(Research statements from 23 students:)

Scott Applebaum

Interactions between thyroid and corticosteroid systems during the early life history of an estuarine dependent fish

During the transformation from embryo to larva to juvenile, fish acquire characteristics necessary for adult life (e.g. complete fins, digestive tract, and scales). A genetically programmed pattern of thyroid hormone production plays an important role in these transformations. However, there is some flexibility in this process and accelerations or delays of development do occur. It is presumed that this developmental flexibility helps larvae adapt to variations in food availability, predation, and physical parameters that place stress on development. However the mechanism by which this adaptation is achieved is not known. Evidence from amphibians, as well as fishes suggests that alterations in corticosteroid levels may play a role in fine tuning development through interaction with the thyroid hormone system.

My dissertation research addressed the hypotheses that a) the thyroid and corticosteroid systems interact to influence growth and development during the early life history of red drum (*Sciaenops ocellatus*) and b) the nature of this interaction (i.e. stimulatory, inhibitory, synergistic) changes during ontogeny thereby influencing growth and development during the early life history.

I have cloned thyroid hormone and glucocorticoid receptors from red drum, as well as portions of several corticosteroidogenic enzymes. In a series of experiments using laboratory reared larvae I am determining the pattern of receptor and steroidogenic enzyme mRNA expression during larval development of red drum using quantitative polymerase chain reaction. I am using pharmacological inhibitors of hormone production and action, to determine the nature (stimulatory, inhibitory) and intensity of interactions between the thyroid and corticosteroid systems during distinct portions of embryonic through juvenile development as indicated by changes in gene expression, hormone concentration, growth and development. In the past my work has emphasized the period from hatching onward. However, in the past year I have put increasing emphasis on the development and interaction of thyroid hormone and corticosteroid systems in pre-hatch embryos.

DORSAL-VENTRAL PATTERNING IN *XENOPUS LEAVIS*

Danny Ben-Zvi
Graduate student
Weizmann Institute of Science

I study the dorsal-ventral patterning in the *Xenopus leavis* embryo. This patterning process has been highly conserved in evolution, and many of its central components also function in the dorsal-ventral patterning of *Drosophila melanogaster*, a system that has been extensively studied in our lab. My study approaches this question using a combination of computational and experimental tools. I formulated a mathematical model which allows for a rigorous and quantitative analysis of the network underlying the dorsal-ventral patterning. A key result of our theoretical study is that the regeneration capacity, as well as size compensation and the double-axis induction, can be accounted for by a particular mechanistic explanation. This extended model can now account for more elaborated features found in the *Xenopus* embryo, and can explain the results of the famous Spemann experiments from the early 20th century.

Investigations of Epidermal Positional Identity in the Induction of Limb Regeneration

Leah Campbell

The ability to regenerate tissues and body parts is a common trait in the animal kingdom except among vertebrates, in which the urodele amphibians are unique due to their regeneration capacity throughout their entire lifespan. Urodele amphibians are able to regenerate many body parts, but it is the limb that has become a model system for studying regeneration in vertebrates. The process of limb regeneration is initiated immediately after amputation with migration of the epidermis over the wound to form the wound epidermis (WE). While many studies have demonstrated that the WE is essential for regeneration, little is known about the requirements for proper WE formation. We hypothesize that the WE functions to induce blastema formation as a direct result of the contact of migrating epidermal cells from different positions around the circumference of the limb (e.g., dorsal, ventral, anterior, posterior). In order to test this hypothesis, four patches of skin from different radial positions of the limb were grafted together onto a lateral wound area of an axolotl limb. At the wound/graft site an ectopic bud formed that stained positively for markers of regeneration, suggesting that the regeneration response can be induced by a wound and contact of skin from different positional identities. This experiment is currently being expanded to dissect the roles of epidermis and dermis positional identity. In addition, we are using microarray analyses of both axolotl and human epidermis to identify differential gene expression between dorsal and ventral positions. Finally, we are setting up an *in vitro* culture system to study the interaction of skin tissues from different positional identities.

Justin Crocker, Albert Erives Lab

Gene regulation of insect neuroectoderm

The Erives lab is interested in understanding how cis-regulatory systems have scaled over macroevolutionary time scales. My thesis work is focused on characterizing the *Drosophila* neuroectodermal enhancers (NEEs) and the rules underlying their evolution. The NEE structure is found in four presumably unrelated gene loci: *rho*, *vnd*, *brk* and *vn*. The *vnd* and *brk* genes encode unrelated homeobox and generic helix-turn-helix transcriptional repressors. The *rho* gene encodes a serine protease that is thought to process an EGF-receptor ligand encoded by the *vn* gene. All 4 NEEs occur in various positions and orientations relative to the genes they regulate. Additionally, all 4 NEEs share a common ~300 bp structure involving a specialized E-box, a Dorsal binding motif, and a specialized Su(H) binding site. The organization of these binding sites appears to follow phasing rules in each of the enhancers. I am exploring the function of the NEE organization by assessing the extent of functional conservation between the NEEs in various dipteran lineages. Current work using an orthologous set of *Drosophila virilis* enhancers, diverged approximately 50 mya, reveals that despite substantial sequence turnover (> 60%) the enhancers drive similar patterns of gene expression when placed into *Drosophila melanogaster*. However, the enhancers collectively drive different heterotopic modes of gene expression. It is apparent that the current studies within the diptera are already contributing towards an understanding of the underlying NEE architecture. I am currently testing predictions of how changes in NEE motif organization contributes to the modified patterns of gene expression and how these changes relate to both *cis* and *trans* changes throughout holometabolous insect evolution.

Mary-Lee Dequéant

The segmentation clock: a complex oscillating network of signaling genes

My research focuses on the segmentation clock, a molecular oscillator that regulates the formation of the segments of the vertebrate body during development. The segmented organization of the body plan observed in the adult, as illustrated by the repetition of the vertebrae along the body axis, derives from the sequential and periodic formation of embryonic segments called somites that form every 120 minutes in mouse, 90 minutes in chick and 30 minutes in zebrafish. During the late 1990s, Olivier Pourquié's laboratory identified a molecular oscillator associated with this process called the "segmentation clock." This clock drives periodic waves of transcription of a group of genes now referred to as "cyclic genes" in the tissue precursor of the somites, the presomitic mesoderm (PSM). Until very recently, only a handful of cyclic genes, mostly linked to Notch signaling, had been identified. The goal of my Ph.D. work was to apply a genome-wide approach to identify systematically the cyclic genes and to provide insights into the regulatory network of the segmentation clock.

To accomplish this, we generated a microarray time series dataset of mouse PSMs during one clock oscillation cycle. Then, we performed a mathematical (Lomb-Scargle) analysis to identify periodic expression profiles. As a result, we identified a large number of new cyclic genes linked to several signaling pathways and participating in negative feedback loops. When clustering the periodic profiles by phase, the cyclic genes linked to Notch and FGF signaling appeared to oscillate in antiphase with the ones related to Wnt signaling, suggesting a tight temporal coordination of these oscillating pathways. Finally, the candidate cyclic genes were validated experimentally, and their inactivation often correlated with segmentation defects.

In conclusion, this quantitative and systematic microarray analysis of the segmentation clock system identifies a complex oscillating network. Based on recently generated microarray data, we are now comparing the clock regulatory networks across species (fish, chick and mouse). We also are collaborating with physicists and mathematicians to test different mathematical methods of pattern detection as well as network analysis.

Martin Distel
GSF Munich, Germany

Research Topic:
Analyzing the cell biology of neuronal migration in the living zebrafish embryo

The research focus of our lab is on the development of the zebrafish cerebellum.
In early developmental stages cerebellar precursors start to migrate from the rhombic lip, a proliferative zone, tangentially to the mid-hindbrain boundary (MHB), where they turn ventrally to reach their final destination.
Migration of neurons is of immense importance to form a functional brain.
Failures of neuronal migration can lead to severe malformations of the brain as it is the case in human lissencephaly. Lissencephaly patients often have mutations in genes involved in nucleokinesis, such as Lis1 or DCX. The parts of the brain most affected in these patients are the cortex and the cerebellum.
Time-lapse movies indicate that precursor cells in the zebrafish cerebellum also migrate via nucleokinesis. Therefore the aim of my PhD project is to characterize the migratory behaviour of these cells in detail on a subcellular level to lay the foundations for a lissencephaly model in zebrafish.
I am particularly interested in the interplay of structures like the microtubules, the centrosome and the nucleus during this migration. I have already established a labelling system, which allows me to visualize three cellular components at the same time for *in vivo* time-lapse imaging.
Using this labelling system as a read-out, I am planning to investigate the role of Lis1 in nucleokinesis *in vivo* on a subcellular level.

Ben Ewen-Campen

Beetle horns: developmental patterning and the mechanisms of dimorphism

Since I graduated college a year ago, I have been working as the technician in Doug Emlen's lab at the University of Montana. Doug has spent his career working on horned beetles, and has put out a ton of fascinating work on many of the "macro" aspects of these animals, including the phenotypic plasticity of horns (horn size is determined by larval nutritional state, not allelic differences), the alternate mating strategies of large horned males versus small hornless males, and the developmental trade-offs involved in producing these massive structures.

My work in the lab, however, has been a part of Doug's transition towards an evo-devo perspective on horn development. Specifically, we are working on two projects: the developmental genetics of horn development and patterning, and the mechanisms underlying dimorphisms in horn development (both between large males and small males, as well as between males and females). This second project is currently focused on the insulin pathway, which is believed to be involved in translating a larva's nutritional state to developing insect appendages, and can lead to different scaling relationships between body size and appendage size for different appendages. For these projects, we have been working with an enormous Japanese rhinoceros beetle, *Allomyrina dichotoma*, a species in which males produce a gigantic, four-pronged horn on their head and a smaller, two-pronged horn on their thorax. In this species, both large and small males produce horns (which is not the case in many beetle species), and females produce a rudimentary horn which is only present during the pupal stage and disappears during the molt to the adult stage.

We are approaching these projects in several ways. Primarily, I have been using immunohistochemistry to localize protein expression in sectioned beetle horns over a range of body sizes and developmental stages. We are hoping to localize the expression of a range of genes which are involved in arthropod appendage patterning in general, as preliminary studies in other species suggest that many of these patterning genes are conserved in beetle horns, and we hope to look at many basic questions about the nature of horn development and its differences in males and females. We are also using these methods to explore the expression of insulin pathway members. In addition, we are working to create cDNA libraries of animals of all sizes and sexes in order to use quantitative PCR to explore differential expression of both patterning and insulin-related genes. We are working with several collaborators on the protein biochemistry of the insulin signalling cascades in males and females in an attempt to determine where in the developmental network the growth of horns is truncated in females. In the future, we plan to perturb the expression of several genes thought to be important in horn development. These projects are very much in their infancy, and proceed quite slowly as I'm sure much work does in non-model systems, but also offer huge, uncharted territories in which to try out many exciting ideas.

MyoD Recruits the cdk9/Cyclin T2 Complex on Myogenic-Genes Regulatory Regions. New perspectives in skeletal muscle regeneration.

CRISTINA GIACINTI*, LUIGI BAGELLA, PIER LORENZO PURI, ANTONIO GIORDANO AND CRISTIANO SIMONE*

During skeletal myogenesis, muscle-regulatory factors bHLH and MEF2 promote the expression of muscle-specific genes by recruiting several chromatin-modifying complexes on specific DNA regulatory sequences. A number of MyoD-interacting proteins have been reported, but whether they are recruited to the chromatin of myogenic loci, and the relationship with other chromatin bound proteins is unknown. CDK9, cyclin-dependent kinase also referred to as PITALRE, is a serine-threonine cell division cycle (cdc) 2-related kinase which is widely expressed in human and murine tissue with high levels in terminally differentiated cells. Cdk9 function depends on its kinase activity and also on its regulatory units: the T-family cyclins (cy) and cyclin K (Peng). Cdk9 associated with its regulator factor cyT2a associates and functionally cooperates with MyoD to activate myogenic transcription.
We show that MyoD recruits cdk9/cyclin T2, together with the histone acetyltransferases p300 and PCAF, and the chromatin remodeling complex SWI/SNF, on promoters and enhancers of muscle-specific genes, and that this event correlates with the acetylation of histone tails, remodeling of chromatin, and phosphorylation of the C-terminal domain (CTD) of the RNA polymerase II at these elements. New perspectives outcomes for the study of muscle regeneration in vivo.

The development, evolution and homology of deuterosome pharyngeal arches

Andrew Gillis

I am interested in a number of questions surrounding the development and evolution of the vertebrate pharynx. I study the patterning and embryonic origins of the branchial skeleton in chondrichthyans using the little skate, *Raja erinacea*, as a model system. *R. erinacea* is a suitable model for addressing these questions, as it possesses a more elaborate branchial endoskeleton than that of any extant osteichthyan taxon, and provides a discrete and easily interpretable morphological readout when studying (and experimentally perturbing) branchial skeletogenesis. Using this system, I'm investigating a putative signaling center in pharyngeal epithelium that is involved in establishing the antero-posterior axis of the gill endoskeleton.

In addition to this, I am also interested in the evolutionary origin of the chordate pharynx. Pharyngeal gill slits have long been regarded as a chordate synapomorphy, though substantial evidence exists that they are an even more general feature of deuterostomes. Gill slits are present in members of the non-chordate deuterostome phylum Hemichordata, and the pharyngeal gill slits of vertebrates and hemichordates arise via similar morphogenetic processes. In addition, certain pharyngeal patterns of gene expression are also shared between developing chordates and hemichordates. These similarities hint at a homology between these structures in hemichordates and chordates, despite the absence of a neural crest-derived gill skeleton in the former, and imply that some components of the pharyngeal gill slit developmental program predate the divergence of deuterostome taxa from their last common ancestor. By comparing gill slit morphogenesis and patterns of pharyngeal gene expression between the hemichordate *Saccoglossus kowalevskii* and various chordate taxa, I am elucidating the fundamental components of this program that are shared by all deuterostomes (with a particular focus on the conserved patterning role of pharyngeal endoderm).

da23 is required for endodermal pouch morphogenesis and craniofacial skeletal patterning in zebrafish

Christopher W. Johnson

My research is currently focused on characterizing the role of a gene, da23, in craniofacial development. Morpholino injections to knock down expression of da23 in zebrafish embryos have demonstrated an essential role for this gene in craniofacial development; Morphants had defects in pharyngeal pouch morphogenesis and later (5 dpf), neural crest cell derived cartilages of the face were deformed or absent. I've performed expression analysis of da23 in the developing zebrafish embryo and found it to be expressed prominently in the pharyngeal endoderm and ectoderm, tissues known to be the origins of important signals required by adjacent neural crest cells for their patterning, differentiation, and survival. Using a small molecule inhibitor, I have shown that FGF signaling is required for da23 expression in the pharyngeal arches. I would like to further characterize da23's role in zebrafish craniofacial development and determine if this role is conserved in other vertebrates, such as Xenopus, chick, or the mouse.

Netrins in Zebrafish Motoneuron Development
Laura Hale

In vertebrates, more motoneurons are born than ultimately survive to innervate their target, muscles. The motoneurons that do not innervate muscle die, probably because trophic support is in limited supply or it is inaccessible. I am studying the development of two identified zebrafish motoneurons, CaP and VaP, in an attempt to determine the mechanisms that underlie normal cell death of vertebrate motoneurons. CaP and VaP form an equivalence pair early in development but VaP typically dies during embryonic development whereas CaP persists through adulthood. In the developing embryo, CaPs are present in all trunk spinal cord hemisegments and have long axons that extend into ventral muscle. In contrast, VaPs are present in about half of the segments, and are situated either rostrally or caudally to CaP. VaP axons are short and stop at the horizontal myoseptum, the boundary between dorsal and ventral muscle. The inability of the VaP axon to extend beyond the horizontal myoseptum may prevent it from accessing a trophic factor located in ventral muscle required for cell survival. CaP may survive because its axon extends into ventral muscle, enabling it to access the necessary trophic factor.

For my dissertation project, I am seeking the molecular signals that cause the VaP axon to stall at the horizontal myoseptum and the signals that contribute to VaP cell death. Preliminary data from morpholino knockdown experiments suggests that Netrin1a, and its receptor, Dcc, are required for VaP axons to stall at the horizontal myoseptum. Interestingly, Netrin and Dcc may also contribute to VaP cell death, by functioning as a survival factor and dependence receptor, respectively. I have begun testing the role of Netrin1a and Netrin2 in VaP cell death by knocking down their functions with morpholinos.

Limb and Somitogenic Heterochrony in Marsupial Mammals
Anna Keyte

My primary research interest is the evolution of development and how changes in developmental programs have contributed to the species diversity we see today. My dissertation research has focused on the role of heterochrony in the morphological evolution of mammals. I have used early limb development and somitogenesis in the Brazilian short-tailed opossum, Monodelphis domestica, as a model to study the genetic and developmental origins of heterochrony. The forelimbs of newborn opossums are well enough developed to allow them to crawl unaided from the opening of the birth canal to the teat. In contrast, the hindlimbs are strikingly smaller, non-functional, and relatively under-developed. Changes in the timing of somitogenesis have also occurred, presumably to allow precocial development of anterior somitic derivatives required at birth. My research aims to uncover the developmental and genetic bases of limb and somitogenic heterochrony in these neonates.

Development of Anthozoan Cnidarians
Heather Marlow

I am broadly interested in cnidarian developmental biology, particularly that of the anthozoans (corals, sea anemones, and sea pens). The emerging model animal, *Nematostella vectensis*, has provided a particularly interesting opportunity for me to explore the evolution and development of the nervous system. While cnidarians are considered to have relatively simple nerve nets, we've found that many of the same pathways employed by flies, mice and nematodes are conserved during neural development in anthozoan cnidarians. Studying specification mechanisms, neural morphology, and cell-type specific markers such as the opsins of photoreceptors has provided additional insight into our understanding of early animal nervous systems. Hawaii has also been the perfect setting for another ongoing project, the development of symbiotic relationships in coral embryos. Adult corals and their skeletons are host to numerous other organisms, notably their intracellular zooxanthellae and bacterial populations. These symbionts are necessary for the adult, and must be properly acquired and localized within the animal. I am currently studying how coral embryos selectively acquire and properly segregate their symbionts.

Ana Mateus

Membrane dynamics during morphogenesis

Epithelial morphogenesis contributes to organ formation and body shape through mechanisms such as cell shape changes, cell intercalation, fusion and cell migration. Dorsal Closure is a model of epithelial morphogenesis that occurs halfway through embryogenesis. It closes a gap that exists in the dorsal epidermis of the *Drosophila* embryo, which is bridged by the extraembryonic amnioserosa cells. During this process, epidermal cells change shape and acquire planar polarity, which led me to focus on the organization of vesicle trafficking in these cells.

I am using genetics, the hallmark of *Drosophila*, to selectively perturb the system. The readout is carried out using two different angles: a genetic approach, focused on large scale phenotypes, to assess the role of trafficking in epithelial morphogenesis; and a more cell biological approach, focused on subcellular events. In the latter, I am exploring the structure and dynamics of the trafficking apparatus in cells with planar polarity and how it interacts structurally and functionally with cell adhesion and the cytoskeleton.

Hopefully, these studies focused on the interface between cell and developmental biology will help to understand how ubiquitous cellular processes, such as intracellular transport, are differentially regulated in diverse cell types, driving several cell behaviors that shape the embryo.

Catherine McCusker

Maintaining the balance: The role of Cadherin-11 and ADAM13 in Cranial Neural Crest Migration.

My graduate research project focuses on understanding the molecular interactions that play a role in the large-scale migration of the Cranial Neural Crest (CNC). The CNC is a population of cells that form between the epidermis and the anterior neural plate of vertebrate embryos. During early embryogenesis, the proper migration of the CNC is imperative to craniofacial development. The ability of these cells to migrate requires tight control over cell adhesion molecules such as integrins, and Cadherins. One member of the Cadherin superfamily, Cadherin-11 is specifically expressed in the *Xenopus laevis* CNC before and during migration. While most cadherins maintain cell-cell cohesion and prevent migration, Cadherin-11 (considered a mesenchymal cadherin) is expressed in cells that migrate. However, the overexpression of Cadherin-11 blocks migration of the CNC in embryos as well as migration in tissue culture cells, suggesting that the levels of this molecule at the cell surface must be balanced. My experiments have shown that endogenous Cadherin-11 extracellular domain is cleaved during CNC migration. This post-translational control of Cadherin-11 surface levels is directly regulated by ADAM13 (A Disintegrin And Metalloprotease), a molecule that is also specifically expressed in the CNC before and during migration. Furthermore, this interaction appears to be functionally relevant, as overexpression of ADAM13 can rescue CNC migration in embryos that overexpress Cadherin-11. Additionally, my studies indicate that Cadherin-11 cleavage does not affect its interaction with β-catenin or stimulate canonical Wnt signaling. My working hypothesis is that ADAM13 cleaves Cadherin-11's extracellular domain to promote single-cell migration during the second phase of CNC migration.

Biophysical dynamics in tissue morphogenesis

Celeste M. Nelson

I am interesting in answering the following fundamental questions: How are the final architectures of tissues and organs determined? Specifically, how do individual cells -- the building blocks of these materials -- integrate complex biological signals (both biochemical and mechanical) dynamically and spatially within tissues to direct the development of organs? I work at the interface of cell biology, developmental biology, and bioengineering to develop tools to engineer organotypic culture models that mimic tissue development, enabling rigorous quantitative analysis and computational predictions of the dynamics of morphogenesis. My current focus is on sophisticated mammalian cell culture and mouse models of normal branching morphogenesis (ie, the developmental process that builds the lung, kidney, and mammary gland) and abnormal neoplastic growth.

Does Fibroblast growth factor 10 regulate the embryonic proliferation/differentiation switch throughout the murine endoderm?

Pia Nyeng

Terminal differentiation of epithelial cells in the endodermal organs is crucial for proper function of the adult organ. I have studied the epithelial lining of the murine stomach and intestine because the continuous cell turnover here makes it a useful model in studies of a proliferation/differentiation switch. I analyzed the transition from an undifferentiated homogenous epithelium to the highly specialized and compartmentalized mature columnar epithelium that takes place during late embryonic development.
Using a transient transgenic mouse model I find that ectopic overexpression of the mesenchymal fibroblast growth factor 10 in the epithelium leads to arrest of differentiation and failure to form a defined progenitor niche, resulting in a very dramatic change of both gastric and intestinal morphogenesis. Endogenous FGF10 is highly expressed early in development and it is likely that it plays a role in progenitor maintenance at this time, and maybe also in setting up and maintaining a progenitor niche in the adult gut. I have currently expanded my focus to the lung and am employing transgenic mouse models and tissue explant culture to find out whether my conclusions on FGF10s role in the stomach and intestine can be extended to the lung.

Cell sorting and cell type analysis of stem cell-derived neural cell suspensions for transplantation

Jan Pruszak, Center for Neuroregeneration Research, Harvard Medical School, McLean Hospital, Belmont, MA 02478, USA

Clinical neurotransplantation studies using fetal midbrain tissue have shown that nigral (A9) dopamine (DA) neurons can alleviate symptoms in patients with Parkinson's disease (PD). Derivation of this specific cell subtype from human embryonic stem cells (hESC) in vitro and its usage in vivo are complicated by the presence of unwanted cell populations such as immature stem cells and other neural and non-neural subsets.
Specific labeling of cell types by either promoter-driven fluorescence or by surface antigens, in combination with cell sorting methodologies for neuronal cells, enable the selection, detailed analysis and application of better-defined neural cell suspensions, which may be critical for establishing future hESC-based therapies of PD.

Tania Rozario,
DeSimone Lab, University of Virginia

Exploring the relationship between adhesion mediated cell behaviors and cell fate specification during gastrulation in *Xenopus*

I am interested in how the microenvironments in embryos comprising of mechanical forces, extracellular matrix and cell interactions impact gene expression and ultimately cell fate specification. As we learn more about morphogenetic gradients that create spatial heterogeneity of signaling cues that instruct cell differentiation, we often ignore the fact that cells of the gastrula are undergoing dynamic changes in shape, polarity and motility. What are the consequences of these critical cell behaviors, on tissue specification? Do cell behaviors provide cues that can refine and/or modify signaling cues from secreted morphogens? These are the questions that form the basis of my research interest.

The focus of my PhD research will be to understand how integrin and cadherin mediated cell adhesions impact mesodermal gene expression. I will use microarray analysis to determine how different adhesive conditions influence mesodermal patterning by the morphogen activin in order to identify genes that are differentially expressed when integrins and cadherins are engaged. Furthermore, I will characterize the functions of selected adhesion-dependent activin-induced genes on gastrulation and specification of mesoderm derived structures such as notochord and somites using a combination of embryological, cell biological and biochemical techniques.

Gap genes in spiders **Evelyn Schwager**

In my PhD project my research focuses on the regulation of segmentation and its evolutionary origin. I work on these questions studying spiders, which as basally branching arthropods display features of both insect segmentation and vertebrate somitogenesis. For the last two years I used the wandering spider *Cupiennius salei* as a model organism, and last year we additionally established the common house spider *Achaearanea tepidariorum* in our lab.

I am investigating a number of candidate genes known to have roles in the segmentation of *Drosophila*, other insects and vertebrates. I study these genes by analyzing their expression patterns as well as their function by RNAi.

I am currently concentrating my research on spider orthologs of the *Drosophila* gap genes, which are thought to not have a role in segmentation outside of insects. I found out that for at least *hunchback*, this is not true in *Achaearanea*. In this spider, pRNAi knockdown leads to missing segments in the prosoma. Unlike in lower insects, this is not due to *hunchback* repressing posterior hox genes in the anterior regions, which makes this spider's gap gene phenotype for *hunchback* more similar to the one seen in *Drosophila* than to the *hunchback* phenotypes seen in other insects.

MBL embryology course

Role of Slit/Robo signaling during trigeminal gangliogenesis and function of cell adhesion molecule, Cadherin 7, during cranial neural crest migration

Celia Shiau

My research interests lie in cell-cell interactions of the cranial neural crest with other cells and among themselves as they migrate and form derivatives of the peripheral nervous system in vertebrates. As a Ph.D. student in Dr. Marianne Bronner-Fraser's lab at Caltech, I am using the chick embryo as my model system to pursue two main projects. First, I have been investigating the role of axon guidance molecules, Slits and their Robo receptors, in formation of the trigeminal ganglion which is responsible for the somatosensation of much of the head. I found complementary expression patterns of Slit1 and Robo2 in the neural crest and placode cells, respectively, that suggest a ligand-receptor relationship between the two cell types. Consistent with a role in cell-cell interactions, perturbation of Slit/Robo signaling using a dominant-negative Robo2 construct caused severe ganglion defects. Data reveal an essential role for Robo2 during placode ingression and ganglion assembly, mediating interactions between neural crest and placode cells. Loss-of-function experiments using RNAi techniques to knock down Slit1 and Robo2 will further reveal the function of these genes. Secondly, I am studying the role of the cell adhesion molecule, Cadherin 7, during cranial neural crest migration. Both expression pattern and preliminary gain- and loss- of- function experiments suggest an early role of Cadherin 7 in promoting intercellular connections in the migratory crest streams and perhaps a later role in organization of the cranial ganglia. Further experiments using time-lapse imaging analysis to study the function of Cadherin 7 will better establish its role in the dynamic process of neural crest cell migration.

Andrea Wills
(Richard Harland lab, UC Berkeley)

Persuing the roles of BMP antagonists in *Xenopus tropicalis* and *Xenopus laevis*: more than just redundancy?

In the frogs *Xenopus laevis* and *Xenopus tropicalis*, several secreted BMP antagonists are expressed in the Spemann/Mangold Organizer, the dorsal-most region of prospective mesoderm, and these antagonists are necessary for the formation of dorsal structures and definitive neural tissue. Two molecules that have been implicated in the BMP signaling pathway have controversial roles: Twisted gastrulation (Tsg), and Xenopus nodal-related 3 (XNR3). My research seeks to clarify the roles of these molecules with respect to BMP signaling, and to identify whether XNR3 may also have activity in other signaling pathways. I have used loss-of-function studies based on morpholino oligos to provide new evidence that Tsg acts as a BMP antagonist, which cooperates with the known BMP antagonist chordin to specify the forebrain and other dorsal structures. A similar loss-of-function approach combined with overexpression and biochemical data support a BMP antagonist role for XNR3, but also suggest that XNR3's function cannot be entirely compensated for by other BMP antagonists. The apparently unique BMP antagonist role of XNR3 helps to illustrate that the many BMP antagonists expressed in the frog may not all have equivalent or redundant functions, and raises the interesting question of how different BMP antagonists partition their roles.

More recently I've also become interested in how embryos cope with serious manipulations to key developmental pathways (especially BMP signaling) early in development. On the one hand, frog embryos seem to be able to tolerate absences of apparently key regulatory molecules (eg. noggin, chordin, or follistatin individually) with relatively minor effects on later patterning, while at the other extreme, loss of too many BMP antagonists (eg noggin, chordin, and follistatin all at once) results in embryonic suicide. How does the embryo compensate for seemingly major changes in BMP ligand/antagonist concentrations, and how does too drastic a change become converted into a cell-death cue?

Mike Wosczyna
Center for Regenerative Biology / Dept. of Molecular and Cell Biology
University of Connecticut

Muscle development: Understanding a complex developmental program

MyoD, a myogenic regulatory factor (MRF), initiates a complex series of molecular events responsible for cell determination and differentiation during embryonic and postnatal muscle development. Transcriptional control of this master regulatory gene has been traced to two regulatory regions located in the 24kb preceding the translational start site (Goldhamer et al. 1995, Tapscott et al. 1992). Factors that target these enhancers are not well defined. A yeast one-hybrid screen identified two zinc finger proteins, Gig1 and Pbf, that interact with a key regulatory element of *myoD*. The characterization of these genes and their putative cofactors through in-vitro interaction and in-vivo expression assays are being completed. Although much of the in-vivo work thus far has been in mice, additional bioassays were conducted in zebrafish and are presently being developed in chickens to further assess gene functions.

In addition to the transcriptional control experiments, we are also investigating cell commitment to the myogenic lineage during embryogenesis and postnatal development. MyoD and an additional MRF, Myf-5, are known to have compensatory functions with respect to the regulation of skeletal muscle development. Mice null for either MyoD or Myf-5 encounter delayed hypaxial or epaxial muscle growth respectively, but eventually present normal skeletal muscle (Kablar et al. 1997, 1998). Conversely, double null mice are completely devoid of skeletal muscle and die shortly after birth (Rudnicki et al. 1993). In an attempt to rescue muscle development in the embryo of MyoD/Myf-5 double null mice and assess MyoD and Myf-5 functions in adult myogenesis, a transgenic mouse has been developed to conditionally express MyoD in the embryo but not in the adult. Presumptive myogenic precursor cells in adult muscle can then be traced and lineage commitment assessed through the use of muscle lineage specific markers. The transgene's functionality has been confirmed in-vitro and currently, the transgenic mice are being assayed for fidelity of expression.