide readers with new insights into the developmental mechanisms underlying organogenesis. However, one important organ system that could have been given more illustrative attention is the placenta. Whereas the text more than adequately covers this essential mammalian organ, there is not one figure. The only figures provided are four contemplating the development of the mouse's belly button (umbilical cord). Mammalian embryos can develop without a head, lungs, kidneys, limbs, or gonads but won't proceed very far without a placenta.

Embryos, fetuses, and their anatomy are beautiful and fascinating to observe. In addition, whole-mount gene expression patterns reveal the beauty of tissue differentiation at the molecular level. This makes developmental biology one of the most visually appealing of all of the biomedical sciences. Thus, before opening the *Anatomical Basis of Mouse Development*, I had anticipated finding beautifully illustrated color figures. To my great disappointment the authors have purposely chosen to illustrate the anatomy using simple black-and-white line drawings. They state that the primary reason for this was to provide simple figures to be used in conjunction with the previous histological *Atlas of Mouse Development* (M. Kaufman, 1994) but also they say it was to keep the price of the book down. In one sense the authors are correct that these simple line drawings, although for the most part immediately boring, do simply convey the necessary information needed to understand the anatomy. Whereas some figures such as the bones of the skull are nearly useless, there are others such as the figure of the floor of the oropharynx (go figure) that are exquisitely rendered, giving the impression of three dimensions and transparency so that the anatomy is absolutely clear. On balance, though, I am still disappointed that the authors did not produce illustrations like the lush pieces of art found in the wonderful new book *Heart Development* edited by Harvey and Rosenthal or the visually appealing figures in *Principles of Development* by Wolpert. Though well intentioned, it appears that the authors' choice to illustrate their text as they did has not helped create a new excitement for anatomy.

In the text, the anatomical terms are in bold so that the reader can quickly and easily find the information they need. These terms are generally familiar and easy to find with the help of the indexes. Unfortunately, one of the tissues that I used to test the usefulness of this book was the visceral endoderm because of the renewed interest in this extraembryonic tissue in body patterning. Surprisingly, I had to dig through the text to find this term because it hadn't been bolded nor was it present in the index of tissues. Perhaps it was a minor editorial oversight. Sometimes the reader will find unfamiliar (perhaps dead) anatomical terms. For example, the mesoderm generated during gastrulation is referred to as intra-embryonic mesoderm, a term rarely if ever mentioned in the current literature. Perhaps the authors were trying to make a statement about standardizing mouse anatomical terminology using original terms. The authors rightly caution readers in the introduction about the limitations of their book, which is essential to keep in mind with something that will almost certainly be considered an information bible.

Ultimately, an anatomy book, even one focused upon developmental anatomy, can only go so far to help one understand the reasons why a mouse with a mutation develops abnormalities. What the mouse molecular genetics field also needs is a user-friendly book that will provide a stepwise guide to researchers on how to analyze their mouse mutants. For example, if my mouse mutant dies at 9.5 days postcoitum, what causes for this lethality should I consider? . . . the placenta! Clearly, we need many more text, CD, and web-based resources in the mouse genetics field such as the *Anatomical Basis of Mouse Development* to help us understand the phenotypes of the mutants we are generating. It is clear to me that every laboratory using the mouse in biomedical research should have at least one copy of this book. It will become an instant classic for the field.

Finally, genetic studies of the mouse are sparking a renewed interest in mammalian developmental anatomy. As more and more mutant mice are being analyzed with increasing detail, new insights into anatomy will become apparent. This will in turn almost certainly lead to a revision of developmental anatomy. Thus, dear Mousie, it is you who is now causing anatomy's dominion to change.

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Diving into *Danios*

Methods in Cell Biology
Volume 59—The Zebrafish: Biology
Volume 60—The Zebrafish: Genetics and Genomics
Edited by H. William Detrich III, Monte Westerfield, and Leonard I. Zon
San Diego, CA: Academic Press (1998). Vol. 59, 391 pp. $64.95; Vol. 2, 396 pp. $64.95

In his 1981 article describing the production of clonal zebrafish (Nature *291*, 293–296, 1981), George Streisinger extolled the value of the tropical, freshwater *Danio rerio* for its short generation time, high egg production, and rapid embryonic development. He realized the potential for large-scale mutagenesis and mutant screens because "the free-swimming 7 day fish exhibit many behavioral and morphological traits of the parent but

are only a few millimetres long." In fact, teleost fishes have often been organisms of choice for the experimental embryologist, but Streisinger heralded their entry into the "model system" category with the lure of genetics.

Not quite 20 years later, with numerous scientists trained at the "Oregon mecca" for zebrafish research started by Streisinger, and the labors of two intensive mutational screens in Boston and Tübingen culminating in rapid gene identification, the time was ripe for a guidebook to this piscine paragon. The intention of the two volumes on *Zebrafish Biology* (vol. 59) and on *Genetics and Genomics* (vol. 60) in the *Methods in Cell Biology* series was to not only serve as an updated resource for the zebrafish connoisseur, but to convince other researchers of the advantages of this vertebrate genetic system. In many ways the editors have succeeded, particularly through their selection of contributors, some of those who first applied the described techniques and tools to the zebrafish. Overall, the volumes present an interesting mix of biology review and practical know-how.

For developmental biologists, the greatest strength of the zebrafish continues to be the clarity of the embryo, a property that facilitates optical imaging, real-time cell tracking, recognition of mutant phenotypes, and visualization of gene expression, by methods that are typically less practicable in other embryos. For example, mutations that produce morphologically subtle or unrecognizable phenotypes have been successfully recovered by altered gene expression as the criteria, with cocktails of RNA probes or antibodies (as described in vol. 59, chapter 14 and vol. 60, chapter 4). The ease and reliability of in situ hybridization on intact embryos also enables large-scale, rapid screening of random cDNAs or ESTs for temporal and spatial patterns of gene expression, thereby providing an important component of genomic analysis.

While studies of early development first relied on injections of fluorescent lineage tracers or the transplantation of labeled cells into unlabeled hosts, more recent efforts to exploit the transparency of the embryo have coupled creative, vital labeling techniques (i.e., bodipy derivatives to outline cell shape and patterns of movement [vol. 59, chapter 11], or photoactivation of caged fluorescent dextrans for precise cellular fate mapping [vol. 59, chapter 19]) with confocal imaging. This brings a new level of sophistication to the analysis of cell behavior as tissues form and differentiate in the vertebrate embryo and in response to specific mutations. Moreover, through the generation of green fluorescent protein expressing lines and the application of other visual methods to assay protein activity and cellular physiology, the potential for biologists who like to watch development in action is unlimited.

In contrast, most of the described techniques for molecular genetics or genomics found in volume 60 (i.e., gene expression, misexpression, library construction, positional cloning, resolving chromosomal linkage by meiotic or radiation hybrid mapping, etc.) are not novel or uniquely applicable to the zebrafish. However, the ability to obtain hundreds of individual genomes for PCR analysis from the progeny of a single pair mating, or to manipulate ploidy, has permitted some new twists to old approaches and has expedited the production of a relatively high-resolution genetic map (vol. 60, chapter 8). Determining map positions and centromere linkage is simplified using haploid genomes produced by fertilizing eggs with UV-irradiated sperm to inactivate the paternal genome, or by applying pressure to such newly fertilized eggs to create gynogenetic diploids. Several strategies adapted from plant and animal genomic studies are explained in detail (vol. 60, chapters 9–12) for isolating and analyzing DNA markers (RAPDs, SSLPs, AFLPs, SSCPs), providing a much-needed clarification for those previously wary of mapping zebrafish genes or mutations, or embarking on cloning a mutated gene (vol. 60, chapter 15). Similarly, mutagenesis schemes are well covered (vol. 60, chapters 1–5), although a summary of the advantages and limitations of the various insertional, chemical, and irradiation approaches would be useful for the newcomer contemplating a genetic screen.

A commonly held view is also tackled concerning the decreased utility of the zebrafish for developmental genetics due to the presumed extra round of genome duplication compared to mammals. Closer inspection indicates that many but not all loci have extra copies, that essential functions partially shared by duplicated genes may be easier to dissect through mutation, and most importantly, reveals a high degree of conserved organization between the zebrafish and human genomes (vol. 60, chapter 8). Testing candidate genes from other vertebrates has been an extremely productive way to identify zebrafish mutations (as described in vol. 59, chapter 10), and with an increased knowledge of syntenic chromosomal regions, going back and forth between human DNA sequences and fish mutations could be a general mechanism for gaining insight into gene function. Although progress has been made in zebrafish transgenesis (vol. 60, chapters 6 and 7), for such comparisons to become a routine approach will require improved gene inactivation methods on par with the elegant, targeted strategies used in the mouse.

As with any book that attempts to summarize the current state of a research field, it is nearly impossible to be comprehensive in scope, and biases surface. The vascular system is admirably dealt with in several chapters (volume 59, chapters 17–19), but little information is provided on studies of other important tissues and organ systems (e.g., muscle, kidney, pancreas, or gut). Also notably lacking is recent progress on auditory and olfactory sensory systems and there is little mention of behavioral studies (Streisinger's passion). Potential strategies to identify mutations or assess tissue function in the larva or adult are also limited in these volumes, where the focus is centered on the embryo and what has already been accomplished.

Despite these omissions and minor repetition between some chapters, the two volumes are packed with useful tidbits and should serve as a handy reference for researchers in the zebrafish mainstream, as well as for those planning to plunge into this powerful genetic system.

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Initiating Development

Advances in Developmental Biochemistry: Volume 5
Edited by Paul M. Wassarman
Stamford, CT: JAI Press (1999). 280 pp. $128.50

An annual series devoted to advances in developmental biochemistry takes on a daunting task, given the vast repertoire of potential topics. It is difficult, but highly desirable, to choose topics that blend together in a synthetic and cohesive fashion, such that each volume serves as a source book for students and investigators alike, rather than being simply a collection of unrelated essays. Since its inception in 1992, *Advances in Developmental Biochemistry* has offered a broad spectrum of contributions discussing gene regulation, cellular determination, and fertilization, to name just three recurring topics. *Volume 5* follows a similar format, presenting well-written, comprehensive reviews on a variety of topics, but with no overall cohesion intended. Nevertheless, most of the seven chapters deal with some aspect of reproduction and early development.

The salient features of fertilization in higher order species encompass virtually all of the key disciplines of modern cell biology and biochemistry: cellular differentiation and remodeling, activation of motility, cell–cell and cell–matrix recognition, stimulus-coupled secretion, intracellular signaling, chromatin remodeling, as well as others. In recent years, all of these aspects of fertilization have witnessed significant and, in some instances, remarkable advancement. A volume that brings together these advances in fertilization is overdue. Whether intended or not, *Volume 5* partially fills this void, but falls short of being the comprehensive resource so badly needed.

A perspective of gametogenesis not normally seen in volumes such as this is reflected in the chapter by Olson, which discusses the role of nitrous oxide in ovarian physiology. Nitrous oxide and its synthases are critical mediators of vasodilation, prostaglandin synthesis, and possibly steroidogenesis, which in turn impact the regulation of ovulation, oocyte maturation, and luteal function. More often the subject of endocrine and physiology reviews, a presentation of the role of this novel signaling molecule in a developmental context is a welcome addition.

Two chapters present comprehensive, although somewhat overlapping, reviews of our emerging understanding of the events that prepare sperm for fertilization. Mammalian sperm must undergo a process called capacitation, which results in alterations in sperm motility as well as the ability of sperm to undergo acrosomal exocytosis in response to binding the egg coat. Despite the overwhelming importance of capacitation, and despite our ability to replicate some of these events in vitro, our knowledge of the underlying molecular mechanisms is embarrassingly poor. Kopf and his colleagues have undertaken a systematic approach to define the biochemical pathways by which specific media constituents that are required for successful capacitation in vitro lead to changes in membrane fluidity, calcium channel regulation, cAMP metabolism, and protein tyrosine phosphorylation—known effectors of capacitation. Attempts to develop a comprehensive framework by which these effectors elicit capacitation reflect one of the first coherent approaches to this seemingly impenetrable black box. In a related chapter, Florman and colleagues present a synthesis of the varied intracellular signals elicited by sperm binding to the egg coat, including activation of G proteins and cation channels, which in turn lead to elevations in intracellular pH, calcium, and cAMP. Each of these second messengers then impacts an array of downstream targets, such as voltage-gated calcium channels that release calcium from intracellular stores required for acrosomal exocytosis.

Although these two chapters are worthy contributions, they only scratch the surface of the many active areas that address how sperm are prepared for fertilization. How do sperm reach the site of fertilization (Cohen-Dayag et al., 1995)? How do sperm pass through the follicular cells that surround the egg coat (Myles and Primakoff, 1997)? Does each wave of sperm released from the storage crypts in the female disperse the follicular cells as well as activate development, or are these tasks accomplished by successive waves of sperm? These as well as other questions reflect our overwhelming ignorance regarding the relationship of in vitro assays of sperm–egg binding to what really happens in the female reproductive tract. An acknowledgment of these areas of renewed interest would have been excellent additions to this volume.

Despite our ignorance, the sperm seem to know where they are going. Nevertheless, we appear to be having an awfully difficult time of determining what molecules sperm rely upon to recognize the mammalian egg coat in a species-specific manner (Snell and White, 1996; Wassarman, 1999). Unfortunately, there are no chapters that discuss this rapidly moving field. In the recent past, candidate sperm receptors for the egg coat have been the subject of homologous recombination (Lu and Shur, 1997) and overexpression in transgenic animals (Youakim et al., 1994) with surprising results that suggest previously unrecognized receptor–ligand pathways. There has been continued progress in the identification and function of sperm glycoproteins thought to act as primary and/or secondary receptors for the mammalian egg coat (Hardy and Garbers, 1995; Foster et al., 1997; Ensslin et al., 1998), but as is the case here, they are often overlooked in volumes of this type.

Similarly, a chapter devoted to recent progress in the structure and function of the egg coat glycoproteins would have been an important addition. It is now clear that, contrary to traditional thinking, the murine zona pellucida is a heterogeneous structure, since many glycoside residues are confined to distinct layers of the egg coat although the protein backbones appear uniformly distributed (Aviles et al., 1997). This has striking implications for how sperm interact with the egg coat during various stages of binding and penetration. Mutagenesis of the egg coat glycoproteins has revealed structural features important to their recognition by sperm (Chen et al., 1998), as well as the ability of heterologous egg coat glycoproteins to support species-specific binding (Rankin et al., 1998). These studies begin to address

the biochemical basis of species specificity of binding, something we know virtually nothing about in mammals.

The complexity of the mammalian system has precluded real progress into the provocative issue of how gamete receptors evolve and how this polymorphism leads to, or impacts, reproductive isolation and speciation. In *Volume 5*, Vacquier and colleagues make a compelling argument that lower systems, such as free spawning marine invertebrates, are better suited for this investigation, since their simpler mechanism of sperm–egg recognition is devoid of the behavioral and endocrinological complexities of internally fertilizing mammals. This is well supported by Vacquier's elegant studies of the marine abalone, whose sperm release only two polypeptides (16 kDa and 18 kDa) during the acrosome reaction. The 16 kDa protein, lysin, is responsible for species-specific binding and generation of a pore in the egg coat through which the sperm penetrates. The function of the 18-kDa protein remains unclear, but appears to participate in membrane fusion between the gametes. A comparison between lysins cloned from five abalone species allows Vacquier to build models accounting for species-specific binding and lysis of the egg coat. The polymorphism found among the lysins suggests a "positive Darwinian selection" (p. 74), which hypothesizes that heterogeneity in the egg coat ligands determines the best fit among the polymorphic sperm lysin populations, leading to reproductive isolation and speciation.

Studies of abalone sperm are but one example of how our understanding of the mechanism of sperm penetration through the egg coat has recently changed. In mammals, new insight into sperm penetration comes from studies that have challenged old paradigms and would have been a valuable contribution to this volume. It has been generally accepted that the acrosomal enzyme, acrosin, is the protease primarily responsible for penetration of the mammalian egg coat. However, the unexpected observation that acrosin-null sperm still penetrate the egg coat, albeit at a lower efficiency than wild-type, has forced a reexamination of the components of the acrosome and their role in penetration (Baba et al., 1994).

In any event, the penetrated sperm finds itself in the perivitelline space, the void between the inside of the egg coat and the egg plasma membrane. Herein occurs the next critical recognition event between the fertilizing gametes, binding and fusion between the sperm and egg plasma membranes. The first real insight into this issue came from the pioneering studies of Myles and Primakoff who identified a sperm surface glycoprotein, fertilin, that was one of the first clear mediators of fusion (Primakoff et al., 1987). Fertilin turns out to be the founding member of a new class of multifunctional proteins called ADAMs (polypeptides that contain *a d*isintegrin and *m*etalloprotease domain), which are the focus of a chapter by Blobel. ADAMs are now known to participate in two apparently independent, but no doubt functionally-coupled, protein–protein interactions: (1) metalloprotease processing of secretory and cell surface glycoproteins and (2) the binding of a disintegrin domain on one cell to its integrin receptor on an adjacent cell. Blobel cites examples where ADAMs participate in cellular recognition, as occurs between the sperm and egg plasma membrane, and where processing of secretory and/or cell surface glycoproteins release polypeptides that impact a range of cell fate decisions during development.

The culmination of fertilization is the activation of the egg leading to zygote formation. Recent focus has turned to what, if anything, the sperm delivers to the egg that might initiate activation. Although the subject of an earlier volume, the topic has fueled much excitement recently by the identification of an insoluble sperm perinuclear-associated factor, as well as an insoluble sperm cytosolic factor, both of which have been reported to induce egg activation (Parrington et al., 1996; Kimura et al., 1998).

Around the two-cell stage, the zygote becomes dependent upon its own transcriptional and translational machinery replacing the need for maternally derived products. The regulation of this "maternal to zygotic gene activation" (ZGA) (p. 130) is the subject of an excellent discussion in *Volume 5* by Schultz, who classifies the two main functions of ZGA as (1) the destruction of residual maternal RNA and (2) reprogramming of gene expression to generate zygotic-specific transcripts. Of the 1500 polypeptides detectable by 2-D PAGE in two-cell embryos, 85% show at least a 2-fold change in the level of their expression, in addition to those transcripts unique to the two-cell embryo (p. 132). Schultz presents evidence that this reprogramming results in a transcriptionally repressed state, due in large part to chromatin remodeling, in which enhancers relieve the repression of zygotically important genes.

The remaining chapter (actually the first in this volume) is a tour de force in its own right, but unfortunately, may be lost in a volume in which most chapters are devoted to reproductive issues. Frasch presents a comprehensive review of the cell biological and genetic cascades that are responsible for mesoderm specification in *Drosophila*. The invagination of mesoderm during gastrulation, its delineation into tissue precursors, and their subsequent differentiation into mesodermally derived tissues have been elegantly dissected in *Drosophila*. Some of these gene products, such as tinman, are known to participate in vertebrate mesoderm patterning. Given that the chapter is the only one in this volume to discuss flies, a more comparative discussion of mesoderm specification in other species would have been helpful earlier in the chapter.

Volume 5 of *Advances in Developmental Biochemistry* continues the tradition of bringing the reader excellent reviews on topics in development. Although all of the chapters are worthy contributions in their own right, series such as *Advances in Developmental Biochemistry* have the opportunity to be invaluable source books for students and investigators. Unfortunately, the overlap between some chapters, the presence of unrelated chapters, the lack of discussion pertaining to many important advances, and the random order of the constituent chapters, make the volume a collection of excellent essays without the cohesion needed to make it the resource it could have been.

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The Heart as a Model Organ

Heart Development
Edited by Richard Harvey and Nadia Rosenthal
San Diego, CA: Academic Press (1998). 530 pp. $159.95

Heart Development Slide Set
San Diego, CA: Academic Press (1998). 88 figures $99.95

The heart has long had a prominent place in human psyche and folk lore. A number of recently published books, including *Heart Development* (R. Harvey and N. Rosenthal, eds.), mark the emergence of the heart as a leading paradigm for organ patterning and morphogenesis in the molecular biological era.

Historically, the visually dramatic embryonic development of a beating organ drew attention of early anatomists and developmental biologists. These studies laid the groundwork for many fascinating questions of cardiac developmental biology still extant today. What are the origins of the intrinsic pacemaker activity of the heart? How do cardiac primordia become specified to be heart? What discriminates the heart field, cells that have the potential to form beating tissue when explanted in vitro, from the more restricted heart fate map, those cells which actually go on to form the heart? How do cardiac primordia regulate to form a complete organ when one side of the primordia have been excised? How is the anterior–posterior polarity of the heart, as evidenced by early-fate mapping studies, established? What determines the situs of the heart—its right-sided looping, the earliest morphological manifestation of organ asymmetry? The fusion of developmental biology with molecular biology is beginning to shed light on molecular mechanisms at play in these intriguing processes.

As developmental–molecular biologists move from questions of cell type specification to the intertwined and more complex issues of organogenesis, the major questions that emerge are those of tissue patterning and morphogenesis. A plethora of recent studies have begun to establish the heart as a model paradigm for addressing these questions (reviewed by Fishman and Chien, 1997; Fishman and Olson, 1997). There are several reasons for the emergence of the heart as an intense focus of study. As the vertebrate heart is the first organ formed during embryogenesis, and its function is critical for embryonic survival, many investigators have found themselves unwittingly or otherwise studying genes that affect cardiogenesis. Phenotypic effects of interfering with the function of genes required for cardiogenesis and perhaps elsewhere are first visible in the developing heart. Furthermore, several discoveries by investigators from diverse disciplines have begun to define genes that may be involved in the fundamental biological phenomenon of left–right asymmetry (reviewed by Mercola, 1999). Heart looping is the first overt evidence of this asymmetry, and therefore these investigations have further focused attention on the heart. A prevalent anthropocentric viewpoint from within and without the research community suggests that research performed in various developmental systems should further our understanding of human development, and impact on human well-being. As congenital heart disease constitutes a substantial proportion of birth defects, and heart disease is a leading cause of adult mortality in developed nations, there has been a strong impetus from both public and private sectors to focus research effort on heart development and cardiac disease.

As more investigators have turned their attention to the development of the heart and cardiovascular system, our initially naive view of the heart has radically changed. Perhaps what we are learning now should have been obvious, as things often are in retrospect. As we entered the molecular biological age, the heart was viewed by most as largely a muscular organ, with the myocyte the premier cell type. There were two obvious types of myocytes, ventricular and atrial myocytes. Conduction cells were critical for function, and understanding cardiac endothelium was essential to understanding valve development. Neural crest cells were known to be critical for normal development of the cardiac outflow tract. We now know that there is greater cell complexity and interdependence of cell types which comprise the heart than previously appreciated. Our “myocentric” viewpoint has been altered.

Although early lineage studies had fairly well established the embryonic origin of cardiomyocytes, embryonic origins of cardiac endothelium, conduction cells, and epithelium were a subject of speculation. Although

questions still remain, modern methods of lineage analysis and molecular biology now demonstrate that ventricular myocytes and ventricular conduction cells share a common precursor, that a subset of endocardial cells are likely to arise from a common progenitor shared with myocytes, and that the epimyocardial organ contributes epicardial cells, coronary vascular endothelial cells and cardiac fibroblasts. Based on a number of studies, cardiac myocytes themselves cannot readily be assigned two phenotypes, that of atrial or ventricular cells, but rather display a complexity of gene expression in a regionalized manner that suggests a greater diversity of muscle cell phenotypes than previously suspected. Our studies are beginning to reveal the dynamic and critical nature of the interplay between diverse cell types within endocardium, myocardium, and epicardium. These tissue–tissue interactions, involved both at the earliest stages of specification, and later in subsequent growth and phenotypic remodeling of specific cell types within the heart, typify interactions that occur in the development and function of any organ.

Superimposed on the sorting out of specific cell types, and the coordination of those cell types into a functioning organ, is the concerted morphogenesis of the composite tissues to adopt their final form. Mechanisms of morphogenesis represent a largely unexplored frontier for molecular biology. As the morphogenesis of the heart is particularly dramatic, and occurs relatively early on in embryonic development, a great number of anatomical studies have been performed to describe cardiac morphogenesis. Despite this, the driving forces and genes involved in heart morphogenesis remain largely unknown. Perhaps our most detailed understanding of cell movements involved in morphogenesis derives from work by Ray Keller and colleagues on *Xenopus* gastrulation (Moore et al., 1995; Winklbauer and Keller, 1996). These studies may point the way to similar kinds of experimental approaches for organ morphogenesis. As cardiac morphogenesis involves cell movement, and undoubtedly cell–cell and cell–extracellular matrix interactions, several cell adhesion and extracellular matrix molecules have recently been implicated in this process (Radice et al., 1997; Tsuda et al., 1998; Haag et al., 1999). Further study of these molecules may provide a molecular handle with which to gain insight into processes of cyto-architectural remodeling. Another interesting aspect of cardiac morphogenesis is that the heart is functioning while it is taking form. How ongoing function affects heart formation remains an issue to be explored.

The manner in which genes that establish the major embryonic axes, including the left–right axis, are involved in determining cardiac morphogenesis and directional looping of the heart is a subject of intense investigation. Normal heart looping obeys a predictable left–right bias. However, in some situations where left–right axis information is perturbed, the heart will still undergo looping, either with a reverse or random left–right bias. That is, the process of looping morphogenesis can occur independently of at least some inputs from the left–right pathway.

The recent focus of investigative attention on the heart has resulted in an explosion of information that has answered some long-standing questions, and has set the stage for the next set of questions to be addressed. In *Heart Development*, the editors have assembled a number of chapters by highly qualified authors that describe our current understanding of heart development, with an emphasis on the heart itself, rather than the larger context of the cardiovascular system. The text is particularly strong and detailed in its description of what we know and are learning about cardiac cell fate specification, the origin of diverse cardiac lineages, muscle transcription factors, and cardiac muscle–specific genes. Information derived from a number of model systems, including *Drosophila*, zebrafish, amphibia, mouse, and rat, is discussed. Cardiac asymmetry is discussed within the larger issue of left–right asymmetry, and several chapters are devoted to human congenital heart disease. Paradigms from skeletal muscle are presented for purposes of comparison. The information presented in *Heart Development* is not readily garnered elsewhere. It provides a unique compilation of current information that should be invaluable to those interested in cardiogenesis, a rapidly expanding audience. A particularly pleasing aspect of this text is the frequent and beautifully rendered illustrations that clarify the written word. And the publisher has made available a slide set of these figures. Indeed, many of these illustrations are already appearing in numerous seminars and classrooms as issues of cardiac development are discussed.

Although introduced in *Heart Development*, topics of tissue–tissue interactions within the heart, the overall issue of looping morphogenesis, and the interplay between the vascular system and the developing heart are not fully developed themes. For the student or investigator who is interested in morphogenesis, a more thorough treatment of this subject is presented in *Living Morphogenesis of the Heart* (de la Cruz and Markwald, 1998).

As the heart and cardiovascular system begin to function prior to the completion of heart development, physiological function and development are inextricably entwined. An important area of synthesis is the merging of a physiological perspective with that of developmental biology. Physiologists have focused for decades on the heart as a functioning secretory organ. Developmental biologists have tended to focus on the building blocks of the heart without considering their physiological endpoint. A textbook that moves toward a synthesis of these two disciplines is *Development of Cardiovascular Systems* (Burggren and Keller, 1998), where emphasis is placed on the anatomy and physiology of cardiovascular systems in commonly utilized invertebrate and vertebrate model systems.

To understand mechanisms of cardiovascular disease, cross-fertilization of cardiac physiology, pathophysiology and cardiac development promise to be particularly fruitful. There are a number of examples where pathophysiology of the adult cardiovascular system is characterized by the reexpression of genes characteristic of embryonic states, suggesting that an understanding of cardiac development will shed light on these disease processes. Such pathophysiological states include cardiac hypertrophy and the formation of atherosclerotic lesions. The inverse is also undoubtedly true, that insight into pathophysiological mechanisms may focus our attention on the role that similar mechanisms play in development. Traditional physiologists have long

suspected that calcium is a key mediator of cardiac pathophysiology, a hypothesis that is being reconfirmed by molecular pathophysiologists. The pivotal role of calcium for cardiac physiology and pathophysiology suggests that calcium-mediated mechanisms may also play key roles in cardiac development, and is worthy of consideration. A recently published textbook, the *Molecular Basis of Cardiovascular Disease* (Chien, 1999), provides an overview of molecular pathophysiology of the cardiovascular system, and is highly recommended for the reader interested in further developing intersections of cardiac development and disease.

In summary, in response to a growing interest in the heart as a model organ, there have been a number of recently published texts that update and summarize our current understanding of cardiovascular development, physiology, and disease, each text complementing the others in its emphasis. These texts should provide the framework for a future interdisciplinary approach to understanding organogenesis of the heart. In this context, *Heart Development* occupies a unique and important niche.

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Unraveling the Endocrine Control of Growth

Handbook of Physiology, Section 7: The Endocrine System
Volume 5: Hormonal Control of Growth
Edited by Jack L. Kostyo
New York: Oxford University Press (1999).
831 pp. $179.50

One needn't be an endocrinologist, or a cell biologist, to marvel at the tightly controlled process whereby we grow. To be a parent, or a close observer of children, is quite enough. The "sacred" spot on the wall of our home where our children's growth is plotted tells it all. Even more so when the data are plotted on a standard pediatric growth chart, revealing, under circumstances of normal health, monotonous hugging of a percentile, with an acceleration at the time of puberty, and soon thereafter, the reaching of a mature height (if not a mature personality!). Of course, the slowly clearing mystery behind the mechanisms that explain how a single cell gives rise, through successive cell divisions, to a fully differentiated organism, whatever its size, motivates an army of developmental biologists. The insights so obtained also help address the general problem of growth. But what about size itself? Why are mice about 1/3000th the mass of humans, and more importantly to endocrinologists, why are some mature humans much larger (and longer) than others? These questions relate to an enormous amount of biology, and given the social implications of variations in height, they deserve serious attention. And they receive such attention in this recent volume of the *Handbook of Physiology*, on the *Endocrine Control of Growth*, edited by Jack L. Kostyo.

If one factors out variations in size due to obesity, which unfortunately modifies only certain dimensions through enlargement of adipocytes with stored triglyceride, the major variations in size between individuals and species generally relates to the number of cells of which they are comprised. At the level of cell biology, therefore, the issue of growth (of an organ, tissue, or organism) is determined by the growth and division of its cells, and their capacity for survival. That these processes are tightly regulated cannot be questioned, as even a 1% imbalance between production of new cells and death of old cells would cause a liver to increase to the size of an entire human over a period of 8 years. The past several years have witnessed an explosion of new knowledge about the genes and encoded proteins that regulate cell cycle control, cell division, and programmed cell death. This new knowledge, so critical at the level of the cell, has yet to provide major insights into the genes and pathways that determine why a mouse is smaller than a human, and why some humans are smaller than others. And perhaps for that reason, these pathways are not the subject of this book, which examines, in great depth, the endocrine control of growth, with greatest attention to the human.

To the endocrinologist, the study of growth has revolved around the identification of hormones, growth factors, and more recently neuropeptides that regulate postnatal growth. Dysregulation of these molecules will produce disease, with disordered growth as a major feature. The modern history of this field has been nothing short of spectacular, and the resulting discoveries have had impact far beyond the domains of growth, or endocrinology. This is well illustrated through the discovery and characterization of the aptly named growth hormone (GH), deficiency of which in early life produces a form of dwarfism, and excess of which in youth or adulthood, produces gigantism or acromegaly, respectively. The ability of anterior pituitary lobe extracts to promote growth in rats dates to 1921, and although demonstration that pituitary lesions retard growth and pituitary extracts can restore growth followed rapidly,

GH was not isolated until 1956, or measured in blood by radioimmunoassay until 1963. It has been administered as a therapeutic agent to individuals with GH deficiency since 1957. Separate chapters recount this history and deal with the chemistry of GH, the factors regulating GH gene expression, GH physiology in vivo, and GH signaling. The GH receptor was the first cloned member of the large class 1 hematopoietic cytokine receptor gene family that includes receptors for erythropoietin, IL-6, and leptin, among many others. This subject is particularly well reviewed in chapters by Smit, and Schwartz and Carter-Su.

Growth hormone is but one component of a complex endocrine circuit that influences growth. Exciting stories are well recounted in this volume both upstream and downstream of GH in the physiological pathways that regulate growth. Demonstration that hypothalamic lesions impaired linear growth in rats, and that stimulation of hypothalamus provoked GH secretion suggested CNS regulation, but the actual degree of complexity was not anticipated, with both positive and negative factors now identified. The identity of growth hormone–releasing hormone (GHRH), which resisted purification directly from hypothalamus, benefited from astute endocrinologists, who surmised that a lung tumor in a patient with acromegaly (but no GH-producing pituitary tumor) might be ectopically expressing the peptide. The frozen tumor was the starting material from which the labs of Vale and Guilleman purified GHRH, in a satisfying bed-to-bench collaboration. The initially unsuccessful search for GHRH did however lead to the identification of somatostatin (SS), an inhibitory neuropeptide for GH secretion. The complex regulation of GH secretion via GHRH, SS, and additional growth hormone–releasing peptides (GHRPs), involves numerous feedback loops, has pulsatile, diurnal, and sexually dimorphic features, and is well reviewed in a series of authoritative chapters.

Events downstream of GH are also given prominent play in this worthy tome. Did GH act directly on cells in the body, including those related to bone growth, to promote growth? Or was its action indirect? Efforts to answer this vexing question have occupied endocrinologists since 1953, when Salmon and Daughaday presented evidence that GH stimulated cartilage growth indirectly, via an unknown factor that it induced in the circulation, rather than via direct action on cartilage cells. Thus was the "somatomedin" hypothesis of GH action on growth born. Initially confused by the variations in assays employed to investigate the putative factor, and by the inherent difficulty of purifying a factor from plasma (especially in the '60s and '70s), this field matured rapidly with the identification of IGFs 1 and 2 as peptide growth factors in part under GH control. These archetypical growth factors, found to be members of the insulin gene family, contribute critically to the GH effect on tissue growth via both endocrine and paracrine mechanisms. The biology of IGFs and their receptors, binding proteins, and signaling pathways are critically reviewed in a series of chapters by eminent authorities. Of course, GH and IGFs are not the whole endocrine story of growth. Other hormones, including insulin and thyroid hormone, are critical, and these are reviewed in separate chapters as well.

This volume illustrates just how much has been learned about the hormonal control of growth over the past several decades. It now appears that the major endocrine controllers have been identified, and this permits us to understand, often in molecular detail, many diseases of growth that were only mysteries before. But major questions remain unanswered, especially as relates to fundamental mechanisms, certainly genetic, that explain why people are bigger than mice, and some healthy people are taller than others. For answers to these questions, we may require additional insights, perhaps more likely to be derived from studies of simpler and more genetically tractable organisms, such as worms and flies. Until these arrive, the *Hormonal Control of Growth* provides an invaluable resource to anyone with a major interest, be it clinical or basic, in growth and its endocrine control.

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Regeneration of the Central Nervous System, A Reemerging Field

CNS Regeneration
Edited by M. H. Tuszynski and J. Kordower
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Traumatic lesions of the brain or spinal cord destroy nerve cells as well as fiber tracts. They often lead to functional deficits and disabilities, and para- and quadraplegia in the case of spinal cord lesions. Severe lesions, in particular disruptions of fiber tracts, lead to life-long loss of functions due to the very restricted capacity of the central nervous system (CNS) for spontaneous repair and regeneration. The term regeneration has two meanings in the context of the CNS: regrowth of lesioned nerve fibers and reconstruction of the lost tissue. Until recently, it was thought that neither axon growth nor nerve cell division is possible in the adult CNS. This view has changed radically within the last few years. Many fascinating scientific questions and a high level of medical interest are attracting top researchers, and the field has become a major focus of present day neuroscience.

Several crucial findings have spurred development of this field. The first was the demonstration that an adult, lesioned nerve cell can reinitiate growth and grow an axon over a very long distance if supplied with a peripheral nerve transplant as a bridge (Bray et al., 1991). (Peripheral nerves are known to regenerate spontaneously, a property that is largely due to the growth support of the special peripheral glial cells, the Schwann cells). Subsequently, axonal regeneration over long distances was demonstrated in the spinal cord itself under conditions where an endogenous growth inhibitory factor was neutralized by specific antibodies (mAb IN-1; Schwab

and Bartholdi, 1996; Olson, 1997). Very recently, pluripotent stem cells were identified in the adult brain and spinal cord; if taken into cell culture, these stem cells divide and generate new nerve cells and glial cells (Temple and Alvarez-Buylla, 1999).

The essential questions in CNS repair at present are: (1) elucidation of the mechanisms of neuronal degeneration and tissue loss in trauma, ischemia, and in diseases like Parkinson's, Alzheimer's or amyotrophic lateral sclerosis (ALS); (2) design of neuroprotective strategies; (3) replacement of crucial, lost cell populations, e.g., by transplantation of stem cells or embryonic tissue; (4) neurotrophic and growth factor delivery to enhance survival or neurite outgrowth. When fiber tracts are disrupted by a lesion, axonal regeneration depends on the activation of a growth program in the lesioned neurons, the suppression of growth inhibitory factors, and the presence of growth-promoting substrates and factors. At the trauma site itself, holes (caverns) need to be bridged, and scars that contain additional inhibitory signals have to be overcome or circumvented. Regenerated fibers then need to link up with target neurons to reestablish functional neuronal circuits; mechanisms of target finding, synapse formation and stabilization of new connections seem to exist in adult CNS but their molecular nature is largely unknown. Finally, compensatory axon growth by spared fiber systems and plastic changes in existing circuits are probably major contributors to functional restoration in the lesioned, developing human and animal CNS. The stimulation of these processes in the adult stage could greatly extend the repair capacity of a damaged brain or spinal cord.

Significant progress has been made in answering many of the questions outlined above within the last 5 to 10 years. Therefore, many scientists and clinical researchers believe that new therapies for neurological diseases based on a deeper understanding of the underlying cellular and molecular processes can be developed in the near future.

CNS Regeneration, edited by Mark H. Tuszynski and Jeffrey Kordower, contains a collection of fine chapters under the headings of "CNS responses to injury," "Promoting CNS recovery" and "Promoting recovery in neurological disease" (the largest chapter). The focus of the book is on neurotrophic factors, and on transplantations (embryonic tissues, or stem cell–derived neurons). The neurotrophic factors are introduced in a condensed, very useful chapter with many crucial references. Neural stem cells and their use for CNS repair and as vehicles for transgenes are presented in three excellent chapters written by pioneers of the field. The identification of pluripotent neural stem cells in the developing and adult CNS, and the possibility of propagating and expanding these cells in vitro open up possibilities for reconstructive interventions that were just dreams only a few years ago.

More than half of the book is devoted to chapters describing experimental interventions mostly in animal models of human neurological diseases, with emphasis on Parkinson's disease. The results of transplantations of fetal cells in rodents, monkeys, and in human Parkinson's patients are reviewed. Several chapters address techniques and results of trophic factor delivery from pumps, implanted cells, encapsulated sources, or transfections of the CNS tissue using viral vectors. These chapters summarize a scientifically exciting area of high future medical relevance in a very informative and useful way.

Trophic factor effects and the use of Schwann cells to bridge spinal cord lesions are examples given for interventions in spinal cord injury. Although well written, the chapter on this topic provides a small window into a large field, leaving the reader who is interested in axonal regeneration quite unsatisfied. Stroke and demyelination lesions (including the recent possibility for myelin repair by oligodendrocyte precursor transplantation) are, unfortunately, lacking from the book. One reason for this imbalance may be the fact that a second book titled *Nerve Regeneration* (Ingoglia and Murray, 1999) is currently in press and may have "absorbed" many potential authors (expected appearance, end 1999). Together, these books will cover this rapidly evolving, exciting field very well, and the excellent choice of authors in both volumes makes these books very valuable sources of information and references.

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Behind the Veils: Uncovering the Biology of Dendritic Cells

Dendritic Cells: Biology and Clinical Applications
Edited by Michael T. Lotze and Angus W. Thomson
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Although it is unpleasant to consider, we are all walking nutrient sources for the sea of microorganisms that cohabit our world. To a great extent, we resist offering up 3-star meals through the combined efforts of barrier tissues (skin, mucous membranes) and the operation of innate and adaptive (antigen-specific) immune defenses. With respect to the latter, both immunologists and nonimmunologists alike now understand that B and T lymphocytes express in a clonal manner a vast number of

distinct cell surface receptors for antigen. But this well-accepted feature of vertebrate immune systems raises a central question—how do rare T or B cells specific for pathogen antigens initially localized to a small region of the body find the ligands necessary for their receptor-mediated activation? It is difficult to imagine this task being efficiently conducted if each lymphocyte must survey every nook and cranny of host tissues. The time lag in getting the right lymphocyte to the right place would be enormous with respect to the replication rate of invading microorganisms and time is of the essence in fighting infection (that is, in avoiding becoming today's "blue plate special"; Cohn and Langman, 1990).

An anthropomorphic analog of the strategy that has evolved to deal with this problem can be found in the design of the Great Wall of China. More than 1500 years of effort generated a vast brick and granite structure forming a physical barrier to potential invaders. This was of course not a perfect defense and therefore observers were positioned at suitable intervals. The location of any breach was communicated by the watchmen to locally garrisoned troops, who were recruited back to the site of the problem to repel the intruders. Likewise, specialized hematopoietic components called dendritic cells (DC) reside within barrier epithelial structures, such as Langerhans cells in the skin. Upon tissue invasion by a pathogen, local signals activate the DC, which follow chemokine gradients into lymphatics and then as "veiled cells" migrate to draining lymph nodes, where lymphocytes congregate. Within the lymph node (and other secondary lymphoid tissue sites such as the spleen or Peyer's patches), DC take up residence in specific locations among collections of T cells, possibly along specific channels or paths followed by the T cells as they migrate between blood and lymph. This allows each lymphocyte to scan the surrounding DC for the presence of its specific ligand, and if it is found, to initiate differentiation into an effector cell that can emigrate out into the infected tissue, to fight the invader at the point of attack. Trafficking of antigen-laden DC to a few specific sites through which most T lymphocytes recirculate markedly improves the efficiency of matching receptor to ligand.

In the book *Dendritic Cells: Biology and Clinical Applications* edited by M. T. Lotze and A. W. Thomson, the mammalian version of this "wall, watchtower, and garrison" scheme is reviewed in extenso. A collection of nearly 40 chapters written by many of the investigators who have helped define the critical role of DC in immunity lays out the developmental pathways, membrane phenotypes, tissue distribution, roles in normal and pathologic responses, methods for isolation and growth, and potential medical utility of these cells. The study of DC has gone through two major phases since the seminal report by Steinman and Cohn (1973) of a rare population of cells within lymphoid tissues possessing a distinctive morphologic appearance. Between then and 1992, when methods for growing DC in culture were first reported, the analysis of these cells was limited to a handful of persistent investigators willing to deal with their small number and difficulty of isolation. Since then, there has been explosive growth in work on DC, as they have become available in numbers more suitable for biochemical, functional, and clinical examination.

A central (perhaps too oft repeated) theme of the book is how the physiologic changes that accompany maturation of DC are so well suited to their role in providing antigenic information and activating signals to T cells. To appreciate the substantial appeal of this model, one must first understand that the receptors (TCR) of these lymphocytes do not recognize native antigen. Rather, they interact with short peptides or glycolipids displayed on cell surfaces tightly bound to either classical class I and II major histocompatibility complex (MHC) molecules or to class I–like CD1 proteins, respectively (reviewed in Germain, 1994; Porcelli et al., 1998). For effective antigen presentation, a cell must first acquire the source material in sufficient quantity, bind the desired peptides or lipid moieties to nascent or recycling MHC or CD1 molecules, and then maintain these complexes on the plasma membrane until a suitable T cell happens along. This method of antigen display provides information about foreign molecules both independent of their intended function and even if they are present only inside an infected cell, where direct recognition by cell surface receptors is impossible. Immature DC are actively phagocytic, show a high level of macropinocytosis, and express many scavenger receptors, permitting the efficient gathering of antigen. The immature cells also have their MHC class II processing pathway in low gear, with most newly synthesized molecules held intracellularly in an inactive state by invariant chain. Stimulation of immature DC leads to removal of invariant chain, the loading of a large cohort of class II molecules with proteolytic fragments of internalized antigen, and the expression of these ligand complexes on the cell surface. As this maturation process proceeds, antigen uptake and class II molecule synthesis diminish, converting the cell from one that actively acquires and processes antigen to one that efficiently displays material derived from these antigens.

Whereas the first part of the maturation model is focused on efficient creation of ligands for the TCR, the second aspect of the model deals with activating the receptor-engaged T cells. In addition to TCR signals, full development of effector responses and clonal growth of T cells requires signals through non-antigen-specific receptors, a process globally termed "costimulation" (reviewed in Lenschow et al., 1996). Immature DC have a low membrane display of ligands for CD28, the major costimulatory receptor on T cells. Upon receiving a suitable inflammatory, infectious, or "danger" signal, dendritic cells markedly increase the surface level of CD80 and CD86, and they also augment expression of integrin ligands such as ICAM-1. This modification of costimulatory and adhesive molecule display accompanies the transition from immature antigen-acquiring to mature antigen-presenting status. All of these changes occur in the context of altered chemokine receptor expression, which promotes entry of the activated, maturing DC into the lymphatic system and their migration to local lymphoid tissues. There the DCs' rich surface display of ligands for the T cell receptor surrounded by a sea of costimulatory proteins and adhesive molecules is highly, and perhaps uniquely, effective in activating T lymphocytes, especially naive cells. The regulation of DC costimulatory molecule expression by inflammatory signals is a key feature of this scheme that links the

consequences of pathogen invasion with antigen-specific T cell activation.

Whereas the data supporting this maturation model are extensive, persuasive, and well described in this volume, other aspects of DC biology are less clear and not always subject to rigorous analysis. Most investigators believe in the existence of two distinct lineages of DC. One derives from committed lymphoid progenitors that do not require GM-CSF for differentiation in vitro and that typically express the surface protein CD8α as mature DC. The other lineage involves myeloid precursors that give rise to DC lacking CD8α expression and requiring GM-CSF for development. The dissonance between the general acceptance of this two-lineage model and the actual data on this issue is striking in the chapters by Galy et al. and by Maraskovsky et al.

Ikaros is a member of the Kruppel family of zinc finger DNA-binding proteins. Mice that express a dominant-negative (DN) form of Ikaros following gene targeting lack T, B, and NK (natural killer) cells, but have normal numbers of myeloid cells such as macrophages, monocytes, and neutrophils. These data thus suggest a critical role for Ikaros gene family members in lymphoid differentiation (Georgopoulos et al., 1994). Based on the two-lineage model, these mutant mice would be expected to lose just the CD8α^+ "lymphoid" DC, but in actuality they lack all DC except for Langerhans cells. How can one reconcile these data with the "two flavors of DC" proposal? Are both CD8$^+$ and all CD8$^-$ DC except for Langerhans cells actually "lymphoid" and none myeloid, despite the GM-CSF dependence of some DC and the evidence of DC development from purified myeloid precursors? Most investigators would of course disagree with this suggestion, but then one surely cannot use the Ikaros mutant data to argue that any DC are of lymphoid origin. In a related vein, Maraskovsky et al. point out that flow cytometric analysis of the DC populations expanded by Flt-3 ligand fits most readily with a single pathway of maturational differentiation from the CD8α^- state the CD8α^+ state. Yet the authors stick to the "two-lineage" model despite lacking evidence in its favor from their own staining studies. This important subject in DC biology receives little critical discussion throughout the book, except perhaps from J. Austyn in his extremely lucid chapter, where he clearly points out that no one has yet seen a DC and a lymphocyte arise from a single precursor cell in clonal culture. An alternative view of DC differentiation that fits with the maturation model and with the diversity of conditions that generate these cells in culture is that they arise from a single lineage, but with various specialized differentiation states induced by combinations of environmental factors acting at diverse points in the maturation scheme.

The absence of probing tests of the two-lineage model carries over to a related topic of increasing importance, namely the role of DC in promoting tolerance rather than immunity. Not all of the changes associated with "maturation" of DC need occur in parallel. Increases in antigen presentation capacity and in migration may occur without concomitant upregulation of costimulatory proteins, or these changes may be accompanied by secretion of inhibitory cytokines (TGFβ, IL-10). When antigenic ligands on such DC are recognized by the receptors of T cells, they induce a state of unresponsiveness or even apoptotic death. The possibility that DC, long considered the perfect immunogen, might also be the perfect tolerogen is receiving a great deal of attention for its medical implications in transplantation and autoimmune disease settings. Fas-L has been reported to be expressed exclusively on CD8α^+, that is, "lymphoid" DC. In vitro, these DC seem less able to induce sustained responses by T cells, presumably due to Fas-mediated apoptotic death of the lymphocytes. This is an intriguing model, but it lacks clear confirmation in the literature and poses several paradoxes as well. More recent studies have shown that IL-12 is produced almost exclusively by CD8α^+ DC in vivo (Reis e Sousa et al., 1997; Maldonado-Lopez et al., 1999). This cytokine plays a central role in the generation of IFNγ-producing effector CD4$^+$ T cells critical to host defense. If the antigen-bearing DC uniquely able to make IL-12 are also Fas-L positive and kill the T cells recognizing ligand on their membranes, how do strong Th1 (IFNγ-producing) responses develop? The simultaneous expression of both this regulatory cytokine and a death-inducing molecule by CD8α^+ DC makes little sense in a physiologic context. A more interesting possibility is the counter-regulation of these two molecules in this DC subpopulation, but no evidence for this yet exists. Little attention is paid to this conundrum in the book, in part because the IL-12 story has emerged after the 1997 date of the most recent references in the chapters (one of the main limitations of this volume is the lag between initial composition of the chapters and the book's publication). The evidence that DC can contribute to T cell hyporesponsiveness is thus substantial, but the mechanisms underlying this "yang" to the more widely appreciated stimulatory "yin" of DC remain to be understood. Overall, a careful reading of this book argues that the lineage/tolerance issues are just two of many aspects of the currently accepted dogma of DC biology that need to be challenged by new experiments.

A last note. Several chapters in the book are devoted to discussions of the medical utility of DC. This is not unexpected, as the editors are clinicians as well as scientists and are involved in several of the ongoing trials of DC-based vaccines for cancer or tolerance-inducing regimes for transplantation. This growing use of DC as cellular drugs thus makes this book's information on their basic biology not only of value to bench scientists interested in the fundamental properties of the immune system, but also to those seeking to exploit the properties of these cells to the patient's benefit. Though there are lapses and omissions, though the writing styles range from the highly lucid to the occasionally impenetrable, and though it is already becoming dated in a few areas, *Dendritic Cells* provides a rich and accessible assemblage of information about the whys and wherefores of these central players in immunity.

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The Complement System Rises Again

Complement Regulatory Proteins
By B. Paul Morgan and Claire L. Harris
San Diego, CA: Academic Press (1999). 382 pp. $99.95

In the 1890s, experimental pathologists were astonished to observe bacteria imploding upon exposure to a thermostable (it still worked after being left on bench top overnight) blood substance and a thermolabile (it did not work the next day) blood substance. The former was antibody, which is specific and acquired, and the latter was complement, which is nonspecific and innate because most everybody has it in abundance. Neither part of this two-component system could independently accomplish the job. Indeed, the labile serum factor was felt to "complement" the specific substance, hence its name. A few years later, the first autoimmune disease that was understood as such was described. In this form of autoimmune hemolytic anemia (Donath-Landsteiner antibody) the same combination of players was responsible for lysis of the human red blood cells. This scenario was in part responsible for Ehrlich's famous dictum "horror autotoxicus." In Ehrlich's words, "It would be exceedingly dysteleologic, if in this situation self poisons, autotoxins were formed" (Silverstein, *A History of Immunology*, Academic Press, 1989). In view of these remarkable observations, it is not surprising that the study of antibody and complement dominated immunology for the next 70 years.

In the 1970s and 1980s, cellular immunity, especially B and T cell function and cell–cell cooperation among immune cells, came to dominate immunologic research as it does today. Interest in the complement system paled by comparison and the field was considered pretty much dead for two reasons. First, through some terrific protein chemistry, by this time the 20 or so known proteins that constitute this system in plasma had been purified and their place in the activation cascade rigorously defined. Consequently, the general feeling was that there just wasn't much new to be learned. Furthermore, connections of the complement to the development of an immune response seemed to be minimal. This line of reasoning occurred despite two contrary points. First, individuals deficient in complement proteins regularly developed the two scourges of an abnormal immune system, infections and autoimmunity. Second, the rediscovery in the 1970s of the properdin or alternative pathway as an antibody-independent means of activating the complement system provided an additional wing of the complement system to consider. However, a complement renaissance began, albeit slowly, in the 1980s piloted by the discovery of ten novel membrane proteins. Six of these are structurally related proteins that belong to a gene cluster at 1q32 and that interact with C3 and C4 products to regulate complement activation. The structural and functional characterization of these receptor and regulatory proteins led to a renewed interest in and appreciation of the role of the complement system in immunity. Such investigations have led complement back to its immunologic roots and to an influx into the complement field of microbiologists, reproductive immunologists, biotechnology companies, and clinicians.

First, an essential role for complement component C3 in enhancing an immune response was defined in the early 1970s but the significance of this observation was initially unappreciated (see review by Fearon, Semin. Immunol. *10*, 355–361, 1998). We now know that the covalent tagging of an antigen with complement activation fragments promotes localization of the antigen to complement receptor–bearing cells in the lymph nodes and the spleen and also facilitates the signaling of B lymphocyte antigen receptors. Complement receptors, by binding the end product of C3 degradation deposited on an antigen (known as C3d), lower the threshold for B lymphocyte activation and thus lead to an earlier and more efficient adaptive immune response. Thus, the complement system is a major player in three pathways of innate immunity; namely, the alternative pathway, the classical pathway (through activation by natural antibody), and the mannose-binding or lectin pathway. By these cascades the complement system identifies foreign materials and helps to guide the adaptive immune response. Thus, in addition to its well-recognized roles as an opsonin for phagocytosis and a promoter of the inflammatory response (effector arm of humoral immunity), the complement system is an important player in triggering an immune response.

The second reason for renewed interest in complement relates to the discovery of its membrane-bound inhibitors. Considering the destructive potential of complement, it is no surprise that nearly half of the system's approximately 30 proteins are involved in its regulation. Present in plasma and expressed on virtually all cells of the body are these protectors of self-tissue. Inhibitors must ensure unimpeded, rapid activation of complement on a microbial target but with strict limitations in time and space. Nature solved this antecedent problem through the evolution of plasma and membrane regulatory proteins, often related structurally and with overlapping functional repertoires. These complement system proteins prevent two highly undesirable events: activation in the fluid phase (in other words, no target) and on host tissue (the wrong target). Thus, acute activation is uninhibited, yet rapidly arrested subsequently. As a corollary, if not properly checked, the system would

cycle to exhaustion, as it does if the regulatory proteins are lacking.

In addition to their role in the complement system, regulatory proteins are now interfacing with three other fields of investigation—microbiology, reproduction, and therapeutics. Each of these areas will be briefly addressed.

For reasons yet to be understood, complement receptors and membrane regulatory proteins seem to have been preferentially chosen by certain microbes as their receptors; a few examples being viruses such as Epstein-Barr, measles, Coxsackie and ECHO, and pathogenic bacteria such as *Neisseria* and *Streptococcus pyogenes*—to name just a few of an ever-growing list. These discoveries have facilitated our understanding of these infections, fostered new transgenic mouse models of disease, and prompted intriguing questions. Is this abuse of complement membrane proteins by chance or does the microbe gain an advantage and, if so, what is it? Does the regulatory protein in turn protect the infecting organism as it does host tissue? Is there a signaling function that is co-opted by the microbe for its benefit?

Many viruses, bacteria, and protozoan parasites either produce a functional mimic of a complement regulatory protein or during their life cycle hijack the host's regulatory proteins for their membranes. Perhaps the champ here is *Streptococcus pyogenes*, which binds to three human complement regulatory proteins so as to block activation on its surface. Another well-studied example is the vaccinia virus complement regulatory protein (VCP) that has approximately 40% homology to a human regulatory protein, and presumably the DNA for this protein was snared from a former host. In a guinea pig model system, mutant virus lacking this protein produced smaller and shorter-lived pox, establishing the complement inhibitory protein as a virulence factor for this and other pox viruses. Many other microbes undoubtedly have devised even more clever ways of defeating the complement system.

Membrane complement regulatory proteins are densely expressed on spermatozoa and placental trophoblast. Several are specifically located on the inner acrosomal membrane of spermatozoa; interestingly, these proteins lack the N- and O-linked sugar moieties expressed on other tissues. In this locale, they presumably prevent complement activation on the head of spermatozoa during fertilization. But, there is also evidence they play a role in sperm–egg attachment. On trophoblast tissue the story seems to be even more intriguing. Mice lacking the Crry complement regulatory protein have an embryonic lethal phenotype. The placenta in this situation is atrophic with a prominent inflammatory infiltrate and abundant complement deposition. However, if these deficient mice are bred to C3-deficient mice, viable pups result and the placenta is normal (Molina, Mol. Immunol. *35*, 382, 1998). The interpretation is that, by not allowing complement action through C3, the phenotype has been rescued.

As yet there is no therapeutic agent for use in the clinic that blocks the pathologic consequences of unwanted complement activation. However, complement receptors and regulatory proteins, produced recombinantly as soluble forms or expressed in transgenic animals, are effective inhibitors of complement activation. Thus, the xenotransplantation field has moved forward because organs from transgenic pigs expressing human complement membrane regulatory proteins are not hyperacutely rejected by primates. Also, clinical trials are underway with a recombinantly produced soluble complement inhibitor known as TP10 (Avant Immunotherapeutics, Needham, MA). The availability of a regulatory protein that inhibits the C3 and C5 cleaving enzymes has already led to unanticipated observations in animal models. For example, complement inhibition reduced tissue damage in ischemia-reperfusion injury such as that which accompanies heart attacks and strokes. Complement may be pivotal in the clearance of dead and dying tissue, including cells undergoing apoptosis. These agents have also permitted confirmation of the destructive capacity of complement in many autoantibody and immune complex excess syndromes. Much remains to be learned about the beneficial and destructive behavior of the complement system in clinical situations.

As these tantalizing tidbits point out, the complement field has been reawakened, in part, due to the discovery of its membrane receptors and regulatory proteins. Since these proteins are relevant to diverse fields such as reproduction, ischemia-reperfusion injury, apoptosis, therapeutics, and multiple infectious diseases, a timely and readable review of the field for both the complement specialist and for the interested reader from other fields would be quite useful. Paul Morgan and Claire Harris have prepared a review on complement regulatory proteins that fills this bill. In an engaging and authoritative manner (as the book jacket correctly points out), the authors have done a splendid job of providing us with an informative, in-depth, easy to read overview of the field. The authors are not bashful in pointing out controversial issues and are even handed in their appraisals of such contentious areas. Simple (but without the glitz of many new science books) and helpful diagrams plus an exceptionally complete bibliography are other useful features. This book compares favorably with two other recent publications on this subject (Liszewski, Adv. Immunol. *61*, 201–283, 1996, and *The Complement System in Health and Disease*, Volanakis and Frank, eds., Marcel Dekker, 1998). It is the most comprehensive and up-to-date of the lot. It has a coherent feel from the first page to the last, largely probably due to only two authors preparing the text. In my laboratory, it passed the "utility test" in that my first two copies quickly disappeared (subsequently found in a cranny of a graduate student's desk while the other was at home with a senior research associate). So, more copies were ordered.

To provide such a comprehensive, reader-friendly, and accurate review of complement regulatory proteins is no small feat. Morgan and Harris initially overview the complement system and the history of its control. They describe both the regulation of the pathways and also introduce the reader to its rare, but biologically informative, inhibitor deficiency states. Each protein's structure and function is described in a clear fashion. The authors then expertly summarize the remarkable connections of these inhibitory proteins to the immune response, microbial infections, reproduction, and clinical medicine. The pertinent references relevant to these issues

and my foregoing discussion can be readily accessed in this text.

The complement and clotting systems are remembered infamously by students for their overwhelming complexity and archaic nomenclature. After teaching the complement aspect of immunology now for more than 20 years to graduate and medical students, I was heartened when, this past fall after I once again gave my half-hearted apology for the complexity of the complement system, a medical student told me "not to worry because complement was just a simple little proteolytic cascade and besides you won't believe what we have to learn about really complicated parts of the immune system such as cytokines and signal transduction." Long live a simple little proteolytic cascade!

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Attempting Arrest or Aiding and Abetting?

Chemokines and Cancer
Edited by Barrett J. Rollins
Totowa, NJ: Humana (1999). 320 pp. $125.00

Chemokines and cancer are linked, at the very least by metaphor. The chemokine system seems ever growing; it is difficult to control and hard to classify; is frequently invasive of established bodies of investigation, metastasizing into important areas of basic biology and medicine. Chemokines are an enormous superfamily of cytokines, comprising some 50 or so distinct but related human gene products, with 6–8 more encoded by human viruses. All of these interact, frequently interchangeably, with at least two dozen receptors expressed on a variety of cell types in multiple tissue compartments. The fact that chemokines can act as "motiligens"—directing the migration of leukocytes and other cells—as well as observations that these molecules might, directly or indirectly, regulate cytoproliferation, makes them candidates for supporting roles in neoplastic transformation.

An exquisite and intricate body of knowledge has amassed concerning tumorigenesis. Despite profound complexity at the molecular, cellular, and organismal levels, the natural history of neoplasms might be thought of in simple concepts. Envision a model where in the host tumors are "fueled," "fed," and "fought." "Fueled" might indicate direct growth promotion, including the action of soluble proteins (and their cell surface receptors) acting as autocrine or paracrine growth factors. Dysregulation of the temporal or spatial elaboration of such growth factors, or alteration of their structures, could lead to constitutive cell proliferation. Once established as a growing entity, a tumor needs to be "fed." Necessary tissue infrastructures include neovasculature, promoted by angiogenic factors, which supply nutrients to the tumor locale. Lastly, it might be thought that an aberrant tissue form in an otherwise well-articulated tissue space might be homeostatically abhorrent and "fought" by an appropriate host response. Immune reaction might be predicated by the migration of host defense cells to the site of the tumor. So-called tumor-associated leukocytes, or TAL, have long been observed to be a part of solid tumor masses. TAL have been speculated to constitute an anti-tumor response, but their role is now being reexamined. Other cells such as natural killer (NK) cells are also known to possess anti-tumor functions in model systems.

Historically chemokine biology has touched upon all of these areas. A potential role in the fight response may be indicated, since chemokines can control the migration of NK cells and other leukocytes known to infiltrate tumors. Some chemokines may also act as fuel: as growth factors that favor tumor mitogenesis or which control apoptotic events. Others may modify the feed structure via angioregulation or by altering the extracellular matrix. Less well considered, but no less important, is the possibility that chemokines acting directly on tumors could promote tumor metastasis. In terms of hard proof of in vivo causal connections, however, it is fair to say that so far speculation exceeds demonstration. Thus, there are compelling reasons to investigate the link between chemokines and cancer in a focused body of work.

The challenge is daunting. One presupposition is that the highly complicated and ever evolving system of chemokine biology can be made accessible and cogent to both novice and aficionado. In *Chemokines and Cancer*, editor Barrett Rollins gamely tackles this imposing task, attempting to knit the areas of chemokine biology and neoplastic pathology into a synthetic whole. In all he and his contributors deliver an informative, if at times uneven, treatment of the subject matter.

One opens this work with anticipation. The book has a satisfying heft and presents a well-organized format of six separate sections. Early enthusiasm is somewhat tempered by a cursory inspection. Surely a few color plates would have enhanced the presentation, and there are other perplexing anomalies, such as the lack of a standard citation format and evidence of imperfect proofreading. These small items are emblematic of a sometimes frustrating unevenness to this work. Informative, provocative, and even inspired concepts are present, but they soar up from a frequently quotidian platform. When the book "works" it does so beautifully; when it doesn't, the contrasts are marked. An otherwise well-constructed preface presages some unfortunate tendencies that run through the body of the work. The first is an inclination toward leukocyte chauvinism, i.e., the predilection to define chemokine biology almost solely in terms of the control of white blood cells, thus classifying chemokines exclusively as inflammatory mediators. This "leukocentric" prejudice is simply not helpful, particularly in consideration of such intricate biologies as malignant transformation in complex tissue environments. The preface also foreshadows a tendency of the contributors to be "articulate axiomists": creating paradigms with words, not necessarily evidence.

Much of what is right—and wrong—about this book can be discovered in part I, an introduction to chemokine biology entitled "Chemokine Physiology." This section

comprises two chapters, one excellent and one almost wholly inadequate. Presumably these are intended to be prolegomena to the concepts examined in the remainder of the book. Here the reader might be well advised to start not at the beginning but at chapter 2, "Chemokine Receptors and Ligand Specificity: Understanding the Enigma." This is more than a pithy overview of chemokine receptors and ligand specificity; it is an exemplary and succinct primer of many major concepts in modern chemokine biology. More accuracy can be gleaned from the simple introduction than in the ponderous leukocentric rhetoric that is rife elsewhere. We are reminded that where chemokines were "Originally thought to be limited to bone marrow derived cells, we now recognize that many epithelial, endothelial, parenchymal, mesothelial, and neuronal cells express chemokines and their receptors" (p. 21). This is a valuable but ignored notion in many of the other chapters.

Chapter 2 is fashioned around the important, frustrating, and endlessly intriguing question of how chemokine receptors "sort information." At issue is why there is so much apparent redundancy in the chemokine system: many different chemokines can bind to a common receptor, and different receptors can bind a given chemokine. These observations, coupled with fairly crude analyses that different chemokines can affect the "same" cell types in vitro, has led to a prevailing prejudice in the field. There exists a "cult of chemokine redundancy": a canon of dubious dogma that maintains that the chemokine system safeguards its many functions by having multiple players effectively filling the same role.

Chemokine redundancy is embraced unthinkingly by many, but few have seriously evaluated the issue. Moreover, the posited degree of functional redundancy is not necessarily supported by observations from animals with targeted gene deletions of various chemokine elements. Chapter 2 confronts this, synthesizing established observations with new data and drawing parallels from a related field of seven transmembrane (7TM) receptors, the tachykinin receptors. The analogy of tachykinin receptor function is used to refute key beliefs underlying the cult of chemokine redundancy, and to argue that such redundancy is likely to be more apparent than real. This is no mere clever eristic. The author presents a strong case, historically and theoretically, that just because two ligands bind to the same receptor, it does not follow that the "information content" of those binding events is at all similar. Each is apt to have its own unique spectrum of functional sequelae. Common binding spectra therefore do not engender redundancy, but rather promote diversity.

Chapter 2 is a gem, but not an altogether flawless one. Figure 1 (p. 21) suffers from typographical errors and is already a bit outdated; Figure 2 is an object lesson in unfulfilled promise. Nonetheless, this chapter stands in rich contrast to a starkly pedestrian first chapter. A musty and obsolete tedium, "Chemokines in Health and Disease" (chapter 1) provides only a wan simulacrum to the dynamic and beautiful complexity of real world chemokinetics. This chapter suffers from extreme leukocentrism. Of course the control of leukocytes by chemokines is extremely important, but it is by no means the entire biology of the system. This makes for a poor introduction to a work that aims to meld together two complicated fields. Regrettably, this chapter, which might have been erudite even if overly focused, reads like recyclate from a not too recent textbook on inflammation. Indeed, reference is made to "recent scientific advances" (p. 4) that the authors describe in the previous paragraph as having occurred in the 1960s. The tables are incomplete and out of date, redundant yet exasperatingly noncomprehensive, and chemokines mentioned in the text are not listed in the tables. The otherwise thorough section on animal models would have been strengthened by comparison with gene knockout and other genetic models. Mystifyingly, no reference whatsoever is made to chemokine receptors, even to cross-reference the topic to chapter 2. So it is with much of this work, going from engaging to entropic in a few pages.

Fortunately the editor's skills ensure that the overall package is useful. In part II, entitled "Tumor Infiltration by Leukocytes," complementary reports discuss the phenomenon of TAL. It is here that the central theme of the book emerges: do TAL represent a call to action or Trojan horse in tumor development? And, by extension, do chemokines generally help or hinder tumor establishment and encroachment in human tissues?

Part II encompasses chapters 3 and 4, and chapter 6 is also thematically linked. All discuss TAL with respect to (mostly) monocyte association with solid tumors. How do monocytes get to a tumor, and what are they doing once they are resident? Although the reports are all overfocused on the biology of MCP-1, they provide excellent historical background, comprehensive reference lists, and in chapter 4 an extensive (if somewhat misplaced) list of chemokines with many of their multiple confusing designations (p. 56). The works are all too leukocentric; axioms also abound. Such statements as "the spectrum of action of chemokines tend to be restricted to leukocytes," (p. 40) and "It is clear that [the] total TAL load at any moment results from the concerted action of the differential temporospatial expression of chemokine mixtures" (p. 51), suggest a level of proof and a consensus of opinion that have not yet been achieved. Other overly broad statements can be found in the chapter.

Nevertheless the three chapters make for valuable reading. The countercurrent model of chemokines in tumor cell seeding and establishment is lucidly examined (see pp. 36 and 55). Enlightened speculation is entertained about the actual role of TAL in tumor biology: tumors produce chemokines, and therefore tumors may actively attract TAL, but why? Do TAL induce tissue destructive reactions, and thus promote an anti-tumor response? Or rather do TAL promote a tumor-friendly environment by producing necessary growth and angiogenic factors, help invasion and metastasis via ECM remodeling, and produce decoy elements that lead to the clearance of effector cytokines? These issues are examined intelligently, and the authors admit that definitive answers do not yet exist. Interesting correlations might be drawn with the contributions in part V, "Chemokines in Specific Malignancies," examining chemokines and cancer in the clinical realm. Again too biased toward the biology of MCP-1, chapters 11–14 still represent an important effort to bring clinical chemokinology into perspective.

The theme of chemokines as tumor help or hindrance is extended in part III "Modulation of Host Tumor Responses to Cancer" (chapter 5–7) and part IV "Chemokines and Tumor Growth Metastasis and Angiogenesis" (chapters 8–10). A very good overview of NK cell function and chemokine regulation of these cells is provided in chapter 5, despite some surprising errors (e.g., SDF-1 is not an abbreviation for stem cell differentiation factor, and fractalkine and neurotactin are the same molecule, p. 76), and a rather personalized tone that may be off-putting to some. Additionally, "Interactions between Chemokines and Other Cytokines in Host Responses to Tumor" (chapter 7) is an excellent and succinct contribution that might have been more useful in part I. In chapter 8, be amused by statements on page 129 that are mostly contradicted a page later.

The lively issue of whether chemokines are true regulators of angiogenesis is covered in chapter 9. It is probably fair to say that despite observations in model systems that certain chemokines act angiogenically while others are angiostatic, the in vivo relevance for human tumor growth remains to be established. Indeed, little is known of the molecular mechanism behind this effect; debate rages even as to whether it is a direct effect of chemokines. Models using tumors transduced with chemokines don't provide much cross-validation for a role in angiogenesis; almost all show tumor inhibition. So it is also with the effects of chemokines on the regulation of hematopoietic precursor cells: are they "on" or "off" signals, and are they relevant at all in vivo? Chapters 15 and 16 (part VI), "Chemokines and Stem Cell Proliferation," provide excellent discussions of concepts and controversies in this field.

Chemokines and Cancer is an ambitious work. It succeeds in bringing to the fore the important notion of whether chemokine action in tumor development should be antagonized or promoted for therapeutic purpose. To be fair, the work also comes up short in certain areas. Data regarding chemokines in the control of apoptosis would have been relevant, as would evaluations of the HHV8-encoded receptor ORF74, which has been reported to have direct transforming activity. Lastly a focused discussion regarding the immunotherapeutic potential of chemokines would have been instructive. The editor wisely describes his work as no more than a "snapshot" (p. vi) capturing current views of chemokines and cancer. In this information age the metaphor is a dangerous one, indeed, as some of the chapters in this work are more akin to a daguerreotype than any more modern image. That major shortcoming notwithstanding, the rest of the work provides a perspective that is both fresh yet familiar. *Chemokines and Cancer* will not be universally accepted, but it should be well read.

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Hunting for Carcinogens

Carcinogenicity: Testing, Predicting, and Interpreting Chemical Effects
By Kirk T. Kitchin
New York: Marcel Dekker (1998). 986 pp. $195.00

The concept that occupational and lifestyle factors play a role in human cancer causation dates back to the 18th century. Astute clinical observations were made by Percival Pott of the association of scrotal cancer in chimney sweeps with chronic exposure to soot, by John Hill of the association of polyps of the nose with the use of snuff, and by Bernardino Ramazzini of the association of breast cancer in nuns with reproductive factors. However, the field of experimental carcinogenesis did not develop until the early part of the 20th century and was heralded by the pioneering studies of Yamagiwa and Ichikawa who in 1918 demonstrated that the repeated painting of tar or soot on the skin of rabbits or mice led to the progressive development of carcinomas. Within recent decades there has been a virtual explosion in the development of assays for chemical carcinogens, including the standardization of long-term cancer assays in rats and mice, as well as the design of innovative medium-term and short-term assays for mutagenicity or epigenetic effects that appear to be predictive of carcinogenicity. This book provides a comprehensive description and appraisal of these various assays through a series of 29 chapters written by experts in the respective subjects. It also provides an extremely valuable summation of the current database on chemicals that are known to display carcinogenic activity in experimental animals and/or in humans.

The subject matter in this book is highly relevant to public health and disease prevention since, while exciting advances are being made in our understanding of the genetic determinants of cancer, several types of evidence indicate that environmental determinants, including dietary and lifestyle factors, play a decisive role in the causation of the majority of human cancers. The high incidence of lung cancer caused by cigarette smoking provides convincing evidence of the susceptibility of the human species to certain chemical carcinogens. Furthermore, over 70,000 chemicals are currently used in commerce and each year a number of newly synthesized chemicals enter our workplaces, our diets, or the general environment. Therefore, there continues to be an urgent need to have a battery of assays that determine the potential carcinogenicity of numerous types of chemicals, especially since epidemiologic studies are usually retrospective and often have limited sensitivity.

This book is divided into three sections, (1) Testing, (2) Predicting, and (3) Interpreting Carcinogenicity. The five chapters in the first section provide a historical perspective as well as a detailed overview of the various current approaches for identifying chemical carcinogens in humans, and in experimental animals. The methods for human investigations include epidemiologic studies and case reports, while the experimental methods include long-term bioassays (usually in mice and rats), midterm in vivo assays, short-term in vivo and in

vitro assays (for example mutagenicity assays), transgenic models, structure–biological activity relationships, artificial intelligence systems, and mechanism-based inference. The advantages and disadvantages of these procedures are critically evaluated. Several issues related to standard long-term carcinogen bioassays that employ mice and rats, used by the National Toxicology Program and other agencies, are also discussed. These issues include: selection of agents for testing, choice of species and strains of animals, diet, caging, route of administration, dose selection, duration, pathology studies, ancillary biochemical and pharmacologic assays, and data interpretation. The controversial issue of using the maximum tolerated dose (MTD) in rodent bioasssays and the question of the relevance of the induction of mouse liver tumors are discussed, emphasizing that in both situations parallel mechanistic studies are essential for interpreting the significance of the results obtained. This section of the book also contains an extremely valuable compilation and summary of 11 major information sources on chemical carcinogenicity data. According to the International Agency for Research on Cancer (IARC), 69 agents, mixtures, and exposure circumstances are known to be carcinogenic to humans (group 1), 57 are probably carcinogenic (group 2A), and 215 are possibly carcinogenic to humans. Compilations of data on mutagenicity and other short-term assays are also provided. A testimony to the validity of the long-term carcinogenicity assays is the fact that about 30 agents that cause cancer in humans were first found to do so in animals; all of the chemicals known to cause cancer in humans that could be properly tested also do so in animals as well. At the same time there are many chemicals that are positive in one or more assays for which there is, as yet, no evidence of carcinogenicity in humans; this may reflect the limitations of the clinical and epidemiologic data in humans or true differences in species specificity. Much more research is required to expand our knowledge about species specificity with respect to the metabolism of various xenobiotics and also to provide greater insights into the mechanisms of actions of various classes of chemicals, in order to clarify these issues.

The section on "Predicting Carcinogenicity" consists of eight chapters that provide detailed discussions of assays in which the endpoint is an effect that appears to be predictive of carcinogenicity, rather than the actual induction of tumors. These assays include mutagenicity (bacterial, mammalian, and cytogenetic assays), structure–activity relationship (SAR) models, the K_e test (a physicochemical measure of electropholicy), inhibition of gap junctional intercellular communication (GJIC), a panel of in vivo biochemical parameters that are markers of tumor promotion in rat liver (for example, induction of ornithine decarboxylase or pyruvate kinase), assays for induction of peroxisome proliferation, and bioassays for the induction of preneoplastic foci of hepatacytes in rat liver. This spectrum of assays encompasses the effects of genotoxic (i.e., DNA-damaging agents) as well as nongenotoxic tumor-promoting-type agents. Although these predictive tests have the virtue of being relatively simple and rapid, at the present time it is difficult to extrapolate the significance of the results obtained to humans, in the absence of ancillary data. Hopefully, somewhat similar assays on human blood or tissue samples can be developed and used in molecular epidemiology studies to bridge this gap (Weinstein et al., In *Molecular Basis of Cancer*, Mendelsohn et al., eds., Philadelphia, W. B. Saunders, pp. 59–85, 1995).

The final chapter in this section discusses the emerging and very promising use of transgenic animals as predictive models for identifying carcinogens, based on the progressive acquisition of mutations in cellular oncogenes and tumor suppressor genes that occurs during the multistage process of carcinogenesis. Recent studies on two genetically engineered strains of mice, p53-deficient mice and mice carrying a mutated v-Ha-ras oncogene (Tg.AC mice), appear very promising as assay systems for carcinogens, since they have responded appropriately to a panel of test chemicals and they require fewer animals and shorter exposure times than assays using conventional mice. It seems likely that other types of genetically engineered mice will be developed to identify specific classes of chemical carcinogens, carcinogens that act on specific organs or through specific molecular mechanisms, and to assess the roles of specific polymorphisms in influencing interindividual variations in susceptibility to specific carcinogens.

The final section of this book is titled "Interpreting Carcinogenicity." Within its 14 chapters, each reviews current knowledge and hypotheses of the causative factors for cancers of specific organs in humans and carcinogenicity data in these same sites in animals. It is of interest that certain organs, for example the mammary gland, display a high incidence of cancer in both experimental animals and humans, whereas others such as the prostate, display a low incidence in experimental animals and a high incidence in humans. The final chapter by Drs. Clayson and Kitchin, entitled "Interspecies Differences in Response to Chemical Carcinogens," reviews the highlights of the above chapters reiterating the quantitative and qualitative differences in responses to individual carcinogens when one compares experimental animal species and humans. Using the definition that a carcinogen is "an agent that leads to a statistically significant increase in the number of benign and/or malignant tumors in a tissue," Drs. Clayson and Kitchin state that we know four major mechanisms by which chemical carcinogens exert their effects. These are: (1) the generation of electrophilic compounds that form covalent adducts with DNA and thereby cause mutations, (2) the formation of oxygen radicals that damage DNA, (3) interactions with specific cellular receptors (for example the estrogen receptor) that modify gene expression, and (4) increases in cellular proliferation, a characteristic of tumor-promoting agents.

As we move into the 21st century, the spectacular current advances being made in our understanding of the cellular and molecular events that occur during the multistage process of carcinogenesis will provide more profound insights into the mechanisms of action of carcinogens and anticarcinogens. Recently revealed potential cellular targets include: membrane-associated growth factor receptors; intracellular pathways of signal transduction; nuclear receptors and transcription factors; genes that control DNA replication and repair, the cell cycle, differentiation, or apoptosis; and genes that control extracellular functions (adhesion molecules,

proteases, and angiogenic factors) (Weinstein et al., Clinical Cancer Res., *3*, 2696–2702, 1997). We can also anticipate that within a few years novel mechanism-based methods that utilize recent advances in biotechnology will play an increasingly important role in assays for carcinogens. These will include the use of DNA chip technology and microassays that can efficiently score for mutations or alterations in profiles of gene expression that predict carcinogenic effects.

Finally, although the types of carcinogen assays described in this book have played, and will continue to play, an important role in our understanding of mechanisms of carcinogenesis and in the prevention of an important subset of human cancers, they have not thus far revealed the causes of several major forms of human cancer, especially cancers of the breast, prostate, and colon. This limitation may reflect the likelihood that the causation of these cancers is due to complex interactions between multiple factors, including genetic susceptibility, dietary factors, lifestyle factors, and/or yet to-be-discovered viral or bacterial agents. The interaction between the dietary chemical carcinogen aflatoxin and chronic infection with hepatitis B virus in the causation of liver cancer, and the interaction of dietary factors and chronic infection of the gastric mucosa with the bacterium *Helicobacter pylori* in the causation of stomach cancer, provide instructive examples of these multifactor interactions in human cancer causation. A major challenge in the field of experimental carcinogenesis is to develop innovative approaches that can provide further insights into these types of multifactor interactions. The above-described use of genetically engineered mice and innovative biotechnology-based assays should facilitate progress in this area. Obviously innovative epidemiologic studies, in particular those that employ the emerging techniques in molecular epidemiology, will also play a crucial role in furthering our understanding of cancer causation. Hopefully by combining these approaches, together with well-controlled intervention studies, the 21st century will bring more effective strategies for reducing cancer incidence and mortality.

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Using Genes to Cure Cancer

Gene Therapy of Cancer
Edited by Edmund C. Lattime and Stanton L. Gerson
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Breakthroughs in our understanding of the genetic alterations that lead to cancer bring with them the ultimate hope that these may be translated into definitive progress in the treatment of human cancer. This may take the form of direct introduction of genetic material into tumors, as well as novel strategies that take advantage of genetic differences between normal and cancer cells. The recently published book *Gene Therapy of Cancer* presents an excellent, detailed, and comprehensive review of the many therapeutic approaches currently under investigation, their underlying rationale, and areas for further development. The book is multi-authored, and most chapters are well written and thorough, although some redundancy is unavoidable. The concept of gene therapy is taken broadly, including all forms of gene-based treatments, with heavy emphasis on immunological approaches. A weakness is the limited discussion of molecular genetic targets and evolving strategies using inhibitory peptides and small molecules. The book will be of interest to those who are new to the field and who would benefit from an excellent review of current approaches to gene-based therapy of cancer.

Gene therapy with the goal of treating cancer differs fundamentally from that aimed at correcting inherited genetic diseases. Rather than achieving long-term, physiological expression of a gene within a few stem cells, the success of genetic therapy in cancer requires the destruction of each and every cancer cell within a tumor, without undue toxicity to surrounding normal cells. This daunting task may be facilitated by secondary in vivo effects, including immunological responses and the so-called "bystander" effect (the death of uninfected cells adjacent to infected cells) (Freeman et al., 1993). However, true success is likely to come from an improved understanding of critical targets that distinguish cancer cells from surrounding normal cells, including both molecular alterations within cancer cells themselves and essential supportive elements such as tumor vasculature.

The design of optimal vectors remains a major limitation for attempts at human gene therapy, and a number of chapters describe current approaches used in the treatment of cancer. Most trials have involved retroviruses, whose ability to selectively infect proliferating cells provides an immediate rationale for their use in cancer, particularly those arising in tissues with a low proliferative index (for example, brain). Chromosomal integration of retrovirally encoded genes provides for long-term expression, but in vivo infection efficiency is low. In contrast, adenoviral vectors allow high infectivity of both quiescent and proliferating cells; viral genes remain episomal resulting in high levels of transient expression. However, adenovirus triggers a significant immunological response that limits attempts at repeated infections. More recently, adeno-associated virus (AAV), a parvovirus-derived vector that affords both high infectivity and chromosomal integration, has been incorporated into clinical trials. In addition to viral vectors, liposomes or even direct injection of naked DNA can lead to ectopic gene expression in animal models. Ex vivo transfection of cultured cells, followed by inoculation into tumors has been useful in strategies aimed at immunological modulation.

The many approaches to cancer gene therapy described in this book can be grouped into three broad classes: (1) the use of suicide genes, (2) modulation of the immune response to cancer cells, and (3) specific targeting of molecular genetic alterations. The chapters describe experimental strategies tested in cell culture, animal models, and in a few cases, clinical trials. The rationale for the use of suicide genes is based on the selective retroviral infection of proliferating cancer cells.

Traditionally, these studies have used the herpes simplex virus thymidine kinase (HSV-TK) gene, whose product phosphorylates the synthetic nucleoside analog ganciclovir with much greater efficiency that the mammalian thymidine kinase, resulting in disruption of DNA replication. Effective killing of infected cancer cells has been reported in trials of patients with brain and ovarian cancer (Freeman et al., 1995). The need for direct inoculation of a circumscribed tumor and the low infectivity of retroviruses remain significant limitations that are currently being addressed. However, these studies have led to the observation of the so-called bystander effect. In vitro experiments have suggested that infected cells might release toxic metabolites that enter neighboring uninfected cells through gap junctions or as a result of their engulfment by neighboring cells. In vivo studies suggest a more complex mechanism, including possible effects on tumor vasculature and the initiation of inflammatory responses. By whatever mechanisms it operates in vivo, the bystander effect is essential for the potential effectiveness of suicide gene therapy in cancer. In an interesting twist, one of the most useful applications of suicide gene therapy may be modulation of the immunological response in bone marrow transplantation. Ex vivo transduction of T lymphocytes with HSV-TK or other suicide genes allows for attenuation of graft-versus-host disease when these engineered T cells are reintroduced, along with heterologous bone marrow, into patients whose own marrow cells have been ablated (Tiberghien et al., 1997).

The second approach addressed in a number of chapters is that of using gene therapy to modulate the host immune response to cancer. This includes experiments in which tumor cells are transfected ex vivo with growth factors, such as IL4 and GM-CSF, and then reinoculated directly into the tumor, where they generate a local inflammatory response (Tepper et al., 1989; Dranoff et al., 1993). Other chapters deal with immunization using tumor cells, tumor-derived peptides, and dendritic cells that have been transfected in vitro with plasmids encoding tumor-derived antigens. These specialized antigen-presenting cells are isolated from individual patients and reinjected following in vitro manipulation, or used to generate an expanded pool of cytotoxic lymphocytes prior to reinoculation. These attempts at establishing tumor epitope–specific reactive lymphocytes extend the principles of adoptive T cell immunity, a concept initially based on the in vitro coculturing of tumor cells and cytotoxic lymphocytes followed by in vivo reinfusion of tumor-reactive lymphocytes along with IL2 (Rosenberg et al., 1986). Eventually, transduction of lymphocytes with chimeric T cell receptors may be used to redirect immune reactivity, effectively targeting these engineered cells toward tumor antigens. Elements of the humoral immune response have also been marshalled in the treatment of cancer, and chapters describe the generation of "humanized" monoclonal antibodies aimed at circumventing the generation of human anti-mouse antibodies, the use of unconjugated antibodies that mediate tumor lysis through Fc-mediated effectors or by direct induction of apoptosis, and the use of antibodies conjugated to cellular toxins or radionuclides. A recent therapeutic success in this regard is the antibody herceptin, reactive against a member of the epidermal growth factor receptor family, HER2/neu, whose expression on the cell surface of breast cancer cells is associated with a poor clinical prognosis. The combination of anti-HER2/neu antibodies with standard chemotherapeutic regimens appears to offer improved clinical responses in the treatment of human breast cancer (Pegram et al., 1998). Another effective unconjugated antibody recently introduced into clinical practice is reactive against the CD20 epitope, carried on the surface of many lymphoma cells (Maloney et al., 1994). Further successes may emerge from analysis of cancer cell expression profiles, which may identify additional antigenic targets that distinguish cancer cells from surrounding normal tissues.

The third general approach to gene-based treatment for cancer directly targets the molecular alterations in the cancer cell. This includes attempts at using retroviruses or AAV to reintroduce wild-type tumor suppressor genes, such as *p53* and *RB*, into cells whose endogenous genes have been mutated during malignant transformation. The use of ribozymes, antisense oligonucleotides, and single-chain intracellular antibodies to target specific molecules thought to drive malignant transformation is also discussed in a number of chapters. As expected, these approaches have been limited by the need to infect all cancer cells and by the efficiency of the methods used to disrupt the selected molecular targets. More recently, the engineered adenovirus Onyx 015 has provided a new paradigm for the targeting of an aberrant molecular pathway linked to malignant transformation (Bischoff et al., 1996). Lysis of human cells by adenovirus requires the viral product E1B 55K, which inactivates cellular p53 and prevents the triggering of apoptosis before viral replication has been achieved. Deletion of E1B 55K therefore produces a virus capable of replication and lysis only in cells lacking endogenous p53 (i.e., approximately half of all cancers). Some controversy exists about the true molecular specificity of this engineered adenovirus, which is complicated by the fact that cancer cells lacking p53 mutations may have enhanced degradation of p53 resulting from overexpression of MDM2 or deletion of $p19^{ARF}$, and hence may be functionally p53-null. In combination with standard chemotherapy regimens, Onyx 015 is being used in clinical trials, by direct inoculation of tumors in head and neck cancer and by intraperitoneal injection in ovarian cancer, with some promising results (Heise et al., 1997). Of particular interest is the intense inflammatory reaction that accompanies infection of tumors, suggesting that direct cellular lysis by the virus may be greatly augmented by an immunological reaction to this antigenic virus.

The ability to translate knowledge about critical genetic lesions in tumor cells into molecularly targeted cancer treatment is likely to present one of the most exciting challenges for the future. This represents an extension from traditional concepts of gene replacement therapy in cancer toward gene-based therapeutic strategies using drugs, peptides, and other small molecules. Here the book is not as comprehensive as perhaps it could have been. Recent discoveries suggest a number of pathways that may constitute effective targets, including aberrant expression of the catalytic subunit of telomerase, hTRT, leading to cellular immortalization, and the synthesis by tumors of angiogenic factors that allow their growth beyond a minimal size. The majority of cancers have a genetic alteration in one of the components of the retinoblastoma pathway essential to

cell cycle regulation, including pRB itself, $p16^{INK4a}$, cyclin-dependent kinase 4 (CDK4), and cyclin D1. In some tumor types, such as colorectal cancer, specific signaling pathways appear to be critical to maintain cellular proliferation, including those mediated by transforming growth factor β (TGFβ) and the products of the familial polyposis gene *APC*, β-catenin, and the TCF/LEF transcriptional regulators. Additional genetic targets are likely to emerge from ongoing efforts at expression and mutational profiling of different tumor types, eventually providing a possible "genetic fingerprint" that may guide therapeutic efforts (Kononen et al., 1998). These altered genetic pathways might be tackled by direct reintroduction of wild-type cDNAs to replace tumor suppressor genes that have been inactivated, or by antisense and ribozyme strategies to target aberrantly expressed gene products. However, the design and testing of small molecules or peptides may in the end prove to be most effective for efficient targeting of a critical gene product within every cell of a human cancer, as discussed in this book. Synthetic CDK inhibitors, telomerase antagonists, and angiogenesis inhibitors are likely to enter the clinical arena in the not-so-distant future. Their success, like that of gene therapy strategies involving suicide genes, immunological modulation, and molecular targeting approaches, would signal important progress in the rational therapy of human cancer.

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What Makes Breast Cancer Cells Tick?

Breast Cancer: Molecular Genetics, Pathogenesis, and Therapeutics
Edited by A. M. Bowcock
Contemporary Cancer Research Series, Volume 3
(Series Editor, J. A. Nickoloff)
Totowa, NJ: Humana Press (1999). 582 pp. $145.00

Breast cancer is not only the most common malignancy in women, but it also accounts for roughly a third of all cases of cancer. Although the overall 5-year survival rate following diagnosis has increased to nearly 80% in recent years, the disease that will affect one in every eight women still exacts considerable social and economic costs. It is therefore the continuing focus for a research effort that spans disparate (and divided) fields ranging from DNA repair to cell adhesion. Their relevance to breast cancer has not effectively been considered together in a single volume. The rationale behind this book—to encapsulate from a genetic viewpoint contemporary research on disease pathogenesis and management—should be welcomed, and not only by those who study or treat breast cancer.

The book consists of reviews that explore three main themes. What are the genetic changes associated with breast cancer? How does metastasis occur? How may diagnosis and therapy be improved by better understanding of these phenomena? The reviews are, in general, clear, concise, and authoritative. They discuss a broad spectrum of interesting but distinct topics, from the biological functions of hereditary breast cancer genes, to the surgical treatment of breast cancer, to environmental risk factors. On the flip side, this accentuates the uneven coverage inevitable in a small volume, particularly evident in the first and last sections (dealing with genetic changes and therapeutics). For example, genetic changes associated with sporadic breast cancer are reviewed primarily in relation to the growth factors and receptors that regulate the proliferation of breast cancer cells, whereas by contrast, cancer-associated alterations in oncogenes and tumor suppressor genes receive relatively scant attention. The book's laudable aim to introduce current thinking in breast cancer genetics, and to provide a background of ideas to spark future research, is diminished by this and other imbalances. As a collection of discrete reviews, this book works. What is missing is an integrative treatment that brings together experimental information from different fields that is of relevance to key issues in pathogenesis and treatment. This book, then, is one I would happily consult for information on many specific topics, particularly those with a clinical flavor, but not one that could be read cover-to-cover to gain an overall view of contemporary breast cancer research.

The reviews in this book bring home the important point that a better understanding of epithelial cell biology is vital to synthesize information from disparate fields of breast cancer research into a common conceptual framework. Breast cancer arises in the ductal and lobular epithelium of the mammary gland. Current understanding of how these epithelia develop, how their

activity is regulated, and how they may interact with surrounding stromal tissues and vasculature, is surprisingly limited considering the centrality of these issues to disease pathogenesis and management. This situation is not unique to breast epithelia, but also pertains to the epithelial tissues from which the other common cancers (prostate, colon, and lung) arise.

The point is particularly exposed by the reviews in the first part of the book, which discuss cancer-associated genetic alterations in growth factors and their receptors. Multiple hormones and cytokines—including estrogens, members of the fibroblast and epidermal growth factor families, *Wnt* gene products, and insulin-like growth factors—affect the growth and differentiation of breast epithelial cells. The intracellular signaling pathways they excite have been elaborated in some detail. Yet we do not clearly understand how their effects can be integrated into the programs that specify organ development and function. Even the most basic elements—the identity of the progenitor cells that undergo different developmental fates, and their lineage relationships during development, for example—have not been precisely established. Perhaps as a result of these gaps in current knowledge, most of the reviews fail to place information regarding cancer-associated alterations in growth factors and their receptors within a common context. I would nevertheless have welcomed more assiduous attempts to do this, a task best accomplished in the excellent reviews on the insulin-like growth factor network, and apoptosis pathway genes.

The importance of developmental physiology to breast cancer pathogenesis also deserves more explicit discussion. In the breast epithelium, developmental programs are activated periodically throughout life, and not only during embryogenesis as in most organs. Full differentiation of rudimentary ductal structures into ducts and lobules is achieved early in pregnancy, followed by involution through apoptosis when lactation ceases. Waves of epithelial proliferation and apoptosis are triggered, albeit to a lesser extent, with each estrous cycle. Many of the known risk factors for breast cancer (e.g., early menarche, late menopause, and age at first pregnancy) are likely linked to aspects of these developmental programs. But their relationship to breast cancer pathogenesis remains obscure. A better understanding of its mechanistic, or preferably genetic, basis will be central to the long-term goal of screening the general population for women at high risk.

Several reviews in the therapeutics section address the relevance of epithelial cell proliferation to breast cancer therapy, as marked by the established success of estrogen antagonists in the treatment of receptor-positive breast cancers, and the potential value of newer therapies directed to the inhibition of EGF-like molecules. The articles make clear that these strategies are in part limited by a basic problem: how to tease apart the developmentally regulated effects of the multiplicity of growth stimuli, the stromal tissues, and the vasculature to achieve an overall picture of the control of proliferation? This renders empirical, to a greater or lesser degree, current and emerging approaches to specifically target proliferation pathways in breast epithelial cells, making it difficult to overcome drug resistance and limiting our ability to design new strategies.

It is self-evident, but worth repetition, that these and related questions will be resolved when epithelial physiology is better understood. It is more difficult to anticipate how progress will come about. Robust in vitro systems in which to study mammary epithelial development and function at the molecular level are not well established. Techniques for the differential analysis of gene expression are impeded by the inability to isolate appropriate target and control cells. The clinical relevance of conclusions from murine gene targeting or transgenesis experiments is questionable until proven otherwise. It seems fair to add, though, that the molecular biology of epithelial development and function in the breast has yet to be systematically studied, and several recent insights have been spin-offs from work directed to other ends. For example, confirmation that the Stat5a transcription factor involved in prolactin signaling is essential for epithelial morphogenesis and function (Liu et al., 1997) may make it feasible to identify the downstream target genes that mediate these developmental processes. Involvement of the p63 tumor suppressor in breast development (Mills et al., 1999; Yang et al., 1999) provides insight into the mechanisms that specify epithelial stem cell fate in this developmental setting. The identification of wholly unexpected participants such as the Lats1 tumor suppressor (St John et al., 1999) or the c-Cbl protein kinase (Murphy et al., 1998) may reveal new signals that regulate development. Discoveries like these should help to accelerate progress in building up a more complete picture of epithelial development at the molecular level, if only the first insights are pursued.

A section devoted to the biology of tumor progression and metastasis is a highlight of this book. In particular, the role of integrins and matrix metalloproteinases in the interactions between stroma and cancer cells is thoughtfully and clearly summarized. The importance of these interactions to breast cancer initiation and progression is brought home by observations too recent for mention in the reviews. A stromal-derived matrix metalloproteinase, stromelysin-1 (Str1), when expressed in mammary epithelial cells induces their conversion to an invasive, mesenchymal form, resembling aggressive human breast cancer cells (Sternlicht et al., 1999). Expression of Str1 in the breast epithelium of transgenic mice suffices to initiate neoplastic transformation, which can be blocked by coexpression of the Str1 inhibitor, *TIMP1*. Remarkably, lesions that arise in this setting exhibit consistent loss or amplification of specific chromosomal regions, suggesting that the disruption of normal stromal–epithelial interactions can foster the genetic changes that result in tumor development (reviewed in Tlsty, 1998). The relevance of this experimental conclusion to breast cancer in humans has not been established, but is perhaps presaged by observations that link alterations in the stromal microenvironment to an increased cancer risk (Jacobs et al., 1999).

Despite wide interest, it is unclear if the study of hereditary breast cancer genes will enlarge our understanding of breast cancer pathogenesis in general. Two thorough reviews, on their genetic epidemiology and biological functions, recognize this uncertainty. *BRCA1* and *BRCA2* mutations account for roughly two-thirds of inherited predisposition to breast cancer, only about 6% of all cases. Somatic mutations in these genes are not found

in tumors from the remaining 90% of (nonfamilial) cases, undermining (but not excluding) the conjecture that a common pathway involving BRCA1 and BRCA2 will be dysfunctional in the majority of breast cancers. *BRCA1* and *BRCA2* encode large, nuclear proteins that are ubiquitously expressed and appear to have functions in DNA repair, cell cycle progression, and transcription, that are altered by tumor-associated mutations. Why should mutations affecting processes fundamental to all tissues confer an excess of breast and ovarian cancer risk in particular? This is a key lacuna in our current knowledge, which is insufficiently discussed in the reviews. Perhaps functions in DNA repair and in cell cycle progression are of especial relevance to cells that must periodically undergo multiple rounds of growth factor–induced expansion and involution. More specific functions for BRCA1 and BRCA2 in breast epithelial development or function are also possible. For example, disruption of murine *Brca1* renders abnormal the development of ductal structures in the breast (Xu et al., 1999). BRCA1 may modulate the response of breast epithelia to proliferative signaling through estrogen receptors (Fan et al., 1999). In either case, further elucidation of the roles played by BRCA1 and BRCA2 in breast cancer pathogenesis is likely to require a better understanding of their functions in the physiology of epithelial cells in the breast.

Thus, much of the material presented in the book, and touched upon here, underscores the urgent necessity for better understanding of the molecular biology of organ development and function as a means to improving the management of the most common forms of human cancer. Genes altered in breast cancer cells are being identified at an increasing rate, and their functions in fundamental cellular processes are quickly being revealed. But as long as our knowledge of the interactions between breast epithelial cells, their surrounding stroma, extracellular matrix, and vasculature remains imperfect, it will remain difficult to correctly interpret this information and ensure that it is efficiently translated into new strategies to improve diagnosis and treatment. In short—we need to learn what makes breast cancer cells tick.

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Viruses—In No Short Supply

HIV and the New Viruses
Edited by Angus G. Dalgleish and Robin A. Weiss
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There are few things that so catch the public's eye in biomedicine as the subject of viruses. As our understanding of viral drug resistance, virus spread, virus strains, and viral cell receptors have become standard parlance in the lay media, so has our appreciation of the tragedies and efforts it took to get us to this juncture. Viruses have become, in the late 1900s, a repository for our fears of a disordered natural world. This book, however, shows us viruses have, as well, become vehicles for deeper understandings of the ways in which our bodies work. The issues covered within the text illustrate that—in the face of viral diseases of global significance to human health—the groundwork can be laid for readily determining the nature of new viruses and acting more quickly to prevent their spread.

The most deeply understood of the pathogenic viruses, human immunodeficiency virus (HIV-1), is the subject of more than half the book. The matters discussed about HIV-1 cover the range of possible virological topics from molecular biology to epidemiology, virus mutation and resistance, as well as immunological consequences of HIV-1 infection. These chapters form an excellent basis for a new, or established, investigator in related fields to get a pre-2000 glimpse at state-of-the-art concepts used to define the HIV-1 pandemic.

From here the book moves into discussions of the primate lentiviruses and then two chapters on some of the non-lentiviral retroviruses of primates, including human T cell leukemia/lymphotropic virus and certain simian retroviruses, as well as certain endogenous retroviruses. Conceptually linked, and appropriately discussed are the more recently described human herpesviruses 6 and 8. Both were discovered as being prevalent in immunocompromised HIV-1-infected individuals. Later these viruses were etiologically linked as endemic infections in the human population and capable of causing severe morbidity in their own rights. The book ends with chapters on the several hepatitis viruses, a chapter mostly focused on a retrovirus proposed by some to be causal in multiple sclerosis (namely the multiple sclerosis–associated retrovirus, MSRV), and a brief discussion

of other viruses of some import (hantavirus and ebola being the most well known).

I will discuss, then, the book in terms of the conceptual groupings of the chapters (with no attendance to the actual numerical orderings of the chapters). Throughout I will try to provide a measure of the excitement clearly indicated by the authors in their subjects and for the potentials of where they hope to move with this current knowledge.

The most fascinating aspect of the HIV-1 pandemic (and its most terrifying) is the virus' rapid adaptation to individual, local, and global niches. Several chapters explore this rich area. Chapter 2, "Viral Evolution and Variation in the HIV Pandemic", provides excellent examples of how molecular typing has allowed us to chart the course of the virus through an infected patient over time, through local populations, and across continents. The reader is first introduced to the concepts of HIV-1 strains or subtypes (some call them clades). Molecularly defined on the basis of sequence comparisons and relatedness, the strains or subtypes form the basis for mapping virus spread within individuals and though regions of countries. A discussion of the spread of the virus by intravenous drug users in Scotland demonstrates the ability of single strains of the virus to rapidly become established within a connected community with little or no need for apparent sequence divergence (i.e., adaptation). The doubling time of new infections within the affected population was as short as 8 weeks in one example of a described cluster of infections. Little or no variation occurred in sequence, perhaps related to the rapidity of the spread and lack of need to diversify. This is in contrast to examples from Thailand or Uganda wherein the B and E subtypes are discussed, and the A and D subtypes, respectively, relative to their infections of different communities and townships. Here the virus strains show considerably more sequence variation and offer us the means to readily track virus spread. The lesson provided, perhaps, is that each community seems to select for viral subtypes whose prevalence can be described by founder effects, selection, individual variation, and niche exploitation. But how then do we find the commonalities between the apparently divergent examples described in the three main examples above? In answer to this begged question, this chapter culminates by underscoring a need for a mathematical understanding of HIV-1 population genetics and frequency estimates that allow for a more precise enunciation of how the virus uses its environment. Without having to explain itself, this chapter lays out a cornerstone for several others in the book.

Two major discussions directly relate to the latter point. The first discussion opens in the chapter by Schuitemaker on "Biological Properties of HIV-1 and Their Relevance for AIDS Pathogenesis." This chapter delves into variation of the virus, with an emphasis on the envelope protein, within the host as disease progresses and attempts to link virus change (from non-syncytia-forming to syncytia-forming virus) to disease pathology. Underlying these changes are virus cellular tropism variations that confer different potential for pathogenesis on the virus depending upon the cell types it infects. There are relations in this chapter to aspects of changes that occur in the face of antiviral therapies and the immune changes during viral infection (discussed below). Shuitemaker makes good use of the literature to point out changes that occur to the viral envelope and certain accessory genes during the course of the infection. A main thesis is that adaptations to different coreceptors (reflecting changes in tropisms) coupled to progression to syncytia induction phenotypes correlates positively with disease progression. This chapter is well complemented by the contribution of a thorough review of the chemokine literature by Norcross, "Chemokine Receptors and HIV-1 Pathogenesis." The main distinction of this chapter is the provision of a detailed analysis of envelope coreceptor interactions with virus envelope. Norcross, as well, underscores the importance of variation during pathogenesis. Finally, the evidence Schuitemaker outlines for changes in the regulatory genes of HIV-1, though less extensive as the data for the envelope, are well-served by the chapter by Alonso and Peterlin, "HIV-1: Control of Gene Expression by the Viral Regulatory Proteins Tat and Rev." Here the authors outline just how far we have come in a molecular understanding of virus regulation of its production from the provirus state. A theme in their text is the potential for identifying host proteins as targets, as well as other modalities, for future antiretroviral therapies. These latter points cannot be overemphasized given that vaccine research is still not where it needs to be and standard antiretrovirals still have significant shortcomings.

A second major thrust of the book is the emphasis on molecular typing of viral isolates. Though discussed mathematically in chapter 2 on what one could do with the data, it is left to Berry and Tedder in "HIV-1 and HIV-2 Molecular Diagnosis" to explain some of the current methods by which sequence information is gathered. The applications in primary diagnosis (in perinatal or acute infections) are discussed. Mathematical discussions are outlined in which the discerning reader could apply the information to quantitation of virus levels in the field to look for specific virus mutations (i.e., drug resistance) or for subtype analysis. Appropriate cautions on the limitations of the techniques, as presented in this chapter, should be required reading for health care workers hoping to use these methods to describe viral load. The last chapter of the book (by Paul Kellam, "Emerging Viruses") takes off on a different tangent from this, though it is related, in explaining generic approaches one might apply to identify new viruses where no protein sequence data or physical data might exist.

Connected to this subject of variation then, and curiously spread into three different parts of the book, are chapters on antiretroviral therapies, viral resistance, and their impact upon HIV-1 pathogenesis. One discussion, by Phillips, "HIV-1 Dynamics: Lessons from the Use of Antiretrovirals", deals with the subject of how can we learn about virus production in the changing environment of new cell infection, cell turnover, virus production, etc. By observing the course of virus load over time after antiretroviral treatment, coupled to population dynamic estimates of potential cell reservoirs, one can make estimates about the numbers of cells infected or potentially infectable during the course of the disease and the potentials for antiretroviral therapies to succeed. The second chapter on the subject, by Weller, "The Impact of Antiviral Therapy on HIV-1 Disease", is a

primer for medical students, health-care providers, and physicians interested in obtaining background information on the various treatments that can be provided (even given that new potential drugs against HIV-1 appear to be introduced with some regularity). The clear lessons from prior antiretrovirals are enunciated as measures for future antiretroviral therapies. Finally there is a chapter on the molecular nature of changes that occur in the face of the reverse transcriptase inhibitors in the chapter by Wainberg "HIV-1 Resistance to Antagonists of Viral Reverse Transcriptase."

A third area of significant interest covered is that of immune system response, or lack thereof more to the point, to infection by HIV-1. It is also the area about which we understand the least. Two chapters detail the unfortunate inability of the immune system to mount an effective T cell response to HIV-1 infection. The first, by McAdam and Gotch, "The Cytotoxic T Lymphocyte Response to the Immunodeficiency Virus", provides a brief survey of HIV-1 epitopes recognized and several of the extant theories to explain the lack of an effective cellular response against HIV-1. The second chapter "T Helper Cells Specific for Retroviral Epitopes" is similarly brief. Some reasons for why the T cell response to HIV-1 infection might be so deplorable are given in the chapter by Gougen and Debatin. The first of these focuses on mechanisms for induced T cell death (apoptosis) through the Fas receptor family of proteins. Dysregulations of the Bcl2 family of proteins are similarly discussed, as are other mechanisms for disruption of T cell signaling leading to T cell death. The second chapter by Clerici and colleagues focuses on dysregulations of cytokine production, complement, and immune responsiveness. The chapter provides a good introduction to the secondary systems that could function to inactivate T cell function. Though the hypotheses explored here have not always been central to our discussions of HIV-1 pathology, they deserve the discussions merited by these chapters.

These are followed with a chapter by Mann about "HLA and HIV-1 Infection." Here, Mann reviews the fascinating differences in disease progression that correlate to HLA haplotypes. The implications for vaccine research and disease outcome prediction are an undertone throughout the chapter. For those not intimate with the area of HLA-dependent immune responses and T cell recognition of antigen there is also a well-done section on HLA functions in immune response. An interesting chapter on molecular mimicry by Dalgleish and colleagues rounds this area out by describing how evolution of the virus toward certain immune system structures (MHC epitopes, CD4 mimicry, etc.) could either provide camouflage for the virus under some circumstances or induce a form of autoimmune disease that feeds an inflammatory response. Given the nature of the virus and the transcriptional mechanisms on which it depends, this chapter proposes the interesting notion that immune suppression, or anti-inflammatory therapy might be in order as an adjuvant therapy against HIV-1.

Other chapters on primate non-lentivirus retroviruses fill out the discussion suggesting where new retroviral diseases might eventually emanate. The HTLV virus discussion outlines a significant disease that is already of considerable importance in some parts of the world. Though HTLV does not represent a pandemic of the same magnitude as HIV-1, the corollaries of how it originally was introduced to the human population, its origination in primate species, its spread as a disease, its retroviral nature, and the severity of the diseases it causes merit the excellent discussion provided by Gessain and Mahieux. Then, a chapter by Rosenblum and McLure takes a step away from the human pathogenic retroviruses into the realm of the academically interesting viruses in search of clues to the origins of retroviral diversity. A good discussion of the taxonomy of the viruses is provided (and might have been useful near the beginning of the book to put the retroviruses in general in perspective). Next is another interesting chapter on endogenous retroviruses of no specific viral class (ERVs). The intriguing discussion here is of these genetic elements in various animal hosts, their probable devolution from exogenous "free-living" retroviruses, and their contribution to host genomic organization as well as to host evolution. Their danger as sources for variation to new retroviral outbreaks (both in natural infections and in gene therapy) are aptly considered. Finally, there a chapter on pathologies related to the nervous system potentially implicating a retrovirus in the etiology of multiple sclerosis. Garson and Kerr discuss MSRV as a disease agent hypothesized to be causal in MS and the curious associations of reverse transcriptase activity associated with MS disease monocytes. Other data relating to this hypothesis and discussion of borna virus as an agent in other neurological diseases is outlined.

Arising from the legion of disorders in HIV-1 immunosuppressed patients come two new human herpesviruses, HHV-6 and HHV-8. Williams and Lusso detail the discovery of HHV-6 from a B cell lymphoma from an HIV-1 patient that led to its recognition as a potential pathogen in children (exanthema subitum) and in certain immunocompromised individuals. A frank discussion of the attempts to link HHV-6 to other diseases is demonstrative of how tenuous our understanding of this virus remains (as well as the diseases to which we have attempted to link it). Following this are chapters devoted to the important hypothesis that HHV-8 is a likely candidate causal in the induction of Kaposi's sarcoma (KS). Though KS is a complex disease composed of multiple cell types, the chapter by Talbot and Whitney is a nice review describing the appropriate history of KS in human populations and HIV-1-infected individuals. This is a very good chapter for those wishing a balanced introduction to the field. This chapter is supported by a second chapter on KS lesions, "Pathogenesis and Cell Biology of Kaposi's Sarcoma" that focuses primarily on the biological nature of the lesion itself (by Monini and Sgadari). This chapter provides more details on the cytokine profiles and cell types that drive the tumor's progression, while the former chapter is more critically determined to show that Kaposi's sarcoma has an infectious cause and linking that cause to HHV-8.

Finally, the hepatitis viruses are considered in two chapters whose subject matter could fill volumes by themselves. For those who find miniaturization a marvel, the HBV genome—and the chapter by Carmen and Trautwein—is a miracle of information packing. A lengthy, though excellent chapter by them on "Hepatitus B and Deltaviruses" provides a detailed entry into most

major aspects of the biology of these viruses and our attempts to control its spread mainly through vaccine strategies. The vaccine prevention outcomes with this virus stand in contrast to the failures with HIV-1. Interestingly though, and perhaps one positive outcome of our experience with HIV-1, is a discussion here of the expected breakthrough of HBV vaccine–nonresponding viruses in the human population and the ways in which one might deal with this future problem (as it will happen). A second chapter on hepatitis viruses, this time HCV and hepatitis G virus, ends on a more somber note, detailing extensive knowledge of genome variability and structure but showing our inadequate appreciation of how to devise a vaccine against this pathogen and the genome of hepatitis G virus. There are significant similarities herein to the story with HIV-1. Given the pathologies associated with infection by these latter agents, and the human and economic consequences, a chapter on these agents is appropriate in a book of this nature.

This is a book for those wanting a modern update on where the HIV-1 epidemic is heading. Those studying new viruses or even bacterial diseases would find considerable intellectual stimulus in the discussions of HIV-1 evolution, host response, viral adaptation to host, antiviral therapy, and the importance of molecular taxonomy. Though a complete reading of the book is something a virologist could truly enjoy, I can find several chapters in here that would gain good following in any course on graduate level virology. Certainly a course wanting to focus on HIV-1 pathology should count this among the reading list. As mentioned above there are also several chapters relevant for medical students or physicians seeking an academic introduction to subject areas of broad medical concern (HIV resistance mechanisms and discussions of immune consequences of infection). Though meant as an introduction to a variety of concepts, the book succeeds by connecting the subject areas and showing their underlying relationships. One feature that could use some consideration in future editions is tying the chapters together into subject areas so the more casual reader can understand which chapters are related to each other more directly without have to read the entire text of the book. Chapters could be listed as falling under several headings to further direct the reader to chapters that support others (where not obvious from the titles). The last chapter discussing detection of new viruses leaves off where a new edition of this book might provide future chapters. These include threats from other intriguing viruses (ebola, hantavirus, etc.), new methods for their detection, and strategies for determining the host proteins on which they depend for their replication. I could truly look forward to reading that book as well.

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