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ABSTRACTS

1. **Teaching Evolution through Evo-Devo.** Scott F. Gilbert. Swarthmore College, Swarthmore, Pennsylvania 19081.

Evolutionary developmental biology has presently reached a level of maturity wherein it can offer evidence as to how major changes in body plans have occurred. These insights can be integrated into the teaching of evolution to undergraduate and other lay audiences. Moreover, in such a presentation, examples form evo-devo can illustrate that biologists (a) have excellent evidence for evolution at the molecular level, (b) have hypotheses as to the causal mechanisms of morphological change, and (c) can provide plausible explanations for macroevolution. Moreover, in such a presentation to lay audiences, one does not need the mathematics of population genetics to provide mechanisms for evolution. In such a talk, one can mention homologies (of Pax6 and Hox), descent with modification (FGF and Hox genes), the roles of hereditary changes these genes' expression patterns and structures (limb development in snakes and insects), and the idea that analogous structures (e.g., human eyes and fly eyes) can be generated form homologous sets of genetic instructions. The lecture will be one such presentation.

2. **Getting the Point: Using PowerPoint for Teaching and Research.** Rebecca Beach* and Karen Crawford.† *Hollins University, Roanoke, Virginia; and †St. Mary's College of Maryland, St. Mary's City, Maryland.

The aim of this workshop is to facilitate routine incorporation of multimedia technology for classroom and research presentations. Since many textbooks now provide an image bank upon request, it is possible to prepare computer-based lectures using Microsoft PowerPoint, provided that the technology is available in the classroom. The benefits of using such technology are multifold: (1) The teaching of biology is greatly enhanced by the use of color figures; (2) time spent preparing lectures can be reduced once the instructor becomes familiar with some basic PowerPoint tools; (3) lecture preparation is streamlined in subsequent semesters as previous lectures can be quickly reviewed and updated from saved files; and (4) expenditures from teaching funds are reduced if the classroom technology is available, or alternatively, high-quality color overheads can be made using an ink-jet printer and transparencies, bypassing the use of a photocopier. Technical elements useful to either classroom or research presentations that will be presented include optimizing image quality of textbook figures; digital photos or scanned images; effective use of animation tools; incorporation of video, audio, and Web sites; avoiding cross-platform conflicts (Windows vs Mac OS); selecting fonts and backgrounds; building research poster presentations using PowerPoint; and guiding students in the use of information technology for oral presentations and research posters. Strengths and weaknesses of PowerPoint in the classroom will be discussed.

3. **Career Opportunities in Developmental Biology and Related Fields.** Ida Chow. Society for Developmental Biology, Bethesda, Maryland 20814-3998.

Developmental biology is one of the fields where major strides are being made and are likely to continue to be made through this new millennium. We study all types of organisms, from slime mold to humans, going through worms, flies, fish, frogs, chicks, and other mammals. Some of us are conducting studies on microbes and others are trying to relate the process of evolution with development (and vice versa) throughout the natural history of the Earth. This is a broad field pulling together just about all the different traditional disciplines, from molecular biology to morphology and physiology, as well as emerging disciplines such as bioinformatics, genomics, and proteomics. Although most of us perform research and/or teach at universities or research institutions, there are increasing numbers of people trained in this area making significant impacts in the industry (biotechnology, pharmaceutical, plant, farm animals, food), in the legal arena (patent laws, forensics, health care, medicine, legislation), and in science education (from precollege to the public at large). The panelists representing many of these careers will discuss specific tasks involved, training and advancement outlook, and "what it takes" to be in respective jobs. Extensive time for questions and answers will follow the presentations. (Workshop co-sponsored by NIGMS.)

4. **Science and Mathematics in an Urban School System from a Developmental Biology Perspective.** Toby Horn. DCACTS/DCPS, Washington, DC 20002.

DC ACTS is an NSF-funded collaborative effort of the DC Public Schools, the American Association for the Advancement of Science, and the Carnegie Institution of Washington. Consider this a three-parent chimera to produce an offspring school system with these traits: (1) Teachers with strong grounding in their science or mathematics teaching subject. (2) Teachers with comfort in using their textbooks only as a resource and using the Internet critically for updates. (3) Teachers who can provide their students with a variety of ways to show what they are learning. (4) Teachers with comfort in managing a classroom and in facilitating inquiry learning of science and mathematics, where students are actively engaged in learning. (5) A school system that provides the funding for materials and gets them into the appropriate classroom in a timely manner. (6) A school system that provides the needed data in time to help teachers modify their teaching to provide an effective learning environment for all their students. (7) Students who enjoy learning science and math and are willing to apply the effort needed to succeed. Oak trees and humans all start small. Scientists may help by: (1) providing the necessary growth factors such as cyber-mentoring, both of teachers and of students, and class visits; (2) donating transcription factors such as your outdated labware or equipment; and (3) offering a nurturing womb such as opening your lab for teacher and/or student internship. And you will see science and math understanding and interest grow teacher-by-teacher, student-by-student, classroom-by-classroom.

5. **Lending a Hand, Axolotls Generate Excitement for Science.** Karen Crawford. St. Mary's College of Maryland, St. Mary's City, Maryland 20686.

Over 1 month, 20 students in a combined first, second, and third grade Montessori class observed normal hand regeneration on a Mexican axolotl, *Ambystoma mexicanum*. The first day, while the animal was being anesthetized (0.007% Benzocaine in 10% Holtfreter's solution), we discussed using anesthetic, regeneration in

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amphibians and other animals, general laboratory safety, feeding and animal care, and making regular observations. Since they had drawn the phases of the moon over 1 month's time in the fall, I distributed a calendar to each student for them to draw the phases of regeneration. Once the animal was anesthetized, it was passed in its container for the students to observe. Next, it was placed on a plate and its right front hand was removed using small scissors. Returning the animal to 10% Holtfreter's solution the students observed it as it "woke up." The axolotl remained in the classroom for 1 month; each student took a turn caring for it, drew pictures of the regeneration process, and compared his or her pictures to figures I provided. At 2 weeks I returned to the classroom to check on how things were going and to answer questions. The limb blastema was at late bud and by 1 month, four digits had formed. The students kept the animal until everyone had a chance to care for it. At 6 weeks I met with the class again to review their data and outcome of our experiment. Benefits? Their observations and questions were amazing, the students and parents now seek me out to tell me extraordinary things and science is alive in the classroom.

6. **Developing an Integrated Scientific Understanding through Identifying Tradeoffs in the Genetically Modified Food Controversy.** Sherry Seethaler and Marcia Linn. U.C. Berkeley, Berkeley, California.

Exploration of scientific controversy is rare in the science classroom. Our work with 8th-grade and undergraduate students involves assessing the impact of innovative science controversy curricula on important critical thinking skills such as the ability to use evidence appropriately and recognize and weigh tradeoffs. This work is guided by a pedagogical framework which seeks to help students develop a rich, connected scientific understanding (Linn and Hsi, 2000). The SCOPE Genetically Modified Food Project supported 8th-grade students as they explored Internet evidence on genetic engineering of food and defended the form of agriculture which they concluded should be used in their geographical region. Pre- to posttest gains indicate that students made significant progress in their understanding over the course of the curriculum. Furthermore, in their final papers students were able to describe evidence about the risks and benefits of their chosen agricultural method; however, we did identify three areas of difficulty which give insight into what further curricular supports might be beneficial. The undergraduate students we have studied, tend, as expected, to develop more sophisticated arguments about genetically modified food, but like the younger students sometimes fail to be explicit about how they are weighing risks and benefits in tradeoffs. Interestingly, these students often expressed surprise about the complexity of science controversy and discussed how their views of science shifted over the course of a semester-long course on science controversy.

7. **Alphatome: A Letter-Based Sectioning Simulation.** Elizabeth E. LeClair. DePaul University, Chicago, Illinois.

Histological exercises for beginners face two educational obstacles: students do not know embryonic anatomy, nor do they have the cognitive skills to process both the 2D and 3D information conveyed by serial sections. This lab isolates the cognitive component by training students to build, slice, and analyze serial sections through the letters of the alphabet. Letter shapes are simple, already memorized, and yet diverse enough to be challenging when presented at unfamiliar angles. Four related exercises occur in sequence: (1) Students rearrange magnet letters on a board to explore how specimens can lie at different angles. (2) Students use disposable foam letters, scissors, an inkpad, and stamping paper to demonstrate how the choice of section angle is both arbitrary and infinite. Choosing different cutting planes leads to nonintuitive but entirely predictable 2D shapes. (3) Students make Play-Doh "blocks" containing letters and then serially section these and draw the surprising results. (4) Finally, students prepare a mystery block for another team whose goal is to identify the enclosed specimen and its orientation. A great ice-breaker for your introductory histology course or embryology lab, the exercise also demonstrates many real-world aspects of sectioning including artifacts (e.g., sections compress and do not form ribbons) and Humpty's law: all the king's horses and all the king's men, can't put your Play-Doh together again.

8. **Use of *Dictyostelium* in an Introductory-Level, Investigative Laboratory Course.** Margaret K. Nelson. Department of Biology, Allegheny College, Meadville, Pennsylvania.

A 4-week module involving *Dictyostelium* was designed for use in an introductory-level, investigative laboratory course. In the first week students carry out a simple chemotaxis assay, in which they use a dissecting microscope to observe the behavior of starved cells deposited on nonnutrient agar at varying distances from a cAMP or AMP source. This initial exercise introduces students to the concepts of dose dependence and receptor specificity. In the two subsequent weeks students, working in groups of three or four carry out independent experiments of their own design. Student-designed experiments in the Spring 2002 semester included explorations of the effect of starvation time on the response to cAMP and folic acid, measuring the response of cells to cAMP analogs, assessing the consequence of various mutations on chemotaxis, and analysis of caffeine's effect on chemotaxis and development. There is also time in the second and third weeks for presentation of preliminary data, discussion of effective table and graph design, and assistance in locating and interpreting scientific literature. In the final week students make a formal oral presentation as a group and also prepare individual written lab reports. This exercise has proven to be an effective way to introduce students to basic concepts in cell and developmental biology, expose students to *Dictyostelium* as an experimental system, and prepare students for advanced biology courses by providing first-hand experience in experimental design, use of scientific literature, and effective scientific communication. (This project was funded in part by NSF CAREER Grant IBN-9985265.)

9. **Kidney Organogenesis in *Xenopus* Embryos: *In vivo* Observations and Modulation by Retinoic Acid.** Barbara Lom, Whitney Christian, Sarah Hooper, and Robert Zsoldos. Biology Department, Davidson College, Davidson, North Carolina.

We have developed a fluorescent labeling technique to visualize *Xenopus* kidney organogenesis *in vivo*. This technique is amenable to developmental biology laboratory exercises and undergraduate research projects because students can quickly master microinjection, fluorescence microscopy, and quantitative morphometric analysis techniques while investigating the dynamics of kidney organogenesis. To visualize the developing *Xenopus* kidney, rhodamine dextran is microinjected into the anterior region of developing tadpoles. Cells at the injection site internalize the dye, while excess dye accumulates rapidly and specifically within the developing pronephros. Within hours of injection, pronephric tubules are

clearly and brightly delineated. Kidney morphology can be visualized in anesthetized tadpoles over several days with conventional fluorescence microscopy. Consequently, students can follow the development of individual kidneys, observing and quantifying morphological parameters of kidney organogenesis in live or fixed tissue. Further, this technique allows students to examine kidney development in response to teratogens such as retinoic acid. Exogenous application of retinoic acid is a potent modulator of anterior–posterior specification in the *Xenopus* body plan. We find that excess retinoic acid dramatically alters kidney morphology. Pronephric tubules in retinoic-acid-treated embryos are significantly wider and less coiled than in controls, suggesting that retinoic acid signaling plays a critical role in patterning the embryonic kidney.

10. **Using Human Adult Mesenchymal Stem Cells in an Undergraduate Teaching Laboratory.** J. Doctor. Department of Biology, Duquesne University, Pittsburgh, Pennsylvania.

The current excitement and promise of stem cell research was brought directly into an undergraduate lab course at Duquesne University. Commercially available human adult mesenchymal stem cells (hAMSC) were the focus of a 4-week-long module in an advanced (junior/senior level) lab course in cellular and molecular biology. The lab meets twice a week for 3–4 h per session, and once a week for a 1 h recitation. The 14 students in the course during the spring of 2002 learned the basics of cell culture and aseptic technique, and set up experiments to examine the developmental potential of hAMSC. Cell culture was a component of all eight lab sessions over the 4 week period. The hAMSC (from Clonetics/Poietics/BioWhittaker) are readily passaged in medium provided by the supplier. Several experiments by small teams of students evaluated hAMSC morphology, proliferation and osteogenic differentiation via fluorescent microscopy, cell counting, MTT vital dye staining/colorimetric quantitation, alkaline phosphatase histochemistry, and chemical determination/alizarin red staining to assess of calcium deposition. The students in the lab also applied these methods in a set of preliminary experiments on the attachment, proliferation, and differentiation of hAMSC on novel bone tissue engineering scaffold materials. Several recitation sessions were devoted to seminal publications in stem cell research and journal club discussions. The students analyzed and presented their research results in written papers, via the course Web site, and at a poster session for the lab course.

11. **Stem Cell Research: A Case Study for Undergraduates.** Elizabeth R. McCain. Muhlenberg College, Allentown, Pennsylvania.

Case studies are frequently used at all levels of education and are typically employed to teach students how to synthesize disparate information and critically evaluate opinions versus facts, to understand that ethical problems can have many "right" answers, and to work collaboratively. The outcome usually involves students presenting their "case" and discussing the topic with the class. Currently, the most ethically charged issue in developmental biology is stem cell research. I wrote a case study on this topic entitled "The Case of Eric, Lou Gehrig's Disease, and Stem Cell Research" to facilitate my undergraduate developmental biology students' learning about this subject. The case outlines the life of a 29-year-old man, Eric, who experiences the initial symptoms of, and then is diagnosed with, this devastating disease. At the end of the story, he sets out to talk to six individuals about his treatment options, including the products of stem cell research. The six people were two prominent stem cell researchers, Drs. John Gearhart and James Thomson, a clergy, a politician/supporter of President Bush, an ALS specialist, and Dr. Leon Kass, a bioethicist and current Presidential advisor. Each pair of students was assigned to investigate the background and opinions of one of these people. In addition, they were expected to learn about Lou Gehrig's disease, the basic tenets of stem cell research, and the difference between embryonic stem cells (ES) and germ cells (EG). Each pair presented its case and "advised" Eric during the pair's 12 to 15-min oral presentation and also wrote a paper outlining the argument. After the presentations, the class discussed the entire topic. Students were graded on all of the components.

12. **Incorporating Group Projects into Developmental Biology Courses.** Joyce J. Fernandes and Amanda Robinson. Department of Zoology, Miami University, Oxford, Ohio.

Group projects can be valuable experiences that enable students bring ideas together in a collaborative approach. This allows development of an appreciation for the subject matter and learning to better connect classroom concepts to current themes in biomedical research. Here we present the development of effective strategies to incorporate group projects in the college biology classroom. A group project called, "Model Organisms in Developmental Biology," was incorporated into a 200-level course, Patterns in Development. We present the content of the group project, how it was carried out, and an assessment of the project carried out using student evaluations that spanned two semesters. We hope that this research will assist in creating a useful, simple design for the structure of group projects in the college biology classroom.

13. **Collaborative Research with Undergraduate Students: An Assembly Line Model.** Mary K. Montgomery. Macalester College, St. Paul, Minnesota.

Working with undergraduates in a research program can be rewarding but also presents some unique challenges. Although senior students are capable of carrying out fairly sophisticated research projects, beginning students lack technical expertise, lack a deep understanding of the discipline, and have limited time to devote to research. Faculty at predominantly undergraduate institutions learn how to divide up their research programs into small digestible problems that undergraduate researchers can more easily tackle. A complementary approach is to introduce students to just one or two different techniques initially; then, after performing small projects that allow them to acquire some expertise, let students work collaboratively to carry out larger projects from beginning to end, in an assembly-line-like fashion. For example, my research program is focused on a comparative study of genes regulating early development using RNAi and a variety of other techniques. I will train one student in some molecular techniques, another to perform immunocytochemistry and/or *in situ* hybridization, and a third student to do microinjections. A typical research project in my lab will involve (1) one student cloning a gene and making dsRNA that (2) another student injects into worms with (3) a third student antibody staining the progeny of the injected worms to reveal the nature of the resulting RNAi phenotype. Each student is respected for his/her contribution and everyone is invested in analyzing the results. Eventually, the students learn many of the other techniques as well, mostly from looking

over the shoulders of each other during the inevitable laboratory "down time." Thus students learn from me initially, become experts by repeating the same protocol several times, and then are able to teach each other. Students learn about shared responsibility and the true nature of collaboration. Eventually students develop a deeper understanding of the nature of the research and can begin to both generate and pursue their own experimental questions.

14. **Four-Dimensional Views of Frog Gastrulation: A Teaching Resource for the SDB.** Andrew J. Ewald,* John B. Wallingford,† J. Michael Tyszka,* Richard M. Harland,† and Scott E. Fraser.* *Beckman Institute, 139-74, Caltech, Pasadena, California 91125; and †Department of Molecular and Cell Biology, 401 Barker Hall, University of California, Berkeley, Berkeley, California 94720.

We have developed a novel method of imaging embryonic development, surface imaging microscopy. This technique yields high-resolution images of both small and large embryos (from 0.5–8 mm in lateral extent) with high contrast and resolution. We have applied this technique to generate high-resolution images of the process of frog gastrulation, resulting in "digital frogs" at each stage of frog gastrulation. These data sets are fully interactive and allow a three-dimensional exploration of any region of the embryo, with cellular resolution throughout the entire embryo. We will be presenting three-dimensional reconstructions from these data sets and interpreting the underlying cell movements, as well as distributing instructions on how users can download these data sets and images for use in developmental biology courses.

15. **CAMBIO: Computational Algorithms for Multidimensional Biological Image Organization.** Kevin W. Eliceiri, Charles Thomas, Curtis Rueden, Narfi Stefansson, Lisa Peterson, Fong-Mei Lu, Vincent Chu, Amos Ron, and John G. White. University of Wisconsin–Madison, Madison, Wisconsin.

To understand fundamental biological processes such as the development of an embryo or the movement and division of cells, three-dimensional structures that change over time must be visualized, a process referred to as four-dimensional (4D) imaging. A need for 4D imaging permeates many areas of bioscience ranging from the scientist attempting to comprehend developmental dynamics by analysis of spatiotemporal datasets obtained from a multifunctional microscope, to the elementary school teacher trying to convey the beauty of biological processes to young minds. To facilitate and advance these research and educational activities we have established the CAMBIO initiative. The goal of the CAMBIO Initiative is to develop an integrated software suite that will be used to capture, archive, visualize, analyze, and distribute multifocal plane, time-lapse (4D) recordings of embryonic development. Advanced visualization aids including roaming in space and time, 3D rendering and arbitrary plane slicing will facilitate perception of complex structural dynamics. A comprehensive annotation system enables extensively labeled canonical developmental sequences to be established in a database thereby providing a powerful educational resource for students of embryology. At our Web site http://www.loci.wisc.edu, online modules that exploit the power of 3D and 4D imaging to promote interactive learning may be accessed, enabling students and researchers to study dynamic biological events, such as the development of an embryo, intuitively by observing the event unfold.

16. **Journeys: Great Experiments in Developmental Biology, as Told by Those Who Performed Them.** Mary S. Tyler,* Ronald N. Kozlowski,* and Scott F. Gilbert.† *Department of Biological Sciences, University of Maine, Orono, Maine; and †Biology Department, Swarthmore College, Swarthmore, Pennsylvania.

The field of developmental biology is changing so rapidly that it would be easy to lose track of our roots, and yet it is of great value to preserve a knowledge of where we came from and how we got where we are today. In an effort to contribute to this record of past accomplishments, we have been creating short, 10- to 20-min, videos of some of the well-known experimental scientists in the field. From original video-taped interviews by Scott Gilbert, we have been creating short documentaries incorporating portions of the interview with illustrated explanations to show the work that was done and its importance to the field. Designed as an educational tool, the videos contain the important message to students that the great names they read about belong to people who are very human, who often started out in modest circumstances, and who had the courage to see what others had not. Videos to be presented will include Jay Lash, From Somites to Thalidomide; Nicole Le Douarin, Chimeric Grafts, Following the Pathways of Migrating Cells; Lauri Saxén, Primary Induction, The Double-Gradient Hypothesis; Walter Gehring, Homeosis in the Fly, a Story of Transdetermination; and John Saunders, Growth and Pattern in the Vertebrate Limb. We are creating the videos in QuickTime and DVD formats and will be presenting them in their DVD format. This is part of a larger educational project in developmental biology to develop a Web site (www.developmentalbiology.net) integrated with our CD-ROM, "Vade Mecum: An Interactive Guide to Developmental Biology." (Supported by NSF-DUE-CCLI Grant 0087657.)

17. **A More Inclusive Course in Development?** Judith E. Heady. University of Michigan–Dearborn, Dearborn, Michigan.

For many years I have been using current literature and student-directed original research projects as important parts of my upper-level undergraduate course on development. I moved away from textbooks when even I became overwhelmed with the amount of information they presented. In addition, no matter how current or inclusive the author is, new material and some topics that might enhance understanding will be missing. For the first time I am planning to use one short text and two specialty books in addition to some current literature. The reasons for this change include: (1) Many students seem less interested in investing energy and time in the study of primary research and less capable of translating the details into overall concepts. (2) Many topics are so timely that they will not be incorporated into large texts until they are already outdated. (3) Some topics might not be expected to be incorporated into texts including the influence of nongenetic factors on development. My detailed plans and reasoning will be displayed to encourage discussion of the philosophy and the content of this course.

18. **Recovering the Classical Tradition in Comparative Embryology.** Jonathan Wells and Paul A. Nelson. The Discovery Institute, Seattle, Washington.

It is an irony of the history of comparative embryology that the flawed diagrams of Ernst Haeckel ever came to be widely adopted in biology textbooks, when historians of science such as Jane Oppenheimer knew that the diagrams were flawed and said as much in their publications. The irony deepens, however, when one

considers that in 1894—over a century before the work of Michael Richardson and his colleagues reawakened interest in the problem—the embryologist Adam Sedgwick had warned that Haeckel's representations were inaccurate at best. We argue that fresh attention should be given to what E. S. Russell in 1916 called the "classical tradition" of comparative embryology—i.e., to such workers as Sedgwick, W. His, and O. Hertwig—to help redress long-standing imbalances in interpreting and understanding the patterns of developmental biology. There is much to be learned from these workers that is of great relevance today.

19. **Role of Learning and Teaching Centers in Transforming and Sustaining Excellence in Teaching.** Susan R. Singer. Department of Biology and Perlman Center for Learning and Teaching, Carleton College, Northfield, Minnesota.

What are learning and teaching centers (LTCs) and why are they vital to educational reform? Centers for learning and teaching are appearing at a rapid rate on many campuses (a 300-fold increase in 30 years), complemented by virtual centers and other resources shared on the Web. LTCs vary in function based on campus needs, but all are development centers that help improve the quality of learning. The following elements contribute to the success of an LTC: (1) not being perceived as remedial, (2) confidentiality separating the center's activities from any faculty review process, (3) faculty ownership of the LTC and administrative support, (4) credible leadership, (5) quality resources disseminated in nonintrusive but engaging ways, and (6) collaboration with others on campus and nationally.

20. **An Extraordinary Dinosaur Nesting Site from Patagonia: Understanding the Reproductive Behavior and Early Development of the Largest Land Animals.** Luis M. Chiappe. Natural History Museum of Los Angeles County, 900 Exposition Boulevard, Los Angeles, California 90007.

The extensive Late Cretaceous dinosaur nesting site of Auca Mahuevo (Patagonia, Argentina) provides the only available evidence of sauropod dinosaur embryos. At this site, *in ovo* remains are abundant, some even preserving fossil casts of the embryonic skin. The cranial anatomy of these embryos indicates they belong to titanosaurs, a cosmopolitan clade of sauropods that includes the largest known dinosaurs. Although comparisons with adults are hampered by the paucity of their fossil skull material, the Auca Mahuevo embryos provide paleontological examples of how developmental data inform hypotheses of character evolution based on adult morphology. Furthermore, the unprecedented wealth of *in situ* egg clutches preserved at Auca Mahuevo renders a unique basis for understanding the nesting behavior of sauropod dinosaurs. The stratigraphic, sedimentary, and taphonomic context of the eggs provides solid ground for inferring: (1) a gregarious nesting behavior, (2) site fidelity, (3) nest construction, and (4) absence of parental care involving specific nests and their parents.

21. **Evolution of Developmental Novelty: The Proliferative Phase of Polyembryonic Development.** Miodrag Grbic, Tomislav Terzin, Vladimir Zhurov, and Peter Dearden. Department of Biology, University of Western Ontario, London, Ontario N6A 5B7, Canada.

Polyembryony is a form of reproduction where multiple embryos develop from a single egg. The polyembryonic parasitic wasp *Copidosoma floridanum* represents one of the most extreme examples of polyembryony producing up to 2000 embryos from a single egg. After initial egg cleavages the *Copidosoma* embryo enters the proliferative phase. This proliferative phase is initiated by the split of the primary morula and the creation of many proliferative morulae. Individual proliferating morulae contain several hundreds of tightly packed round cells. The proliferation ultimately results in 2000 morulae consisting of 20–30 cells which form a single embryonic primordium. This proliferative phase does not have a counterpart in monoembryonic insects and thus represents a novel phase in polyembryonic development. To understand the evolution and regulation of the proliferative phase we have constructed a proliferative cDNA library. Using a combination of homology screens for genes that regulate cell proliferation, EST screens, cell biology approaches, and time-lapse microscopy we have determined that maternal developmental asymmetries are maintained during the proliferative phase. We have cloned a *Copidosoma vasa* homolog and examined the expression of *vasa* RNA and protein during polyembryonic development. The Vasa expression pattern suggests that in the *Copidosoma* germ line is specified maternally and that a complex parceling system precisely distributes germ line cells in each of the future embryos.

22. **How Conserved Is Polyembryony? Development of Independently Evolved Polyembryonic Wasp *Macrocentrus grandii*.** Koen Vandenberghe, Chun-Che Chang, and Miodrag Grbic. Department of Biology, University of Western Ontario, London, Ontario N6A 5B7, Canada.

The developmental constraint is one of important factors that could limit possible evolutionary trajectories. The conservation of convergently evolved developmental program under different selection regimes could be interpreted as an indication of developmental constraint. Polyembryony in parasitic wasps evolved four independent times in families Encyrtidae, Braconidae, Driiinidae, and Platygasteridae. Our goal is to understand whether independent evolution of polyembryony is associated with the reuse of the same regulatory cascades or whether multiple regulatory pathways can lead to the same result? We are characterizing development of the polyembryonic braconid, *Macrocentrus grandii,* and comparing its development with our model polyembryonic wasp, encyrtid, *Copidosoma floridanum*. We find changes in *Macrocentrus* ovarial structure, proliferative phase of development, and morphogenetic patterning relative to *Copidosoma*. However, analysis of germ line development using the *Macrocentrus vasa* homolog indicates conservation of the germ line specification in polyembryonic development.

23. **Proliferation during Polyembryonic Development.** Laura S. Corley,* Cristina K. Rubio,* and Michael R. Strand.† *Washington State University, Pullman, Washington; and †University of Georgia, Athens, Georgia.

Copidosoma floridanum is a polyembryonic parasitoid wasp that produces two castes during development from a single egg. Polyembryonic development proceeds by a process of cellular proliferation resulting in a single brood containing approximately 950 reproductive larvae that develop into adult wasps and 50 precocious larvae that function as soldiers. We examined how the single egg proliferates and responds to environmental factors to produce two castes in *C. floridanum*. Our results indicate that the earliest stages of wasp morulae have the highest proliferative capacity and produce both larval castes. The older wasp morulae produced significantly fewer larvae and could produce one caste or

the other, but not both. Host environmental affects did not significantly change the proliferative capacity or caste fate of the morulae. Embryonic cellular proliferation is intrinsically regulated and is linked to totipotency in *C. floridanum*. Currently we are cloning several candidate genes that may be involved in polyembryonic proliferation or could act as markers of larval caste fate.

24. **How Cellularization Affects Patterning? Pattern Formation in Related Syncytial and Total Cleaving Wasps.** Vladimir Zhurov, Kyle Martin, and Miodrag Grbic. Department of Biology, University of Western Ontario, London, Ontario N6A 5B7, Canada.

Nearly all insects undergo a syncytial phase during the initial cleavages of embryogenesis. During this syncytial phase, nuclear divisions are not followed by physical separation of a cell membrane, resulting in thousands of nuclei in a single compartment. This syncytial phase is crucial for *Drosophila* axial patterning, as it allows for the distribution of proteins involved in establishing embryonic polarity without the barrier imposed by cell membranes. Phylogenetic analysis suggests that parasitic wasps evolved from a long germband ancestor with a large and yolky egg that underwent syncytial development. However, in certain parasitic families some derived wasps evolved total egg cleavage. To understand how cellularization affects patterning machinery we have compared pattern formation in two related parasitic wasps from the subfamily Aphidiinea. Both wasps are aphid parasites where *Praon* is a representative of primitive species that undergoes syncytial cleavage and *Aphidius ervi* exhibits a derived mode of development undergoing total cleavage. To determine how changes in cleavage pattern have affected establishment of axial polarity and pattern formation we analyzed expression patterns of homologs of *Drosophila* maternal coordinate, gap, and pair-rule genes in *Praon* and *Aphidius.* Our preliminary results suggest that some elements of pair-rule patterning are retained in *Aphidius* despite dramatic changes in the early embryogenesis. However, portions of the pair-rule cascade are modified suggesting that cellularization affects the patterning process.

25. **Axillary Meristem Development in the Branchless Zu-0 Ecotype of *Arabidopsis thaliana.*** Vojislave Grbic, Anna Kalinina, Nela Mihajlovic, and Esther Hidber. Department of Plant Sciences, University of Western Ontario, 1151 Richmond Street, London, Ontario N6A 5B8, Canada.

Axillary meristems form in the leaf axils during postembryonic development. To initiate the genetic dissection of axillary meristem development, we have characterized the late-flowering branchless ecotype of *Arabidopsis*, Zu-0. The oldest rosette leaves of Zu-0 plants all initiate axillary meristems, but leaves from the upper part of the rosette remain branchless. Alteration in the meristem development is axillary meristem specific because the shoot apical and floral meristems develop normally. Scanning electron microscopy, histology, and RNA *in situ* hybridization with SHOOTMERISTEMLESS (STM), a marker for meristematic tissues, show that a mound of cells form and STM mRNA accumulates in barren leaf axils, indicating that axillary meristems initiate but arrest in their development prior to organizing a meristem proper. A genetic analysis suggests that the branchless phenotype arises due to a single recessive allele whose effect on the branching pattern of Zu-0 plants can be suppressed by early flowering.

26. **Analysis of Limburg, an *Arabidopsis* Late-Flowering Aerial Rosette-Bearing Ecotype.** Branislava Poduska, Tai Wai Yeo, Tania Humphrey, and Vojislava Grbic. Department of Plant Sciences, University of Western Ontario, 1151 Richmond Street, London, Ontario N6A 5B8, Canada.

Flowering time is regulated by a complex genetic network that integrates environmental cues with the developmental state of the plant. To elucidate the molecular mechanism of flowering in *Arabidopsis* we investigated the genetic basis of aerial rosette formation in late-flowering *Arabidopsis* ecotypes such as Limburg (Li). Delayed transition to reproductive development in Li plants can be suppressed by vernalization, and the transition to reproductive development can be completely abolished if plants are grown under a short photoperiod. These physiological responses suggest that Li carries genes acting in the autonomous flowering pathway. Genetic analyses indicates that dominant alleles of two loci are responsible for the late-flowering aerial rosette-bearing phenotype. These loci have been identified as new alleles of the late-flowering genes FRI and FLC. There is evidence that an additional genetic factor (ART3, aerial rosette3) may be required for the extreme late-flowering phenotype of Li plants. This evidence includes the extended linkage between the late-flowering aerial-rosette phenotype to the distal 60 cM of chromosome V and variable flowering behavior of the fri-Ler fri-Ler FLC-Li FLC-Li lines (they either flower early, producing 15 leaves, as typical for other FLC-containing lines; or they flower after producing ~40 leaves). Results of the experiments aimed at identifying and characterizing the ART3 locus are presented.

27. **The Role of LEAFY in Determination of the Primordia Initiation Rate and Activation of Axillary Meristems.** Anna Kalinina, Nela Mihajlovic, and Vojislava Grbic. Department of Plant Sciences, University of Western Ontario, 1151 Richmond Street, London, Ontario N6A 5B8, Canada.

At the transition to reproductive development several processes occur concomitantly. First, the primary shoot apical meristem ceases initiation of leaf primordia and starts initiation of floral meristems; second, axillary meristems initiate in the axils of the youngest leaf primordia in a basipetal pattern; and third, the rate of organ initiation increases. Simultaneous occurrence of these processes in *Arabidopsis* raises the possibility that they are coordinately regulated. The transcriptional factor LFY promotes floral meristem development by activating floral meristem identity genes. This study uses lfy1, a loss-of-function allele, and gain-of-function the 35S::LFY construct to investigate whether LFY also plays a role in establishment of the elevated primordia initiation rate and activation of axillary meristems. Our results indicate that LFY is required for maintenance of the higher rate of primordia initiation and that LFY, together with the independently acting factor, can activate axillary meristem development.

28. **Unraveling the Flower with Pea Developmental Mutants—Homologies and Hidden Potentials.** John D. Sollinger and Susan R. Singer. Southern Oregon University, Ashland, Oregon; and Carleton College, Northfield, Minnesota.

A widely accepted paradigm for the derivation and nature of the angiosperm flower is that it represents a single determinate stem with modified leaves. To investigate the underlying character of the flower in a phylogenetic context, we undertook a study of developmental mutants in pea (*Pisum sativum*). Our interpretation

of several pea flower and leaf mutant phenotypes disputes or modifies botanical axioms regarding the nature of the flower and its derivation from vegetative structures as follows. (1) Flowers may result from the condensation of multiple axes rather than a single stem, as suggested from the phenotypes of two mutants. The pim (proliferating inflorescence meristem) mutation increases the complexity of branching by replacing flowers with infloral triads. A multiple-stem origin of the flower is further supported by the substitution of leaf axillary shoots with single stamen in the veg2-1 (vegetative2-1) mutant. (2) If stamens are shoots, as implied above, then not all floral organs are modified leaves. That is, the axillary placement of the veg2-1 stamens indicates that stamens (at least the filaments) are homologous with stems. (3) Carpels may have only leaf (i.e., and not stem) homology, as suggested by the substitution of leaflets with carpels in veg2-1 mutants. A second mutation, veg2-2, of the VEG2 gene globally conditions stem indeterminacy, including postcarpel shoot development. This, too, suggests that carpels are lateral (leaf) and not terminal (shoot) organs. (4) Finally, the leaflet/carpel homology observed in the veg2-1 mutant is consistent with the notion that floral organs are homologous with specific leaf regions, as also indicated by the phenotype of pim st (stipulata) double mutants. The st mutation genetically defines the stipule by constricting stipule width while leaving other leaf regions unaltered. When coupled with the pim mutation, bracts and abnormally wide sepals also narrow down considerably. This suggests that there is serial homology between stipules, bracts, and sepals. In conclusion, default phenotypes resulting from the dysfunction of developmentally critical genes may unmask hidden potentials and homologies, leading us to a reevaluate the constitution of flowers.

29. **Molecular Characterization of a Deficiency in the Homeotic Complex of the Red Flour Beetle *Tribolium castaneum*.** Elizabeth A. Richardson and Susan J. Brown. Kansas State University, Manhattan, Kansas.

Homeotic genes determine developmental fate along the anterior–posterior axis in animals. Homeotic mutations alter developmental fate and result in the misspecification of segmental identity in insects. Work in *Drosophila* suggests that in their normal expression domains, some homeotic genes repress antennal development while directing segment-specific identity during adult development. Embryonic analysis of *Tribolium* homeotic mutants indicates that repression of antennal development by homeotic genes is conserved and occurs throughout the gnathos, thorax, and abdomen. A deficiency that removes the homeotic genes *Dfd* through *abd-A* (*DfHOMC*) results in the development of antennae on all postoral segments through the eighth abdominal segment. *DfHOMC* mutants arise spontaneously at low frequency in the *Antennagalea(Ag)/Abdominal*$^{Extra\text{-}sclerite\ (Es)}$ balanced strain. Since such events can result from recombination between large overlapping inversions, we hypothesize that both *Ag* and *Es* are such inversions. Thus we predict that one of the *DfHOMC* breakpoints originated in the *Ag* mutant and the other in the *Es* mutant. We are molecularly characterizing the chromosomal breakpoints in *DfHOMC* and will correlate them with the mutations associated with *Ag* and *Es*.

30. **Axial Patterning in Polyembryonic Development: Pattern Formation in the Polyembryonic Wasp *Copidosoma floridanum*.** Tomislav Terzin, Vladimir Zhurov, and Miodrag Grbic. Department of Biology, University of Western Ontario, London, Ontario N6A 5B7, Canada.

In the fly, two main processes underlie polarity and patterning of the embryo. First, oocyte polarity determines the future axial polarity of the embryo. Second, diffusion of transcription-factor morphogens in a syncytial environment initiates the patterning cascade and specifies the fate of nuclei. The parasitic wasp *Copidosoma floridanum* represents one of the most extreme cases of polyembryony in metazoans, producing 2000 embryos from a single egg. The polarity of the *Copidosoma* egg does not reflect future axial polarity of the embryos. During the 8 days of random proliferation prior to morphogenesis, any remnants of global polarity derived from the egg must have been lost. Each embryo forms from approximately 20 cells, lacking a syncytial stage. All 2000 simultaneously formed embryos have randomly orientated embryonic axes, suggesting that axis specification occurs later in development, independently in each embryo. To understand axis specification and patterning in *Copidosoma* we have constructed a cDNA library from morphogenetic stage embryos. Using PCR, low-stringency screens with heterologous probes, and EST screening we have isolated homologs of maternal coordinate, gap, and pair-rule genes as well as homologs of cell-signaling genes from other metazoans that might be involved in axial patterning.

31. **Hoxc13 Orthologs in Zebrafish.** R. Thummel, Li Li, M. P. Sarras, Jr., and A. R. Godwin. University of Kansas Medical Center, Kansas City, Kansas 66160.

Antennapedia-class homeobox-containing (*Hox*) genes are involved in anterior–posterior axis patterning. In addition, as cell- and region-specific selectors, they often play special roles in organ systems or in organogenesis. Zebrafish contain 47 *Hox* genes. This large number of *Hox* genes is due, in part, to a duplication of each *Hox* cluster. The result is 7 clusters in zebrafish (versus 4 clusters in mammals), and often, two zebrafish orthologs have diverged in structure, expression pattern, and function. We have chosen to analyze the two *Hoxc13* orthologs in zebrafish for such divergence. We cloned and sequenced both genes and find regions of protein divergence, especially in the N-terminal region. We will also show that both *Hoxc13* orthologs are expressed in the developing tail bud and will discuss differences in timing and apparent transcript level observed between both orthologs. Finally, to test the functional significance of both zebrafish *Hoxc13* orthologs in the developing zebrafish tail bud, we have started experiments using morpholino-mediated gene knockdown.

32. **Dorsal–Ventral Axis of Polyembryonic Wasp *Copidosoma* Is Zygotically Regulated.** Gang Chen and Michael R. Strand. Department of Entomology, University of Georgia, Athens, Georgia.

The polyembryonic wasp *Copidosoma floridanum* produces up to 2000 individuals from a single egg. During production of these embryos, the original dorsoventral (DV) axis is lost and axial patterning must subsequently be reestablished within each embryo. In long germ insects like *Drosophila*, the DV axis is established during oogenesis and is activated along the ventral side of the egg through an extraembryonic signaling cascade. This extracellular signal is transduced via the Toll pathway leading to a Dorsal (Dl) gradient along the entire ventral side of the egg with concomitant repression of decapentaplegic (dpp) and activation of twist (twi). A syncytial phase during early development is also considered essential for establishing morphogenetic gradients in *Drosophila*. However, polyembryonic wasps lack a syncytial phase of development as early egg cleavage and subsequent proliferation

of embryos occurs in a totally cellularized environment. Despite the absence of a syncytial phase, we report here that *C. floridanum* retains elements of the *D. melanogaster* DV patterning hierarchy. PCR-based cloning strategies resulted in identification of homologs of the genes dl, dpp, and twi. Analysis of expression patterns suggests that dl, dpp, and twi are involved in DV axis formation in *C. floridanum*. However, the absence of dl expression in unlaid eggs also suggests that DV axis formation is regulated zygotically rather than maternally.

33. **Development of the Stylet Mouthparts of a Hemipteran Insect, *Oncopeltus fasciatus,* the Large Milkweed Bug.** David R. Angelini and Thomas C. Kaufman. Howard Hughes Medical Institute, Indiana University, Bloomington, Indiana 47405.

The Hemiptera are a large and ecologically diverse group of insects, which share a distinctive, highly derived mouthpart morphology. Unlike *Drosophila,* the young of the Hemiptera reach adulthood through a series of nymphal stages resembling the adult. In adult Hemiptera, the mandibles and maxillae exist as bristles or setae, which interdigitate to form a set of tubes, supported by the labium. In the large milkweed bug, *Oncopeltus fasciatus,* the maxillary anlagen also give rise to the maxillary plates, which form part of the head capsule but do not directly function in the mouth parts. We are currently investigating the roll of conserved limb patterning genes in the development of *Oncopeltus* mouth parts. The expression patterns of the genes homothorax (hth), Dachshund (Dac), and Distal-less (Dll) define proximal-to-distal domains along the appendages of diverse arthropods. Previous work by Rogers *et al.* (2002) has shown that during development in *Oncopeltus* the protein products of Dll appear in the maxillary setae, but not in the mandibular setae, which are morphological very similarity. This is a paradox, if Dll is assumed to function as in other known arthropod appendages, and we are exploring Dll function using double-strand RNA-mediated gene suppression. We also want to determine the origin of the material that gives rise to the maxillary plate, as well as test the hypotheses for the conserved function of hth, Dac, and Dll in the limbs of *Oncopeltus.* By examining the expression patterns and function of these genes we hope to understand hemipteran mouth part development in comparison to other insects.

34. **Ectopic Expression of *maxillopedia* in *Tribolium.*** Katherine M. Ruyle, Teresa D. Shippy, and Robin E. Denell. Division of Biology, Kansas State University, Manhattan, Kansas.

Hox genes specify regional identity along the anterior–posterior axis of most, if not all, animals. *proboscipedia* (*pb*) is a *Drosophila* Hox gene that is required for specification of the adult proboscis (labial appendages). Ectopic expression of *pb* in *Drosophila* during adult development causes transformation of legs toward labial appendages as well as antennae toward maxillary palps. The latter observation suggests that *pb* function is sufficient to specify palp identity in the absence of other Hox genes. The *Tribolium castaneum* ortholog of *pb* is *maxillopedia* (*mxp*). Unlike *Drosophila pb*, which has no embryonic function, *mxp* is required for maxillary and labial appendage identity in both embryos and adults. Thus, we want to examine the effects of ectopically expressing *maxillopedia* in *Tribolium* to determine whether *mxp* function is sufficient to transform embryonic and/or adult antennae toward palp identity. We are using a piggyBac construct carrying the *Drosophila hsp70* promoter driving *mxp* expression. Eventually, we plan to perform site-directed mutagenesis to identify functional domains of Mxp.

35. **Muscle Formation in Dendrobranchiate Shrimp Embryos and Larvae.** Philip L. Hertzler and Daniel A. Kiernan. Department of Biology, Central Michigan University, Mt. Pleasant, Michigan 48859.

Little is known about cell lineage and organogenesis in crustaceans. In dendrobranchiate shrimp, eggs are freely spawned in large numbers and undergo holoblastic cleavage to a nauplius larva. Previous studies have revealed two sources of mesoderm: the naupliar mesoderm (ectomesoderm) that forms the musculature of the naupliar appendages and the teloblastic mesoderm (endomesoderm) which forms the posterior muscles. In *Sicyonia ingentis,* the naupliar mesoderm arises from nine founder cells at the 62-cell stage embryo while the teloblastic mesoderm is derived from the ventral mesendoblast, which forms at the 32-cell stage. Early larvae swim using their cephalic appendages, while late-stage larvae swim using thoracic and, later, abdominal appendages. *S. ingentis* embryos and larvae were stained with fluorescently labeled phallacidin to study the formation of muscle. Confocal microscopic analysis showed that foci of muscle fiber formation were first detected in embryonic limb buds at 12 h postspawning. These longitudinal fibers grew in length during the formation of the antennules, antennae, and mandibles during later embryogenesis and became joined to other fibers that formed in the trunk. Few muscle fibers were present in the postnaupliar region. Muscular contractions began just before hatching of the nauplius 1. Visceral muscle formation occurred during the development of the gut during nauplius 4 and 5 stages. Muscle development in later larval stages was examined in *Litopenaeus vannemei.* Posterior limb muscles were correlated with locomotion by more posterior appendages in protozea, mysis, and postlarvae.

36. **Bat Wings and the Diversity of Vertebrate Limb Development.** S. D. Weatherbee,* C. J. Cretekos,† R. R. Behringer,† J. J. Rasweiler IV, and L. A. Niswander.* *Memorial Sloan Kettering Cancer Center, New York, New York 10021; and †M. D. Anderson Cancer Center, Houston, Texas 77030.

One of the most exciting discoveries of the past two decades is that animals share large numbers of genes that regulate major aspects of body pattern. Yet the diversity of animal forms clearly demonstrates that animals are not made the same. Dissecting the molecular genetic mechanisms underlying development in different species may elucidate common principles of development but it will also shed light on the divergence of animal form. One of the most productive adaptations of the vertebrate limb has been for flight. While bats are the only mammals capable of sustained flight, they comprise almost one-quarter of all mammalian species. Bat wings are composed of a membrane that extends between the forelimb digits and between the limbs and body wall. Bat wings also exhibit elongated zeugopod and autopod skeletal elements that support the wing membranes. We are pursuing molecular embryological studies in the short-tailed fruit bat *Carollia perspicillata.* Our goal is to identify genetic differences that contribute to the morphological diversity of vertebrate limbs. We have begun cloning and characterizing the expression of a number of genes from *Carollia,* with particular interest in those known to regulate limb growth or patterning to better understand the unique aspects of bat limb development. We propose to test the functionality of changes in gene expression by swapping bat regulatory sequences into the mouse using embryonic stem cell and gene targeting techniques.

37. **On the Molecular Hierarchy Regulating Cartilage and Bone Formation.** B. F. Eames, P. T. Sharpe, and J. A. Helms. UCSF, San Francisco, California; and Guy's Hospital, London, United Kingdom.

During dermal bone formation, transcription factors that enhance chondrogenesis (e.g., *Sox9*) are initially expressed, but become down-regulated simultaneously with the up-regulation of factors (e.g., *Cbfa1*) that enhance osteogenesis. We hypothesize that *Sox9* down-regulation is essential for osteoblast differentiation. We used a gain-of-function approach, overexpressing *Sox9* in chick craniofacial mesenchyme via retroviral infection. This manipulation dramatically promotes chondrogenesis, resulting in widespread ectopic cartilage nodules throughout the head. To determine the effects on osteogenesis, we examined various stages of dermal bone formation for gene expression and histological changes. Between Stages 26 and 38, the *Cbfa1* domains appear equivalent in size and shape in control and treated embryos. Likewise, the onset and location of ossification are similar. These data suggest that the initiation of dermal bone formation is unaffected by *Sox9* overexpression. Mineralization, however, is reduced or absent in Stage 36 treated embryos. In addition, all ectopic chondrocytes express *Collagen type IIa* and stain with Safranin O, but only those chondrocytes within dermal bone periosteum express *Collagen type X* and are replaced by bone in treated embryos. These data indicate that later stages of dermal bone formation are affected by *Sox9* overexpression. We speculate that misexpressing Sox9 in Cbfa1-positive cells inhibits osteoblast maturation and thus hampers dermal bone formation. These data may have implications for understanding the evolutionary switch between dermal and cartilage replacement bone.

38. **Growth Properties of the Deutocerebral Cells of the Silkworm *Bombyx mori*.** Hun Hee Park, So Young Na, Kang Min Kim, and Bong Hee Lee. Department of Biological Science, Korea University, Seoul, Korea.

Primary cell culture carried by dissociated specific cerebral cells of five-stage pupae of the silk moth *Bombyx mori*. The primary cultures leaded to classification of these neurons into several morphological types, including monopolar, bipolar, and multipolar cells. In this primary culture, most of these cells survive for about 2 weeks. A method for culturing insect deutocerebral cells, which includes time-lapse recording, is the labeling technique. Also, director factor of steroid hormone of 20-hydroxyecdysone and neuromodulator of 5-hydroxytryptamine added to normal medium. The result shows that recording time lapse of deutocerebral cells shows differentiation of new neurites. The deutocerebral cells showed strong expressed anti-HRP immunoreactivity and contained light-refracting inclusion. Both 20-HE and 5-HT stimulate the cells bodies to increase lengthy extension and number of branches. On the other hand, the 20-HE remarkably increased neurite outgrowth and also decreased survival days of these neuronal cells; the 5-HT caused little increase of neurite outgrowth and did not affect the survival of those neurons. These results provide an initial important step for further investigation on developmental study in neurons system.

39. **Identification and Characterization of Novel Zebrafish Brain Development Mutants by Large-Scale Mutagenesis Screening.** Christiane Klisa,* Philippa Bayley,† David A. Lyons,† Tübingen 2000 Screen Consortium,‡ Christiane Nüsslein-Volhard,‡ Jonathan D. W. Clarke,† Michael Brand,* Stephen W. Wilson,† and colleagues. *MPI-CBG, Pfotenhauerstrasse 108, Dresden, Germany; †Department of Anatomy and Developmental Biology, UCL, London, United Kingdom; and ‡MPI for Developmental Biology, Spemannstrasse 35, Tübingen, and Artemis Pharmaceuticals, Tübingen, Germany.

The zebrafish is an excellent model for studying the development of the vertebrate nervous system, because of its relative simplicity, genetic accessibility, and stereotyped pattern of development. The forward genetic approach of mutagenesis screening has proved to be extremely successful in elucidating the genetic basis of developmental processes. Here we report the identification and preliminary characterization of a number of potentially novel zebrafish mutants generated in a new large-scale screen—the "Tübingen Screen 2000." After screening 2205 genomes, we have found at least 48 mutant lines with heritable brain development phenotypes. These phenotypes were originally identified both morphologically and by immunostaining the early axon scaffold with anti-tubulin and opsin antibodies. In addition we now present gene expression analysis and a more detailed morphological characterization of some of these mutants, as well as the results of genetic complementation with previously known mutants. A range of phenotypes have been observed, including alterations to both axonal patterning and regional morphology, affecting all areas of the CNS. We hope to begin mapping some of these novel mutations in the near future.

40. **Cell Adhesion Molecule, Neuroglian, Directs Axon Guidance, in Concert with Sema 1a in Mushroom Body Development in *Drosophila* Brain.** Y. Y. Kang,* R. Hiesinger,† S. Natasha,* and P. Callaerts.* *Department of Biology and Biochemistry, University of Houston, Houston, Texas; and †HHMI/Baylor College of Medicine, Houston, Texas.

Cell adhesion molecules are critical for normal development and function of the brain and are involved in growth cone guidance and axon outgrowth and fasciculation. Cell adhesion molecules comprise cadherins, integrins, selectins, and members of the immunoglobulin superfamily. L1 CAM-related genes are members of the immunoglobulin superfamily important for normal brain development. We are studying the role of *Drosophila* homolog of L1 CAM, Neuroglian, in adult brain. We have studied and molecularly characterized three *neuroglian* alleles, two of which correspond to the brain structure mutant *central brain deranged* (*ceb*). All alleles showed similar phenotypes, affecting axon outgrowth, axon branching, and axon guidance in mushroom body, which is involved in olfactory learning and memory. Sema 1a, belonging to a large family of phylogenetically conserved secreted and transmembrane glycoprotein, can function as an axonal repellent and mediates motor axon defasciculation in concert with the cell adhesion molecule Fasciclin II. Here, we have shown that Sema 1a also is involved in mushroom body development and functions conversely with Neuroglian in axon guidance in *Drosophila* brain. Overexpression of Sema 1a in mushroom body neurons mimics the mutant phenotype of *neuroglian* alleles, whereas a combination of *neuroglian* and *sema 1a* mutation partially suppresses the mutant phenotypes in *nrg* mutants in mushroom body development.

41. **Tracing the Ancient History of Animal Signaling and Adhesion Proteins.** Nicole King and Sean B. Carroll. HHMI and University of Wisconsin, Madison, Wisconsin.

Multicellular animals, from sponges to arthropods to vertebrates, are thought to have descended from a unicellular protozoan ancestor. This shared ancestry has been revealed in part by the discovery of common themes in the early development of all Metazoa. Many fundamental processes in early development, including cell adhesion and cell signaling, are known to be regulated by a shared set of conserved proteins acting at the cell membrane. Furthermore, comparisons between the genomes of model Metazoa, Fungi, and Plants have revealed that many signaling and adhesion genes are apparently restricted to the animal lineage and may shed light on the evolutionary transition to multicellularity. While much is known about the function of these genes during animal development, their origins have remained a mystery. Recent molecular phylogenetic analyses support Haeckel's historic hypothesis that a group of single-celled protozoa, the choanoflagellates, represents the closest living relatives of animals. Using comparative genomics, we have identified in choanoflagellates a set of signaling and adhesion proteins thought previously to reside solely within Metazoa. Many of the proteins combine diverse domains or "modules" in a way reminiscent of the modularity common to animal signaling proteins, and there are indications of the ancient expansion of at least two protein families. These observations suggest that important genetic underpinnings of animal multicellularity predate the origin of Metazoa. In addition, they reveal choanoflagellates to be a useful group of organisms in which to study the ancient function of such proteins in the single-celled ancestor of animals. To test hypotheses about signaling and adhesion protein function in choanoflagellates, we are developing systems for manipulating protein function in laboratory cultures.

42. **GXD: The Gene Expression Database for Mouse Development.** I. McCright, D. Begley, D. Hill, T. Hayamizu, C. Smith, J. T. Eppig, J. Kadin, J. Richardson, and M. Ringwald. The Jackson Laboratory, Bar Harbor, Maine.

The Gene Expression Database (GXD) is a community resource of gene expression information of the laboratory mouse. GXD integrates many different types of expression data, such as RNA *in situ* hybridization, immunohistochemistry, Northern blot, Western blot, and RT-PCR data. Our aim is to provide comprehensive information about where and in what amount transcripts and proteins are expressed and how their expression varies in different mouse strains and mutants. We standardize the description of expression patterns using terms from the anatomical dictionary that has been developed in collaboration with our Edinburgh colleagues (1). Database records are linked to digitized images of original data. GXD is available at http://www.informatics.jax.org. The database is updated daily as new expression data are acquired. To facilitate electronic submissions, we have developed the Gene Expression Notebook (GEN). Implemented in Microsoft Excel, GEN is a tool to manage expression data in the laboratory, including images, and to submit these data to GXD. Submitted data will receive accession numbers that can be cited in publications. GXD places the expression data in the larger biological context. A combined analysis of genotype, expression, and phenotype data is possible due to integration of GXD with the Mouse Genome Database. The use of standardized classification schemes for gene products and extensive interconnections with other databases further extend GXD's utility for the analysis of gene expression information. 1. Edinburgh collaborators: J. Bard, R. Baldock, D. Davidson, and M. Kaufman. (GXD is supported by NIH Grant HD33745.)

43. **Evolution of Developmental Mechanisms in Chelicerata: Genomic of Spider Mite *Tetranychus urticae*.** Peter Dearden,* Cameron Donly,† and Miodrag Grbic.* *Department of Zoology, University of Western Ontario, London N6A 5B7, Canada; and †Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, Ontario, Canada.

Despite being the second largest animal group, the developmental genetics of chelicerates is poorly understood. The main obstacle for future progress in this field is the lack of a chelicerate model organism. *Tetranychus urticae* belongs to class Chelicerata, subclass Acari (mites and ticks). The rapid generation time (7 days) makes *T. urticae* a favorable laboratory organism for potential genetic studies. *T. urticae* also has small eggs (150 mm) that are surrounded by a transparent chorion. Transparency of embryos allows visualization of complete embryonic development. Finally, *T. urticae* has only three chromosomes and a smaller genome than the insect model system, *D. melanogaster*. We are using a combination of large-scale sequencing and automated *in situ* screens with classical embryology to systematically isolate developmentally important regulatory genes that direct the spider mite embryogenesis. So far we have sequenced over 4000 ESTs isolating orthologs of genes involved in anterior–posterior, dorsal–ventral, and segmentation patterning cascade described in *Drosophila*. Our results indicate that while some changes in expression pattern and function of segmentation genes have occurred in the lineage leading to spider mites, many of the genes involved in insect segmentation are also regulating segmentation in spider mites, despite 600 million years of independent evolution.

44. **Multiple Regulatory Changes Contribute to the Evolution of the *Caenorhabditis lin-48 ovo* Gene.** Xiaodong Wang and Helen M Chamberlin. Department of Molecular Genetics, Ohio State University, Columbus, Ohio 43210.

Changes in gene expression patterns play an important role in the differences between species. To understand the molecular changes responsible for the evolution of gene expression patterns, we have investigated the function and expression of the *lin-48* gene in two *Caenorhabditis* nematode species. *lin-48* encodes an OVO-related zinc finger transcription factor important for the development of several cells in *C. elegans*. In a comparison of *C. elegans* and *C. briggsae*, we have found that *lin-48* functions in some cells in both species, but it is expressed and functions in one cell (the excretory duct cell) only in *C. elegans*. In *C. elegans*, *lin-48* is required for normal morphogenesis of the excretory duct. In *C. briggsae*, the excretory duct exhibits a morphological similarity to *C. elegans lin-48* mutants. Interspecific experiments using both *C. elegans* and *C. briggsae lin-48::gfp* reporter transgenes indicate that only the *C. elegans* gene can be expressed in *C. elegans* animals. Consequently, the differences between the two species result both from changes in the *lin-48 cis*-regulatory sequences and in proteins that mediate *lin-48* expression. We have tested reporter transgenes containing chimeric upstream regulatory sequences that include portions of *lin-48* from each species. The results from these tests indicate that the *cis*-regulatory sequences from the *C. elegans* gene responsible for the expression difference correspond to multiple, dispersed sequence changes rather than alteration at a single site. Furthermore, we have identified that the bZip transcription factor CES-2 is one protein that mediates *lin-48* expression through the *C.*

elegans-specific duct cell regulatory elements. Our results show the accumulation of several regulatory changes affecting both *cis* elements and *trans*-acting factors can contribute to gene evolution.

45. **Developmental Plasticity in the Sex Determination Mechanism of *Caenorhabditis elegans.*** Veena Prahlad,* Dave Pilgrim,† and Elizabeth B. Goodwin.* *Department of Genetics, University of Wisconsin, Madison, Wisconsin; and †Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada.

Phenotypic plasticity, the ability of an organism to express diverse phenotypes in different environments, is thought to maximize adaptive fitness and to influence evolutionary diversification of organisms. We found that the sex determination mechanism of *Caenorhabditis elegans* expressed such developmental plasticity. Sex in *C. elegans* is thought to be determined during embryogenesis by an X chromosome arithmetic: XX embryos develop as hermaphrodites, XO as males. We find that environmental signals, such as bacterial secretions, can postembryonically alter the sex of XX larvae. For example, growth medium from early log phase bacterial cultures induce 20% (n = 350) of XX larvae to develop as males whereas medium from lag phase bacterial cultures induces 0% (n = 315) to develop as males. Remarkably, this plasticity is seen only in larvae that are a product of sexual reproduction. XX self-progeny from hermaphrodites show no such flexibility (0% males; n = 500). These results suggest that one advantage of sex may be to confer developmental plasticity. Thus, XX cross-progeny that develop as males under the described conditions might enjoy a selective advantage because of the superior reproductive capabilities of *C. elegans* males. Surprisingly, genetic and PCR analyses indicate that sex reversal of XX larvae is often accompanied by the loss of one X chromosome. The observation that an X chromosome can be lost from sex reversed cross-progeny suggests that phenotypic plasticity might influence genotype.

46. **Doublesex-Related Genes Regulate Vertebrate Sexual Development.** Shinseog Kim, Christopher Raymond, Jae Kettlewell, Vivian Bardwell, and David Zarkower. Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, Minnesota 55455.

In contrast to many developmental processes, sex determination mechanisms appear to be highly variable between phyla. A potential exception to this lack of conservation is the similarity of the *Drosophila* doublesex (dsx) and *Caenorhabditis elegans* mab-3 genes. The Doublesex and MAB-3 proteins contain a zinc finger-like DNA-binding motif called the DM domain, perform several related regulatory functions and are at least partially interchangeable *in vivo*. We identified vertebrate genes with DM domains and showed that one of these, Dmrt1, plays a widely conserved role in male gonad development. Mice lacking Dmrt1 exhibit defective testes with defects in Sertoli and germ cell differentiation similar to the effects of human deletions removing the gene. Recently we have found six other mammalian genes with DM domains. In the mouse, three of these genes are expressed in the embryonic gonad during the time that the male and female gonads become distinct. Each gene exhibits a different expression pattern. One is expressed strongly in female gonads, a second is expressed in male gonads, and the third one is expressed equally in both. These genes are good candidates to play roles in mammalian sexual development. DM domain genes also seem to be involved in other biological processes. One gene is involved in somite development and two genes appear to be expressed brain specifically during embryogenesis. We are currently making targeted deletions of these genes and hope to define their roles in the regulatory network controlling mammalian sexual development.

47. ***In vitro* Fertilization to Hatching, a Novel Culture Method for Embryos of the Long-Finned Squid, *Loligo pealei.*** Karen Crawford. St. Mary's College of Maryland, St. Mary's City, Maryland 20686.

While much of what we know about development in cephalopods has been drawn from observing naturally laid embryos within jelly strings and egg capsules, reliable *in vitro* fertilization and culture would greatly enhance our ability to study the molecular and cellular events of development in these organisms. Currently, there are very few reported culture methods for raising *in vitro* fertilized cephalopod embryos to hatching. Normal embryonic development for *Loligo pealei* was reported when embryos were cultured in Millipore filtered seawater on cushions of 0.2% agarose (Klein and Jaffe, 1984), although expansion of the chorion (the membrane surrounding the embryo did not always occur. More recently, a considerable advancement in culture success was reported for other cephalopod embryos when their seawater was supplemented with fresh or freeze-dried oviductal gland jelly (Sakai and Brunetti, 1997). Here, this study presents a novel culture method for routinely obtaining *in vitro* fertilized hatchlings of the long-finned squid, *Loligo pealei*, and describes the effects of several commercially available sera and serum proteins on chorion expansion and embryonic development. Embryos from these cultures undergo significant, and with some treatments, normal chorion expansion to develop and hatch after 21 days at 17°C. This work was supported by a faculty development grant to KC from SMC. (Laboratory space and resources at the Marine Biological Laboratory Woods Hole, MA, were generously provided by Dr. R. Baker.)

48. Abstract #48 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

49. **Receptor for Activated C Kinase (RACK1) Required for *Drosophila* Dorsal Closure.** Jennifer A. Chapin and Robert A. Holmgren. Northwestern University, Evanston, Illinois.

Using a yeast two-hybrid screen, we found that a highly conserved subdomain of Cubitus interruptus (Ci) interacts with the receptor for activated C kinase (RACK1). This interaction was also observed using the corresponding region from Ci's closest vertebrate homolog, Gli3. The RACK1 protein consists of seven WD-40 repeats and has some similarity to the trimeric G-protein β-subunit. It was originally characterized in vertebrates for its ability to stabilize and translocate the active conformation of PKC to the cytoskeleton. To study *RACK1*'s role in fly development, mutant alleles were generated. A P-element 400-bp upstream of the *RACK1* start codon was imprecisely excised generating a deficiency in the locus and an adjacent gene. The deletion was then used to identify noncomplementing mutations from a large-scale EMS mutagenesis. Several alleles were identified in the *RACK1* locus, at least one of which we predict to be a protein null. Homozygous null mutants do not complete embryogenesis and frequently have dorsal holes indicative of failure to complete dorsal

closure. Germ line clones of a hypomorphic allele of *RACK1*, arrest slightly earlier in development. We are currently analyzing *RACK1*'s role in the dorsal closure pathway.

50. **The Role of TAK1 in Mouse Developmental Cell Death.** Joy Jadrich, Maria Danos, Michael O'Connor, and Electra Coucouvanis. Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, Minnesota.

Programmed cell death (PCD) plays an essential role in vertebrate development. Known examples include digit separation, proamniotic cavity formation, and removal of excess cells in the nervous system. Our studies are aimed at determining the signals that induce or repress the initiation of PCD during mouse embryogenesis. During pregastrulation development, the mouse embryo undergoes a cavitation event by which it is converted from a solid bud to a hollow tubular structure consisting of a single layer of columnar epithelium surrounding the proamniotic cavity. The ES-cell-derived embryoid body is an *in vitro* model of the early embryo, that mimics this early cavitation event. Our previous work has demonstrated that cavitation is the result of spatially restricted PCD, and that BMP signaling is required for cavitation to occur. Here we focus on the pathway functioning downstream of BMPs to initiate cell death. TGFβ-activated kinase 1 (TAK1) has been shown in *Xenopus* to act downstream of BMPs in a pathway leading to activation of PCD. Here we show that a dominant-negative version of TAK1 prevents cavitation in embryoid bodies. We also determined the expression pattern of TAK1 during mouse development. Our results demonstrate that TAK1 expression corresponds temporally and spatially with several tissues and cell types previously shown to exhibit developmental cell death. Our results establish a role for TAK1 in mediating PCD during cavitation in embryoid bodies *in vitro* and suggest TAK1 acts downstream of BMPs and/or other signaling molecules to initiate PCD in multiple tissues during development.

51. **Identification of Downstream Effectors of EphA4 Signaling Using a *Xenopus* Embryo Assay System.** Robert S. Winning. Department of Biology, Eastern Michigan University, Ypsilanti, Michigan 48197.

The Eph family of receptor tyrosine kinases and their ligands, the ephrins, regulate cell and tissue interactions via a repulsive mechanism. These repulsive interactions are involved in axonal targeting, somitogenesis, gastrulation, and vasculogenesis. Little is known, however, about the cellular events in Eph RTK signaling. Previous work established a convenient assay system in *Xenopus* embryos for testing possible downstream effectors of Eph signaling. This assay involves ectopically expressing a chimeric receptor (named EPP) that is activated by epidermal growth factor but has EphA4 catalytic activity. Activation of EphA4 in *Xenopus* blastulae causes loss of cell adhesion, change in cell shape, and loss of cell polarity. Candidate molecules that may act in the EphA4 signaling pathway are coexpressed (in wild-type or mutant forms) with the chimeric receptor to determine if they affect the EPP phenotype. Using this assay system, it has been shown that constitutive Rho GTPase activity rescues *Xenopus* blastulae from the effects of EPP, suggesting that at least some of the effects of EphA4 activation are brought about by inhibition of Rho. Further investigation using this assay system suggests that Rac1, another member of the Rho family of small GTPases, is also inhibited by EphA4 activity. Rho-activated kinase (p160ROCK), because it acts downstream of Rho, has also been examined for a possible role in the EphA4 pathway. Y27632, a selective inhibitor of p160ROCK, when injected into embryos, mimics the phenotype of EphA4, suggesting that p160ROCK plays a role in EphA4 signaling.

52. **A Segmentation-Specific Role of Small GTPases Rac 1 in Mesenchymal–Epithelial Transition.** Yoshiko Takahashi,*,† Shinya Kuroda,‡ Kozo Kaibuchi,§ Kunio Yasuda,* and Yukiko Nakaya.* *Nara Institute of Science and Technology, Nara, Japan; ‡The University of Tokyo, Tokyo, Japan; §Nagoya University, Nagoya, Japan; and †Center of Developmental Biology, RIKEN, Saitama, Japan.

In vertebrates the segmented somites, which give rise to skeletal muscles and axial bones, are sequentially and periodically produced from the anterior end of the presomitic mesoderm (PSM). When segmentation occurs, mesenchymal cells located at the next boundary site of PSM undergo stereotyped epithelialization. Because of a simple architecture of the somite where epithelial and mesenchymal cells are geometrically aligned, this tissue serves a good model to investigate cytoskeletal dynamics that control cell shapes in three-dimensional multicellular organization. We investigated the roles of Rho family of small GTPases, Rho, Cdc42, Rac1, and one of their effectors, N-WASP, in somitic cell epithelialization. Overexpression of mutant forms of Rac 1 (dominant-negative or dominant active) in a developing chicken somite by electroporation caused a failure of cell epithelialization and consequently, DNRac1-expressing cells sorted to stick together. DNRac1-expressing somite also displayed aberrantly organized actin filament when cultured *in vitro.* These effects were specific to Rac1, and loss of function of Rho and Cdc42 did not show defects in somite epithelialization. Our observations suggest that Rac1 plays a primary role in mesenchymal–epithelial transition in the course of somite segmentation by regulating cytoskeletal dynamics and also that changes in cell shape in multicellular organization are precisely regulated by tissue specific roles of Rho family members.

53. **Signaling via Phosphoinositide-Dependent Pathways in Fetal Mouse Submandibular Glands.** M. Kashimata,* N. Koyama,† and E. W. Gresik.† *Department of Pharmacology, Asahi University, Gifu, Japan; and †Department of Cell Biology and Anatomy Science, CUNY Medical School, New York, New York.

Branching morphogenesis of fetal mouse submandibular glands (SMGs) depends in part on the epidermal growth factor receptor (EGFR) that activates at least three intracellular signaling pathways involving (1) ERK-1/2, (2) phospholipase Cγ1 (PLCγ1), and (3) phosphatidylinositol 3-kinase (PI3K). PLCγ1 activates protein kinase C (PKC) isozymes directly; PI3K stimulates Akt/PKB and ultimately PKCs. We showed that phosphorylation of ERK-1/2 in response to EGF varies with fetal age in SMGs and that blockade of the ERK-1/2 pathway partially inhibits branching (*Dev. Biol.* **229,** 183, 2000). Here we report on components of the PLCγ1, PI3K, and PKC cascades in SMGs from the 13th day of gestation (E13) to postnatal ages. Immunoblotting revealed that (1) PLCγ1 is present from E13 to E18, but falls to negligible levels at birth, and (2) PI3K, Akt, and several PKC isozymes are expressed from E13 and through adult life. PLCγ1 and PI3K are phosphorylated in response to EGF. Inhibition of PLCγ1 by U73122 or of PI3K by LY294002 also inhibited branching. Western blotting showed that the concentrations of eight PKC isozymes vary with fetal and postnatal age. General inhibition of PKCs by calphostin C increased branching. Go 6976, Rottlerin, or Ro-32-0432, specific inhibitors of PKCs α, δ,

and ϵ, respectively, also stimulate it. These data indicate that these phosphoinositide-dependent pathways are also involved in regulation of branching morphogenesis in fetal mouse SMGs. (Supported by grants from Ministry of Education, Science and Culture of Japan and NIH DE 10858.)

54. **Transcriptional Coactivation of C/EBPα by a β-Catenin/TCF-4N Complex.** J. A. Kennell, E. E. O'Leary, F. Schaufele, and O. A. MacDougald. Cellular and Molecular Biology and Department of Physiology, University of Michigan, Ann Arbor, Michigan; and Department of Medicine, University of California–San Francisco, San Francisco, California.

Wnt signaling through the activation of TCF/LEF transcription factors inhibits differentiation of 3T3-L1 preadipocytes. We have identified and characterized TCF-4N, an alternative splice product of the mouse Tcf-4 gene, which lacks the DNA-binding domain due to a premature stop codon. We hypothesized that TCF-4N would act as an inhibitor of β-catenin activity. In reporter gene studies using a TCF-responsive promoter, TCF-4N acts to decrease induction by β-catenin. However, TCF-4N potentiates β-catenin activation of a cyclin-D1 promoter even in the absence of TCF-binding sites, suggesting that TCF-4N acts to increase β-catenin coactivation of transcription factors other than TCF/LEFs. We have identified a functional interaction between the β-catenin/TCF-4N coactivator complex and the transcription factor C/EBP α, a positive regulator of adipogenesis. To confirm data from reporter gene assays, enforced expression of TCF-4N partially relieved the block on 3T3-L1 adipocyte differentiation by enforced expression of a dominant stable mutant of β-catenin. Although the canonical Wnt pathway suggests that β-catenin coactivates TCF/LEF transcription factors alone, our findings with C/EBPα suggest that β-catenin can act as a coactivator independent of DNA-bound TCF/LEF transcription factors. This suggests a possible role for β-catenin during adipogenesis outside of the inhibitory role during Wnt signaling.

55. **Regulation of Wnt Signaling during Adipogenesis.** C. N. Bennett, S. E. Ross, K. A. Longo, L. Bajnok, N. Hemati, K. W. Johnson, S. D. Harris, and O. A. MacDougald. Department of Physiology, University of Michigan, Ann Arbor, Michigan; and Chiron Corporation, Emeryville, California.

The decision to undergo adipogenesis is controlled by a balance of factors that either repress or stimulate differentiation. We have identified Wnt10b as a potent, endogenous inhibitor of adipogenesis. Wnt 10b acts as an adipogenic switch that must be shut off for preadipocytes to differentiate *in vitro*. Here we have investigated the window of time during which adipogenesis is inhibited by Wnt signaling and have characterized specific members of the Wnt signaling pathway through which this repressive signal is mediated. We demonstrate that a specific inhibitor of glycogen synthase kinase 3, CHIR 99021, mimics Wnt signaling in preadipocytes. CHIR 99021 stabilizes free cytosolic β-catenin and inhibits adipocyte conversion by blocking induction of C/EBPα and PPARγ. Differentiation is inhibited when 3T3-L1 cells are exposed to CHIR 99021 for 24-h periods of time during the first 3 days of adipogenesis. Consistent with this time frame, expression of Wnt10b mRNA is suppressed upon induction of differentiation. Of the agents used to induce differentiation, exposure of 3T3-L1 cells to IBMX or cAMP is sufficient to suppress expression of Wnt10b mRNA. Inhibition of adipogenesis by Wnt10b is likely mediated by Wnt receptors Frizzled 1, 2, and/or 5. These receptors, like Wnt10b, are highly expressed in preadipocytes and stromal vascular cells, but not in 3T3-L1 or primary adipocytes. Furthermore, Wnt coreceptors, LRP5 and LRP6, may also play a role in Wnt signaling as both of these genes are expressed in 3T3-L1 preadipocytes and stromal vascular cells.

56. **The Sys Pathway Synergizes with Wnt Signaling to Set Up the Proximal–Distal Axes of the Gonad in *Caenorhabditis elegans*.** Kellee R. Siegfried, Trey Kidd, and Judith Kimble. Laboratory of Genetics, Program in Cellular and Molecular Biology, Department of Biochemistry, and HHMI, University of Wisconsin, Madison, Wisconsin.

The proximal–distal axes of the *Caenorhabditis elegans* gonad are established by the asymmetric division of the two somatic gonadal precursor cells. We have isolated a group of mutations that cause defects in this asymmetric cell division, thereby failing to specify the proximal–distal axes of the gonad. These genes have been dubbed "*sys*" for symmetrical sisters. One of these mutations corresponds to the *pop-1* locus, which is the *C. elegans* TCF/Lef-1 homolog, implicating Wnt signaling in gonadogenesis. Through mutant analysis and RNAi, we have found that several key Wnt signaling components indeed function in this process. However, none of the five *sys* genes map near known Wnt signaling members, which suggests that they either encode novel or highly diverged components of the pathway or encode a distinct pathway that interfaces with the Wnt signaling pathway. To investigate the relationship between the Sys pathway and the Wnt pathway, we have conducted genetic tests between *sys-1* and the other *sys* genes and Wnt signaling components. Our analysis is consistent with the idea that the *sys* genes and *pop-1* function together in a developmental pathway during gonad development. We suggest that the Sys and Wnt pathways converge at or above the level of POP-1 regulation. We are further investigating this hypothesis by asking if the sys genes regulate subcellular localization of POP-1 in gonadal cells using a POP-1::GFP reporter.

57. **Regulation of Wnt Signaling by Disheveled and Frodo.** J. Gloy, K. Itoh, H. Hikasa, B. Brott, M. Ratcliffe, and S. Y. Sokol. Department of Microbiology and Molecular Genetics, Harvard Medical School, and Beth Israel Deaconess Medical Center, Boston, Massachusetts.

Disheveled (Dsh) is a key mediator of Wnt signal transduction that regulates cell fate and polarity during development. We report that Dsh protein accumulates in the nucleus when *Xenopus* embryonic cells are incubated with inhibitors of nuclear export. Point mutations in the conserved amino acid sequence that is essential for nuclear localization of Dsh impair the ability of Dsh to activate downstream targets of Wnt signaling and stabilize β-catenin. When this sequence is replaced with the SV40 nuclear localization signal, full Dsh activity is restored. These findings demonstrate that nuclear localization of Dsh is required for its function in the canonical Wnt pathway. In the nucleus, Dsh interacts with Frodo, a novel conserved protein that synergizes with Dsh in secondary axis induction. Antisense oligonucleotide-mediated depletion of Frodo and a dominant inhibitory Frodo construct inhibited signaling by Dsh and Wnt8 and interfered with normal eye and neural tissue development. Frodo RNA suppressed these loss-of-function phenotypes. These findings establish a role for Frodo as an essential positive regulator of Wnt signaling and provide further insight into how Wnt signaling controls neural development.

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60. **Investigating the Domains Critical for MBC Function in Myoblast Fusion in *Drosophila*.** L. Balagopalan, B. Galletta, and S. M. Abmayr. Pennsylvania State University, 459 North Frear, University Park, Pennsylvania 16802.

myoblast city (*mbc*) was identified on the basis of defects in myoblast fusion in the *Drosophila* embryo. It belongs to the CDM family of proteins, which includes human DOCK180 and DOCK 2 and *Caenorhabditis elegans ced-5*. In vertebrates and *C. elegans* these molecules function in a pathway linking upstream signals from the SH2–SH3 adaptor protein CRK to the cytoskeleton. The interaction between members of the CDM family and CRK is thought to occur through the binding of CRK's SH3 domain to one or more PXXP motifs in the C-terminus. Consistent with such a mechanism, we previously identified the *Drosophila* homolog of CRK on the basis of an interaction with the C-terminus of MBC, which contains a conserved CRK-binding site. To gain insight into the mechanism and pathway through which *mbc* might function in myoblast fusion, identification of essential domains of MBC is in progress. To address whether the consensus C terminal PXXP motifs of MBC are required, and reflect a biochemical interaction between CRK and MBC that is essential *in vivo*, we have generated site-directed mutations in these motifs. Transgenic flies containing these mutant constructs have been analyzed for protein expression and their ability to rescue the myoblast fusion defect when ectopically expressed in *mbc* mutant embryos. We have also observed that MBC is recruited to the cell membrane in adhesion-induced aggregates of S2 cells. Numerous adhesion molecules, including those involved in myoblast fusion, mediate this localization. Experiments are in progress to identify domains of MBC that are essential for this recruitment.

61. **Investigating the Role of the Cytoplasmic Domain of Sticks and Stones in Myoblast Fusion in *Drosophila*.** R. Banerjee and S. M. Abmayr. Department of Biochemistry and Molecular Biology, Pennsylvania State University, 459 North Frear, University Park, Pennsylvania 16802.

Sticks and stones (SNS) is a member of the immunoglobulin superfamily (IgSF) that is specifically expressed in fusion competent cells of the *Drosophila* embryo and is essential for myoblast fusion. In contrast to many members of the IgSF, SNS has an unusually long cytoplasmic domain of 385 amino acids. To investigate whether this cytoplasmic domain is essential, and required for signal transduction events mediating myoblast migration and fusion, we have generated constructs with modifications in this domain. Truncations include deletion of a majority of the cytoplasmic domain and fusion of the extracellular domain of SNS to a GPI anchor. After confirming that proteins were being expressed we examined the ability of the proteins to rescue *sns* mutant embryos. Forms in which the cytoplasmic domain contained only 18 amino acids past the transmembrane region and the extracellular domain of SNS with GPI were unable to rescue *sns* function when ectopically expressed in the mesoderm of *sns* mutants. These data suggest that the cytoplasmic domain is essential for SNS function. By contrast, flies expressing a truncated form without the last third of the cytoplasmic domain did exhibit reduced myoblast fusion, when expressed in *sns* mutants. In addition to the rescue experiments, we have examined the consequences of overexpression of these constructs in the mesoderm. The construct deleting the majority of the cytoplasmic domain appeared to function as a dominant negative inhibitor, disrupting myoblast fusion whereas other constructs had no effect. Details of these experimental results will be presented.

62. **Identification of Proteins That Interact with the Cytoplasmic Domain of Sticks-and-Stones.** S. J. Hong, K. E. Smith, and S. M. Abmayr. Department of Biochemistry, Microbiology, and Molecular Biology, Pennsylvania State University, 459 North Frear, University Park, Pennsylvania 16802.

In the animal kingdom, the genetically tractable fruitfly, *Drosophila melanogaster*, plays an important role in providing with new insights into how the complex muscle pattern is established. The muscles of a *Drosophila* larva are the result of a fusion process between founder myoblasts which endow the muscles with their specific characteristics and surrounding fusion-competent myoblasts. Several important genes involved in the fusion process have recently been identified including *sticks-and-stones* (*sns*). *sns* is specifically expressed in the fusion-competent cells and is essential for the fusion. *sns* encodes a cell adhesion molecule in the immunoglobulin superfamily with homology to human Nephrin and *Drosophila* Hibris. The long cytoplasmic domain of SNS with several motifs associated with the activity of signaling molecules suggests a possible role for SNS in signal transduction pathways essential for myoblast fusion. To identify proteins that interact with SNS, we performed a yeast two-hybrid screen using a part of SNS cytoplasmic domain as a bait. Preliminary results have identified 237 Ade^+, His^+, $LacZ^+$ colonies from approximately 4×10^6 yeast transformants screened. All 237 colonies activated the *lacZ* gene within 2 h. Sequencing analysis followed by BLAST database searches revealed 31 possible candidate genes which include genes involved in cell signaling. In this report, we present data characterizing genes belonging to the selected pool.

63. **SpADAM Is Required for Cell Fate Determination in Early Sea Urchin Development.** Robert D. Burke, Greg Murray, and Matt Rise. Biology and Biochemistry/Microbiology, University of Victoria, Victoria, British Columbia, Canada.

SpADAM is a sea urchin ADAM related to vertebrate ADAMs 12, 13, and 19, which is expressed during early cleavage on blastomere surfaces. We have used morpholino antisense oligonucleotides to block expression. Antisense injected embryos form normal blastulae and primary mesenchyme ingresses, but embryos fail to gastrulate normally. Using tissue-specific markers we have determined that primary mesenchyme does not pattern properly, nor do they fuse to from syncytia. A small rudiment of endoderm forms, but a gut does not differentiate. Fewer pigment cells and neurons form and pigment cells fail to migrate into the ectoderm. All of the ectoderm expresses the oral ectoderm marker, EctoV. When eggs are coinjected with morpholino and RNA encoding full-length SpADAM lacking the morpholino target sequence, normal larvae result. As all vegetal cell types are under represented, we conclude SpADAM appears to function in the signaling pathways that establish these lineages. We speculate that it may function as a sheddase or a necessary ligand for integrins.

64. **A Deficiency Screen for Genetic Regulators of *Drosophila* Imaginal Leg Imaginal Disk Morphogenesis.** Laurie von Kalm, Troy Camarata, and Amanda Leppert. University of Central Florida, Orlando, Florida.

The *Drosophila* Stubble locus encodes a type II transmembrane serine protease with an extracellular catalytic domain. The Stubble protease is required for cells to change shape during elongation of prepupal leg imaginal disks. Stubble mutant alleles interact genetically with mutations in RhoA, Rho kinase, DRhoGEF2, and zipper, the gene encoding nonmuscle myosin II, suggesting a role for Stubble in regulating actin cytoskeletal dynamics during leg development. To date the mechanism by which a protease with an extracellular catalytic domain might regulate the actin cytoskeleton is unknown. To help clarify the relationship between Stubble and the RhoA signaling pathway we have conducted a genetic screen on all chromosomes for deletions that interact with Stubble and RhoA mutant alleles. Strongly interacting deletions have been tested in secondary screens using smaller chromosomal deletions. In addition, we have tested mutations in candidate genes uncovered by the deletions for genetic interactions with Stubble and RhoA mutants. The results from our screen will be compared to a previously published screen for deletions that interact with zipper mutations (Halsell and Kiehart). Deletions that interact genetically with Stubble, RhoA, and zipper mutants are likely to uncover genes with important roles in mediating a hypothetical Stubble-RhoA signaling pathway leading to myosin activation and cell shape changes in developing leg imaginal disks.

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67. **aph-1 in Notch Signaling Pathways of *Caenorhabditis elegans.*** Nora Sullivan and Caroline Goutte. Amherst College, Amherst, Massachusetts.

The Notch signaling pathway mediates a number of distinct cell interactions during the development of *C. elegans*. The molecular components responsible for mediating these events are well conserved with those involved in Notch signaling events in other systems. Signaling by the GLP-1 Notch receptor is critical for proper cell fate specification in the early embryo and for proliferation of the germ line in the larva and adult. Signaling by the LIN-12 Notch receptor is critical for the proper development of the vulva, including both epidermal and uterine cell fate specifications. We are investigating the role of a new Notch pathway component, the product of the aph-1 gene (Goutte *et al.*, 2002). This novel multipass protein has been evolutionarily conserved, but its function remains unknown. Disruption of aph-1 function results in phenotypes that resemble those associated with mutations in glp-1, namely embryonic lethality and sterility, as well as a phenotype that is reminiscent of that caused by certain mutations in lin-12, a defect in vulval development. These mutant phenotypes are also observed in worms that are defective in the presenilin genes hop-1 and sel-12. Here we present further analysis of the aph-1 gonadal and vulval phenotypes and compare and contrast them to those of glp-1 and presenilin mutants. Although the sterile gonads of aph-1-deficient worms are similar to those of glp-1 mutants, we found one aspect in which they differ: the aph-1 gonads contain excess sperm in a manner very similar to what we observe in presenilin mutants. The fact that aph-1 mutants share similarities with both glp-1 and lin-12 mutants suggests that it may have a general role in Notch signaling pathways; however, we do not know whether it acts before, after, or within the steps involved in receptor activation. To begin to address this question we are performing epistasis analysis with activated versions of GLP-1 and LIN-12 and will present these results.

68. **A Novel Role of the Notch Signaling Pathway in Dauer Maintenance in *Caenorhabditis elegans.*** J. Ouellet and R. Roy. Department of Biology, McGill University, Montréal, Quebec, Canada, H3A 1B1.

During unfavorable growth conditions *Caenorhabditis elegans* can execute an alternative diapause-like larval stage referred to as dauer. Dauer larvae can recover from this stage and resume reproductive development without developmental consequence when environmental conditions become favorable. The integration of environmental signals during the first larval stage is required to trigger the execution of dauer development and at least three parallel inputs affect this decision including a TGF-β, an insulin-like and a cGMP signaling pathway. The mechanisms for dauer formation have been well characterized, but little is known about maintenance or exit from this stage. Our results suggest that the Notch signaling pathway is necessary to maintain this developmental stage. Using a transcriptional *lag-2*::GFP reporter (a DSL-like ligand) we found that *lag-2* was strongly and specifically expressed in the IL1 neurons at the onset of, and throughout, dauer. Genetic analysis using different components of the Notch signaling pathway and various mutants in the dauer signaling pathway (Daf) confirmed the requirement of Notch signaling in maintaining dauer. Although two Notch receptors are present in the *C. elegans* genome, only *glp-1* is directly involved in dauer maintenance. Our data suggest that TGF-β signals repress Notch signaling in the IL1 neurons during reproductive development and when these signals are downregulated during dauer formation a LAG-2/GLP-1 signaling cascade ensures the correct maintenance of the dauer stage during adverse growth conditions.

69. **A Suppressor Screen of *egl-38* Egg-Laying Defect to Study the Vulva to Uterus Signaling Pathway in *Caenorhabditis elegans.*** Vandana Rajakumar and Helen M. Chamberlin. MCDB program and Department of Molecular Genetics, The Ohio State University, Columbus, Ohio 43210.

Communication between the tissues in an organ system during development is vital to their functioning as a unit. To better understand how tissues coordinate their development during organogenesis, we are studying the *Caenorhabditis elegans* egg-laying system. This process involves interaction first between the anchor cell (AC) of the somatic gonad and the vulva and then reciprocally from the vulva to the uterus. Both interactions utilize the LIN-3 EGF signal. The AC to vulva pathway has been well characterized, whereas the vulva to uterus pathway is less well defined. Some genes are common in both pathways whereas others function in one pathway and not the other. To identify additional genes in the vulva to uterus reciprocal pathway, we have exploited the fact that the expression of textitlin-3 requires the PAX transcription factor EGL-38 only for this pathway. We carried out F2 genetic screens for temperature sensitive suppressors of the egg-laying defect of *egl-*

38(n578) animals. From over 48,000 mutagenized gametes screened, we can identify 6 strong and 13 weak suppressor mutations. Currently we are completing genetic mapping and complementation tests for the six strong suppressors.

70. **MAB21L2 Relocalizes to the Nucleus in Response to the Msx Genes.** Rebecca L. Y. Wong, Gene T. C. Lau, and K. L. Chow. Department of Biology, Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong.

The Mab21 gene was first identified in *Caenorhabditis elegans* through the characterization of sensory ray mutants. The gene encodes a novel protein with no known protein function. Functional studies in worm, mouse, and frog models revealed common defects in neurulation and tissue differentiation processes. The regulation of Mab21 is largely unknown. Genetic studies in worms have however indicated that a BMP-like pathway negatively regulates this gene. In search of a modulator in this BMP-Mab21 relationship, we have identified the Msx genes as potential modulators of MAB21 activity. There are two Mab21 genes in mouse. In this cell culture study, we demonstrated that MAB21L2 was relocalized into the nucleus in response to MSX1. Such an induction of the MAB21L2 nuclear translocation process was specific between MSX1 and MAB21L2. Deletion analysis uncovered many critical regions on both proteins for this translocation. MAB21L2 and MSX1 did not interact in the nucleus and neither did MAB21L2 associate with MSX1 in the cytoplasm for nuclear entry. The mechanism of the induced translocation was elucidated with the use of transcriptional and translational inhibitors. The results suggest that the induced translocation requires synthesis of new proteins. Our data here not only demonstrate the modulation of the MAB21 products but also a novel indirect role of the Msx genes on its target genes. (This study was funded by Research Grants Council, Hong Kong.)

71. Abstract #71 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

72. **Growth and Differentiation Factor-8 (GDF-8) Induces Dorsal Mesoderm Formation in *Xenopus* Explants and Stimulates Erythroid Differentiation of K562 Human Myelogenous Leukemia Cells.** E. Carter, R. Hao, E. Etter, H. Lellman, M. Tsang, and M. Breitenfeldt. R&D Systems, Inc., Minneapolis, Minnesota.

Members of the TGF-β superfamily show diverse function in development, including early embryo patterning and the development and differentiation of many organs and tissues. Myostatin, also known as GDF-8, is a member of the TGF-β superfamily and is characterized as a negative regulator of muscle growth. GDF-8 is most closely related to BMP-11, with 90% homology at the amino acid level in the biologically active carboxy-terminal region. GDF-8 is first expressed in the myotome of developing somites and continues to be expressed throughout development in myogenic tissue. We show that GDF-8 induces dorsal mesoderm elongation in *Xenopus* ectodermal explants and that follistatin, capable of binding and blocking the activity of several TGF-β superfamily members including activin and several BMPs, inhibits this elongation. Because of its activin-like activity in *Xenopus* explants we examined GDF-8 activity in an established activin assay. Like activin, GDF-8 stimulates erythroid differentiation of K562 human myelogenous leukemia cells, as measured by the psuedoperoxidase activity of hemoglobin. Follistatin fully blocks GDF-8 activity in the K562 assay. GDF-8 activity in the K562 assay can also be blocked by exposure to soluble activin receptors and by exposing the cells to activin receptors antibodies. These results indicate that GDF-8 can act through activin receptors type IIA and IIB.

73. **Tob Proteins Enhance Inhibitory Smad–Receptor Interactions to Repress BMP Signaling.** Yutaka Yoshida,* Andreas von Bubnoff,† Naoko Ikematsu,* Ira L. Blitz,† Eri Yoshida-Hosoda,* Hisashi Umemori,* Kohei Miyazono,‡,§ Tadashi Yamamoto,* and Ken W. Y. Cho.† *Department of Oncology, The Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108, Japan; †Developmental Biology Center and the Department of Developmental and Cell Biology, University of California, Irvine, California 92717-2300; ‡Department of Molecular Pathology, Graduate School of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan; and §Department of Biochemistry, Cancer Institute, Toshima-ku, Tokyo 170-8455, Japan.

Tob inhibits the bone morphogenetic protein (BMP) signaling pathway by interacting with receptor-regulated Smads in osteoblasts. Here we provide evidence that Tob also interacts with inhibitory Smads (Smad6 and Smad7). A yeast two-hybrid screen identified Smad6 as a protein interacting with Tob, and Tob colocalizes with Smad6 at the plasma membrane. In addition, Tob enhances the interaction between Smad6 and activated BMP type I receptors. Furthermore, we find that Tob and Tob2 cooperate with Smad6 in inducing secondary axes when expressed in the ventral marginal zone of early *Xenopus* embryos. Finally, Tob and Tob2 cooperate with Smad6 to inhibit endogenous BMP signaling in *Xenopus* embryonic explants and in cultured mammalian cells. Taken together, our results provide both *in vitro* and *in vivo* evidence for a novel mechanism of how Tob inhibits endogenous BMP signaling: by facilitating inhibitory Smad functions.

74. **Embryonic Erythropoiesis Is Dependent on a Smad-Mediated Signaling Pathway in the Ventral Blood Islands.** Matthew Schmerer and Todd R. Evans. Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, New York.

The bone morphogenetic proteins (BMPs) are known to pattern ventral mesoderm in *Xenopus* embryos. In *Xenopus,* primitive erythropoiesis begins in a region of the tailbud embryo called the ventral blood island (VBI). The VBI is derived from ventral mesoderm. Experiments in our lab have shown that when BMP signaling is blocked using a dominant negative BMP receptor expressed at the midblastula transition, primitive blood differentiation is blocked. This block occurs prior to gastrulation, so it is impossible to rule out mesoderm patterning defects as the cause of the lack of blood. So, it was our goal to create an inducible system to block BMP signaling in a controlled fashion at later time points in development. The Smad proteins function downstream of the BMP, activin, and TGF-β receptors and serve to transduce signaling from these receptors. Two of the Smads, Smad 6 and Smad 7, are inhibitory Smads and their function is thought to serve as a biofeedback mechanism to dampen signaling downstream of the receptors. We made one of these inhibitory Smads, Xsmad6, inducible by fusing it with the ligand-binding domain of the estrogen receptor, so as to create a tool to block BMP signaling in a controlled fashion. Our data show that ER-SMAD6 is inducible

both at early time points and at late time points and gives the same phenotype as overexpression of native Xsmad6. We also show that we can use this construct to block primitive blood differentiation, when ER-SMAD6 is induced either during or after gastrulation. This suggests that (1) our construct is a useful tool for blocking BMP signaling in a controllable fashion and (2) BMP signaling is required after gastrulation for primitive blood differentiation. To dissect the molecular character of the phenotype, RT-PCR analysis was carried out. This analysis suggests that induction of ER-SMAD6 caused an inhibition of erythropoietic progenitor proliferation via downregulation of xGATA-2, a known BMP-responsive gene necessary for primitive erythropoiesis. Using xGATA-2 as a readout for BMP signaling, we will be able to determine whether BMP signaling is required in stromal cells for proper erythropoietic differentiation or in erythropoietic progenitors themselves.

75. **Expression of Timeless in Mouse Lung Development.** J. Xiao. Department of Pediatrics, School of Medicine, University of Southern California, Los Angeles, California 90033.

The Clock gene *timeless* regulates circadian rhythms in *Drosophila,* but its mammalian homolog does not appear essential for circadian clock function. However, recent studies have reported that the mammalian timeless plays a key role in embryonic development. We generated a polyclonal antibody to mouse Timeless (mTIM) to study the distribution of mTIM in whole early embryos and during various stages of lung development. The results showed that mTIM can be localized in all main organs, especially in neural epithelium of the mouse embryo in early stage (E10) of development. mTIM is present uniformly in both endodermally derived epithelium and splanchnic mesenchyme at the onset of lung morphogenesis in E9.5 mouse embryos. Subsequently in E15 lungs expression of mTIM decreased in the mesenchyme, but remained pronounced in the epithelium of both large and smaller airways. Further development of the lung in E18 and postnatal stages was accompanied by restricted localization of mTIM in a specific subset of epithelial cells with alveolar type II (or precursors) phenotype. In contrast, alveolar type I-like cells obtained by long-term culturing of alveolar type II cells were devoid of mTIM. In the proximal airways, mTIM was colocalized with CC-10 a product of the nonciliated columnar epithelial cells. The pattern changes of mTIM in lung embryonic development and cell specificity suggest that mTIM plays a key role in lung development.

76. **Dual Roles of Cripto as a Ligand and Coreceptor in the Nodal Signaling Pathway.** Yu-Ting Yan,*,† Jan-Jan Liu,*,† Yi Luo,‡ E. Chaosu,*,† Robert S. Haltiwanger,‡ Cory Abate-Shen,*,§ and Michael M. Shen.*,† *Center for Advanced Biotechnology and Medicine, †Department of Pediatrics and §Neuroscience, UMDNJ–Robert Wood Johnson Medical School, 679 Hoes Lane, Piscataway, New Jersey 08854; and ‡Department of Biochemistry and Cell Biology, SUNY–Stony Brook, Stony Brook, New York 11794.

The *EGF-CFC* genes *Cripto, Cryptic, FRL-1,* and *oep* encode extracellular proteins that contain a divergent epidermal growth factor (EGF) motif and a novel conserved cysteine-rich domain termed the CFC motif, with most of the sequence similarity occurring in the central EGF and CFC motifs. Molecular genetic studies in fish, mouse, and frog have shown that EGF-CFC genes are essential for signaling by the transforming growth factor-β (TGF-β) ligand Nodal. Recent experiments in frog embryos indicate that Cripto can function as a coreceptor for Nodal by forming a membrane-associated complex with activin receptors. In contrast, earlier studies in mammalian cell culture have implicated Cripto as a secreted growth factor-like signaling molecule. To reconcile these apparently disparate models of Cripto function, we have established a mammalian cell culture assay to investigate the signaling activities of Nodal and EGF-CFC proteins. Using a luciferase reporter assay, we find that Cripto has activities consistent with a coreceptor for Nodal. However, Cripto can also function as a secreted signaling factor in cell coculture assays, suggesting that it may also act as a coligand for Nodal. Furthermore, we find that the ability of Cripto to bind to Nodal and mediate Nodal signaling requires the addition of an O-linked fucose monosaccharide to a conserved site within EGF-CFC proteins. We propose a model in which Cripto has dual roles as a coreceptor as well as a coligand for Nodal and that this signaling interaction with Nodal is regulated by an unusual form of glycosylation. Our findings highlight the significance of extracellular modulation of ligand activity as an important means of regulating TGF-β signaling pathways during vertebrate development.

77. **Mechanisms of Calcium Signaling in Zebrafish Development.** D. C. Slusarski, B. Hjertos, and J. Humbert. University of Iowa, Iowa City, Iowa.

In zebrafish, we have the unique capability of visualizing the spatial and temporal changes in cytoplasmic calcium in living cells of the intact embryo. We observe several dynamic and distinct phases of calcium release during zebrafish development. After fertilization, there are dramatic calcium increases associated with the forming cleavage furrow during the first few cell cycles. During early development, subsets of cells initiate rapid aperiodic calcium fluxes that persist until the midblastula transition stage. Additional calcium dynamics are observed in the marginal zone during gastrulation and during neural development. Each phase has a distinct pattern of calcium release, highlighting the complexity of Ca^{2+} ion and cellular physiology. Modulation of calcium release during cleavage stage impacts axis formation and gastrulation-specific calcium release has been proposed to coordinate movements of populations of cells. The noncanonical Wnts (Wnt-5A and Wnt-11) modulate calcium release, and in loss of function mutations, we observe alterations in the subcellular localization of β-catenin resulting in ectopic expression of β-catenin target genes. Alterations of the distinctive calcium release events in several different mutant strains and in treated zebrafish embryos demonstrate that calcium release acts in part, through negative regulation of canonical Wnt signaling.

78. **Exogenous Amino Acids Regulate Trophoblast Cell Differentiation through a mTOR-Dependent Pathway.** P. M. Martin and A. E. Sutherland. Cell Biology and Biotechnology Training Program, University of Virginia, Charlottesville, Virginia.

Trophoblast cells are essential in the process of mammalian embryo implantation. Just prior to implanting quiescent, epithelial trophectoderm cells differentiate into postmitotic mesenchymal trophoblast cells that mediate implantation. We demonstrate that embryos cultured in defined medium lacking amino acids cannot form trophoblast cell outgrowths on fibronectin, an *in vitro* model of implantation. The inability of trophoblast cells to spread correlates with absence of their normal protrusive activity. We have determined that the requirement for amino acids is developmentally regulated and is confined to a narrow window of time. Regulation of trophoblast cell development by exogenous amino

acids correlates with data form other systems. It has been demonstrated in cultured cell lines an animal models that protein translation and cell cycle progression are controlled by amino acid availability through regulation of mTOR (mammalian target of rapamycin) activation. To determine whether trophoblast cell differentiation requires mTOR activation we cultured embryos in the presence of amino acids and rapamycin, a specific inhibitor of mTOR, and found that rapamycin treatment completely inhibited the ability of amino-acid-containing medium to promote trophoblast cell differentiation. Embryos treated with rapamycin or cultured in amino-acid-lacking medium do not display specific threonine phosphorylation that is a hallmark of the mTOR signaling cascade. Additionally embryos cultured in amino acid-free medium or in the presence of rapamycin have a morphology similar to that of embryos in diapause, a phenomenon that delays implantation. To date, no cellular mechanism has been established for the regulation of diapause. Our data suggest that embryonic diapause may be regulated through an mTOR-dependent signaling pathway. (Supported by NICHD HD034807.)

79. Abstract #79 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

80. **Molting in Free-Living and Parasitic Nematodes: A Role for Nuclear Receptors?** Puneet Gandotra, Maureen Luschini, Kelly Kraus, Sarah Joyce, and Kirsten Crossgrove. Loyola College, Baltimore, Maryland.

Nematode development is punctuated by a series of molts in which the organism synthesizes a new cuticle, sheds its old cuticle and grows in size. In parasitic nematodes, molting occurs in response to the movement of worms from one host to the next. Parasitic nematodes are responsible for severe and debilitating disease in humans and domestic animals. An understanding of how molting is controlled may lead to new ideas for drug development. The signals required for molting are not well understood. However, there is some evidence that at the genetic level molting in nematodes may be controlled by some of the same genes that control molting and metamorphosis in *Drosophila*. For example, two *Caenorhabditis elegans* genes that encode members of the nuclear receptor superfamily of transcription factors (*nhr-23* and *nhr-25*) show molting defects when inactivated by dsRNAi (M. Kostrouchova *et al.*, 1998, *Development* **125,** 1617–1626; C. R. Gissendanner and A. E. Sluder, 2000, *Dev. Biol.* **221,** 259–272; M. Kostrouchova *et al.*, 2001, *Proc. Natl. Acad. Sci. USA* **98,** 7360–7365). Both of these genes have putative homologs in *Drosophila* (*DHR3* and *βFTZ-F1*, respectively) that have known roles in controlling gene expression during metamorphosis. We have identified a gene in the parasitic nematode *Dirofilaria immitis*, the causative agent of dog heartworm disease, that encodes a putative homolog of the *Drosophila E75* gene. In *Drosophila*, *E75* is required for molting and metamorphosis. The protein encoded by the *D. immitis E75* homolog, Di-nhr-6, is 83% identical to the E75A protein in the DNA-binding domain, the most highly conserved region of nuclear receptors. Northern blot analysis suggests that *Di-nhr-6* encodes multiple isoforms and is female specific in adults. We are currently using mobility shift assays to see if the Di-nhr-6 protein binds to DNA in a similar manner to E75A. We are also working with putative *E75* homologs in both the human parasite *Brugia malayi* and the free-living nematode *C. elegans*. In particular, we are using tools available in *C. elegans*, such as transgenics and dsRNAi, to ask questions that are difficult to ask in parasites. We discuss the potential of using *C. elegans* to help understand parasitic nematode development.

81. **Hormonal Regulation of a *Manduca sexta* Cuticular Protein Gene, MSCP14.6.** Dayton Petibone and John Rebers. Northern Michigan University, Marquette, Michigan.

The tobacco hornworm, *Manduca sexta*, is currently being used to investigate how genes are regulated to result in the synthesis of different cuticular proteins throughout metamorphosis. During larval stages, epidermal cells secrete a flexible cuticle over the entire surface of the larva. In adulthood, some of the same epidermal cells produce the rigid regions of the exoskeleton, while some cells retain the ability to produce the flexible cuticle in other regions. The expression of a 14.6-kDa *M. sexta* cuticular protein (MSCP14.6) gene is the focus in this set of experiments. One interesting feature of the MSCP14.6 gene is the modification of its spatial pattern of expression during development. The MSCP14.6 mRNA is found throughout the cuticular segments during larval development, but is restricted to flexible intersegmental cuticle in pupal and adult stages. Two hormones, juvenile hormone (JH) and 20-hydroxyecdysone (ecdysone), are believed to control expression of the MSCP14.6 gene during metamorphosis. In the presence of JH, ecdysone will induce a larval to larval molt; while at low concentrations or in the absence of JH, a larval to pupal molt is induced. A segment of the MSCP14.6 gene 5′ region containing putative ecdysone response elements has been cloned into a reporter vector designed to study the way gene response elements regulate expression. This reporter construct will be transfected into insect tissue culture cells, the cells will be treated with ecdysone and JH, and the level of reporter gene activity will be measured. In this experiment, the hormonal signals and gene response elements needed for the synthesis of MSCP14.6 will be determined.

82. **mRNA-Controlled Gene Expression in Development, the Epigene.** Mannchiang Niu. Department of Biology, Temple University, Philadelphia, Pennsylvania 19122; and The Niu Laboratory in Institute of Development Biology, Academia Sinica, Beijing 100800, China.

The substance of epigenetic effect on nucleus in fertilized egg is likely protein/RNA. The role of mRNA was tested by the injection of goldfish eggs with/without rabbit globin mRNA. Only red blood cells (RBC) from the injected fish developed rabbit globin. The lactate dehydrogenase (LDH) of the RBC was analyzed by starch gel electrophoresis and found that the LDH of the injected is a hybrid of goldfish and rabbit. This result implied that homologous globin mRNA of the injected rabbit globin is present in the egg. Both globin mRNA act as the gene for the formation of the hybrid LDH. Accordingly they are being designated EPIGENE. mRNA-transcribed cDNA was reported first in goldfish egg. The cDNA from enucleated goldfish and carp eggs was hybridized with the probe, P32 cDNA, transcribed respectively by the mRNA from goldfish heart, kidney, liver, and testis. A band was found between the goldfish enucleated egg cDNA and each of the four probes and ^{32}P-labeled cDNA but none in the carp enucleated egg cDNA and the probes. These four specific egg mRNA were injected separately into goldfish eggs. The fish developed the organ corresponding to the mRNA donors of the mRNA injected and uninjected which was subjected to PAGE. An extra band appeared only in each of the injected, indicating the presence of the injected mRNA-translated proteins. The LDH of the organ homologous of mRNA donor

excised from goldfish, injected fish, and respective rabbit, mouse, or chicken was analyzed by starch gel electrophoresis. It was found that the LDH of the injected fish is a hybrid of goldfish and respective rabbit, mouse, or chicken.

83. **Sequence and Expression of BET Family Genes in Zebrafish.** K. J. Bee, J. J. Andahazy, and A. J. DiBenedetto. Villanova University, Villanova, Pennsylvania.

Drosophila fsh, human RING3, and rat mud6 are homologous members of the BET family, proteins containing dual bromodomains and an extraterminal (ET) domain. Bromodomain proteins are involved in transcriptional regulation; both bromo and ET domains participate in protein–protein interactions. fsh is a maternal effect gene affecting segmentation, RING3 is a mitogen-activated, nuclear kinase that transactivates E2F-dependent genes, and mud6 is upregulated in rat neurons undergoing apoptosis. These data implicate fsh/Ring3/mud6 sequences in early development, cell proliferation, and cell death. To study these sequences in early vertebrate development, the zebrafish homolog (zRing3) was cloned using a probe generated by PCR amplification of the conserved bromodomain. Twelve of the resulting 25 positive clones from a zebrafish embryonic library were sequenced based on unique restriction enzyme digest patterns. Each shows similarity at one or both ends to Ring3. Two categories of Ring3-related cDNAs have been identified. The first contains what appear to be true zRing3 cDNAs, from the structural homolog in zebrafish, and is represented by two size classes of cDNA (zRing3.6 and zRing3.19), encoding proteins that differ only in the N-terminal half of their ET domains. This is the first example of a BET protein with a modified ET domain. The second category contains Ring3-related cDNAs that show sequence similarity only at their 5′ ends (zrRing3.9). The chromosomal locations of these cDNAs will be determined to verify the common origin of zRing3.6 and zRing3.19 and to assess whether cDNAs map to regions containing known genetic mutations. The expression pattern of zRing3.6, determined by Northern blot and *in situ* hybridization, suggests that zRing3 plays a role in the development of head and tail, fin buds, heart, kidney, gut, and regions of the brain. Probes specific to zRing3.6 and zRing3.19 are being used to assess transcript-specific expression patterns during development.

84. **Genetic Analysis of Hypoxia Signaling and Response.** Chuan Shen and Jo Anne Powell-Coffman. Department of Zoology and Genetics, Iowa State University, Ames, Iowa 50011-3260.

Hypoxia-inducible factors (HIFs) regulate important physiological responses to low environmental oxygen (hypoxia). HIFs are heterodimeric transcription factors consisting of two subunits, HIFα and ARNT. Under normoxic conditions, HIFα binds the von Hippel–Lindau tumor-suppressor protein (VHL) and is rapidly degraded by a ubiquitin–proteasome pathway. In addition, several studies suggest that cell-type-specific factors or signaling pathways may modulate HIFα activity by VHL-independent mechanisms. We are studying hypoxia signaling and response in *Caenorhabditis elegans*, a powerful genetic model organism. We recently identified *hif-1*, the *C. elegans* homolog of HIFα. *hif-1* mutants have decreased ability to adapt to hypoxic conditions (Jiang *et al., PNAS,* 2001). Others have shown that degradation of *C. elegans* HIF-1 under normoxic conditions is dependent upon *vhl-1*, the *C. elegans* homolog of VHL (Epstein *et al., Cell,* 2001). Thus, some transcriptional targets of HIF-1 are expressed at decreased levels in *hif-1* mutants and are expressed at constitutively high levels in *vhl-1* mutants. In collaboration with S. Kim and colleagues at Stanford, we are using microarray analyses to analyze hypoxia-dependent changes in gene expression. Three short-term goals are (1) to test the hypothesis that most transcriptional responses to hypoxia are mediated by *hif-1;* (2) to test the hypothesis that HIF-1 activity is regulated by both VHL-independent and VHL-dependent mechanisms; and (3) to identify direct transcriptional targets of the HIF-1 complex.

85. **An Essential Role for bHLH-PAS Proteins in *Caenorhabditis elegans*.** Huaqi Jiang, Shu Wu, and Jo Anne Powell-Coffman. Department of Zoology and Genetics, Iowa State University, Ames, Iowa 50011-3260.

The basic helix–loop–helix–PAS (bHLH-PAS) proteins are a family of transcription factors that mediate diverse processes, including cellular adaptations to environmental signals and developmental cell fate decisions. The *Caenorhabditis elegans* genome encodes five bHLH-PAS proteins. These include AHR-1, HIF-1, and AHA-1. The AHA-1 protein dimerizes with multiple bHLH-PAS partners and is expressed in most, if not all, somatic cells. Although *ahr-1, hif-1* double mutants are viable, animals homozygous for null mutations in *aha-1* have feeding defects and arrest development as young larvae. To understand the essential functions of *aha-1,* we examined the expression of the two remaining bHLH-PAS genes. A T01D3.2:GFP reporter is expressed in two interneurons. CKY-1:GFP is expressed in most nonneuronal pharyngeal cells. The pharynx is a neuromuscular feeding organ at the anterior end of the worm. The *aha-1* mutant phenotype and the expression data suggest that the function of an AHA-1/CKY-1 heterodimer might be required for feeding and viability. In support of this model, AHA-1 and CKY-1 form a DNA-binding complex *in vitro.* To determine whether this complex might be important *in vivo,* we constructed a *cky-1:aha-1* chimeric gene, in which *cky-1* 5′ regulatory sequences directed the expression of the *aha-1* transcript. We injected *cky-1:aha-1* into the *aha-1* mutant background, thereby creating animals mosaic for AHA-1 expression. The transgenic animals were viable and fertile. We conclude that AHA-1 function is essential, and we propose that AHA-1 and CKY-1 have a role in pharyngeal function.

86. **A Pharyngeal Muscle-Specific Enhancer from *ceh-22* Is Targeted by PHA-4 and Other Factors.** Tomas Vilimas, Alin Abraham, and Peter G. Okkema. University of Illinois at Chicago, Chicago, Illinois.

Caenorhabditis elegans pharyngeal muscle development involves *ceh-22,* an NK-2 family homeobox gene structurally and functionally related to genes controlling heart development in other species. *ceh-22* expression is the earliest marker of pharyngeal muscle differentiation, and it requires the panpharyngeal Forkhead factor PHA-4. However, we do not know how PHA-4 activates muscle specific expression of *ceh-22.* We have identified a transcriptional enhancer 1.4 kb upstream of *ceh-22* that is required for full *ceh-22* promoter activity and is active in the pharynx when *ceh-22* expression initiates. This “distal enhancer” sequence is conserved in the nematode *C. briggsae,* and we believe that it responds to cues specifying pharyngeal muscle fate. The distal enhancer contains multiple subelements that contribute to activity in an additive manner. One subelement, *DE3,* is highly active in the pharyngeal muscles, and within this subelement we have identified two short oligonucleotides (*de199* and *de209*) sufficient to reproduce *DE3* activity. Mutational analyses suggest that each of these oligonucleotides contains multiple sites contributing to enhancer activity. The *de209* oligonucleotide is necessary

for *DE3* activity, and it can independently enhance transcription specifically in the pharyngeal muscles. *de209* binds PHA-4 *in vitro* and responds to ectopic PHA-4 expression *in vivo,* suggesting that PHA-4 directly regulates *ceh-22* expression through *de209.* Using a yeast one-hybrid screen, we have identified several factors binding *de209* and *de199,* and we are currently examining these factors for a role in pharyngeal muscle development.

87. **mab-21 Gene Expression Is Regulated by Forkhead- and Homeodomain-Containing Transcription Factors.** Stephen S. H. Ho and King L. Chow. Department of Biology, Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong.

In *Caenorhabditis elegans* male tail, the morphological and functional identity of each sensory ray is tightly under genetic control. mab-21 determines the ray 6 identity. We dissected the regulatory elements in the mab-21 locus with both rescue and reporter assays. Of the different tissue-specific enhancer elements uncovered, only the 3′ enhancer is essential for ray 6 differentiation. Both the hypodermal and the neuronal enhancers are dispensable. Serial deletion of this 1.3-kb 3′ enhancer identified a 120-bp fragment to be sufficient for expressing the mab-21 gene in the structural cells and for the ray 6 differentiation, which led to the conclusion that the ray 6 identity is primarily determined by its structural cells. We tried to identify *trans*-acting factors interacting with this 3′ enhancer. Sequence analysis revealed two homeodomain and two forkhead transcription factor-binding sites in this 3′ enhancer. We hypothesize that three homeodomain-containing genetic partners of mab-21, egl-5, mab-5, and mab-18, may regulate mab-21 directly. unc-130 encoding a forkhead transcription factor may also act upstream of mab-21, since mutants of unc-130 displayed a mab-21 like phenotype. Testing of the binding of these transcription factors on the 3′ enhancer is in progress. The outcome would offer insight of the upstream regulation of mab-21 gene. (This study was funded by Research Grants Council, Hong Kong.)

88. **A Genetic Screen Identifies osa as a Dominant Interacter with the *Drosophila* Pax-6 Homolog Eyeless.** Maisa Meziou and Patrick Callaerts. University of Houston, Houston, Texas.

The *Drosophila* Pax-6 homolog *eyeless* is expressed in the eye-antennal disk and the central nervous system. Research has proven *eyeless* essential for normal brain and eye development in *Drosophila.* Heterozygous *eyeless* mutants exhibit normal eye phenotype, as opposed to homozygous flies with abnormal eyes. Yet in past screens, some heterozygous flies did exhibit a subtle eye phenotype, indicating that there might be other genes whose normal function involves an interaction with *eyeless.* This project involved screening chromosomes 1, 2, and 3 for genes that may interact with *eyeless.* Heterozygous *eyeless Drosophila* were crossed with flies heterozygous for deficiencies spanning chromosomes 1, 2, and 3 and screened for transheterozygotes exhibiting eye phenotypes, indicating that the deficiency contains a gene that may indeed interact with *eyeless.* One such gene that came out of the screen is *osa,* also known as *eyelid,* which encodes a putative DNA-binding protein. *In vivo* and *in vitro* data will be presented showing that *osa* plays an important role in *eyeless* function.

89. **Molecular Screen Identifies Fasciclin II as a Transcriptional Target of Eyeless.** B. Gafford, H. Sun, and P. Callaerts. Department of Biology and Biochemistry, University of Houston, Houston, Texas.

Eyeless, a *Drosophila* Pax-6 homolog, is a transcription factor involved in the development of the eye and central nervous system. Eyeless contains two DNA-binding domains: a paired domain and a homeodomain. Eyeless is expressed in undifferentiated precursor cells in the developing eye and in neuroblast, ganglion mother cells, and postmitotic neurons in the brain. Mutations in eyeless result in a reduction or loss of the eye and in severe defects in different neuropils of the brain. This information bolsters the idea that Eyeless is an indispensable part of gene circuits that regulate normal development of the eye and brain. To analyze the gene circuits controlled by Eyeless a strategy was developed to discover target genes. A modified PCR-selection assay was used to screen the *Drosophila* genome for segments of DNA that are directly bound by the Eyeless paired domain. A number of genomic DNA fragments were isolated and subsequently analyzed further *in vitro* (yeast one-hybrid, EMSA, and footprinting) and *in vivo.* One fragment that was isolated multiple times corresponds to a portion of the second intron of fasciclin II. The expression pattern of this fragment shows partial overlap with eyeless expression suggesting that other factors contribute to establishing the correct fasciclin II expression pattern. *In vitro* and *in vivo* data will be presented that confirm that fasciclin II is a target gene of Eyeless.

90. Abstract #90 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

91. **Targeted Disruption of a Mouse Homolog of the *Drosophila* Asx Gene Leads to Bidirectional Axial Skeleton Transformations and Spermatocyte Defects.** Cynthia Fisher,* Cheryl Helgason,† Caroline Bodner,‡ Keith Humphries,‡ and Hugh Brock.* *Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, British Columbia, V6T 1Z4 Canada; and †Department of Cancer Endocrinology and ‡Terry Fox Laboratory, BC Cancer Research Centre, 601 West 10th Avenue, Vancouver, British Columbia, V5Z 1L3 Canada.

Polycomb Group (PcG) proteins maintain transcriptional repression of homeotic HOM-C genes during development, leading to posterior homeotic transformations in the absence of gene product. Conversely, trithorax Group (trxG) proteins maintain activation of HOM-C genes and trxG null mutants exhibit anterior transformations. Mutations in some PcG genes, termed Enhancers of trx and Pc (ETP), result in both posterior and anterior transformations and hence mediate both silencing and activation of HOM-C genes. The *Additional sex combs (Asx)* gene of *Drosophila* is an ETP gene; here we present its murine homolog called *Asxl1 (Asx-like-1).* Primary sequence conservation between the two proteins is limited to an N-terminal region and a C-terminal region containing a PHD zinc finger which mediates interaction with the SET domain of trx/MLL in flies and mammals. Homozygous *Asxl1* −/− mice exhibit partial perinatal lethality. Surviving adult *Asxl1* mice are reduced in size. Reduced numbers of secondary spermatocytes and an increase in the number of primary spermatocytes of *Asxl1* −/− males is observed. *Asxl1* −/− mice show both posterior and anterior axial skeletal transformations in the same animal. Since bidirectional homeotic transformations are also observed in fly Asx nulls, this indicates that Asxl1 is a true functional homolog of Asx. This unique phenotype suggests a role of Asxl1 in maintaining Hox gene repression and activation, depending on the specific target

locus and context, and suggests that Asxl1 belongs to a new class of conserved ETP proteins in mammals.

92. Abstract #92 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

93. **Identification of Differentially Regulated Novel Genes during Embryo Development.** In-Taek Hwang, Yun-Jee Kim, and Jong-Yoon Chun. Seegene Life Science Laboratory, Seoul, Korea.

To study embryo development, we have isolated differentially expressed genes (DEGs) during embryo development using mouse conceptus tissues from 4.5 to 18.5 day old. Six DEGs have been isolated from E4.5, E11.5, or E18.5. Sequence analysis revealed that four DEGs are known genes such as tropomyosin 2β, troponin T2, hydroxylacyl–coenzyme A dehydrogenase (Hadh), and testis-derived transcript (Tds) and two are novel genes, named DEG1-8 and DEG4-2. Their expression patterns were confirmed by Northern blot analysis. The expression of tropomyosin 2β was very strong at E4.5, and almost disappeared at E11.5, but recovered at E18.5. Tds transcripts were strongly expressed at E4.5 and gradually reduced during embryo development. However, the expression of Troponin T2 and Hadh was weakly detected at E4.5 and gradually increased. The expression of the novel DEG1-8 was detected only at E4.5 but not at E11.5 and E18.5. From the conceptus cDNA library screening using DEG1-8 as a probe, a 1.5-kb insert cDNA clone was isolated. Sequence analysis of this clone showed that there was no homology to the BLAST database except an Alu sequence at the C-terminal region. The expression of another novel DEG4-2 was analyzed during the full stages of embryo development. It was interesting that its transcript illustrated a very unique pattern; at E4.5, a transcript, about 1.2 kb, was strongly detected and gradually decreased until E9.5 but gradually increased from E13.5 to E18.5, suggesting that DEG4-2 plays an important role at the early and late stages of embryo development. DEG4-2 transcripts were weakly detected in most of mouse adult organs including brain, heart, lung, spleen, kidney, stomach, small intestine, muscle, skin, thymus, testis, uterus, and placenta. However, its expression level was relatively high in heart, lung, and liver.

94. **A Role for a Mouse Polycomb Group Gene in Imprinting.** Jesse Chisolm Mager, Nathan D. Montgomery, Fernando Pardo-Manuel de Villena, and Terry Magnuson. Department of Genetics University of North Carolina, Chapel Hill, North Carolina.

A subset of both the mouse and the human genome is expressed only from one allele in a parent of origin-specific manner, including the imprinted X chromosome as well as autosomal imprinted loci. Targeted disruption of the Dnmt3L locus prevents maternal DNA methylation specifically at imprinted loci resulting in activation of maternally silenced genes (Bourc'his *et al.*, 2001). However, very little is known about how this methylation instructs proper imprinted expression patterns or what proteins may be involved in silencing mechanisms. Recently we have shown that *eed*, a Polycomb Group gene, is involved in stable repression of the imprinted X chromosome, possibly through associations with histone deacetylases. Based on these findings, we investigated whether *eed* is also required for maintenance of imprinted expression patterns at autosomal loci. Examination of both parent-of-origin gene expression and DNA methylation patterns at imprinted loci in *eed* null embryos implicate Polycomb Group genes in the silencing of autosomal imprinted loci.

95. Abstract #95 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

96. **Transcription Factor AP-2—A Gatekeeper at the Checkpoint Proliferation/Differentiation?** Uwe Werling, Richard Jäger, Petra Pfisterer, Julia Ehlermann, and Hubert Schorle. Institute for Pathology, Department of Developmental Pathology, University of Bonn Medical School, Sigmund-Freud Strasse 25, 53127 Bonn, Germany.

Transcription factors of the AP-2 family have been implicated to exert multiple functions during proliferation and differentiation based on its expression pattern in trophoblast, neural crest, and ectoderm cells in murine embryos. Using a subtractive cloning approach we identified a set of genes repressed by AP-2α which are described to retard cellular proliferation and induce differentiation and apoptosis. We show that these target genes are prematurely expressed in AP-2α mutant mice. One of the genes isolated, KLF-4, implicated in induction of terminal differentiation and growth regulation, is found expressed in mutant embryonic fibroblasts which display retarded growth but no enhanced apoptosis. Similar to these findings, generation and analysis of mice deficient for AP-2γ revealed that the trophectodermal cells from the knockout mice fail to proliferate leading to failure of labyrinth layer formation. As a consequence, the embryo suffers from malnutrition and dies at days 7–9 during mouse embryonic development. Analysis of knockout trophectoderm cultures suggests that AP-2γ is involved in regulation of ADA, a gene required in purine metabolism. To test whether a forced expression of AP-2 genes leads to hyperproliferation and eventually to cancer, a transgenic mouse line was established and is currently being analyzed. Based on these data we suggest that AP-2 genes might be required for cell proliferation by suppression of genes inducing terminal differentiation apoptosis and growth retardation.

97. **Abnormal Function of Astroglia and Vestibular Dysgenesis in Mice Lacking Abr and Bcr Cdc42/RacGAPs.** Vesa Kaartinen, Ignacio Gonzalez-Gomez, Jan Willem Voncken, Andre Nagy, Leena Haataja, John Groffen, and Nore Heisterkamp. Department of Pathology, Children's Hospital, Los Angeles, California.

The small Rho-related GTPases (Rho, Rac, and CDC42) have been suggested to mediate a wide variety of cellular functions. Their activity is regulated by different classes of modifiers, guanidine nucleotide exchange factors function as activators, whereas inactivation is promoted by GTPase-activating proteins (GAPs). To determine the developmental role of two closely related GAPs for Cdc42/Rac GTPases, Abr and Bcr, we generated a mouse strain deficient in both of these proteins. Double null mutant mice exhibit hyperactivity and persistent circling and are unable to swim. These phenotypic features are caused by specific abnormalities both in cerebellar and inner ear development. Mutants show cerebellar granule cell ectopia concomitant with foliation defects. We demonstrate that this phenotype is causally related to functional and structural abnormalities of glial cells. Bergmann glial processes are abnormal and astroglial cells were aberrantly present

on the pial surface. Double null mutant astroglia are hyperresponsive to growth factor stimulation and exhibit constitutively increased phosphorylation of p38 kinase. In addition to cerebellar defects, double null mutants display abnormal dysmorphic structures of both the saccule and the utricle as well as loss of otoconia. These results suggest that Abr and Bcr play important complementary roles during cerebellar and vestibular morphogenesis and that a function of Cdc42/RacGAPs and, therefore, that of the small Rho-related GTPases is critically important for balance and motor coordination.

98. **Dissecting the Regulatory Regions of the Mouse Fgf3 Gene and the Requirement for Sonic Hedgehog Signaling for Some Domains of Expression.** Nicola Powles,* Heather Marshall,† Androulla Economou,‡ Chin Chang,§ Akira Murakami,¶ Clive Dickson,‖ Robb Krumlauf,† and Mark Maconochie.* *Mammalian Genetics Unit, MRC, Harwell, Oxon OX11 ORD, United Kingdom; †Stowers Institute for Medical Research, Kansas City, Missouri 64110; ‡Developmental Neurobiology, National Institute for Medical Research, Mill Hill, London, United Kingdom; §Department of Cell Biology, Vanderbilt University School of Medicine, 116121 ST Avenue South, Nashville, Tennessee 37232; ¶Institute of Virus Research, Kyoto University, Shogion, Sakyo-ku, Kyoto 606-8507, Japan; and Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX, United Kingdom.

The fibroblast growth factors (FGFs) comprise an important family of intercellular signaling molecules required for normal development in many different embryonic contexts. In this study we set out to identify the mouse enhancer which confers the normal spatial and temporal pattern of expression of Fgf3 *in vivo*. We have scanned the Fgf3 locus for enhancer activity *in vivo* in transgenic mice by using different DNA fragments in lacZ reporter constructs, and we have now delimited an enhancer responsible for much of the embryonic Fgf3 expression pattern. We have sequenced the functional Fgf3 enhancer and have performed DNA sequence comparisons between the mouse sequence and an equivalent region upstream of the human FGF3 gene. No obvious blocks of homology corresponding to conserved regulatory regions were detected. In addition, in contrast to the human region, the mouse sequence is largely devoid of repeat elements. To examine the precise localization of reporter expression, we used neurofilament antibody staining along with X-gal staining on 9.5 and 10.5 dpc transgenic embryos carrying the Fgf3-enhancer-reporter. Reporter staining was seen extensively but not exclusively in the motor and sensory nerve components of the developing nervous system and in subsets of the proximal cranial sensory ganglia. Reporter activity was also noted in restricted areas outside the developing nervous system during early embryogenesis. Finally, to begin to dissect out the regulatory cascade upstream of Fgf3, we crossed the reporter line into the Shh mutant background. Transgenic mice heterozygous for both reporter and Shh mutant alleles were used to generate doubly transgenic mutant embryos. In all cases, 10.5 dpc Shh −/− mutant embryos carrying the Fgf3 reporter illustrated specific loss of some domains of reporter expression, revealing a part dependency of the Fgf3 enhancer on Shh signaling.

99. Abstract #99 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

100. **Translation and Polyadenylation of BMP7 mRNA Are Regulated by Novel Mechanisms in *Xenopus* Embryos.** Brian R. Fritz and Michael D. Sheets. Department of Biomolecular Chemistry, University of Wisconsin–Madison, Madison, Wisconsin.

The translational activation of maternal mRNAs is an important regulatory mechanism for controlling protein expression during vertebrate embryogenesis. Our results demonstrate that translation of the mRNA encoding the bone morphogenetic protein-7 (BMP7) growth factor is specifically activated during the early cleavage stages of *Xenopus* development and this activation is controlled by the regulated addition of poly(A). The results of our recent experiments demonstrate that this embryonic polyadenylation occurs by a novel mechanism. In particular, the sequence elements in the 3′UTR of the BMP7 mRNA that control polyadenylation are distinct from the previously described cytoplasmic polyadenylation elements (CPEs) or embryonic CPEs (eCPEs). Furthermore, polyadenylation of the BMP7 mRNA does not depend upon the AAUAAA sequence, unlike most previously defined cytoplasmic polyadenylation reactions. We are currently analyzing the interaction of regulatory proteins with the BMP7 mRNA's polyadenylation elements using a combination of UV crosslinking, *in vitro* binding assays, and *in vitro* polyadenylation extracts from *Xenopus* embryos. Collectively, these results suggest that translation and polyadenylation of the BMP7 mRNA are regulated by novel activities that function after fertilization.

101. **Inducible Control of Tissue-Specific Transgene Expression in *Xenopus tropicalis* Transgenic Lines.** Jeiwook Chae, Lyle B. Zimmerman,* and Robert M. Grainger. Department of Biology, University of Virginia, Charlottesville, Virginia 22903; and *Division of Developmental Biology, National Institute for Medical Research, The Ridgeway, Mill Hill, NW7 1AA, London, United Kingdom.

The *Xenopus laevis* system has been remarkably useful in studying gene function by injection of synthetic mRNA for mis- or overexpression studies. This approach, however, is hampered by lack of spatial and temporal control of expression of the introduced gene product. This problem can be resolved by using nuclear-transfer-mediated transgenic technique, but functional analyses are complicated by variability and background abnormalities in primary transgenic embryos. The closely related species *Xenopus tropicalis* is more suitable for genetic manipulations and multigeneration experiments by virtue of its diploid genome and shorter generation time (3 months for males), allowing us to overcome these limitations, by adapting the GAL4/UAS system. The GAL4/UAS system permits establishment of stable lines and elimination of nuclear-transfer-associated abnormalities, through generation of separate UAS "effector" and GAL4 "transactivator" transgenic lines. When the GAL4 is combined with a steroid hormone ligand-binding domain, this system allows full temporal regulation of transgene expression by introduction of an exogenous steroid analog, the progesterone antagonist RU486. We show here that by crossing stable transgenic lines, one bearing a UAS-GFP reporter construct, and the other with a GAL4-progesterone receptor fusion driven by the retina-specific *Rx* promoter, reporter expression in the resulting embryos can be induced with RU486 in a tissue-specific manner. These results suggest that the inducible binary system, in which the target gene expression can be controlled in a stage- and tissue-specific pattern, should be readily applicable for gene function studies at all stages of development. Using this

system, we are currently investigating the developmental functions of genes that are potentially important in eye development, such as the homeobox Otx2 and noggin, an antagonist of BMP-4 signaling, by targeting expression of their wild-type or dominant negative constructs to eye tissue.

102. **Modulation of Smad-Induced Collagen Gene Expression by p53 in Skin Fibroblasts.** Asish Ghosh and John Varga. University of Illinois at Chicago College of Medicine, Chicago, Illinois 60607.

TGF-β is a potent inducer of collagen gene expression and has been implicated in the excessive collagen accumulation in scleroderma. We previously demonstrated that in skin fibroblasts, the cooperation of Smads and transcriptional coactivators p300/CBP is required for TGF-β-stimulated collagen synthesis. As tumor suppressor p53 is present in fibrotic lesions and interacts directly with p300, here we examined the role of p53 in regulation of collagen synthesis. Overexpressed p53 inhibits basal collagen synthesis in human skin fibroblasts. Furthermore, p53 suppressed the TGF-β- and Smad3-induced COL1A2 promoter activity and collagen synthesis. Overexpressed p53 also abrogates the TGF-β-induced Smad-binding element containing TK minimal promoter activity further implicates the p53 as a modulator of Smad signaling. The inhibition of Smad3-induced COL1A2 promoter activity or SBE-Luc activity is not due to alteration of Smad3 expression in p53 overexpressing cells. Treatment of fibroblasts with etoposide, a potent inducer of cellular p53, blocked TGF-β stimulation of COL1A2 promoter activity in a dose-dependent manner. Exogenous p300 partially rescues TGF-β-stimulated COL1A2 promoter activity in fibroblasts overexpressing p53. The inhibitory effect of p53 on Smad-dependent collagen gene expression may be due to (i) recruitment of HDAC to the transcriptional complex by p53 or (ii) p53-induced repressor of Smad signaling. To our knowledge this is the first demonstration that the tumor suppressor p53 is a potent and selective endogenous suppressor of Smad/TGF-β induced cellular responses in normal skin fibroblasts. Alterations in p53 expression level or posttranslational modifications may therefore be involved in deregulated TGF-β responses in scleroderma fibroblasts. (This work was supported by grants from Scleroderma Foundation (005/01 to A.K.G.) and from National Institute of Arthritis and Musculoskeletal and Skin Diseases (AR-46390 to A.K.G.; AR-42309 to J.V.)

103. Abstract #103 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

104. **Functional Characterization of EPS, a Novel Lineage-Specific Transcription Factor.** Yanfei Xu,* Erica D. Smith,* Brian Kennedy,† Yuko Fujiwara,† Stuart H. Orkin,† and John D. Crispino.* *Ben May Institute for Cancer Research, University of Chicago, Chicago, Illinois; and †Harvard Medical School, Boston, Massachusetts.

Hematopoiesis is regulated by multiple transcription factors, including the basic helix–loop–helix (bHLH) protein SCL (stem cell leukemia), which plays an essential role in pluripotent hematopoietic stem cells. We recently identified a novel SCL-interacting protein, termed erythroid partner of SCL (EPS). EPS is expressed in a similar spatial and temporal manner as SCL in mid-to-late embryonic development. EPS functions as a transcriptional repressor when expressed in yeast, likely through an interaction with the transcriptional corepressors Sin3p and Rpd3p. In mammalian cells, EPS interacts with SCL and a related bHLH protein, E12, and negatively regulates E12-mediated transcriptional activity. This repression is most likely achieved through recruitment of histone deacetylases, since the addition of trichostatin A alleviates the repression. Overexpression of EPS in proerythroblasts enhances GATA-1-mediated erythroid differentiation. Indeed, EPS represses the activation of an SCL target gene, c-kit, which is consistent with the downregulation of c-kit expression during erythroid differentiation. To define the role of EPS *in vivo*, we generated mice with a targeted disruption of the EPS locus. While the loss of SCL results in lethality around embryonic day 8.5 (E8.5), the EPS-null embryos die around E5.5, prior to blood cell development and gastrulation. This result suggests that EPS plays a vital role, independent of SCL, in very early embryonic development. EPS $-/-$ blastocysts cultured *in vitro* fail to form an outgrowth of the inner cell mass, indicating that EPS is required for the survival and/or proliferation of embryonic stem cells. Thus, we have identified a novel transcription factor that is critical for survival/proliferation of embryonic stem cells and likely hematopoietic stem cells as well.

105. **Initial Localization of a Neurogenic Response Element in the Promoter of the Human Zfhep Transcription Factor Gene.** K. L. Hapney, R. P. Stearman, and D. S. Darling. University of Louisville, Louisville, Kentucky 40292.

Zfhep is a member of the Zfh family of transcription factors having a homeodomain-like sequence and multiple zinc fingers. We have used immunocytochemistry and *in situ* hybridization to demonstrate that Zfhep is expressed in the ventricular zone of the embryonic brain and spinal cord. However, Zfhep mRNA or protein is not expressed in cells that have undergone neurodifferentiation. The goal of this study was to localize promoter element(s) responsible for this pattern of expression of Zfhep during neurogenesis. The human Zfhep promoter was isolated by PCR amplification. Luciferase reporter clones were made in pGL3-basic using 1000, 400, or 196 bp of the promoter. Each clone was transfected using Fugene6 and CMV-β-galactosidase cDNA as a control for differences in transfection efficiency. We used a well-characterized method for inducing neurogenesis of P19 (embryonal carcinoma) cells. Pretreatment of P19 cells with retinoic acid (RA) allows subsequent neurodifferentiation. We have reported that, after RA pretreatment, Zfhep is highly expressed in P19 neuroblast cells, but expression is lost during *in vitro* neurogenesis. We compared transfections of untreated cells to differentiating P19 cells. Normalized expression of each clone was significantly reduced ($P < 0.001$) in RA-treated cultures showing neurite formation. In contrast, the mouse NeuroD2 promoter had similar activity in both control and differentiating cultures. Therefore, element(s) sufficient for normal regulation are localized within –196 bp of the Zfhep promoter. (Supported by NIH DE07171 and DE13614.)

106. **Unique Roles for E2F1 in the Mouse Ocular Lens.** R. K. Hyde and A. E. Griep. Department of Anatomy, University of Wisconsin, Madison, Wisconsin.

During lens development, cell cycle withdrawal is necessary for the transition from proliferating epithelial cells to terminally differentiated fiber cells. Previously, we determined that the pRB family is required for cell cycle withdrawal using transgenic mice with lens-specific expression of the viral oncoprotein HPV-16 E7,

which binds to and inactivates pRB and its family members. We also showed previously that *E2F1,* a member of the E2F transcription factor family whose activities are modulated by pRB proteins, is only partially responsible for E7-induced defects in the lens. This suggests that other E2Fs contribute to the *E7* phenotype, but that certain functions are unique to *E2F1.* To address this hypothesis, we determined expression levels of *E2F*s in lenses from mice of various genotypes using Northern and *in situ* analyses. We showed that expression levels of *E2F2* and *E2F3a* are increased in the *E7* lens. Increased expression of *E2F2* was *E2F1* independent. However, increased expression of *E2F3a* was *E2F1* dependent, indicating that *E2F3a* is a unique target of *E2F1* in the absence of functional pRB proteins. Expression levels of various direct and indirect E2F target genes showed increased expression in the *E7* lens. Expression of one of these targets, *p19ARF,* required *E2F1* indicating that it is also a unique *E2F1* target when pRB is inactivated. Expression levels of most of the target genes examined were only partially dependent on *E2F1.* Interestingly, two of the target genes examined, *b-myb* and *cdc2,* are predicted to be targets of *E2F4* and *5,* but not of *E2F1.* Presently, we are addressing the role of E2Fs in the regulation of *b-myb* and *cdc2* expression using chromatin immunoprecipitation.

107. **Mechanisms Underlying Region-Specific Expression of the δ-Crystallin Gene in Chick Lens Development.** Naoko Shimada, Tomoko Murata-Aya, and Kunio Yasuda. Nara Institute of Science and Technology, Ikoma, Japan.

L-Maf (Lens-Maf) binds to lens-specific enhancer element αCE2 of the chick *αA-crystallin* gene. Expression of L-Maf begins at the lens placode-forming stage and is maintained in lens cells. Misexpression of L-Maf induces the expression of lens-specific markers, such as *crystallin* genes and *fillensin* gene. Interestingly, L-Maf can convert chick retinal cells into lens-like structures. These properties of L-Maf raise the possibility that L-Maf plays an important role in lens induction and differentiation *in vivo.* To assess this possibility, we misexpressed L-Maf in various regions of the head ectoderm in the lens placode-forming stage. In the course of the study, we found that expression of δ-crystallin, an early molecular marker for lens development, was induced only in a region surrounding the lens, but not in other regions of the head ectoderm. To explain the mechanism of the restricted expression of δ-crystallin, we tried to find factors that alter the expression domain of δ-crystallin in cooperation with L-Maf. Among factors that show these expression in presumptive lens, a transcription factor Sox2 is thought to be a candidate key factor because of its transactivation of the *δ-crystallin* gene on a lens-specific enhancer, DC5. When L-Maf and Sox2 were comisexpressed, the δ-crystallin-inducible region was expanded. Next, we performed in ovo reporter assay using a *β-gal* reporter gene bearing DC5. Coelectroporation of L-Maf and Sox2 synergistically transactivated the reporter gene. These results led to the suggestion that L-Maf and Sox2 cooperatively regulate the lens development.

108. **Understanding the Regulation of Hedgehog Genes during Eye Development.** Andrea R. Morris and Kevin Moses. Department of Cell Biology, Emory University School of Medicine, Atlanta, Georgia.

In *Drosophila,* retinal pattern formation is progressive and associated with a moving indentation in the developing eye disk called the morphogenetic furrow. The furrow functions as a moving boundary that separates posteriorly localized differentiating photoreceptor neurons from the undifferentiated cells that lie anterior to it. The movement of the furrow thus, directs a "wave of neural differentiation" and a propagation of information that specifies photoreceptor cells. The segment polarity gene hedgehog (hh) encodes the primary signal for furrow movement and thus for photoreceptor cell determination. The mechanism for furrow progression, however, remains unknown. Here, we examine the eye-specific regulation of the hh gene and test whether hh is regulated at the transcriptional level by an eye-specific enhancer that allows hh to be genetically upstream of itself in the eye. To study this question, we have focused on mutations which affect only hh function in the eye—hh[bar3] (= hh[1]) and hh[fse]. The hh[bar3] mutation causes an arrest of the furrow in the developing eye while not affecting other aspects of *Drosophila* development. The hh[fse] mutation has similar effects. Both alleles were found to have deletions in the same region of intron I. We propose that these deletions may affect the hypothetical eye specific hh transcriptional enhancer. We are also investigating the conservation of hh enhancers in vertebrate eye development, as well.

109. **Identification and Characterization of Male-Specific Sexual Regulators and TRA-1 Target Gene.** Kara Thoemke,* Woelsung Yi,* Valerie Reinke,† Marc Sohrmann,‡ and David Zarkower.* *Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, Minnesota 55455; †Department of Genetics, Yale Medical School, New Haven, Connecticut 06520; and ‡The Sanger Center, Wellcome Trust Genome Campus, Hinxton, Cambs CB101, United Kingdom.

Caenorhabditis elegans is highly sexually dimorphic, with about 30% of the somatic cells in the adult hermaphrodite and 40% of the somatic cells in adult male being sexually specialized. All aspects of somatic sexual development are controlled by TRA-1, a zinc finger protein that is active in hermaphrodites and can repress transcription. TRA-1 probably directly regulates multiple genes involved in restricted aspects of sexual differentiation, and an important goal is to identify these genes. We have identified a large number of sex-enriched mRNAs, mostly male-enriched, using microarray analysis. We compared N2 XX hermaphrodites and *tra-2(ts)* XX pseudomales from the L2 through the L4 stages to generate a developmental profile of sex-specific gene expression. Many of these genes likely function as male-specific sexual regulators. We have also identified potential TRA-1 binding sites in the *Caenorhabditis elegans* genome using a hidden Markov model (HMM) computer algorithm and weight matrix searches. Combining these two approaches, we seek to identify and characterize male-specific TRA-1 target genes in an effort to understand the mechanisms by which *C. elegans* achieves sexual dimorphism. We are currently conducting functional analysis of these putative male sexual regulators using RNAi and GFP

110. **Molecular Control of Testis Development by Dmrt1.** Umut Fahrioglu,* Chris Raymond,† David Zarkower,‡ and Vivian Bardwell.‡ *Graduate Program in Molecular, Cellular, Developmental Biology and Genetics, †Graduate Program in Biochemistry, Molecular Biology and Biophysics, and ‡Department of Genetics, Cell Biology and Development University of Minnesota, Twin Cities, Minneapolis, Minnesota 55455.

Mammalian sexual development is a very complex process with many key regulators yet to be identified. DMRT1 encodes a protein related to the products of invertebrate sexual regulators *Dsx* and

mab-3 which share a DNA binding motif called the DM domain. In all vertebrates examined, Dmrt1 expression suggests a role in sexual development and/or sex determination. In humans, DMRT1 is located at 9p24.3, a region required for testis development. Dmrt1$^{-/-}$ XY mice display a testicular dysgenesis phenotype. The presence of a zinc-finger-containing DM domain suggests a transcriptional regulatory role for DMRT1/Dmrt1. Our aim is to identify genes that are regulated by Dmrt1. Testicular dysgenesis in Dmrt1$^{-/-}$ animals begins around postnatal day 7 (P7). Labeled cDNA is being made from P6 (and possibly earlier stages) wild-type and Dmrt1$^{-/-}$ testes and cDNA will be hybridized to gene arrays. Genes reproducibly enriched in wild-type or knockout testes will be examined further.

111. **Analysis of Muscle- and Tissue-Specific Expression of MRP in *Drosophila.*** John Daley, Mea Gentile, Weitao Sun, David M. Standiford, and Charles P. Emerson Jr. Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104.

Myosin rod protein (MRP) is a 155-kDa *Drosophila* muscle protein encoded by a gene internal to the myosin heavy chain (MHC) locus that consists of a novel amino terminus conjoined to the MHC rod domain. The amino terminus of MRP has a putative actin-binding domain and is hypothesized to tether MRP to the thin filament to modify the contractile properties of specialized muscles. The pattern of MRP expression is complex and previous studies have shown that a 1.4 kb promoter element is sufficient to direct the expression of MRP in specific somatic, visceral, and cardiac muscles as well as in the male germline. We have analyzed the regulatory sites in the MRP promoter in an effort to better understand the mechanisms that direct muscle and tissue-specific gene expression in *Drosophila.* Sequence analysis revealed two domains that are highly conserved in *D. hydei* (CDI and II) and a domain that contains a number of E-boxes. We have used site-directed mutagenesis and transgenic analysis of a LacZ-containing reporter gene to determine the function of these domains in directing the expression of MRP. These assays show that elements in CDI and CDII affect the expression of MRP in direct flight muscle 49, but are not required for expression in other muscles. This activity has been further localized to a 30-nt element within CDII that is sufficient to direct MRP expression in DFM49. This element will be a useful target for the identification of specific transacting factors that collaborate to direct the muscle-specific gene expression in *Drosophila.*

112. **Analysis of Myosin Heavy Chain Expression in Tadpole Hindlimb and Tail Muscle during Spontaneous Metamorphosis.** K. D. Martin, B. G. Atkinson, and P. A. Merrifield. Department of Zoology, University of Western Ontario, London, Ontario, Canada.

The North American bullfrog, *Rana catesbeiana,* has been the subject of many studies into the mechanical properties of vertebrate muscles. In other Ranid species, muscle fibers have previously been analyzed and classified into four distinct fiber types. The present study examines the muscle fiber types present in the hindlimb muscle of adult *Rana* and in the hindlimb and tail muscle of premetamorphic tadpoles of this species. Fiber types were first identified using fiber size, morphology, and myosin ATPase activity following acid or alkali treatment. This resulted in the determination of four distinct fiber types (1, 2, 3, and tonic). Monoclonal antibodies (Mabs) against myosin heavy chain (MHC) were then tested and several were found to display fiber-type specificity in this species. These antibodies were used to examine changes in MHC expression in developing hindlimb and tail muscle of stage XI–XX tadpoles. It was possible to visualize the formation of muscle groups using the 1G3 antibody and no embryonic- and/or larval-specific MHCs were detected. An increase in 1G3 and 212F antibody reactivity in the older tadpoles was apparent. Expression patterns of MHC in the tail revealed that slow, tonic fibers are isolated to a one-cell-thick layer around the outside of the tail muscle similar to the pattern seen in trout. One of the antibodies tested showed unique reactivity to tadpole tail, suggesting the possibility of a tail-specific isoform. These findings demonstrate the potential of MHC-specific Mabs to examine muscle fiber development during the thyroid hormone-dependent metamorphosis of this amphibian.

113. **Structure and Regulation of an Amphibian Muscle-Specific Creatine Kinase Gene.** L. F. Petersen and B. G. Atkinson. Department of Zoology, University of Western Ontario, London, Ontario, Canada.

Creatine kinase (CK) isozymes catalyze the reversible transfer of a phosphate ion from ATP to creatine. In mammals, four CK isoforms, encoded by separate genes and expressed in different temporal and spatial patterns, have been characterized. Two of these isoforms are cytosolic proteins that are restricted to the brain (CK-B) or muscle (CK-M) tissue. The fact that little is known about the CK genes or the regulatory mechanisms governing their tissue-specific expression in the amphibians prompted studies focused on (1) isolating and characterizing the gene encoding the CK-M isoform in an amphibian and (2) determining which elements in its promoter are required for its tissue-specific expression in this more primitive group of vertebrates. We isolated and characterized a full-length cDNA and the gene encoding it from an amphibian, *Rana catesbeiana,* and determined, by RT-PCR analyses and Northern blot hybridization, that it encoded the cytosolic, muscle-specific isoform of CK. The putative promoter region of this gene (RcCK-M) was analyzed for regulatory motifs, and a 915-bp fragment of it was inserted into the pEGFP-1 expression vector and assessed for its ability to drive GFP expression in a tissue-specific manner in transgenic *Xenopus* tadpoles. The transgenic tadpoles expressed GFP in somites at stage 33/34 and in skeletal and cardiac muscle by stage 43. Deletions of the 5′-end and mutagenesis of putative regulatory elements are being conducted. Whatever the case, these results demonstrate that all of the elements required for the muscle-specific expression of RcCK-M are present in this 915-bp fragment of the RcCK-M promoter.

114. **A Transcriptional Profile of Development in Wild-Type and in Mutant *Dictyostelium discoideum* Cells.** Nancy Van Driessche, Chad Shaw, Miroslava Ibarra, Adam Kuspa, and Gad Shaulsky. Baylor College of Medicine, Houston, Texas 77030.

A distinct feature of development in the simple eukaryote *Dictyostelium discoideum* is an aggregative transition from a unicellular to a multicellular phase. Using genomewide transcriptional analysis we show that this transition is accompanied by a dramatic change in the expression of more than 25% of the genes in the genome and that altogether, about 40% of the genes are regulated during development. Analysis of the two differentiated cell types, spores and stalk cells, and their precursors revealed a large number of differentially expressed genes as well as unex-

pected patterns of gene expression that shed new light on the timing and mechanism of cell-type divergence. One of the patterns was consistent the activity of the cAMP-dependent protein kinas, PKA, which participates in most stages of *Dictyostelium* development. We generated mutations in many of the genes that regulate PKA activity and found corresponding transcriptional patterns that distinguish the various activities of the PKA pathway during aggregation, cell-type divergence, and terminal differentiation. We also show that the transcription profile can be used as a developmental phenotype in epistasis analysis.

115. **A Search for Targets of the *Drosophila* Neuroblast Temporal Network.** Thomas Brody, Chad Stivers, and Ward F. Odenwald. Neurogenetics Unit, Laboratory of Neurochemistry, NINDS, NIH, Bethesda, Maryland.

During *Drosophila* neuroblast (NB) lineage development, temporally ordered transitions in gene expression have been shown to accompany the changing repertoire of functionally diverse cells generated by NBs. For example, we have described a transcription factor network, the Hb → Pdm → Cas → Gh cascade (1, 2) that regulates temporal transitions in gene expression during CNS lineage development. We have initiated a search for targets of the temporal network by taking a candidate gene approach based on the presence of presumed binding sites for temporal factors in the promoters of known CNS determinants and other uncharacterized genes. We will described use of multiple approaches for in silico promoter analysis, including the use of Cis-Analyst, a program for identification of *cis*-regulatory modules (CRMs) in the fly genome (3), and reiterative BLAST. Using Cis-Analyst, we have verified our ability to distinguish known targets of the temporal network, based on the arrangement of presumed binding sites of temporal factors. Reiterative BLAST also reveals the presence of conserved modules throughout the genome that may serve as CRMs targeted by temporal factors. We have identified over 100 potential targets of the temporal network and are currently screening those genes for their expression dynamics in wild-type embryos and temporal network mutants. 1. Kambadur *et al.*, 1998, *Genes Dev.* **12,** 246–260; 2. Brody and Odenwald, 2000, *Dev. Biol.* **226,** 34–44; 3. Berman *et al.*, 2002, *Proc. Natl. Acad. Sci. USA* **99,** 757–762.

116. **Insertional Mutagenesis in *Xenopus* using Retroviruses and Transposons.** Emin Kuliyev, Andrea M. Proctor, Joanne R. Doherty, Haiqing Zhu, Michelle J. Hamlet, and Paul E. Mead. Department of Pathology, St. Jude Children's Research Hospital, Memphis, Tennessee.

Genetic maps and genomic tools for *Xenopus* are still in their infancy and thus we have decided to investigate insertional mutagenesis strategies in the frog. The advantage of insertional mutagenesis is that a heritable tag is introduced into the genome at the site of DNA disruption and thus genetic maps are not required to clone the insertion site. We have focused our studies on two methods for introducing genetic alterations in the frog genome: retroviral and transposon insertions. *Retroviruses:* We have successfully used high-titer MSCV-GFP VSV-G pseudotyped virus to introduce GFP into the frog genome. Injecting virus at the four- to eight-cell stage resulted in the generation of tadpoles that expressed high levels of GFP. *Transposons:* We have successfully used Sleeping Beauty transposase to introduce GFP-expressing transposons into the frog genome. Coexpression of Sleeping Beauty with a transposon substrate gave efficient transgenesis with high-level expression of GFP in the resulting tadpoles and frogs. Integration site analysis by Southern blotting tadpole genomic DNA and hybridizing with transposon-specific probes indicated that we had achieved stable, very low copy number integration. We will show examples of transposon-mediated transgenics, enhancer traps (minimal promoter transposons), and gene traps (splice acceptor transposons). Chimeric mRNA from gene trap animals can be readily cloned and sequenced using RT-PCR strategies. We will discuss the potential use of retroviral and transposon vectors for genetic screens in *Xenopus* and will compare the two insertional strategies we have studied.

117. **An ENU-Induced Hypomorphic Allele of Smad2 Identifies Novel Functions in Murine Embryonic Development.** Jay L. Vivian, Yijing Chen, and Terry Magnuson. Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina.

An efficient means of creating an allelic series of subtle mutations in the mouse, nonnull mutations in particular, would greatly aid the analysis of complex gene function. Our laboratory has recently described a technique to mutagenize mouse ES cells with the chemical mutagen ENU. We have extended this mutagenesis strategy to identify mutations in nonselectible genes. A cryopreserved bank of mutagenized ES cell clones has been produced and used in a genotype-based screen to identify mutations in genes of interest. Using this methodology we have identified several mutations in the mouse Smad2 locus. The early lethality of targeted Smad2 mutations, along with the various protein interactions and signaling pathways mediated by this factor, suggested that Smad2 would be an excellent candidate for the development of an allelic series of subtle mutations. *In vivo* analysis of one of these mutations identifies the change to be hypomorphic in character, allowing for functional analysis of Smad2 in later embryonic development. A variety of defects are observed in these Smad2 mutants by E8.5, including defects in the formation of anterior neurectoderm, dorsal aorta, foregut, heart, and anterior notochord. Our work demonstrates that a large number of mutations in nonselectible genes can be readily identified by combining ENU mutagenesis and high-throughput mutation detection. Further analysis of this as well as other subtle Smad2 mutations will allow us to dissect the roles of this factor in various biological processes.

118. **Identifying Novel Relationships among RNA Expression Patterns in Microarray Data.** Fredrick D. Oakley. Department of Zoology and Genetics, Iowa State University, Ames, Iowa 50011.

In a developing organism, gene expression is regulated by many factors, including receptor–ligand interactions, extracellular matrix contacts, developmental determinants, or environmental stresses. Cells integrate these signals and adjust gene expression in a coordinated manner. Microarray experiments provide vast amounts of information about mRNA expression in differing developmental or environmental contexts. A challenge for researchers is to develop statistical methods to identify patterns of gene expression that are informative to gene function and regulation. One method used to identify relationships between mRNA expression data sets is to calculate their linear correlation coefficient over a large number of experiments. While linear regression has proven informative, this method potentially overlooks expression patterns that are not obviously linear or patterns that are not linear in nature. A developmental or environmental stimulus may initiate a cascade of positive and negative regulatory interactions,

each of which may influence mRNA levels. I have devised an algorithm that systematically looks at pairs of genes to identify patterns indicative of certain linear and nonlinear relationships. To test my algorithm I have been analyzing recently published data derived from hundreds of experiments using *Caenorhabditis elegans* mRNAs (Kim *et al.*, 2001, *Science* **293,** 2087–2092). My analyses identify many previously reported sets of coregulated genes, and they reveal some possible new relationships.

119. **DNA Microarray Optimizations: Increasing Spot Accuracy and Automated Identification of True Microarray Signals Using *Xenopus laevis* as a Model System.** Daniel Peiffer,* Yongchol Shin,* Andreas von Bubnoff,* Peter Tran,* Makoto Mochii,† Atushi Kitayama,† Naoto Ueno,† and Ken W. Y. Cho.* *Department of Developmental and Cell Biology, University of California, Irvine, California; and †National Institute for Basic Biology, Aichi, Japan.

DNA microarray technology has opened the door for large-scale gene expression screening, functional analysis, and genomic profiling. The flood of biological information produced by these experiments is anticipated to revolutionize genetic analysis. Microarray hybridization technology has been extensively tested and the measurement between two samples using fluorescence intensity ratios is particularly robust; however, more investigation is still needed to fully mine these data. Here, we describe improved techniques to analyze fluorescent microarray images, which will improve the microarray data acquisition process. These techniques allow for the automatic quantitative determination of accurate and reliable microarray signals. We are also currently using microarray technology to identify novel players involved in TGF-β superfamily (e.g., BMP and nodal) signaling pathways active in the early embryonic development of the amphibian *Xenopus laevis.* We successfully spotted over 42,000 *Xenopus* cDNAs representing 15,000 unigenes expressed in early embryos onto glass slides using high-throughput robotic techniques. Our preliminary results suggest that microarray analysis can provide useful information about novel candidate factors functioning in signal transduction and gene regulation in early *Xenopus* development.

120. **Microarray Gene Expression Profiling Reveals Novel Tissue Relationships and Coordinately Regulated Genes in Mouse Development.** M. D. Bates, L. C. Schatzman, M. A. Betzel, and B. J. Aronow. Children's Hospital Medical Center, Cincinnati, Ohio.

Large-scale comparisons of gene expression can provide insights into relationships between tissues and genes. We used microarray technology to test the hypothesis that the greatest similarities in gene expression patterns among developing mouse tissues would be between those of similar developmental origin. As part of an institutional consortium, cDNA was prepared from embryonic day (E) 16.5 intestine, E14.5 liver, E13.5 and E16.5 lung, E18.5 kidney, E14.5 heart, E16.5 skin, E18.5 brain, and more than 70 postnatal and adult tissues. Gene expression profiles were obtained by competitive hybridization of Cy5-labeled cDNA from the various tissues versus Cy3-labeled cDNA from whole postnatal day 1 mouse with Incyte GEM1 cDNA microarrays (containing 8638 cDNAs) and analyzed using GeneSpring (Silicon Genetics). Hierarchical clustering of the profiles showed that prenatal kidney and lung were closely related to each other and distinct from their corresponding adult tissues, perhaps reflecting similar processes of branching morphogenesis. In contrast, the other prenatal tissues clustered separately from each other and most closely to their corresponding adult tissues. We found a group of 14 genes highly expressed in embryonic tissues derived from endoderm or mesoderm that were coordinately expressed across the entire database, including developing and adult tissues. These genes encode cell cycle regulators, nuclear proteins, and unknown proteins. Gene expression profiling thus helps define functional relationships between tissues and provides opportunities for gene discovery relevant to growth and development.

121. **Centrosome Rotation and Tubulin in the Early *Caenorhabditis elegans* Embryo.** Amanda J. Wright and Craig P. Hunter. Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts 02138.

We isolated a *Caenorhabditis elegans* mutant, called *qt1,* which fails to undergo centrosome rotation events in the early embryo. Centrosome rotations are required to orient mitotic spindles with respect to the developmental axis. We determined that *qt1* is an allele of *tbb-2,* a β-tubulin gene. Interestingly, *qt1* complements *t1623,* another allele of *tbb-2.* Since a deletion allele of *tbb-2* has no obvious phenotype and RNAi of *tbb-2* in the mutants restores normal centrosome rotation, *qt1* and *t1623* are gain of function mutations. Microarray analysis indicates that two β-tubulin and two α-tubulin genes are expressed in the early embryo. Gene specific RNAi demonstrates that the α- and β-tubulins are functionally redundant and that the four possible kinds of tubulin heterodimers are functionally equivalent. Despite this functional equivalence, RNAi of one α-tubulin suppresses the *qt1* mutant phenotype. Staining *qt1* and *t1623* embryos with an anti-tubulin antibody reveals subtle differences in their phenotypes. In both, spindle orientation is disrupted due to a lack of centrosome rotation, but *t1623* embryos have stunted astral microtubules and spindle microtubules often fail to make kinetochore attachments. Thus these two β-tubulin mutations appear to be affecting microtubule structure in different ways, perhaps by altering the dynamic instability of the microtubules or interfering with microtubule-binding partners.

122. **A Microtubule Array Precedes the Formation of the Cleavage Furrow during the First Two-Cell Division Cycles in Zebrafish Embryos.** Karen W. Lee, Sarah E. Webb, Steven M. Ho, Carey H. Wong, and Andrew L. Miller. Calcium-Aequorin Imaging Laboratory, Department of Biology, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, People's Republic of China.

It has been clearly established that microtubules play a crucial role during cell division. Until recently, their functional role was restricted to the formation of the mitotic spindle and the astral array (1). However, recent evidence suggests that they might have additional roles in furrow formation following karyokinesis. It was recently reported that in zebrafish an array of parallel microtubules is present on either side of the cleavage furrow during furrow deepening (b). It was suggested that this array might function as a transportation mechanism to bring new membrane components to the expanding daughter cell membranes. Here we present new data showing the presence of a cytokinetic microtubule array that *precedes* the formation of the cleavage furrow and thus suggest that it plays a role in furrow positioning. This prefurrowing microtubule array was seen during the first two-cell division cycles. Following its positioning role we suggest that this array develops into furrow deepening array described previously. 1. R. Rappaport, 1996, Cyto-

kinesis in Animal Cells. Chapter 7. The Stimulus–Response System, pp. 186–229, Cambridge Univ. Press; 2. S. Jesuthasan, 1998, *J. Cell Sci.* **111,** 3695-3703.

123. Abstract #123 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

124. **Wnt3a Is Required for Establishment of L-R Asymmetry.** Terry P. Yamaguchi, Kristin Biris, and Jaime Greear. NCI-Frederick, NIH, Frederick, Maryland.

The alignment of the left–right (L-R) body axis relative to the anteroposterior (A-P) and dorsoventral body axes is central to the organization of the vertebrate body plan. The mouse node/organizer plays a key role in the formation of the L-R axis. The node first becomes morphologically evident at the anterior end of the primitive streak 1 day into gastrulation and gives rise to the axial mesendoderm. Several members of the Wnt family of secreted signaling molecules are expressed during gastrulation; however, Wnt3a is not expressed until just prior to node and L-R axis formation. The Wnt3a expression domain in the midline primitive streak is symmetrical and the anterior limit lies in the posterior node suggesting that Wnt3a may have a function in the node. To assess the role of Wnt3a in L-R asymmetry, we have examined embryos carrying null alleles of Wnt3a for laterality defects. Forty-eight percent of the E9.5 homozygous Wnt3a mutants examined displayed heart looping defects consistent with situs inversus or situs ambiguus. Asymmetric expression of left-determining genes in the node and/or LPM is either not detected or significantly delayed and then bilaterally expressed. SEM analysis of mutant nodes indicates that node epithelial cells are monociliated. Our results place Wnt3a upstream of asymmetrically expressed left-determining genes and downstream, or in parallel with, genes essential for node cilia structure. Taken together with a previously established role in A-P axis extension, Wnt3a appears to function as a molecular link between the A-P and L-R axes. Furthermore, we suggest that Wnt3a may play a critical role in breaking bilateral symmetry during the development of the L-R axis.

125. **BMP Signaling through ALK2 Plays a Role as Tight Determinant in the Establishment of Left–Right Asymmetry in Mouse Embryos.** Satoshi Kishigami, Castranio Trisha, and Yuji Mishina. Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina 27709

Bone morphogenetic proteins (BMPs) were originally found for their bone-inducing activities, but recent findings suggest that they also play critical roles during mammalian development. A mutant mouse line deficient for a BMP type I receptor known as Alk2 showed embryonic lethality around the gastrulation stage. To rescue these defects, we generated Alk2 homozygous mutant embryonic stem cells (Alk2 −/− ES cells) to inject into wild-type blastocysts. In the resulting chimeric embryos, the gastrulation defects were rescued suggesting that signaling through ALK2 is essential in the extraembryonic tissues during gastrulation. The chimeric embryos with a low contribution of Alk2 (−/−) cells developed normally, although few Alk2 (−/−) cells populated the eyes and the heart, particularly the myocardium. On the other hand, high contribution chimeras which had Alk2 (−/−) cells that evenly populated the heart showed abnormal heart looping, a poorly developed myocardium, and disorganized heart tissues. The marker genes that are expressed specifically in the left lateral plate mesoderm were abnormally expressed bilaterally in the chimeras in high contribution chimeras. Asymmetric expression of nodal in perinodal region was randomized. A current model determining left–right body asymmetry taken together with these data allows us to propose that Alk2 is the BMP receptor necessary for establishing right identity as an upstream factor of nodal.

126. **Morphological Landmarks of Anteroposterior Development in Prestreak Mouse Embryos.** Jaime A. Rivera-Perez, Jesse Mager, and Terry Magnuson. University of North Carolina–Chapel Hill, Chapel Hill, North Carolina.

The discovery of molecular asymmetry in the anterior visceral endoderm has produced a renewed interest in this layer of the embryo because of its potential role in rostral neural development. The anterior visceral endoderm can be distinguished at prestreak stages using a variety of molecular markers additionally, a morphological distinction of particular regions of the visceral endoderm has been suggested based on analysis of fluorescent transgenic lines (Kimura *et al., Dev. Biol.* **225,** 304). Furthermore, lineage analysis using DiI has suggested a transformation of the proximodistal axis into the anteroposterior axis of the embryo by virtue of cell movements (Beddington and Robertson, *Trends Genet.* **14,** 277–284). To investigate the role of the visceral endoderm in anterior patterning, we conducted a comprehensive morphological analysis of wild-type embryos as well as a lineage analysis using iontophoresis at pregastrula stages between 5.5 and 5.75 days postcoitum. We found that the visceral endoderm as well as the epiblast provide clues to distinguish the anteroposterior axis of the embryo before there are any signs of primitive streak formation. Lineage analysis of individual cells suggests that the distal to proximal rotation of visceral endoderm is completed by 5.75 days postcoitum. Other amniotes show similar molecular, morphological, and cell movements in the extraembryonic endodermal components at pregastrulation stages suggesting that common mechanisms pattern the anterior portion of the embryo (Foley *et al., Development* **127,** 3839). Our results provide additional tools to allow further investigation of the mechanisms responsible for rostral development in mammals.

127. **The Role of *bruno-like* in Early Zebrafish Development.** Shannon M. Byrd and Robert K. Ho. The University of Chicago, Chicago, Illinois.

Posttranscriptional gene regulation is important during oocyte maturation and early embryonic development for many species. During these early stages of development, the genome is transcriptionally repressed and changes in gene expression are dependent on differences in the stabilization, localization, and translational regulation of maternal stores of mRNA. One conserved function of the posttranscriptional regulation of maternal transcripts is the establishment of the embryonic axes. In *Drosophila*, the translational repressor Bruno is required for anterior–posterior, and most likely dorsal–ventral, polarity in the oocyte and embryo through regulation of the maternal transcripts *oskar* and *gurken*. In zebrafish there is evidence that supports the existence of a vegetally localized maternal determinant that, shortly after fertilization, is transported to the marginal blastomeres via microtubules and is responsible for the positioning of the organizer and hence the basic body plan. The zebrafish ortholog of *Drosophila* Bruno is maternally expressed at the vegetal cortex in the early embryo. This

localization raises the possibility that *bruno-like* genes may have a conserved function in axis specification. We have cloned three maternally expressed splice variants of the *brul* gene and are currently investigating the role of these isoforms in early zebrafish development.

128. **Axis Duplication and Neural Specification in *Xenopus* Embryos Overexpressing the Novel Gene *Ashwin*.** T. B. Alexander,* S. S. Patil,* J. A. Uzman,† and A. K. Sater.* *Department of Biology and Biochemistry, University of Houston, Houston, Texas; and †Department of Natural Science, University of Houston Downtown, Houston, Texas.

The novel gene *Ashwin* was identified in a differential display RT-PCR screen for genes upregulated in response to neural induction. Overexpression of *Ashwin* in whole embryos leads to the formation of a secondary axis as well as a reduction in head structures and ventral tissues. These phenotypes are seen in embryos where *Ashwin* expression is driven by a promoter that is activated at the onset of zygotic transcription indicating that Ashwin can modulate patterning of the ectoderm and mesoderm downstream of early signaling pathways. Expression of *Ashwin* in isolated ectoderm results in expression of both anterior and posterior neural genes. *Ashwin* is maternally expressed throughout the embryo and is also observed in both anterior and posterior neural ectoderm in tailbud stage embryos. Myc-tagged Ashwin localizes to the nucleus in all tissues and stages examined. Overexpression of an N-terminal deletion construct does not produce significant defects in whole embryos, although coexpression with *Noggin* leads to the formation of a secondary axis. Ashwin shares significant sequence similarity to putative proteins identified in multiple vertebrate species. Overexpression of the human clone in *Xenopus* embryos mimics many effects of *Ashwin* overexpression, suggesting that these are functional homologs. These findings implicate Ashwin in neural specification and axis determination in normal *Xenopus* development.

129. **Chordin Mediates Pronephros Induction by the Trunk Organizer.** Tracy S. Mitchell and Michael D. Sheets. University of Wisconsin–Madison, Madison, Wisconsin.

Spemann's organizer is an essential inducing center that regulates cell fate decisions during vertebrate embryogenesis. The organizer consists of two distinct domains: a trunk organizer and a head organizer. Trunk-organizer signals pattern the mesoderm into specific cell types such as somitic muscle, pronephros, and heart. We recently demonstrated that FGFR1 is an important regulator of the *Xenopus laevis* trunk organizer. Disruption of FGFR1 eliminates trunk-organizer signaling and the tissues dependent upon this signaling. FGFR1 regulates the trunk organizer in part by controlling the expression of the trunk-inducing protein chordin. We hypothesize that the defects in the pronephros-inducing functions of FGFR1-inhibited trunk organizers are due to defects in chordin expression. To test this hypothesis, we analyzed ectopic trunks generated by chordin mRNA injection into the presumptive posterior cells of *Xenopus* embryos. These ectopic trunks contained fully differentiated pronephric ducts, tubules, and somitic muscle. Pronephros differentiation appeared dependent on somitic muscle since pronephros only formed in the presence of somitic muscle but somitic muscle developed in the absence of pronephros. These observations oppose the simple BMP gradient model for mesodermal patterning and support a more complex model in which chordin induces anterior somitic muscle, which induces pronephros. We obtained similar results with noggin and a DN-BMPR, suggesting that the ability to induce pronephros differentiation and anterior somites is a general feature of BMP inhibitors. Our results further suggest that chordin and other BMP inhibitors function as inducers of anterior dorsal cell fates.

130. **Withdrawn.**

131. Abstract #131 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

132. **LvTbx2/3, a T-box Family Transcription Factor That Patterns the Dorsal/Ventral Axis of the Sea Urchin Embryo.** Jeffrey M. Gross, Robert E. Peterson, and David R. McClay. DCMB Group, Duke University, Box 91000 LSRC, Durham, North Carolina 27708.

We report here the identification and characterization of a member of the Tbx2/3 subfamily of T-box genes, LvTbx2/3, during development of the sea urchin embryo. LvTbx2/3 is expressed solely in the dorsal territories of all three embryonic germ layers. The linkage of the dorsal-specific expression of LvTbx2/3 within all three germ layers is examined through perturbing the events thought to be involved in the patterning of the D/V axis of the sea urchin embryo and examining the effects on LvTbx2/3 expression. LvTbx2/3 expression is dependent on β-catenin or β-catenin downstream genes as shown through expression of the cytoplasmic tail of cadherin and thereby preventing β-catenin nuclear localization. Dorsal LvTbx2/3 expression is also prevented by ventralization with $NiCl_2$, with overexpression of LvBMP2/4, through the disruption of the extracellular matrix, and through abrogating vegetal–animal cell–cell interactions indicating that D/V axis specification is indeed molecularly linked in all three embryonic germ layers. Furthermore, we then demonstrate that LvTbx2/3 functions in D/V patterning of the sea urchin embryo through a variety of molecular perturbations.

133. **Spatial Regulation of Proteolysis in Patterning the *Drosophila* Embryonic D/V Axis.** Ellen K. LeMosy. Department of Cellular Biology and Anatomy, Medical College of Georgia, Augusta, Georgia.

The D/V axis of the *Drosophila* embryo is established by ventral activation of the Toll signaling pathway. The Toll ligand is generated by proteolysis, an event requiring four serine proteases—Nudel, GD, Snake, and Easter—acting in a cascade of zymogen activation. A spatial cue is provided indirectly by the ventral activity of an apparent heparan sulfate 2-*O*-sulfotransferase, Pipe, on an unknown substrate. Based on published data, this spatial cue might function to promote proteolytic activation or might stabilize the activated Toll ligand. I previously showed that early events in the protease cascade, activation of Nudel and cleavage of GD, are not regulated by *pipe* and appear to occur uniformly. I hypothesized that the protease cascade provides a means of integrating multiple inputs, e.g., temporal and spatial, at distinct steps, and thus that at least one of the downstream proteolytic activation events would be ventrally restricted. To test this hypothesis, I have begun studies of Snake and Easter. I will present evidence, involving a rapid assay of protease activation, that Easter activation depends on *pipe* and thus is presumptively ventrally restricted. Studies of Snake activation are in progress. These results demonstrate that spatial regulation

occurs within the protease cascade, as predicted. Future experiments will center on using active-form-specific reagents to visualize protease activation and to isolate complexes of active proteases with cofactors that might include the spatial cue. The ultimate goal of this work is to understand the molecular mechanism for spatial regulation of D/V axis establishment.

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137. ***eomesodermin* Antagonizes Nodals to Pattern the Organizer.** Ashley E. E. Bruce, Cristin Howley, and Robert K. Ho. University of Chicago, Chicago, Illinois.

The Nodal-like molecules *squint* (*sqt*) and *cyclops* pattern the shield in the zebrafish embryo. In the shield, *floating head* (*flh*)-expressing cells give rise to the notochord while cells expressing *goosecoid* (*gsc*) give rise to the prechordal plate. Injection of *sqt* into the animal pole demonstrates that *sqt* acts as a morphogen, inducing *gsc* expression at high concentrations close to the source and *flh* expression at lower concentrations at a distance. We show that the T-box gene *eomesodermin* (*eom*) appears to play a complementary role to the Nodals in patterning the shield. Overexpression of *eom* results in ectopic expression of *gsc* and *flh* near the margin. This suggests that *eom*, like *sqt*, plays a role in specifying the shield; indeed *eom* requires Nodals to effect these inductions. However, *eom* induces *flh* expression cell autonomously whereas it induces *gsc* expression at a distance, the reciprocal of *sqt* overexpression. The interactions between *eom* and *sqt* were further investigated by animal pole injections. Injection of *sqt* induces a patch of *gsc* while *flh* is induced in a ring several cell diameters away from the *sqt* source. Coinjection of *eom* and *sqt* results in expression of *flh* in a patch closer to the source, while *gsc* expression is inhibited centrally. These data suggest that the presence of *eom* modulates the effects of *sqt* induction.

138. **Analysis of Wnt Pathway Function in *Tribolium* Segmentation.** Laila Farzana, Leslie Peterson, and Susan Brown. Division of Biology, Kansas State University, Manhattan, Kansas.

Cell-to-cell communication is an important aspect of many developmental processes. The Wnt signal transduction pathway is required for normal segmentation in *Drosophila.* The long germ mode of embryonic development (all segments formed simultaneously) displayed by *Drosophila* is highly derived. Most insects, including *Tribolium*, display some form of short germ development (some or all segments form sequentially). Comparative expression analyses suggest that the Wnt pathway plays a similar role in segment boundary formation in both types of segmentation. However, there are no functional studies to confirm these observations. Thus, we are using RNAi to examine the function of the Wnt pathway components *wingless* (*wg*) and *pangolin* (*pan*) in *Tribolium*. *wg* and *pan* are positive regulators of the Wnt pathway in *Drosophila,* and loss of gene function results in similar global effects on segmentation. The most strongly affected *Tcwg* RNAi embryos also display severe global effects on segmentation, suggesting a conserved role for *wg* in insect segmentation. However, the most severely affected *Tcpan* RNAi embryo lack abdominal segments, but display clearly recognizable thoracic segments. In addition, Engrailed stripes, which fade in *Tcwg* RNAi embryos (and in *Drosophila wg* and *pan* mutants), are not only stable in *Tcpan* RNAi embryos, they are actually wider than in wild type. These results suggest that *Tcpan* may have a novel function during segmentation.

139. **Regulation of Wg Signaling by *Drosophila* Sulfated.** May Lai, Xinbin Ai, Weitao Sun, Charles Emerson, Jr., and David M. Standiford. Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104.

An extracellular sulfatase has been recently shown to be required for normal development in vertebrate embryos and to promote Wnt signaling in culture (Dhoot *et al., Science* **293,** 1663). These properties are believed to be mediated by the ability of such proteins to remove specific sulfate groups from cell-surface heparan sulfate proteoglycans (HSPG) to influence the transmission, presentation, and activity of secreted signaling molecules during development. We have examined this hypothesis through the analysis of Sulfated, which encodes an extracellular sulfatase in *Drosophila.* Molecular and genetic assays show that Sulfated expression is associated with secreted signals in the embryo and both the RNAi-mediated repression and the overexpression of Sulfated induce a loss of naked cuticle in the ectoderm. These results indicate that Sulfated is required for embryonic patterning and suggest a role in regulating Wingless signaling during development. This possibility was tested using a heparin-dependent Wnt-signaling assay in cultured C2C12 cells. The expression of wild-type Sulfated in this assay increased the activity of a TCF-Luciferase reporter gene 2.5-fold over the basal response, while a catalytically inert form had no affect. Further, the Sulfated-mediated enhancement of Wnt signaling was found to be dose dependent and competed by catalytically inert protein. These findings indicate that Sulfated regulates Wg signaling and supports a model where extracellular sulfatases affect signal transduction during development by modifying the sulfation state of extracellular HSPGs.

140. **Basal Repression of Wnt Target Genes by Hdl and Tcf3b Helps Define the Low End of a Wnt Activity Gradient in the Neurectoderm.** Motoyuki Itoh,* Richard I. Dorsky,† Randall T. Moon,‡ and Ajay Chitnis.* *LMG, NICHD NIH, Bethesda, Maryland 20892; †Department of Neurobiology and Anatomy, University of Utah, Salt Lake City, Utah 84132; and ‡Howard Hughes Medical Institute/Department of Pharmacology and Center for Developmental Biology, University of Washington, Seattle, Washington 98195.

Analysis of *headless* and *tcf3b* function in zebrafish has revealed the essential role of these *tcf3* homologs in providing basal repression of Wnt target genes during early neural development. Exaggerated Wnt signaling in maternal zygotic (MZ) *hdl* mutants leads to posteriorization of the neural tube, which is characterized by loss of the forebrain and expansion of the midbrain–hindbrain boundary (MHB) domain. We report here the cloning of a second zebrafish *tcf3* homolog, *tcf3b*. This gene is expressed during early

development like *hdl* but has clear differences and is notably absent during early gastrulation when patterning is most affected in *hdl* mutants. Functionally, *tcf3b* can substitute for *hdl* function in neural patterning and reduction of *tcf3b* function in a MZ *hdl* background produces a more severe posteriorized phenotype where expansion of the hindbrain can be accompanied by a reduction in the size of the MHB domain. The range of phenotypes that accompany progressive loss of tcf3 function suggests that additive repression of Wnt target genes provided by Hdl and Tcf3b helps define the low end of a Wnt activity gradient in the neurectoderm. Computer simulations help visualize how the gradient of Wnt activity may be interpreted to define distinct domains of gene expression during normal development and when tcf3 function is reduced. Furthermore, specific changes in the shape of "caudal" expression domains in posteriorized embryos, that can be predicted by simulations, suggest that the shape of the Wnt activity gradient is likely to be influenced by Wnt antagonists in the prechordal plate.

141. **The RNA-Binding Protein Hermes Is Essential for Embryonic Development.** Miranda E. George, Li-Juan Duan,* and Thomas A. Drysdale. Lawson Health Research Institute, Departments of Paediatrics and Physiology, University of Western Ontario, London, Ontario, Canada N6A 4V2; and *Department of Physiology, Center for Vascular Biology, University of Connecticut Health Center, 23 Farmington Avenue, Farmington, Connecticut 06030.

Hermes (Heart, RRM Expressed Sequence) is a member of the RRM (RNA recognition motif) family of RNA-binding proteins. It is predominantly expressed in the myocardium of the developing heart in mouse, chick, and *Xenopus*. Later in development, hermes is expressed in the ganglion layer of the retina and pronephros. Transfection of mammalian cells with a GFP-tagged hermes indicates that it is primarily found in the cytoplasm suggesting a role in translation or RNA transport. To understand a specific role for hermes in embryogenesis, homologous recombination was used to generate mice that lack hermes by removing a 360-bp region spanning most of exon 1, including the translational start site. Mice that are heterozygous for the hermes mutation are viable and fertile. No hermes −/− were born from matings of heterozygotes, indicating that hermes is essential for life. Preliminary analysis of embryos from heterozygote matings indicates that the hermes −/− embryos are dying prior to e9.5. Hermes −/− embryonic stem (ES) cells were generated by exposure of +/− ES cells to high G418 and these were viable suggesting that the problem with the hermes −/− embryos is one of embryonic development rather than cell viability. Experiments are ongoing to precisely determine the cause of embryonic lethality. These results underscore the importance of RNA processing in early embryogenesis.

142. **Identification of an Organizer-Specific Lim1 Regulatory Element in the Mouse.** William Shawlot and Irina Leaf. University of Minnesota, Twin Cities, Minneapolis, Minnesota 55455.

Lim1 is a homeobox gene required in the visceral endoderm and the organizer region for the development of anterior neural structures in the mouse. The factors that regulate *Lim1* transcription in these regions are unknown. To begin to identify upstream regulators we sought to define *Lim1 cis*-regulatory sequences using transgenic mice. To locate potential regulatory sequences we compared mouse and human *Lim1* locus DNA sequences. We identified 14 conserved modules of 200 bp or greater that were over 75% conserved between mouse and human. We focused on a module in intron 3 because we were unable to detect *lacZ* expression in E6.5 embryos that contain a knockin allele that removes intron sequences. We found that a DNA fragment containing the intron module linked to hsp68*lacZ* could direct *lacZ* expression to the anterior primitive streak (early organizer) in early and mid-streak stage embryos. We also observed ectopic expression in the parietal endoderm. Sequence analysis of this module identified a 10-bp motif CACAATC(A/T) (G/C)C that is repeated three times. This sequence is a potential binding site for GATA transcription factors. *GATA4* and *GATA6* are expressed in the visceral and parietal endoderm and the primitive streak. These results suggest that GATA factors may play a role in regulating early *Lim1* transcription. Further characterization of this regulatory element may facilitate the definition of the molecular pathways that establish the anterior visceral endoderm and the organizer in the mouse.

143. **Defective Forebrain Patterning in Embryos of Diabetic Mice.** D. M. Liao, Y. K. Ng, S. S. W. Tay, E. A. Ling, and S. T. Dheen. Molecular Neurobiology Laboratory, Department of Anatomy, Faculty of Medicine, National University of Singapore, Singapore 117597.

Several clinical studies have demonstrated that maternal diabetes produce various congenital malformations including neural tube defects in human embryos. To study the maternal diabetes-induced changes in the developing neural tube, embryos were obtained from mice made diabetic by injection with streptozotocin before pregnancy. In some of these embryos, severe malformation in the presumptive forebrain was observed at 11.5 dpc. These embryos were examined for the molecular basis of forebrain malformations using various markers by *in situ* hybridization and RT-PCR. Our findings indicate that the expression of Shh, which is known to establish ventral midline of the brain, is upregulated and extends dorsally in the forebrain of embryos from diabetic mothers. Similarly, expression of Nkx2.1, which encodes a homeodomain transcription factor expressed in the ventral forebrain region, also extends dorsally in the forebrain. Moreover, the expression of Bmp4, a secreted signaling molecule of the BMP subfamily of the TGFβ superfamily required for early patterning of the dorsal forebrain, is reduced in the dorsal forebrain of embryos from diabetic mothers. Cell proliferation analysis by BrdU labeling indicates that the cell proliferation index is significantly higher in the ventral forebrain in the embryos of diabetic mothers compared to that of normal embryos. These results suggest that specific genes, which are involved in dorsoventral patterning of the forebrain, are altered in the embryos of diabetic mothers.

144. **Analysis of Postaxial Limb Malformations in Mice Induced by Gestational Ethanol Exposure.** David P. Gardner, Lawrence Suchocki, Tanya Thal, and Y. Gloria Yueh. Basic Science Department, Midwestern University, Glendale, Arizona.

Administration of ethanol to mice during gestation has been shown to induce birth defects and behavioral symptoms in mice that are similar to those seen in humans. One aspect of gestational ethanol exposure is the presentation of skeletal malformations, in particular, limb defects in the offspring. The most common limb defect is a postaxial malformation involving the loss of digits 3–5. Our working hypothesis is that this malformation is the result of alteration in the signal transduction feedback loop that occurs in

the limb between the apical ectodermal ridge (AER) and the zone of polarizing activity (ZPA). We have found that ethanol exposure of pregnant mice at 9 days and 8 h of gestation induces postaxial digit loss in a high percentage of embryos analyzed at 15–18 days of gestation. This same time point of ethanol exposure also induces a loss of the postaxial AER when embryos are analyzed at 11.25 days of gestation for expression of a marker β-gal transgene expressed in the AER as well as expression of FGF8. We are currently analyzing ethanol-exposed embryos for expression of both Sonic hedgehog (Shh) and FGF8 by *in situ* hybridization with the hypothesis that an ethanol-induced downregulation of either gene could produce the malformations seen. Further evidence for an involvement of Shh is the observation that exposure of pregnant females to higher concentrations of ethanol induces a more extreme phenotype in embryos that closely resembles the limb phenotype seen in embryos with a targeted deletion of the Shh gene (C. Chiang *et al.*, 2001, *Dev. Biol.* **236,** 421–435).

145. **The Differentiation of Dopaminergic Neurons Is Inhibited by Estrogen.** J. Roffers and R. D. Heathcote. Department of Biological Sciences, University of Wisconsin–Milwaukee, Milwaukee, Wisconsin 53201.

During development of the frog *Xenopus laevis,* a population of dopaminergic (DA) neurons differentiates in the ventral spinal cord. These neurons form a nonrandom dispersed pattern in two columns on either side of the midline. The determination of spinal cord cell fates involves a cascade of inductive mechanisms mediated by diffusible factors. Retinoic acid (RA) can influence this process by altering differentiation and patterning along the anterior–posterior axis. Both RA and estrogen are lipophilic molecules that signal using nuclear hormone receptors. Several pieces of evidence support the idea that estrogen might regulate DA neuron patterning and differentiation. Estrogen can reduce the numbers of DA cells in adults. It can also inhibit the activity of tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, in the test tube and in cultured cells. We treated embryos with estrogen and found that it dramatically decreased the numbers of DA spinal cord neurons. The greatest decrease occurred when the embryos were treated around the time of gastrulation. This is when many neurons become postmitotic, but well before the start of axon outgrowth and the appearance of other characteristics of neuronal differentiation. The maximum effect was elicited with an extremely short period of treatment. The effect of estrogen during this critical period of development suggests that signaling through the estrogen receptor may play a role in the embryonic development of dopaminergic neurons and perhaps other populations of CNS neurons.

146. **Central and Peripheral Pattern Formation of Primary Sensory Neurons.** S. Wieczorek and R. D. Heathcote. Department of Biological Sciences, University of Wisconsin–Milwaukee, Milwaukee, Wisconsin 53201.

In *Xenopus laevis,* the primary sensory Rohon–Beard (RB) neurons differentiate rapidly to provide sensory input to the embryonic and larval nervous system. RB neurons originate during gastrulation and form two longitudinal columns after the completion of neurulation. We have examined the patterning of these neurons from the emergence of a transcription factor that marks presumptive RB neurons during neurulation to the appearance of the mature central pattern during the tail bud stage and the eventual innervation of peripheral targets. The transcription factor Islet-1 is expressed in two columns of presumptive dorsal neurons during midneurulation (st 17). The cells form continuous columns unlike the discontinuous columns of RB neurons which appear after the completion of neurulation (st 22) and express the HNK-1 epitope. There are always twice as many Islet-1 cells as RB neurons indicating that not all differentiate into RB neurons. RB neurons seldom contact other RB neurons and form a nonrandom, dispersed pattern within each column. This pattern could be generated by lateral inhibitory interactions between Islet-1 cells. Once the central pattern is established, RB neurons send axons to the periphery to innervate the skin. Growth cones enter the skin near the RB cell bodies and rapidly begin to ramify in the epidermal cell layer. The conical cells are the first cells completely surrounded by axons. By hatching, almost every epidermal cell (conical, ciliated, and mucus-secreting) is entirely encircled by sensory axons. At this point, the cutaneous innervation is a dense plexus of axons originating from the RB neurons.

147. **Signal Synergy of the Dpp and Scw Pathways Occurs at the Level of the Type I Receptors.** D. G. Stathakis, S. Park, and K. Arora. Developmental and Cell Biology, University of California, 4150 McGaugh Hall, Irvine, California 92697.

The use of multiple, structurally similar ligands to elicit distinct biological responses is a striking feature of many cell signaling pathways. Two BMP-related ligands, Decapentaplegic (Dpp) and Screw (Scw), act in concert to establish all dorsal cell fates. A nonreciprocal relationship exists between these ligands in that Scw has no biological effect in the absence of Dpp and instead acts only to amplify the Dpp signal. Both ligands act through independent type I receptors. Thick veins (Tkv) transduces the Dpp response, while Saxophone (Sax) mediates the Scw signal. The receptors maintain pathway nonreciprocity since Sax signaling has no effect in the absence of Tkv, although Tkv can signal independently of Sax. Simultaneous activation of both receptors results in a strong synergistic response essential for establishing all dorsal cell fates. While synergy allows the cell to integrate signals from these two pathways, the exact mechanism remains ill-defined. One possibility is that synergy is controlled by receptor complex formation/activity. A second alterative is that synergy occurs by the differential activation/stability of a common R-Smad. A third synergy model involves the recruitment of a second R-Smad that mediates different nuclear responses. We have used a combined molecular genetic and biochemical approach to distinguish between these alternatives. We will present data indicating that both receptors signal through the same R-Smad and that Tkv and Sax can form heteromeric complexes. These data favor a model in which both signal integration and synergy occurs at the receptor level.

148. **The Putative RNA-Binding Proteins MEX-5, MEX-6, and SPN-4 Regulate MEX-3 Localization and Activity to Control PAL-1 Spatial Patterning.** Nancy N. Huang and Craig P. Hunter. Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts 02138.

Spatial and temporal patterning of the homeodomain protein PAL-1 is dependent on the KH domain protein MEX-3 and the *pal-1* 3′-UTR. While *pal-1* mRNA is present throughout the oocyte and early embryo, PAL-1 protein is only detected in posterior blastomeres starting at the four-cell stage. MEX-5, MEX-6, and SPN-4 are required for proper MEX-3 localization and activity in the embryo and subsequent spatial patterning of PAL-1. *mex-5* and *mex-6* encode homologous and functionally redundant CCCH zinc

finger proteins that are localized to the anterior, required to prevent premature degradation of anteriorly localized MEX-3 protein, and required to maintain PAL-1 repression in the anterior. *spn-4* encodes an RNA recognition motif protein present throughout the early embryo that contributes to PAL-1 repression in the anterior and is required for the timely degradation of MEX-3. MEX-6 and SPN-4 interact with MEX-3 in yeast, suggesting that these proteins form a complex *in vivo*. Genetic analysis suggests that MEX-5, MEX-6, and SPN-4 act in combination to link initial cell polarity to the asymmetric distribution of the cell fate determinant PAL-1 by controlling MEX-3 stability and activity. As MEX-3, MEX-5, MEX-6, and SPN-4 all contain RNA-binding motifs, an appealing hypothesis is that these proteins interact on the *pal-1* 3′-UTR. Ongoing *in vivo* and *in vitro* biochemical studies aim to verify and characterize protein–protein interactions to better understand how these putative RNA-binding proteins regulate the spatial pattern of *pal-1* expression.

149. Abstract #149 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

150. **Do Morphogen Gradients Arise by Diffusion?** Arthur. D. Lander, Qing Nie, and Frederic Y. M. Wan. Department of Developmental and Cell Biology, Developmental Biology Center, and Department of Mathematics, University of California, Irvine, California 92697.

Many patterns of cell and tissue organization are specified during development by gradients of morphogens, substances that assign different cell fates at different concentrations. For several polypeptide morphogens, it has been established that gradients form by transport from a localized site of synthesis. Exactly how transport occurs is hotly debated. Some researchers favor simple diffusion, whereas to others more elaborate mechanisms are required ("bucket brigades," transcytosis, cytonemes, argosomes, etc.). We have attempted to resolve this controversy by mathematically analyzing recent published data—particularly results obtained in *Drosophila* wing disks—in ways that appropriately capture the complexity of systems in which transport, receptor interaction, endo- and exocytosis, and degradation occur together. We find that diffusive mechanisms of morphogen transport are much more plausible—and nondiffusive mechanisms much less plausible—than has generally been argued. Moreover, we show that a class of experiments—endocytic blockade—thought to effectively distinguish between diffusive- and nondiffusive transport models actually fails to draw any useful distinctions. This analysis underscores the importance of appropriate mathematical descriptions in predicting the behaviors of complex biological systems. (Supported by NIH Grants NS26862 and HD038761.)

151. **Computational Analysis of Cell Communication *Drosophila* Oogenesis.** Stanislav Y. Shvartsman and Cyrill B. Muratov. Princeton University, New Jersey Institute of Technology, Newark, New Jersey.

We have developed and computationally analyzed a model of cell communication in *Drosophila* egg development. Our model accounts for EGFR-mediated interaction between the oocyte and the follicle cells. We focus on the mechanisms by which an oocyte-derived Gurken signal is modulated in space and time by the Rhomboid/Spitz/Vein/Argos network within the follicular epithelium. We have determined the conditions under which a mechanism based on the spatially distributed positive and negative feedback loops can convert a single-peaked Gurken input into a two-peaked pattern of signaling activity across the follicular epithelium. We have used our model to test the sufficiency of the proposed patterning mechanisms, to interpret the observed phenotypic transitions, and to predict the effects of new genetic manipulations. We will present (1) the model-based analysis of the original mechanism in one and two spatial dimensions, (2) analysis of the patterning induced by more complex spatial inputs (such as the ones realized in egg chambers binuclear oocytes), and (3) analysis of the mechanism with an additional positive feedback loop.

152. **Remodeling of Motor Neuronal Contacts into Functional Synapses at Developing Adult Neuromuscular Junctions in *Drosophila*.** Sarita Hebbar and Joyce Fernandes. Zoology Department, Miami University, Oxford, Ohio.

The *Drosophila* neuromuscular junction (NMJ) is a powerful system to elucidate the physiology and development of synapses. We focus on the development and remodeling of embryonic NMJs into adult counterparts. The adult NMJs carry out a distinctive repertoire of motor functions: walking, flight, reproduction compared to hatching, feeding, and crawling in the embryo. The nervous system and musculature are restructured during metamorphosis to bring about these different behaviors. At the onset of metamorphosis, 8–12 h after puparium formation (hAPF) larval NMJs are withdrawn. This is followed by the outgrowth and elaboration of adult-specific innervation by 24 h APF. Some of the neuromuscular contacts established during the elaboration phase are later withdrawn, a process we refer to as pruning which is evident at 38 h APF. Pruning of neuronal arbors is a well-known feature of developing vertebrate nervous systems. We are interested in developing the adult NMJs of *Drosophila* as a model to investigate mechanisms of pruning. Another aspect of our study is directed at understanding how these contacts mature into functional synapses. We are taking two approaches. The first involves a developmental profile of expression of mature synaptic markers. These include Fas II (cell adhesion molecule), csp (synaptic vesicle protein), glutamate receptor, and Dlg (postsynaptic anchor protein). The second approach involves following changes in ultrastructure of these contacts as they mature into synapses. Our preliminary studies identify the first 48 h to be important in the formation of functional contacts.

153. **VAB-9 Is a Claudin-like Adherens Junction Protein That Regulates Epithelial Morphology and Adhesion in *Caenorhabditis elegans*.** Jeffrey S. Simske and Jeff Hardin. Rammelkamp Center, Cleveland, Ohio; and Department of Zoology, Madison, Wisconsin.

To understand how epithelial cell shape and adhesion regulate organismal morphology in *Caenorhabditis elegans*, we are analyzing the gene *vab-9*. *vab-9* mutants have tail and body shape defects resulting from defects in either the attachment or distribution of circumferential actin filaments at the adherens junctions. In wild-type animals, contraction of circumferential actin filament bundles results in the elongation of the embryo. *vab-9* encodes a putative four-pass transmembrane protein similar to the claudin family of tight junction proteins, yet VAB-9 localizes to the adherens junctions (AJ) of all *C. elegans* epithelia. VAB-9 localization is dependent on other adherens junction components. In the absence of HMR-1 (cadherin), VAB-9 fails to localize to the cell membrane,

whereas in the absence of HMP-1 (α-catenin) or HMP-2 (β-catenin), VAB-9 localizes at junctions, but the distribution around the periphery of the adherens junction is disrupted. Conversely, *vab-9* mutations do not obviously disrupt AJ protein localization. These results suggest that VAB-9 is closely associated with the AJ and that VAB-9 affects actin organization by regulating the interaction between actin and the cadherin complex. *vab-9* mutants have no obvious cell adhesion defects; however, *vab-9* mutations enhance the cell adhesion defects of *ajm-1* mutants and *dlg-1(RNAi)* animals. Since VAB-9 localizes apically to DLG-1 and AJM-1, one possibility is that VAB-9 and DLG-1/AJM-1 independently regulate epithelial adhesion.

154. **Two Ram Genes Interact with Genes Guiding Axonal Migration and Matrix Formation during Sensory Ray Morphogenesis.** Joanne C. N. Tam, Y. Man Lam, and King L. Chow. Department of Biology, Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong.

The morphology of sensory ray structures in *Caenorhabditis elegans* is governed by a number of genes, mutations of which give rise to lumpy rays of abnormal morphology (Ram). Cloning and characterization of a number of the ram genes reveal that their gene products are interacting *in situ* in the extracellular compartment in a ray. Both ram-2 and ram-3 are acting in the glycosylation-independent pathway for ray differentiation. The ram-3 phenotype was the most severe one among all ram mutants. All the rays are indistinguishable and ray cell nuclei are trapped inside the fan. A similar but less severe trapping was also observed in ram-2 mutants. We showed that ram-3 mutation affect the anterior dorsal migration of the ray cells. We further demonstrated that there exist genetic interactions between these two ram genes with genes involved in axonal migration and collagen matrix formation. In combination with unc-5 or unc-6, ram-2 and ram-3 could lead to dramatic change of embryonic viability. The male tail Ram phenotypes also became more severe. On the other hand, the ram-3 phenotype could be suppressed by a number of dpy mutations, arguing that the matrix composition had a significant impact on the cellular migration in the male tail. Whether the suppression is directly due to the matrix protein or simply due to body morphology change is currently under investigation. (This study was funded by Research Grants Council, Hong Kong.)

155. **A Novel Secretory Protein, MAB-7, Is Involved in Sensory Ray Morphogenesis of *Caenorhabditis elegans*.** Horace S. W. Tsang and King L. Chow. Department of Biology, Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong.

Male sensory ray morphogenesis in *Caenorhabditis elegans* is a complex process that occurs in a simple cellular context. It requires a number of ram genes, mutations of which lead to the transformation of tapered sensory rays into lumpy rays. mab-7 males share the same phenotype as the ram mutants, except that the swelling is localized at the proximal part of the rays. In this study, it was found that all ray components in mab-7 mutant showed abnormal features, including nodules associated with the neuronal processes, swelling in the hypodermis, and structural cells. The results suggest that mab-7 is required for differentiation of all three cell types. mab-7 was cloned and was found to encode a novel secreted protein with a EGF domain, a ShKT domain, and a long C-terminal tail. These domains are being analyzed to determine their roles in mab-7 biological activity. Temporal and spatial expression revealed by reporter transgenes suggested that mab-7 was active in the hypodermis, body seam, and structural cells. Ectopic expression of mab-7 cDNA showed that mab-7 was primarily required in the hypodermis. Furthermore, suppressor and enhancer mutations that interact with mab-7 were identified. The implication of their interactions relevant to ray morphogenesis will be discussed. (This study was funded by Research Grants Council, Hong Kong.)

156. **Synthesis of Specific Cuticular Collagen in *Caenorhabditis elegans* Male Tail Modulates Sensory Organ Morphogenesis.** Raymond Y. L. Yu, Daisy W. S. Hui, and King L. Chow. Department of Biology, Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong.

Collagen being an ECM protein functions as a structural component and plays a regulatory role. Cuticular collagen with a general structure similar to the vertebrate nonfibrillar FACIT collagens is the largest collagen family predicted in the *Caenorhabditis elegans* genome. Genes within this family exhibit distinct expression profile indicative of their functions in determining body morphology. ram-4 mutant shows a male specific phenotype: abnormal morphology of male sensory organs, amorphous shape (lumpy) in the 9 pairs of sensory rays. All ray cell components, including two neuronal cells, a structural cell and the hypodermis, showed abnormal morphology, suggesting that ram-4 has impact on the differentiations of all three ray cell types. Rescue of the ram-4 mutants by a minimal genomic fragment reveals that it encodes a cuticular collagen. The specific developmental function of ram-4 in males is further corroborated by its novel expression profile. This gene is turned on only in the male posterior hypodermal syncytium at the onset of tail retraction. This temporal spatial expression implicates the presence of an induction signal. The physical association of RAM-4 with the RAM-5 transmembrane protein on the structural cell surface indicates that RAM-4 may modulate cellular communication events during ray morphogenesis. (This study was funded by Research Grants Council, Hong Kong.)

157. **The Para-Hox Gene Caudal Is Required for Tail Formation in Zebrafish.** Isaac Skromne,* Yutaka Kikuchi,† Didier Stainier,† and Robert K. Ho.* *Department Organismal Biology and Anatomy, Chicago University, 1027 East 57th Street, Chicago, Illinois 60637; and †Department Biochemistry and Biophysics, University of California–San Francisco, 513 Parnassus Avenue, San Francisco, California 94143.

In vertebrates the tailbud is responsible for the formation of the posterior body at the end of gastrulation. To date, however, very little is known about the molecular mechanisms involved in tail bud outgrowth and differentiation. In zebrafish as in other vertebrates, expression of the homeobox gene caudal has been correlated with the establishment of posterior cell lineages. To address the role of Caudal function in zebrafish tail development, we have used morpholino antisense technology to specifically block translation of this gene. Morpholino-injected embryos undergo gastrulation and develop normal head and trunk structures, but fail to develop a postanal tail. Morphological and molecular analysis of affected embryos suggest that this apparently simple phenotype arises from combined defects in tissue specification, anterior/posterior axis specification, and cell movements. We are extending our analysis by undertaking cell labeling and transplantation experiments between wild-type and morpholino-injected embryos to further un-

derstand the role Caudal may play in posterior body formation and growth.

158. **Analysis of the Cellular Behaviors Driving Cardiac Fusion in Zebrafish.** Nathalia S. Glickman and Deborah Yelon. Developmental Genetics Program, Skirball Institute, New York University School of Medicine, New York, New York 10016.

Organ development is a complex process involving a reciprocal relationship between morphogenesis and patterning. A cell's position can affect its fate specification, and its fate can affect its movements. Thus it is critical to understand cellular behaviors and their genetic regulation during organogenesis. Although some of the genes involved in heart tube formation have been identified, the cell behaviors required for this process are unknown. By driving expression of GFP in cardiac precursors, we can follow precursor behaviors during cardiac fusion and heart tube assembly. In particular, we can examine cell polarity, cell shape, and rate and direction of cell movement. It will be especially interesting to compare the behaviors of ventricular and atrial precursors. These data will provide an important foundation for the analysis of cell behavior changes in zebrafish mutants with cardiac defects.

159. **Pronephric Duct Morphogenesis in *Ambystoma mexicanum* and *Xenopus laevis* Compared.** Rebecca Lumpkins, Christopher Meighan,* Mary E. Kite, and Julie Drawbridge. Rider University, Lawrenceville, New Jersey; and *Princeton University, Princeton, New Jersey.

In the axolotl, *Ambystoma mexicanum*, pronephric duct (PND) morphogenesis is accomplished by active posterior migration of the bilateral PND primordia from the ventral border of somite seven, along the ventral margin of the somitic mesoderm, to the cloaca. We have shown that treatment of axolotl embryos with PI-PLC prevents posterior migration of the PND due to removal of the GPI-linked receptor GFRα1. However, in *Xenopus* embryos, PND morphogenesis is more complex. Extension of the *Xenopus* PND rudiment occurs through both migration of PND cells into more posterior territory and *in situ* recruitment of lateral mesoderm into the forming PND. Furthermore, the *Xenopus* PND does not extend all the way to the cloaca. Instead, cloacal diverticulae extend rostrally from the cloaca to meet and fuse with the two PNDs at approximately the level of somites 14–16. We show here that PI-PLC treatment prevents extension of the *Xenopus* PND from somites 10–14 as well as preventing the cloacal diverticulum from reaching and fusing with the PND. A summary of the conserved and unconserved processes of PND morphogenesis in the two amphibian species will be presented.

160. **The Role of the Rho Family of GTPases in Gastrulation Cell Movements.** Raymond Habas,*,† Yoichi Kato,* Igor Dawid,† and Xi He.* *Division of Neuroscience, Department of Neurology, Children's Hospital, Harvard Medical School, Boston, Massachusetts; and †Laboratory of Molecular Genetics, NICHD/NIH, Bethesda, Maryland.

Gastrulation cell movements are critical for the establishment of primary germ layers and primary axis development. The Wnt family of signaling molecules has emerged as essential ligands for the control of the convergent extension during gastrulation. The molecular nature of this pathway has however remained poorly defined. We have biochemically investigated the role of small GTPase of the Rho family in the Wnt signal transduction pathway and demonstrate an essential role of the Rho GTPase family. Inhibition of Rho GTPase function with dominant-negative constructs disrupt cell movements and impede gastrulation of *Xenopus* embryos. We further define a signaling cascade that involves Wnt, Frizzled, Disheveled, and a novel formin-homology containing protein, Daam1, which regulates these cell movements. This cascade is distinct from the canonical Wnt/β-catenin pathway that controls cell fate determination and proliferation in establishing the primary axis formation. These studies highlight a pathway similar to the planar cell polarity pathway of *Drosophila* for the regulation of gastrulation and define a biochemical pathway for the Wnt regulation of cell polarity and gastrulation movements.

161. **Membrane Protrusive Activity and Cleavage Furrow Closure in *Xenopus* embryos.** Mike Danilchik, Elizabeth Brown, and Kimberly Ray. Biological Structure and Function Department, OHSU Portland, Oregon 97201.

During each of the first several cell-division cycles in eggs of *Xenopus laevis*, more than 1 mm^2 of new plasma membrane is added to the cleavage plane by localized exocytosis of stored vesicles. The surface thus produced constitutes a new basolateral membrane domain that eventually defines the blastocoel surface. This basolateral domain becomes isolated from the outside when the furrow margins—boundaries between new and old membrane domains—zip together, later to become stabilized via tight junctions. In this study, we investigate the role of membrane protrusive activity in this zippering process. During the latter half of furrowing, numerous deep folds—classically referred to as stress folds—develop in the egg surface flanking the furrow margins. Rhodamine–phalloidin staining reveals prominent bands of cortical f-actin at each margin that is clearly distinct from the contractile ring, and which close inspection reveals to be collections of long, branched actin-filled protrusions. Confocal time lapse of eggs labeled with fluorescent lipid dyes or a membrane-anchored GFP construct confirms that these protrusions are actively motile. They are apparently mutually adherent, as collections of thin, cytoplasmic extensions come to stretch across the cleavage furrow from one furrow margin to the other. These cell-to-cell extensions are clearly contractile: as their bases migrate toward the furrow margins, they exert tension on the two new cell surfaces and may thus play a role in bringing the two blastomeres' new surfaces together at the end of cytokinesis. (Supported NSF IBN-0110985, DBI-0070391, and NASA NAG2-1199.)

162. **Control of Tension across the Chorioamniotic Membrane.** R. Pulver, Y. Evrard, P. Tilkens, and B. Holton. Department of Biology/Microbiology, University of Wisconsin, Oshkosh, Wisconsin.

The avian chorioamniotic membrane begins as a fold of tissue in the blastodisk, anterior to the head. The fold expands down the sides of the embryo as well as across the body. The lateral folds zip together, enclosing the embryo. Tension exists across the membrane during this process and is seen by the stretched morphology of cells along the leading edge of the membrane and its line of fusion (where zipping occurs). Cells elsewhere in the membrane are more or less cuboidal. We propose that the degree of tension is controlled by the amount of apoptosis, not the mitotic rate. Our initial results indicated that the number of mitotic cells appeared constant as the membrane enclosed the embryo and did not visibly change when tension was released by membrane wounding. In contrast, using TUNEL stain, we observed few to no apoptotic cells

in the membrane as it first formed, even after fusion had begun. Tension appeared low at this stage as evidenced by the flaccid nature of the leading edge and fold. By the time the membrane reached past the heart, tension was high; hundreds of apoptotic cells could be detected in the leading edge of the fold, along the line of fusion, and in areas just posterior to the leading edge. The amount of apoptosis at additional stages between the time the head fold forms and the membrane moves posterior to the heart is being quantified. Apoptosis is also being measured in the membrane after tension has been released (by wounding). We predict that levels of apoptosis should increase dramatically if apoptosis is the mechanism for establishing and controlling tension.

163. **Arterial Blood Vessel-Specific Expression of the ALK1 Gene.** Tsugio Seki, Jihye Yun, Christopher Hughes, and S. Paul Oh. Department of Physiology, University of Florida, University of California, Irvine, California.

ALK1 is one of the type I receptors for TGF-β family proteins and has shown to be linked to an inherited multisystemic vascular disorder, hereditary hemorrhagic telangiectasia 2 (HHT2). We have previously shown that targeted insertion of the neomycin resistant gene into the ALK1 locus ($ALK1^{neo}$) caused embryonic lethality around E10.5 due to severe defects in angiogenesis. In this study, we created a novel null mutant mouse line for ALK1 by replacing its exons, including the one that encodes the transmembrane domain, with the LacZ gene ($ALK1^{lacZ}$). E9.5 homozygous $ALK1^{lacZ}$ mutant embryo showed severely dilated vasculature and died around E10.5, identical to previous $ALK1^{neo/neo}$ mouse, showing that the $ALK1^{neo}$ allele is indeed null. Exploiting the LacZ insertion, we visualized the ALK1 gene expression in various stages of embryo and adult $ALK1^{+/lacZ}$ mice by X-gal staining. The ALK1 expression was detected primarily in blood vessels of $ALK1^{+/lacZ}$ embryos throughout development and postnatal life. Compared with evenly distributed Flk1 expression, however, ALK1 expression was detected only in a subset of blood vessels. Furthermore, detailed analyses revealed that the ALK1 expression was found predominantly in arterial blood vessels. It has been shown that bHLH protein Gridlock determines the arterial endothelial cell fate in angioblasts. As a preliminary study, we show here that human Gridlock homolog HESR1 upregulates the ALK1 expression in HUVEC cells. These results suggest that ALK1 may play a pivotal role in remodeling of arterial vessels as a down stream factor for Gridlock.

164. **Defective Placental and Yolk Sac Vascularization in Mice Lacking LBP-1a, a Member of the NTF Family of Transcription Factors.** V. Parekh,* A. McEwen,* V. Barbour,* Y. Takahashi,† S. M. Jane,‡ and J. M. Cunningham.*,† *Department of Hematology/Oncology and †Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee 38105; and †Rotary Bone Marrow Research Laboratory, Royal Melbourne Hospital Research Foundation, Parkville, Victoria 3050, Australia.

Gene disruption studies have frequently resulted in unexpected insights into the role of regulatory factors in development. LBP-1a, a ubiquitously expressed mammalian transcription factor, modulates transcription initiation of various viral and cellular genes including HIV-1 and cytochrome P450 gene family. Gene targeting of the highly related factor CP2 has suggested that LBP-1a may compensate for the loss of CP2 function. To address this hypothesis, we have targeted the LBP-1a gene in murine ES cells and generated LBP-1a-deficient mice. Although mice heterozygous for the LBP-1a null allele were phenotypically normal, no LBP-1a−/− animals were identified. Homozygous embryos grew normally till E9.5, after which they showed growth retardation. Histological examination revealed apparently normal development until E10.5 except for growth retardation. However, the yolk sac in homozygous concepti showed reduced vascularity. Moreover, the placental labyrinth was drastically reduced in thickness and allantoic blood vessels failed to penetrate deep and branch out in the labyrinth. Tetraploid rescue experiment failed to rescue placental defect, suggesting a primary defect in allantois. Efforts are under way to determine whether primary defect resides in allantoic angiogenesis pathways or in allantoic signaling pathway(s) responsible for normal branching morphogenesis of labyrinthine trophoblasts.

165. **Role of Nope and DCC in Otic Development.** Lily Francis and Suzanne L. Mansour. Eccles Institute of Human Genetics, University of Utah, Salt Lake City, Utah 84112.

The mammalian inner ear develops from a thickening of the surface ectoderm, the otic placode, which invaginates to form the otic vesicle. The vesicle gives rise to sensory, nonsensory and neuronal cells, and morphogenetic processes determine the complex and stereotypical arrangement of these distinct classes of cells within the mature inner ear. The role of neural cell adhesion molecules (NCAMs) in this process is largely unknown. A gene trap screen showed that the punc gene, which encodes a new NCAM, is expressed in the developing otic vesicle and in neurons of the otic ganglion. Punc is closely related to the axon guidance receptors, Deleted in Colorectal Carcinoma (DCC), Neogenin, and Neighbor of PuncE (Nope). Mice that lack punc have impaired motor coordination, but no detectable inner ear abnormalities (W. Yang, *et al.*, 2001, *MCB* **21,** 6031–6043). We hypothesized that punc is redundant with a related gene with respect to inner ear development and we are surveying the expression patterns of related genes to identify the best candidates. We performed *in situ* hybridization and RT-PCR studies of the nope gene in developing and mature murine inner ears and the results show that Nope is expressed in these tissues. We are now generating gene targeting constructs designed to mutate nope as well as both nope and its neighbor, punc simultaneously. Mice that lack the DCC ligand, Netrin-1, have inner ear abnormalities (M. Salminen *et al.*, 2000, *Development* **127,** 13–22) and we are currently assessing the expression pattern of DCC in the developing inner ear as a prelude to studies of its role in inner ear development. Progress toward these goals will be presented.

166. **Mesenchyme–Epithelial Transformation during Corneal Endothelial Morphogenesis.** T. Mgwebi and S. H. Kidson. University of Cape Town, Cape Town, South Africa.

The corneal endothelium (CE), a monolayer of cells on the inner surface of the cornea, forms by a process of mesenchyme–epithelial transformation. Experimental evidence suggests that this is triggered by interactions between the lens and the ingressing neural crest-derived cranial mesenchyme. To explore the lens–corneal interactions at a molecular level, it is necessary to accurately define the timing of morphological changes that occur during the transformation of mesenchyme into endothelium. We used SEM to monitor morphological changes during CE mouse development from 12 dpc to adults. Expression of zonula occludens-1 protein (ZO-1), a tight junction (TJ) marker, was mapped using ICC to correlate the timing of TJ formation with changes in cellular morphology. Results show that the change from a dendritic to a flattened epithelial-like phenotype starts be-

tween 12.5 and 13.0 dpc. At 12.5 dpc, the presumptive CE appears as an irregular meshwork of mesenchyme. By 13.0 dpc flattening occurs and there is a loss of dendritic morphology and establishment of cell–cell contact. At 13.5 dpc junctions between the cells become evident as confirmed by expression of ZO-1. The formation of TJs coincides with the separation of the cornea from the lens and the formation of the anterior chamber. This suggests establishment of a functional barrier for paracellular transport of water and ions across the endothelial layer. We conclude that the transformation of mesenchyme to endothelium begins earlier that previously reported and suggest that signaling between the lens and the cornea occurs between 12.5 and 13.0 dpc.

167. Abstract #167 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

168. **Asymmetric Limb Malformations Induced by Transgene Integration into a Novel Gene.** S. M. Bell, C. M. Schreiner, B. Aronow, and W. J. Scott. Children's Hospital Medical Center, Cincinnati, Ohio 45229.

Footless is a mouse line characterized by the preferential loss of posterior skeletal elements of the hindlimb and anterior skeletal elements of the forelimb. The left hindlimb is more severely affected than the right and the right forelimb is affected whereas the left forelimb is normal. We have attributed the limb malformations to abnormal formation of the apical ectodermal ridge (AER). Characterization of the transgene integration site has revealed that integration resulted in the deletion of ~7 kb of intronic sequence and that the transgene interferes with normal splicing of the *Ftl* gene. This gene spans over 150 kb and encodes a novel gene product. Consistent with the observed limb abnormalities, the *Ftl* gene is expressed in the AER of the day 10–11 embryo. During embryogenesis other domains of expression include the telencephalon, genital tubercle, lung, lateral and medial nasal processes, and branchial arches.

169. Abstract #169 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

170. **Wnt3 Is Necessary for Proper Limb Development.** Benjamin Arenkiel, Kirk Thomas, and Mario Capecchi. University of Utah, Salt Lake City, Utah 84112.

Wnt genes encode a large family of secreted proteins that have been shown to be key regulators in animal development. Acting through Frizzled receptors to regulate the activity of β-catenin signaling, Wnts have been implicated in processes such as axis formation, segmentation, CNS patterning, cell migration, and cell fate specification. In this study we describe a role for Wnt3 in vertebrate limb development. Previous work in the mouse has demonstrated that Wnt3 is important during gastrulation. Homozygous null mutations in the Wnt3 locus result in early embryonic lethality due to the failure to generate ectodermal and mesodermal derivatives. To overcome the effects of early lethality and study further roles of Wnt3 beyond gastrulation, we have taken advantage of a Cre/loxP-based conditional mutagenesis system. By targeting deletion of the Wnt3 locus in specific somatic tissues, we have found that Wnt3 is necessary for proper limb development. Conditional ablation of Wnt3 results in mice with abnormal forelimbs with asymmetric variability and that are completely missing hindlimbs. Phenotypic and molecular analysis suggest that Wnt3 acts early during limb development, affecting both induction and patterning events, revealing a novel role for Wnt genes in animal development.

171. **Spatial and Temporal Aspects of SHH Signaling during Limb Patterning.** L. Panman, P. te Welscher, G. Soete, O. Michos, R. Zeller, and A. Zuniga. Department of Developmental Biology, University of Utrecht, Utrecht, The Netherlands.

SHH is the morphogenetic signal which patterns the vertebrate limb by long and short range involving extensive signal relay. Our and other studies show that the nascent limb bud mesenchyme is patterned by an intricate interplay of Wnt, BMP, and FGF signals, which both control and are themselves responsive to SHH signaling. A major challenge is now to understand how the different signaling cascades interact over space and time. To this aim we have studied the differential mesenchymal response of Shh targets such as Gli, Gremlin, BMP, and Hox genes by combining loss and gain-of-function mutagenesis in the mouse with rescue of several mouse mutant strains with disrupted, attenuated, or ectopic SHH signaling. The first major conclusion from these studies is that the nascent limb bud mesenchyme is prepatterned prior activation of SHH signaling by mutual genetic antagonism of GLI3 and dHAND (1). This prepatterning mechanism establishes differential mesenchymal responsiveness to future SHH signaling, which in turn is essential for maintenance and propagation of the limb bud organizer (ZPA). Second, SHH signaling seems to activate secondary mesenchymal signals in two waves. Initial mesenchymal response results in transcriptional up-regulation of Gli1 and the secondary signal Gremlin while other genes such as 5′ HoxD genes are up-regulated with much slower kinetics (15–22 h). We have began to isolate the signals which might mediate the temporal aspects of patterning the mesenchyme in response to morphogenetic SHH signaling. 1. te Welscher *et al.,* 2002, *Genes Dev*. **16,** 421–426.

172. **Retroviral Expression of a Constitutively Active Epidermal Growth Factor Receptor Leads to Alterations of Limb Patterning in Developing Chick Embryos.** M. Omi, N. Maihle,* and C. N. Dealy. Department of BioStructure and Function, University of Connecticut Health Center, Farmington, Connecticut; and *Tumor Biology Program, Mayo Clinic Foundation, Rochester, Minnesota.

Epidermal growth factor (EGF) and transforming growth factor-α previously have been implicated in the outgrowth and patterning of developing chick limbs. Here, we have utilized an RCAS retroviral vector to misexpress a constitutively active truncated form of the chicken EGFR (E1; vErbB) in developing chick limbs *in ovo*. This mutant receptor consists of the intracellular carboxyl and transmembrane domains, but lacks the extracellular ligand-binding domain and has constitutive kinase activity. Injection of this E1-RCAS vector into the prospective limb-forming mesoderm of the lateral plate of stage 11–14 chick embryos results in severe limb defects typified by syndactyly, formation of ectopic autopod cartilages and/or digits, and abnormal limb curvature. Less frequently, E1 infection may also lead to hypodactyly, oligodactyly, "lobster-claw" appearance, and supernumerary AER/limb formation. We have found that cell death in the interdigital webbing of E1-infected limbs is reduced compared to control limbs, suggesting that EGFR signaling may be involved in regulating the balance between cell death and cell proliferation during digit morphogenesis. We have

also examined the expression of genetic markers of limb patterning in E1-infected limbs and results suggest potential roles for EGFR signaling in additional aspects of limb morphogenesis including limb outgrowth and initiation and AER formation. (This work was supported by NIH Grant CA79808 to N.J.M. and HD22610 to C.N.D.)

173. **The Role of Delta-like 3 in Maintaining the Notch Pathway Somite Clock.** Mizuho S. Mimoto,* Kelly L. Covello,† Stacey A. Stevens,* and Kenro Kusumi.*,† *Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, and †University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-4318.

The notch ligand, delta-like 3 (*Dll3*), is disrupted in the pudgy (*Dll3*^{pu}) mutant mouse and in the human vertebral segmentation disorder, spondylocostal dysostosis (MIM277300). Previously, we have shown that *Dll3*^{pu} mutant embryos have defective epithelial somite segmentation and fail to form normal rostral–caudal somite domains, leading to vertebral malformations. In addition, maintenance but not initiation of the somite cycling gene, lunatic fringe (*Lfng*), is disrupted. To differentiate between the role of *Dll3* in initiation versus maintenance of somitogenesis, we are studying notch pathway cycling genes in *Dll3*^{pu} embryos. We are characterizing *Hes1*, *Hes7*, *Hey2*, and textitHey3, in *Dll3*^{pu} mutant embryos, both near the onset of somitogenesis at 8.25 dpc and in midsomitogenesis at 9.5 dpc. In examining *Hes1*, we observed loss of expression in caudal *Dll3*^{pu} paraxial mesoderm, but development of dynamic rostral expression by 9.5 dpc. This contrasts with *Dll3*^{pu} effects on *Lfng* expression. In addition, to study genetic interactions, we are generating *Dll3* and *Notch1* double mutants and examining them for segmental defects.

174. **Consequences of the Lack of Aggrecan in Epiphyseal Growth Plate Signaling.** Miriam S. Domowicz, Melissa M. Mueller, Sean M. Ferguson, Judy G. Henry, Lauren E. Schwartz, and Nancy B. Schwartz. Departments of Pediatrics and Biochemistry and Molecular Biology, University of Chicago, Chicago, Illinois 60637.

Mutations in the aggrecan gene result in gross skeletal abnormalities, including extremely shortened and broad limbs in both the nanomelic (nm) chick and the cmd mouse. We have assessed the functional consequences of the lack of aggrecan expression on the cartilage phenotype during growth plate formation in these mutants by analyzing expression of chondrocytic markers and signaling molecules considered essential for chondrogenesis and endochondral bone development using mRNA *in situ* hybridization. In wild-type (wt) tibia growth plate, which expressed high levels of aggrecan, there is clear distinction of the areas expressing collagen X (Col X), characteristic of hypertrophic chondrocytes and Indian hedgehog (Ihh), characteristic of prehypertrophic chondrocytes. In contrast, in the absence of aggrecan there is a significant reduction of the hypertrophic zone in E12 nm limb growth plates compared to wt and both markers are expressed in an overlapping area, making it impossible to distinguish hypertrophic from prehypertrophic zones. Osteopontin (OSP), a marker of late hypertrophic chondrocytes and osteoblasts, also colocalizes with Col X and Ihh and is found at increased levels associated with the secondary ossification sites in the nm epiphyseal cartilage. At early stages (E7), a significant reduction in the level of Ihh and an increase in Col X and OSP is observed, indicating acceleration of hypertrophy early in the establishment of the growth plate. Furthermore, there is mislocalization and reduction of SOX9 expression in nm cartilage while levels of collagen IX and tenascin-C expression remained comparable between nm and wt. Because SOX9 is considered a regulator of the chondrocytic phenotype, this result suggests feedback from the matrix surrounding the chondrocytes that modulate levels of matrix component production. These observations reveal deregulation of several genes critical to the maturation process of hypertrophic chondrocytes.

175. **Differentiation and Patterning of Vertebrate Tendons.** Tim Riordan, Nicholas Murchison, and Ronen Schweitzer. Department of Research, Shriners Hospital for Children, 3101 SW Sam Jackson Park Drive, Portland, Oregon 97210-3095.

Tendons are essential for embryonic patterning and the later functionality of the musculoskeletal system. Despite their importance, surprisingly little is known about the embryonic formation of tendons, largely due to the absence of histological staining methods or specific molecular markers for this tissue. We have recently demonstrated that Scleraxis, a bHLH transcription factor, is a specific and unique marker for all the connective tissues mediating the attachment of muscle to bone, including both tendons and ligaments. Scleraxis expression persists in tendons from an early progenitor stage to the postembryonic mature tendons. Using Scleraxis as a marker, we describe the locations of tendon progenitor pools and present an initial characterization of the signals regulating the establishment of tendon progenitors in the limb bud. Our analysis shows that tendon formation is a multistep process in which the early progenitors are induced by an ectodermal signal and restricted by mesenchymal BMP signaling. Tendon formation in mouse has not been addressed to date. To facilitate these studies we have established a transgenic mouse expressing the GFP protein in the expression domain of the Scleraxis gene (ScxGFP). Characterization of normal tendon formation and initial studies of mouse mutations affecting tendon formation, including a conditional mutation in the Scleraxis gene, will be presented.

176. **Epimorphin Promotes Cartilage Condensation/Sorting during Vertebral Skeletogenesis.** Yumiko Oka,* Yuki Sato,† Yohei Hirai,* Hokari Tsuda,* and Yoshiko Takahashi.†,‡ *Osaka R and D Laboratories, Sumitomo Electric Industries, LTD., 1, Taya-cho, Sakae-ku, Yokohama, 244-8588, Japan; †Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5, Takayama, Ikoma, Nara, 630-0101, Japan; and ‡Center of Developmental Biology, RIKEN, Saitama, Japan.

Epimorphin was originally discovered as a morphoregulator that governs lung morphogenesis by mediating epithelial–mesenchymal interactions (Hirai *et al.*, *Cell* **69,** 1992). In the present study we isolated avian homologs of Epimorphin cDNA and examined expression patterns during early embryogenesis. Among tissues displaying Epimorphin transcripts, we focused on the vertebral cartilage primordium. The onset of Epimorphin expression was initiated by an influence of the notochord. Implantation of Epimorphin-producing COS cells into an early chicken embryo caused enhanced accumulation of collagen type II, an early cartilage marker, leading subsequently to a formation of supernumerary cartilage. We further analyzed the function of Epimorphin in cartilage formation directly by using *in vitro* aggregation assay and found that Epimorphin promoted condensation/sorting of precartilage cells. We propose a model in which Epimorphin at the tip of

the expanding vertebral cartilage promotes recruitment/condensation of the adjacent sclerotomal mesenchymal cells and it might reflect a driving force of progressive expansion of the vertebral cartilage which forms in a dorsolateral sequence.

177. **Changes in Gap Junction Communication between Human Mesenchymal Stem Cells during Differentiation and Senescence.** Rita A. Meyer. Department of Biomedical Sciences, Creighton University, Omaha, Nebraska 68178.

We are using human mesenchymal stem cells (hMSC) derived from bone marrow that are multipotent to investigate the roles of gap junction communication in cell differentiation and senescence (Poietics/Biowhittaker). The hMSC are normal cells with a finite life span that can form chondrocytes, osteocytes, and adipocytes when grown in medium that promotes that cell lineage. The cells in chrondrogenic or osteogenic medias form aggregates or nodules with characteristics of either forming cartilage (aggecan and type II collagen positive) or forming bone (alkaline phosphatase and Alizuran RedS positive). Cells in adipogenic media have lipid deposits that are Oil RedO positive. We show that hMSC grown in the growth medium are less dye coupled (6-carboxyfluorescein) than cells induced to differentiate in either the chrondrogenic or osteogenic media. The cells form small nodules that have more gap junctions than internodular cells. With longer times in culture the cells at the nodule periphery are better coupled than those that have differentiated in the center of the nodules. Early passage cultures (passages 2 or 3) are better coupled than older passage cultures (4 or 5) and form more cartilage or bone nodules. Adipocytes form in the medium that promotes adipogenesis and in cultures treated with chrondrogenic and osteogenic media for 3 or more weeks. Adipocytes are well couple to adjacent nondifferentiated cells and remain well coupled regardless of culture passage number or the type of medium present. The gap junction protein connexin 43 is present by immunostaining in the internodular cells and in the periphery of the differentiating nodules. Connexin 45 is seen in the center of the nodule. Neither appears to be present between adipocytes suggesting another connexin protein is present in these cultures. The hMSC provide a model system where changes in cell communication can be examined during differentiation of the cells into several different cell types and with aging in culture. (This work was supported by funds by Creighton University and the Health Future Foundation.)

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179. **G1 Regulation in *Caenorhabditis elegans.*** Julián Cerón and Sander van den Heuvel. MGH Cancer Center (Harvard Medical School), Building 149, 13th Street, Charlestown, Massachusetts 02129.

Control of cell proliferation during development of multicellular organisms is a highly regulated and evolutionarily conserved process. In general, extracellular and intracellular cues appear to converge during G1 phase to determine whether a cell enters a new division cycle or exits from the cell cycle (Sherr, 1994) Studies of mammalian cells in tissue culture have indicated that the retinoblastoma tumor suppressor protein (pRb) plays a central role in controlling progression through G1 phase. pRb acts as a negative regulator of cell cycle entry when it is hypophosphorylated. Phosphorylation by cyclin-dependent kinases (CDKs) during G1 phase inactivates pRb and allows in entry into a new cell cycle. In *Caenorhabditis elegans*, lin-35 is the single Rb-related gene (Lu and Horvitz, 1998). Single lin-35 mutants do not display a cell cycle phenotype. However, when lin-35 inactivation is combined with loss of function of other negative regulators of G1 progression, a synergistic increase in ectopic cell divisions is observed (Boxem and van den Heuvel, 2001). Our results are consistent with a model in which lin-35 Rb and cki-1,2 Cip/Kip act in parallel to repress cell cycle entry, downstream of cyd-1 Cyclin D and cdk-4 CDK4/6. Genetic analyses of lin-35 function in *C. elegans* support the idea that additional genes and regulatory pathways must be involved in the control of cell cycle entry. To identify others components of this G1 regulatory network, we are performing a genetic screen for genes that cooperate with lin-35 Rb during development of *C. elegans.* The details of this screen and the mutants identified thus far will be presented at the meeting.

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181. **Possible Role for PDZ Domain Containing Proteins in Lens Development.** M. M. Nguyen and A. E. Griep. University of Wisconsin, Madison, Wisconsin.

During normal ocular lens development, precise regulation of cell proliferation is essential. However, how this regulation is maintained, especially in the less differentiated cells of the epithelium, is not well understood. Recent studies in our lab have provided some evidence that, in addition to the pRb family of tumor suppressors, PDZ domain containing membrane-associated guanylate kinases (MAGUKs) may play a vital role in cell cycle control and in the maintenance of cell–cell adhesion and cell polarity in the mouse lens. Among these PDZ proteins are the *Drosophila* tumor suppressors DLG, Scrib, and LGL. Null mutations of these genes in *Drosophila* lead to hyperplastic growth and loss of cell–cell adhesion and polarity in some epithelial tissues. Interestingly, we see a remarkably similar phenotype in lenses of transgenic mice (E6wt mice) that express HPV16-E6, a viral oncogene, in the epithelium and newly differentiating cells of the lens. E6 can bind and inactivate several proteins including DLG and Scrib, through binding their PDZ domains. Using RT-PCR and *in situ* hybridization we determined that DLG, Scrib, and LGL are expressed in the lens. To determine if E6′s binding to these PDZ proteins was the basis for the E6wt phenotype, we examined lenses from transgenic mice expressing E6 mutants that either retain (E6I128T) or lose (E6Δ 146-151) their ability to bind PDZ proteins. Histological and BrdU analyses showed that while the E6I128T mutant largely retained the E6wt phenotype, the E6Δ 146-151 mutant largely lost the phenotype. These results suggest that member(s) of the PDZ family of proteins may be required in normal lens development.

182. **Inhibition of Cell Division Is Required during Vertebrate Gastrulation.** Walter F. Leise and Paul R. Mueller. The University of Chicago, Chicago, Illinois 60637.

The coordination of cell division and morphogenesis is critical for proper development. In *Xenopus,* cell divisions are rapid and synchronous early in development, but then slow and become spatially restricted with the onset of gastrulation. This switch is

dependent on the transition from maternal to zygotic control of the cell cycle. The cell cycle and rate of cell division is ultimately controlled by the activity of the cyclin-dependent kinases (Cdks). Our work focuses on the regulation of the Cdks through inhibitory phosphorylations mediated by the Wee1 and Myt1 kinases. We are interested in the roles Wee1 and Myt1 play in coordinating cell cycle progression with morphogenetic events such as gastrulation and cell type specification. We have found that in *Xenopus* there are at least two Wee1-like kinases. The first is a maternal gene product that disappears during gastrulation. The second is a zygotic gene product that is expressed after the midblastula transition and into adult stages of development. The related Myt1 kinase is expressed throughout development, albeit at varying levels. Concurrent with the changing levels of these Cdk inhibitory kinases, the pattern of embryonic cell division becomes asynchronous and spatially restricted in the *Xenopus* embryo. The zygotic XWee1 is expressed in regions of the embryo that are devoid of mitotic cells such as the involuting mesoderm. In contrast, XMyt1 is expressed in regions of the embryo that have high levels of proliferation such as the developing neural tissues. We have found that zygotic Wee1 is required for the morphogenetic process of gastrulation. Knockdown of zygotic XWee1 expression inhibits gastrulation and blocks further development. Our results suggest that active cell proliferation is incompatible with the cell movements associated with gastrulation in *Xenopus.* Together, the existence of multiple Wee kinases may help explain how distinct patterns of cell division arise and are regulated during development.

183. **Specificity Assay for Cell Binding to Derivatized Beads.** Arash Razi, Maria R. Khurrum, David Khatibi, Stephanie Gipson, Mehdi Sina Khadiv, Puria Parsa, Evelyn S. Soriano, Emely Garcia, Kiana Keyvanjah, Karolin Abedi, Tristan Clark, Mandy Sidhu, Sam Meshkinfam, Manya Khoddami, Oliver Badali, and Steven B. Oppenheimer. California State University, Northridge, California 91330-8303.

Here we describe a new component of a novel assay that we have developed to survey cell surface properties of many cell types including those in developmental and cancer cell systems. We used five different normal and malignant human cell lines (CRL 2049, CRL 1539, CRL 1459, CCL 220, CCL 224, from ATCC, Manassas, VA) as models for testing this new specificity component of our assay that examines cell surface properties by assessing cell binding to agarose beads derivatized with over 100 different compounds. In many repeated experiments, we examined bead binding to each of the five cell types, in the presence and absence of hapten inhibitor compounds or nonspecific compounds, at defined concentrations, under the standard assay conditions. The results showed that in many, but not all cases, the specific compound most effectively inhibited cell-bead binding. This assay component will help us learn more about the nature of the cell surface receptors detected with the assay system (Supported by grants and fellowships from NIH MBRS, NIH RISE, NIH MARC, ONR RISE, NSF ESIE, and the Joseph Drown Foundation.)

184. **Sonic Hedgehog Signaling Activates Stromal *Gli1* Expression and Accelerates Prostate Cancer Xenograft Tumor Growth.** L. Fan, M. Lamm, C. Hebner, W. Catbagan, R. Laciak, D. Barnett, and W. Bushman. Northwestern University Medical School, Evanston, Illinois.

Gli1 Shh The epithelial–mesenchymal interactions of prostate cancer represent a promising target for therapies to arrest tumor growth. During fetal prostate development, *Shh* expression by the urogenital sinus epithelium activates *Gli1* expression in the adjacent mesenchyme. Blockade of Shh signaling prevents *Gli1* activation and inhibits prostate ductal development. *Shh* and *Gli1* are expressed in the human fetal prostate and, as in the mouse, are down-regulated in the adult. However, both *Shh* and *Gli1* are frequently reexpressed in human prostate cancer, suggesting that the prostate cancer (epithelial) cells commandeer this embryonic pathway to recruit stromal cells to support tumor growth. We used the human prostate cancer (epithelial) cell line LNCaP to engineer tumor cells which overexpress human *Shh* (approximately 10× by western blot). Growth rate in culture was identical to the parent line. Xenograft tumors generated in nude mice with the control and overexpressor cells demonstrated: (1) *Shh* expressed by the parental human LNCaP tumor cells activates *Gli1* expression in the mouse tumor stromal cells; (2) engineered *Shh* overexpression drives stromal *Gli1* expression; and (3) *Shh* overexpression dramatically accelerates tumor growth (approximately sixfold) without altering the histology of the tumor. These data suggest that the hedgehog signaling between the tumor cells and stroma can function as a critical regulator of tumor growth and is a potential target for cytostatic therapy.

185. **Interplay of Receptors, Coreceptors, and Molecular Diffusion in the Regulation of Developmental Signaling.** A. D. Lander, K. Ding, E. Kanakubo, A. Kumbasar, Q. Nie, L. Pham, J. A. Sanchez, and F. Y. M. Wan. Department of Developmental and Cell Biology and Department of Mathematics, University of California, Irvine, California 92697.

Much of development is orchestrated by molecules that are produced at one location and act on cells elsewhere. Along the way, such molecules are influenced by binding sites on other cells, soluble antagonists, enzymes, extracellular matrix, and the physics of diffusion. On responding cells, their effects are further modified by coreceptors. The interplay of such phenomena can lead to unexpected behaviors, especially in morphogen gradient systems. We have shown that this interplay alone can explain peculiarities in morphogen gradients (e.g., Dpp in *Drosophila* wing disks) that have led others to invoke nondiffusive morphogen transport (see poster, this meeting). Here, we extend these studies: We show that ancillary binding sites (proteoglycans, extracellular matrix, etc.) need not interfere with morphogen gradient formation, but are also unlikely to "promote diffusion," as some have suggested. We explain how receptor density can inhibit morphogen spread in some systems, yet enhance it in others. We argue that a widely accepted mechanism of coreceptor action—stabilization of ligand–receptor complexes—is unlikely to be of much value in morphogen gradient systems, and instead we propose mechanisms such as (a) catalyzing binding and (b) increasing signal duration by signaling complexes. We present *in vitro* evidence for the existence of both types of mechanism, in one case involving a novel coreceptor for BMP2/4 signaling, in the other case involving the role of glypicans in signaling by fibroblast growth factors.

186. **FGF Signaling Is Governed by Distinct Heparan Sulfate Domains during Mouse Development.** Benjamin L. Allen and Alan C. Rapraeger. University of Wisconsin–Madison, Madison, Wisconsin.

FGFs are a family of 23 growth factors which regulate a number of developmental processes, including lung morphogenesis, limb development, and cardiogenesis. FGFs signal through a family of

four receptor tyrosine kinases (FR1-4). The formation of high affinity FGF/FR signaling complexes requires the interaction of both FGF and FR with heparan sulfate (HS). Although there is speculation that the interaction of specific FGF/FR pairs is determined by specific sulfation patterns present on HS and that these patterns vary in both a tissue-specific and a developmental stage-specific manner, there are few experiments which directly test this hypothesis. We have used an *in situ* binding assay using both soluble FGFs and FRs to probe for tissue-specific and developmental stage-specific HS in the developing mouse embryo. In this study we examine the ability of two FGFs, FGF1 and FGF8b, and two FRs, FR2c and FR3c, to bind developmental stage-specific HS. We find that while heparin, a highly sulfated form of HS, mediates both FR2c and FR3c binding to FGF1 and FGF8b, there are distinct differences in FR recognition of these FGFs when bound to HS. In particular, we find that although FGF1 and FGF8b bind HS throughout multiple developmental stages, FR2c and FR3c recognition of these FGF/HS complexes changes dramatically at different stages of development. Furthermore, we use selectively desulfated heparin to identify specific sulfation requirements necessary for FR recognition of these FGFs. These results suggest that FR recognition of FGF is developmentally controlled by the specific expression of distinct HS sulfation patterns in the mouse embryo.

187. **Syndecan 2 Is Asymmetrically Regulated by PKC during Early *Xenopus* Left–Right Development.** K. L. Kramer and H. J. Yost. Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah.

We have recently demonstrated that the heparan sulfate proteoglycan Syndecan 2 functions in the *Xenopus* animal cap ectoderm as a cell nonautonomous cofactor that transmits left–right information to migrating mesoderm during gastrulation. Here, we extend those observations by demonstrating that asymmetric phosphorylation of two highly conserved serines in the cytoplasmic domain of Syndecan 2 is essential for this activity. A full-length syndecan 2 construct with the two cytoplasmic serines mutated to alanines randomized situs when specifically expressed in the right but not left ectoderm. In contrast, situs was randomized when a full-length syndecan 2 construct with the two cytoplasmic serines mutated to glutamates (phosphomimetic) was specifically expressed in the left but not right ectoderm. Syndecan 2 phospho-specific antibodies illustrated that Syndecan 2 is asymmetrically phosphorylated in the right animal cap ectoderm at a very early stage in development. Using multiple protein kinase C (PKC) inhibitors, we have identified a specific PKC that mediates the phosphorylation of Syndecan 2 in left–right development. We further demonstrate that this PKC activity is required for left–right development during the same gastrula stages that Syndecan 2 functions. Finally, expression of phosphomimetic Syndecan 2 on the right side of the embryo can rescue normal situs in embryos expressing a specific dominant-negative PKC. This work supports a model wherein cytoplasmic phosphorylation of ectodermal Syndecan 2 regulates how it transmits early left–right information to migrating mesoderm.

188. **Regulation of Cell Polarity during Zebrafish Gastrulation.** Lilianna Solnica-Krezel, Florence Marlow, Jacek Topczewski, Jason Jessen, and Diane Sepich. Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee.

During the vertebrate gastrulation movements of convergence and extension (C&E) the entire embryo and most organ rudiments narrow along the mediolateral axis while extending their anterior–posterior dimension. In zebrafish C&E movements are driven by directed cell migration and mediolateral intercalation of polarized cells and require noncanonical Wnt signaling. Mutations in knypek gene encoding a member of the glypican family of heparan sulfate proteoglycans impair C&E movements due to abnormal cell polarity, as mutant cells fail to elongate and align mediolaterally. Knypek potentiates Silberblick (Wnt11) signaling mediating C&E via a noncanonical signal transduction cascade. Mosaic analyses demonstrate that knypek has both cell-autonomous and nonautonomous roles in cell polarity determination. Zebrafish Rho kinase α (Rok2) is expressed in zebrafish gastrula and overexpression of a dominant-negative Rok2 (dnRok2) disrupts C&E, phenocopying noncanonical Wnt signaling mutants. Epistatic analyses place Rok2 downstream of Wnt 11 in regulation of C&E. Impaired dorsal convergence movements in Rok2-deficient embryos are associated with defective cell polarity and consequently inability of a cell to migrate persistently dorsally along straight path. Transplantation experiments reveal both cell-autonomous and cell nonautonomous roles for Rok2 function during cell elongation and orientation.

189. **Xlefty Antagonizes both Nodal and Wnt Signaling during Gastrulation.** W. W. Branford and H. J. Yost. Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah 84112.

Nodal signaling is an important determinant of cell fate and behavior. However, the mechanisms modulating Nodal signaling are poorly understood. The ability of the Nodal protein Squint to act at a distance suggests that signaling modulation might occur via a diffusion-dependent gradient. Nodal antagonists, such as Lefty, likely superimpose further regulation over the diffusion gradient. Here we demonstrate that *Xenopus* Lefty modulates the Nodal diffusion gradient during gastrulation. Xlefty-deficient gastrulae were created by injecting anti-Xlefty morpholino oligos into 2- to 4-cell embryos. Surprisingly, these embryos exogastrulated and exhibited an anterior expansion of both Nodal-responsive (xnr2, gsc, xbra, cer, xlefty) and Wnt-responsive (xnr3, gsc) gene expression at the dorsal lip. These results suggested that Xlefty functions as a barrier against both Nodal and Wnt signaling during gastrulation. To test this, nodal (xnr1) or wnt (xwnt8) RNAs were injected, with or without Xlefty RNA, into the animal poles of 8- to 16-cell embryos. The ability of Xlefty to reduce the area of ectopically induced xbra (Nodal) and xnr3 (Wnt) expression further suggested that Xlefty acts as a boundary against Nodal and Wnt signaling. Additionally, the repression of endogenous xbra, xnr3, and gsc expression, following the injection of Xlefty RNA into the animal pole of 32-cell embryos, suggested that Xlefty can antagonize both Nodal and Wnt signaling via diffusion. In summary, Xlefty forms a barrier against the Nodal and Wnt signaling pathways during gastrulation. In the absence of Xlefty, these signals are not attenuated and gastrulation is severely perturbed by localized alterations in cell fate and behavior.

190. Abstract #190 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

191. **β-Catenin Is Differentially Degraded along the Animal–Vegetal Axis of Early Sea Urchin Embryos in a GSK-3β-Dependent Manner.** Heather E. Weitzel and Charles A. Ettensohn. Carnegie Mellon University, Pittsburgh, Pennsylvania.

Differential nuclear accumulation of β-catenin along one axis of the early embryo is required for mesoderm and/or endoderm formation in deuterostomes and is important in the development of early signaling centers. The mechanisms underlying this critical developmental step, however, are unknown. We microinjected mRNA-encoding *Xenopus* β-catenin-GFP into fertilized eggs and observed the dynamics of β-catenin localization *in vivo* using 3-D time-lapse confocal microscopy. β-Catenin-GFP initially accumulated in the nuclei of all cells. Beginning at the 32-cell stage, we observed a dynamic loss of β-catenin-GFP from the animal two-thirds of the embryo. Embryos injected with hyperstable β-catenin-GFP mRNA maintained nuclear β-catenin in all cells, supporting the hypothesis that putative GSK-3β phosphorylation sites are important for β-catenin degradation. Embryos coexpressing kinase-dead GSK-3β and β-catenin-GFP also retained nuclear β-catenin in all cells. To quantitate β-catenin-GFP half-life in specific cell lineages, we measured changes in fluorescent intensity in tiers of blastomeres along the animal–vegetal axis. We found, on average, an eightfold difference in β-catenin stability when comparing the animal and vegetal-most cells. This difference would lead to a 500-fold difference in protein levels within 2 h time. Together, these data provide the first experimental evidence for a gradient of β-catenin stability along an axis and support the view that degradation of β-catenin occurs via a GSK-3β-dependent mechanism.

192. **Characterization of the *in vivo* Substrate Activities of the Mammalian BMP-1/Tolloid-Related Metalloproteinases: Analysis of the Bmp1/Tll1 Double Knockout.** William N. Pappano* and Daniel S. Greenspan.*,† *Department of Biomolecular Chemistry and †Department of Pathology and Laboratory Medicine, University of Wisconsin Medical School, Madison, Wisconsin.

Vertebrate bone morphogenetic protein 1 (BMP-1) and *Drosophila* tolloid (TLD) are prototypes of a family of metalloproteinases with important roles in various developmental events. The mammalian BMP-1/TLD-like proteases are multifunctional regulators of extracellular matrix deposition and growth factor signaling. Mice null for two of these family members, Bmp1 and Tll1, have previously been generated. The Bmp1 −/− and Tll1 −/− mouse strains have developmental defects that lead to perinatal and embryonic death, respectively. However, as has been shown *in vitro*, the phenotypes of these single null mice suggest that the products of the Bmp1 and Tll1 genes are capable of functional substitution for each other *in vivo*. In this study, we have generated mouse embryos null for both the Bmp1 and the Tll1 genes in an attempt to negate the functional substitution of their gene products. These Bmp1/Tll1 "double knockouts" have served as an excellent genetic tool to analyze the functions of the BMP-1/TLD-like protease family *in vivo*. Substrates affected by the loss of these two genes include proteins necessary for extracellular matrix deposition (such as type I procollagen) as well as proteins involved in early embryonic patterning (such as the BMP antagonist chordin).

193. **Functional Genomic Analysis of Cellular Morphology Using High-Throughput RNAi Screens.** A. Kiger,* B. Baum,*,[1] S. Armknecht,* M. Chang,* M. Jones,† A. Coulson,† S. Jones,‡ B. Sönnichsen,‡ C. Echeverri,‡ and N. Perrimon.* *Department of Genetics, HHMI/Harvard Medical School, Boston, Massachusetts; †The Wellcome Trust Sanger Institute, Cambridge, England; and ‡Cenix BioScience GmbH, Dresden, Germany. [1]Current address: Ludwig Institute for Cancer Research, University College of London, England.

The sequencing of the *Drosophila* genome provides us with an unprecedented resource. New technologies, however, are required to systematically analyze the functions of the ~14,000 predicted genes. The simple addition of double-stranded RNA (dsRNA) to *Drosophila* cells in culture reduces or eliminates the expression of target genes by RNA interference (RNAi), efficiently phenocopying loss-of-function mutations. Thus, genomewide screens using RNAi methodology offers an approach to systematically identify genes with cell-based functions. Here we describe our efforts at developing high-throughput RNAi screens using *Drosophila* cells cultured in 384-well plates and automated microscopic imaging. For pilot screens, we generated a set of 1000 dsRNAs from genes, predicted to encode regulators central to many fundamental cellular processes, including all kinases, phosphatases, small GTPases, and GTPase regulators. We screened this collection to identify genes that control cell morphology and cytoskeletal organization in different cell assays. By visualizing actin filaments, microtubules, and DNA, we identified many distinct cellular defects that allowed us to group the genes into phenotypic classes. In this way, we identified components of linear signaling pathways that differentially affect cell form and function. To identify additional components in these pathways, we then carried out RNAi modifier screens. This work shows that RNAi screens in cell culture assays are ideal for the identification of genes and additional pathway components that affect specific cell functions, including aspects of cell morphology.

194. **Genetic Analysis of the Roles of BMPs and FGFs in Forebrain Patterning.** S. K. McConnell and J. Hébert. Department of Biological Sciences, Stanford University, Stanford, California 94305.

During development, the embryonic telencephalon is patterned into different areas that give rise to distinct adult brain structures. Several secreted signaling molecules are expressed at putative signaling centers in the early telencephalon. Multiple Bmp genes are expressed at the dorsal midline and have been hypothesized to pattern the medial–lateral axis, whereas Fgf8 is expressed at the anterior end of the telencephalon and may pattern the anterior–posterior axis. Using a Cre/loxP genetic approach to disrupt genes during telencephalic development, we addressed the role of BMP and FGF signaling *in vivo* by abolishing expression of the receptors Bmpr1a and Fgfr1. In the absence of Bmpr1a, cells of the choroid plexus (the most medial/dorsal telencephalic derivative) fail to be specified or differentiate, and instead remain as proliferative cells. These cells do not adopt the fate of neighboring cells, but do express dorsal midline markers. These results suggest that BMPs induce the formation of the choroid plexus and play an essential role in patterning the medial–lateral axis of the telencephalon. In the Fgfr1-deficient telencephalon, striking morphological defects are observed at the anterior end of the telencephalon, where the olfactory bulb fails to form normally. Examination of the proliferation state of anterior telencephalic cells supports a model for initial olfactory bulb formation in which a decrease in proliferation is required for bulb evagination. Together the results demonstrate an essential role for Fgfr1 and Bmpr1a in patterning and morphogenesis of the telencephalon.

195. **Dual Roles for FGF Signaling in Promoting Zebrafish Hindbrain Development.** Lisa Maves and Charles B. Kimmel. Institute of Neuroscience, 1254 University of Oregon, Eugene, Oregon 97403-1254.

The segmentation of the vertebrate hindbrain into rhombomeres is highly conserved, but how early hindbrain patterning is established is not well understood. We have found that rhombomere 4 (r4) is an early-differentiating rhombomere that functions as an organizing center in the middle of the zebrafish hindbrain. Two fibroblast growth factor (FGF) signals, FGF3 and FGF8, are expressed early in r4 and are together required for the development of r5 and r6. Transplantation of r4 cells can induce expression of early r5/r6 markers, demonstrating that r4 tissue has organizing activity. Misexpression of FGF3 or FGF8 can also induce expression of r5/r6 markers, showing that FGF signals mediate the organizing activity of r4. These findings underscore the significance of organizing centers in patterning the vertebrate neural plate. Additionally, FGF3 plays an earlier role in hindbrain development by promoting posteriorization of the neuroectoderm during gastrulation. FGF3 signaling from mesendoderm promotes expression of hindbrain markers. In particular, misexpression of FGF3 in anterior mesendoderm is sufficient to posteriorize the forebrain and thus likely mediates the posteriorizing activity previously attributed to nonaxial mesendoderm. Our findings thus support dual, sequential roles of FGF signaling in promoting hindbrain development.

196. **Generating the Mammalian Neocortical Area Map.** E. A. Grove. Department of Neurobiology, Pharmacology and Physiology, University of Chicago, Chicago, Illinois 60637.

The mature mammalian neocortex is the most functionally and anatomically complex structure of the brain, yet the cortical primordium is a simple hollow vesicle. Thus, the cortical primordium is a ready target for gene transfer via microelectroporation. This approach was used in mouse to test a new model in which the neocortical area map is generated, not by unique mechanisms, as previously proposed, but by patterning strategies and gene families used elsewhere in the embryo. Surprisingly, the neocortex is therefore a useful new system for elucidating patterning mechanism. Putative cortical signaling centers express members of the fibroblast growth factor (FGF), bone morphogenetic protein (BMP), and WNT protein families. Findings indicate that FGF8, from a source at the anterior pole of the cortical primordium, specifies anterior–posterior (A/P) positional identity in the area map. Areas are shifted posteriorly in the map by augmenting the endogenous embryonic source of FGF8 and shifted anteriorly when the FGF signal is reduced. Introducing a second, posterior source of FGF8 reverses A/P polarity locally, eliciting partial area duplications. Duplicates appear mirror reversed with respect to native areas. Further findings suggest these manipulations shift the thalamic innervation of the neocortex in an area-appropriate manner and that area duplicates are functional. How FGF8 contributes to patterning here or at other embryonic sites remains unclear. Thus, current experiments, combining electroporation and mouse mutant analysis, focus on identifying transcription factors that act downstream of FGF8 to pattern the area map or that regulate the position of the FGF cortical patterning source.

197. Abstract #197 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

198. **Genetic Analysis of Axonal Guidance in the Zebrafish Embryo.** Jing Zhang, Shuxia Zhao, and Michael Granato. Department of Cell and Developmental Biology, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania.

In the vertebrate embryo, different populations of spinal motor neurons initially share common paths, but at choice points diverge onto a cell-type-specific path. Elegant embryonic manipulations have provided evidence that motor growth cones respond to cues along their trajectory to select their cell-type-specific path. However, little is known about guidance cues that direct pathway selection of motor growth cones locally at choice points. The zebrafish embryo is an excellent model system in which to study pathway selection of spinal motor neurons. As in other vertebrates, zebrafish spinal motor neurons have distinct identities and targets. Each somitic hemisegment is typically innervated by three pioneering motor neurons. On their way toward their synaptic targets, their growth cones encounter choice points, where they are confronted with trajectory choices. In unplugged mutant embryos, growth cones reach the somite choice point, but make inappropriate pathway decisions. We have previously shown that the unplugged gene acts as an adaxial cell-derived cue controlling pathway choice of motor axons. As the first motor growth cones approach the choice point, adaxial cells migrate away, suggesting that unplugged activity influences growth cones by a contact-independent mechanism. Our results suggested a model by which the unplugged gene encodes or is essential to produce a somite derived signal that elicits differential guidance decisions in motor growth cones. To test this model and to identify the molecular nature of the unplugged gene, we positionally cloned the unplugged gene. We will present ongoing molecular analysis of the unplugged gene to provide insights how unplugged activity controls motor axon guidance.

199. **EphA4–Ephrin Interactions in Axon Pathfinding.** C. E. Krull, J. Eberhart, M. E. Swartz, and E. B. Pasquale. Biological Sciences, University of Missouri, Columbia, Missouri; and The Burnham Institute, La Jolla, California.

Motor axons extend precisely from the neural tube to muscle targets in the hindlimb. We are interested in the role of Eph family members in motor axon pathfinding. Previously, we showed that certain EphA members were expressed dynamically by motor neurons, their axons, and limb mesoderm. Motor neurons in the medial lateral motor column (LMC) project to ventral muscle in the hindlimb and do not normally express EphA4. In contrast, motor neurons in the lateral LMC extend EphA4-positive axons to dorsal limb muscle. To examine the role of Eph–ephrin signaling, we ectopically expressed EphA4 and GFP primarily in LMC(m) neurons and subsequently analyzed their projections. Ectopic EphA4 in LMC(m) neurons alters the trajectories of their axons: these neurons now project aberrantly via the dorsal nerve trunk into the hindlimb. In control embryos, LMC(m) neurons expressing GFP project normally into the ventral hindlimb. Expression of Lim family transcription factors is unaltered in ectopic EphA4 motor neurons, suggesting that motor neuron identity is unchanged. However, axon projection errors do appear to occur while axons sort into dorsal versus ventral nerve trunks. Our results thus far suggest that EphA4 promotes dorsal axon projections by LMC neurons. Furthermore, these data offer a testable model by which Eph–ephrin signaling controls axon pathway selection in the limb, by either axon–axon or axon–limb mesoderm interactions. Experiments are in progress to test this model by expressing kinase-dead EphA4 in LMC(l) neurons or by ectopically expressing ephrins in dorsal limb mesoderm and analyzing subsequent effects on axon pathway selection.

200. **Identification of a Cell Autonomous Neuronal Function for Commissureless in Axon Guidance.** Vicki L. McGovern and Mark A. Seeger. Department of Molecular Genetics and the Neurobiotechnology Center, The Ohio State University, Columbus, Ohio 43220.

Commissureless (Comm) is required for proper guidance of commissural axons across the midline of the CNS in the *Drosophila* embryo. In the absence of Comm, axons that would normally project across the midline fail to do so and instead project ispilaterally on their respective sides of the midline. *In situ* hybridization reveals strong *COMM* mRNA accumulation at the midline during development of the embryonic axon scaffold. Immunohistochemistry reveals strong COMM protein expression along the tracts of commissural axons. Previously, this difference in mRNA and protein expression patterns was explained by the "transfer hypothesis." It was proposed that COMM protein is somehow transferred to commissural axons as they project across the midline. However, more recent *in situ* hybridizations reveal neuronal mRNA accumulation in addition to midline accumulation. Confocal microscopy also shows a more equal distribution of COMM staining in punctate vesicles and along commissural axon pathways compared to previous HRP immunohistochemical-based detection of COMM protein. Moreover, when a myc-tagged version of COMM was expressed at the midline in an otherwise wild-type *comm* background no transfer of COMM-MYC to the axon tracts was detected. These observations led us to ask the question: is Comm functioning in a cell autonomous fashion in neurons? We have addressed this issue by using the MARCM (mosaic analysis with repressible cell marker) system to generate and label individual *comm*− clones in a *comm*+ background. Clones that were wild type for *comm* crossed the midline 85% ($n = 101$) of the time. In comparison, clones homozygous for a hypomorphic allele or a null allele of *comm* crossed the midline 70% ($n = 122$, $P = 0.015$) and 58% ($n = 132$, $P < 0.0001$) of the time, respectively. Therefore, cell autonomous neuronal expression of Comm is important for formation of commissural projections in *Drosophila*.

201. **Global Analysis of Gene Expression in *Caenorhabditis elegans*.** S. K. Kim. Stanford University, Stanford, California 94305.

The genomes for three animals have now been sequenced, providing biologists with a wonderful and powerful tool to explore the genetic basis of metazoan life. Nearly all of the genes have been identified in worms, flies, and humans, and the next step is to uncover their function. We have produced DNA microarrays containing essentially every gene in *Caenorhabditis elegans* and are using them as powerful functional genomics tools to examine expression changes on a global scale and thus speed up the process of genetic analysis. The use of DNA microarrays has allowed us to enter a new age in molecular genetics in which we can dissect cell, developmental, and disease pathways more completely and more sensitively than ever before. We are using global approaches to study development, behavior, and aging in *C. elegans*. We have developed a method to isolate RNA from specific tissues and used it to determine gene expression profiles for the germ line, muscles, and neuronal tissues. We have scanned the entire genome for genes regulated by Ras signaling, Hox transcriptional control, and sex regulation. We are conducting a large study of gene expression changes during aging, both in normal worms and in long-lived mutants. Not only have these projects identified hundreds of new tissue-specific genes, but they have yielded several surprising results that only became apparent using a whole-genome approach. We have found that the entire X chromosome is inactivate in the germ line and that expressed genes appear in bunches along the chromosome possibly due to chromatin domains. We have performed more than 1200 *C. elegans* DNA microarray experiments and have assembled them into a large gene expression database. One can now profile gene expression changes and find coregulated genes not only from one experiment but from a wide variety of developmental and growth conditions. We visualized coexpressed genes using a three-dimensional gene expression topomap, in which coregulated genes appear close together on one of 44 gene expression mountains. The expression topomap can be used to ascribe functions to the large fraction of genes in the genome whose functions were previously unknown.

202. **Unraveling the Genetic Hierarchy of Muscle Development Using Genetics and Genomics.** Eileen E. M. Furlong,* Ruben D. Artero,† Mary K. Baylies,† and Matthew P. Scott.* *Department of Developmental Biology, Genetics and HHMI, Stanford University Medical School, Stanford, California 94305; and †Department of Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021.

Muscle progenitors arise from groups of cells in the mesoderm. In *Drosophila* these are composed of two distinct myoblast populations, the founder cells (FCs) and fusion-competent myoblasts (FCMs). Once specified, FCs and FCMs become attracted to each other and fuse together to form a multinucleated myotube. The events of myoblast specification, fusion, and differentiation are highly regulated to ensure that each resulting muscle has its correct size, shape, attachment, and innervation. Many or all of these unique properties are imparted by specific transcription factors expressed in the muscles founder cell. We are using genomic and genetic approaches to understand what genes are required to develop a functional muscle. Using a combination of an embryo sorter and DNA microarrays, we compared the transcription patterns in normal embryos to embryos that lack all muscle or, alternatively, that have ectopic myoblasts, at different developmental stages. This approach successfully identified hundreds of new genes involved in muscle development. One new muscle-specific transcription factor identified is a homolog of vertebrate Gli. We and others have shown that this protein is both essential and sufficient to induce the transcription of muscle fusion genes. The founder cells represent a very small percentage of the cells within the embryo making it difficult to detect gene expression changes within these cells. To address this we have used ectoptic expression of activated Ras or Notch, in a mesoderm-enriched genetic background, to drive cells to adopt either a FC or a FCM cell fate, respectively. We identified 83 genes with differential expression between FC-enriched embryos and FCM-enriched embryos. These genes provide the molecular basis for the different developmental properties of these two types of muscle cells. One newly identified FC-enriched gene is *phyllopod*. We show through loss and gain of function experiments that *phyllopod* plays a critical role in determining the identity of a subset of muscles.

203. **Common Mechanisms Underlying Growth Cone Guidance and Axon Branching.** K. Kalil, F. Tang, and E. W. Dent. University of Wisconsin–Madison, Madison, Wisconsin.

During development growth cones direct growing axons into targets. Axon guidance also occurs through interstitial branching from the axon shaft. How do such branches form? High-resolution

imaging of rodent cortical neurons showed that the growth cone demarcates sites of axon branching by lengthy pausing behaviors. Interstitial axon branches later develop from remnants of the large paused growth cones. To investigate how the cytoskeleton reorganizes during new growth at axon branch points and in growth cones, microtubules and actin filaments were fluorescently labeled and imaged in living cortical neurons. In both regions of the axon microtubules reorganize by splaying apart and fragmenting. Splaying of looped or bundled microtubules is accompanied by focal accumulation of f-actin. In the transition regions of growth cones and at axon branch points dynamic microtubules interact with actin by copolymerization. Drugs that attenuate microtubule or f-actin dynamics prevent axon branching and cause undirected axon outgrowth. The inhibitory axon guidance molecule Semaphorin 3A reduces axon branching and in the growth cone depolymerizes actin filaments and disrupts microtubule organization. Calcium imaging in cortical growth cones revealed that high-frequency calcium transients are associated with growth cone pausing and branching whereas rapidly growing growth cones exhibit few calcium transients. These results show that growth cone pausing is closely related to axon branching and suggest that common mechanisms regulate axon growth from both regions.

204. **Using Multiphoton Microscopy to Explore the Dynamics of Embryonic Development.** Jayne M. Squirrell. University of Wisconsin–Madison, Madison, Wisconsin.

A major challenge for fluorescence imaging of living cells is maintaining viability during, and subsequent to, prolonged exposure to excitation illumination. This is particularly true when imaging embryo development in which processes of interest may occur on the order of hours or even days. Furthermore, development often involves complex three-dimensional changes of a thick specimen. One promising technique for addressing these two potential problems, namely maintenance of specimen viability and deep optical sectioning, is multiphoton laser scanning microscopy (MPLSM). MPLSM utilizes lower-energy photons and restricts fluorophore excitation to the focal plane, thus reducing the total photodamage in thicker specimens. Furthermore, the longer wavelengths used for MPLSM can penetrate deeper into tissue with less scattering than comparable confocal laser scanning microscopy. Additionally, MPLSM can be used in conjunction with other experimental optical techniques, such as laser ablation. Examples of data from a number of organisms will be presented that demonstrate a variety of applications of MPLSM to the analysis of embryo development. These examples emphasize the potential advantages of MPLSM imaging for studying embryonic development.

205. **Single-Molecule Physiology under an Optical Microscope: How Molecular Machines May Work.** Kazuhiko Kinosita, Jr. Cener for Integrative Bioscience, Okazaki National Research Institutes, Okazaki 444-8585, Japan.

A single molecule of protein (or RNA) enzyme acts as a machine which carries out a unique function in cellular activities. To elucidate the mechanisms of various molecular machines, we need to observe closely the behaviors of individual molecules, because these machines, unlike man-made machines, operate stochastically and thus cannot be synchronized with each other. By attaching a tag that is huge compared to the size of a molecular machine, or a small tag such as a single fluorophore, we have been able to image the individual behaviors in real time under an optical microscope. Stepping rotation of the central subunit in a single molecule of F1-ATPase has been videotaped, and now we can discuss its detailed mechanism (1, 2). RNA polymerase has been shown to be a helical motor that precisely tracks the right-handed double helix of DNA (3), whereas myosin V has been shown to proceed as a left-handed spiral around an actin filament which is a right-handed double helix (4). Huge tags such as micrometer-sized plastic beads also allow the manipulation of individual molecules with, e.g., optical or magnetic tweezers (3–6). I personally believe that molecular machines operate by changing their conformations. Thus, detection of the conformational changes during function is our prime goal. 1. K. Kinosita Jr. *et al.,* 2000, *Phil. Trans. R. Soc. Lond. B* **355,** 473–489; 2. R. Yasuda *et al.,* 2001, *Nature* **410,** 898–904; 3. Y. Harada *et al.,* 2001, *Nature* **409,** 113–115; 4. M. Y. Ali *et al., Nature Struct. Biol.,* in press; 5. Y. Arai *et al.,* 1999, *Nature* **399,** 446–448; 6. T. Nishizaka *et al.,* 1995, *Nature* **377,** 251–254.

206. **Size Control.** M. Raff. University College London, London WC1E 6BT, United Kingdom.

The size of an organ or organism depends mainly on the number and size of the cells it contains. We have been studying rodent myelinating glial cells to determine how cell size and cell number are controlled. Remarkably little attention has been paid to the controls on cell growth (cell enlargement). This neglect is surprising, as it is cell growth that ultimately limits the growth of all organisms. Most proliferating cells grow before they divide, but it is not known how growth and division are coordinated to ensure that the cells do not get too large or too small. In studies on purified rat Schwann cells, we have found that extracellular signals can control growth and cell-cycle progression separately and that the size of proliferating cells depends on the rates of both cell growth and cell-cycle progression, rather than on a cell-size checkpoint that monitors cell size. Cell numbers depend on controls on cell proliferation and cell death. Most types of differentiated cells develop from dividing precursor cells that divide a limited number of times before they terminally differentiate. It is not known what stops cell division and initiates differentiation. We have found that oligodendrocyte precursor cells have an intrinsic timing mechanism that limits how long they divide for. The timing mechanism depends on a number of intracellular proteins, whose concentrations increase or decrease progressively as the precursor cells proliferate, as well as on the mitogen PDGF and thyroid hormone.

207. **The Control of Body Size in *Manduca sexta.*** Fred Nijhout. Duke University, Durham, North Carolina.

Because adult insects do not grow, adult body size is determined by the size a larva has achieved when it begins metamorphosis. Larval growth and body size in the tobacco hornworm, *Manduca sexta,* are controlled by the interplay of two developmental hormones, juvenile hormone and ecdysone. During the last larval instar, juvenile hormone inhibits the secretion of ecdysone and this inhibition is relieved only after a larva reaches a critical body size. The relief of inhibition requires both the cessation of juvenile hormone secretion and the synthesis and activity of juvenile hormone esterase. Evolutionary changes in body size in *Manduca* have been shown to be explained by genetic changes in only three factors: the critical weight, the rate of juvenile hormone decay, and the growth rate of the larva. Experimental manipulations of these regulatory mechanisms and how they relate to body size regulation in other insects will be discussed.

208. Abstract #208 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

209. ***Drosophila* Inhibitor of Apoptosis (DIAP1) Affects the Actin Cytoskeleton and Is Required for Border Cell Migration.** Erika R. Geisbrecht and Denise J. Montell. Johns Hopkins School of Medicine, Baltimore, Maryland.

One of the primary roles of the GTPase Rac is regulation of the actin cytoskeleton. This holds true for processes during *Drosophila* development, including axon guidance and border cell migration. The border cells are a group of 8–10 cells that delaminate from an epithelial monolayer and migrate in between nurse cells to the nurse cell/oocyte border during stages 8–10 of oogenesis. Expression of a dominant-negative form of DRacA in the border cells causes a migration defect in 90% of egg chambers. Other genes that are known to interact with Rac (*trio, PAK, dock*) are not required for border cell migration. Also, of the known genes that are required for this process (*E-cadherin, slbo, taiman*), none has been found to interact with Rac. To identify such candidates, a targeted mis-/overexpression screen using 2300 EP lines (Rorth *et al.*, 1999) was carried out. About 30 genes were identified that when overexpressed in a dominant-negative Rac background rescued the migration defect. One of the lines isolated contains a P-element inserted upstream of the *thread* gene, which encodes *Drosophila* Inhibitor of apoptosis (DIAP1). Loss-of-function mutations cause border cell migration defects in mosaic clones, but surprisingly, no apoptosis. However, filamentous actin in lost from the lateral and basal regions of outer follicle cells lacking *thread* function. In addition, the localizations of a few proteins known to regulate actin are also disrupted. This suggests a new role for DIAP1, independent of apoptosis, in regulating the actin cytoskeleton perhaps in association with Rac.

210. **Cell Biological Studies of Ephrin-B1 Signaling in Avian Neural Crest Cell Migration.** Andrew J. Ewald and Scott E. Fraser. Beckman Institute, Caltech, 139-74, Pasadena, California 91125.

Neural crest cells emerge from the dorsal neural tube in early vertebrate embryos. They migrate over hundreds of micrometers to their eventual location within the adult, giving rise to a diverse range of cell types, including neurons, glia, and cartilage. The mechanisms that pattern this cell migration remain largely unknown, although recent work suggests the involvement of several distinct mechanisms ranging from chemotaxis and haptotaxis to cell repulsion. To better test the cell biological role of the ephrin-B family of repulsive guidance molecules in guiding neural crest cells in avian embryos, we have developed two *in vitro* assays. The first approach involves photolithographically binding proteins on glass coverslips to build well-defined, reproducible patterns with which the neural crest cells can interact. Time-lapse confocal microscopy allows us to record the reactions of migrating neural crest cells to these patterns. By pharmaceutically perturbing cells during the assay, and imaging the resulting change in responsiveness to ephrin-B signals, we are determining the cellular requirements for ephrin-B signal transduction. Our second assay involves the focal application of ephrin-B proteins to the cell surface, with very precise control of the timing and cellular location of the stimulus. This enables us to determine the kinetics of neural crest cell responses over a range of experimental conditions. To elucidate the *in vivo* role of guidance molecules we identify *in vitro*, we are imaging the normal migration of neural crest cells with confocal and two-photon microscopy, revealing their three-dimensional pathways within the trunk of chick embryos. This allows a careful coregistration of migration pathways with the distribution of putative guidance molecules in the intact embryo.

211. **Two-Component Circuitry in *Arabidopsis* Cytokinin Signal Transduction.** Ildoo Hwang and Jen Sheen. Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts.

Cytokinins are essential plant hormones that control cell division, shoot meristem initiation, leaf and root differentiation, chloroplast biogenesis, stress tolerance, and senescence. Together with another plant hormone auxin, cytokinins can reprogram terminally differentiated leaf cells to stem cells and support shoot regeneration indefinitely in plant tissue culture. Thus, cytokinins are master regulators of plant growth and development that is highly plastic and adaptive as well as remarkably resilient and perpetual. Recent rapid advances have discovered hybrid histidine protein kinases (AHKs) as cytokinin receptors, histidine phosphotransfer proteins (AHPs), and nuclear response regulators (ARRs) as transcription activators and repressors in the *Arabidopsis* cytokinin signal transduction pathway. Similar components are also found in maize, suggesting a conservation of the cytokinin signaling mechanism in plants. There are four major steps in the cytokinin phosphorelay: AHK sensing and signaling, AHP nuclear translocation, ARR transcription activation, and a negative feedback loop through cytokinin-inducible ARR gene products. Analyses of mutants and transgenic tissues and plants support the importance of this central signaling pathway in diverse cytokinin responses, including promoting shoot meristem proliferation and leaf differentiation, as well as delaying leaf senescence.

212. **Rac and Rho Act in Parallel in Signaling Pathways That Ultimately Converge to Control Convergent Extension during *Xenopus* Gastrulation.** E. Tahinci and K. Symes. Boston University School of Medicine, Boston, Massachusetts.

Rho GTPases are known regulators of the actin cytoskeleton and their role in developmental processes that require cell movements has recently been demonstrated. To understand the role of Rho GTPases in cell movement during frog development, constructs that encode constitutively active or dominant inhibitory Rac or Rho were microinjected into *Xenopus* embryos. Culture of axial mesoderm explants from these embryos showed that Rho and Rac affected the movements of convergent extension. Further examination of axial mesoderm cell behavior by confocal microscopy revealed that cell intercalation was blocked in Rho GTPase-injected explants. Cells in these explants were still able to move but in an uncoordinated way. Examination of cell behavior in these explants revealed that both Rho and Rac signaling are necessary to establish the polarity of cytoplasmic protrusions around the cell body. Rho and Rac also influence the protrusive activity of the cells and are required for the formation of lamellipodia. Rac signaling alone, however, contributes to the formation of filopodia and Rho is important for the mediolateral elongation of intercalating mesodermal cells. These findings suggest that Rho and Rac control convergent extension movements by eliciting specific changes in the actin cytoskeleton during gastrulation. They further support a model for Rho and Rac in which they participate in parallel signaling pathways that ultimately converge to control convergent extension.

213. **Finding Their Way—Role of PI3K in Directional Sensing.** R. A. Firtel, S. Funamoto, and R. Meili. Section of Cell and Developmental Biology, CMG, UCSD, La Jolla, California 92093-0634.

Phosphatidylinositol 3-kinase (PI3K) is a key regulator of chemotaxis in leukocytes and *Dictyostelium* cells. We have demonstrated that the downstream PI3K effectors Akt/PKB and PhdA are required for proper cell polarity by regulating (1) PAKa and myosin assembly and (2) actin polymerization, respectively, during chemotaxis. Our findings suggest a model by which the translocation of PH-domain-containing proteins, which bind the PI3K lipid products, to the leading edge results from the localized activation of PI3K at this site on the membrane. We demonstrated that PI3K-GFP translocates to the membrane upon chemoattractant stimulation and localizes to the leading edge during chemotaxis and is mediated by specific N-terminal domain. Constitutive localization of PI3K to the membrane via a myristoylation site results in simultaneous activation of downstream effector pathways along the entire plasma membrane when cells are placed in a chemoattractant gradient. The phosphoinositide 3′ phosphatase PTEN attenuates downstream PI3K signaling. PTEN is on the membrane of unstimulated cells, is transiently released from the membrane coincident with PH domain membrane localization, and is reduced in concentration at the leading edge of migrating cells but not at other regions of the membrane. PTEN's subcellular localization during chemotaxis and redistribution in response to chemoattractants are consistent with a role in establishing and maintaining a phosphoinositide-enriched domain at the leading edge.

214. **A Secreted Cell-Number Counting Factor Represses Intracellular Glucose Levels to Regulate Group Size in *Dictyostelium*.** Wonhee Jang, Binh Chiem, and Richard H. Gomer. HHMI/Biochemistry, Rice University, Houston, Texas 77005.

Little is known about tissue size regulation. When the simple eukaryote *Dictyostelium discoideum* starves, the cells use relayed pulses of cAMP as a chemoattractant to aggregate into dendritic streams, which then break up into evenly sized groups of 2×10^4 cells. Each group then forms a fruiting body. We found that a secreted 450-kDa protein complex called counting factor (CF) regulates the group size. Computer simulations indicated that stream breakup could be induced by decreasing cell–cell adhesion and/or increasing cell motility, causing the stream to tear apart, and that having a secreted diffusible factor regulating this could regulate group size. We find that CF regulates group size in part by repressing internal glucose levels. Adding 1 mM exogenous glucose negates the effect of high levels of extracellular CF and mimics the effect of depleting CF on glucose levels, cell–cell adhesion, expression of the adhesion molecule gp24, and motility. The decreased glucose level increases the size of the cAMP-induced cAMP pulse. This then increases the level of actin polymerization and myosin heavy chain phosphorylation. The latter decreases the extent of myosin polymerization at the cell cortex, allowing actin-rich pseudopodia to form. CF increases the expression of the actin-crosslinker ABP-120, which stabilized the pseudopodia. The extended pseudopodia then cause the cell to move. These results suggest that CF regulates group size by regulating specific aspects of adhesion and motility and that glucose is a downstream component in part of the CF signaling pathway.

215. **Feedback Loops and Gradients Determine Cortical Domains during Planar Cell Polarity Signaling.** Dali Ma, Dave Tree, Chung-hui Yang, Mike Simon, and Jeff Axelrod. Stanford University School of Medicine, Stanford, California.

Many epithelial cells acquire a polarity orthogonal to their apical–basal axes, known as planar cell polarity (PCP). PCP may be manifested by arrays of asymmetrically organized cellular structures or multicellular developmental units. In *Drosophila,* PCP is evident in the parallel arrays of cuticular hairs, sensory bristles, and the repeating units of the eye. The system works with remarkable precision: of the 30,000 cells in a wild-type fly wing, all are correctly oriented. PCP is regulated by a pathway utilizing Frizzled as a receptor and Disheveled as a signal transducer, but is otherwise distinct from the Wnt/Wingless signaling pathway. Wing cells position a single prehair at the distal vertex by selecting and marking this cortical site for prehair growth. Frizzled and Disheveled determine the cortical mark by accumulating at the distal side of wild-type cells. We have shown that Frizzled and Disheveled are positioned by the action of a Prickle-dependent feedback loop that functions between adjacent cells. One consequence of this non-cell-autonomous mechanism is that polarization propagates by causing cells to align with their neighbors. We will provide evidence that the global cue for this alignment is the opposing expression gradients of Dachsous and Four-jointed and that their input is transduced through the cadherin Fat. The interaction between the global Fat signal and the propagation resulting from the feedback loop produces an exceptionally high fidelity response.

216. **Scents and Sensibility: Development of Chemosensory Neurons in *Caenorhabditis elegans*.** P. Sengupta. Biology Department, Brandeis University, Waltham, Massachusetts 02454-9110.

The ability of animals to sense the rich aromas of their environment is due largely to the presence of many different chemosensory neuron types with characteristic sensory response profiles. We are interested in understanding how complexity of function is generated among chemosensory neurons. Our system of choice is the chemosensory nervous system of the nematode *Caenorhabditis elegans*. These animals navigate their environment using a small number of chemosensory neurons, each of which responds to distinct subsets of chemicals. We have taken advantage of the availability of specific chemosensory cell-type markers and performed genetic screens to identify mutants in which the development of individual sensory neurons is affected. This approach has allowed us to identify complex networks of spatially and temporally regulated transcription factors that play roles at different steps in the developmental cascades. Moreover, by performing such screens using different cell-type-specific markers, we have determined that sensory neuron diversity is generated through the use of similar transcription factor modules, with specificity provided by the particular genes expressed, the cellular context, and the lineal history of the cell type. Our results also suggest that most, if not all chemosensory neurons in *C. elegans* share a common developmental default state. This default fate may be used as a basic building block upon which the distinct fates of different sensory neurons are elaborated by the action of specific gene cascades. Ongoing work is aimed at further dissecting these developmental pathways and also determining how the functional properties of these chemosensory neurons are additionally modified by experience and the environment.

217. Abstract #217 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

218. **BMP Gradient and Tbx Genes Determine the Dorsal–Ventral Polarity of Eye.** Kazuko Koshiba-Takeuchi, Jun Takeuchi, Takayuki Suzuki, and Toshihiko Ogura. Nara Institute of Science and Technology, Nara, Japan.

Recently, Tbx5 and Vax genes have been shown to play key roles during pattern formation of eye along its dorsal–ventral (D-V) axis. Misexpression of these genes induces both morphological alterations and aberrant retinotectum projection. Careful analysis has revealed that Tbx5 and Vax genes are expressed in dorsal and ventral sides of retina, leaving the middle portion negative for both Tbx5 and Vax. This prompts us to explore factor(s) that are supposed to be expressed in the middle part and specify the continuous positional values along the D-V axis of retina. During development of chick eye, three Tbx genes, Tbx5, Tbx3, and Tbx2, are expressed dorsally, yet in different gradients with distinct ventral limits. This indicates that the retina can be divided into four domains: Tbx5/3/2-positive, Tbx3/2-positive, Tbx2-positive regions, and Tbx-negative/cVax-positive region. This combinatorial expression (Tbx code) in the retina was found to be established by the action of BMP4 emanating from the dorsal side of the retina. Implantation of BMP4-soaked beads has shown that BMP4 affects both the Tbx code and the retinotectum projection in a dose-dependent manner; a terminal zone of ventral retinal axons was shifted from dorsal to ventral side of tectum in proportion to the increase of applied BMP4 concentration. This shift was accompanied by the distinct induction of Tbx genes in the retina. These results suggest that the combinatorial expression of Tbx genes (Tbx code) is established by the action of BMP4, specifying the positional identity of the retina along its D-V axis.

219. **Establishing the Preplacodal Region.** Anna Litsiou, Keith W. Mc Larren, and Andrea Streit. Department of Craniofacial Development, King's College, Guy's Hospital, London SE1 9RT, United Kingdom.

In vertebrates, cranial sensory ganglia and vital parts of the sensory organs arise from transient ectodermal thickenings, the cranial placodes, next to the anterior neural plate. These develop in a multistep process through inductive interactions with surrounding tissues, beginning at late gastrulation and continuing until after neural tube closure. It has been proposed that all cranial placodes are derived from a single, common region—the pre-placodal region (PPR)—and that their initial induction involves a common mechanism. However, the tissue interactions and molecular mechanisms that establish the PPR have not been investigated. We have constructed a fate map of the chick ectoderm over several developmental stages showing that indeed otic and epibranchial placode precursors arise from a common region and are initially interspersed with future neural, neural crest, and epidermal cells. Video time lapse analysis reveals that extensive cell movements accompany placode formation and constant cell rearrangements occur. We then compared the fate map with several molecular markers (ERNI, dlx5, GATA3, Six4, msx1) to determine which, if any, coincides with the position of placode precursors. We next investigated the tissue interactions that establish the PPR and found that the apposition of neural and epidermal tissue is sufficient to induce some PPR markers, while others are dependent on signals from the underlying mesoderm. Finally, we have studied the functional role of some PPR specific genes by misexpression in different regions of the primitive streak stage embryo.

220. **Lamina-Selective Synapse Formation in the Visual System.** Joshua R. Sanes, Joshua A. Weiner, and Masahito Yamagata. Washington University Medical School, St. Louis, Missouri.

Many regions of the vertebrate central nervous system are divided into multiple laminae parallel to the pial and ventricular surfaces. The dendritic and axonal arbors of multiple neuronal subpopulations are restricted to specific laminae. This laminar restriction is a major determinant of synaptic specificity. In the chick, for example, retinal ganglion cells (RGCs) project to just 3 of 16 laminae in the tectum, and each ganglion cell's axon arborizes in just a single lamina. Similarly, the dendrites of many RGC subclasses arborize in just 1 or 2 of the >10 sublaminae within the retinal inner plexiform layer, where they receive inputs from amacrine and bipolar cells whose processes are also lamina restricted. To learn how laminar specificity is encoded, we have identified genes expressed in subsets of RGCs whose axons or dendrites share laminar destinations. After describing strategies for molecular fingerprinting of RGC subsets, we will focus on sidekick-1 and -2, homologous transmembrane immunoglobulin superfamily molecules that we have identified. We will present evidence that sidekicks are concentrated in the synaptic clefts that connect complementary lamina-restricted subsets of amacrines and RGCs and that sidekicks help direct these processes to appropriate sublaminae during development.

221. **Formation of the Rod Photoreceptor Cell Mosaic.** James M. Fadool. Department of Biological Science, Florida State University, Tallahassee, Florida.

The zebrafish *Danio rerio* has become a powerful model for the genetic analysis of retinal development. During photoreceptor cell development, cone differentiation progresses as a wave across the retina forming a crystalline-like mosaic, while rod differentiation has been described as scattered or random. However, in the present study, a novel pattern of rod differentiation in relation to the cone mosaic was uncovered using a line of transgenic zebrafish. Zebrafish demonstrating high-level expression of enhanced green fluorescent protein (EGFP) in rod photoreceptors under the control of the *Xenopus* principal opsin promoter were crossed with *albino* fish. The spatial and temporal pattern of rod differentiation was investigated in live embryos or retinal whole mounts maintained in teleost saline by confocal and multiphoton microscopy and by immunochemistry. As previously reported, in 5-day-old transgenic larvae, the pattern of rods appeared random. However, by 9 days, EGFP expression revealed the rods to be positioned in regularly spaced, parallel rows. At later stages and in young adults, the arrangement was evident at the rod terminals, myoids, and ellipsoids. Immunochemistry revealed the rod pattern to be centered on the UV-sensitive cone. Formation of the rod pattern was examined at the retinal margin, where newly differentiated rod inner segments appeared to project around the UV-sensitive cones, and the rod outer segments coalesce at the position of the immature UV-cone outer segment. The rod mosaic revealed in the transgenic line provides a novel model to explore the genetic mechanisms underlying photoreceptor cell development.

222. Abstract #222 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

223. **Analysis of *Arabidopsis* Root Pattern Formation: Tissue-Specific Ectopic Expression of the "Moving" Putative Transcription Factor Short-Root.** G. Sena, K. Nakajima, J. Jung, and P. N. Benfey. Department of Biology, New York University, New York, New York.

The *Arabidopsis* root radial pattern is formed and maintained by well-defined asymmetric divisions of a set of stem cells (initials)

located in the apical meristem. While it has been shown that this process is primarily dependent on positional information, little is known about the actual signaling mechanism. The *short-root* (*shr*) mutant is defective in one asymmetric division in the root meristem, so that the resulting radial pattern is missing one tissue (endodermis). The SHR gene, a putative transcription factor, in the root is expressed in the central tissue (stele), but not in the stem cells nor in the endodermis. We have shown (1) that the SHR protein appears to move from the stele into all the "nearest-neighbor" adjacent cells, where it is localized in the nuclei. No SHR is detectable in tissues more distant from the source stele. Nothing is known about the mechanism responsible for such nearest-neighbor movement. Moreover, it has also been shown (1) that ectopic expression of SHR can result in altered cell fates and multiplication of cell layers. Tissue-specific competence to regulate SHR movement and to respond to SHR with alteration of cell fates and/or cell divisions seems to be part of the regulation of the positional information required for the establishment of the root radial pattern. Here we present preliminary data about ectopic expression, through tissue-specific promoters, of a fully functional protein fusion SHR-GFP. Its effect on radial pattern modification, cell fate alteration, and SHR-GFP movement will be discussed. 1. K. Nakajima *et al.*, 2001, *Nature* **413,** 307-311.

224. Abstract #224 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

225. **Functional Consequences of Epidermal Patterning in *Arabidopsis* Leaves.** J. L. Croxdale, M. L. Spletter, and T. D. Sharkey. University of Wisconsin–Madison, Madison, Wisconsin.

Stomata regulate gas exchange between the interior of the leaf and the atmosphere. These specialized epidermal cells are essential for terrestrial plants; mutant plants without stomata survive only a few days. Patterns of stomatal distribution-random, ordered, and clustered-in *Arabidopsis* are under genetic control. We looked for functional consequences to pattern differences by measuring photosynthesis in three genotypes. Wild-type (Nossen ecotype) plants and mutant plants of GL1 and TRY, genes identified as transcription factors and known to affect initiation of trichomes, another specialized epidermal cell type, were used. Gas exchange studies showed that leaves with an ordered distribution of stomata (*gl1* plants) have a greater photosynthetic rate than do leaves with a random (WT plants) or a clustered (*try* plants) pattern. The time to steady-state carbon exchange parallels the photosynthetic rate data implicating impaired stomatal or chloroplast function or organization and number of interior leaf cells may affect gas exchange rates. We have studied selected aspects of stomatal and chloroplast function in the three genotypes to evaluate them as agents responsible for gas exchange characteristics. Since wild-type leaves do not have the optimal photosynthetic rate, their leaves have no evolutionary advantage and present a dilemma.

226. **RNAi of *Deformed* Ortholog *Lox6* Leads to Axonal Patterning Defects in the Leech Nervous System.** M. E. Mercado-Pimentel and G. O. Aisemberg. Lehman College and Graduate Center of CUNY, New York, New York.

The Hox genes code for transcription factors that play important roles in the specification of anterior–posterior polarity in animals. *Lox6,* an ortholog of *Drosophila* is expressed at early stages of development in the central and peripheral nervous systems of the leech *Hirudo medicinallis. Lox6* expression starts at embryonic day 6 (E6), when development of the nervous system begins, and declines at E10, when axonal pathways are well established. As development proceeds, *Lox6* expression extends from the posterior aspect of rostral neuromere 2 (RN2), to the last caudal neuromere. To examine the role of *Lox6* in nervous system development, we injected double-stranded RNA (dsRNA) of *Lox6* into embryos at E6-8 and dissected them 1–4 days after the injection. The *Lox6* dsRNA blocked *Lox6* expression in over 90% of the embryos injected. The phenotypes observed in these embryos included reduced size of the longitudinal connectives, axonal defects in the bipolar neurons, a reduced number of axons in Faivre's nerve and in the anterior and posterior root of the ganglia, and misrouting of axons. These defects were not observed in control embryos. These results show that *Lox6* is involved in establishing normal axonal pathways. (Supported by NIH 5S11NS37519 and 2S06GM08225.)

227. **A Link between Developmental Timing and Circadian Rhythms.** Heather Gardner, Mili Jeon, and Ann Rougvie. University of Minnesota, Minneapolis, Minnesota.

Cells of a developing animal must integrate temporal, spatial, and sexual cues to adopt the appropriate fate. The heterochronic genes of *Caenorhabditis elegans* specify temporal information during postembryonic development. Mutations in these genes alter the timing of the terminal differentiation of the lateral hypodermis. For example, hypodermal cells in *lin-42* mutants terminally differentiate one stage earlier than in the wild type. We cloned *lin-42* and found that the predicted *lin-42* protein is most similar the PERIOD (PER) family of proteins from *Drosophila* and other organisms. This similarity is especially interesting since PER is involved in a second type of biological timing mechanism, the control of circadian rhythms. This connection is further highlighted by experiments that demonstrate that *per* mutants exhibit defects in growth rate. The region of similarity between LIN-42 and PER is mostly restricted to a protein interaction domain known as a PAS domain. We are investigating other similarities between LIN-42 and PER. A hallmark of *per* expression is that its mRNA levels oscillate with a 24-h periodicity. We have found that, like *per, lin-42* message levels oscillate, but with a faster rhythm that is synchronized to the molting cycles. In flies, the PAS domain of PER mediates an interaction with TIMELESS. We have identified a timeless homolog in *Caenorhabditis elegans, tim-1,* but to date have no evidence for interaction between LIN-42 and TIM-1. Screens to identify factors that interact with LIN-42 are in progress, and analysis of candidate LIN-42-interacting proteins will be described.

228. **A Screen for Factors Affecting the Expression Pattern of *lin-48* in *Caenorhabditis elegans.*** Rong-Jeng Tseng,* and Helen M. Chamberlin.*,† *Program in MCD Biology and †Department of Molecular Genetics, The Ohio State University, Columbus, Ohio 43210.

lin-48 encodes an Ovo-like zinc finger protein. It is important for normal development of the *Caenorhabditis elegans hindgut.* Genetic and expression data show that *lin-48* is a hindgut-specific target for EGL-38, a Pax transcription factor important for development of the hindgut, the egg-laying system, and structures in the male tail. The *lin-48::gfp* expression in the hindgut is significantly reduced in *egl-38* mutants. Likewise, mutations of EGL-38-binding elements in the *lin-48* promoter disrupt hindgut expression. How-

ever, *lin-48::gfp* expression in other cells is unaffected in these experiments. Since EGL-38 functions and is expressed in cells in addition to the hindgut, we predict that it functions in combination with another factor to regulate *lin-48* specifically in hindgut cells. To identify additional factors regulating the tissue-specific expression of *lin-48*, we performed an F2 genetic screen to isolate mutants with altered *lin-48::gfp* expression pattern. Following EMS mutagenesis 24 mutant lines have been isolated from a screen of 18,000 mutagenized gametes. These mutants can be classified into two major classes. Mutants in the first class have reduced GFP expression in the hindgut. The responsible genes in this class might include genes that function with *egl-38* in the hindgut, genes important for EGL-38 expression or activity, or *egl-38* itself with new mutations. In the second class, mutants have ectopic or altered GFP expression pattern. The mutated genes in the second class might include negative regulators of *egl-38* or factors that affect *lin-48* expression in other pathways. Currently, we are working on complementation tests and mapping to identify the genes. Four complementation groups were found in the first class including a new *egl-38* allele. Meanwhile, we have characterized some mutant lines exhibiting altered growth rate, viability, and morphology in the hindgut or vulva.

229. **Identification of Cofactors That Act with EGL-38, a Pax Transcription Factor, to Activate *Caenorhabditis elegans lin-48* Gene Expression.** Sama F. Sleiman and Helen M. Chamberlin. MCDB Program and Department of Molecular Genetics, Ohio State University, Columbus, Ohio 43210.

Pax transcription factors play an important role in development of organs and cells. They can activate different target genes in different cells and can act as monomers or in a complex with other transcription factors. In *Caenorhabditis elegans*, EGL-38 is a Pax factor that plays a key role in the development of several organs. To better understand its function, we decided to characterize the regulation of a target gene, *lin-48*. Previously, two *cis*-regulatory elements important for *lin-48* expression (lre1 and lre2) were shown to be sensitive to EGL-38 activity *in vivo*. *In vitro*, lre2 binds EGL-38, while lre1 does not. To explain this difference, we hypothesize that EGL-38 may act through lre1 in combination with other unidentified factors as part of a protein complex. To better characterize lre1, we employed molecular and biochemical strategies. We mutagenized DNA sequences in the lre1 area to define sequences necessary for its activity. We generated mutant transgenes consisting of *lin-48* promoter-driving green fluorescent protein (GFP) and tested them for expression in the animals. These experiments show that lre1 activity requires a region of approximately 33 bp. Since Pax factors bind to only 20 nucleotides, this is consistent with additional factors acting at this site. To test whether EGL-38 binds lre1 *in vivo*, and to identify potential interacting proteins, we are in the process of creating a FLAG-tagged EGL-38, which we plan to use for coimmunoprecipitation experiments with embryonic extracts.

230. **A Role for the Polycomb Group in Development of the *Caenorhabditis elegans* Male Nervous System.** Jennifer Ross and David Zarkower. Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, Minnesota 55455.

Development of the *Caenorhabditis elegans* male-specific nervous system depends on interplay between two regulatory pathways, one involving the *Hox* genes *mab-5* and *egl-5* and the other involving the conserved sexual regulator *mab-3*. Males harboring *Hox* or *mab-3* mutations lack V rays, sensory structures required for mating. The Hox proteins activate the bHLH gene *lin-32* to specify the V rays, while the *doublesex* homolog MAB-3 synergizes with the *Hox/lin-32* pathway to promote V ray differentiation. V rays can be restored in Mab-3(−) males by overexpression of *lin-32*, suggesting that LIN-32 acts as a primary determinant of V ray fate, while MAB-3 potentiates LIN-32 activity. To better understand the mechanism by which the *mab-3* and *Hox/lin-32* pathways converge to direct V ray development, we performed a screen to identify mutations that suppress the Mab-3 V ray defect. Three mutations identified in this screen are alleles of *mes-3*, a novel gene that functions with Polycomb group (PcG) homologs *mes-2* (*enhancer of zeste*) and *mes-6* (*extra sex combs*) to maintain silencing in the *C. elegans* germline. In flies and vertebrates, the PcG of chromatin-interacting proteins maintains domains of *Hox* repression during development. We find that *mes-2*, *mes-3*, and *mes-6* act upstream of the *Hox* genes during V ray development and are likely to suppress *mab-3* by increasing *Hox/lin-32* activity. Patterning of the V rays is disrupted in *mes* mutants, providing further evidence that the MES proteins modulate *Hox* expression in the male soma. Thus in nematodes, as in other phyla, the PcG acts in somatic cells to regulate *Ho*.

231. **Temporal Control of Pattern Formation by LIN-57/HBL-1, a *Caenorhabditis elegans* Hunchback-like Protein.** Ming Li, Aric Daul, Mandy Volk, and Ann Rougvie. University of Minnesota, Minneapolis, Minnesota.

The heterochronic genes of *Caenorhabditis elegans* are global temporal regulators that control the relative timing and sequence of diverse events during postembryonic development. One of these events is the terminal differentiation of the lateral hypodermal "seam" cells, an event that occurs during the final (L4) molt in wild-type animals. During the L4 molt, seam cells exit the cell cycle, fuse, and synthesize a morphologically distinct adult cuticle. Mutations in heterochronic genes advance or retard the timing of hypodermal terminal differentiation, resulting in larvae with adult-type hypodermis or adults with larval-type hypodermis. Mutations in *lin-57* fall into the former class, causing hypodermal cells to terminally differentiate abnormally early, during the third molt. Cloning of *lin-57* revealed that it is allelic to *hbl-1* and encodes a hunchback-related protein. *lin-57::gfp* fusions are down-regulated during the L4 stage and this down-regulation is mediated by the *lin-57* 3′UTR. Examination of the 3′UTR reveals multiple binding sites for the 21 nt microRNA encoded by *let-7*. The temporal expression pattern of *let-7* (late L3 through adult) is consistent with its proposed role in *lin-57* down-regulation.

232. **Genetic Dissection of the Ci Signaling Complex.** Mark A. Lefers, Qun T. Wang, and Robert A. Holmgren. Northwestern University, Evanston, Illinois.

The Hedgehog (Hh) signaling pathway has been conserved throughout most of the animal kingdom and plays an essential role in the patterning of many organs and tissues. Much of our understanding of this pathway comes from *Drosophila* where Hh regulates the function of the transcription factor Cubitus interruptus (Ci) at three levels: protein stabilization, nuclear import, and activation. Regulation of Ci occurs in a cytoplasmic complex which is known to contain Ci, the kinesin-like protein Costal-2 (Cos2), the serine-threonine kinase Fused (Fu), and the Suppressor of Fused (Su(fu)) protein. By sequentially removing components of

the Ci signaling complex, it is possible to assay their roles in each aspect of Ci regulation. Our results show that Hh regulates the signaling complex at multiple levels. The Cos2–Ci core complex is able to mediate Hh regulated activation of Ci. Addition of Su(fu) to the core complex blocks nuclear import while the addition of Fu restores Hh regulation of Ci nuclear import and proteolytic cleavage. Fu participates in two partially redundant pathways to regulate Ci nuclear import; the kinase function plays a positive role by inhibiting Su(fu) and the regulatory domain plays a negative role in conjunction with Cos2. This complex pattern of regulation allows Hh signaling to establish distinct domains of Ci activity.

233. **Redox Regulation of DNA Binding by *Drosophila* Ultrabithorax 1b.** Sarah E. Bondos, Shiyama Mudali, Meredith Hanson, and Kathleen S. Matthews. Department of Biochemistry and Cell Biology, Rice University, Houston, Texas 77005.

DNA binding by many transcription factors is regulated by redox conditions. Here, we investigate the effects of oxidation and reduction on DNA binding by the *Drosophila* Hox protein Ultrabithorax 1b (Ubx1b). The full-length protein does not bind DNA in the presence of oxidized glutathione or hydrogen peroxide. Binding by full-length Ubx1b by oxidants was restored by dilution into buffer containing 5 mM DTT. Since the isolated homeodomain binds DNA both under reducing or under oxidizing conditions, this effect requires amino acid sequences outside the homeodomain. The redox effect on Ubx is likely mediated by disulfide bond formation between cysteine residues. Full-length Ubx1b is protected from oxidization by pretreatment with *N*-ethylmaleimide (NEM), which alkylates cysteine residues. Further, the Ubx1b contains one cysteine residue in the homedomain and two in the N-terminus, a region required for the redox effect. Therefore, cysteines are located in regions likely to mediate a redox effect on DNA binding. Mutations of the six cysteine residues to alanine have been generated, and identification of the cysteine residues involved in disulfide bond formation is under way. Interestingly, cysteine residues are frequently conserved in the homeodomain of Hox proteins and always located on the surface of the homeodomain. We hypothesize that the DNA-binding effects demonstrated *in vitro* may be modulated *in vivo* by changes in the half cell potential of the tissue during cell division and development or mimicked by interactions with heterologous proteins.

234. **A Potential Network of Proteins Interacting with Ultrabithorax.** Xin-Xing Tan, Sarah E. Bondos, and Kathleen S. Matthews. Department of Biochemistry and Cell Biology, Rice University, Houston, Texas 77005.

Eukaryotic gene expression appears to be regulated by multicomponent regulatory complexes which allow a variety of signals to be integrated into specific transcriptional responses through protein–protein interactions. We have applied yeast two-hybrid screens and phage display assays to identify protein partners for Ultrabithorax 1b (Ubx1b). This protein is a member of the Hox/Hom-C family of transcription factors that play a critical role in determining segmental identity and generating morphological diversity in developing organisms. A mutant of Ubx1b, with three amino acid changes that abrogate constitutive transcription activation by the Ubx1b-LexA hybrid, was employed for screening. Interactions were confirmed by yeast two-hybrid screens, in which wild-type Ubx1b was hybridized with the B42 activation domain. Interactions between putative partners and wild-type Ubx1b were also checked by phage display, which indicated that the strength of most protein interactions was comparable to the known Ubx–Exd interaction. Putative partners can be classified into four major functional categories: transcription regulation, RNA binding/processing, DNA repair, and cell cycle regulation/signaling pathways. The physiological significance of the observed interactions has been postulated by known genetic interactions, subcellular location, expression patterns, or functions of the proteins involved. These results suggest that Hox/Hom-C proteins may integrate a broad spectrum of cellular signals during early development.

235. **Transcription Control by Hunchback in the Early *Drosophila* Embryo.** I. Brun, V. Napolitano, J. Lin, and C. Desplan. New York University, New York, New York.

The morphogen hunchback (hb) plays a critical role during *Drosophila* early development, as well as during nervous system development. It encodes a zinc finger transcription factor and can function both as an activator and a repressor. Hb has been very well studied; however, little is known about the molecular mechanisms of hb function which, we suspect, are likely to involve cofactors. To identify such proteins, we performed an interaction screen using the yeast two-hybrid system. Using hb fused to the GAL4 DNA-binding domain as a bait, we screened a *Drosophila* embryonic cDNA library fused to the GAL4 activation domain. We identified 15 different known genes and 26 different unknown genes. Sixteen of these genes are described as transcription factors or putative transcription factors. This screen also revealed that hb was able to homodimerize. We used the two-hybrid system to map hb dimerization domain and showed that a 50-amino-acid fragment containing the last two zinc fingers motifs is able to interact with the full-length hb protein. Interestingly, the same domain of Ikaros, the putative textithb homolog in vertebrates, is involved in its homodimerization. We also identified a repression domain located in the last 150 C-terminal amino acids of hb and an activation domain located in the 145 N-terminal amino acids. Now, we would like to test if the repression activity and the dimerization are linked. If this is the case, we would like to propose a model where, at high concentration, hb can form homodimers and act as a repressor. At lower concentration, it would associate with other proteins to acquire different specificities.

236. **Patterning of *Drosophila* Leg Sensory Bristles through Coordinate Function of the Hedgehog, Dpp, and EGFR Pathways.** C. Kwon,* R. Hays,† J. Fetting,* and T. Orenic. *Department of Biological Sciences, University of Illinois at Chicago, Chicago, Illinois; and †Department of Molecular Biology and Pharmacology, Washington University, St. Louis, Missouri.

The microchaetae of the *Drosophila* leg are organized into a series of longitudinal rows found at specific positions around the leg circumference. This orderly arrangement of leg sensory organs requires the position-specific expression of the hairy (h) gene, which is expressed in two pairs of longitudinal stripes, one pair that traverses the D/V axis (D/V-h) and another pair that runs along the A/P axis (A/P-h). h patterns the leg bristle rows by regulating the periodic expression of the proneural gene achaete (ac). In the pupal leg disk, a stripe of ac expression on either side of each h domain defines the primordia of the leg bristle rows. In the absence of h function, ac expression expands into the regions normally occupied by H, resulting in disorganized bristle rows in the adult. Thus, proper regulation of h expression is fundamental to patterning of the leg bristle rows. D/V-h expression is directed by dorsal (D-h) and ventral (V-h) specific enhancers that integrate signals from the

Hedgehog (Hh), Decapentaplegic (Dpp), and Wingless (Wg) pathways. The D-h enhancer consists of two subelements, a Hedgehog response element (HE) and another which functions in dorsal restriction of the D-h stripe, a Dpp/EGFR-responsive element (DE). We have identified a site in the DE that modulates the function of the HE by mediating ventral repression and defining stripe width. The sequence of this site suggests that it responds to Dpp, to EGFR, and to the transcriptional repressor Brinker.

237. **Involvement of the MADS Domain Transcription Factor Mef2 in Vein Formation in the *Drosophila* Wing.** Hitoshi Matakatsu,* Young Soeck Lee,† Jaeseob Kim,† and Seth S. Blair.* *Department of Zoology, University of Wisconsin–Madison, Madison, Wisconsin; and †Department of Biological Science, BMRC, Seoul, Korea.

Mef2 is a MADS domain-containing transcription factor. Previous data from vertebrate and *Drosophila* indicate that Mef2 is expressed in and required for the proper differentiation of muscle cell lineages. Our work indicates that Mefs is also involved in the development of an ectodermal tissue, the wing vein of *Drosophila*. The *Drosophila* wing is composed of two ectodermal cell sheets, dorsal and ventral, that differentiate a stereotyped pattern of longitudinal and cross-veins during late larval and early pupal life. We identified two transposable element insertion lines that gave patterns of ectopic longitudinal and cross-veins in adult wings. Both were allelic to *mef2* and had inserted 5′ to the known *mef2* transcripts. Antibody staining identified that Mef2 protein was expressed in vein and intervein cells in pupal wings, but not at larval stages of wing development. In the hobo element insertion line hb330 this pupal expression was missing. This is in agreement with the changes we observed in the expression of vein-specific and intervein-specific markers. *rhomboid* (*rho*) encodes a transmembrane protein that is expressed in developing veins, where it helps activate the *Drosophila* EGF receptor. In hb330 flies *rho* expression is normal in early stages of vein formation, but ectopic *rho* is observed in pupal stages. Thus, Mef2 suppresses *rho* expression in pupal intervein cells. We will also discuss the results of overexpression and genetic mosaic experiments.

238. **Klumpfuss, the *Drosophila* Wilm's Tumor Suppressor 1 (WT-1) Ortholog, Regulates Programmed Cell Death in the Developing Retina by Modulating Activity of the EGFR/Ras Pathway.** J. C. Rusconi and R. L. Cagan. Washington University Medical School, St. Louis, Missouri.

Programmed cell death (PCD) plays a central role in the sculpting and maturation of developing epithelia. In adult tissue, PCD also plays a vital role in the prevention of malignancy though the removal of damaged cells. Here, we report that mutations in klumpfuss, a nuclear factor implicated in cell fate decisions in the nervous system and other tissues, result in an excess of support cells during maturation of the developing *Drosophila* pupal retina. These ectopic cells are the result of a partial and specific failure of apoptotic death during normal cell fate selection. We also provide both genetic and biochemical evidence that klumpfuss regulates apoptosis through down-regulation of the epidermal growth factor/Ras signaling pathway. klumpfuss represents the closest *Drosophila* ortholog of the Wilm's tumor suppressor-1 nuclear factor, and our results suggest a potential mechanism by which mutations in Wilm's tumor suppressor-1 may result in a number of cancer types including pediatric kidney tumors.

239. **Molecular Characterization of Seven Eyeless Alleles with eyeless GAL4 Activity in *Drosophila melanogaster* and Evidence for Tissue-Specific Regulation of Eyeless Protein Transport into the Nucleus.** Jason Clements and Patrick Callaerts. Department of Biology and Biochemistry, University of Houston, Houston, Texas.

The Pax-6 homolog eyeless has been shown to have an essential role in the development of the *Drosophila* eye and brain. To further elucidate the role of Eyeless in these tissues, we mutagenized the GAL4 enhancer trap line OK107, which reproduces the eyeless expression pattern in the eye and brain with high fidelity. This mutagenesis resulted in seven eyeless mutants that maintained the OK107 GAL4 activity, capable of driving any UAS line in an eyeless pattern. These seven alleles will allow us to perform experiments to rescue the eyeless phenotype in a homozygous mutant background. Furthermore, the molecular characterization of these alleles provides new insights into the function and requirements of the various domains of the Eyeless protein. One of these alleles, ey107/6B.1, results in an amino acid substitution within the third helix of the homeodomain. Interestingly, this allele does not seem to affect Eyeless function in the brain, where it localizes normally, but fails to be transported to the nucleus in the eye, suggesting the possibility of an eye-specific nuclear localization signal in the Eyeless protein. Furthermore, this allele is the only described eyeless allele that affects the Eyeless protein which is homozygous viable, suggesting that Eyeless function in the brain is unaffected.

240. **Fibroblast Growth Factors-3, -8, and -10 in Mouse Inner Ear Development.** T. J. Wright and S. L. Mansour. University of Utah, Salt Lake City, Utah 84112.

Otic development initiates early in development when signals from the hindbrain and mesoderm induce a region of ectoderm to thicken, forming the otic placode. The placode then invaginates and forms a vesicle that undergoes cellular differentiation and morphogenesis, resulting in a mature inner ear. Studies of mice, chick, and zebrafish have implicated several signaling molecules, including fibroblast growth factors (Fgfs)-3, -8, and -10 in these processes. Expression analyses suggest that Fgf-3 and Fgf-10 could signal to the ectoderm from the hindbrain and the mesoderm, respectively. To determine the combinatorial roles of these molecules, we have generated mice that lack both Fgf-3 and Fgf-10. Surprisingly, the Fgf-3/-10 double mutant embryos lack otic vesicles. These embryos do not show otic expression of pax-2 and expression of additional placode markers is mislocalized. Hindbrain patterning in rhombomeres 4–6 in the double mutants is normal. In addition, an intermediate otic phenotype is present in embryos with three mutant alleles. These results suggest that fgf3/10 signaling plays a direct role in placode specification. Otic development in zebrafish requires fgf-3 and fgf-8. To determine if fgf-8 plays a similar role in mice, we have generated mice that lack Fgf-3 and have half the normal dose of Fgf-8. At E9.5, loss of Fgf-3 leads to a small, ventrally localized otic vesicle. In embryos lacking fgf-3 and missing one copy of fgf-8 the vesicle is further reduced in size. These data suggest that fgf-8 has a function in mouse otic development. Characterization of this phenotype using markers of otic and hindbrain development will be presented.

241. **Conditional Inactivation of the Rx Homeobox Gene Results in Viable Anophthalmic Animals.** Vera A. Voronina,*′† Serguei V. Kozlov,† Peter H. Mathers,* and Mark Lewandoski.† *Sensory Neuroscience Research Center, West Virginia University, Morgantown, West Virginia; and †Cancer and Developmental Biology Laboratory, National Cancer Institute, Frederick, Maryland.

Inactivation of the mouse Rx gene in the germline results in anophthalmia and perinatal death due to a variety of neural defects. We sought to elucidate Rx gene function at various developmental stages using Cre-lox technology. We generated mouse lines carrying an allelic series at the Rx locus: a null allele lacking exon 2, a putative hypomorphic allele due to insertion of neomycin resistance gene into intron 1, and a conditional allele with wild-type activity but subject to Cre-mediated inactivation. Null homozygotes recapitulate the previously published phenotype, including early postnatal lethality, anophthalmia, brain defects, and cleft palate. Hypomorphic homozygotes are anophthalmic and die soon after birth. Removal of the neomycin resistance gene (via Flp-mediated recombination) from the putative hypomorphic allele rescues both lethality and proper eye development, allowing this allele to be used for the conditional deletion. To specifically knock out Rx after the initiation of retinal development begins, the conditional allele was inactivated in mice carrying the Foxg1-Cre transgene, which is expressed in the anterior neural ridge at E8.5 and later in the nasal retina. These animals lack eyes and optic nerves but otherwise appear to be normal, making them an ideal model for true anophthalmia as seen in the patient population. Molecular analysis of these animals is currently under way.

242. **Analysis of *GBX* Genes during Neurogenesis.** Samuel T. Waters,* Catherine Wilson,* Rhonda Anderson,† and Mark Lewandoski.* *CDBL, CCR, NCI-Frederick, NIH, Frederick, Maryland; and †SAIC-Frederick, Frederick, Maryland.

The *GBX* class of homeobox genes consist of *Gbx1* and *Gbx2* which are nearly identical throughout their homeodomains and carboxy termini. The importance of *Gbx2* in the specification of the anterior hindbrain is well established via studies of embryos homozygous for a targeted null allele. Analysis of the brain in these mutant embryos detected an absence of a normal cerebellum. Here, we present analysis of a *Gbx2* hypomorphic allele, in which expression is compromised due to the presence of a neo-resistance cassette (neo-r). RT-PCR analysis demonstrates that the neo-r interferes with normal *Gbx2* splicing: a hybrid RNA is formed with a portion of the neo-r spliced into exons 1 and 2, thus reducing wild-type *Gbx2* mRNA. Hypomorph homozygotes display a less severe phenotype than null homozygotes in that only the cerebellum midline (vermis) is deleted. Molecular analysis reveals that the normal refinement of the expression pattern of genes controlling the mid-/hindbrain organizer fails to occur in hypomorph homozygotes. In contrast to *Gbx2*, little is known about the expression of *Gbx1* during embryogenesis and its role in the development of the neuronal system. We have isolated a nearly full-length *Gbx1* cDNA from an E12.5 mouse library. *In situ* analysis reveals that *Gbx1* is expressed in the allantois by E8.0 and in the intermediate mesoderm at E8.5. During neurogenesis *Gbx1* is expressed in a dynamic pattern starting at E8.0. *Gbx1* is not upregulated in embryos that lack *Gbx2*.

243. **Role of GAP-43 in Early Cerebellar Patterning.** Rashmi Mishra,* Libby Donahue,† Yiping Shen,† Karina Meiri,† and Shyamala Mani.* *National Brain Research Center, SCO 5, 6, and 7, Sector 15/II, Gurgaon, India–122001; and †Department of Anatomy and Cellular Biology, Tufts University School of Medicine, Boston, Massachusetts 02111.

GAP-43, a nervous system specific protein, is a major determinant of central nervous system (CNS) cytoarchitecture *in vivo*. Greater than 95% of GAP-43(−/−) mice die perinatally. Death is associated with two distinct defects in neuronal development: a defect in neurogenesis and errors in axon pathfinding. A defect in cerebellar foliation is one of the phenotypes that the GAP-43(−/−) mice exhibit. We are looking at the mechanisms by which loss of GAP-43 leads to specific defects in folial patterning. We hypothesize that defects in the proliferation of cerebellar neuron precursors underlie this phenotype. Initial results show that at E12.5, GAP-43(−/−) mice show a reduced rate of cell proliferation compared to wild-type controls. Furthermore, neurons in the GAP-43(−/−) mice appear to differentiate prematurely. This suggests that premature differentiation could result in a reduction in the pool of precursors that are still cycling, leading to an overall reduction of granule neurons in the cerebellum. How can this in turn affect patterning of the folia? One way could be by affecting the expression of patterning molecules such as En-2 and Wnt7b that may determine the position of fissures in the cerebellum. We are asking the question whether deregulation of cell proliferation can affect the expression of these patterning molecules. To this end, we are comparing the expression of En-2 and Wnt7b in the developing cerebellum in wild-type and GAP-43(−/−) mice.

244. **Roof Plate Formation and Function in the Brain.** Anne Lindgren, Richard Roberts, and Kathleen Millen. University of Chicago, Chicago, Illinois.

The roof plate is a central nervous system (CNS) structure required for patterning of the dorsal/ventral axis. Located along the entire dorsal midline of the embryo, it is composed of a specialized group of neuroepithelial cells that secrete soluble factors including Wnt and Bmp family members. Numerous studies have demonstrated a pivotal developmental role for the roof plate in the dorsal spinal cord. The role of the roof plate in the brain, however, is poorly understood. Our aim is to define the role of the roof plate in anterior CNS development by manipulating roof plate formation using both spontaneous and targeted mouse mutants. We previously demonstrated that the LIM-HD transcription factor Lmx1a is mutated in the spontaneous mouse mutant dreher and found that loss of Lmx1a function results in loss of roof plate in the developing spinal cord. In contrast, however, the dreher roof plate remains intact in anterior CNS regions. We hypothesize that Lmx1b, a closely related gene with overlapping dorsal midline expression, is functionally redundant to Lmx1a in the brain. Preliminary data from double null mutant embryos support this hypothesis in the developing cortex. Analysis of roof plate function in the developing midbrain and cerebellum is not possible using null mutants of Lmx1b due to the requirement of Lmx1b function for isthmus function. Through a combination of gene targeting and transgenic experiments, we will specifically eliminate both Lmx1a and Lmx1b from the developing roof plate in the mid-/hindbrain region, leaving Lmx1b isthmus expression intact. These experiments will address if cerebellar and midbrain neuronal specification is dependent on roof plate formation and function.

245. **Genetic Control of Dorsal–Ventral Identity in the Telencephalon: Cooperative Roles for Pax6 and Tailless (Tlx) in the Establishment of the Pallioganglionic Boundary.** Jan Stenman,*,† Ruth Yu,‡ Ron Evans,‡ and Kenneth Campbell.* *Division of Developmental Biology, Children's Hospital Medical Center, Cincinnati, Ohio 45229-3039; †Wallenberg Neuroscience Center, Department of Physiological Sciences, Lund University, Solvegatan 17, S-223 62 Lund, Sweden; and †Salk Institute, La Jolla, California 92037.

Previous studies have shown that Pax6 is crucial for correct dorsoventral patterning of the telencephalon. Homozygous Small eye (Pax6) mutants exhibit ectopic expression of ganglionic eminence markers, such as GSH2 and DLX, in the dorsal part of the telencephalon (i.e., the pallium). Here, we examine the role of Tailless (Tlx), an orphan nuclear receptor, in the establishment of dorsal ventral identity. Tlx is expressed at high levels throughout the telencephalic ventricular zone with the exception of the most dorsomedial and ventromedial portions. We show that $Tlx^{-/-}$ mutants have ectopic expression of ganglionic eminence markers in the most ventral part of the pallium. This mispatterning of the telencephalon is similar to that seen in Pax6 mutants; however, it is less severe. Interestingly, Pax6 is expressed in Tlx mutants, and vice versa, indicating that Pax6 and Tlx are part of two genetically convergent pathways. Analysis of compound Pax6/Tlx mutant embryos strengthen this conclusion. Indeed, $Tlx^{-/-}$;$Pax6^{+/-}$ embryos show a more pronounced dorsal expansion of ganglionic eminence markers into the pallium than Tlx mutants, however, a less severe expansion than that observed in Pax6 mutants. These findings indicate that Tlx and Pax6 cooperate genetically in the establishment of the pallioganglionic boundary.

246. **Incomplete Rescue of the Tbx6 Mutation Generates an Allelic Series of Phenotypes in the Mouse.** E. E. McFadden, D. R. Hamburger, and D. L. Chapman. Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania.

The transcription factor Tbx6 is absolutely required for the development of posterior paraxial mesoderm in the mouse, as evidenced by Tbx6 mutant embryos in which posterior somites are replaced by ectopic neural tubes. During our studies to identify the *cis*-acting regions responsible for directing Tbx6 expression, we generated transgenic mice bearing the Tbx6 regulatory regions driving the expression of a lacZ reporter gene. As this transgene includes the entire coding region of Tbx6, we have attempted to rescue the Tbx6 mutant phenotype with the transgene. The two separate transgenic lines express Tbx6 at different levels and variably restore somite formation. One line restores posterior somite formation when present in a single copy transgene; however, the rescue is incomplete, as revealed by severe fusions of the vertebrae and ribs. Marker gene analysis reveals that the somites of these "rescued" embryos lack anterior identity. A second transgenic line also fails to rescue the mutant phenotype, but this "rescue" is even less successful, with only a few vertebrae and ribs formed. Combinations of the different transgenic lines reveal a more robust rescue of the mutant phenotype, with only a few fused ribs present. Through our attempts to rescue the Tbx6 mutant phenotype by adding back Tbx6 via a transgene, we have generated an allelic series of Tbx6 phenotypes that resemble Notch signaling pathway mutants, in particular Delta3. We believe that mutations in the regulatory region of Tbx6, resulting in lowered levels of Tbx6 expression, could therefore lead to birth defects in humans.

247. **Foxc1 and Foxc2 Are Involved in Medial–Lateral Patterning of the Nonaxial Mesoderm in the Mouse Embryo.** Bettina Wilm* and Brigid Hogan.† *Department of Cell Biology, Vanderbilt University School of Medicine, and †Howard Hughes Medical Institute, Nashville, Tennessee 37232.

The closely related forkhead genes *Foxc1* and *Foxc2* are required for somitogenesis in the mouse embryo, since in compound homozygous null mutants no somites are formed (Kume *et al.*, 2001). We therefore investigated the fate of those cells originally fated to become somites. We have found that a number of genes normally expressed in the intermediate mesoderm are medially expanded in *Foxc1;Foxc2* compound mutant embryos, while a paraxial marker is strongly downregulated. Our data indicate that mesoderm cells located adjacent to the neural tube have become lateralized. While paraxial cells switch to intermediate fate, they do not assume a lateral plate fate, since expression of several lateral plate mesoderm genes appear to be more or less normal in *Foxc1;Foxc2* compound mutant embryos. Analysis of the allelic series of *Foxc1* and *Foxc2* compound mutant embryos revealed a gene dosage effect for the lateralization process. A weak lateralization is found in *Foxc2* single homozygous embryos, while it is more pronounced in *Foxc1*+/−;*Foxc2*−/− mutant embryos. Therefore, in *Foxc1*+/−; *Foxc2*−/− mutant embryos, somites coexpress paraxial and intermediate markers. In addition, lateralization occurs also within the somites, as we find expansion in the expression of a lateral dermomyotomal lip marker, *Lbx1*. We will discuss these data and present a model for the role of Foxc1 and Foxc2 in medial–lateral patterning of the nonaxial mesoderm.

248. **Withdrawn.**

249. ***Prx-Hox* Transgenic Mice: A Model for Dissecting the Molecular Basis for Functional Dominance of Posterior Hox Genes.** Melissa E. Williams and Jeffrey W. Innis. Department of Human Genetics, University of Michigan, Ann Arbor, Michigan 48109.

Hox genes encode highly conserved transcription factors that are expressed in specific domains during development. Posterior HOX proteins expressed ectopically in more anterior or proximal domains generally exert functional dominance over Hox gene products normally expressed in those regions, giving rise to malformations or "posterior" transformations. This interesting aspect of HOX function, first described in *Drosophila* and subsequently in mice, is termed posterior prevalence. Previous work in mice has shown that ectopic zeugopodal expression of *Hoxd13* or *Hoxd12* leads to shortening of skeletal elements. Thus, HOXD13 and HOXD12 are capable of exerting profound negative effects on limb growth; however, the molecular requirements for this property are incompletely understood. To explore the genetic requirements for posterior prevalence we have utilized the mouse *Prx-1* promoter to drive transgenic expression of Hox genes throughout the mesenchyme of developing limbs. The use of this promoter allows examination of overexpression proximally in the stylopod and zeugopod, as well as the autopod where the posterior Hox genes normally function. Transgenic *Hoxd13* misexpression leads to shortening of proximal limb elements and occasional preaxial polydactyly. This system provides a model to determine domains of HOXD13 that are necessary and sufficient to cause functional dominance as well as whether paralogs of the group 13 Hox genes have equivalent posterior prevalence functions on gene expression and skeletal pattern.

250. **A Role for the Polycomb Group Gene TPD in the Regulation of Establishment of Left–Right Asymmetry in the Chick.** Shusheng Wang, Xueyan Yu, and YiPing Chen. Department of Cell and Molecular Biology, Tulane University, New Orleans, Louisiana.

In the vertebrate animal body plan, the left–right (L-R) axis is specified in accord with the development of the anterior–posterior

(A-P) and dorsal–ventral (D-V) axes. The first morphological indication of L-R asymmetry is exhibited by the dextral looping of the developing heart. Left-sided signaling pathways and right-sided signaling pathways have been identified to interact each other in regulating correct L-R patterning. Multiple growth factors have been observed to express asymmetrically in Hensení's node of gastrulating chick embryo prior to the appearance of morphological L-R asymmetry. But how these signals are mediated by transcriptional regulators is still unknown. In an effort to identify new components in the signaling pathways regulating L-R axis development, we cloned a novel chick Polycomb Group gene TPD that exhibits a right-sided asymmetric expression in the node beginning from HH stage 5. Antisense TPD treatment of chick embryo showed a stage-specific effect on randomizing heart looping direction. Shh was ectopically expressed in the right side of Hensení's node in 25% of the antisense TPD-treated samples. Interestingly, ectopic expression of TPD by retrovirus in developing chick limb resulted in inhibition of limb outgrowth and down-regulation of Shh expression in the ZPA. Based on the results above, we propose that TPD function to mediate the repression of Shh expression in the right side of Hensení's node in the regulation of L-R axis development, which is consistent with the repressive effect of TPD on transcription in an *in vitro* assay. (Supported by NIH grants.)

251. **Tbx Genes and the Digit Identity.** Takayuki Suzuki, Jun Takeuchi, Kazuko Koshiba-Takeuchi, and Toshihiko Ogura. Nara Institute of Science and Technology, Nara, Japan.

Vertebrate digits are arranged along the AP axis of limb. Chick leg possesses four distinctive digits that are characterized by both number of phalanges and their morphology. Despite the extensive studies on the AP axis formation of limb bud, the molecular mechanism that specifies the distinct digits along the AP axis has remained unsolved. Tbx genes are expressed in temporally and spatially restricted manners and play essential roles during development of various tissues and organs. In addition, recent genetic analyses have revealed that mutations of Tbx genes cause specific morphological alterations. For example, mutations of human TBX3 cause the ulnar-mammary syndrome, which is characterized by the absence of digit 4 and/or 5. Since Tbx3 is expressed in the posterior side of limb bud, we speculate that Tbx3 specifies the identity of posterior digits, which develop in the Tbx3-positive area. To explore this hypothesis, we first studied expression patterns of Tbx2 and Tbx3 genes in developing chick limb bud. Anterior limits of posterior Tbx2 and Tbx3 expression coincide well with the positions of digit 4 and digit 3, respectively. These observations suggest that the identity of digit 4 and digit 3 is specified by Tbx2 and Tbx3, respectively. To explore further, we misexpressed these genes in developing chick limb. Interestingly, misexpression of Tbx3 induced transformation of digit 2 to digit 3. In addition, misexpression of Tbx2 transformed digit 3 to digit 4. These results strongly suggest that the identity of posterior digits is specified by Tbx2 and Tbx3 genes, highlighting the pivotal roles of Tbx genes in specification of digit identity.

252. **Modulation of BMP Activity by Heparan Sulfate Proteoglycans during Limb Cartilage Differentiation *in Vitro*.** M. C. Fisher, M. R. Seghatoleslami, C. N. Dealy, and R. A. Kosher. Department of Biostructure and Function, University of Connecticut Health Center, Farmington, Connecticut.

Bone morphogenetic protein 2 (BMP-2) contains a heparin-binding domain, suggesting that BMP-2 activity may be modulated by heparan sulfate proteoglycans (HSPGs). HSPGs could modify BMP-2 activity by restricting the amount of BMP-2 available for signaling or by facilitating the interaction between BMP-2 and its receptors. To explore these possibilities, we examined the effects of exogenous heparan sulfate or heparitinase treatment on the ability of BMP-2 to modulate the differentiation of limb mesenchymal cells in micromass culture. Exogenous BMP-2 supplied to micromass cultures before the onset of chondrogenesis suppressed chondrogenic differentiation and promoted apoptosis. However, BMP-2 added after the initiation of chondrogenesis stimulated cartilage differentiation. Exogenous heparan sulfate (HS) had little or no effect on the ability of BMP-2 to induce apoptosis. In contrast, HS dramatically enhanced the ability of BMP-2 to promote chondrogenesis and cartilage-specific gene expression. Pretreatment with heparitinase also potentiated the chondrogenic activity of BMP-2. HS or heparitinase alone had little effect. These results suggest that exogenous HS and heparitinase facilitate the ability of BMP-2 to stimulate chondrogenesis by preventing its interaction with endogenous HSPGs. Thus, endogenous HSPGs at the cell surface or in the extracellular matrix may normally modulate BMP activity during limb chondrogenesis by limiting its bioavailability. (Supported by NIH Grant HD22610.)

253. **Expression of *Xenopus* Zinc-Finger Transcription Regulator *sal* (*Xsal-3*) during Limb Development and Regeneration.** Mark W. Harty,* Trent Nguyen,† Michael W. King,‡ Anthony L. Mescher,* Michael C. Muzinich,* Rosamund C. Smith,§ and Anton W. Neff.* *Medical Sciences Program, IU School of Medicine, Bloomington, Indiana 47405; †M. D. Anderson Cancer Center, Houston, Texas 77030; ‡Terre Haute Center for Medical Education, IU School of Medicine, Terre Haute, Indiana 47809; and §Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285.

We show for the first time that a member of the *spalt* family (*Xsal-3*) is expressed at the right place and time to play a regulatory role in epimorphic regeneration. *Xsal-3* was identified in a competitive subtractive hybridization screen of regeneration blastemas minus regeneration-competent stage (st.53) hindlimbs. *In situ* hybridization showed *Xsal-3* expression in dynamic patterns in the limb mesenchyme throughout early limb development (stages 48–55). Expression peaks at stage 50. By stage 53 expression is restricted to subdomains in distal limbs. Stage 54 limb expression is in the interdigital spaces of the autopod. Expression declines through stage 55 and by stage 57 (regeneration-incompetent) *Xsal-3* is not expressed. Following limb amputation at stage 53 *Xsal-3* is reexpressed in the dedifferentiating region of the distal limb stumps with the highest level of expression 3 days postamputation. By day 7 the major expression is in the blastema. *Xsal-3* is not expressed in distal limb stumps after amputation at stage 57. In light of the recent findings that murine homolog of *Xsal-3, sall1,* acts as a transcriptional repressor we speculate that *Xsal-3* acts as a transcriptional repressor in the early limb bud, in the interdigital tissues, and in the dedifferentiation zone and blastemas of regenerating limbs.

254. **Developmental Functions of XKaiso, a Transcriptional Repressor Associating with the Xp120 Catenin in *Xenopus laevis*.** Si Wan Kim, Xiang Fang, Hong Ji, and Pierre D. McCrea. Department of Biochemistry and Molecular Biology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030.

Catenin proteins participate in cadherin-mediated cell–cell adhesion and additionally in nuclear signaling. p120 catenin

(p120ctn), a substrate of Src and receptor tyrosine kinases, binds to the membrane-proximal domain of cadherins. A novel transcription factor, Kaiso, interacts with p120ctn, suggesting that p120ctn may possess a nuclear function. We isolated the *Xenopus* homolog of Kaiso, XKaiso, from the hybridization screening of a *Xenopus* stage 17 cDNA library. XKaiso contains a N-terminal POZ domain involved in protein–protein interactions and a C-terminal zinc finger domain known to bind DNA. Coimmunoprecipitation of Xp120ctn and XKaiso has demonstrated their mutual association, while related experiments employing differentially epitope tagged-XKaiso constructs suggest that XKaiso may exist as a dimer. The XKaiso transcript is present maternally and expressed throughout early embryonic development, while its spatial expression was defined via *in situ* hybridization revealing localized expression in the brain, eye, ear, branchial arches, and spinal cord. Luciferase reporter assays employing a chimeric GAL4BD fusion of XKaiso indicate that XKaiso is a transcriptional repressor. To assess the developmental functions of XKaiso and Xp120ctn in *Xenopus*, XKaiso was exogenously expressed, resulting in ectodermal cell shedding. We are currently examining phenotypes resulting from XKaiso loss of function analyses in the developing embryo. Further, misexpression of XKaiso's POZ or zinc finger domains in isolation will be used to interfere with its function in a dominant-negative fashion. Ultimately, we hope to reveal the molecular pathway in which the Xp120ctn/XKaiso complex functions, which will require the identification of presently unknown upstream signaling components and downstream gene targets.

255. **A Double-Negative: FoxD3 Regulation of Nodal in *Xenopus* Mesoderm Formation.** M. E. Engleka, J. L. Lefebvre, A. B. Steiner, J. Walters, S. Yaklichkin, E. J. Craig, P. A. Labosky, and D. S. Kessler. Department of Cell Developmental Biology, University of Pennsylvania, Philadelphia, Pennsylvania.

Establishment of the mesodermal germ layer is a critical step in vertebrate embryogenesis. We report that FoxD3, a forkhead family gene expressed in Spemann's organizer, regulates *Xenopus* mesoderm formation and acts upstream of Nodal signals. In explants and embryos, FoxD3 induced mesodermal gene expression and mesodermal differentiation. The transcriptional activity of FoxD3 required for mesoderm induction was determined by fusing defined regulatory domains to the FoxD3 DNA-binding domain. Like FoxD3, Engrailed-FoxD3 induced mesoderm, while VP16-FoxD3 did not, suggesting that FoxD3 functions as a transcriptional repressor to induce mesoderm. To determine the requirement for FoxD3 in mesodermal development, embryos were injected with VP16-FoxD3 to antagonize FoxD3 function or an antisense morpholino oligo that inhibits FoxD3 translation. FoxD3 loss-of-function resulted in a block to axis formation, a failure to form axial mesodermal tissues, and an absence of mesodermal gene expression, indicating that FoxD3 is required for mesodermal development. In addition, FoxD3 functions in a non-cell-autonomous manner, via secreted factors, to induce mesoderm. Consistent with this result, FoxD3 was necessary and sufficient for Nodal gene expression and mesoderm induction by FoxD3 was dependent on Nodal signaling. Therefore, transcriptional repression by FoxD3 promotes Nodal expression and this function is required for mesoderm formation. The results suggest a disinhibition model of *Xenopus* mesoderm formation involving transcriptional repression of an inhibitor of mesoderm induction.

256. **Detection of Myogenic Transcription Factors in Electrocytes Lacking Sarcomeric Proteins in *Soriculus macrurus*.** Graciela A. Unguez, Colleen B. Jonsson, and Jung A. Kim. Departments of Biology and Chemistry Biochemistry. New Mexico State University, Las Cruces, New Mexico.

The MyoD family of basic helix–loop–helix (bHLH) transcription factors modulate muscle gene expression. However, the set of target genes whose expression depends on a distinct bHLH factor or a combination of these bHLH factors is not known. In the electric fish *Soriculus macrurus*, mature cells of the electric organ (EO) reveal a partial muscle phenotype characterized by the expression of desmin, actin, α-actinin, and acetylcholine receptors and the absence of expression of myosin heavy chain and tropomyosin. We hypothesize that the unique protein profile of the EO is modulated by a combination of bHLH proteins to create an expression pattern different from that of skeletal muscle. Using RT-PCR, we have begun to isolate genes for the four MyoD proteins from *S. macrurus*. Total RNA was isolated from EO, skeletal muscle and 2-week regenerating tails. Primer sequences were designed from alignments of heterologous sequences from teleosts and murine mRNAs. We have cloned partial MyoD and myogenin cDNAs from regenerating tails. The deduced amino acid sequence for the 290-bp cDNA fragment of MyoD contained 96 amino acid residues including the DNA-binding domain, whereas the 312-bp fragment of myogenin contained 104 amino acid residues outside the DNA-binding domain. Northern blots revealed the *S. macrurus* MyoD and myogenin cDNAs to be about 2370 and 1926 nt in length, respectively. Homologous primers were designed for RT-PCR experiments in which we measured the expression of these genes in EO, skeletal muscle, and brain. MyoD and myogenin were expressed in EO and muscle, but not in brain. The presence of myogenic transcription factors MyoD and myogenin in EO is intriguing in view of its complete absence of sarcomeric proteins, i.e., myosin heavy chain and tropomyosin. (Supported by NIH Grant SO6-GM08136-27.)

257. **Roles of the Nlz Zinc Finger Protein in Zebrafish Development.** A. P. Runko and C. G. Sagerstrom. Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical Center, Worcester, Massachusetts.

Our laboratory studies anteroposterior axis formation in the zebrafish embryo. A subtractive hybridization-based cloning approach has identified the *nlz* (nocA like zinc finger) gene, which is first expressed in the blastoderm margin and then in a broad dorsoposterior domain that extends from the rhombomere (r) 3/r4 boundary to the caudal end of the embryo. During somitogenesis, expression becomes restricted to the hindbrain where it progressively expands anteriorly to encompass both r3 and r2, while expression caudal to r4 is reduced. Ectopic expression of *nlz* in zebrafish embryos leads to a reduction in r3 gene expression (*krox20*, *ephA4*) that is at least partially replaced by the expansion of r4 gene expression (*hoxb1a*, *ephrinB2a*). To elucidate functional domains within *nlz*, a series of deletions within the *nlz* construct have indicated that the C2H2 zinc finger and an unidentified domain conserved among the Sp1 family of transcription factors are important for the r3/r4 phenotype and the C-terminus is required for nuclear localization. Defects in cellular movements of convergence were exhibited either with the injection of *nlz* constructs with N-terminal deletions or with a VP16 activation domain fused to full-length *nlz*. This phenotype is reminiscent of the expression of soluble Eph receptor tyrosine kinases and their ephrin ligands.

We hypothesize that *nlz* is involved in the coordination of cell migration and the proper formation of r3 and r4 through the regulation of Eph and ephrin signaling.

258. **Functions of Zebrafish Hox Paralog Group 2 and 3 Genes in Hindbrain and Pharyngeal Arch Development.** Michael Hunter and Vicky Prince. Departments of Molecular Genetics and Cell Biology and Organismal Biology and Anatomy, University of Chicago, Chicago, Illinois.

The hindbrain and pharyngeal arches are series of reiterated segments that form along the anterior/posterior (A/P) axis during early vertebrate head development. Proper specification of A/P identity of these segments is important for overall head patterning. Hox genes encode homeodomain-containing transcription factors that play an important role in specifying segment identity within the hindbrain and arches. We have used both loss-of-function (morpholino-mediated knock-down) and gain-of-function (misexpression) approaches to study the roles of Hox paralog group (PG) 2 and PG3 genes during head patterning. The two zebrafish PG2 genes, hoxa2 and hoxb2, are both expressed in neural crest cells that migrate into the second pharyngeal arch. We show that these genes function redundantly to specify second pharyngeal arch identity. Loss of Hox PG2 function causes second arch structures to phenocopy those of the first arch, whereas gain of function causes a reciprocal phenotype. In both cases duplicated arch structures form a mirror-image copy of endogenous structures suggesting the existence of an "organizer" between the arches. We are currently testing candidate genes for organizing activity. The zebrafish hoxa3 and hoxb3 genes are expressed in hindbrain rhombomeres (r) 5 and 6 and in neural crest cells that migrate into the third and posterior arches. We show that knock-down of these two genes prevents differentiation of the r5- and r6-specific abducens motor neurons. We are currently investigating additional phenotypes that result from loss and gain-of-function of Hox PG3 genes.

259. **Analysis of *meis* Genes Expression in Zebrafish Suggests a Role in the Development of Organs Derived from the Endoderm.** Frédéric Biemar,*,† Francesca Baraldi,* Nathalie Devos,* Jochen Holzschuh,† Joseph A. Martial,* Wolfgang Driever,† and Bernard Peers.* *University of Liège, Allée du 6 Août, 4000 Liège, Belgium; and †Universität Freiburg, Hauptrasse 1, 79104 Freiburg, Germany.

Hox genes encode homeodomain-containing transcription factors that function to establish body organization along the anterior–posterior axis in arthropods and vertebrates. Members of the TALE superclass of homeodomain proteins (i.e., Pbx/Exd and Meis/Hth) have been shown to act as cofactors of Hox protein function. By forming dimeric and trimeric complexes with Hox proteins *in vitro*, these partners provide enhanced DNA-binding affinity and specificity to the otherwise not very fussy Hox monomers. Pdx-1, another homeodomain protein whose gene does not lie on the *hox* gene clusters, has also been shown to interact with Pbx/Exd and/or Meis/Hth cofactors. Pdx-1 is essential for pancreas development during embryogenesis and for pancreas-specific gene expression in adulthood. We showed previously that Pdx-1 synergizes with Pbx1a and Prep1 on the UE-A element of the somatostatin promoter. Using the zebrafish as a model, we are employing a combination of gain and loss of function approaches to address the possible role of such trimers in the patterning of the endoderm and/or the development of endodermally derived organs. We have identified several members of the *meis/prep* family in zebrafish and studied their expression pattern during the embryonic development. We show that *meis3* is expressed specifically in a subset of endodermal cells during somitogenesis. Recent progress of our functional study will be presented.

260. **Meis Proteins Are Essential for Hindbrain Development in the Zebrafish.** Seong-Kyu Choe, Nikolaos Vlachakis, and Charles G. Sagerstrom. Department of Biochemistry and Molecular Pharmacology and Program in Neuroscience, University of Massachusetts Medical School, Worcester, Massachusetts.

Hox proteins together with their cofactors function in cell fate specification during embryogenesis. Of the Hox cofactors, Pbx and Meis proteins are believed to participate in Hox-containing transcriptional regulation. Recently, we reported that Meis proteins promote hindbrain fates at the expense of more anterior fore- and midbrain. To clearly demonstrate the function of Meis, we performed a dominant-negative approach with which all Meis family members can be interfered. We observed that expression of Pbx4 N-terminus efficiently sequesters Meis proteins in the cytoplasm, thereby keeping Meis out of transcriptional complex in the nucleus. Gene expression and neuronal specification in zebrafish hindbrain region were specifically impaired by the expression of Pbx N-terminus. Our results suggest that Meis proteins are essential for proper hindbrain patterning during embryonic development. On the other hand, even though a function of Meis proteins together with Pbx has been postulated to help improve DNA-binding specificity of Hox proteins, it is possible that Meis protein may have other *in vivo* activities than merely stabilizing Pbx/Hox complexes. Through a series of Meis deletion constructs and fusion constructs, we found that the Meinox domain of Meis protein is sufficient for its *in vivo* activity. More importantly, this activity is separable from Pbx binding. This result suggests that Meis proteins contribute an activity to the multimeric complex possibly by recruiting an unknown factor.

261. **Yeast Two-Hybrid Analysis Provides New Information about Interaction of HOX Proteins with Meis-Family Cofactors.** Thomas M. Williams and Jeffrey W. Innis. Department of Human Genetics, University of Michigan, Ann Arbor, Michigan 48109.

Hox genes are a group of evolutionarily conserved transcription factors that play an instructive role in patterning body structures along the anteroposterior axis. Humans and mice have 39 Hox genes clustered in four groups, and the genes in each group are numbered 1–13 depending on their position in the cluster. Groups 9–13 are collectively known as *AbdB*-like, referring to their orthology to the *Drosophila* homeotic selector gene *abdominal-B* (*Abd-B*). Each HOX protein contains a 61-amino-acid homeodomain that functions in DNA binding. These proteins specify different developmental fates by controlling expression of downstream genes through *cis*-acting regulatory sequences. Evidence is accumulating that target site specificity is aided by interactions of HOX proteins with cofactors. Among those identified are proteins from the Meis family, which belong to the TALE class of homeodomain proteins. Previous work has shown that AbdB-like HOX proteins form DNA-binding complexes with both Meis1a and Meis1b. In addition to Meis-HOX dimers, trimeric complexes also may form that include a second TALE class protein, PBX. By utilizing yeast two-hybrid methods to study protein–protein interactions, we have identified domains involved in the interaction between various

Meis and AbdB-like HOX proteins. This enhanced knowledge provides a basis to investigate the functional importance of the Meis-HOX interaction.

262. **The Roles of LXRα in Adipocyte Differentiation and Metabolism.** I. Gerin,* S. E. Ross,* R. L. Erickson,* P. M. DeRose,* L. Bajnok,* K. A. Longo,* D. E. Misek,† R. Kuick,† S. Hanash,† K. B. Atkins,‡ S. M. Andresen,§ H. I. Nebb,§ and O. A. MacDougald.* *Department of Physiology, †Department of Pediatrics and Communicative Diseases, and ‡Department of Internal Medicine, University of Michigan; and §Institute for Nutrition Research, University of Oslo, Oslo, Norway.

Adipocyte differentiation involves complex and coordinate regulation of gene expression. Here we used microarray analyses to define patterns of gene expression during adipogenesis and to identify subsets of genes whose expression is aberrant when differentiation is blocked through enforced Wnt signaling. Clustering of genes based on their patterns of expression revealed five major nodes, each representing a phase of adipocyte differentiation. Our studies suggest that Wnt signaling inhibits adipogenesis in part through dysregulation of the cell cycle. Analysis of transcripts that are specifically expressed in adipocytes identified numerous interesting genes, hitherto uncharacterized in adipocyte biology, including the transcription factor LXRα. Thus, we investigated potential roles of LXRα in adipocyte differentiation and metabolism. Experiments herein reveal that, unlike C/EBPα and PPARγ, LXRα is not adipogenic, but rather inhibits adipogenesis if inappropriately expressed and activated. However, LXRα has several important roles in adipocyte function. Our studies show that this nuclear receptor increases basal glucose uptake and glycogen synthesis in 3T3-L1 adipocytes. In addition, LXRα increases cholesterol synthesis and release of nonesterified fatty acids. These findings demonstrate new metabolic roles for LXRα and increase our understanding of adipogenesis.

263. **The Role of a William–Beuren Syndrome Associated HLH Domain-Containing Transcription Factor in Activin/Nodal Signaling.** Colleen Ring,* Souichi Ogata,* Lauren Meek,* Jihwan Song,* Tatsuru Ohta,† Kohei Miyazono,† and Ken W. Y. Cho.* *Department of Developmental Cell Biology and Developmental Biology Center University of California, Irvine, California 92697-2300; and †Department of Biochemistry, The Cancer Institute of the Japanese Foundation for Cancer Research (JFCR), 1-37-1 Kami-ikebukuro, Toshima-ku, Tokyo 170-8455, Japan.

We have investigated the regulation of the activin/nodal-inducible distal element (DE) of *Xenopus* goosecoid (gsc) promoter. On the basis of its interaction with the DE, we isolated a *Xenopus* homolog of the human Williams–Beuren syndrome critical region 11 (WBSCR11) and further, show that it interacts with pathway-specific Smad2 and Smad3 in a ligand-dependent manner to modulate gsc induction. Interestingly, we also find that XWBSCR11 functions cooperatively with FoxH1 (Fast-1) to stimulate DE-dependent transcription. We propose a mechanism in which FoxH1 functions together with Smads, as a cofactor for the recruitment of transcription factors like XWBSCR11 in the process of activin/nodal mediated gsc-specific induction. This mechanism provides considerable opportunities for modulation of transcription across a variety of activin/nodal-inducible genes, increasing diversity in promoter selection, thus leading to the differential induction of activin/nodal target genes.

264. **The Requirement for JAK/STAT Signaling in Ovarian Cell Migration.** Debra L. Silver and Denise J. Montell. Johns Hopkins School of Medicine, Baltimore, Maryland.

Cell migration is essential for embryonic development and when disregulated can result in both congenital disease as well as the progression of a variety of cancers. Our lab uses the *Drosophila* ovary as a model to identify new genes essential for this process. During oogenesis, two polar cells at the anterior of the egg chamber recruit about four to six epithelial follicle cells to form a border cell cluster. This cluster undergoes an epithelial to mesenchymal transition, migrating between the germline cells and reaching the oocyte after about 6 h. We have recently shown that JAK/STAT signaling from the polar cells to the surrounding follicle cells is essential for the recruitment and migration of border cells. Furthermore, ectopic activation of JAK, STAT, or UPD (the only known ligand) is sufficient to cause extra epithelial cells to become invasive. This signaling activates expression of several essential migratory genes, including *shotgun* (DE-Cadherin) and *slbo* (C/EBP). This relationship is supported by the finding that STAT exhibits specific dominant genetic interactions with *shg* and *slbo* mutants, but not all border cell mutants. We are currently investigating other downstream targets of STAT in the border cells using expression of border cell markers and dominant genetic interactions. In addition, we have observed genetic interactions between the JAK/STAT pathway and other signal transduction pathways, suggesting a cytoplasmic signaling role for JAK and STAT in border cells. We have begun to examine a more general role for JAK/STAT signaling in cell migration of ovarian cancer cells.

265. **AHR-1, the *Caenorhabditis elegans* Homolog of the Aryl Hydrocarbon Receptor, Regulates Neuronal Migration.** Hongtao Qin and Jo Anne Powell-Coffman. Department of Zoology and Genetics, Iowa State University, Ames, Iowa 50011-3260.

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor. In mammals, AHR mediates the toxic effects of dioxin and related environmental pollutants. Exposure to dioxin can cause cognitive deficiencies, but the neuronal function of AHR during normal development is not understood. We are investigating the function and regulation of *ahr-1*, the *Caenorhabditis elegans* homolog of the aryl hydrocarbon receptor (AHR). An AHR-1:GFP reporter is expressed transiently in many neurons. During the first larval stage, AHR-1:GFP is expressed in the Q neuroblasts and their descendents as they migrate. One of these cells is the SDQR interneuron. In wild-type animals, SDQR migrates dorsally to a position on the ALMR-associated nerve and its axon projects dorsally. In *ahr-1* mutants, SDQR migrates inappropriately: the SDQR cell body moves ventrally, and the axon projects anteriorly. UNC-6/Netrin and SAX-3/Robo are also required during SDQR pathfinding. To determine whether *ahr-1* mediates UNC-6 or SAX-3 signaling during the migration of SDQR, we analyzed double mutants. A null mutation in *ahr-1* enhances the SDQR migration defects exhibited in *unc-6* or *sax-3* mutants. We conclude that AHR-1 has a role in SDQR migration and that it functions in parallel to the UNC-6 and SAX-3 pathways. The AVM touch receptor neuron is the sister of SDQR, and AVM cell and axon pathfinding is disrupted in *ahr-1* mutants. We are currently examining interactions between *ahr-1*, *unc-6*, and *sax-3* during AVM migration.

266. **A Role for Semaphorin 3D, a Secreted Cell-Signaling Molecule, in Zebrafish Cranial Neural Crest Cell Delamination and Migration.** J. D. Berndt, A. Isadore, and M. C. Halloran. University of Wisconsin, Madison, Wisconsin 53706.

The appropriate migration of cranial neural crest cells is critical for the correct formation of the craniofacial skeleton and sensory ganglia of the head. Relatively little is known about the molecular factors regulating neural crest cell delamination from the neural keel and migration to the pharyngeal arches and sensory ganglia. *sema3D* mRNA is expressed in the dorsal region of the hindbrain and in specific clusters of neural crest cells, beginning at the onset of and continuing throughout neural crest cell migration. Based on this expression pattern, we hypothesize that Sema3D acts in a repulsive manner to promote delamination and to separate adjacent streams of migrating crest cells. Morpholino antisense-mediated knockdown of Sema3D expression results in a dose dependent decrease in a subpopulation of cranial neural crest cells that normally express *sema3D*. Furthermore, when morpholino injected embryos are observed later in development there is a significant disruption in the organization of the craniofacial cartilage. We are currently analyzing the dynamics of neural crest cell delamination using DIC microscopy and time-lapse video analysis *in vivo*. Preliminary results suggest that in morpholino-injected embryos fewer cells may be undergoing the epithelial to mesenchymal transformation necessary to initiate migration. We are also analyzing the pattern of migration to the pharyngeal arches using fluorescent tracer dyes and confocal imaging. Preliminary data from these experiments suggest that Sema3D may play a role in the structural organization of the pharyngeal arches.

267. **The Zebrafish *diwanka* Gene Controls Multiple Aspects of Motor Axon Migration.** Valerie Schneider, Joerg Zeller, and Michael Granato. University of Pennsylvania, Philadelphia, Pennsylvania.

During development, axons rely upon surrounding tissues to provide guidance cues necessary for their migration to appropriate targets. Our previous studies showed that primary motor neurons require *diwanka* activity provided by adaxial cells (1). We have now extended our analysis of the roles of *diwanka* and adaxial cells to secondary motor neurons, whose axons migrate along the same pathways, but at later developmental stages. Using transgenic lines that express GFP in different populations of secondary motor neurons, we find that in *diwanka* embryos, secondary motor axons exhibit migration defects both similar to, and distinct from, those of primary motor axons. Like primary motor neurons, secondary nerves that normally project dorsally stall at spinal cord exit points. In contrast, ventral projecting nerves exit, but often at ectopic locations, are poorly fasiculated and frequently extend inappropriately into adjacent somites. To determine whether adaxial cell cues also play a role in secondary motor axon migration, we have analyzed *you-too* embryos, which lack this cell population. We find that, in contrast to *diwanka* embryos, the exit of all classes of secondary motor axons from the spinal cord is impaired in *you-too* embryos. Furthermore, the migration of secondary motor axons that do exit the spinal cord is frequently arrested prematurely. Our results suggest that adaxial cells are likely to be the source of multiple guidance cues that control different aspects of secondary motor axon migration. We will present evidence supporting this hypothesis and our progress in the cloning of *diwanka*. 1. Zeller and Granato, 1999, *Development* **126,** 3461–3472.

268. **Domains of EphA4-Mediating Dissociation of Cadherin Adhesion Complexes.** Josh Bonis, Jaime L Malcore, and Jon B. Scales. University of Wisconsin–Eau Claire, Eau Claire, Wisconsin 54701.

It is becoming well established that members of the Eph receptor tyrosine kinase family play crucial roles in the establishment of boundaries and the regulation of cell migrations during embryogenesis in vertebrates. An initial finding was that misexpression of Eph A4 kinase activity in early *Xenopus* embryos causes loss of cell adhesion implying a molecular mechanism involving cadherin-based cell adhesion. By coexpressing a chimeric Eph A4 and various cadherin constructs having C-terminal truncations, we have shown that a small portion of the intracellular juxtamembrane domain of cadherin is all that is required to rescue cell-dissociation caused by Eph A4 signaling. This finding presumably precludes a requirement for catenins and other components of the cadherin adhesion complex since the binding sites within cadherin for these proteins have been removed. Further studies have demonstrated downstream signaling through members of the rho/rac family of GTPases mediates EphA4′s disruption of cytoskeletal elements. However, the *in vivo* down-regulation of cell adhesion by EphA4 must ultimately be linked to specific sites of cell adhesion and cell–cell contact such as adherens junctions and focal adhesions. We are currently undertaking studies to define potential domains of EphA4 necessary for mediating these interactions with adherens junctions based on our previous studies demonstrating dominant-negative cadherin's ability to inhibit EphA4-induced dissociation of wild-type cadherin-containing adherens junctions.

269. **Syndecan-1-Mediated Cell Spreading Requires $\alpha v\beta 3$ ($\alpha v\beta 3$) Integrins.** DeannaLee M. Beauvais and Alan C. Rapraeger. Department of Pathology and Laboratory Medicine, University of Wisconsin–Madison, Madison, Wisconsin.

Syndecans are heparan sulfate proteoglycans with roles in regulation of cell adhesion and differentiation. While it is clear that the syndecan heparan sulfate chains are essential for matrix binding, less is known about the signaling role of their core proteins. The focus of this work is to investigate molecular interactions of the syndecan-1 core proteins that regulate cell adhesion and spreading. To mimic syndecan-specific cell adhesion, MDA-MB-231 human mammary epithelial cells were plated on antibodies against syndecan-4 or syndecan-1. While cells adherent via syndecan-4 spread, cells adherent via syndecan-1 do not. However, cells adhering via syndecan-1 can be induced to spread by Mn^{2+}, suggesting that activation of a $\beta 1$ ($\beta 1$) or $\beta 3$ ($\beta 3$) integrin partner is required. Surprisingly, function activating $\beta 1$ antibodies do not induce spreading, whereas function blocking $\beta 1$ integrin antibodies trigger a response, indicating that a $\beta 1$ to $\beta 3$ integrin cross-talk may be involved. Indeed, blockade of $\beta 1$ integrin activation induces $\alpha v\beta 3$ integrin activation, as detected by fibrinogen binding, and this activity is required as treatment with $\beta 3$ blocking antibody, LM609, blocks cell spreading. Mutation of the syndecan-1 core protein indicates spreading requires an intact ectodomain. This suggests that binding to an organized extracellular matrix initiates formation of a syndecan-1-$\alpha v\beta 3$ integrin signaling complex that transduces a signal to the actin cytoskeleton. This signaling complex is negatively regulated by $\beta 1$ integrins and this phenom-

enon may be a critical regulator of cell migration and invasiveness in development and in cancer.

270. **Overexpression of Syndecan-1 Extracellular Domain Disrupts Adhesion and Blocks Invasion of T47D Mammary Carcinoma Cells.** Brandon J. Burbach and Alan C. Rapraeger. Department of Pathology and Laboratory Medicine, University of Wisconsin–Madison, Madison, Wisconsin.

The cell surface heparan sulfate proteoglycan (HSPG) syndecan-1 is thought to regulate epithelial cell differentiation and migration during development and carcinogenesis. We hypothesized that syndecan-1 may function within signaling complexes that serve to modulate cellular adhesion and invasion. To characterize these putative activities of syndecan-1, we monitored cell adhesion, morphology, and migration following overexpression of this HSPG in T47D human mammary carcinoma cells. T47D cells overexpressing syndecan-1 exhibit disrupted adhesion in serum culture, resulting in a poorly adherent, rounded cell morphology compared to the normal fused and spread morphology of control cells. A similar response is noted following overexpression of the syndecan-1 extracellular domain fused to the GPI lipid anchor domain of glypican-1 (GPI-mS1ED), but not with constructs lacking 160 membrane-proximal amino acids of the extracellular domain, tracing the effect to the ectodomain core protein. The adhesion phenotype is a direct consequence of syndecan overexpression, since treatment with the GPI linkage inhibitor 2-fluro-2-deoxyglucose reduces expression of GPI-mS1ED and coordinately restores normal morphology. Furthermore, migration of GPI-mS1ED cells across type I collagen or fibronectin is sharply reduced compared to control cells. These data support a model where the syndecan-1 extracellular domain core protein participates in the formation of a cell surface signaling complex via interaction with an unidentified coreceptor, functioning to regulate epithelial cell adhesion and invasion.

271. **Characterization of the *Dictyostelium* Protein FbiA, a Potential Target of Ubiquitin-Mediated Degradation.** Kelly A. McFeaters, Seth C. Houwer, Elizabeth A. Wilson, Carla More, Jennifer A. Christman, Tomoaki Abe,* and Margaret K. Nelson. Department of Biology, Allegheny College, Meadville, Pennsylvania; and *Wellcome Trust Biocentre, University of Dundee, Dundee, Scotland, United Kingdom.

The FbiA protein of *Dictyostelium discoideum* was identified via yeast two-hybrid analysis in a search for proteins capable of interacting with the WD-40 repeat region of the F-box/WD-40 repeat-containing protein FbxA. Based on analogy to FbxA homologs in other systems, this FbiA–FbxA interaction suggests that FbiA is likely to be targeted for FbxA-dependent, ubiquitin-mediated degradation. We describe here our preliminary characterization of FbiA, including the developmental phenotypes of *fbiA*$^-$ null mutants and FbiA overexpressors and the effect of altered FbiA levels on the prespore:prestalk ratio. The C-terminal region of FbiA is homologous to proteins in humans, mice, *Drosophila, Caenorhabditis elegans, Arabidopsis, Schizosaccharomyces pombe, Neurospora crassa,* and *Plasmodium falciparum*. The function of these FbiA homologs is, however, unknown. Hence, further characterization of FbiA's role in *Dictyostelium* development may shed light on the function of this evolutionarily conserved protein family. (This research was supported in part by NSF CAREER Grant IBN-9985265.)

272. **Metamorphic Remodeling in Frog Cranial Cartilages Is Specified before Neural Crest Cell Migration.** C. A. Rose, A. L. Johnson, and K. A. Pomeroy. James Madison University, Dalhousie University, Halifax, Nova Scotia, Canada.

The postembryonic shape and arrangement of neural crest-derived cartilages are specified before neural crest cells leave the neural fold, and this aspect of fate appears to be a product of Hox gene patterning. Metamorphic vertebrates complicate this picture by developing distinct larval and postlarval cranial configurations, the latter of which results from TH-mediated remodeling. In frogs, this remodeling involves expansion of the lower jaw (Meckel's cartilage or MC) by cell proliferation and resorption of the branchial arch cartilages (BA) by apoptosis. This study investigates whether these two responses to TH in *Xenopus laevis* are specified before crest migration. Neural fold segments were transplanted from mandibular crest regions of GFP-labeled embryos to branchial crest regions of unlabeled embryos and vice versa. Tadpoles that developed GFP-labeled ectopic cartilages, i.e., MCs in their gill baskets or BAs in their mouth regions, were immersed in a T3, BrdU solution. Ectopic cartilages were then compared with untransplanted MCs and BAs in T3-treated and control specimens. Observations of BrdU and TUNEL labeling, cell size and arrangement, and Alcian blue staining suggest that ectopic MCs and BAs respond to T3 in the same manner as untransplanted MCs and Bas, respectively. These results indicate that the cell responses to TH that generate the postlarval configurations of neural crest-derived cartilages are specified prior to neural crest migration. This is consistent with the embryonic formation and metamorphic remodeling of neural crest-derived skeleton being specified by the same patterning mechanisms.

273. **FGF10 and SMAD in the Chick Otic Vesicle.** Berta Alsina, Encarna Ulloa, and Fernando Giraldez. CEXS-Universitat Pompeu Fabra, Dr. Aiguader 80, 08003 Barcelona, Spain.

The family of fibroblast growth factors (FGFs) play significant roles in cell fate specification in development. Recently, it has been reported that FGF-10 knockout mice show a reduction in auditory neurons (Pauley *et al.*, 2001, Abstract 20455, ARO Meeting). We have explored the role of FGF10 in the specification of the neurosensory territory of the chick otic placode. Expression of FGF10 was detected in 14-somite embryos and was restricted to the anterior region of the placode. During otic cup, FGF10 was expressed in the anterior and ventral aspect, with sharp medial and lateral boundaries. In the otic vesicle (stages 17 to 20), it was restricted to two anterior and posterior patches, resembling the expression domains of BMP4 in the nascent sensory patches. FGF10 domain coincided with that of Delta1, an early marker of the site of neurogenesis (Adam *et al.*, 1998, *Development* **125,** 4645–4654). Contrary to Delta1, FGF10 was expressed in all cells of the neurocompetent region. Delaminating neuroblast labeled with TUJ1 and Islet2 originated from the FGF10 expression domain. Recent studies have revealed that FGFs induce neural fate through repression of bone morphogenetic proteins (BMPs) (Wilson and Edlund, 2001, *Nature Neurosci.* **4**(Suppl.), 1161–1168). We have monitored BMP4 activated cells by the detection of the phosphorylated form of SMAD1, a transducer protein of the BMP signaling pathway. Double staining with TUJ1 and anti-SMAD1-P showed that SMAD1-P was down-regulated in delaminating young neuroblasts, while still present in scattered cells within the FGF10 domain. The results indicate that FGF10 may be important for specification of the neurosensory domain and that neural fate may

require the down-regulation of BMP signaling. (Supported by MCYT PM98-0145.)

274. **Requirement for *Fgf8* in Olfactory Neurogenesis.** S. Kawauchi, J. Shou, and A. L. Calof. Department of Anatomy and Neurobiology and Developmental Biology Center, University of California, Irvine, California.

The ability of mouse olfactory epithelium (OE) to generate neurons throughout life suggests that endogenous proliferative signals drive OE neurogenesis. Studies in our lab have shown that fibroblast growth factors (FGFs) promote proliferation of both OE stem cells and immediate neuronal precursors (INPs), cells that give rise to olfactory receptor neurons (ORNs). Here, we sought to determine if the hypothesized endogenous stimulatory signal in OE is FGF8. Fgf8 is expressed in a "rim" of epithelium outlining the developing olfactory pit at E10.5; by E14.5, *Fgf8*+ cells are found throughout the OE. Since this pattern is suggestive of a role for *Fgf8* in OE neurogenesis, we performed tissue culture assays in which OE explants were treated with recombinant FGF8. The results indicate that FGF8 stimulates proliferation of OE stem cells and INPs *in vitro*. To determine if *Fgf8* regulates neurogenesis *in vivo*, we generated mice with *Fgf8* inactivated in the Bf-1 (*Foxg1*) domain. Pronounced defects in forebrain and facial structures were observed from E9.5 onward. Normal numbers of neuronal progenitors (*Mash1*+ and *Ngn1*+) and ORNs (*Ncam*+) were present in olfactory pit at E10.5. However, at E17.5 no neuronal cells were evident in the epithelium lining the nasal cavity, itself much smaller than normal. These results suggest that *Fgf8* is not required for determination of the OE neuronal lineage, but is necessary for neurogenesis to be maintained. Current experiments seek to identify the developmental stage at which the requirement for *Fgf8* becomes evident. (Supported by NIH (DC03583 and HD38761) and March of Dimes. S.K. is a Human Frontier Science Program Fellow.)

275. ***In Vivo* Monitoring of Neurogenesis in the Vertebrate Neuroepithelium.** David A. Lyons and Jonathan D. W. Clarke. Department of Anatomy and Developmental Biology, University College London, Gower St., London WC1E 6BT, United Kingdom.

We use the zebrafish hindbrain to model neurogenesis in the vertebrate neuroepithelium. Our aim is to provide an accurate description of the modes of division which progenitor cells undergo as they generate cells which will contribute to a functional nervous system and to provide information on the overall dynamics of neurogenesis in a vertebrate. We label single neuroepithelial cells with fluorescent dextrans and monitor development of the clone over the course of embryogenesis by time-lapse microscopy. Furthermore, by applying this technique in a transgenic animal that expresses GFP in all neurons soon after they are born we can verify the identity of specific cells *in vivo* as they develop. Our findings suggest that the lineage of the majority of progenitor cells is quite stereotyped. A large proportion of neurons are generated by lineages exclusively comprised of symmetric divisions. In other cases we see that individual neurons are generated following an asymmetric divisions but we see no examples of stem cell-like modes of division. Quantification of birth dates, neuronal number, and total cell number suggests that the modes of division we observe account for the generation of most cells in the fish hindbrain. By studying Bodipy-stained embryos using time-lapse microscopy we have determined that the orientation of almost all progenitor divisions occurs in the plane of the ventricular zone. Our results suggest that asymmetric divisions play a minor role in neurogenesis and that stem cell progenitors are a rare cell type if they exist at this stage at all.

276. **Asymmetric Cell Division and Numb Segregation in the Developing Mammalian Retina.** Michel Cayouette and Martin Raff. MRC Laboratory for Molecular Cell Biology, University College London, London, United Kingdom.

How do individual neuroepithelial cells choose between alternative fates? Asymmetric segregation of cell-fate determinants during mitosis plays an important part in cell-fate choice in invertebrate development, but it is uncertain how important this mechanism is in vertebrate development. We have recently shown that most neuroepithelial cells in the developing rat retina divide horizontally, with their mitotic spindle aligned parallel to the plane of the neuroepithelium, but that a substantial number divide vertically, with their spindle oriented 45°–90° off this plane. The proportion of vertical divisions is influenced by the underlying retinal pigment epithelium (RPE) and changes with developmental age. We also found that the Numb protein, which influences cell-fate choice in *Drosophila*, is concentrated at the apical side of retinal neuroepithelial cells and is preferentially inherited by the apical daughter cell in vertical divisions. As Numb is thought to inhibit Notch signaling and Notch signaling has been shown to promote glial development, we speculated that basal daughters of vertical divisions would have unopposed Notch signaling and may therefore develop as Muller glial cells. Videomicroscopy of GFP-labeled neuroepithelial cells in newborn rat retinal explants supports this idea: the two daughter cells of vertical divisions acquire different fates, with the basal daughter usually becoming a Muller-like cell; by contrast, the two daughters of horizontal divisions acquire similar fate and usually become rod-like cells. These results demonstrate that asymmetric cell division contributes to cell-fate decisions in the developing mammalian retina.

277. **Ath5 Acts in the Embryonic Mouse Retina to Specify Retinal Ganglion Cell Fate.** Nadean L. Brown, Tien Le, and Emily Wroblewski. Northwestern University Medical School and Children's Memorial Institute for Education and Research, Evanston, Illinois.

During vertebrate retinal development, the appropriate numbers and types of neurons must form correctly. Retinal cell-fate determination is orchestrated by intrinsic and extrinsic factors, consistent with the rest of the nervous system. Basic helix–loop–helix (bHLH) proteins are transcription factors that intrinsically regulate neuronal specification. Several of these factors, *Ath5, Mash1, Ngn2,* and *NeuroD,* participate in the determination of particular neural and glial cell fates in the developing mouse retina. Of these, progenitors express *Ath5* first and require it for retinal ganglion cell (RGC) fate. *Ath5* mutant mice completely lack RGCs, optic nerves, and chiasmata. Interestingly, the loss of RGCs is accompanied by a corresponding increase in cone photoreceptor or amacrine neurons. This suggests that retinal progenitors change their fate when *Ath5* function is removed. We compared the earliest stages of RGC development in wild-type and *Ath5* mutant embryos by retinal birth dating and molecular marker experimentation. These studies further demonstrate that *Ath5* functions within retinal progenitors to specify RGC fate. We also observed derepression of *Ngn2* and *NeuroD* in *Ath5* mutants, suggesting interdependence among bHLH genes within the developing retina. We propose that alter-

ation of this cross-regulatory network may be sufficient to cause cell fate changes in *Ath5* mutant retinae.

278. **Information Provided by the Extraocular Muscle Has a Role in Retinal Differentiation.** B. Kablar. Dalhousie University, Halifax, Nova Scotia, B3H 4H7, Canada.

Retinas of mutant mouse embryos lacking extraocular striated muscles are employed to study the epigenetic role of fetal eye movements in the genesis of retinal cell diversity. While retinal lamination and the total number of cells were unaffected in amyogenic fetuses, the absence of cholinergic amacrine cells was revealed. By contrast, the amounts of other amacrine cell subpopulations were increased, whereas the amounts of retinal ganglion cells were decreased. Surprisingly, it was not possible to detect any change in proliferation or cell death. Consistently, the number of ganglion progenitor cells was increased, whereas the amounts of amacrine cell precursors were decreased. Together, these data suggest that epigenetic cues have an essential role in the determination of retinal cell fates. (Supported by NSERC Canada.)

279. Abstract #279 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

280. **Characterization of Different Populations of Motoneurons in Regenerating and Adult Spinal Cords of the Weakly Electric Fish *Soriculus macrurus.*** Marian N. Viveros, Karima White, and Graciela A. Unguez. Department of Biology, New Mexico State University, Las Cruces, New Mexico.

In all electric fish, some muscle fibers lose their contractile apparatus and transform into noncontractile electrocytes, i.e., the cells of the electric organ (EO). Muscle fibers, as in other vertebrates, are innervated by somatomotoneurons (SMNs), while electrocytes are innervated by electromotoneurons (EMNs). Our goal is to determine how different EMNs and SMNs arise and how they innervate distinct myogenically derived cell types during regeneration. We have begun to study anatomical differences between EMNs and SMNs in adult fish using horseradish peroxidase (HRP) and immunohistochemistry. HRP was injected into the ventral musculature ($n = 2$) and EO ($n = 1$) of adult fish. After a 4- to 7-day survival period, serial cross-sections of the spinal cord were processed for HRP histochemistry. Preliminary results show that SMNs are located in the ventral horns, whereas EMNs are located more dorsally and centrally in the spinal cord. Antibodies against nestin (Rat-401), islet-1 (39.4D5), and HuC/HuD (HU) label distinct motoneuronal populations in adult spinal cords ($n = 3$). Rat-401 labels larger, more centrally located neurons. 39.4D5 and HU label a broader population of neurons with a larger range in size and distributed throughout the ventral and central spinal cord. Each antibody also shows a distinct labeling pattern of neurons in spinal cords of regenerating tails. These data suggest that mature EMNs and SMNs differ in their anatomical and biochemical phenotype, and these differences may be detected early in regeneration. (Supported by NIH Grants GM61222-01 and S06-GMO8136-27.)

281. **Identification of Cells That Give Rise to Slow and Fast Muscle during Postembryonic Growth in Zebrafish.** Joel A. D'Angelo, Domenick Acquista, Michael J. F. Barresi, and Stephen H. Devoto. Biology Department, Wesleyan University, Middletown, Connecticut 06459.

Vertebrate muscle development begins when signals from adjacent tissues pattern the paraxial mesoderm. In zebrafish, Hedgehog signaling from the notochord induces the most medial cells in the paraxial mesoderm to become adaxial cells, which subsequently differentiate into embryonic slow muscle fibers. We propose that lateral presomitic cells give rise to embryonic fast muscle (1) and a population of germinal cells which will generate new slow and fast muscle fibers during postembryonic growth. The dorsal and ventral regions of the myotome express muscle regulatory factors and have a higher percentage of proliferating cells, suggesting that the dorsal and ventral extremes are the site of germinal cells (2). These germinal zones can be identified in living embryos by position and morphology. Small-diameter slow and fast fibers are found adjacent to the germinal zones suggesting that germinal zone cells give rise to both cell types. To test this hypothesis we are ablating and fate mapping these cells. Hedgehog induces the differentiation of paraxial mesoderm into embryonic slow muscle fibers. Overexpression of Hh induces all of the paraxial mesoderm to differentiate into adaxial-derived slow muscle. We have shown that the overexpression of Hh leads to the loss of germinal zone cells. 1. S. H. Devoto *et al.,* 1996, *Development* **122,** 3371–3380; 2. M. J. F. Barresi *et al.,* 2001, *Curr. Biol.* **11,** 1432–1438.

282. **Wnt7a Regulates Multiple Aspects of Limb Bud Development in the Mouse Embryo.** Brian A. Parr, Amy P. Nunnally, and Jennifer H. Olson. University of Colorado, Denver, Colorado.

Previous research has demonstrated that a secreted Wnt7a signal is required for normal development of the limbs, reproductive tract, and cerebellum in mice. We have examined the expression of frizzled-10 (Fz10), a putative Wnt7a receptor, to further characterize the mechanisms of Wnt7a signaling. Fz10 expression closely tracks the expression of Wnt7a, as would be expected for a Wnt7a receptor. Analysis of Fz10 expression suggests an explanation for the distal restriction of dorsal–ventral patterning defects in the limbs of Wnt7a mutant mice. Fz10 expression patterns also suggest a more complex mechanism for Wnt7a function in reproductive tract development than indicated by earlier studies, as well as providing insights into the regulation of Müllerian duct regression. To further characterize the role of Wnt7a in limb bud development, we have examined the generation of limb muscles in Wnt7a mutants. By analyzing the expression of marker genes for limb muscle precursor cells in Wnt7a mutant embryos, we have found that Wnt7a is required for the production of a subset of limb muscles. This aspect of Wnt7a signaling appears to affect the limb muscle precursors prior to their migration into the limb bud. It may not require a Wnt7a signal in the limb bud itself. Therefore, Wnt7a regulates the development of limb structures by at least two distinct mechanisms.

283. **Withdrawn.**

284. **Distalization of the *Drosophila* Leg by Graded EGF-Receptor Signaling.** Gerard Campbell. University of Pittsburgh, Pittsburgh, Pennsylvania.

The evolution of appendages in animals required mechanisms to establish a novel axis: the proximodistal (P/D) axis. Here we show the mechanisms required for patterning the distal region of the *Drosophila* leg are very similar to those used in vertebrate limbs indicating either a remarkable convergence of patterning mechanisms used to generate the P/D axis in both arthropods and vertebrate appendages or possibly a common evolutionary origin to

outgrowths from the body wall in both groups. Previous studies in *Drosophila* indicated that patterning along the P/D axis of the leg is controlled directly by two signaling molecules, Wg (a Wnt) and Dpp (a TGF-β). However, we show that Wg and Dpp pattern the distal region of the leg, the tarsus, only indirectly by establishing a P/D organizer at the presumptive distal tip. This organizer is the source of EGF-receptor (EGFR) ligands and P/D patterning of the tarsus is regulated by graded EGFR activity: high levels are required for development of the distal-most tissue while progressively lower levels are required more proximally. EGFR signaling functions to activate genes such as aristaless and Bar that regulate differentiation of distal regions of the leg and to repress other genes, including daschous that are absent from distal regions. Specification of the P/D axis in vertebrate limbs is also regulated by the presumptive tip, the AER, which is the source of ligands for a tyrosine kinase receptor, in this case FGF-receptors, raising the possibility that graded activity of the same intracellular signaling pathway may be involved in generating the P/D axis in insects and vertebrate limbs.

285. ***nerfin-1*, a Member of a Conserved Zn-Finger Gene Subfamily, Is Required for Proper Neuronal Cell Fate Specification.** Alexander Kuzin, Chad Stivers, Thomas Brody, and Ward F. Odenwald. Neurogenetics Unit, LNC, NINDS, NIH, Bethesda, Maryland.

We have identified a new *Drosophila* Zn-finger gene, *nerfin-1* (nervous fingers-1) from a cDNA library screen for genes expressed during CNS development (1). *nerfin-1* belongs to a conserved Zn-finger gene subfamily, with human, mouse, and nematode cognates. Loss-of-function RNAi studies reveal that Nerfin-1 is essential for proper lineage development in both the CNS and the PNS. In addition to the aberrant expression of multiple neuronal identity regulators, RNAi experiments indicate that *nerfin-1* may also be required for proper axonal outgrowth. During embryonic CNS development, *nerfin-1* mRNA expression shifts from early delaminating neuroblasts to most, if not all, ganglion mother cells. However, immunolocalization studies reveal that Nerfin-1 protein accumulates only in the nucleus of neuronal precursor cells that are poised to undergo a single final division that generates neurons. To determine the functional significance of its role in CNS development, we have targeted Nerfin-1 misexpression to different temporal and spatial windows of development. An array of lineage markers is being used to study the effects of Nerfin-1 misexpression. In addition, we have generated stable loss-of-function mutations by homologous recombination and we are now analyzing the phenotype. More specifically, we will determine if *nerfin-1* function is required for precursor cells and/or their nascent neurons to exit the cell cycle and undergo terminal differentiation. 1. Stivers *et al.*, 2000, *Mech. Dev.* **97,** 205–210.

286. **The Cysteine-Rich Domain-Containing Protein Crossveinless 2 Is Required for BMP-like Signaling in the Developing Crossveins of *Drosophila*.** Amy Ralston and Seth S. Blair. Department of Zoology, University of Wisconsin, Madison, Wisconsin.

Members of the *Drosophila* bone morphogenetic protein (BMP) family of signaling molecules are involved in many aspects of wing imaginal disk patterning. Signaling by the *Drosophila* BMP4 homolog Decapentaplegic (Dpp) is required for the maintenance of longitudinal vein (LV) fate during pupal wing development. In addition, our lab has found that BMP-like signaling is required for the initiation of crossvein (CV) patterning. We have monitored BMP-like signaling in the pupal wing using an antibody which recognizes the phosphorylated form of the Dpp effector molecule Mad (pMad). Beginning at 19 h after pupariation, pMad is present specifically in the two presumptive CVs which develop at stereotyped anterior and posterior positions. Interestingly, early Mad phosphorylation within the wild-type posterior CV (PCV) occurs in the absence of detectable cell-autonomous *dpp* expression, in contrast to the situation in the anterior CV (ACV). This result suggests that BMP-like signaling within the presumptive PCV may involve a mechanism distinct from other vein structures. We previously identified Crossveinless 2 (Cv 2), a cysteine-rich domain-containing protein required for early Mad phosphorylation within the PCV. Cv 2 bears homology to the vertebrate BMP signaling antagonist Chordin and its invertebrate homolog Short gastrulation (Sog). Our genetic analyses suggest that Mad phosphorylation within the early PCV is sensitive to levels of Sog. Similarly, Mad phosphorylation within the PCV is sensitive to levels of the BMP7 homolog encoded by *glass bottom boat* (*gbb*). We will discuss the differential roles of BMP pathway members within the two developing CVs further. We will also discuss results of other experiments aimed at understanding the role of Cv 2 in BMP signaling in the presumptive CVs.

287. **Three GLH Interactors: A Tale of Two Mutants. Great Expectations for the Third?** April Orsborn, Ruth Montgomery, Pliny Smith, Emily Coberly, Regan Barnes, and Karen Bennett. University of Missouri, Columbia, Missouri.

A family of four germline-specific RNA helicases, the GLHs, has been identified in *Caenorhabditis elegans*. The GLHs are similar to the *Drosophila* RNA helicase Vasa, except that the GLHs also contain CCHC zinc fingers. The GLHs are components of P granules, nonmembranous aggregates of protein and RNA, localized to the germ cell lineage throughout the life of the worm. A yeast two-hybrid screen identified three proteins that interact with the GLHs: KGB-1, ZXY-1, and CSN-5. These proteins are the first members in each of their conserved families reported to associate with germline helicases. We used reverse genetic screens and isolated deletion strains in two of these genes, kgb-1(um3) and zyx-1(um4). The size and location of each deletion indicate that both are likely protein nulls. KGB-1 is a novel putative MAP serine/threonine kinase that GLHs bind. The kgb-1 mutant strain exhibits a temperature-sensitive, sterile phenotype, characterized by oocytes that undergo endoreplication. ZYX-1 is a LIM domain protein most related to vertebrate Zyxin, a cytoskeletal-associated, actin adaptor found in focal adhesions. Although zyx-1 is a single-copy gene in *C. elegans*, the zyx-1 deletion strain exhibits no obvious phenotype. CSN-5 is closely related to the subunit 5 of signalosomes, conserved multiprotein complexes found in plants and animals. Anti-CSN-5 antibodies reveal a distinct nuclear as well as a diffuse cytoplamic location. GST-tagged CSN-5 pulls down GLHs from worm homogenates. RNAi with csn-5 results in sterile worms with small gonads and no oocytes, a defect similar to glh-1/glh-4(RNAi) (Kuznicki *et al.*, 2000, *Development* **127,** 2907).

288. Abstract #288 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

289. **The *outsiders* Gene Is Required for the Programmed Cell Death of *Drosophila melanogaster* Germ Cells.** Yukiko Yamada, Rachel C. Strohm, and Clark R. Coffman. Department of Zoology and Genetics, Iowa State University, Ames, Iowa 50011-3260.

Precise control of programmed cell death is essential for the generation of normal body patterns in the developing embryo and for maintaining homeostasis in adults. To elucidate the mechanisms that regulate cell migration and programmed cell death, we are studying *Drosophila* germ cell development. In the early *Drosophila* embryo, approximately 50% of the germ cells successfully migrate from the posterior midgut to the developing gonads. Germ cells that fail to reach the target tissue are efficiently eliminated by programmed cell death. Here, we describe six alleles of the *outsiders* (*out*) gene. In *out* mutants, the correct number of germ cells reach the developing gonads. However, the germ cells ectopic to the gonads fail to undergo cell death and continue to exhibit germ cell characteristics. Animals that are hemizygous for a deletion of *out* also exhibit a germ cell death defect. We conclude that *out* regulates the programmed cell death of germ cells, but it is not required for proper germ cell migration. We have mapped *out* to a small region of the X chromosome, and it does not colocalize with any known regulators of programmed cell death. Analysis of the *out* gene will provide insights into the mechanisms regulating cell death.

290. **The JAK Pathway Ligand Unpaired Acts as a Putative Morphogen to Determine Fates in the Follicular Epithelium.** Doug Harrison, Rongwen Xi, and Jen McGregor. Department of Biology, University of Kentucky, Lexington, Kentucky.

The Janus kinase (JAK) pathway is an integral part of signaling through a variety of ligands and receptors in mammals. The extensive reutilization of this pathway in vertebrate development is conserved in other animals as well. In *Drosophila*, JAK signaling is required in processes including embryonic patterning, sex determination, hematopoiesis, wing venation, and spermatogenesis. We have also uncovered roles for JAK signaling in oogenesis. The JAK pathway ligand gene, *unpaired* (*upd*), is expressed in the polar follicle cells, two pairs of somatic cells at the anterior and posterior poles of the developing egg chamber. Consistent with upd expression, reduced JAK pathway activity results in the fusion of developing egg chambers. A primary defect of these chambers is the expansion of the polar cell population and concomitant loss of interfollicular stalk cells. In addition to this early role, JAK activity is also necessary in epithelial follicle cells. A mirror image prepattern of multiple fates is established from the termini of the developing chamber. JAK signaling is required for the determination of these follicle cell subpopulations. Furthermore, ectopic JAK pathway activity causes epithelial follicle cells to adopt a more terminal identity. These data are consistent with a model in which Upd secreted from the polar cells prepatterns the follicular epithelium in a concentration-dependent manner. This provides the first evidence of potential morphogenic function for the JAK pathway. The relationship of JAK signaling to other pathways in follicular patterning will also be discussed.

291. **Withdrawn.**

292. **Antiapoptotic Effects of SCF and IGF-1 on Fetal Mouse Oocytes.** Francesca Gioia Klinger and Massimo De Felici. Department of Public Health and Cell Biology, Section of Histology and Embryology, University of Rome Tor Vergata, Rome, Italy.

Mouse oocytes isolated from 16.5 days postcoitum (dpc) fetal ovaries progress *in vitro* through the stages of meiotic prophase I and undergo a wave of apoptosis revealed by TUNEL histochemistry (about 60–70%) at the stage of pachytene/diplotene. The addition of 50–100 ng stem cell factor (SCF) to the culture medium reduced oocytes apoptosis of 20–30% and induced a significant oocyte growth. The addition of 50–100 ng/ml IGF-1 resulted in a similar reduction of oocyte apoptosis, but did not induce oocyte growth. RT-PCR and immunohistochemistry showed that at the pachytene/diplotene stage oocytes *in vitro* as *in vivo* up-regulate the expression of the SCF receptor c-kit while they express constant level of IGF-1 receptor throughout meiotic prophase I. IGF-1, but not SCF, was able to completely abolish apoptosis activated in 16.5 dpc oocytes by 200 nM adriamycin (ADR). Taken together these results indicate that apoptosis of fetal oocytes can partly occur following growth factor deprivation. Whereas some growth factors (i.e., IGF-1) might be necessary throughout the entire prophase I others (i.e., SCF) appear required at specific stages. In addition to reducing the apoptotic wave of oocytes in culture, IGF-1 is able to protect them from apoptosis triggered by DNA damaging compounds, suggesting that growth factors can positively influence the oocyte DNA repair ability.

293. **Involvement of Fas/FasL in Spermatogenic Cell Apoptosis Induced by Experimental Autoimmune Orchiditis.** Zhenying Nie and Bin Liu. Department of Pathology Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania; and Department of Histology and Embryology, Peking University Medical Center, Beijing, China.

Autoimmune orchiditis cause azoospermia or oligozoospermia and is the common reason for infertility. The objectives of this study were to investigate the role of apoptosis in experimental autoimmune orchiditis (EAO) and whether this process involves Fas/FasL system. By surgically induced autoimmune orchiditis in KM mice cellular changes in the testis were studied 1, 2, 3, 4, 5, and 6 weeks after the operation by histochemistry, immunohistochemistry, TUNEL, flow cytometry, and Western blotting. The mice of experimental groups had low-weight testis and inactive sperm 4 weeks after surgery and were infertile. In the serum of those mice the anti-sperm antibody was positive. Flow cytometry and TUNEL demonstrated that there were two- to threefold increases of apoptotic cells in testis of experimental group than that of control group. The cell types that undergo apoptosis most were spermatogonia and some spermatocyte. Immunohistochemistry stain and Western blotting demonstrate the expression level of FasL in Sertoli and leydig cells decreased, whereas the expression level of Fas, which confined to leydig cells and sporadic spermatocytes increased. The giant nucleus cells, which located near the center of seminiferous tubule, were also TUNEL positive and had high expression of Fas. Our results indicate that apoptosis of germ cell is involved in the infertility of autoimmune orchiditis and Fas/FasL signaling pathway play an important role in regular the germ cell apoptosis of autoimmune orchiditis.

294. **Rat Embryos Cloned with Cumulus Cells and Fibroblasts.** Yue Zhou, Michael Bader, Vasiliy Galat, and Philip Iannaccone. Children's Memorial Institute for Education and Research, Northwestern University, Evanston, Illinois; and Max Delbrück Center for Molecular Medicine, Berlin, Germany.

Genetic manipulation of animals is critical to the study of biological aspects of disease and an understanding of normal development. Although ES-like cells have been isolated from blastocysts of several mammalian species, developing targeted

mutations using ES cells has only worked in the mouse to date. Accomplishing targeted mutations in the rat has proven to be problematic, for while isolating ES-like cells is possible, the cells are not capable of generating germ line mosaic chimeras. Recently, nuclear transfer (NT) has been shown to produce viable offspring of several mammalian species. Cultured cells, even with targeted mutations, including those isolated from sheep and pigs have developed into viable offspring when fused to enucleated eggs. We report procedures for *in vitro* development of fertilized and NT rat zygotes from one-cell to blastocyst stages, the enucleation of rat oocytes; NT by injection of nuclei from adult rat cumulus cells, rat primary embryonic fibroblasts, and genetically modified rat fibroblasts; and activation resulting in advanced preimplantation development. Blastocyst-stage rat embryos were obtained after *in vitro* culture of nuclear transfer zygotes at similar frequencies with each of these nuclear donor cell types. Transfer of NT embryos to surrogate mothers leads to implantation of 24% of the zygotes although without postimplantation development.

295. **Bead Analysis of Sea Urchin Sperm.** Lylla Ngo, Marcela Barajas, Gayani Weerasinghe, Gregory Zem, and Steven B. Oppenheimer. California State University, Northridge, California 91330-8303.

The surface properties of live and fixed sperm from two sea urchin species (*Strongylocentrotus purpuratus* and *Lytechinus pictus*) were surveyed by assessing the ability of the sperm to bind to agarose beads derivatized with over 100 different compounds in many repeated experiments under the standard conditions of the assay system. While both species of sperm bound to many of the same beads, there were substantial, statistically significant binding differences to some beads. For example, only *L. pictus* sperm bound to beads derivatized with *Phaseolus vulgaris* agglutinin and paraaminobenzyl β-D-glucopyranoside, while only *S. purpuratus* sperm bound to beads derivatized with one form of concanavalin A, *Lens culinaris* hemagglutinin, aprotinin, globin, histamine, phosphodiesterase 3,5 cyclic nucleotide activator, and protein A. Seasonal sperm-bead binding differences were also observed. The results indicate that the assay can detect species-specific and seasonally specific surface properties that could be the subject of further investigations into their functional importance. (Supported by grants and fellowships from NIH MBRS, NIH RISE, NIH MARC, ONR RISE, NSF ESIE, and the Joseph Drown Foundation.)

296. **Allurin, a *Xenopus* Sperm Chemoattractant: Sequence Confirmation and Immunovisualization.** Andrew Kittelson, Alan Rawls, Douglas Chandler, and Allan Bieber. Molecular and Cellular Biology Program, Arizona State University, Tempe, Arizona 85287-1501.

The amino acid sequence of allurin, a 21-kDa sperm chemoattractant protein from *Xenopus laevis* egg jelly, was determined by a combination of Edman degradation and cloning of cDNAs coding for the protein. The sequence revealed homologies to mammalian AEG and TPX proteins, both members of the cysteine-rich secretory protein (CRiSP) family and known to bind to mammalian sperm. Allurin was the first vertebrate sperm chemoattractant to be sequenced and the first member of the CRiSP family to be isolated from the female reproductive tract. In this report sequence confirmation of allurin was carried out by MALDI-TOF MS analysis of peptides resulting from trypsin digestion of the protein. The observed cleavages matched those predicted from the sequence, and the observed molecular weights of the fragments correlated well with predicted molecular weights. Two pieces of allurin, peptides 12 amino acids in length, were synthesized and conjugated to keyhole limpet hemocyanin for the production of rabbit antibodies (Affinity BioReagents, Inc.). Polyclonal antibodies to allurin were also obtained by rabbit immune response to purified allurin (Rockland Inc.). These antibodies have been used in Western blotting analysis of egg jelly extracts as well as immunocytochemistry of the allurin-sperm binding event. (This study was supported by NSF Grants IBN-9807862 and IBN-0130001 and by a grant from the Beckman Foundation.)

297. **Expression and Purification of Recombinant Allurin, a 21-kDa Sperm Chemoattractant Protein from *Xenopus laevis* Egg Jelly.** Hitoshi Sugiyama,*,† Alan Rawls,* Allan Bieber,* and Douglas Chandler.* *Molecular and Cellular Biology Program, Arizona State University, Tempe, Arizona 85287; and †Department of Physiology, St. Marianna University, 2-16-1 Sugao, Miyamae-ku, Kawasaki 216-8511, Japan.

We have purified to homogeneity and sequenced the sperm chemoattractant protein allurin, a 21-kDa protein isolated from the egg jelly of *Xenopus laevis*. A full-length cDNA for allurin was prepared and amplified by PCR and then inserted into the expression vector pCR T7/NT-TOPO (Invitrogen). The expression of recombinant allurin in *Escherichia coli* was induced by 1 mM IPTG, the bacteria were lysed, and expressed recombinant allurin was isolated from inclusion bodies. Inclusion bodies were solubilized using 6 M guanidinium chloride and the His-tagged protein purified by elution from a nickel-chelated column. Purified recombinant allurin appeared as a single band of the expected size on SDS–PAGE gels and exhibited a molecular mass of 25,056 by MALDI-TOF MS, a value within 28 atomic units of the theoretical predicted from the sequence. In addition, Western blotting showed that recombinant allurin was recognized by anti-allurin rabbit antiserum. These results confirm that the purified recombinant protein has an identical primary structure with that of native allurin except for addition of the N-terminal His tag and enterokinase cleavage site. Preliminary observations suggest that proteolytic removal of the His tag may produce an active protein. (This study was supported by NSF Grants IBN-9807862 and IBN-0130001.)

298. **Allurin, a 21-kDa Sperm Chemoattractant from *Xenopus* Egg Jelly, Is Expressed in a Hormone-Dependent Manner in the Pars Recta Region of the *Xenopus* Oviduct.** Xueyu Xiang, Alan Rawls, and Douglas E. Chandler. Molecular and Cellular Biology Program, Arizona State University, Tempe, Arizona 85287-1501.

Previously we have cloned and sequenced a 21-kDa sperm chemoattractant from the oviducts of *Xenopus* laevis (*Proc. Natl. Acad. Sci. USA* **78,** 11205–11210). This chemoattractant, allurin, shares homology with the mammalian cysteine-rich secretory protein (CRISP) family some members of which bind to sperm. Northern blotting was carried out to detect the expression of allurin mRNA in a panel of *Xenopus* tissues including reproductive tissues (e.g., ovary, oviduct, uterus) and nonreproductive tissues (e.g., liver, muscle, heart). We found that allurin was expressed only in the oviduct and not in other tissues. Subsequently, dot blotting was performed to observe the expression of allurin mRNA in HCG-primed oviduct compared to that in nonprimed oviduct. It was found that expression is HCG dependent and occurs largely in the upper third (pars recta region) of the oviduct. To determine the

time course of allurin mRNA accumulation after HCG treatment, mRNA was isolated from the oviducts of frogs pretreated with HCG for different time periods (12 and 14 h in a preliminary study). Northern blotting indicated that allurin mRNAs were equally expressed in oviduct at 12 and 14 h after HCG suggesting that this mRNA accumulates prior to these time points. The time course study will be refined, and the distribution of allurin mRNA expression was determined in mammals as well. (This study was supported by NSF Grants IBN-9807852 and IBN-0130001.)

299. **Withdrawn.**

300. **Isolation and Identification of Potential Dental Pulp Stem Cells from Adult Rat.** Shuping Gu, Yiqiang Song, Zunyi Zhang, and YiPing Chen. Department of Cell and Molecular Biology, Tulane University, New Orleans, Louisiana.

Dental repair in adult animals takes place through the activity of odontoblasts that differentiate from a precursor cell population existing in dental pulp tissue. Several odontoblast-like cell lines have been established from bovine or mouse dental papilla. Recently human dental pulp stem cells have been isolated and identified. Here we report isolation and identification of potential dental pulp stem cells from adult rat. The dental pulp was isolated from adult rat molars by separating from the crown and root. Single-cell suspensions of dental pulps were obtained by digestion with pancreatin and trypsin and then plated in 35-mm tissue culture dishes in DMEM with 20% FBS at a density of 1×10^4. Clonogenic and highly proliferative cells were observed in the primary culture which, even after seven passages, remains rapid proliferation and colony-forming capability. The characters of the dental pulp colony-forming cells (DPCFC) were compared with rat bone marrow stromal stem cells. Similar to rat bone marrow stromal cells, the DPCFCs were able to produce sparsely scattered calcified nodules after a 2-week culture in the presence of inorganic phosphate, L-ascorbate-2-phosphate, and dexamethasone. These results demonstrated that the DPCFCs are potential dental pulp stem cells. Experiments to test capability of the DPCFCs to differentiate into odontoblasts and participate in tooth forming are in progress. (Supported by NIH Grant R01DE12329 and the Millennium Trust Health Excellence Fund from the Louisiana Board of Regents.)

301. **The Regulation of the Epithelial Stem Cell Compartment in the Continuous Growing Molar of the Vole by the Notch Signaling Pathway and fgf10.** Mark Tummers and Irma Thesleff. Developmental Biology Program, Institute of Biotechnology, Viikki Biocenter, P.O. Box 56, University of Helsinki, FIN-00014 Helsinki, Finland.

Notch signaling and fgf10 regulate the epithelial stem cell compartment of the continuous growing incisor of the mouse. Here we look at a different model system for continuous growth, the molar of the sibling vole, another rodent. Unlike the incisor the vole molar has a very complex shape as can be seen by the intricate zigzag cusp pattern. This pattern is created by the actions of the primary and secondary enamel knots, the embryonic organizers of the tooth, during early development. Here we show that notch signaling and fgf10 are also involved in the regulation of the stem cell compartment in the vole molar. Because of the complex structure of this tooth we also analyzed some of these expression patterns and the shape by means of three-dimensional reconstructions. The 3D shape revealed a simple nonpatterned base running along the entire length of the molar, possibly containing the stem cells. Just above it is the proliferative zone, but this zone already has the complex zigzag folding pattern as has been laid down by the actions of the enamel knots earlier during development. We propose that this zone acts as a template for the complex shape above it and is fed by stem cell progeny underneath it. The vole molar acts therefore as a structural proliferative unit that maintains the complex shape laid down during early development, by means of a template.

302. Abstract #302 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

303. **Hedgehog Signaling Promotes the Differentiation of ES Cells into Neurons.** J. Thorne, P. Maye, S. Becker, N. Byrd, H. Siemen, A. Soucy, and L. Grabel. Wesleyan University, Middletown, Connecticut 06459.

The discovery of human embryonic stem (ES) cells and their prospective use in cell transplantation therapies heightens our need to identify the parameters that regulate differentiation of ES cells into various progenitor populations. A number of protocols have been defined that promote the differentiation of mouse ES cells into neurons *in vitro,* most of which start with the formation of embryoid bodies, aggregates of ES cells grown in suspension culture, consisting of an inner ectoderm/epiblast epithelial core surrounded by an outer visceral endoderm layer. We have used mouse ES cells to examine the role of Hedgehog signaling in early embryogenesis and have demonstrated that the outer endoderm layer expresses *Indian hedgehog* (*Ihh*) and the core responds by upregulating downstream genes such as *Ptc1* and *gli1*. We now show that *Ihh*-deficient and *Smoothened* (*Smo*)-deficient ES cells (*Smo* encodes a membrane receptor essential for Hedgehog-mediated signaling) form embryoid bodies that have decreased levels of cavitated ectoderm and express lower levels of ectoderm and neurectoderm markers in comparison with embryoid bodies derived from heterozygous mutant ES cells. We also show that treatment with retinoic acid promotes a transition in embryoid body ectoderm from nestin negative to nestin positive in cultures derived from heterozygous mutant cells, but not in cultures derived from *Ihh*- or *Smo*-deficient cells. In addition, neuron outgrowth cultures derived from the *Ihh* or *Smo* heterozygous ES cells demonstrate ample differentiation of nestin-positive neural stem cells as well as postmitotic neurons, while homozygous mutant cultures are severely lacking in these cell types.

304. **Withdrawn.**

305. ***In Vitro* Differentiation of Human Embryonic Stem Cell Lines: Derivation of Progenitors and Differentiated Progeny of Pancreatic Islet Lineages.** B. W. Kahan, L. M. Jacobson, K. Lang, J. Ochoada, D. A. Hullett, and J. S. Odorico. Department of Surgery, University of Wisconsin, Madison, Wisconsin 53792.

Transplantation of the pancreas or isolated islets of Langerhans, a promising treatment for diabetic patients, is restrained because of the limited availability of appropriate donor tissue. Another approach to diabetes that seeks to overcome this problem is the generation of functional islet cells from embryonic stem cells. Toward this aim, we have used human embryonic stem cells to derive differentiated cells having characteristics of progenitor and

differentiated pancreatic islet cells. This system should facilitate identification of: (1) characteristics of pancreatic progenitor cells that generate specific differentiated progeny, (2) proliferative signals that can expand specified progenitor cells, and (3) instructive signals that induce the differentiation of progenitor cells into fully functional β cells. We first identified progenitor and differentiated pancreatic cells and the time course of their appearance, using RT-PCR and antibody staining. To initiate differentiation, stem cell colonies growing as monolayers were suspended with mild proteolytic treatment and transferred to bacteriological petri dishes. Suspended intact colonies gradually transformed into spherical bodies that resemble murine EBs. Two-week suspension cultures were plated onto gelatinized dishes and allowed to differentiate further for up to 10 additional weeks. By antibody staining, cells expressing IPF-1/PDX-1 were observed after 4 weeks of differentiation. Other markers of pancreatic progenitor cells (YY and IAPP) were detected subsequently. Glucagon and somatostatin expression was detected at 8–10 weeks and insulin-expressing cells appeared last, after 3 months total *in vitro* differentiation. A variety of growth conditions and factors have been tested for their potential to promote selective growth of pancreatic progenitor cells from human ES cells.

306. **Evaluating Microcarriers for Delivering Human Adult Mesenchymal Stem Cells in Bone Tissue Engineering.** J. Doctor, C. Petraglia, A. Loveland, M. Dietz, E. Minich, J. Leung, J. Hollinger,* and P. Campbell.* Department of Biology, Duquesne University, Pittsburgh, Pennsylvania; and *Bone Tissue Engineering Center, Carnegie Mellon University, Pittsburgh, Pennsylvania.

We are pursuing novel approaches using two different microcarriers as vehicles for the delivery of human adult mesenchymal stem cells (hAMSC) for tissue engineering. As one vehicle for cell delivery, we are using CultiSphers, commercially available, porous, collagen microcarriers (www.percell.se). Because CultiSphers are made from denatured collagen, they provide a biocompatible and biodegradable system for delivering cells. CultiSphers loaded with cells (hAMSC from Poietics/BioWhittaker) offer the advantage of being able to readily place large numbers of cells directly and controllably in a wound site or on a tissue engineering scaffold. hAMSC readily seed over 95% of the available CultiSphers, and the hAMSC will proliferate to yield 50–100 hAMSC per CultiSpher. hAMSC on CultiSphers cultured in the presence of an osteogenic-inducing supplement differentiate as indicated by the expression of alkaline phosphatase and the deposition of calcium. We are also evaluating hollow hydroxyapatite spheres (www.capbio.com) for the culture of cells for application *in vitro* and *in vivo* in bone tissue engineering. We have examined the attachment, proliferation, and differentiation of several cell types including hAMSC. Spheres made of hydroxyapatite and hydroxyapatite/tricalcium phosphate blends allow the attachment and proliferation of hAMSC *in vitro* and support their differentiation in response to an osteogenic supplement in the culture medium. (Supported by a grant from the Pittsburgh Tissue Engineering Initiative.)

307. **Withdrawn.**

308. **Functions of the Septins in *Caenorhabditis elegans* Development.** Fern P. Finger, Kevin R. Kopish, and John G. White. University of Wisconsin, Madison, Wisconsin.

Septins are a family of GTPases involved in cytokinesis in diverse organisms. They are also expressed in postmitotic cells, suggestive of other cellular functions. *Caenorhabditis elegans* has two septins, encoded by the *unc-59* and *unc-61* genes. Loss of septin function results in approximately 20% embryonic lethality. Up to 50% of those larvae that hatch do not survive the first larval (L1) stage due to defective formation of the pharynx. Some pharynges appear to not have properly elongated and are not attached to the buccal cavity, while others appear morphologically normal, but are also unattached. Preliminary studies reveal no obvious early failures in cell division, indicating a potential role for septins in pharyngeal organogenesis. UNC-59 appears to be associated with junctional complexes in embryonic pharyngeal cells. In contrast, UNC-61 is enriched in the embryonic nervous system, particularly in the nerve ring and ventral cord. UNC-61 localization to the nervous system requires *unc-59* function. Assays of locomotory behavior on newly hatched L1s reveal that mutations in either septin frequently cause uncoordination. As all of the ventral cord neurons are present, these defects cannot be attributed to cytokinesis failures in the ventral nerve cord. This is the first *in vivo* evidence of a role for septins in neuronal development or function. We are using neuronal drugs and GFP reporters to determine if septins are required for neuronal development, function, or both. Septin mutants also have protruding vulvae and extrude their gonads. Abnormalities in the pattern of vulval cell junctions are also being characterized.

309. **BMP Signaling Is Important for Mesoderm Induction and Germ Layer Development in Mouse Embryogenesis.** Shigeto Miura,* Michelle D. Tallquist,† Philippe Soriano,‡ and Yuji Mishina.* *Molecular Developmental Biology Group, LRDT, NIEHS/NIH, Research Triangle Park, North Carolina 27709; Department of Molecular Biology, UT Southwestern Medical Center, 6000 Harry Hines Boulevard, NA5.508A, Dallas, Texas 75390; and ‡Program in Developmental Biology and Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109.

Bmpr encodes a BMP type IA receptor (ALK3) for bone morphogenetic proteins (BMPs) 2 and 4. Bmpr mutant embryos die before gastrulation stage. To dissect the mechanism of how BMP signaling functions to initiate gastrulation, we established a Bmpr-conditional allele. MORE mice that express Cre in epiblast generate an epiblast-specific mutation of Bmpr. At E7.5, mutants showed normal phenotype by histology and marker analyses. Thus, BMP signaling is critical in the extraembryonic tissues for normal gastrulation. Histological analyses of mutant embryos at E8.0–8.5 revealed that (1) neurectoderm highly proliferated; (2) paraxial mesoderm expanded toward ventral side; 3) heart, notochord, and lateral mesoderm did not develop; and (4) gut was not formed. Expression of paraxis suggested paraxial mesoderm expands soon after gastrulation. However, rostral–caudal polarity was established within somites normally, and sclerotome and myotome were formed. Heart marker analyses showed that heart mesoderm developed normally at E7.5 but did not differentiate to form heart tissue in mutants. Expression of axial and lateral mesoderm markers in mutants suggested that notochord and lateral plate mesoderm may be aberrantly formed. These observations indicate that BMP signaling is important for normal germ layer development in mouse embryogenesis.

310. **Two-Stage Patterning of the Avian Intermediate Mesoderm.** Richard G. James and Thomas M. Schultheiss. Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts.

All amniote kidney tissue is derived from the intermediate mesoderm (IM), a strip of mesoderm that lies between the somites and the lateral plate. While many previous studies have examined the inductive interactions which lead to differentiation of the IM into the mammalian metanephros (from which the adult kidney is derived), relatively little is known regarding how the IM itself is specified. In the current study, the avian pronephros is used as a model system to study the specification of the intermediate mesoderm. *In situ* hybridization and immunohistochemical studies were conducted to determine the expression patterns of the kidney transcription factors lim-1, pax-2, c-ret, and wt-1 during chick pronephros development. Next, a fate map of the pronephros was constructed using the lipophilic dye DiI. This information was then used to conduct a series of quail/chick chimeric transplant and explant experiments to identify tissues that play a role in patterning the pronephric IM and the critical time periods during which such patterning events take place. Based on these results and those of others, we propose a two-step model for the patterning of the IM. While tissue is still in the primitive streak, the prospective IM is relatively uncommitted. After cells leave the primitive streak, signals from medial tissues (including Hensen's node and perhaps other axial tissues) generate a field of cells competent to give rise to IM in the medial part of the lateral mesoderm. Subsequently, competition between permissive signals from the somite and a previously uncharacterized inhibitory signal from the lateral plate modulates the number of cells that commit to an IM fate.

311. **Molecular Genetic Analysis of the Mouse Orofacial Cleft Mutation, Dancer.** Jeffrey O. Bush and Rulang Jiang. Department of Biology and Center for Oral Biology, University of Rochester, Rochester, New York 14642.

Cleft lip and cleft palate are common birth defects that affect 1 in 700 to 1 in 1000 infants worldwide. Whereas it is clear that orofacial clefts are genetic diseases, the genes contributing to the cause of most human cleft defects have so far eluded linkage mapping and identification. However, much progress has been made in recent years in understanding the embryological mechanisms leading to orofacial clefts in animal models, particularly mice. Since the embryological developmental processes leading to the formation of the orofacial structures are strikingly similar in mice and humans, characterization of mutations that predispose mouse embryos to orofacial clefting will help elucidate the molecular genetic basis of such birth defects. We have been characterizing a mutation, Dancer (Dc), which causes cleft lip and cleft palate in mice. The Dc mutation arose spontaneously in a heterogeneous mouse colony. Dc/+ heterozygous mutants exhibit circling and head-tossing behavior due to developmental defects in the vestibular structures. Dc/Dc homozygous mutants die perinatally and show cleft lip and cleft palate. Interestingly, Dc/+ heterozygous mice show a genetic background-dependent cleft lip phenotype and exhibit significantly increased susceptibility to teratogen-induced clefting. Using an intersubspecific genetic mapping strategy, we have localized the Dc mutation to within a 2.2-Mb region of proximal Chromosome 19. We have characterized the developmental expression patterns of one candidate gene and are carrying out direct mutation screening of the candidate genes in Dc mutant mice.

312. **FGF10 Functions as a Survival Factor during Mouse Palatogenesis.** Sylvia Alappat,* Zunyi Zhang,* Kentaro Suzuki,† Xiaoyun Zhang,* Gen Yamada,† and Yiping Chen.* *Department of Cell and Molecular Biology, Tulane University, New Orleans, Louisiana; and †Center for Animal Resources and Development, Kumamoto University, Honjo 2-2-1, 860-0811, Kumamoto, Japan.

FGF10 signaling is essential for the development of multiple organs. This is consistent with widespread agenesis or dysgenesis of organs observed in *Fgf10* knockout mice. Recent reports have demonstrated that FGF10 is required for the outgrowth of the limb bud, morphogenesis of the lung and pancreas, and the establishment of stem cells in incisor germs. In these organs, FGF10 regulates proliferation, differentiation, cell fate specification, and survival of tissues. With regard to craniofacial development cleft palate phenotype was not reported in *Fgf10* null mutants. However, further examination revealed a wide open cleft palate phenotype in the *Fgf10* mutant. The current study addresses the role of *Fgf10* in palatogenesis. The expression of *Fgf10* in the developing palate from E11.5 to E14.0 was examined by *in situ* hybridization. *Fgf10* expression was initially detected in the palatal mesenchyme immediately adjacent to the medial edge epithelium (MEE) at E11.5 and shifted laterally and was no longer seen in close apposition to the MEE at E12.5. By E13.5 the level of expression diminished. Histological analysis of the wild-type and mutant embryos revealed clear morphological differences between the two at E12.5 and E13.5. BrdU and TUNEL assays demonstrated a significant level of apoptosis in the MEE but unaltered cell proliferation in the *Fgf10* mutant palatal shelves. *Fgf10* expression in the palatal mesenchyme is thus essential for the survival of cells in the MEE during mammalian palatogenesis. (Supported by NIH Grant RO1DE14044 and P60DE13076.)

313. **Msx1 Controls Alveolar Bone Formation through Bmp4, Dlx5, and Cbfa1.** Zunyi Zhang, Yiqiang Song, Xiaoyun Zhang, and YiPing Chen. Department of Cell and Molecular Biology, Tulane University, New Orleans, Louisiana 70118.

The cranial neural-crest-derived dental mesenchyme consists of dental papilla and dental follicle, with the dental papilla giving rise to odontoblasts and dental pulp, while the dental follicle gives rise to the periodontium including osteoblasts that contribute to alveolar bone formation. Alveolar process is a specialized intramembraneous bone that forms the primary supportive structures for the developing dentition. The homeobox gene Msx1 controls many aspects of craniofacial development, as evidenced by Msx1 mutant mice that exhibit arrest of tooth development, cleft palate, and absence of alveolar bone. Our previous studies demonstrated that ectopic expression of Bmp4, a downstream gene of Msx1, to Msx1 mutant dental mesenchyme was able to partially rescue alveolar bone formation. In this study, we demonstrated that expression of Cbfa1 and Dlx5, two critical transcription factors for bone differentiation that are normally overlapped with Msx1 and Bmp4 in dental mesenchyme and alveolar process, is down-regulated in Msx1 mutant dental mesenchyme. Although ectopic transgenic Bmp4 restored the expression of Dlx5 but not Cbfa1in the dental papilla, expression of the two latter genes was restored in the alveolar bone of Msx1 mutant carrying the Bmp4 transgene.

Together with *in vitro* studies, our results demonstrate that Msx1 regulates Dlx5 but not Cbfa1 in dental papilla through Bmp4. Bmp4 functions upstream to both Dlx5 and Cbfa1 in the development alveolar bone. Ectopic Bmp4 can bypass Msx1 function to induce alveolar bone formation, which might be mediated through induction of Dlx5 and Cbfa1. (Supported by NIH Grant R01DE12329.)

314. **Evidence for Prepatterned Odontogenic Neural Crest.** Yanding Zhang,*,† Shusheng Wang,† Jun Han,‡ Yang Chai,‡ and YiPing Chen.† *College of Bioengineering, Fujian Teachers University, Fuzhou, China; †Department of Cell and Molecular Biology, Tulane University, New Orleans, Louisiana; and ‡Center for Craniofacial Molecular Biology, University of Southern California, Los Angeles, California.

Mammalian tooth develops through sequential and reciprocal interactions between cranial neural crest (CNC) derived ectomesenchymal cells and the stomadial epithelium. Classic tissue recombination studies demonstrated that premigratory CNC and its derived ectomesenchymal cells possess odontogenic capacity and can respond to oral epithelial signals to form tooth, suggesting that the CNC contributing to odontogenic tissue is not prespecified or prepatterned. Here, we showed that, in mice, the CNC has populated the forming first branchial arch before the 9-somite stage and continues to migrate into the arch by the 13-somite stage. Grafts of the first arch from the 10-somite embryo or earlier yielded membranous bone and cysts but no teeth after subrenal culture. However, grafts of the first arch with its dorsally extended tissue containing migrating neural crest from the same aged embryos gave rise to teeth. In contrast, teeth formed in the first arch grafts that do not contain migrating neural crests from embryos with 12 or more somites. Interestingly, the acquisition of tooth forming capability in the first arch coincides with the onset of Fgf8 expression in the oral epithelium. These results suggested that odontogenic neural crest cells are prepatterned. Cells that migrate into the first arch between 10- and 12-somite stages possess the initial odontogenic potential. These cells, once they migrate in and make contact with oral epithelium, may also be responsible for the induction of Fgf8, one of the earliest molecular markers of the dental epithelium. (Supported by NSF of China and grants from NIH and Louisiana Board of Regents.)

315. **Fibroblast Growth Factor Receptors-1 and –2(IIIb), FGF7, and FGF10 Regulate Branching Morphogenesis of Developing Mouse Submandibular Glands in Organ Culture.** M. P. Hoffman, B. L. Kidder, Z. Steinberg, M. Larsen, and H. K. Kleinman. CDBRB, NIDCR, NIH, Bethesda, Maryland.

The embryonic mouse submandibular gland develops by branching morphogenesis, which is regulated by multiple growth factors and their cell surface receptors. There are 23 FGFs with four known receptors. FGFR1, 2, and 3 each have two alternatively spliced isoforms. We hypothesize that FGFR isoforms and their ligands regulate branching morphogenesis during gland development. RT-PCR identified FGFRs 1, 2, 3, and 4 in developing mouse submandibular glands, and branching of the gland in organ culture was reduced using antisense oligonucleotides to FGFR1 and 2. In addition, by inhibiting FGFR1 signaling with SU5402, a FGFR1 tyrosine kinase inhibitor, branching morphogenesis was reduced. Soluble recombinant extracellular domains of FGFR1, 2, and 3 isoforms were used to compete the endogenous growth factors and define the role of individual FGFR isoforms. FGFR2(IIIb) significantly decreased bud formation and branching; FGFR1(IIIb) and FGFR3(IIIc) had lesser effects. BrdU incorporation into glands treated with SU5402 or FGFR2(IIIb) revealed that the mechanism for the reduction in branching is decreased cell proliferation. FGFR2 immunolocalized in the epithelial cells of the terminal buds and the receptor was still present in the glands treated with rFGFR(2IIIb). Analysis of gene expression after SU5402 and rFGFR2(IIIb) treatment suggests that FGFR signaling may regulate the expression of FGF1, -7, and -10 and BMP1 and -7. The addition of FGF7 or FGF10 to glands cultured with SU5402 or rFGFR2(IIIb)was able to rescue the inhibition in branching. Taken together, these data suggest that FGFR1 and 2 gene expression and signaling regulate the expression of genes important for early salivary gland branching morphogenesis, including both FGF7 and FGF10.

316. **Identification of Genes Preferentially Expressed in Distal Endoderm during Lung Branching Morphogenesis.** Yuru Liu, Haiyan Jiang, and Brigid L. M. Hogan. HHMI and Department of Cell and Developmental Biology, Vanderbilt University Medical School, Nashville, Tennessee.

The early development of the embryonic mouse lung involves stereotypic branching morphogenesis. During this process, members of several conserved families of signaling molecules are expressed specifically in the distal tips of the developing lung bud. In this location, they act coordinately to regulate the morphological changes of the lung buds as well as proximal–distal patterning. To know more about the mechanism of lung branching morphogenesis, we carried out a subtractive hybridization and differential screen for genes preferentially expressed in the epithelium at the distal tips of E11.5 lung buds versus more proximal regions. Twenty genes were identified and their expression patterns were examined by whole mount *in situ* hybridization. Sequence analysis identified them as members of different functional groups. One distally expressed gene encodes an ETS domain transcription factor named Erm (Etv5). The function of Erm during lung development was studied by transgenic expression of wild-type, truncated, and repressor forms of the protein. Our results suggest that Erm and possibly the closely related protein Pea3 are new players in the regulatory loops of signaling pathways at the distal tip regulating proliferation and/or proximal–distal patterning.

317. **Tissue Interactions Pattern the Mesenchyme of the Embryonic Mouse Lung.** Molly Weaver,* Mildred Stahlman,† and Brigid L. M. Hogan.†,‡ *Department of Cell and Developmental Biology, †Department of Pathology, and ‡HHMI, Vanderbilt University, Nashville, Tennessee 37232.

Tissue interactions are critical for the development of lung mesenchyme, but the molecular mechanisms controlling patterning and differentiation in this tissue are not well defined. We therefore examined the expression of several patterning genes in the pseudoglandular lung using lacZ reporter alleles. We find that $Bmp4^{lacZ}$ is expressed in a broad domain throughout the lung mesenchyme. The Bmp antagonist $Noggin^{lacZ}$ is expressed in a more restricted fashion in the parabronchial smooth muscle, as well as in individual cells scattered in the distal mesenchyme. This suggested that inhibition of Bmp signaling by Noggin plays a role in parabronchial smooth muscle differentiation. While we do observe patterning defects in homozygous null *Noggin* mutant lungs, differentiation of smooth muscle appears normal. To further examine signaling networks in the lung mesenchyme, we have also

developed an *in vitro* mesenchyme culture system. If separated from its companion epithelium, growth and differentiation of lung mesenchyme is not sustained. Evidence suggests that Shh, a signaling factor expressed throughout the lung epithelium, promotes growth of lung mesenchyme. We demonstrate that recombinant N-Shh protein added to the culture medium can maintain isolated distal lung mesenchyme, including vascular endothelial cells and smooth muscle. Using this *in vitro* culture system, we have examined the effect of Shh on the expression of $Bmp4^{lacZ}$ and $Noggin^{lacZ}$ and on smooth muscle differentiation.

318. **Heparan Sulfate Proteoglycans Modulate the Epithelial Response to Fibroblast Growth Factors during Lung Morphogenesis.** Konstantin Izvolsky,* Deana Shoykhet,† Matthew Nugent,† and Wellington Cardoso.* *Pulmonary Center Department of Medicine and †Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts.

Lung bud formation requires local activation of FGFR2 signaling in the epithelium by mesenchymal-derived FGF10. FGFR2b is evenly expressed throughout the respiratory epithelium, while FGF10 is expressed in distal mesenchyme at prospective sites of budding. Here we provide evidence that the mechanism by which FGF10 induces localized responses in the lung epithelium likely involves selective interactions with heparan sulfate proteoglycans (HSPG). We show that FGF10 binds with high affinity to HSPG from the lung epithelial cell line 20-3 in contrast to FGF7, another FGFR2b ligand which binds poorly to these HSPG. Immunolocalization of HSPG in day-11 to -12 embryonic lungs using the mouse monoclonal antibody 10E4 shows a dynamic pattern of mesenchymal HSPG expression in regions where branching is active. HSPG expression changes rapidly and appears to be associated with areas of FGF10 expression. We demonstrate that the epithelial response to FGFs can be modulated by altering HSPG sulfation by sodium chlorate in mesenchyme-free lung epithelial cultures. Whole-lung cultures reveal a dramatic decrease in FGF10 binding to distal epithelium and disruption of normal branching as a result of sodium chlorate treatment. We suggest that specific heparan sulfate motifs predominantly expressed in distal lung epithelium and mesenchyme are essential for establishing proper FGF10 gradients and for FGF10–FGFR2 signaling during lung morphogenesis.

319. **Gene-Dosage-Sensitive Genetic Interactions between iv, Nodal, and ActRIIB Genes in the Left–Right Asymmetric Patterning.** En Li and S. Paul Oh. Department of Physiology, University of Florida, Gainesville, Florida; and CVRC, Massachusetts General Hospital, Boston, Massachusetts.

We have previously shown that mice deficient in the activin type IIB receptor (ActRIIB) exhibit right isomerism, characterized by mirror-image symmetrical right lungs. We hypothesized that ActRIIB transduces a TGF-β signal which is necessary for the determination of the leftsidedness. To test this hypothesis, we examined laterality defects in mice carrying mutations in both ActRIIB and inversus viscerum (iv) genes, since iv$-/-$ mice display a spectrum of laterality defects including situs inversus, right isomerism, and left isomerism. We found that all mice homozygous for both iv and ActRIIB mutations displayed the right isomerism. Interestingly, the incidence of right isomerism also increased significantly in iv$-/-$;IIB$+/-$ and iv$+/-$;IIB$-/-$ mice compared to homozygous mice carrying either of single gene mutations. A mechanism of the genetic interaction between ActRIIB and iv genes may be that iv modulates the asymmetric expression of a TGF-β family member that signals through activin type II receptors to specify the leftsidedness. Nodal is the most likely candidate. We show here that the penetrance and severity of the right isomerism is significantly elevated in nodal$+/-$;IIB$-/-$ mice, compared to IIB$-/-$ mice. Furthermore, the chimeric mice derived from nodal$-/-$ ES cells displayed right isomerism, indistinguishable from that in (iv$-/-$;IIB$-/-$) mice. We propose that iv functions to establish asymmetric expression of nodal in a gene-dosage-sensitive manner and that nodal signals through the activin type II receptors to specify the leftsidedness via a threshold mechanism.

320. **Inducible Mouse Models in Lung Development.** L. A. Miller, S. E. Wert, and J. A. Whitsett. Children's Hospital Research Foundation, Cincinnati, Ohio 45229-3039.

Shh is thought to play important roles in organogenesis of various tissues including the lung. During early lung development, SHH influences cell proliferation, and branching morphogenesis in humans and mice. To examine the possible role of SHH in lung morphogenesis at various stages of development, a tetracycline-inducible system was developed to overexpress Shh in the embryonic lung. SHH was expressed via a doxycycline-regulated rtTA transgene directed by the SP-C or CCSP promoter. Preliminary observations of lungs from SP-C/rtTA-Shh/tetO and CC10/rtTA-Shh/tetO transgenic mice indicate that Shh overexpression throughout lung development caused lung malformation associated with abnormal airway branching and sacculation. In parallel, a tetracycline-inducible system was generated to knock-out Shh expression during lung development. Since the patterns of Shh expression differ between early and late lung morphogenesis, it is therefore possible that there are separate functions of Shh during these developmental stages.

321. **Do Cardiac Neural Crest Defects in Zebrafish Result in Loss of Cardiomyocytes?** Mariko Sato and H. Joseph Yost. Huntsman Cancer Institute, Salt Lake City, Utah.

Cardiac neural crest is essential for cardiac development in mammals and chick, contributing to conotruncal cushion formation, outflow tract septation, and aortic arch remodeling. We use zebrafish as a tool for studying cardiac neural crest. In zebrafish, we have shown by fate mapping that cardiac neural crest contributes to cardiomyocyte formation as well as pharyngeal arch development. Analyses of several neural crest mutants with heart defects will help us understand the role of cardiac neural crest in cardiac morphogenesis. One of the mutants we are examining is *alyron*, a recessive, embryonic-lethal mutant that has severe defects of both cranial and trunk neural crest cells. At 24 hpf, *alyron* embryos have two chambered, beating hearts; severe pericardiac edema; and no blood circulation. We found that expression patterns of neural-crest-specific genes and cardiomyocyte-specific genes (*cmlc2* and *vmhc*) were altered in *alyron* mutants. Our results suggest that cardiac neural crest contributes to the formation of the atrium and ventricle in zebrafish.

322. **Isolation of Novel Heart and Cardiac Neural Crest Genes by Modified Differential Display.** Brad J. Martinsen, Nathan Groebner, and Jamie Lohr. Department of Pediatrics/Developmental Biology Center, University of Minnesota, Minneapolis, Minnesota.

Cardiac neural crest cells, which are localized in the neural tube from postotic placode to somite 3, are necessary for the normal formation of the heart and great vessels. Unlike other subsets of neural crest cells, the function of cardiac neural crest (CNC) is unique and cannot be replaced by neural crest of other axial levels. The goal of this work is to identify genes which give CNC its unique identity and function and to determine the role of these genes or their downstream targets in cardiovascular development. We have performed two three-way differential display screens in the chick embryo. The screen for early cardiac-specific neural crest genes included differential display of RNA isolated from midbrain, brain at the CNC (BCNC), and full cephalic region minus BCNC, of stage-8 to -10 embryos. A second differential display screen was performed using RNA isolated from 3- to 5-day-old embryonic chick hearts. RNA from distal outflow tract, proximal outflow tract, and atria and ventricles was isolated and compared. Our differential display protocol uses one-base anchored oligo(dT) primers, which reduces the number of reactions required, minimizes redundancy, and improves representation of RNA species. These screens have resulted in the isolation of 10 novel CNC genes and 5 novel heart genes, which have been evaluated for neural tube and cardiac expression patterns. Further studies, including the effects of CNC ablation and misexpression studies will lead to an improved understanding of the role of these genes in the specification of cardiac neural crest and heart development.

323. **An ENU Mutagenesis Screen to Isolate Cardiovascular and Hematopoietic Lethal Mutations Using a Mouse Balancer Chromosome.** Kathryn Hentges,* Yas Furuta,† Colleen Kaiser,* Suzanne Moncrief,* Yingdi Wang,† Randy L. Johnson,† Allan Bradley,‡ and Monica J. Justice.* *Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030; †Department of Biochemistry and Molecular Biology, University of Texas M. D. Anderson Cancer Center, Houston, TX 77030; and ‡Sanger Centre, Cambridge, United Kingdom.

Balancer chromosomes are a useful tool for mutation isolation; however, they have not previously been used in mice. We used a Cre/loxP-engineered Chromosome (Chr) 11 balancer in a genetic screen designed to isolate recessive mouse mutations induced by *N*-ethyl-*N*-nitrosourea (ENU). We have isolated 39 lines of mice on Chr 11 that are lethal before weaning. The time of death has been determined for several mutant lines and ranges from preimplantation to 2 weeks after birth. An examination of mutant phenotypes reveals that many of the mutants are defective in the hematopoetic or cardiovascular system. The phenotypes in these mutants include heart formation outside the amnion, internal hemorrhages late in gestation, and abnormal blood cell counts. Markers of cardiac mesoderm, vascular endothelium, and blood cell development are being analyzed in mutants with phenotypes that suggest cardiovascular or hematopoietic defects. Ongoing histological analysis of late gestation mutants will determine if the embryonic cardiovascular system is abnormal, causing the internal hemorrhage and eventual death of the embryo. Continuing mutation isolation on this and other mouse chromosomes will identify novel members of pathways required for hematopoiesis and cardiovascular development.

324. Abstract #324 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

325. **Effects of TGFβ Proteins on Left–Right Signaling and Cardiac Development.** Jamie Lohr, Allison Arndt, Sarah Wanner, and Maria Breitenfeldt. University of Minnesota and R&D Systems, Minneapolis, Minnesota.

Members of the TGFβ family of cell signaling molecules are involved in the establishment of left–right asymmetry and orientation of the developing heart. While *lefty* and *nodal* appear to have an important and highly conserved role in left–right signaling, the role of other TGFβ family members is less well understood. We investigated the effects of injection of activin, TGFβ1, BMP2, BMP4, and lefty proteins on left–right signaling and cardiac orientation. *Xenopus* embryos were injected with protein in the right lateral plate (RLP) or left lateral plate (LLP) at late neurula stages. Embryos were assayed for Xnr-1, Xlefty, Pitx2, and XNkx2.5 expression and scored for heart development. Activin, TGFβ1, BMP2, and lefty proteins randomized cardiac looping when injected into the RLP. Only lefty protein altered cardiac orientation when injected into LLP. BMP4 injection had no effect on cardiac orientation. RLP activin and TGFβ1 injections resulted in increased right-sided and bilateral expression of Xnr-1, Xlefty, and Pitx2, while lefty protein injected into either LLP or RLP eliminated all expression of Xlefty in the LP mesoderm. No change in domain of XNkx2.5 expression was observed, suggesting that TGFβ signaling does not alter recruitment of cells into the cardiac lineage. In summary, diverse TGFβ proteins can ectopically activate the left-sided signaling pathway when injected into the RLP. Only lefty protein was able to inhibit the normal left-sided signaling pathway, adding evidence for a regulatory role for this protein in the development of normal left–right asymmetry.

326. **Regulation of the Retinoic Acid Signaling Pathway Is Essential for Multiple Events in Early *Xenopus* Cardiogenesis.** Joel A. S. Broomfield, Andrew H. Collop, Roshantha A. S. Chandraratna, and Thomas A. Drysdale. Lawson Health Research Institute, Departments of Paediatrics and Physiology, University of Western Ontario, London, Ontario, Canada N6A 4V2; and Departments of Biology and Chemistry, Retinoid Research, Allergan Inc., Irvine, California 92623.

Retinoic acid receptors (RARs) are essential elements of myocardial differentiation and axis formation. Previously we have observed that retinoic acid can block cardiac differentiation even after heart specification. Here we report that exposure of *Xenopus* embryos to continuous low levels of all-*trans*-RA, starting after gastrulation, results in reduced heart size and a loss of presumptive atrial tissue. Treatments using AGN 194301, a retinoic acid receptor antagonist (Allergan) also resulted in a reduction in heart size, but in this case, presumptive ventricular tissue is lost. The phenotype is similar to, but more severe than, phenotypes observed with various knockout mouse models. Treatment with AGN 194301 also inhibited heart tube formation, leaving the myocardium as a sheet, and this has not been observed in other models of retinoic acid deficiency. Embryos treated with trichostatin A, an inhibitor of histone deacetylases, were found to phenocopy RA-treated embryos suggesting that the RA may be acting by releasing repression of target genes by unliganded RARs. We are currently identifying the downstream targets of RA signaling using a combination of RNA injection and *in situ* hybridization.

327. **Some New FACS about Nkx2-5 and Cardiogenesis.** Mark Solloway, David Elliott, Owen Prall, Christine Biben, and Richard Harvey. Victor Chang Cardiac Research Institute, Darlinghurst, NSW 2010 Australia.

The vertebrate homeobox gene Nkx2-5 sits high in a genetic regulatory hierarchy controlling cardiac morphogenesis. Nkx2-5 is expressed in the paired bilateral myocardial precursor populations in the mouse embryo and expression continues in the myocardial layer of the heart throughout fetal and adult life. Nkx2-5−/− embryos die early in development due to a failure of both cardiac looping and formation of the ventricles. Morphological and gene expression analysis indicates that a primitive myocardial tube develops in these mutants, but the specialized ventricular myocardium does not differentiate. Despite these advances, relatively few targets, either direct or indirect, have been found for Nkx2-5. We are now utilizing the power of FACS and microarray technology to further elucidate these pathways. We have created a strain of mice in which eGFP has been inserted into the Nkx2-5 locus. This renders cardiomyocytes fluorescent from cardiac crescent stages (E7.8), persisting into adulthood. FACS analysis of dissected Nkx2-5GFP/+ hearts shows that even as early as E10.5, cardiomyocytes compromise only 50% of total cells. GFP-positive cells are clearly resolved from nonfluorescent cells, indicating that a 99+% pure population of cardiomyocytes can be obtained for mRNA preparation. We have also targeted lacZ into Nkx2-5GFP/+ cells to produce Nkx2-5−/− ES cells. These tools are now being used to generate developmental profiles of Nkx2-5-dependent genes during embryogenesis and from *in vitro* derived cardiomyocytes.

328. **mRNF4 Is Necessary for Normal Heart Development.** G. E. Lyons, A. Griffin, A. Petrie, E. Lyons, J. Grilley, C. Berrios, L. Bauer, R. Baker, and B. K. Micales. UW Medical School, Madison, Wisconsin 53706.

mRNF4 was identified by a gene trap insertion in a screen for genes required for normal heart development (Baker *et al.*, 1997). A transgenic mouse line was made that carried the gene trap insertion. In this study, mRNF4 homozygous mouse embryos showed heart malformations including ventricular septal defect, hypoplasia of the ventricular wall, and double outlet right ventricle. Peripheral veins were dilated. The neural tube failed to close before E12.5. The mRNF4 mutants died between E12 and E15.5. Cardiac marker analysis by *in situ* hybridization suggested that cardiomyocytes were differentiated. Expression patterns determined by *in situ* hybridization and lacZ staining in heterozygous mouse embryos showed robust expression of mRNF4 in the myocardium, especially in the ventricular septum, ventricular wall, and outflow tract, which correlated well with the locations of defects observed in the hearts of homozygous mice. The gene trap insertion represents a null allele because sequences 3′ to the gene trap are not detected in homozygotes. mRNF4 is known to be a nuclear protein. We have performed a pilot yeast two-hybrid screen for the nuclear proteins with which mRNF4 interacts. We have identified downstream genes that appear to be regulated by the transcriptional coactivation function of mRNF4. The original gene trap insertion was analyzed on a mixed genetic background. We have bred the gene trap onto C57BL6 and BALB/c inbred backgrounds to further analyze the developmental mutations that occur in the absence of the mRNF4 protein. (Supported by NIH HD29471 to G.E.L., who is an Established Investigator of the American Heart Association.)

329. **Defective Heart and Liver Development in Type III TGFβ-Receptor-Deficient Embryos.** Kaye L. Stenvers, Nicole Kountouri, Melinda Tursky, Sue Amatayakul-Chantler, Dianne Grail, Clayton Small, Robert A. Weinberg, and Andrew Sizeland. Ludwig Institute for Cancer Research, Melbourne, Australia; and Whitehead Institute for Biomedical Research, Boston, Massachusetts.

The type III TGFβ receptor (Tβ RIII) binds both TGFβ and inhibin with high affinity and modulates the association of these ligands with the TGFβ and activin type II receptors, respectively. However, the significance of Tβ RIII activity *in vivo* is not known. In this study, we sought to determine the role of Tβ RIII during development. We have identified the predominant expression sites of Tβ RIII mRNA as liver and heart during midgestation by *in situ* hybridization histochemistry and have disrupted the murine Tβ RIII gene by homologous recombination. Consistent with the Tβ RIII mRNA expression pattern, Tβ RIII null mice developed heart and liver defects beginning at embryonic day 13.5 and generally died during late gestation. Tβ RIII null embryos exhibited small liver size, extensive hepatocyte cell death, and loss of liver structure. In addition, half of the Tβ RIII null embryos exhibited a defect in the development of the heart ventricles, resulting in a thin myocardial wall and muscular ventricular septum. The defects observed in Tβ RIII null embryos indicate that Tβ RIII is required during murine development. However, the Tβ RIII null phenotype does not resemble the embryonic phenotypes of the single TGFβ and inhibin null mutations, suggesting that Tβ RIII is dispensable for facilitating certain TGFβ-mediated and inhibin-mediated processes during development.

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332. **BMP Signaling and Patterning of the Liver and Other Endodermal Tissues.** Aidan J. Peterson, Jennifer M. Rossi, and Kenneth S. Zaret. Cellular and Developmental Biology Program, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111.

We are studying the earliest events in murine liver development as a model of endoderm tissue specification. A subset of ventral foregut endoderm cells begins to express liver-specific markers at the seven- to eight-somite stage, and these cells proceed to form a bud and migrate into the surrounding septum transversum mesenchyme. Previous studies have employed a tissue explant system, which recapitulates hepatic gene induction, to identify signaling molecules required for hepatic specification. Treatment of ventral foregut explants with noggin, an extracellular BMP inhibitor, blocks hepatic induction, demonstrating that BMP signals from the neighboring septum transversum are required for endodermal specification. We are seeking to determine if the endoderm is a direct target of BMP signals or if BMP signaling acts through another tissue to effect hepatic specification. To this end, we are studying the expression and intracellular localization of SMAD proteins, which transduce BMP signals. Analysis of SMAD nuclear localization will reveal which cell types are receiving BMP signals at the time of hepatic induction. We are also seeking to identify BMP-responsive target genes by comparing the expression of candidate genes in normal and BMP-inhibited explant samples. In particular, we will assay expression of marker genes of the pancreas and thyroid, which also arise from the ventral foregut endoderm. Together these approaches will define targets of BMP signals at the cellular and molecular levels and provide insights into how BMP signals pattern tissues in the endoderm.

332. **Comparative and Functional DNA-Binding Analyses of the Novel Pharyngeal Factor PEB-1.** Laura Beaster-Jones and Peter Okkema. Department of Biological Sciences, University of Illinois at Chicago, Chicago, Illinois.

The *Caenorhabditis elegans* pharynx is a neuromuscular organ that provides a simple model for studying cell-type specification and organogenesis. Pharyngeal cell-type-specific gene expression is regulated in part by organ-specific mechanisms, and we have recently described the novel factor PEB-1 that binds the enhancer of the pharyngeal muscle myosin gene *myo-2*. PEB-1 is expressed in most pharyngeal cells and may participate in organ-specific regulation of gene expression. We have identified the *peb-1* gene from the nematode *C. briggsae* and shown Cb-PEB-1 is expressed in a pattern similar to that observed in *C. elegans*. Cb-PEB-1 and Ce-PEB-1 share several highly conserved domains that may be important for PEB-1 function. Interestingly the PEB-1 DNA-binding domain (DBD) is not as highly conserved as other DBDs sequenced in both species, suggesting the PEB-1 DBD is evolving relatively rapidly. A match to a Cys/His-rich consensus found in the *Drosophila* Mod(mdg4) proteins is conserved in Cb-PEB-1. Mutations affecting this consensus disrupt PEB-1 DNA binding *in vitro*, indicating that these residues either are directly involved DNA binding or are necessary to maintain PEB-1 structure. We are currently asking if these mutant proteins retain activity *in vivo*. We have also used PCR-assisted binding site selection to determine an 8 bp consensus PEB-1 binding site. χ^2 analysis confirms these bases have highly significant deviations from randomness. Analysis of PEB-1 sites in *myo-2* and other promoters may allow development of a computational algorithm to search the *C. elegans* genome for other PEB-1 target genes.

333. **Lateral Plate Mesoderm Induces the Prepancreatic Domain in a Posterior Dominant Fashion.** Maya E. Kumar, Douglas A. Melton, and Anne Grapin-Botton. Department of Molecular and Cellular Biology, Harvard University, Boston, Massachusetts; and ISREC, Lausanne, Switzerland.

The endoderm gives rise to the epithelium of the gut and associated organs, including the lung, liver, and pancreas, which perform diverse functions from gas exchange to maintaining glucose homeostasis. During embryonic development various organs arise along the gut tube as a series of buds in a stereotyped anterior–posterior (A-P) and dorsal–ventral pattern. How a simple endodermal tube is subdivided into distinct organ domains is unknown. An early sign of patterning, before organ buds emerge along the tube, is the regionalized expression of organ-specific genes. The aim of the present study is to elucidate the interactions governing the induction and maintenance of this regionalized gene expression, with particular attention paid to the establishment of the prepancreatic domain, as marked by the expression of the transcription factor Pdx1. We show that the endoderm that gives rise to the ventral pancreas, as marked by Pdx1, is specified by the six somite stage. We show that lateral plate mesoderm underlying the Pdx1 expression domain sends instructive signals that induce Pdx1. Although specified, the pancreatic domain remains plastic, even after the onset of Pdx1 expression. It can be induced to express posterior markers like CdxA through grafting to a more caudal location. The lateral plate mesoderm appears to pattern the endoderm in a posterior dominant fashion analogous to the patterning of the neural tube. These findings argue for a global A-P mechanism that coordinates the patterning of all three primary germ layers.

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335. **Ectopic Retinoic Acid Induces Three-Dimensional Patterning Defects in the Digestive System.** Kristen J. Lipscomb and Nanette M. Nascone-Yoder. Eckerd College, 4200 54th Avenue South, St. Petersburg, Florida 33711.

The coordination of independent patterning information from the anterior–posterior (AP), dorsal–ventral (DV), and left–right (LR) axes during organogenesis is unknown. Retinoic acid (RA) has been shown to play important roles in AP, DV, and LR patterning in separate developmental contexts. Here we show that *Xenopus laevis* embryos treated with RA at late neurula stages develop anomalies in their digestive systems that result from a perturbation of all three axes of patterning. For example, in the AP dimension, the guts of RA-treated embryos are shortened, with gut marker gene expression being posteriorized along the length of the gut tube. In the LR dimension, RA-treated guts often have a heterotaxic pancreas and/or liver, as well as a malrotated or straight intestine. Accordingly, the normal LR asymmetric expression pattern of *Pitx2* is lost in RA-treated guts. Finally, in the DV dimension, abnormal hepatopancreatic morphogenesis is observed, and aberrant fusion of the dorsal and ventral pancreatic rudiments sometimes results in an annular pancreas. Thus, ectopic RA can independently perturb all axes of gut patterning, and the final phenotype is a composite of altered morphogenesis in all dimensions. These results suggest that multidimensional analyses in RA-treated guts will provide a model system to study the coordination of three-dimensional patterning cues during organogenesis.

336. **Disruption of Pax2/Pax8 Gene Function Reveals Essential Role for Pax8, but Not for Pax2, in Early *Xenopus* Pronephric Kidney Development.** H. Ghanbari and A. W. Brändli. Department of Applied Biosciences Swiss Federal Institute of Technology, Zürich, Switzerland.

Pax genes encode a family of transcription factors that play crucial roles in regulating the development of numerous embryonic organs and structures. In the *Xenopus* pronephric kidney, two members of this family are expressed, Pax2 and Pax8, whereby onset of Pax8 pronephric expression precedes the one of Pax2. To assess the functions of Pax2 and Pax8 during early pronephric development, we chose a morpholino (MO)-based approach to disrupt gene function in *Xenopus*. Pax2-MO or/and Pax8-MO were injected into one blastomere at the two-cell stage. Disruption of Pax2 gene function revealed that pronephric development could apparently proceed normally in the absence of Pax2 expression. Detailed analysis of pronephric marker gene expression revealed largely normal levels and patterns of expression. On the other hand, inhibition of Pax8 function led to the downregulation or complete absence of several pronephric genes. Development of the pronephric kidney was affected from early stages onward, resulting occasionally in the complete absence of pronephric tubules and duct. Impaired kidney function was confirmed by the severe formation of edema in tadpoles, when embryos were injected with Pax8-MO into both blastomeres. Upon double disruption of Pax2 and Pax8 gene functions, the phenotype was identical to the one observed for Pax8-MO alone; however, it occurred at higher frequencies. Taken together, our results suggest that Pax8, but not Pax2, gene function is essential for pronephric development in *Xenopus*.

337. **Notch Signaling in the Developing Kidney.** S. Kuure, K. Sainio, S. Vainio,* and H. Sariola. Developmental Biology Laboratory, Institute of Biomedicine, Biomedicum Helsinki, Helsinki, Finland; and *Biocenter Oulu, Department of Biochemistry, University of Oulu, Oulu, Finland.

We have analyzed the role of Notch pathway during kidney development. The receptors Notch-2 and -4 are expressed in embryonic kidney; Notch-2 in the tubulogenic mesenchyme as well as in the branching ureter; and Notch-4 in endothelial cells. The expression of ligands Delta-1 and Jagged-1 is highest in the epithelialization of induced mesenchyme. Delta-1 expression suggests role for it in the fusion of the future secretory nephron with collecting duct. Jagged-1 transcripts localize to pretubular structures namely cap condensates and aggregates as well as to ureter tips. This might indicate a role for Jagged-1 in sorting out the cell population participating in tubulogenesis or in guidance of the ureteric branching morphogenesis. We have now generated Jagged-1 transgenic mice lines overexpressing the gene under Hoxb7 promoter in Wolffian duct and ureteric bud derivatives. The resulting phenotype is postnatal lethality. These mice die due to either kidney aplasia or severe hypoplasia. The mildest phenotype shows disorganization in overall structure of the kidney having some mislocated glomeruli in the medulla, thin differentiation zone in the cortex, and reduction in the size. These all are possible consequences of ureter branching defect. Mice with several copies of the transgene in their genome have hydroureters with dilated pelvis and often unilateral hypoplasia. *In vitro* such Jagged-1 transgenic kidneys develop poorly showing severe ureter branching defect and supernumerous budding from the Wolffian duct. The most severe phenotype shows complete lack of kidneys already at E13. At the beginning of the kidney development (E11) these animals contain metanephric mesenchyme but the ureteric bud fails to invaginate the mesenchyme leading to degeneration of the kidney rudiment.

338. **Transcriptional Profiling of Tubulogenesis Using Wnt4 Mutant Mice.** M. Todd Valerius and Andrew P. McMahon. Harvard University, Boston, Massachusetts.

The mature nephron contains distinct regions dedicated to specialized physiological functions. While much work in the field has focused on early inductive events and the physiology of mature nephrons, less is known of the molecular processes at work during tubulogenesis to form these complex structures. To identify genes that may play a role in this process we have conducted a transcriptional profiling screen using Wnt4 mutant kidneys. In Wnt4−/− homozygous mice pretubular aggregates are induced; however, epithelial renal vesicles fail to form and subsequent tubulogenesis is blocked. A transcriptional profile comparison between wild-type and Wnt4−/− mutant kidneys at E14.5 was performed using Affymetrix oligonucleotide microarrays. Expected differences between mutant and wild-type kidneys, e.g., Wnt4 itself and Pax8, were also identified by this screen. Several genes expressed in the mature nephron were also correctly identified in comparison analysis as expected. These results suggest that we are effectively identifying genes expressed during tubulogenesis. We are currently validating candidates by *in situ* hybridization. These include regulators of the Wnt signaling pathway, known genes without described kidney function, as well as ESTs.

339. **Altered Cell Adhesive Mechanisms and Signal Transduction during Kidney Development in Bcl-2 −/− Mice.** C. M. Sorenson. University of Wisconsin–Madison, Madison, Wisconsin.

Apoptosis plays a critical role during development and in the maintenance of multicellular organisms. Bcl-2 protects cells from apoptosis initiated by a variety of stimuli including loss of cell adhesion. Bcl-2 is a death repressor that plays an important role during nephrogenesis. Mice deficient in bcl-2 (bcl-2−/−) are born with renal hypoplasia and develop renal cysts by postnatal day 20 (P20). Renal cyst formation in these mice coincides with renal maturation in normal mice. Our research indicates that several signal transduction pathways which are normally down-regulated following renal maturation remain active in cystic kidneys from P20 bcl-2−/− mice. Appropriate regulation of focal adhesion proteins FAK and paxillin is important in cell adhesion, migration, and differentiation. In cystic kidneys, we observe sustained phosphorylation of FAK, paxillin, Src, and ERKs as well as nuclear localization of β-catenin and loss of apical brush border actin staining. Sustained phosphorylation (activation) of these proteins correlated with a reduced expression, a sixfold decrease in activity, and altered distribution of protein tyrosine phosphatases PTP1B and SHP-2. These changes occur in the bcl-2−/− mouse at a time when bcl-2 expression is normally low, suggesting that loss of bcl-2 early in development may alter signaling pathways which ultimately result in the inability of these epithelial cells to terminally differentiate. Thus, renal cyst formation in the bcl-2−/− mice may be the result of an inability to complete differentiation due to continued activation of growth processes including activation of FAK and paxillin.

340. **Identification and Characterization of Male-Specific Gonad Mutants in *Caenorhabditis elegans.*** Weiru Chang, Julie Illi, and David Zarkower. Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, Minnesota 55455.

In *Caenorhabditis elegans,* most tissues are sexually dimorphic between hermaphrodites and males. Notable examples include the musculature, the nervous system, the germline, and the somatic gonad. The sexually dimorphic gonads of both sexes arise from morphologically identical four cell gonad primordia but develop into very different organs. The adult hermaphrodite somatic gonad is two armed and symmetrical with each arm comprised of a uterus, spermatheca, and sheath cells. The male somatic gonad is single armed and asymmetrical with a seminal vesicle and vas deferens. Our goal is to understand how sex-specific cell fates, divisions, polarities, and migrations are controlled and lead to the formation of distinct gonads. Using a GFP marker expressed in the male vas deferens and seminal vesicle (see Thoemke *et al.*, Abstract 109), we performed a F2 screen for male defective gonads. From about 2500 mutagenized haploid genomes we have so far identified about 30 mutants. The mutants preliminarily fall into three classes: gonad with normal male morphology but abnormal differentiation of specific cell types; grossly abnormal morphology but no sign of sex reversal; and abnormal morphology with hermaphrodite cell types present. We are currently focusing on a mutant from the third class. Most mutant males express *lim-7::gfp,* a sheath cell marker, and some mutant males have hermaphrodite vulval structures, indicating a functional hermaphrodite anchor cell. The tail and other male structures appear to be normal. We have mapped the mutant to a small region on chromosome II and are cloning it. We are also further characterizing this and other mutants.

341. **The PDGFα Receptor Is Required for Differentiation of Leydig Cells and Proper Testis Cord Organization in the Embryonic Testis.** Jennifer Brennan, Christopher Tilmann, and Blanche Capel. Duke University Medical Center, Durham, North Carolina.

Primary sex determination is defined as the development of either a testis or an ovary from the bipotential cells of the genital

ridge. This is determined by the presence of absence of the sex-determining gene on the Y chromosome, *Sry*. *Sry* expression initiates a cascade of male-specific cellular and morphological events in the gonad that include proliferation, cell migration, and vascularization. In our lab, we have characterized these events in detail and have begun to identify the molecular factors involved. The platelet-derived growth factor (PDGF) family of ligands and receptors participates in multiple aspects of development and is required for the migration and proliferation of various cell types including smooth muscle and perivascular smooth muscle cells in organs such as the lung and the kidney. Because of the similarities in cell types and developmental events in the testis and the lung and kidney, we investigated the role of PDGF factors in early testis development. Analysis of expression patterns of the PDGF family members (A, B, C, D) and the two PDGF receptors (α, β) demonstrated that PDGF A and the α receptor are expressed in a sexually dimorphic pattern indicating a role in testis development. Using PDGF α receptor null mice, we show that signaling through the α receptor is not required for cell migration as we initially hypothesized, but instead is required in the gonad for the differentiation of fetal Leydig cells. We found that there is a decrease in characteristic XY-specific proliferation at the coelomic surface in null gonads. There are also defects in the organization of the testis cord and interstitial compartments as well as in the organization of the vasculature. This work identifies PDGF signaling through the α receptor as an important event downstream of *Sry* in testis organogenesis and provides insight into the origin and differentiation of fetal Leydig cells.

342. ***Fgf9* Acts Downstream of *Sry* to Induce Proliferation of Sertoli Precursor Cells.** Jenna Schmahl and Blanche Capel. Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710.

The initiation of testis development in mammals depends on the presence or absence of the Y-chromosome-linked gene, *Sry*. It has been shown that one of the earliest effects of *Sry* is the induction of cell proliferation in the XY gonad. The first stage of proliferation gives rise to Sertoli cells, the cell type that expresses *Sry* and directs the formation of testis cords. If the first stage of proliferation is inhibited, Sertoli cells and testis cord formation are also inhibited. Recently, it has been found that XY gonads from *Fgf9* knockout mice have few or no Sertoli cells and often do not form testis cords. We investigated the early proliferation pattern of *Fgf9*−/− XY gonads and found that proliferation is reduced in these mutants during all early stages of development resulting in a decrease in the number of Sertoli progenitor cells. Application of FGF9 to cultured XX gonads induces several male-specific pathways including an increase in proliferation. However, Sertoli markers such as *Sox9* and *Mis* are not induced, suggesting that other elements of the male-specific pathway are required for Sertoli differentiation. These results provide evidence that *Fgf9* acts to build the number of Sertoli precursors to a critical threshold level sufficient to recruit the gonad to the testis pathway.

343. **Sonic Hedgehog Activates Mesenchymal *Gli1* Expression during Prostate Ductal Bud Formation.** Marilyn L. G. Lamm,* Winnie S. Catbagan,* Robert J. Laciak,* Daniel H. Barnett,* Christy M. Hebner,* William Gaffield, David Walterhouse,† Philip Iannaccone,† and Wade Bushman.* *Northwestern University Medical School, U.S. Department of Agriculture, and †Children's Memorial Institute for Education and Research, Northwestern University Medical School, Evanston, Illinois.

Ductal budding in the developing prostate is a testosterone-dependent event that involves signaling between the urogenital sinus epithelium (UGE) and urogenital sinus mesenchyme (UGM). We show here that ductal bud formation is associated with focused expression of Shh in the epithelium of nascent prostate buds and in the growing tips of elongating prostate ducts. This pattern of localized *Shh* expression occurs in response to testosterone stimulation. The gene for the Shh receptor, *Ptc*, is expressed in the UGM, as are the members of the *Gli* gene family of transcriptional regulators (*Gli1*, *Gli2*, and *Gli3*). Expression of *Ptc*, *Gli1*, and *Gli2* are localized primarily to mesenchyme surrounding prostate buds, whereas *Gli3* is expressed diffusely throughout the UGM. A strong dependence of *Gli1* (and *Ptc*) expression on Shh signaling is demonstrated by induction of expression in both the intact urogenital sinus and the isolated UGM by exogenous Shh. A similar dependence of *Gli2* and *Gli3* expression on Shh is not observed. Nonetheless, the chemical inhibitor of Shh signaling, cyclopamine, produced a graded inhibition of *Gli* gene expression (*Gli1* > *Gli2* > *Gli3*) in urogenital sinus explants that was paralleled by a severe inhibition of ductal budding.

344. **Expression Analysis on Genes Involved in the Development of External Genitalia.** G. Yamada, K. Suzuki, Y. Ogino, Y. Sato, H. Ogi, H. Katoh, M. Kamikawa, and R. Haraguchi. Center for Animal Resources and Development (CARD), Kumamoto University, Kumamoto, Japan.

Our group has been analyzing on the roles of several key signaling molecules during murine external genitalia formation. In this presentation, series of gene expression analysis on "putative regulatory genes" for external genitalia formation will be presented. The external genitalia formation has been not elucidated well and left virtually unexplored. Recent gene KO studies have suggested that the developmental process of the anlage, the genital tubercle (GT), has much in common with organs like limb buds or other endodermal organs. Given the complexity of cascades composed with growth factors, transcription factors revealed in other organogenesis, detailed expression analysis will be required in the research field. Morphological divergence for external genitalia (copulatory organ) formation awaits researches on differential gene expressions, functions of regulatory genes. (Haraguchi *et al.*, 2000, *Development* **127,** 2471–2479; Ogino *et al.*, 2001, *Ann. N.Y. Acad. Sci.* **948,** 13–31; Haraguchi *et al.*, 2001, *Development* **128,** 4241–4250; Cohn *et al.*, 1996, *Cell* **80,** 739–746.)

345. **BMP Signaling in Mammalian Neural Tube Development.** Rolf W. Stottmann, Yuiji Mishina,* and John A. Klingensmith. Department of Cell Biology, Duke University Medical Center, Durham, North Carolina; and *NIEHS, Research Triangle Park, North Carolina.

Several lines of evidence implicate bone morphogenetic proteins (BMPs) in neurulation. BMP activity has been shown to affect the forming neural plate and neural crest, as well as pattern the neural tube along the dorsal–ventral axis. The requirements for BMP signaling have been difficult to study in the mouse, however, because many null mutations are lethal before the process of neurulation. We are addressing the role of BMPs in dorsal neural tube development using two different approaches. We have used the Cre-Lox tissue-specific recombination system to inhibit BMP receptor IA (BMPR-IA) signal transduction in the dorsal neural tube. This ablation of BMPR-IA does not result in a neurulation defect. Furthermore, whole mount *in situ* hybridization and histo-

logical analysis of mutant embryos reveals normal patterns of neuronal pattering, as well as neural crest specification, initial migration, and differentiation. BMPR-IA-ablated embryos coexpressing a lineage tracer do show a loss of neural crest in some target tissues. Embryos lacking BMPR-IA in the dorsal neural tube are phenotypically indistinguishable from wild type through E10.5, show circulatory system defects at E11.5 and do not survive to E12.5. To study the consequences of excess BMP signaling on dorsal development, we are studying embryos lacking the BMP antagonist Noggin. *Noggin* is expressed both dorsally and ventrally in the neural tube, and loss of function in the mouse results in severe neural tube defects resembling human malformations. Neurulation defects in *noggin* mutants are readily apparent at E9.5. While *noggin* mutants display appropriate patterning in the dorsal neural tube, initial neural crest specification seems to be expanded. The consequence of this neural crest expansion is under investigation. These complementary approaches lead to a further understanding of BMP signaling in the dorsal neural tube.

346. **Role of Ventral Midline Signals in the Formation of the Trigeminal Ganglion.** Natalia Fedtsova* and Eric E. Turner.*,† *Department of Psychiatry, UCSD, La Jolla, California 92093; and †VA Medical Center, San Diego, California 92122.

The majority of trigeminal neurons originate from the trigeminal placode, a specialized region of the surface ectoderm. The trigeminal precursors then undergo an epithelial–mesenchymal transformation and migrate in a ventral–caudal direction, where after several cycles of division they differentiate into mature sensory neurons. It is not clear what signals mediate this migratory pathway and determine the final position of the ganglion. In previous work, we have shown that placode-derived trigeminal precursors express the POU-domain transcription factor Brn3a and that coexpression of Brn3a and the LIM-domain factor Islet1/2 marks sensory neurons throughout the neural axis. Here we demonstrate that signals from the ventral midline, including Shh, determine the position of the trigeminal ganglia. First, when tissue from the notocord or spinal cord floorplate is transplanted beneath the developing trigeminal placode, ectopic sensory ganglia result. Second, in mice with a targeted deletion of Shh, induction of sensory neurogenesis takes place, but the trigeminal ganglia fuse at the ventral midline. Third, Shh-soaked agarose beads implanted under the surface ectoderm of the 10-somite chick embryo near the presumptive trigeminal ganglion locally disrupt the formation of the ganglion. We suggest that the notochord transforms the local mesenchyme and creates a nonpermissive condition for the passage of migrating trigeminal precursors and that Shh protein mediates this process.

347. **Homeobox Gene Prop1 Is Required for the Response to WNT Signaling in the Pituitary Gland.** M. Brinkmeier,* M. Potok,* K. Bromfield,* T. Gridley,† J. Meeldijk,‡ H. Clevers,‡ and S. Camper.* *Department of Human Genetics, University of Michigan, Ann Arbor, Michigan; †The Jackson Laboratory, Bar Harbor, Maine; and ‡Department of Immunology, University Hospital, Utrecht, The Netherlands.

The wingless family member *Wnt4* and the homeobox gene *Prop1* are both important in establishing the *Pit1* lineage, which is composed of three hormone-producing cell types of the anterior pituitary gland. In a differential expression screen for potential *Prop1* targets we identified several members of the WNT signaling pathway. Canonical WNT signaling activates transcription by releasing β-catenin into the nucleus where it activates TCF transcription factors. We discovered that *Prop1* mutant mice fail to express *TCF4* normally, suggesting that Prop1 is required for the response to WNT signaling. TCFs interact with groucho-like corepressors, such as the transducin-like enhancer of split family, Tle1-4. One of the corepressors, *Tle3*, is dependent upon *Prop1* for appropriate region specific expression. The Tle3 domain is expanded ventrally in *Prop1* mutants instead of being confined to the dorsal aspect of the pituitary primordia. The ectopic expression of Tle3 in *Prop1* mutants may result from altered expression of *Grg5*, a repressor of the groucho-like corepressors that is also known as *Aes*, amino terminal enhancer of split. These data suggest that region specific expression of corepressors plays a role in cell fate specification in the pituitary gland that is similar to that observed in the developing neural tube and that *Prop1* is critical for this process.

348. ***Lhx4* and *Prop1* Are Required for Cell Survival and Expansion of the Pituitary Primordia.** Lori T. Raetzman,* Robert Ward,† and Sally A. Camper.*,† *Department of Human Genetics and †Graduate Program in Cell and Molecular Biology, University of Michigan, Ann Arbor, Michigan 48109-0638.

Pituitary gland development relies on a series of signaling events that lead to the specification and proliferation of the five cell types in the anterior pituitary. Two homeodomain transcription factors, *Prop1* and *Lhx4*, are necessary for this process. Mutations that reduce or ablate *Prop1* or *Lhx4* function cause combined pituitary hormone deficiency (CPHD) in humans and mice with loss of almost all anterior pituitary cell types. Although specification of the somatotropes, thyrotropes, and gonadotropes occurs, there is no evidence of lineage-specific expansion of these cell types. In this study we determined that the hypoplasia in *Lhx4* mutant pituitaries results from increased cell death and that the reduced differentiation is attributable to a temporal shift in LHX3 activation. In contrast, *Prop1* mutants exhibit normal cell proliferation and cell survival but show evidence of defective dorsal–ventral patterning. Molecular genetic analyses reveal that *Lhx4* and *Prop1* have overlapping functions in early pituitary development. Double mutants exhibit delayed corticotrope specification and complete failure of all other anterior pituitary cell types to differentiate. Thus, *Lhx4* and *Prop1* have critical, but mechanistically different, roles in specification and expansion of specialized anterior pituitary cells.

349. **Molecular and Tissue Interactions Controlling Inner Ear Induction.** Andrew K. Groves, Kareen Martin, and Stephen T. Brown. House Ear Institute, Los Angeles, California.

The inner ear develops from ectoderm on either side of the hindbrain. Despite nearly a century of study, the exact tissue interactions that induce the ear and the molecules that mediate these interactions are only just beginning to be understood. We have examined the role of the hindbrain in inner ear induction by examining vitamin A-deficient (VAD) quail embryos. VAD embryos lack rhombomeres 5–7, with both rhombomeres 3 and 4 being enlarged caudally. In the normal embryo, the otic vesicle develops adjacent to rhombomeres 5 and 6. In VAD embryos, the otic vesicle still develops immediately in front of the first pair of somites, adjacent to rhombomere 3. This suggests that if a hindbrain signal is required for ear induction, it is not localized exclusively to rhombomeres 5 and 6. Some molecules previously implicated in inner ear induction, such as Wnt8c, are not expressed

in rhombomere 3 in VAD embryos adjacent to where the ear develops. We have begun to examine a series of Wnt and FGF family members in normal and VAD embryos to try and understand the necessity and sufficiency of these signaling molecules during ear induction.

350. ***Drosophila hibris*, a Gene Related to Human Nephrin, Is Involved in Muscle and Eye Development.** H. A. Dworak, M. Thomas, and H. Sink. Skirball Institute of Biomolecular Medicine, New York University School of Medicine, 540 First Avenue, New York, New York 10016.

We have characterized the gene *hibris* (*hbs*) which encodes a member of the immunoglobulin superfamily that has homology to vertebrate Nephrins and *Drosophila* Sticks-and-stones (Sns). We have focused our attention on the roles of *hbs* in both embryonic muscle and adult eye development. Embryonic muscle formation in *Drosophila* involves the recognition, adhesion, and fusion between two different types of myoblasts—fusion-competent myoblasts and founder cells—to form syncitial muscle fibers. *hbs* is expressed in fusion competent myoblasts during the fusion process. While mutations in *hbs* show that its function is largely redundant during muscle development, overexpression of *hbs* in the mesoderm disrupts myoblast fusion. Recently several other molecular players involved in the fusion process have been identified including the cell surface proteins Sns, Dumbfounded (Duf). and Roughest (Rst). We have shown that Hbs and its homolog Sns can interact with the myoblast attractant Dumbfounded and have characterized the domain requirements necessary for these interactions. *hbs* is also expressed within and posterior to the morphogenetic furrow in the larval eye imaginal disk. Loss- and gain-of-function *hbs* mutants display defects in ommatidial patterning. We present here models of how Hibris functions in cell–cell interactions during both myoblast fusion and eye development.

351. **Network of FGF, Ihh, and BMP Signaling Coordinates Chondrocyte Proliferation and Differentiation.** E. Minina,* C. Kreschel,* M. C. Naski,† D. M. Ornitz,‡ and A. Vortkamp.* *Max-Planck-Institute for Molecular Genetics, 14195 Berlin, Germany; †The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284-7750; and ‡Washington University School of Medicine, St. Louis, Missouri 63110.

During endochondral ossification chondrocyte proliferation and differentiation are regulated by numerous signaling systems, such as fibroblast growth factors (FGF), Indian hedgehog (Ihh), parathyroid hormone-like peptide (Pthlh), and bone morphogenetic proteins (BMP). Mutations in FGF receptor 3 gene lead to the human dwarfism syndrome achondroplasia. We have analyzed the interaction of FGFs with the Ihh/Pthlh and the BMP pathways using an organ culture system of embryonic mouse limbs, which was supplemented with activators or inhibitors of each pathway. In addition we used transgenic mice carrying an activated Fgf receptor 3 (Fgfr3ach mice) or the chicken Ihh gene under control of the ColII-promoter (ColII/Ihh mice). We have shown that FGF signaling regulates the onset of hypertrophic differentiation upstream of the Ihh/Pthlh pathway. Additionally, we found that FGF signaling advances terminal hypertrophic differentiation and reduces chondrocyte proliferation in an Ihh-independent manner. Our results led to a new interpretation of the role of FGF signaling in regulating chondrocyte differentiation: instead of delaying hypertrophic differentiation FGF signaling actually accelerates this process. As the FGF-induced phenotype resembles the phenotype of blocking BMP signaling, we performed coculture experiments with FGF2 and BMP2. We found that both pathways act in an antagonistic relationship. Moreover, BMP signaling can rescue the dwarfism phenotype of Fgfr3ach mice, which is characterized by reduced zones of proliferating and hypertrophic chondrocytes. Finally, we propose a model in which FGF, Ihh, and BMP signaling are integrated into a common network controlling chondrocyte development.

352. **Regulation and Roles for VEGF in Skeletal Development.** Elazar Zelzer,* William McLean,* Yin-Shan Ng,† Patricia A. D'Amore,† and Bjorn R. Olsen.* *Department of Cell Biology, Harvard Medical School, 240 Longwood Avenue, Boston, Massachusetts; and †Department of Ophthalmology, Schepens Eye Research Institute, Boston, Massachusetts.

VEGF signaling plays a pivotal role in development, yet little is known about the factors that control its expression. Tissue-specific loss of VEGF expression and angiogenesis in hypertrophic cartilage as a result of targeting a single gene, Cbfa1, demonstrate that Cbfa1/Runx2 is a necessary component of a tissue specific genetic program that regulates VEGF during endochondral bone formation. Previous attempts to examine the roles of VEGF *in vivo* have been largely unsuccessful because deletion of even one VEGF allele leads to embryonic lethality before skeletal development is initiated. The availability of mice expressing only the VEGF120 isoform (which do survive to term) offered an opportunity to explore the function of VEGF during embryonic skeletal development. Our study of these mice provides new *in vivo* evidence for multiple important roles of VEGF in both endochondral and intramembranous bone formation as well as some insights into isoform-specific functions. There are two key differences in vascularization of developing bones between wild-type and VEGF120/120 mice. VEGF120/120 mice have not only a delayed recruitment of blood vessels into the perichondrium but also show delayed invasion of vessels into the primary ossification center, demonstrating a significant role of VEGF at both an early and a late stage of cartilage vascularization. These findings are the basis for a two-step model of VEGF-controlled vascularization of the developing skeleton, a hypothesis that is supported by the new finding that VEGF is expressed robustly in the perichondrium and surrounding tissue of cartilage templates of future bones well before blood vessels appear in these regions. We also describe new *in vivo* evidence for a possible role of VEGF in chondrocyte maturation and document that VEGF has a direct role in regulating osteoblastic activity based on *in vivo* evidence and organ culture experiments.

353. **Functional Analysis of GDF6 during Skeletogenesis.** Laura Gamer, Karen Cox, and Vicki Rosen. HSDM/Forsyth Institute, Boston, Massachusetts; and Wyeth, Cambridge, Massachusetts.

Growth and differentiation factors (GDFs) play important roles in the embryonic skeleton. During limb development, Gdf5 is expressed in the interzones of all joints while Gdf6 localizes to the elbows, knees, wrists, and ankles. While Gdf5 mutations in mice and humans have identified a direct role for Gdf5 in skeletal patterning, the role of Gdf6 in the skeleton remains unclear. To examine the function of Gdf6, beads soaked in hGDF6 protein were implanted in chick limb buds. Exogenous GDF6 increased the size of limb cartilage elements and caused joint fusions. Chondrocytes around the GDF6 bead were disorganized and had reduced levels of Ihh and collagen X when compared to controls, suggesting GDF6 was delaying chondrocyte maturation. Further analysis of treated

limbs revealed that GDF6 was diverting mesenchymal cells away from muscle and into the chondrocyte lineage. Although expression of Pax3 did not change, strong inhibition of MyoD was detected 24 h after GDF6 treatment, and when examined later, abnormal muscle patterning and decreased muscle size were evident. Our data indicate that Gdf6 induces limb cartilage in two ways. Early on, Gdf6 recruits muscle progenitor cells into cartilage condensations and later it maintains a population of immature chondrocytes within these condensations, resulting in thicker skeletal elements and joint fusions. These Gdf6 effects are distinct from the increases in cell proliferation and adhesion observed with Gdf5 overexpression in chick. Taken together, our data suggest that Gdfs have multiple roles during skeletogenesis, functioning to recruit cells into cartilage and to signal from the interzone to adjacent cartilages to modulate joint formation.

354. **Gene-Dosage-Sensitive Compensatory Mechanism of Activin Type II Receptors for Mediating GDF11 and Nodal Signals for Anteroposterior and Left–Right Patternings.** S. Paul Oh,* Changyeol Yeo,† Youngjae Lee,* Heindrich Schrewe,‡ Sejin Lee,§ Malcome Whitman,† and En Li.¶ *Department of Physiology, University of Florida, Gainesville, Florida; †Harvard Medical School, Boston, Massachusetts; ‡Max-Planck Institute, Berlin, Germany; §Johns Hopkins University, Baltimore, Maryland; and ¶Massachusetts General Hospital, Boston, Massachusetts.

Vertebral bodies manifest the segmentation along the anteroposterior (AP) body axis in vertebrates. The Hox genes have been shown to play an important role in specification of segmental identities. Recent studies have demonstrated that transforming growth factor-β (TGF-β) family protein, growth and differentiation factor 11 (Gdf11), and TGF-β family receptor, Activin receptor type IIB (ActRIIB), are involved in controlling the spatiotemporal expression of Hox genes along the AP axis and that disruption of each of these genes cause anterior transformation of vertebra. Although Gdf11 and ActRIIB belong to TGF-β family ligand and receptor, respectively, a more severe skeletal defect in Gdf11-null than that in ActRIIB-null mice made establishing a ligand–receptor relationship between these two genes elusive. Here we demonstrate using genetic and biochemical studies that ActRIIB and its subfamily receptor ActRIIA cooperatively mediate the Gdf11 signal for the specification of axial vertebra in a gene-dosage-sensitive manner. In addition, we also show genetic evidences that a similar compensatory mechanism of these two receptors may exist in mediating another TGF-β signal, nodal, for the left–right patterning and development of anterior head structure.

355. **Redundancy between Components of the Segmentation Oscillator and the Delta/Notch Pathway Protects the Anterior Somites of Zebrafish from Genetic Perturbation.** Andrew C. Oates and Robert K. Ho. University of Chicago, Chicago, Illinois.

We studied the role of cyclic HER genes, her1 and her7, in segmentation and oscillator function by inhibiting their translation with morpholinos and measuring somite formation, segment polarity, and the wave-like domains of cyclic gene expression. Loss of her1 or her7 function produces distinct defects in somitogenesis, segment polarity, and cyclic expression. Combining the effect of her1 and her7 loss causes more severe defects, including a complete absence of cyclic expression domains, suggesting that her7 and her1 are a central component of the segmentation oscillator. Muscle and bone precursors are nevertheless specified, and erratically positioned epithelial furrows form, indicating that the role of the cyclic expression domains is to position the segmental boundaries, most likely through control of segment polarity. We next studied the interaction of the oscillator with the Delta/Notch pathway by inhibiting HER translation in the deltaD/after eight, notch1a/deadly seven, and beamter mutants. Alone, these mutants show posterior segmentation defects, as does the her7 morpholino-induced loss of function, whereas her1 loss gives isolated, mild segmental defects along the axis. Combining her7 or her1 loss with any of the mutants, or with each other, results in additional defects in the anterior paraxial mesoderm indicating that HER genes and the Delta/Notch pathway have redundant functions in anterior somitogenesis. These results, while not ruling out the existence of anterior or posterior-specific mechanisms, show that redundancy is responsible for the apparent independence of anterior and posterior somitogenesis.

356. ***doubleridge*, a New Mouse Mutant with an Atypical Apical Ectodermal Ridge (AER) Resulting in Postaxial Polydactyly and Syndactyly.** Bryan T. MacDonald, Maja Adamska, and Miriam H. Meisler. Department of Human Genetics, University of Michigan, Ann Arbor, Michigan 48109-0618.

doubleridge (*dblr*) is a recessive viable transgene-induced insertional mutant with abnormal digit morphology. *dblr* mutants consistently show postaxial polydactyly as well as syndactyly of digits 5 and 6 in the forelimbs. Inner digit syndactyly is also observed at lower frequency and is often more severe on the right forelimb. The limbs exhibit normal length and dorsal–ventral characteristics, although ventral footpads are occasionally missing. The phenotype is first identifiable at E10.5 with limb buds displaying a flattened distal tip. At E11.5, two broad elevations divided by a furrow are observed in the posterior forelimb bud, in contrast to the normal raised AER. *Fgf8* is expressed at E11.5 in two distinct posterior domains, bifurcating from a single broad stripe in the anterior limb bud. Occasionally a second *Fgf8* band extends across the entire distal tip. An extra *Shh* expression domain in the posterior mesenchyme is located below the additional *Fgf8* domain. The extra *Shh* domain may be responsible for the postaxial supernumary digit in the forelimb. *dblr* mutants display normal expression of *Wnt7a* and *En1* in the dorsal and ventral compartments, respectively. Members of the notch signaling pathway are currently under investigation. Chromosomal FISH physically mapped the transgene to mouse chromosome 19C. Genetic mapping of the phenotype confirmed the location on Chr 19 and excluded *Fgf8* as a candidate gene. Further studies are under way to identify and characterize the gene disrupted by the *dblr* mutation.

357. Abstract #357 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

358. **Embryonic and Regenerative Forelimb/Hindlimb Patterns Are Controlled by Different Mechanisms.** Hans-Georg Simon, Barbara Linkhart, and Paul Khan. CMIER-Developmental Systems Biology, Northwestern University Medical School, Evanston, Illinois.

The T-domain transcription factors Tbx4 and Tbx5 have been implicated, by virtue of their limb-type specific expression, in controlling the identity of vertebrate legs and arms, respectively.

To study the roles these genes have in developing and regenerating limbs, we have cloned Tbx4 and Tbx5 cDNAs from the newt and generated antisera that recognize Tbx4 or Tbx5 proteins. We show that in two urodele amphibians, newts and axolotls, the regulation of Tbx4 and Tbx5 is different from higher vertebrates. At the mRNA and protein level, both Tbx4 and Tbx5 are expressed in developing hindlimbs as well as in developing forelimbs. The coexpression of these genes argues that additional factors are involved in the control of limb-type-specific patterns. To identify such accessory factors, we have performed two-hybrid screens and isolated proteins containing different classes of protein–protein interaction domains. One novel protein is expressed in developing forelimbs and hindlimbs and interacts specifically with the Tbx4 and Tbx5 transcription factors. While Tbx4 and Tbx5 are coexpressed in the limbs during development, they display a different expression during regenerative pattern formation: Tbx4 is exclusively reactivated in hindlimb blastemas, and Tbx5 is exclusively reactivated in forelimb blastemas. Growth and differentiation of the regeneration blastema resemble that of the embryonic bud; however, we present for the first time clear molecular evidence that the patterning mechanisms in these two structures are controlled by different gene activities.

359. **Expression Patterns of Tbx4 and Tbx5.** Ange Krause, Barbara Linkhart, Luke Sleiter, Paul Khan, and Hans-Georg Simon. CMIER-Developmental Systems Biology, Northwestern University Medical School, Evanston, Illinois.

Two members of the T-box gene family—the forelimb-specific Tbx5 and the hindlimb-specific Tbx4—are thought to control limb identity. These genes encode transcription factors composed of a DNA-binding domain (T-domain) and a transactivator—repressor domain. The encoded proteins reveal remarkable evolutionary conservation among various vertebrates. In humans, point mutations in TBX5 cause Holt—Oram syndrome, a disease characterized by hand, arm, and shoulder malformations, as well as heart septation defects. In higher vertebrates, such as the mouse and chick, various studies (including our own) have demonstrated that the Tbx4 and Tbx5 genes are expressed in the limb fields during the period of limb determination. As an in-depth study of the behavior of these genes, our lab uses two model systems: regenerating urodele amphibia, such as the newt and axolotl, and the chick. In the adult newt, our work has shown that these genes control forelimb and hindlimb shapes during regeneration, similar to limb development in other vertebrates. However, newt embryos exhibit a differing pattern of Tbx4 and Tbx5 expression, as both genes are expressed in the forelimb and hindlimb. To study whether mRNA expression results in the translation of protein, we generated Tbx4-specific and Tbx5-specific antibodies that were designed to detect the respective Tbx proteins in all vertebrates. Protein expression patterns of Tbx4 and Tbx5 are consistent with their respective message, which confirms our previous *in situ* data. This would suggest that although Tbx4 and Tbx5 are good candidates for specification of fore- and hindlimb morphologies, they may not execute this function alone. It is possible that Tbx4 and Tbx5 proteins interact with other cellular proteins, thereby instructing limbs to develop different morphological structures.

360. Abstract #360 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

361. **Decreased Adipose Tissue and Altered Metabolic Function in Wnt-10b Transgenic Mice.** Kenneth A. Longo, Sona Kang, Wendy S. Wright, Peter C. Lucas, Marc R. Opp, and Ormond A. MacDougald. University of Michigan, Ann Arbor, Michigan.

The Wnts are a family of proteins that influence several developmental processes. We recently demonstrated that one member, Wnt-10b, blocks adipogenesis *in vitro.* To test the effect of Wnt-10b *in vivo,* we made a transgenic animal that expresses Wnt-10b in adipose tissue using the 422/aP2 promoter. Transgenic animals have less white adipose tissue (WAT) and transgenic WAT is histologically indistinguishable from wild-type WAT. As expected, the transgenic animals have altered metabolic substrate utilization. However, unlike other lipoatrophic mouse models that develop diabetes, Wnt-10b transgenic mice are not insulin resistant. Transgenic WAT expresses mRNAs for the key adipogenic transcription factors C/EBPα and PPARγ. Lower transgenic levels of SREBP-1c correlate with decreased expression of lipogenic mRNAs for FAS, ACC1, and SCDI. However, levels of HSL and LPL mRNAs are normal. Furthermore, transgenic brown adipose tissue (BAT) histologically resembles WAT and correlates with lower body temperature. The expression of mRNAs involved in differentiation and thermogenic function of BAT (C/EBPα, PPARγ, PGC-1α, PGC-1β, and UCP-1) are lower or completely absent in transgenic mice. These data suggest that Wnt-10b is a powerful modulator of adipocyte differentiation *in vivo.* The Wnt-10b transgenic mouse is a unique lipoatrophic animal free of diabetes, but with impaired thermogenesis.

362. **A Unique System to Study Keratinocyte Proliferation and Differentiation.** Wen-Pin Wang, Yu-Fang Hsu, Ya-Tzu Chen, and Da-Wei Liu. Institute of Molecular and Cellular Biology, Tzu Chi University, Hualien, Taiwan, Republic of China.

The RCAS-TVA system is based on the finding that infection of mammalian cells by a subgroup A avian leukosis virus (the RCAS) vector is not achieved unless the mammalian cell produces the receptor from the avian tv-a gene. Transgenic mice producing the TVA receptor allow infection by RCAS viruses as long as the cells are actively dividing. This system has several advantages over classical transgenic animal models. Particularly, multiple infection of TVA mice, namely simultaneous expression of multiple genes in mice, can be achieved without intensive breeding procedure. We have successfully adapted this novel system to study skin development by generating TVA-expressing transgenic mice under the control of keratin 14 promoter. K14 promoter is strongly active in dividing cells of epidermis, which facilitates the infection of retroviruses. Optimized conditions for retroviral infection into the skin of K174-TVA mice *in vitro* and *in vivo* were established using marker virus. The transduction of dissociated keratinocytes from K14-TVA transgenic mice with RCAS-AP retrovirus showed the impressive AP signal. Organ culture of mouse skin showed abnormal hair growth after infection with RCAS-noggin. Topical application of RCAS-AP showed a nice AP signal in the skin, particularly in the interfollicular region. These results greatly expand the usage of the RCAS-TVA system for studying the impact of various exogenous genes on keratinocyte proliferation, differentiation, and stratification. Furthermore, since K14 promoter is active in the epidermal stem cells, the system can be used to study stem cell properties.

363. **Limb Development in the Absence of Sonic Hedgehog (Shh) and Gli3 Function.** John F. Fallon,* Ying Litingtung,† Yina Li,† Randall D. Dahn,* and Chin Chiang.† *University of Wisconsin, Madison, Wisconsin; and †Vanderbilt University, Nashville, Tennessee.

Current models propose that *Sonic hedgehog* (*Shh*) is the primary determinant controlling anteroposterior (A/P) development of amniote limbs, directing digit formation and specifying digit identities through dose-dependent activation of target gene expression. *Gli3* is thought to negatively regulate *Shh* by restricting its expression to the posterior mesoderm. Using the $Shh^{-/-}$; $Gli3^{-/-}$ null mouse, we show that *Shh* and *Gli3* are not only dispensable for the formation of mouse limb skeletal elements, but normally act to constrain skeletogenic potential. $Shh^{-/-}$; $Gli3^{-/-}$ limbs are distally complete and polydactylous, but completely lack wild-type digit identities. Interestingly, $Shh^{-/-}$; $Gli3^{-/-}$ and $Gli3^{-/-}$ limbs are indistinguishable from one another, demonstrating that *Shh* exerts no effect on skeletal patterning in the absence of *Gli3*. We suggest that *Shh* signaling in the limb has a single primary output, regulating the relative balance of *Gli3* full length (or transcriptional activator) and *Gli3* respressor activities. We propose that the principal developmental function of *Shh* and *Gli3* in normal limb patterning is to refine autopodial morphology, concomitantly imposing pentadactyl constraint on the mesoderm's polydactl potential and organizing the specification of discrete digit identities.

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365. **Initiation and Elaboration of Leaves.** Sarah Hake,* Angela Hay,* Harley Smith,* and Miltos Tsiantis.† *Plant Gene Expression Center, Albany, California 94710; and †Oxford University, Oxford, United Kingdom.

Meristems produce leaves in distinct patterns that allow one to predict the site of the next leaf. These preleaf cells are marked by distinct gene expression patterns, including the loss of *knotted1*-like homeobox (*knox*) gene expression. *STM* in *Arabidopsis* and *kn1* in maize are expressed throughout the meristem, except in the group of cells that will produce the next leaf. In the absence of either *STM* or *kn1*, the shoot meristem fails to progress. We have used an inducible system to investigate the function of *knox* genes. Plants that carry the *kn1* gene fused to the glucocorticoid receptor are normal in the absence of the hormone dexamethasone. Upon dexamethasone addition, the next few leaves that form are deeply lobed. We have followed the morphology of these plants at the same time examining repression and induction of genes in hormone pathways. Our data implicate both cytokinin and gibberellin as downstream of *knox* gene function. *knox* genes encode homeodomain proteins in the TALE superfamily. We have found that KN1, like Meis or Hth, bind to DNA in a complex with other TALE homeodomain proteins. We are combining a biochemical and genetic approach to find targets of KNOX proteins. Whereas *knox* genes are likely to regulate leaf initiation, other genes function during leaf elaboration, which involves establishment of three axis, distal–proximal, dorsal–ventral, and medial–lateral. We have identified a new dominant mutant, *Wab*, which provides evidence of the interconnections between establishment of the distal–proximal and medial–lateral domains.

366. **Flower Development in Pea: Role of *Proliferating Inflorescence Meristem* (*PIM*), an *AP1* Homolog.** S. Singer,* S. Maki,* J. Sollinger,† J. Plotz,* K. Fitzgerald,* J. Fishbach,* and H. Mullen.* *Department of Biology, Carleton College, Northfield, Minnesota; and †Department of Biology, Southern Oregon University, Ashland, Oregon.

PIM (*PROLIFERATING INFLORESCENCE MERISTEM*) is an *AP1* homolog in garden pea that interacts with a suite of genes to regulate floral development. Two alleles, *pim-1* and *pim-2*, alter the fate of the floral meristem resulting in atavistic traits, including development of a triad of structures found in less-derived papilionoid legumes. *PIM* appears to be partially redundant in function to two other genes, *BROC* (*BROOCCOLI*) and *COCH* (*COCHLEATA*). The phenotype of *pim broc* plants resembles broccoli and is phenotypeically similar to *ap1 cal* mutants in *Arabidopsis*. *CAL* and *BROC* must have evolved independently based on the work of Purugganan *et al.* (2000, *Genetics* **155,** 855). No phenotypic differences are observed between wild type and *broc*. The most severe of four *coch* mutants, *coch-w*, affects stipule and flower morphology and symmetry. Under different daylength and light regimes, *coch-w pim* plants remain entirely vegetative. Like *COCH* which functions in both vegetative and reproductive organogenesis, *ST* (*REDUCED STIPULES*) affects both stipule and sepal development. The sepal function is revealed only in the absence of *PIM*. Plants that are *pim st* have small narrow stipules. (Supported by NSF 9977057.)

367. **Regulation of Ectodermal Organogenesis by TNF Signaling.** Irma Thesleff. Institute of Biotechnology, University of Helsinki, Helsinki, Finland.

A new tumor necrosis factor (TNF) pathway has been recently identified which, unlike most TNFs, has an important function in the regulation of embryonic development. Interestingly, three key components of this pathway are previously unknown novel proteins: the TNF ligand ectodysplasin, its death domain-containing receptor EDAR, and the death domain adapter molecule EDARADD. Their functions are nonredundant and their mutations cause similar phenotypes in man and mouse. This pathway was discovered and worked out through cloning of genes behind human ectodermal dysplasia syndromes and the corresponding mouse mutants. We have analyzed the roles of ectodysplasin-EDAR signaling in wild-type and mutant mice and shown that it mediates interactions between ectodermal cell compartments during tooth and hair follicle morphogenesis. This pathway controls the formation and function of ectodermal placodes and is integrated with Wnt and activin signaling. It acts upstream of most other genes expressed in ectodermal placodes and apparently regulates the patterning of hairs and teeth. (Supported by the Academy of Finland.)

368. **The Ventral Midline Endoderm Constitutes a Molecularly Distinct Population of Cells.** Yin-Xiong Li, Marzena Zdanowicz, Harriett Stadt, and Margaret Kirby. Duke University Medical Center, Durham, North Carolina.

The head consists of distinct dorsal (neural) and ventral (frontonasal and pharyngeal) domains. While the head process and notochord are well recognized as dorsal midline organizers, no axial structure has been described that might play such a role in patterning the ventral head. We recently identified a midline craniocaudal stripe of endodermal cells in the ventral floor of the foregut after placing a label in the node. Using quail–chick chimeras, we have been able to show that derivatives of the ventral midline endoderm include endothelium, endocardium, and myocardium. Using laser-capture microdissection and microarray screening of mouse frozen sections we have been able to show that the ventral midline endoderm represents a molecularly distinct population of cells compared to other locations in the endoderm.

Several of the genes with the most abundant expression are associated with human syndromes that include craniofacial and cardiac defects. The location and gene expression of these cells is concordant with the phenotypes seen in human sequences of craniocardiofacial defects such as the DiGeorge and velocardiofacial syndromes.

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370. **Evolution of a Well-Characterized Embryonic Promoter: The *Endo16 cis*-Regulatory System of Sea Urchins.** Gregory A. Wray. Department of Biology, Duke University, Durham, North Carolina.

Changes in the organization and function of embryonic gene networks are clearly a significant component of the genetic basis for metazoan diversity. Relatively little is known, however, about the evolutionary mechanisms that shape *cis*-regulatory sequences and their function. We are currently examining the evolution of the *Endo16* promoter of sea urchins, about which an exceptional level of functional information is available. Detailed SNP maps of allelic variation in full-length promoter sequences from two congeric species, along with sequences from several additional species, provide estimates of the strength of positive selection on transcription-factor-binding sites. Among closely related species, several key binding sites have been eliminated by deletions or overwritten by substitutions, providing evidence for stabilizing selection. Comparisons to more distantly related species reveal extensive divergence in promoter sequences, despite an almost perfectly conserved transcription profile. Transient expression assays demonstrate that this conserved expression profile is generated by regulatory sequences that are functionally incompatible with the *in vivo* array of transcription factors in other species. Together, our results indicate that a variety of evolutionary mechanisms operate on this complex *cis*-regulatory region.

371. **Engrailed Expression in Three Polychaete Annelids: A Possible Role in Chaetogenesis.** Elaine C. Seaver and Mark Q. Martindale. Kewalo Marine Lab, University of Hawaii, Honolulu, Hawaii.

Historically, annelids and arthropods were thought to share a common segmented ancestor. The segment polarity gene engrailed (en) is expressed in a highly conserved pattern of ectodermal stripes in at the posterior boundary of each segment prior to morphological segmentation in arthropods and has been shown to affect patterning within segments in flies. Spatiotemporal patterns of gene expression of genes underlying the segmentation process may be conserved if segmentation has a common origin between these two phlya. To address this possibility, we have taken a comparative approach and analyzed expression patterns of en by whole mount *in situ* hybridization at several stages of development in three different polychaete annelids: Capitella, Chaetopterus, and Hydroides. Each species has a distinct body plan, degree of tagmatization, and life history. None of them show the highly conserved pattern of en seen in arthropods. Rather, staining is observed in the CNS and notably in the developing chaetal sacs in all three species. Chaetae represent a distinguishing feature of the polychaetes and en expression in association with several different chaetal types supports the idea of a general role in chaetogenesis in polychaetes. From our data we propose that 1/en is not utilized in segment formation in the annelid lineage and 2/en may have been coopted in annelids to function in the formation of chaetae, a defining character of annelids.

372. **Antagonism between Bone Morphogenesis Proteins and Noggin in the Branching Morphogenesis of Feathers.** Mingke Yu, Ping Wu, and Cheng-Ming Chuong. Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, California.

The most unique characteristics of feather is that it is a highly ordered hierarchical branched structure. This evolutionary novelty confers flight function to the birds. The molecular and cellular basis for the branching morphogenesis has been studied in the *Drosophila*'s trachea system, mouse lung, and kidney, etc. However, none of them form the three levels of branches, i.e., from rachis to barbs; from ramus to barbules; and from barbules to hooklets with highly ordered spacing, size, and regularity. We have established a novel and efficient gene expression system in the regenerating chicken feather follicles using the replication competent avian sarcoma (RCAS) retrovirus. Study of the expression patterns and overexpression effects of BMP4, BMP2, and Noggin in the chicken feather follicles has indicated that an antagonistic balance between noggin and BMP4 plays critical roles in the first branching level, with BMP4 promoting rachis formation and barb fusion, while Noggin promotes more branching of barbs and rachis. In the second and third branching levels, however, BMP2 and BMP4 determine the formation of rami, barbules, cilia, and hooklets possibly by regulating cell differentiation, shape changes, and organization. The implication of the developmental pathways in the evolution of feather is speculated.

373. **Activation of FLC by ART1, ART2, and FRI Is Required for the Altered Body Plan of the Sy-0 Ecotype of *Arabidopsis*.** Branislava Poduska, Tania Humphrey, Antje Redweik, and Vojislava Grbic. Department of Plant Sciences, University of Western Ontario, 1151 Richmond Street, London, Ontario N6A 5B8, Canada.

The late-flowering behavior of *Arabidopsis* winter-annual ecotypes is mainly conferred by two genes, FRIGIDA (FRI) and FLOWERING LOCUS C (FLC). Sy-0 is a late-flowering *Arabidopsis* ecotype characterized by an enlarged basal rosette, aerial rosettes, and reversion of the inflorescence and floral meristems. Genetic analysis has revealed the requirement of dominant alleles at four loci for the establishment of late flowering in Sy-0. These have been identified as FRI, FLC, and two novel flowering loci designated ART1 and ART2 (aerial rosette 1 and 2). ART1 maps 20 cM proximal to FLC on chromosome V. The ART1 gene has been localized to a 15-kb genomic region and its cloning and molecular characterization are currently under way. Genetic interaction studies show that ART1 and ART2 cause transcriptional activation of FLC independently of FRI. Thus, ART1 and ART2 delineate a novel flowering subpathway that interacts with the FRI-activated pathway at or upstream of FLC. These novel interactions among transcriptional regulators of FLC, discovered in a naturally occurring ecotype of *Arabidopsis*, cause a heterochronic shift in shoot apical meristem development that results in a novel *Arabidopsis* morphology. Therefore, understanding the molecular mechanism of ART1/ART2 action would allow us to gain an insight into the regulation of flowering in *Arabidopsis* and would also uncover one of the mechanisms that lead to the evolution of plant morphological diversity.

374. **Evolution of Vulva Development in Nematodes: From Genetics and Genomics to Gene Function.** Ralf J. Sommer. Department for Evolutionary Biology, Max-Planck Institute for Developmental Biology, Spemannstrasse 37, D-72076 Tubingen, Germany.

The detailed understanding of *Caenorhabditis elegans* biology and particularly its developmental biology makes nematodes an attractive group of organisms for comparative evolutionary studies. More specifically, development of the vulva, the egg-laying structure of nematode females and hermaphrodites, has been studied in great detail. Since developmental processes can be analyzed at the cellular, the genetic, and the molecular levels, the comparative analysis of nematode vulva development can serve as a case study in evolutionary developmental biology. In *Pristionchus pacificus*, vulva formation differs from *C. elegans* with respect to (i) the mechanism of vulva induction, (ii) the evolution of a new signaling center in the posterior body region, and (iii) the fate of nonvulval epidermal cells. More than 100 vulva-defective mutants have been isolated in *P. pacificus* over the years. To allow cloning of the corresponding genes, a genomic approach has been used, generating a genetic linkage map and a physical map of the *P. pacificus* genome. The genetic and molecular analysis of vulva development now indicates that some vulva genes show conservation and change of function simultaneously; that is, they have functions that are conserved while other functions clearly differ between the two species. Other genes are only involved in vulva development in one but not the other species. Thus, the molecular, genetic, and cellular processes can change dramatically during evolution, even when the developmental structure under consideration is homologous between species.

375. **Rapid Coevolution of the Nematode Sex-Determining Genes *fem-3* and *tra-2*.** E. S. Haag, S. Wang, D. Bernstein, M. Wickens, and J. Kimble. Department of Biochemistry and HHMI, University of Wisconsin, Madison, Wisconsin 53706.

In the nematode *Caenorhabditis elegans*, fem-3 encodes a novel protein essential for specification of male fates in both somatic and germ cells. The physical interaction of FEM-3 with TRA-2 has been proposed to be the major mechanism by which TRA-2 promotes female development. In addition, translational control of *fem-3* via its 3′ UTR is required for hermaphrodites to cease spermatogenesis and start oogenesis. To learn more about fem-3 and the evolution of hermaphroditism, we have cloned the *fem-3* orthologs from the two closest relatives of *C. elegans*, *C. briggsae* and *C. remanei*. FEM-3 is the most rapidly evolving protein yet described in *Caenorhabditis*. Despite this, RNA interference demonstrates a conserved requirement for Cb-fem-3 and Cr-fem-3 in the promotion of male somatic fates. Yeast two-hybrid assays indicate that the binding of TRA-2 and FEM-3 is also conserved but is species specific, suggesting that compensatory coevolution has occurred. The significance of the conserved interaction is confirmed by epistasis experiments employing double RNAi. Finally, we provide data consistent with conservation of a translational repressor element in the 3′ UTRs of Cr-fem-3 and Cb-fem-3, suggesting that translational control of fem-3 is not unique to hermaphrodites. These results help to explain how the sex determination systems of more distantly related organisms come to bear little resemblance to each other.

376. **Lineage Compartments and Signaling Boundaries in the Wing of the Fruitfly.** Seth S. Blair. Department of Zoology, University of Wisconsin–Madison, Madison, Wisconsin.

Early in its growth phase the developing wing imaginal disk of *Drosophila* is subdivided into a small number of lineage compartments, between which cells cannot cross. The anterior/posterior (A/P) lineage restriction requires the posterior expression of the Engrailed and Invected (En-Inv) transcription factors, while the dorsal/ventral (D/V) restriction requires the dorsal expression of the Apterous transcription factor. The lineage compartments are known to serve two functions: they separate cells with different identities and terminal fates, and they establish specialized cells at compartmental boundaries by regulating intercompartmental signaling. Cells on the anterior side of the A/P boundary respond to Hedgehog signaling from the posterior by expressing the secreted morphogen Decapentaplegic, and cells on both sides of the D/V boundary respond to reciprocal Notch signaling by expressing the secreted morphogen Wingless. While the purpose of the lineage restrictions is now thought to be understood, little is known about the molecular bases of cellular affinities that maintain those restrictions. Nonetheless, much has been learned by studying the regulation of the lineage boundaries. It was initially thought that En-Inv and Apterous regulated the expression of a compartment-specific form of cellular affinity. However, evidence from my own and other laboratories now indicates that the formation of boundary cells is required for the maintenance of the A/P and D/V lineage restrictions, suggesting the existence of one or more boundary-specific states of cell affinity. I will also present evidence supporting the existence of second form of affinity present in the posterior compartment, likely regulated by En-Inv. Finally, I will discuss evidence for localized differences in the regulation of compartmental affinities.

377. **Linking Morphogen Gradients to Morphogenesis.** Ethan Bier,* Jennifer Trimble,* Karen Lunde,† and Orna Cook.* *University of California, San Diego, California; and University of Freiburg, Freiburg, Germany.

Longitudinal wing veins in *Drosophila* form at boundaries between discrete territories of cells along the anterior–posterior axis of the developing wing primordium. A variety of evidence suggests that a common line drawing mechanism is responsible initiating vein development between adjacent domains of cells. According to this "for-export-only" signaling model, cells in one domain produce a short-range signal to which they cannot respond. As a consequence of this constraint, cells lying in a narrow line immediately outside the signal-producing domain are the only cells that can respond to the signal by activating expression of vein-promoting genes. In response to vein-inducing borders, distinct genes organize different patterns of gene expression on a vein-by-vein basis. In the case of the L2 vein, expression of the knirps (kni) gene is induced along the anterior border of a broad domain of spalt expression. kni in turn organizes gene expression appropriate for the L2 primordium. In the case of the L5 vein, a yet unknown boundary induces expression of the abrupt gene which organizes gene expression in the L5 primordium. A central unresolved question is whether the observed vein-specific gene expression is essential for the proper development of distinct vein characteristics. Progress in understanding the relationships between morphogens, vein-inducing boundaries, vein-organizing genes, and vein morphogenesis will be discussed.

378. **The Establishment of Crustacean Segments.** Nipam H. Patel. HHMI, University of Chicago, Chicago, Illinois.

It is generally accepted that all arthropods are derived from a segmented ancestor and that extant arthropods retain this fundamental aspect of their body plan. We understand the molecular and genetic

mechanisms that establish this pattern of segmentation in *Drosophila* melanogaster, but it seems that there have been evolutionary changes in the mechanisms that establish this same pattern of segmentation in different arthropod species. Our lab has focused recently on trying to understand how segments are established in the amphipod crustacean, *Parhyale hawaiensis*. In this animal, it appears that segmentation is tied to a specific pattern of cell division, but that it does utilize, in a modified fashion, many of the same genes used in *Drosophila* segmentation such as hairy, runt, and engrailed. We will also present our preliminary results on using RNAi and morpholinos to disrupt gene expression in these animals.

379. **MicroRNAs and Heterochronic Genes.** V. Ambros, R. Lee, A. Abbott, L. Sempere, A. Lavanway, N. Sokol, and D. Jewell. Department of Genetics, Dartmouth Medical School, Hanover, New Hampshire 03755.

The postembryonic development of *Caenorhabditis elegans* proceeds through four larval stages to the adult. The proper formation of adult tissues and structures requires a coordinated schedule of the preceeding larval cell division and differentiation events. The timing of these stage-specific developmental events is controlled by a genetic pathway of heterochronic genes. The various heterochronic genes encode regulatory molecules that include transcription factors, RNA-binding proteins, and small antisense RNA translational repressors. Regulatory interactions among the genes of this pathway control the stage-specific expression of developmental programs in diverse cell types. The products of two of these heterochronic genes, *lin-4* and *let-7*, are small noncoding RNAs that repress translation of target messenger RNAs through a mechanism involving antisense base pairing. cDNA cloning of very small transcripts and informatics screens for characteristic secondary structures have identified additional genes of the *lin-4/let-7* class. This class of regulatory RNAs, called microRNAs, is abundant and diverse. There are more than 100 different microRNA genes in *C. elegans*, many of which are conserved in insects and vertebrates. The diversity of microRNA sequences and their developmental patterns of expression suggest that microRNAs play significant roles in the control of development in animals.

380. **Genetic Regulation of Vegetative Phase Change in Plants.** S. Poethig. Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6018.

The transition between discrete stages of shoot development in plants is known as "phase change." At least two such stages exist during the vegetative phase of shoot growth—a juvenile phase and an adult phase. These phases are distinguished both by phase-specific vegetative traits and by the capacity of the shoot to undergo reproductive development. Analysis of mutations affecting the position of the juvenile-to-adult transition along the axis of the shoot reveals that the identity of leaves at a particular position on the shoot is regulated by the interaction between two independent processes—leaf production and the process that regulates leaf identity. We are particularly interested in the latter process and have identified several genes in *Arabidopsis* and maize that regulate the expression of juvenile and adult vegetative traits. One of these genes, HASTY/EARLY PHASE CHANGE, affects both vegetative identity and reproductive competence, whereas other genes (SQUINT, ZIPPY1, and ZIPPY2) affect vegetative identity but do not have a major effect on reproductive development. HASTY/EARLY PHASE CHANGE is a homolog of the nucleocytoplasmic transport receptor, Exportin-5, and therefore probably regulates the transition from juvenile to adult development by mediating intracellular location of key regulatory molecules. SQUINT is the *Arabidopsis* ortholog of cyclophilin-40, a component of the HST90 chaperone complex. Information about the function of these genes will be presented.

381. **Light Control of *Arabidopsis* Development, a Role of Protein Degradation.** Xing Wang Deng. Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut 06520-8104.

We are interested in the molecular mechanism responsible for regulation of development by extracellular stimuli. We used the light control of *Arabidopsis* seedling development as an experimental model. During our dissection of the genetic network involved in light control of *Arabidopsis* development, 11 pleiotropic COP/DET/FUS loci have been identified and revealed to be responsible for mediating light control of *Arabidopsis* seedling developmental program switch. Among them, COP1 is the master repressor of photomorphogenic development and acts within the nucleus as an E3 ligase by directly targeting photomorphogenesis-promoting transcription factors' degradation by the 26S proteasome in darkness. Light inactivates COP1 and causes a reduction in its nuclear abundance. Another gene, COP10, encodes a likely E2 component. While most remaining genes encode subunits of a highly conserved multisubunit protein complex, the COP9 signalosome, which defines a novel regulator of the E3 ligases and promotes deconjugation of NEDD8/RUB1 from the certain E3 ligase. Therefore, this group of regulators defines new cellular machinery in regulating cellular responses to external stimuli or stresses. Currently, we are applying both molecular genetics and genomic approaches to further analyze this novel cellular machinery conserved among all multicellular organisms. Through this combinatorial approach we hope to better understand signaling mechanisms in how extracellular signals regulate this machinery pathway and how this machinery controls the genome expression pattern in response to the environmental signals.

382. **Does the Segmentation Clock Measure Embryonic Time?** Olivier Pourquie, Kim Dale, Mary-Lee Dequeant, Julien Dubrulle, Tadahiro Iimura, Caroline Jouve, Mike McGrew, Pascale Malapert, Miguel Maroto, amd Sandrine Millet. Laboratoire de génétique et de physiologie du développement (LGPD), Developmental Biology Institute of Marseille (IBDM), CNRS-INSERM-Université de la méditerranée-AP de Marseille, Campus de Luminy, Case 907, 13288 Marseille Cedex 09, France.

We have shown that vertebrate somitogenesis is associated with a molecular oscillator, the segmentation clock, whose periodicity matches that of the somitogenesis process. Molecular evidence for the existence of this clock in vertebrates has been obtained on the basis of periodic expression of genes in the presomitic mesoderm (PSM), all of which are implicated in the Notch pathway. Notch signaling is required for cycling gene expression and appears to be part of the mechanism of the oscillator. Recent evidence indicated interactions between the segmentation clock and the spatiotemporal activation of Hox genes suggesting coordination between segmentation and anteroposterior patterning during the development of vertebrate embryos. This led to the suggestion that the segmentation clock could act as a time-measuring device in the PSM progenitor cells and that this temporal information could be converted into anteroposterior information may be via progressive opening of the Hox gene clusters. We will discuss experiments aimed at testing this hypothesis.

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384. **Nuclear Localization of Disheveled Is Required for Wnt/β-Catenin Signal Transduction.** Keiji Itoh, Barbara Brott, Marianne Ratcliffe, and Sergei Sokol. Department of Microbiology and Molecular Genetics, Harvard Medical School and Molecular Medicine Unit, Beth Israel Deaconess Medical Center, Boston, Massachusetts 02215.

Secreted Wnt proteins are implicated in the control of cell fate and polarity in many developmental systems. Wnt signal transduction from Frizzled receptors to different cellular targets is mediated by Disheveled (Dsh), an abundant cytoplasmic protein. Inhibition of nuclear export leads to accumulation of Dsh in the nucleus. Various Dsh deletion constructs were examined for their capability to enter the nucleus. Whereas the three known conserved domains of Dsh, including DIX, PDZ, and DEP, were dispensable, a 40-amino-acid sequence adjacent to the PDZ domain was required for Dsh nuclear import. Site-directed mutagenesis identified specific amino acid residues that are critical for nuclear localization of Dsh. When these were mutated, the ability of Dsh to activate downstream targets of Wnt signaling and stabilize β-catenin was impaired, whereas Frizzled-dependent membrane recruitment of Dsh was not affected. Substitution of the critical residues with the SV40 nuclear localization signal restored full Dsh activity. We gained further insight into the nuclear function of Dsh by showing that it formed a complex with TCF3, a transcription factor required for β-catenin signaling. Moreover, Dsh stimulated the association of TCF3 and β-catenin. These observations suggest a more direct role of Dsh in transcriptional activation of Wnt/β-catenin target genes in the nucleus.

385. **BMP Signals Positively Regulate Nodal Expression during the Early Somite Stage in the Chick Embryo: Implications for Left–Right Development.** M. Elisa Piedra and Maria A. Ros. Departamento de Anatomía y Biología Celularm, Universidad de Cantabria, Cantabria, Spain.

Exogenous application of BMP to the lateral plate mesoderm (LPM) of early somite stage chick embryos had a positive effect on Nodal expression. BMP applications into the right LPM were followed by a rapid activation of Nodal while applications into the left LPM resulted in expansion of the normal domain of Nodal expression. Conversely, blocking of BMP signaling by Noggin in the left LPM clearly interfered with activation of Nodal expression. These results suggest that endogenous BMP signaling is necessary for activation of Nodal expression in the LPM. We have also found that BMP positively regulates the expression of Caronte, Snail, and CFC in both the left and the right LPM. Treatment with BMP impaired the midline causing downregulation of Lefty1, Brachyury, and Shh expression. However, we demonstrate that the molecular alteration of the midline was not sufficient to induce Nodal expression on the right. Thus, our work identifies a positive effect of BMP signaling on Nodal expression, likely mediated by the induction of CFC. This has important implications for our present understanding of left–right development.

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387. **Tracheal Branching Morphogenesis in *Drosophila* as a Model System to Analyze Cell Migration *in Vivo*.** Markus Affolter. Biozentrum, Basel, Switzerland.

Much of our knowledge concerning the intracellular mechanisms involved in cell locomotion has been obtained from *in vitro* studies of cells in culture. Many of the concepts derived from these systems have been partially confirmed *in vivo* but numerous questions regarding the control of cell migration during the development of multicellular organisms remain to be addressed. Tracheal morphogenesis in *Drosophila melanogaster* embryos represents an excellent *in vivo* model system to study the genetic control of cell migration. Many genes that are involved in the process have been isolated and a general scheme for tracheal branching morphogenesis is emerging. More recently, the development of the tracheal system has been studied in wild-type and mutant living embryos using GFP proteins and 4D confocal microscopy. I will summarize these data and incorporate them into a refined model of branching morphogenesis, which relies both on genetic data and on observations made at the cellular level *in vivo*.

388. **Exclusion of Germ Plasm Components from Somatic Lineages by Localized Protein Degradation.** Cynthia Derenzo, Kimberly Reese, and Geraldine Seydoux. Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

In *Drosophila, Caenorhabditis elegans, Xenopus,* and zebrafish embryos, germ plasm components segregate with the germ lineage and are not maintained in somatic lineages. Here we show that, in *C. elegans,* this process involves active degradation of germ plasm components in somatic lineages. PIE-1, POS-1, and MEX-1 are germ plasm components that share a pair of CCCH zinc fingers (ZF1 and ZF2). When fused to GFP, ZF1s from each protein cause GFP to be degraded specifically in somatic blastomeres. Consistent with a role in protein degradation, mutations in ZF1 cause abnormal stabilization of PIE-1 in somatic blastomeres. We have identified a novel protein ZIF-1 that binds to the ZF1 of PIE-1. Depletion of ZIF-1 by RNAi causes PIE-1, POS-1 and MEX-1 to be maintained in somatic lineages and results in embryonic lethality. A yeast two-hybrid screen revealed that ZIF-1 can bind to elongin C. Elongin C in mammals functions with Cul2 as an E3 ubiquitin ligase that targets specific proteins for ubiquitination and degradation. Consistent with this role, RNAi depletion of elongin C, Cul2, or the E2 ubiquitin conjugation enzyme Ubc5 blocks ZF1-dependent degradation in somatic blastomeres. These results suggest that a ZIF-1/Elongin C/Cul2/Ubc5 complex targets ZF1 containing proteins for degradation in somatic cells. We propose that localization of germ plasm components involves both targeting to the germ lineage and rapid degradation in somatic lineages.

389. Abstract #389 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

390. **Separation of the Germ Line at the Eight-Cell Stage—The Invariant Cell Lineage of the Amphipod *Parhyale hawaiensis*.** Matthias Gerberding, William Browne, Sabbi Lall, and Nipam Patel. HHMI, University of Chicago, Chicago, Illinois.

Total cleavage during early embryogenesis often generates blastomeres with different fate. Among the arthropods, total cleavage is found in some primitive insect taxa and several crustacean taxa and is

regarded as a derived mode of development. Previous studies of those taxa described cleavage patterns and correlated cell movement and morphology with cell fate. We have studied the total cleavage pattern of the amphipod *Parhyale hawaiensis.* By injecting blastomeres after the third division, we can track cell division, movement, morphology, and differentiation of the blastomere progeny. We find that one of the blastomeres at the eight-cell stage exclusively contributes to the germ line. The germ line clone migrates from the dorsal side posteriorly along the midline and stops under the ventral germ band. Later, the clone splits into a right and a left cluster. The clusters migrate separately back to the dorsal side until they reach the paired gonad rudiments that are situated on both sides of the gut in the fourth thoracic segment. The lineage restriction of the germ line at the eight-cell stage in *Parhyale* is earlier than that found in other animals with total cleavage. Perturbing cleavage with centrifugation and blocking cell division may be used to tell if there is a germ plasm that can be relocated, and we are also currently looking for molecular markers of potential germ plasm.

391. **Recognition and Rejection of Self in Plant Reproduction.** June B. Nasrallah. Cornell University, Ithaca, New York 14853.

The self-incompatibility (SI) system of crucifers is an intraspecific mating barrier that allows the epidermal cells of the stigma to recognize and reject self-related pollen. This system and other plant SI systems are unique among self-/non-self-recognition phenomena in being based on the recognition of self rather than nonself. In crucifer SI, recognition of self-pollen and subsequent arrest of pollen tube development are based on allele-specific interactions between stigma receptor kinase and pollen ligand, two proteins that are encoded by highly polymorphic and coevolving genes linked in a complex. Data relating to the mechanism of SI will be presented.

392. **Translational Control of Maternal mRNA.** Quiping Cao, Irina Groisman, Joyce Tay, and Joel D. Richter. Program in Molecular Medicine University of Massachusetts Medical School, Worcester, Massachusetts 01605.

Early animal development in probably all metazoans is programmed at least in part by mRNAs inherited by the egg at the time of fertilization. These mRNAs are not translated en masse at any one time or any one place, but instead their expression is regulated both temporally and spatially. For example, various mRNAs are translated only during the pachytene stage of oogenesis, during the oocyte's reentry into the meiotic divisions (oocyte maturation), or during specific stages of embryogenesis. The translation of several key maternal mRNAs is controlled by cytoplasmic polyadenylation. Dormant mRNAs have relatively short poly(A) tails, which when elongated in response to various developmental cues, stimulates translation. Two 3′ UTR *cis* elements and their respective binding factors, CPEB and CPSF, are required for cytoplasmic polyadenylation, which is initiated by the phosphorylation of CPEB by the kinase Aurora. Maskin, a factor that associates with both CPEB and the cap-binding factor eIF4E, controls mRNA-specific translation by modulating the interaction between eIF4E and eIF4G, which is essential for initiation complex formation. Maskin activity is regulated by cytoplasmic polyadenylation. Recent results to be discussed at length show that CPEB-mediated polyadenylation is necessary for oocyte differentiation and synaptonemal complex formation in the mouse and for oocyte maturation and embryonic cell cycling in *Xenopus.*

393. Abstract #393 will be presented as scheduled, but the abstract cannot be published due to lack of license agreement between authors and publisher.

394. **A Mutagenesis Screen to Identify Maternal Factors Required in Early Zebrafish Development.** Daniel S. Wagner, Roland Dosch, Beth A. Holloway, Wen Yan Mei, Keith A. Mintzer, and Mary C. Mullins. Department of Cell and Developmental Biology, University of Pennsylvania Medical School, Philadelphia, Pennsylvania 19104.

We have conducted a four-generation maternal-effect screen to identify maternally supplied factors that are critical for oogenesis and embryogenesis in the zebrafish. We have screened 600 mutagenized genomes and have identified approximately 75 maternal-effect mutants. The mutants we recovered affect a wide variety of developmental processes, including egg synthesis, egg activation, cell division, cell survival, embryonic pattern formation, and morphogenesis. The spectrum of phenotypes we recovered clearly demonstrates a complex maternal contribution to early embryo development in the zebrafish. Maternally supplied factors are not only required for early events such as egg activation and early cell division but also at later periods where maternally supplied factors persist and coordinate with newly synthesized zygotic factors to execute a normal developmental program.

395. **Germline Development in *Drosophila.*** R. Lehmann, L. Gilboa, R. Martinho, and J. Stein. Skirball Inst and HHMI, NYU School of Medicine, New York, New York 10016.

In many organisms primordial germ cells (PGCs) form in a specialized germ plasm. In *Drosophila* PGCs form by budding at nuclear cycle 10 while the somatic cells form by polarized cellularization at nuclear cycle 14 in the early embryo. We have identified a membrane-associated protein, Slow as molasses (SLAM), whose function is only required for polarized cell growth but not for germ cell formation. This suggests that somatic and germ cells already differ by their modes of cell formation. The first molecular manifestation of germ cell specification is the lack of transcriptional activity in PGCs. We have identified transcripts expressed in PGCs and have used these to study transcriptional repression during PGC development. Our analysis suggests that relief of transcriptional repression in PGC is mediated by a series of steps. For example, members of the Brahma chromatin-remodeling complex affect early germ line transcriptional quiescence, while the translational repressor protein Nanos plays a role during later stages. During embryogenesis PGCs migrate from their place of origin at the posterior pole to the somatic gonad in the mesoderm of abdominal segments 4–6. During the subsequent stages of gonad morphogenesis, germ line stem cells (GSCs) are selected among the PGCs. Germ line stem cell maintenance requires cell–cell interaction between the somatic niche and the germ cells. We have studied a gap junction protein, Zero population growth (ZPG), which affects the differentiation of GSCs. The *zpg* phenotype is novel and we used this phenotype to genetically dissect the process of GSC maintenance and differentiation during oogenesis. Our studies suggest that gap-junction-mediated cell–cell interactions direct GSCs to differentiate, that GSCs differentiate upon losing contact with their niche, and that GSC differentiation to a cystoblast is not direct but proceeds through an intermediate.