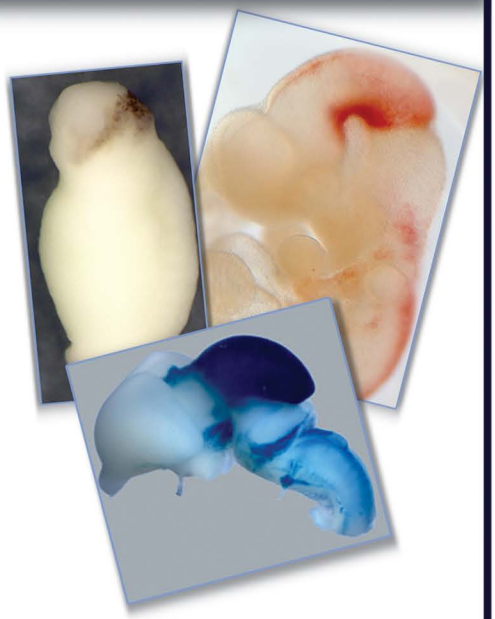
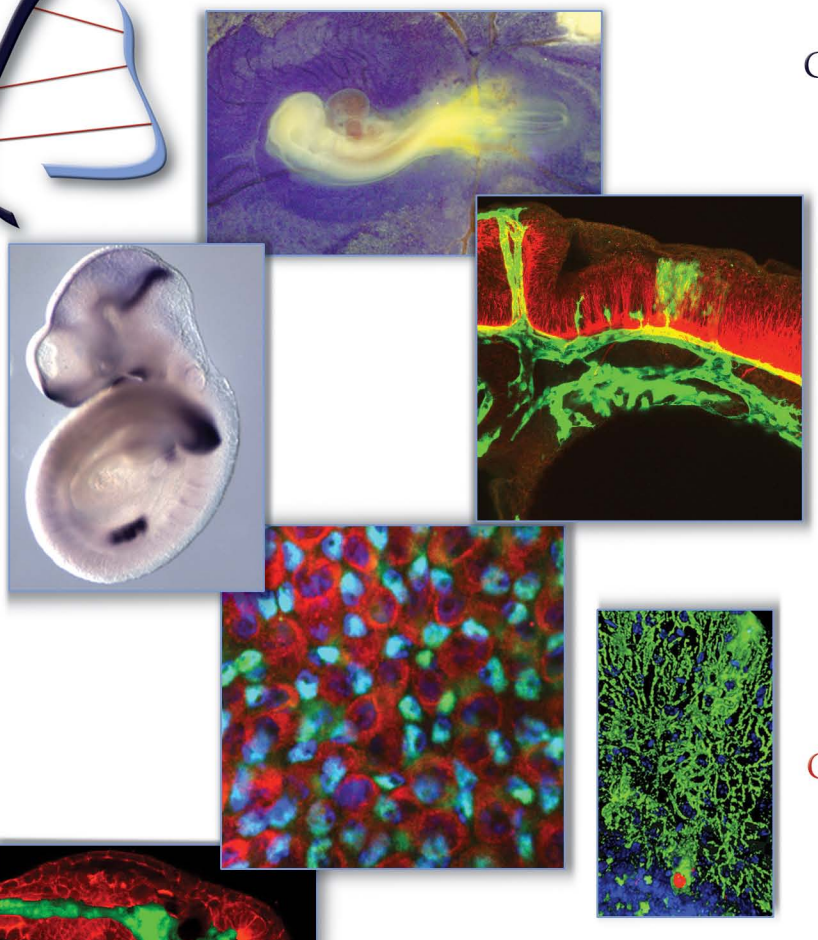
Northeast Regional Meeting of the Society for Developmental Biology

NESDB 2011
March 25th-27th
Marine Biological Laboratory
Woods Hole, MA



Keynote Speaker
Kenneth Birnbaum, NYU



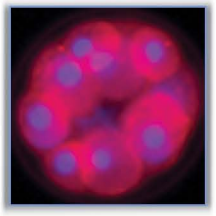
Guest speakers include...

Florence Marlow, AECOM
James Li, UConn Health Center
Steven Q. Irvine, University of RI
Cynthia Bradham, Boston University
Paola Arlotta, Harvard Medical School
Michael Marr, Brandeis University
Alison DeLong, Brown University
Arkhat Abzhanov, Harvard University
Erika Bach, New York University
Eric Morrow, Brown University
Vivian Irish, Yale University
Zheng-Zheng Bao, UMass Medical
Mustafa Sahin, Children's Hospital

and...
additional speakers
chosen from abstracts

Organizers

Jesús Torres-Vázquez, NYU
Mark Zervas, Brown University



Registration and Program
http://www.brown.edu/Conference/NESDB

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SATURDAY MARCH 26TH

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SUNDAY MARCH 27TH

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SUNDAY MARCH 27TH

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BOXED LUNCH: 12:15

SPEAKER ABSTRACTS (SPEAKER NAMES ARE BOLD)

SESSION I

Establishing oocyte asymmetries and the animal-vegetal axis in zebrafish.

Sophie Rothhämel, Amanda Heim, Andreas Jenny, and **Florence Marlow***
Department of Developmental and Molecular Biology, Albert Einstein College of Medicine. Bronx, NY. U.S.A.

Our group is interested in understanding how cell polarity is established and maintained in the vertebrate ovary. The vertebrate animal-vegetal embryonic axis is established during oogenesis. Oocyte polarity is prerequisite for determining the prospective embryonic axes and setting aside the germ cell determinants in non-mammalian vertebrates. The Balbiani body is an evolutionarily conserved oocyte asymmetry present in early oocytes of all animals examined, including humans; however, genes acting in Balbiani body assembly had not been identified in vertebrates. Bucky ball was identified as an essential regulator of oocyte polarity through genetic screens. Lack of maternal Buc causes failure to establish oocyte asymmetry and the embryonic axes through an unknown pathway and mechanism. We are using *buckyball* mutants as a tool to investigate the function of putative interacting proteins and other candidate genes to gain insights into the mechanisms underlying Balbiani body formation and oocyte asymmetries including localization of germ plasm mRNAs in the oocyte. To investigate how Bucky ball regulates mRNA asymmetries in oocytes we are testing the hypothesis that *buc* specifies the oocyte axis upstream or at the level of Balbiani body assembly. To determine the mechanism via which Buc regulates oocyte asymmetry we have identified candidate components of the Buc pathway through yeast genetic and affinity purification approaches. Recently we have identified Bucky ball-binding proteins, which link Bucky ball to RNA binding. To identify potentially functional regions of Buc protein, we are mapping the regions of the Buc protein that are important for engaging binding partners. We are currently using *bucky ball* mutants and transgenic interference approaches to study Buc interacting proteins and to gain insight into the mechanisms underlying Balbiani body formation and oocyte polarity.

Gamete plasma membrane fusion triggers a block to polytubey in *Arabidopsis thaliana*.

Kristin Beale* and Mark Johnson. Department of Molecular Biology, Cellular Biology, and Biochemistry. Brown University. 185 Meeting Street, Box G, Providence, RI. 02912.

Sexually reproducing organisms have mechanisms ensuring that a single sperm fuses with a single egg. Failure of these mechanisms can lead to defects in embryo development due to improper chromosome segregation and/or gene dosage. The mechanisms preventing multiple sperm from fertilizing a single egg are poorly understood in flowering plants. *HAP2-GCS1* is a broadly conserved gene required for gamete plasma membrane fusion and fertilization in multiple eukaryotes. Here, we analyze fluorescently tagged wild type, *hap2-1* (loss of function), and *hap2*^{hypo} (partially functional) mutant sperm during fertilization in the plant model species *Arabidopsis thaliana.* We find that *HAP2* is required for gamete fusion and for triggering a mechanism that prevents additional sperm from being deposited within the ovule (female reproductive structure). Normally, a single pollen tube enters an ovule and deposits two sperm cells; one fuses with the egg to form the embryo, the other with the central cell to form endosperm. When *hap2* mutant sperm are deposited, we find that additional sperm are delivered by multiple pollen tubes entering the ovule. These data suggest that in wild type, additional pollen tubes (polytubey) are prevented from entering the ovule by a mechanism triggered by gamete fusion. Previous models propose that the signal responsible for blocking polytubey occurs at the point of pollen tube entry into the ovule, but we show that gamete fusion is the trigger for this block. Our data lead us to propose that gamete fusion initiates an unknown signal cascade that redirects pollen tubes to an unfertilized ovule.

Yin-Yang1 is required in the epiblast during mammalian gastrulation.

Mary Trask, Jacob Hiller, John B. Pawlak, Kimberly D. Tremblay and Jesse Mager
Dept of Veterinary and Animal Science, University of Massachusetts, Amherst, MA 01003

One of the many ways that the Polycomb Group Gene, Yin-Yang1 (*Yy1*), is thought to regulate gene expression is through direct binding to DNA elements found in promoters or enhancers of target loci. In order to further define the role of YY1 during gastrulation and identify *in vivo* targets we deleted *Yy1* specifically in the epiblast of mouse embryos. *Yy1* conditional knockout (cKO) embryos initiate gastrulation and generate all three primary germ layers. However, the mesoderm and endoderm that are specified have abnormal gene expression of many critical developmental factors including *Brachyury*, *Fgf8*, *Nodal*, *Pitx2, Cdh1* and *Oct4*. *Yy1* mutants fail to undergo proper morphogenic movements typical of gastrulation and do not form a node, notochord or appropriately structured allantois. Our results reveal critical requirements of YY1 in several important developmental processes, including epithelial to mesenchymal transition and expression of OCT4 pluripotency complex. Here we present data defining the developmental and molecular consequences that result from epiblast specific disruption of YY1 function *in vivo*.

Developmental *cis*-regulatory elements: are there rules for their organization?

Steven Q. Irvine, Department of Biological Sciences, University of Rhode Island

Cis-regulatory elements, in concert with their DNA binding transcription factors, are recognized as major regulators of gene expression and players in morphological evolution. Are there rules for the position and orientation of *cis*-, or genomic regulatory elements (GREs) that govern their function and evolution? The classic view of GREs holds that, apart from the basal promoter, their position and orientation relative to the gene they regulate does not affect their function. Some recent findings call these axioms into question. The ascidian *Ciona intestinalis*, a simple chordate, has advantages for studying GREs by reporter transgene experiments. A wealth of empirical data identifying GREs in *Ciona* is now accumulating. This talk summarizes insights into GRE function and the role of genomic position from work in *Ciona*. The *Ciona* data is also compared with data from other vertebrate species showing similarities and differences in GRE organization.

Interplay between intrinsic and extrinsic factors in controlling the formation of the mid-hindbrain boundary

James Li, University of Connecticut Health Center, Assistant Professor

During embryonic development, discrete cellular units called compartments are separated by boundaries that prevent intermingling of cells destined to different developmental fates. The compartment boundary often serves as a signaling center by producing morphogens that pattern cells in the neighboring compartments. Therefore, boundary formation is a fundamental mechanism for coordinating growth and patterning of embryonic tissues. In vertebrates, the common expression border of two homeobox genes, *Otx2* and *Gbx2,* demarcates the prospective mid-hindbrain (MHB) border in the neural plate at the end of gastrulation. Genetic and embryological studies have demonstrated that reciprocal repression between *Otx2* and *Gbx2* results in their mutually exclusive expression in midbrain and hindbrain progenitors, and sorting between *Otx2*$^+$ and *Gbx2*$^+$ cells establishes the MHB boundary. Signaling molecule Fgf8 is subsequently induced in *Gbx2*$^+$ cells immediately posterior to *Otx2*-expression domain. Fgf8 not only acts as an organizer to pattern the MHB region, but also plays an important role in maintaining the MHB boundary by regulating the cell adhesion in *Otx2*$^+$ cells. These stepwise processes leading to the formation of the MHB compartment boundary represents an evolutionary conserve regulatory logic in the formation of a compartment boundary and organizing center.

Role of CK2α in Wnt/β-catenin signaling *in vivo*

Isabel Dominguez, Irene R. Degano, Kathleen Chea
Department of Medicine, Hematology-Oncology Section, Boston University School of Medicine, 02180 Boston, Massachusetts

Wnt/β-catenin signaling is essential for embryonic development and adult tissue homeostasis. Although many Wnt/β-catenin components have been identified *in vitro*, key modulators of Wnt/β-catenin signaling that regulate appropriate mammalian development *in vivo* are still emerging. The highly conserved serine-threonine kinase CK2 is essential for Wnt/β-catenin signaling in *Xenopus laevis* embryos and for β-catenin stability *in vitro*. Since one of the two mammalian CK2 kinases, CK2α, is essential for proper mouse embryo development, we aimed to test whether CK2α is a component of Wnt/β-catenin signaling *in vivo* in mammals. We used somite-matched *CK2α+/+* and *CK2α-/-* embryos to analyze Wnt/β-catenin pathway activation by three complementary approaches. 1) We found that *CK2α-/-* embryos had decreased cytoplasmic and nuclear levels of β-catenin in Wnt responding tissues, but seemly no change in membrane β-catenin compared to wildtype embryos, using immunoblot and immunostaining. 2) We found downregulation of some Wnt/β-catenin-specific target genes in *CK2α-/-* embryos compared to wildtype embryos by RT-PCR. 3) Crossing CK2 mutants to a novel Wnt-reporter that we just characterized, *LEF-eGFP*, we found that *CK2α-/-* embryos, compared to wildtype embryos, had decreased eGFP levels by live fluorescence, immunoblot and FACS analyses. These data indicate that CK2α regulates Wnt/β-catenin pathway activation *in vivo* in mouse embryos. Our results also suggest that the serine-threonine kinase CK2α' could be a contributor to Wnt/β-catenin signaling *in vivo*.

In vivo analysis of epithelial cell polarization

Stephen Armenti and Jeremy Nance
Skirball Institute for Biomolecular Medicine, Developmental Genetics Program NYU School of Medicine 540 1st Ave., New York, NY 10016

Establishing epithelial cell polarity and cell-cell junctions are critical steps in tissue morphogenesis. In order to form mature junctions, epithelial cells must (1) respond to polarity cues and localize junction proteins to the site of future junction formation and (2) coalesce these proteins into fully-formed junctions. This process is essential during organogenesis, where the establishment and maintenance of junctions enables cells to segregate functional membrane domains. Our lab uses *C. elegans* to study the highly conserved process of epithelial polarization and junction formation. We have shown that the scaffolding protein PAR-3 is required to polarize the embryonic intestine but is dispensable for epidermal polarization. However, additional loss of apical membrane determinant EAT-20/Crb in PAR-3 mutant embryos compromises epidermal polarization. We are currently investigating how EAT-20 localizes and functions with PAR-3 in junction formation. Our findings will provide new insights as to how distinct cell types can employ unique methods to polarize. In other systems, PKC-3/aPKC regulates junction stability downstream of PAR-3. However, the molecular mechanism for this process has yet to be fully described. We have performed a genetic suppressor screen to identify genes functioning with *pkc-3* to regulate epithelial junctions. We are cloning two unknown *pkc-3* suppressors identified in this screen and characterizing how they regulate epithelial junctions. We have shown that these suppressors specifically rescue junction defects seen in *pkc-3ts* mutants. Findings from this study will provide new insights into the basic mechanisms of junction formation.

A High-Throughput Sequencing-Based Screen for Skeletal Patterning Genes in Sea Urchin Embryos

Arlene Reyna, Amanda B. Core, Jasmin Coulombe-Huntington, Hajerah Hameeduddin, Christy Li, Michael Piacentino, Finnegan Hewitt, Evan Bardot, David E. Lee, Albert J. Poustka, and **Cynthia A. Bradham**

Sea urchin embryos produce a calcium carbonate endoskeleton that is shaped like an artist's easel. The skeleton is secreted by primary mesenchyme cells (PMCs), but the skeletal patterning information is localized to ectodermal cells, which are contacted by thin filopodia extended from the PMCs. It seems that ectodermal cues somehow regulate the migration of PMCs, which adopt a stereotypic pattern prior to skeletogenesis. To identify ectodermal patterning cues, we used two chemical perturbations. One ventralizes and the other dorsalizes the ectoderm at the top level of the DV specification network; however, both perturbations produce similar, ectodermally-based skeletal patterning defects. We hypothesized that patterning cues are cell-surface or secreted proteins that are absent in both perturbations. We therefore Illumina-sequenced cDNAs from control and treated embryos, then selected genes downregulated in both chemical treatment relative to controls. We next selected genes encoding cell surface or secreted proteins, which were enriched in the mutually downregulated set. This screen identified about 70 candidate patterning genes, including genes implicated in vertebrate neocortical patterning, vasculogenesis, auditory amplification, and metastasis. Functional tests demonstrate that all the candidates tested to date (n= 9) are required for appropriate skeletal patterning. Since sea urchins lack a neocortex, a vasculature, audition (to our knowledge), and cancer, this screen provides us an important glimpse of the ancestral functions of these evolutionarily conserved genes.

KEYNOTE ADDRESS

Built to Rebuild: What Organ Regeneration Tells Us About Organogenesis in Plants

Kenneth Birnbaum
Center for Genomics and Systems Biology, Biology Department, New York University

More than 100 years ago, Gottlieb Haberlandt predicted that differentiated plant cells could exhibit totipotency, the ability of a single cell to produce all the cells of an organism. His prediction turned out to be correct but it took almost 60 years to accomplish the feat. Two important features of plant development needed to be manipulated. The first was learning how to control plasticity and reverse the developmental potential of plant cells. The second was learning how to harness the mechanisms that control organogenesis within the growth centers of the plant, meristems. I will talk about our work and the work of others in gaining an understanding of the mechanisms and operating principles of meristem organization in the root. In many respects, our persistent questions about meristem patterning retrace the landmark steps in the quest for totipotency. Work in cell biology and molecular genetics has uncovered major regulators of organ growth and patterning. We have built on this work using development genomics to order events at the cellular level during regeneration, showing some unexpected features of meristem organization. I will also present some new results on the role of auxin in setting up gradients that mirror cell potential in the plant root and during regeneration.

SESSION II

Molecular development of projection neuron types and building of local microcircuitry in the cerebral cortex

Caroline Rouaux, Simona Lodato and **Paola Arlotta** Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA 02138.

In the developing cerebral cortex, cell-extrinsic and -intrinsic signals govern the establishment of neuron subtype-specific identity and the building of local microcircuitry. By comparing expression profiles of pure populations of cortical projection neurons, we previously identified the transcription factor *Fezf2* as necessary for the fate specification of corticospinal motor neurons (CSMN), a major class of corticofugal projection neurons (CFuPN). Here, we show that the ectopic expression of this single transcription factor within the striatum is sufficient to generate CFuPN from progenitors that are normally fated to become medium spiny neurons. The newly generated neurons express molecular markers of CFuPN and do not express MSN-specific genes. In addition, they acquire pyramidal morphology and connectivity to subcortical and subcerebral targets. Finally, we demonstrate that different subtypes of cortical projection neurons uniquely and differentially determine the laminar distribution of the inhibitory GABAergic interneurons with which they pair. This process is critical for the modulation of projection neuron firing activity and the building of local microcircuitry. Interestingly, we demonstrate that the subtypespecific identity of the projection neurons, rather than strictly their birthdate, determines the interaction with specific classes of interneurons. Together, the data demonstrate that specific populations of cortical projection neurons can be directed to differentiate outside of the cortex by cell-autonomous signaling, and that projection neurons cell-extrinsically affect their interaction with interneurons during the assembly of the local inhibitory circuitry.

Characterizing the role of Eg5 kinesin on mediating neural stem cell division in the developing zebrafish neural tube

Kimberly Johnson[1], Rebecca Bernardos[2], Chelsea Moriarty[2], Alissa Ortman[2], Kim Chi Ngo[2], Kristina DiPietrantonio[2], Michael J. Barresi[2]
[1]Molecular and Cellular Biology Graduate Program, UMass Amherst, Amherst MA 01003.
[2]Department of Biological Sciences, Smith College, Northampton MA 01063

Stem cell proliferation must be tightly regulated, especially during early developmental stages such as neurogenesis. In a genetic screen designed to identify essential genes required for astroglial (neural stem cell) development in zebrafish, we found that a loss of the *kif11* gene caused a significant increase in the presence of radial glial cell bodies in the ventricular zone and floor plate of the neural tube. Kif11, also known as Eg5, is a plus-end directed motor protein responsible for establishing the forces required to stabilize and separate the bipolar mitotic spindle. Our analysis confirms that *eg5* expression occurs in a Gfap+ radial glial population throughout the ventricular zone and floor plate. Furthermore, labeling for anti-phosphohistone H3 and α-tubulin in *kif11* mutants has shown monastral spindles characteristic of mitotic arrest. We have confirmed this phenotype by treatment with S-trityl-L-cysteine, a specific Eg5 inhibitor previously reported to cause mitotic arrest through monastral spindle formation. Lastly, by labeling for sensory, motor, and specific interneuron populations, we show that Eg5 mediated division is required for proper neuronal development within the neural tube. Our findings support a model in which Eg5 is an important mediator of neural stem cell division and neuronal patterning during embryonic CNS development. Currently, we are counting the overall nuclei in the neural tube as well as conducting cell death assays and characterizing other glial populations to test whether the increase in Gfap+ cell bodies following Eg5 inhibition is a compensatory mechanism in response to a decrease found in specific neuron populations.

Wnt1 expression temporally allocates upper rhombic lip progenitors and defines their terminal cell fate in the cerebellum

Nellwyn Hagan[1] and Mark Zervas[2]

[1]Department of Neuroscience, [2]Department of Molecular Biology, Cell Biology and Biochemistry, Division of Biology and Medicine, Brown University, 70 Ship St., Providence, RI 02903.

The cerebellum (Cb) controls movement related physiology using a diverse array of neurons derived from rhombomere 1 (r1), an embryonic compartment patterned by the isthmus organizer. A secreted glycoprotein, WNT1, is expressed at the junction of the mesencephalon (mes) and r1 and plays a pivotal role in maintaining the isthmus organizer and patterning the cerebellar primordia. Mutations in *Wnt1* produce severe Cb defects which have generally been attributed to aberrant organizer activity. Interestingly, *Wnt1* is also expressed in r1, but the distribution and molecular identity of *Wnt1* expressing progenitors have not been described in this region. We used *Wnt1-Venus* transgenic mice to generate a molecular map of *Wnt1*-expressing progenitors in relation to LMX1a and OTX2. Our analysis validated *Wnt1* expression in the upper rhombic lip and revealed molecularly-defined developmental zones in r1. We then used genetic inducible fate mapping to link transient *Wnt1* expression in r1 to cell fates in the developing and mature Cb. *Wnt1* expressing progenitors primarily contributed to deep cerebellar nuclei, granule cells, and unipolar brush cells in distinct but overlapping temporal windows and sparsely contributed to inhibitory neurons and Bergmann glia. The *Wnt1* lineage did not follow a competency model of progressive lineage restriction to generate the Cb or the functionally related precerebellar system. Instead, progenitors initiated *Wnt1* expression *de novo* to give rise to each Cb cell type and precerebellar nuclei. Our findings provide new insight into how lineage and timing establish cell diversity and circuitry within the Cb system.

Multi-level control of gene expression by Foxo transcription factors

Calla Olson & **Michael T. Marr II**
Department of Biology, Brandeis University, Rosenstiel Basic Medical Sciences Research Center 415 South St. Waltham, MA 02454

Insulin receptor is a critical cellular sensor of nutrients and growth factors. This pathway dictates cell growth and proliferation by controlling both protein and RNA synthesis. Downstream targets of the TOR pathway control protein synthesis, while RNA synthesis is modulated by transcription factors such as the **Fo**rkhead-bo**x** family **O** (FOXO) transcription factors. We recently identified direct targets of FOXO in *Drosophila* and uncovered a functional connection between FOXO targets and translation initiation. Several FOXO targets are immune to the regulation imposed by the TOR pathway. This provides a novel mechanism for stimulated protein synthesis from transcripts activated by FOXO. Currently we are working to understand how these transcripts escape the translational regulation and how widespread this type of regulation is in response to insulin signaling.

Early markers of mesoderm and segmental morphogenesis in annelids

Rob Savage, Emily Greenberger, and Elizabeth Brinckley. Williams College
Biology Department, Williamstown, MA. 01267

Our lab investigates the developmental basis and evolutionary origins of segmentation in annelids, the segmented worms. Annelids are an understudied phylum and little is known of their axial patterning mechanisms relative to the model systems. Our objective is to characterize candidate 'segmentation' genes identified from an unbiased expression screen of subtraction libraries derived from the leech *Helobdella* sp (Austin) and the basal polychaete *Capitella teleta*. This bioinformatic approach represents the first unbiased screen for regulatory gene products between classes within a phylum. In this poster, we present gene expression and sequence data on two gene products identified from our screen that are expressed at key stages of segmental morphogenesis. We find that the NK homeobox *Ladybird* (*Hau-Lbx*) and helix-loop-helix *Paraxis* (*Hau-Paraxis*) transcription factors are expressed in a reiterated pattern in the mesodermal lineage before the morphological appearance of segment primordia in leeches. *Hau-Paraxis* is also expressed in an earlier phase. It is restricted to the mesodermal lineage before the left and right sides fuse at the ventral midline, and thus it represents one of the earliest markers of mesoderm development in leech. We also find that *Hau-Paraxis* gene products are required for normal segmental morphogenesis based on the results obtained from our preliminary functional knockdown data using antisense morpholinos. The comparative expression and functional data will be discussed in light of what is known in other bilaterians.

Bioelectric detection and control of carcinogenesis *in vivo*

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Most current cancer studies focus on biochemical signaling factors and gene transcription networks implicated in cell proliferation, differentiation, migration, and apoptosis. Motivated by the known role of endogenous bioelectric cues in regulating pattern formation, and the possibility that cancer reveals a loss of morphogenetic control, we examined steady-state transmembrane potential (V_{mem}) as an important parameter by which tumorigenesis can be detected and controlled. Microinjection of mRNA encoding canonical tumor inducers (*Gli1*, *Rel3*, *KRAS* and $p53^{Thr248}$) or chemical carcinogen treatments (4-nitroquinoline-n-oxide and N-nitrosodimethylamine) in *Xenopus laevis* embryos result in the development of tumor foci; using fluorescent membrane voltage reporter dyes, we defined a physiological signature by which prospective tumor sites could be specifically detected before the tumors became morphologically apparent, revealing depolarization as an early step in neoplastic transformation. Moreover, we demonstrated that the relatively depolarized nature of putative tumor sites is a functionally important (instructive) parameter: misexpression of hyperpolarizing ion transporter mRNA's partially rescued the phenotype. Reduction of tumor incidence from canonical oncogenic inducers by forcing a more normal (polarized) bioelectric state is thus not only a promising approach to tumor normalization but highlights a novel control pathway for induction of cancer in somatic cells. Strikingly, the effect also occurs non-cell-autonomously – tumors can be suppressed by artificially hyperpolarizing cells at considerable distance from the site of oncogene misexpression. These data reveal V_{mem} as a powerful, tractable regulator of cancer and suggest the existence of novel long-range mechanisms by which bioelectric state of the host environment controls cell behavior.

The importance of being dephosphorylated: protein phosphatase 2A regulation of Arabidopsis seedling development

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The serine/threonine protein phosphatase PP2A regulates numerous signaling pathways in plants, but only a few specific proteins have been identified as specific targets for PP2A-mediated dephosphorylation in vivo. Our recent work has focused on analysis of PP2A-mediated regulation of ethylene biosynthesis. The gaseous hormone ethylene is one of the master regulators of plant development and defense. Ethylene biosynthesis is stringently regulated to maintain low levels during normal vegetative growth but allow for rapid production peaks at developmental transitions and under stress conditions. Ethylene negatively regulates cell expansion in most developmental phases; low basal levels of ethylene biosynthesis in dark-grown seedlings are critical for optimal cell expansion during early seedling development. The enzyme 1-aminocyclopropane 1-carboxylate synthase (ACS) performs the rate-limiting biosynthetic step in most tissues, and the expression and turnover of different ACS enzymes are tightly regulated. We find that specific ACS isozymes are targets for regulation by protein phosphatase 2A (PP2A) during Arabidopsis seedling growth, and that reduced PP2A function causes increased ACS activity. Ethylene overproduction in PP2A-deficient plants requires *ACS2* and *ACS6*, genes that encode ACS proteins known to be stabilized by MAPK-mediated phosphorylation. Proteolytic turnover of the ACS6 protein is retarded when PP2A activity is reduced, and PP2A dephosphorylates the ACS6 C-terminal serine residues phosphorylated by MPK6. Surprisingly, we also find that PP2A differentially regulates a second ACS isozyme type. Our data show that PP2A mediates a finely tuned regulation of overall ethylene production by differentially affecting the stability of specific classes of ACS enzymes.

Gpr125 - a novel planar cell polarity pathway component in zebrafish

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Planar cell polarity (PCP) signaling, initially discovered in *Drosophila* mediates the establishment of polarity within the plane of an epithelium. In vertebrates, an evolutionarily conserved Wnt/PCP pathway has been identified that regulates distinct planar-polarized features in various epithelial and mesenchymal cell types. During zebrafish gastrulation, convergence and extension (C&E) movements narrow embryonic tissues dorsoventrally and elongate them anteroposteriorly. Although the Wnt/PCP pathway is a key mediator of C&E, the known components do not fully account for all cell behaviors contributing to C&E. In our search for unknown regulators of zebrafish gastrulation, we have identified Gpr125 as a novel PCP component. As for other PCP genes, excess *gpr125* function results in C&E defects. Diminished *gpr125* function exacerbates the defects of PCP component mutants, including *trilobite (tri)/vang-like2 (vangl2), silberblick (slb)/wnt11* and *landlocked (llk)/scribble1 (scrb1)* in the context of C&E and facial motor neuron migration. In addition, doses of *gpr125* unable to disrupt C&E of wild-type embryos exacerbate C&E defects in *slb/wnt11* mutants. Intriguingly, *gpr125* promotes localized accumulation of Dishevelled (Dvl), the intracellular molecular hub of Wnt/PCP pathway, on the cell membrane in co-expression assays, suggesting Gpr125 may influence PCP upstream or at the level of Dvl. Future experiments will test the biochemical interactions between Gpr125 and Wnt-PCP components and examine the consequences of *gpr125* dysregulation on cell behaviors during C&E.

Single-cell recombination in embryonic tissues mediated by dose sensitivity of a tamoxifen-inducible Cre line.

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Genetic strategies designed to follow the lineage of single cells (clonal analysis) during development are often fairly complex. We have sought to determine whether tamoxifen (TM) inducible Cre-expressing lines in conjunction with Cre-inducible reporters are useful for clonal analysis. Towards this goal we have utilized the endodermally expressed FoxA2^{mcm} line, in which CreER expression is under the control of the *FoxA2* locus, and the R26R line, a ubiquitously expressed floxed-stop LacZ allele, to create embryos that are heterozygous for each allele. High-level TM administration produces functional LacZ expression throughout the *FoxA2* expression domain in such embryos. We sought to determine whether we could use low doses of TM to generate embryos with LacZ marked single cells. We have found that FoxA2^{mcm}+/-;R26R+/- embryos respond to TM in a dose sensitive manner *in vivo,* at doses far below standard administration protocols. Furthermore, we have used a whole embryo culture system to demonstrate that this effect is mimicked under *ex vivo* conditions. Although other labs have used TM induction of CreER to perform lineage analysis, this is the first example that we know of in which precise dose sensitivity of a CreER line has been used to create single labeled cells in a tissue with uniform expression of CreER. In summary our results demonstrate that low doses of TM can be used to create labeled single cells in FoxA2^{mcm}+/-;R26R+/- embryos and suggest that this strategy might be useful for clonal analysis with other TM-inducible Cre lines.

The Na^+K^+ATPAse: a bifunctional protein complex that regulates brain ventricle volume

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The brain ventricular system arises from the central lumen of the neural tube, and is filled with cerebrospinal fluid (CSF). Control of CSF volume is essential for normal brain development. Too much or too little CSF can result in devastating birth defects such as hydrocephalus and anencephaly, respectively. We are the first to demonstrate that the Na^+K^+ATPase (composed of one alpha, beta, and FXYD subunit) controls two aspects of zebrafish brain ventricle inflation. The first is formation of an intact, non-leaky neuroepithelium with correct cell polarity and strong junctions; while the second is production of CSF. We observe that the alpha subunit, (*atp1a1*) and *FXYD1* are required for neuroepithelial polarity and junction formation. Neuroepithelial formation is tightly correlated with low intracellular Na^+ ($[Na^+]_i$), and RhoA activation. Knockdown of *atp1a1* and *FXYD1* increases $[Na^+]_i$ and reduces RhoA-GTP as measured with a RhoA FRET probe, suggesting that $[Na^+]_i$ negatively regulates RhoA activation. Partial loss of Na^+K^+ATPase function does not obviously disrupt neuroepithelial junctions, however, neuroepithelial permeability and "leakiness" to an injected dye are strikingly increased. Conversely, increasing *atp1a1* or *RhoA* leads to "tighter", less leaky junctions. The second function of the Na^+K^+ATPase complex (impact on CSF production), is not mediated by RhoA, requires only *atp1a1* and the beta subunit (*atp1b3a*), and is associated with very low levels of $[Na^+]_i$. Dose response assays show that forebrain ventricle size is mediated by the amount of Atp1a1, which together with RhoA, regulates neuroepithelial permeability, a result with implications for the etiology of hydrocephalus.

SESSION III

Pecking at the Origin of Vertebrate Diversity: Insights from Darwin's Finches and other birds

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The faces of vertebrates display a number of species-specific and adaptive characteristics which they acquired during evolution by natural selection. The classic textbook example of adaptive radiation, natural selection and niche partitioning in animals is the fifteen closely related species of Darwin's finches (Thraupidae, Passeriformes), whose primary diversity is in the size and shape of their beaks. This natural morphological diversity is associated with the exploitation of various ecological resources and its developmental basis is not fully understood. It is likely that this stunning diversity of beaks in Darwin's finches and cranial morphology in vertebrates more generally was produced by alterations in their craniofacial developmental genetics. We continue to discover high degree of modularity in the developing finch beaks. For example, we recently discovered that beak shapes are built by two distinct regulatory and tissue modules producing prenasal cartilage and premaxillary bone skeletal elements. Such levels of modularity help to explain the levels of morphological variation observed in Darwin's finches. Moreover, we began analyzing beak shapes using mathematical approaches followed by developmental studies in both Darwin's finches as well as other related groups of songbirds. Such combined studies allowed us to reveal unexpected levels of flexibility in relation of the beak shape phenotypes with underlying developmental programs.

An interplay between anterior and posterior visceral endoderm controls anteroposterior axis formation in mice

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During early mouse embryogenesis, temporal and spatial regulation of gene expression and cell signaling influences lineage specification and embryonic polarity. Two extra-embryonic tissues, Anterior visceral endoderm (AVE) and posterior visceral endoderm (PVE) cells, located on the opposite sides of the embryo, are thought to establish the anteroposterior body axis.
Wnt3 is expressed in the PVE and the AVE expresses Wnt antagonists, suggesting that AVE cells block *Wnt3* expression in the anterior side of the embryo. To test this possibility, we analyzed the expression of *Wnt3* in *Cripto* mutants in which, the AVE fails to move to the anterior side of the embryo. Our results show that the removal of AVE cells from the anterior side of the embryo allows *Wnt3* expression to expand to the AVE and radialized expression of *Brachyury*, a primitive streak marker.

To determine if the expansion of *Wnt3* expression in these mutants is responsible for radialization of *Brachyury*, we generated double *Cripto/Wnt3* mutant embryos. Ablation of *Wnt3* signaling in *Cripto* mutants completely abolishes the *Brachyury* expression.
These results support a model in which the AVE restricts the expression of *Wnt3* to the PVE, preventing it from expanding to the anterior region of the embryo and leading to the formation of the primitive streak opposite to the AVE.

Quantitative live imaging and function of microtubule plus-end-tracking proteins in the neuronal growth cone

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Proper neural connections, which are essential to nervous system function, depend upon precise navigation by the neuronal growth cone. A fundamental problem in growth cone cell biology is how guidance pathways are integrated to coordinate cytoskeletal dynamics, thus driving accurate steering. To address this question, we focus on the plus-ends of microtubules (MTs), which explore the growth cone periphery and play a role in growth cone steering. MT plus-end dynamics are regulated by a conserved family of proteins called 'plus-end-tracking proteins' (+TIPs). Yet, it is unclear how +TIPs interact with each other and with plus-ends to control MT behavior. Additionally, it is largely a mystery how signaling mechanisms downstream of extracellular cues coordinate +TIPs to guide the growth cone in the right direction. One conserved node of integration for multiple guidance pathways is the Abelson kinase (Abl). Our work identified the +TIP XMAP215 and its co-factor Maskin as potent antagonists of the +TIP CLASP, which is a direct Abl substrate. We also found that accurate axon guidance requires XMAP215 and Maskin in vivo, and that they antagonize Abl function. In order to determine how +TIPs directly affect MTs inside the growth cone, we analyzed and quantified parameters of MT+TIP dynamics using plusTipTracker, a Matlab-based open-source software, following acquisition of high-resolution live-imaging data of +TIPs within cultured *Xenopus* growth cones. Our results tell us how +TIPs differentially behave within the growth cone during axon guidance, thus bringing us one step closer to illuminating how the growth cone cytoskeleton is globally coordinated.

Activated Stat is a supercompetitor that acts independently of Myc and ribosome biogenesis

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Cell competition is a conserved mechanism that regulates tissue size and shares properties with the early stages of cancer and tissue regeneration. Cell competition can be induced between adjacent viable cell populations that differ in metabolic rates; cells with higher ribosomal function become winners and kill their less robust neighbors (called losers). Clonal growth assays in *Drosophila* have revealed that wing cells expressing elevated levels of Myc become supercompetitors that upregulate ribosome biogenesis and kill neighboring wild-type cells. However, no other factors with this transforming ability have yet been identified. Here we demonstrate that sustained activation of the Janus kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway transforms wing cells into supercompetitors without increasing Myc levels. Furthermore, Stat can bypass the requirement for Myc in cell competition as hyper-activation of Stat in loser cells lacking *myc* rescues them from elimination. In contrast to Myc, activated Stat does not induce ribosome biogenesis genes. Despite this difference, both activated Stat and Myc induce apoptosis up to several cell diameters away, suggesting that they act independently and converge on a common signal that non-autonomously kills losers. Since sustained activity of STAT proteins is causal to tumorigenesis and successful niche occupancy of resident stem cells, our results have therapeutic implications for cancer and regenerative medicine.

Faculty development opportunity—learn *C.R.E.AT.E.*—how to use primary literature to demystify science, promote critical thinking and stimulate student engagement

Kristy Kenyon, Biology Department, Hobart and William Smith Colleges, Geneva NY; Leslie M. Stevens, Dept. of Molecular Cell and Developmental Biology, University of Texas, Austin, Austin TX; Sally G. Hoskins, Biology Dept., City College of the City University of New York, New York NY.

The goal of the C.R.E.A.T.E. (***C****onsider,* ***R****ead,* ***E****lucidate hypotheses,* ***A****nalyze and interpret the data, and* ***T****hink of the next* ***E****xperiment)* project is to demystify the research process and humanize the people who choose science research careers, through intensive analysis of primary literature coupled with direct communication with authors. Our original project showed that students at the City College of New York, a Minority Serving Institution, made significant gains in critical thinking and content understanding as well as in attitudes toward science and scientists, during their C.R.E.A.T.E. semester. We subsequently tested C.R.E.A.T.E. in a broader spectrum of students. We trained a subgroup of faculty from a variety of public and private colleges and universities in the New York area in C.R.E.A.T.E. teaching methods during a series of workshops held in Manhattan in Fall 2007. We then followed a subset of faculty as they taught CREATE classes on their home campuses. Learning outcomes parallel to those seen at CCNY (e.g. gains in critical thinking, ability to read/analyze primary literature, positive shifts in attitudes toward science and understanding of the nature of science), were made by students from community colleges, large public universities, small liberal arts colleges and the Ivy League, suggesting that the low-cost C.R.E.A.T.E. approach is broadly applicable in different academic settings.

Faculty Development Opportunity: We are recruiting faculty participants for NSF-supported summer C.R.E.A.T.E. Intensive Workshop at Hobart and William Smith Colleges (June 2012, 2013). Stipends and housing support provided. Contact Kristy Kenyon (Kenyon@hws.edu) or Sally Hoskins (shoskins@ccny.cuny.edu) for details.

SESSION IV

Heterogeneous Genetic Mechanisms in Autism and Related Developmental Disorders

Eric Morrow, Brown University Assistant Professor

Autism is a childhood neurodevelopmental disorder which affects language, cognition and social communication. Recent progress in genetics has identified a large number of individually rare genetic loci in the disorder, including a variety of copy number variants which likely affect the level of gene expression. We will present a variety of new results from genetic studies which have implicated new loci and genes in the autism. In addition, novel bioinformatic strategies have pinpointed potential points of convergence for the variety of genes involved. We will present data that supports a role for axon outgrowth as well as activity-dependent gene regulation as mechanistic points of convergence for the variety of genes implicated. The ultimate goal of this research is to establish these neurodevelopmental points of convergence as if we are able to subtype this heterogeneous disorder based on developmental pathways, strategies for intervention may be tailored to these biologic subtypes of the disorder.

Generation of a genetically encoded measure of outer segment renewal in zebrafish rod photoreceptors.

John Willoughby, **Abbie Jensen**
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Photoreceptors are morphologically specialized cells with distinct functional compartments. The rod outer segment compartment is a modified cilium that contains phototransduction machinery and intramembraneous discs packed with photon-capturing Rhodopsin. Photoreceptors have the remarkable ability to shed and renew the outer segment. Little is known about the cellular and molecular mechanisms that control outer segment shedding and renewal, such as what determines how much outer segment is made each day and what is the composition of the machinery that adds the new material. We are interested in indentifying the genes and cellular mechanisms that control outer segment renewal.

We created a new genetic tool to help identify the genes and cellular mechanisms involved in renewal. We generated a stable transgenic zebrafish line that expresses a transmembrane-bound mCherry (TM-mCherry) construct under the control of the Hsp70 promoter.

Within hours after heat shock, we observed high levels of TM-mCherry in the plasma membrane of the cell body, inner segment and base of the outer segment of all photoreceptors. At later time points, we observed a stripe of red fluorescence in rod outer segments, diffuse fluorescence in cone outer segments, and very low fluorescence in the plasma membrane of photoreceptor cell bodies and inner segments. With time, the red fluorescent stripe is displaced distally. We measured rod outer segment growth rates in young larvae and adult fish.

Regulation of petal organogenesis

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The Arabidopsis petal is an ideal system for investigations into plant organogenesis. Petals are dispensable for growth and reproduction, enabling varied manipulations to be carried out with ease. Furthermore, Arabidopsis petals have a simple laminar structure with a small number of cell types, facilitating the analysis of organogenesis. We are examining the role of several floral homeotic genes required for specifying petal identity, and characterized their downstream effectors. We have shown that a number of genes that are required for photosynthetic cell type differentiation are transcriptionally downregulated in petals, implying that turning off inappropriate developmental pathways is an important facet of petal differentiation. We have also initiated efforts to understand how petal growth is regulated. We have identified a microRNA-dependent pathway that appears to regulate the eventual size of the petal. Through quantitative trait locus (QTL) analyses, we have also identified a LRR receptor-like kinase that is critical for petal shape and size. We are currently characterizing these genes, and their regulatory interactions, and through these efforts we are building a dynamic view of the gene regulatory networks controlling petal organogenesis.

Sonic hedgehog noncanonical signaling pathways and membrane trafficking in directional axon turning

Adrianne Kolpak[1], Daorong Guo[1], Jun Jiang[1], Clive Standley[2], Karl Bellve[2], Kevin Fogarty[2], **Zheng-Zheng Bao[1]**
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Axon pathfinding is one of the important biological processes requiredfor establishment of correct neural networks during embryonic development. However, signaling pathways and cellular mechanisms that mediate the effects of many guidance factors remain poorly understood. We and others have shown that Sonic hedgehog (Shh) protein has dual effects on axonal growth and guidance, acting as a positive guidance factor to direct the retinal ganglion cell (RGC) axon projection toward the optic disc and a negative factor to confine the RGC axons within the borders of the optic chiasm. By in vitro assays, Shh appears to exert the opposing effects on RGC axons through a concentration-dependent manner, increasing axonal growth and eliciting attractive axon turning at lower concentrations but inducing growth cone collapse and repulsive axon turning at higher concentrations. We found that high- and low-concentrations of Shh rapidly activated distinct noncanonical signaling pathways, to regulate cytoskeletal dynamics and membrane trafficking in the growth cones. In particular, a type of clathrin-independent endocytosis induced by high concentration of Shh is critical for mediating the negative effects of Shh on RGC axons including growth cone collapse and repulsive axon turning.

Role of TSC/mTOR pathway in axonal connectivity

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Tuberous sclerosis complex (TSC) provides an ideal model for autism research, given the very high rates of autism spectrum disorders associated with TSC, the early detection of the disease in very young infants, and its increasingly well-characterized genetic and molecular basis. TSC is caused by mutations in either the TSC1 or TSC2 gene. Both genes encode for proteins that are upstream of the mammalian target of rapamycin (mTOR) and mutations in TSC1 or TSC2 lead to upregulation of mTOR. Accumulating evidence suggests that TSC patients have non-tuber abnormalities that contribute to the development of the neurological phenotype. In particular, we have shown that TSC1/2 deficiency leads to aberrant axon specification, guidance and myelination in TSC mouse models. Mice mutants in either gene have neurological and cognitive deficits, which can be blocked or reversed by mTOR inhibitors. Furthermore, an mTOR inhibitor was recently approved by FDA for use in rare brain tumors that affect individuals with TSC. However, the effect of mTOR inhibitors on the cognitive and social deficits that affect individuals with TSC remains unexplored. Clinical trials that address this important question are in progress.

POSTER ABSTRACTS

Listed alphabetically by presenter (bold*)
Number gives poster location

1.) Essential functions of the ADAM13 cytoplasmic domain

Genevieve Abbruzzese*, Hélène Cousin, Dominique Alfandari
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ADAMs are transmembrane metalloproteases that control cell behavior by cleaving both cell adhesion and signaling molecules. The cytoplasmic domain of ADAMs can regulate the proteolytic activity by controlling the subcellular localization and/or the activation of the protease domain. We have recently shown that the cytoplasmic domain of ADAM13 is cleaved and translocates into the nucleus. Preventing this translocation renders the protein incapable of promoting cranial neural crest (CNC) cell migration *in vivo,* without affecting its proteolytic activity. In addition, the cytoplasmic domain of ADAM13 regulates the expression of multiple genes in CNC, including the protease Calpain-8. Restoring the expression of Calpain-8 is sufficient to rescue CNC migration in the absence of the ADAM13 cytoplasmic domain. This study shows that the cytoplasmic domain of ADAM metalloproteases can perform essential functions in the nucleus of cells and may contribute substantially to the overall function of the protein.

2.) V-ATPase-dependent ectodermal voltage and pH regionalization are required for craniofacial morphogenesis

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Despite solid evidence for the importance of ion-based signaling during development, only Ca^{2+}-dependent signals have received extensive attention. Regulated ion flux, or bioelectricity, is a fundamental aspect of physiology, and new reporting dyes promise to reveal the roles of other ions the way Fura and Fluo dyes have done for Ca^{2+}. Using voltage and pH reporters we have discovered a never-before-seen regionalization of the ectoderm, with ectodermal-cell sub-populations delimited by different membrane voltage and pH. We distinguish three courses of bioelectrical activity. Course I is a wave of hyperpolarization that travels across the gastrula. Course II comprises the appearance and evolution of patterns that match shape changes and gene expression domains of the developing face; hyperpolarization marks folding epithelium including the neural tube, stomodeum and sensory placodes; both hyperpolarized and depolarized regions overlap domains of important head patterning genes. In Course III, localized regions of hyperpolarization form at various positions, expand, and disappear. Inhibiting H^+-transport by the H^+-V-ATPase causes abnormalities in: (a) the morphology of tadpole facial structures, especially the branchial arches and eyes; (b) Course II voltage patterns; and (c) the ISH patterns of the genes *sox9*, *pax8*, *slug*, *mitf*, *xfz3*, *otx2*, and *pax6.* We conclude that this bioelectric signal has a role in the development of the face, thus it exemplifies an important, little-studied mechanism of developmental regulation. Understanding how bioelectrical signals intertwine with developmental signaling pathways provides important insight into differentiation and morphogenesis, and suggests novel approaches to treating and preventing birth defects, trauma, and disease.

3.) Indirect development, transdifferentiation and the eumetazoan body plan.

Cesar Arenas Mena*. College of Staten Island, City University Of New York.

The histone variant *H2A.Z* is associated with transcriptional multipotency in indirectly developing sea urchins and polychaetes. The redeployment of *H2A.Z* in differentiated larval regions known to contribute to adult organs of the indirectly developing polychaete *Hydroides* also suggests a role for this histone in transdifferentiation. It is proposed that development through partial dedifferentiation represents the ancestral developmental mode of metazoans, and that adult development of indirect developers is not entirely reliant on multipotent cells. Thus, the evolution and ontogeny of indirect development seems more continuous than echinoderms may suggest. Gene usage in *Hydroides* is characterized and contrasted with prevailing views of metazoan body plan evolution. Hox cluster gene expression in echinoderms and cnidarians suggests an ancestral association with the feeding-dependent development of the reproductive side of metazoans, which proceeds by terminal growth in bilaterians. The synexpression of transcription factors during gastrulation by invagination in indirectly developing deuterostomes and protostomes shows more similarities with cnidarians than with direct developers with epibolic gastrulation, a gastrulation mode that does not result in an epithelial gut. Therefore, gastrulation by invagination and indirect development seem ancestral in eumetazoans and the syncytial "endoderm" of acoelomorph flatworms highly derived.

4.) Molecular organization and timing of *Wnt1* expression define cohorts of midbrain dopamine neuron progenitors *in vivo*.

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Midbrain dopamine (MbDA) neurons are functionally heterogenous and modulate complex functions through precisely organized anatomical groups. MbDA neurons are generated from *Wnt1*-expressing progenitors located in the ventral mesencephalon (vMes) during embryogenesis. However, it is unclear whether the progenitor pool is partitioned into distinct cohorts based on molecular identity and whether the timing of gene expression uniquely identifies subtypes of MbDA neurons. In this study we show that *Wnt1*-expressing MbDA progenitors from E8.5-12.5 have dynamic molecular identities that correlate with specific spatial locations in the vMes. We also tested the hypothesis that the timing of *Wnt1* expression in progenitors is related to the distribution of anatomically distinct cohorts of adult MbDA neurons using Genetic Inducible Fate Mapping (GIFM). We demonstrate that the *Wnt1* lineage contributes to MbDA neurons in the VTA, SNc, and RRF during a seven day epoch and that the contribution to MbDA neurons predominates over other ventral Mb domains. In addition, we show that calbindin-, GIRK2-, and calretinin-expressing MbDA neuron subtypes are derived from *Wnt1*-expressing progenitors marked over a broad temporal window. Through GIFM and quantitative analysis we demonstrate that the *Wnt1* lineage does not undergo progressive lineage restriction, which eliminates a restricted competence model of generating MbDA diversity. Interestingly, we uncover that two significant peaks of *Wnt1* lineage contribution to MbDA neurons occur at E9.5 and E11.5. Collectively, our findings delineate the temporal window of MbDA neuron generation and show that lineage and timing predicts the terminal distribution pattern of MbDA neurons.

5.) Role of meltrin in cranial neural crest cell migration and evolution of their functionalities across species.

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ADAM are single pass transmembrane proteins containing **A** **D**isintegrin **A**nd **M**etalloprotease domain. In vertebrates, the members of this family are mostly expressed either ubiquitously (ADAM10, 17) or in testis (ADAM1 and 2). The members of the meltrins (ADAM 9, 12, 13/33 and 19) are one of the few that are expressed during early development notably in cranial neural crest (CNC) or somites.

We have shown that two members of the meltrin family, ADAM13 and 19, are essential for CNC migration in Xenopus. To investigate wether this function is conserved in other species, we identified these meltrins in the zebrafish genome and investigated their role during zebrafish CNC migration. We report that while these ADAM are indeed involved in craniofacial development, they are involved at different steps.

In Xenopus, the ADAM 13 cytoplasmic tail is cleaved and translocated to the nucleus where it modifies the expression of genes involved in CNC migration (Cousin et al., 2011). Using the Xenopus CNC migration system, we uncovered that this particular function of ADAM13 is conserved from *C.elegans* to marsupial but has been lost in mammals such as mouse and probably humans. We will discuss the reasons for this loss of functionality among ADAM13 orthologs and the implications to the nomenclature.

6.) JNK phosphorylation of hnRNP K is required for axon outgrowth and medium neurofilament expression during nervous system development in *Xenopus laevis*.

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The RNA-binding protein hnRNP K is required for axon outgrowth during development. Its suppression in *Xenopus* embryos causes defects in the nuclear export and translation of mRNAs of multiple cytoskeletal genes, including Type IV neurofilaments (NFs). Studies in cell lines have established that hnRNP K shuttles between the nucleus and the cytoplasm to bind and regulate the fates of its target RNAs, from splicing to export, translation, and degradation. At each step, hnRNP K's actions are regulated through post-translational modifications that alter its nucleic acid and protein interactions as well as its subcellular localization. Precisely how this happens in developing neurons to coordinate NF gene expression with the extracellular signals directing axon outgrowth is unknown. We have identified a JNK phosphorylation site within hnRNP K that is essential for its function during neuronal development; a serine-to-alanine mutation of this site abrogated both NF mRNA translation and axon outgrowth in *Xenopus* embryos. JNK has been implicated in the intracellular signaling pathways that mediate effects of several receptors on axon outgrowth. In addition to its role in neuronal apoptosis, studies in cell lines and brain explants have identified a critical function for JNK in axon outgrowth. Thus, hnRNP K likely serves as a substrate on which JNK acts to effect axonogenesis. These data suggest a role for hnRNP K as a central regulatory component linking extracellular signals that regulate axon outgrowth directly with the expressions of key axonal structural components. *Supported by NSF IOS-951043 and an AAUW predoctoral fellowship (EJH)*

7.) Identifying mechanisms of contact-mediated cell polarization

Dorian Anderson, **Diana Klompstra***, and Jeremy Nance
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During gastrulation, cells move to a position in the embryo that is appropriate for the type of tissue they will form. The directional movements of gastrulation require that cells polarize in order to asymmetrically localize cytoskeletal components. Most cells polarize when PAR proteins develop a restricted cortical localization, which allows them to interact asymmetrically with the downstream effectors that mediate polarity. In *C. elegans* and mammalian embryos, the polarity of early embryonic cells is determined by cell-cell contacts, which restrict PAR polarity proteins to contact-free surfaces. The goal of my project is to determine how cell contacts induce the PAR protein asymmetries that polarize early embryonic cells. We previously identified the RhoGAP protein PAC-1 as an upstream regulator that is required to exclude PAR proteins from contacted surfaces of early embryonic cells. PAC-1 is recruited by cell contacts, where it inhibits the Rho GTPase CDC-42 to direct PAR protein asymmetries. How PAC-1 is able to sense and localize to contacts is unknown. We have identified an N-terminal fragment of PAC-1 that is sufficient for localization to cell contacts, and showed that localization of this fragment depends on HMR-1/E-cadherin. These findings suggest that E-cadherin recruits PAC-1 directly to contacts. However, full-length PAC-1 can localize properly when HMR-1/E-cadherin is knocked down, indicating that a redundant signal functions to recruit PAC-1 to contacts. I will report on our progress in characterizing the linkage between PAC-1 and HMR-1/E-cadherin and in identifying factors that function redundantly with HMR-1 to localize PAC-1 and polarize cells.

8.) *Xl*Myo1d is essential for *Xenopus laevis* embryonic development

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Members of the 18 different classes of myosin molecular motors have been found in all eukaryotes where they carry out myriad cell motility functions. However, examples of the roles they play in developmental processes are few. Members of the myosin I family are single headed, bind calmodulin and exhibit a specific tail homology, TH1, which contains both actin and lipid binding. The *Drosophila* homologue of vertebrate myo1d, *Dm*Myo31DF, is the first actin-based motor shown to play a role in l/r asymmetry determination. Previous work showed that *Xl*Myo1d mRNA is detected by *in situ* hybridization in neural tube, cranial neural crest (CNC) cells, cells surrounding the otic and optic vesicles, pre-somitic mesoderm and in the perinuclear regions of somites. A myo1d isoform-specific tail homology, overlapping the TH1 lipid binding tail homology, was also identified. Two-cell stage embryos injected into both blastomeres with *Xl*Myo1d MO displayed several abnormalities: missing dorsal and tail fin, lack of discernable somites, greatly reduced response to stimuli, failure to hatch from the vitelline envelope and an “L” shaped body. Mortality was not significantly reduced. Developmental delay was first observed at stage 17, coincident with the first detection of localized mRNA expression. Single blastomere injected embryos showed injection side abnormalities, including somites that were disorganized, indistinct and reduced in number and size. Uninjected sides showed normal development. The notochord appeared unaffected.

9.) Chemokine receptor cooperation mediates trigeminal ganglion assembly

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Sensory neurons with related functions must migrate to a common ganglion assembly site in the peripheral nervous system. The chemokine SDF-1 guides the assembly of Cxcr4b-expressing trigeminal sensory neurons in zebrafish. Here, we investigate the role of a second SDF-1 receptor, Cxcr7, in trigeminal sensory neuron migration. We find that *cxcr7b* is expressed in the vicinity of migrating trigeminal sensory neurons and is required non-autonomously for proper ganglion assembly. Intriguingly, loss of *cxcr7b* results in a phenotype that is distinct from loss of *cxcr4b*, while loss of both receptors results in a phenotype that resembles loss of *cxcr4b* alone. This suggests that Cxcr7b interacts with SDF1 upstream of Cxcr4b activity. We also demonstrate that loss of *cxcr7b* phenotypically resembles overexpression of *SDF*-1, while overexpression of *cxcr7b* phenotypically resembles loss of *SDF-1.* These two findings provide *in vivo* evidence for negative regulation of SDF-1 by Cxcr7b. Finally, we show that overexpression of *cxcr7b* in the tissue through which wild-type neurons migrate disrupts ganglion assembly, indicating that Cxcr7b plays an instructive role in this context. Together, our results demonstrate that Cxcr7b cooperates with Cxcr4b to facilitate SDF-1-mediated ganglion assembly in the zebrafish peripheral nervous system.

10.) Identifying transcriptional regulators essential for capacitating pollen tube growth and guidance

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Sexual reproduction culminates in the combination of two haploid nuclear genomes, an egg and a sperm, to form a diploid zygote. While flagellar motile sperm is common throughout many kingdoms of life, flowering plants employ an alternative system of fertilization by encasing their sperm in a protective companion cell known as pollen. The pollen grain's existence is devoted to the development of a polar pollen tube that delivers sperm by boring through the female tissue of the pistil to the egg-bearing ovules. Within the pistil, pollen tubes respond to female-gamete-excreted attractants, which alter tube polarity resulting in the precise targeting of the pollen tube to a targeted ovule. Pollen tubes are dramatically capacitated to reach the ovules only after they have grown through female tissues. Transcriptional activation of the pollen tube by interaction with the female tissues of the pistil has been demonstrated, resulting in a wealth of pollen tube specific unregulated gene products. Included in this transcriptional profile is a family of redundant R2R3-MYB transcription factors that we have shown to be crucial for normal sexual reproduction via mutant analysis. Additionally, gene-fluorophore fusions of these transcription factors specifically localize to the pollen grain nucleus yet are undetectable before tube development. These mark the first pollen grain nuclear genes confirmed to be transcriptionally activated only during pollen tube development in the pistil tissues and this family may regulate a pollen-tube specific capacitating gene network.

11.) A novel in vitro 3D microtissue platform for the study of microenvironment cues' influence on lineage decisions

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Cell lineage decisions are strongly influenced by cell-cell interactions and exogenous factors. Here we present a novel approach to study the cellular and chemical cues involved in lineage decision making using 3D microtissues generated with micro-molded hydrogels. Monodispersed cells are seeded in non-adhesive agarose hydrogels containing round or toroidal microwells, and the cells self-assemble into spherical microtissues (SMT) or toroidal microtissues (TMT). We present two methods to study cell-cell interactions. In the Cell-Cell Interface method, two cell types are co-seeded to form heterotypic SMT. In the Tissue-Tissue Interface method, SMT of the first cell type are formed, TMT of the second cell type are formed, and SMT are placed in the lumen of TMT.

The microtissue approach offers several advantages: 3D, which better mimics the in vivo microenvironment; ease of fabrication of 3D microtissues; contacting co-culture; high degree of control of parameters for each microtissue, such as seeding density, time, exogenous morphogens, and developmental stages of cells of interest; ease of visualization; and high throughput. We have fabricated monotypic SMT of the neural stem cell line NE-4C, neuronal line B104, astroglial line A7, brain endothelial lines bEnd.3 and RBE4; heterotypic SMT of B104/bEnd.3 and A7/bEnd.3; and TMT of A7 and primary cortical cells. We have also placed NE-4C SMT in the lumen of cortical TMT and A7 TMT, and the SMT fused with the TMT within 24 hrs. The preliminary data show the feasibility of using the 3D microtissues to study cell-cell interactions and exogenous morphogens for cell lineage decisions.
Funding: NSF Advance, Career Awards to DHK; NIH R01EB008664-01A1 to JM; NSF GK-12 Fellowship to YL.

12.) Asymmetry randomization by tubulin mutations reveals a conserved cytoplasmic mechanism initiating left-right patterning in vertebrates and plants

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Consistent laterality, a fascinating aspect of embryonic development, has considerable implications for physiology and behavior. The vertebrate body is inherently asymmetric with respect to the left-right (LR) axis. A popular model of LR patterning proposes that vertebrate asymmetry is first established by the movement of cilia during gastrulation. Since numerous species initiate asymmetry prior to (or without) the presence of cilia, it is often proposed that asymmetry generation is highly divergent. In contrast, we have suggested that asymmetry is an ancient, well-conserved property of individual cells arising from the chirality of cytoskeletal structures. Recent findings in *Arabidopsis thaliana* have shown that mutations in α-tubulin and a γ-tubulin associated protein (GCP2) break the wildtype symmetry of the plant's axial organs. Since both tubulin complexes are ubiquitous in eukaryotes and are involved in the formation and nucleation of microtubules, we tested the hypothesis that the same mutations would affect asymmetry in a vertebrate embryo. Our data reveal that the same point mutations made in *Xenopus laevis* tubulin proteins function as a dominant negative to specifically and efficiently randomize the LR patterning of the internal organs. Importantly, this phenotype occurs only when embryos are injected with the mutant mRNA at an early one-cell stage and no later. Taken together, the data suggest that the LR-randomizing effect is not mediated by any problem with nodal ciliary flow, instead implicating tubulin structure in early orientation events and revealing a striking conservation of intracellular laterality mechanisms between kingdoms.

13.) Utilization of the tamoxifen-inducible FoxA2^{mcm} allele to generate single cell recombination in the endoderm

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The liver plays vital exocrine and endocrine roles in the human body, such as detoxifying blood, secreting serum factors, as well as producing and secreting bile in the intestines. While it is clear that the embryonic liver-bud contains all the liver precursors, it is uncertain if the two main liver cell-types, the bile producing hepatocytes and the bile-duct forming cholangiocytes, are derived from a single bipotent precursor cell or if the two cell types are derived from distinct precursors. To answer this and other questions about liver ontogeny, we will test a system to label single liver-bud cells. We propose an endoderm-labeling system utilizing the FoxA2^{mcm} mouse, a tamoxifen (TM)-inducible Cre line inserted into the FoxA2 locus. In our experiments, embryos harboring both the FoxA2^{mcm} and the R26^{R} alleles express beta-galactosidase throughout the liver bud when their mothers are injected with the maximum amount of TM (0.1mg/g body weight) via oral gavage. The FoxA2^{mcm}; R26R embryonic endoderm cells respond to TM by activating beta-galalatosidase in a cell and all its descendants in a dose-responsive manner, and thus we hypothesize that a minimal dose of TM required to activate the reporter allele in a single liver-bud cell can be determined. We will investigate the tamoxifen-inducible system for producing single-cell recombination by conducting dose-response studies *in vivo* and *in vitro*, in order to determine the potency of single liver-bud cells. Our research may provide a novel technique for performing single-cell lineage tracing and insight into the growth of hepatic cells during development.

14.) A Novel Post-differentiation Role for Notch signaling

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Notch signaling has been studied in many contexts, most of which involve cell fate specification during development. Fewer examples can be found in which Notch signaling is required for post-differentiation processes. We have found evidence that Notch signaling plays an important role in *C. elegans* ovulation – a highly regulated, complex process involving multiple germline-soma signaling events that coordinate meiotic maturation, sheath contraction, spermathecal dilation, and fertilization.

Previously we showed that APX-1, a conserved ligand for Notch family receptors, acts in the proximal sheath of the *C. elegans* gonad as a latent proliferation-promoting signal that promotes proximal germline tumors in a variety of mutant backgrounds (McGovern et al., 2009 PNAS). We showed that in this inappropriate “latent niche” role, APX-1 acts in the proximal sheath, signals through the Notch receptor GLP-1, and is required continuously to maintain germline tumors. However, a normal functional role for Notch signaling in the proximal sheath had not been previously characterized. Starting with the observation that APX-1 depletion causes oocyte defects, we determined that APX-1 is required for proper ovulation. Proper ovulation depends on correct somatic cell fate specification during development, as well as a host of signaling events that occur with the ovulation of each oocyte. We found that reducing somatic activity of APX-1 or LIN-12/Notch interferes with ovulation but does not alter cell fate specification. Using conditional RNAi, we found that signaling is required throughout adulthood for continuous ovulation. Together these results suggest a novel post-differentiation role for Notch signaling in ovulation.

15.) Endocardial requirement for myocardial migration and heart tube formation

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Cardiac morphogenesis is a key step in development necessary for correct integration of the heart into the embryos body plan. Previous work has shown that proper endocardial patterning is related to proper myocardium formation. We want to understand the importance of the endocardium for proper myocardial migration and how the interactions between these two populations of cells relate to heart tube formation. We are taking advantage of small molecule drug screening in zebrafish to further explore the interactions between these two populations by identifying endothelial affecters. Because of its multiple destructive effects, we examined the consequence of FK-506 (Tacrolimus) on cardiac morphogenesis. Our data shows that FK-506 significantly disrupts angiogenesis resulting in defects in myocardial migration and consequently a misshapen heart tube forms. Specifically, endothelial cells were present in treated embryos but were reduced in number and irregularly positioned. These endothelial defects result in failure of blood circulation and severe edema. Preliminary observations indicate linear heart tube defects consistent with early loss of endocardial-myocardial interactions. This study supports the finding that a proper myocardium is dependent on a proper endocardium. We plan to examine the cellular behaviors underlying these morphogenesis defects while pursuing other drugs of interest. We have identified another candidate drug that also results in similar endocardial and myocardial defects. We hope that, taken together, these data will provide insight into the molecular mechanisms underlying myocardial morphogenesis.

16.) A novel high-throughput imaging system for automated analyses of behavior in zebrafish larvae

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Early brain development may be influenced by numerous genetic and environmental factors, with long-lasting effects on brain function and behavior. Identification of these factors can be facilitated by behavioral studies in animal model systems. In this study, we present a novel imaging system for high-throughput analyses of behavior in zebrafish larvae. The three-camera system can image twelve multiwell plates simultaneously and is unique in its ability to provide local visual stimuli in the wells of a multiwell plate. The acquired images are converted into a series of coordinates, which characterize the location and orientation of the larvae. The imaging techniques were tested by measuring avoidance behaviors of zebrafish larvae in response to visual stimuli. The system effectively quantified larval avoidance responses and revealed an increased edge preference in response to a ‘bouncing ball’ stimulus. The imaging system and assays for measuring avoidance behavior may be used to screen for genetic and environmental factors that cause developmental brain disorders and for novel drugs that could prevent or treat these disorders.

17.) Assessing the role of YY1 in the visceral endoderm during early embryogenesis.

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Ying Yang-1(YY1), a multifunctional protein essential for embryonic development, is aptly named based on its function as both a repressor and an activator of gene transcription. Even though YY1 is known to control cell proliferation and chromatin remodeling, its function in particular cell types during embryogenesis is largely unexplored. To determine the biological role of YY1 in mouse during yolk sac development, we used a conditional strategy to delete YY1 from the visceral endoderm (VE) and observe dramatic defects in yolk sac vasculature, impaired embryonic growth and lethality by 10.5 dpc. Immunofluorescence analysis revealed the presence of early blood vessels in the mutant yolk sac by E9.5, however, vessels failed to undergo appropriate remodeling (angiogenesis) by E10.5. Because blood vessel development by the yolk sac mesoderm is known to rely on signals from the adjacent VE, we used RT-PCR to demonstrate the both VEGFA expression from the VE and VEGF receptor (Flk-1, Flt-1) expression in the mesoderm is disrupted in mutant embryos. In addition we have found that the nuclei of YY1-deleted VE display abnormal morphology and seem to have disrupted cell-polarity. Taken together, these results suggest that the transcription of YY1 is required for proper morphological and functional development of the murine yolk sac.

18.) CK2 is essential for heart morphogenesis in mice and frogs

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We recently identified CK2 as a potential regulator of mouse heart morphogenesis, as genetic ablation of CK2α results in embryonic lethality displaying defects in heart formation. CK2α-/- embryos showed enlarged heart tubes, hypoplastic right ventricle, and underdeveloped cardiac cushions and ventricular trabeculations. Since CK2 has been implicated in vitro in the regulation of proliferation, survival and differentiation, we tested the role of CK2 in these cellular processes in the CK2α-/- heart phenotype. Proliferation, assessed by anti-phospho histone H3 (serine 10) staining, was decreased while apoptosis, assessed by TUNEL staining, was not significantly increased in CK2α-/- hearts. RT-qPCR analysis showed that proliferation markers, such as Fgf10 and Fgf8, were downregulated in CK2α-/- hearts while differentiation markers were not affected. Progenitor incorporation, assessed by Islet-1 staining, was not significantly different in CK2α-/- hearts compared to wildtype. To determine whether CK2 activity was also required for heart development in other species we used frog embryos. Xenopus laevis embryos were treated with the CK2 inhibitor, TBB, for 5 hours at stage 10 (gastrulation) and cultured until stage 46. Morphological and histological analyses showed that TBB treated embryos had hypoplastic ventricle and abnormal ventricular trabeculations. These data suggest that, in the heart, CK2α regulates cell proliferation and not apoptosis or differentiation. Our results also point to CK2 as an important regulator of heart morphogenesis cross-species.

19.) *buckyball*: oocyte polarity is essential for embryonic axes formation and germ plasm assembly in zebrafish

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In most animals, including humans, developmentally competent oocytes are crucial for reproduction and to provide maternal gene products essential for early development until zygotic genome activation. In many animals germ plasm, which is needed to specify the germ line, is maternally supplied. Despite their capacity to give rise to all cell types of the embryo, we are only beginning to understand the basic mechanisms necessary for formation of competent oocytes. In vertebrates such as zebrafish and *Xenopus* oocyte polarity is known to be essential for determining the prospective embryonic axes and setting aside the germ cell determinants. An evolutionarily conserved asymmetry in the early oocytes of all vertebrates examined is the Balbiani body, which in *Xenopus* and zebrafish contains maternal regulators of axis formation and germ plasm components. In zebrafish *bucky ball* mutants, the Balbiani body does not form and mRNAs are not localized properly. Apart from Bucky ball, the regulators of Balbiani body formation are largely unknown.

To investigate how Bucky ball regulates Balbiani body formation and oocyte asymmetries in oocytes we identified putative Bucky ball interacting proteins using the Yeast-two-hybrid system. Among the 68 proteins identified, was a conserved RNA binding protein that localizes to the Balbiani body by a Bucky ball dependent mechanism. We have confirmed binding to Buc through co-immunoprecipitation experiments, and are currently investigating the essential function of this RNA binding protein through overexpression and loss-of-function analyses. The interaction between these proteins suggests Buc mediates oocyte polarity in part by recruiting RNA binding proteins

20.) Zebrafish as a model to study cardiomyopathy

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It is well established that proper development during early embryogenesis is crucial to embryonic and adult survival. Post-embryonic maturation of the heart in response to environmental and genetic factors is a little studied part of development, but just as important to adult survival. Understanding this continued maturation in normal development is especially important when taking advantage of viable cardiac mutants, such as the zebrafish mutant, *weak atrium,* which, through the reduced function of the atrium, develops a cardiomyopathy-like phenotype in the ventricle.

Form and function of organs effect development and maturation. In the heart, contraction and blood flow are some of the factors effecting the development of the structure of the heart. We examined the maturation of heart structure of *weak atrium* mutants to gain insight into the mechanisms regulating cardiomyopathy. To completely understand the variance between these mutants and wildtype zebrafish, we first examined normal maturation of the zebrafish heart. By removing the hearts through dissection, we took external measurements of the heart and viewed internal morphology by sectioning. Comparing the mutant development with established normal maturation we can obtain a more thorough understanding of the effect of loss of atrial function on the rest of cardiac maturation. We determined that the lack of contraction of the atrium in *weak atrium* mutant causes the ventricle to compensate resulting in a dilated cardiomyopathy evident in fish surviving through adulthood.

21.) Murine Posterior Visceral Endoderm is Functionally Equivalent to the Amphibian Nieuwkoop Center

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Wnt3 mutant mouse embryos lack a primitive streak, mesoderm and endoderm. *Wnt3* is expressed in the Posterior Visceral Endoderm (PVE) at E5.5 and subsequently in the adjacent epiblast. These observations led us to hypothesize that *Wnt3* signaling induces primitive streak formation in mouse embryos. To test this hypothesis, we generated mouse embryos lacking *Wnt3* in the visceral endoderm, but retaining *Wnt3* expression in the epiblast. Embryos with a visceral endoderm-specific knockout of *Wnt3* phenocopy the *Wnt3* null mouse phenotype. These experiments indicate that *Wnt3* function in the PVE is essential for gastrulation in mice. To determine whether visceral endoderm-derived *Wnt3* is sufficient to drive gastrulation, we generated embryos lacking *Wnt3* in the epiblast, but retaining the expression of *Wnt3* in the visceral endoderm. These embryos undergo gastrulation but display delayed expression of primitive streak markers, show abnormal gastrulation and are resorbed at around E9.5, suggesting that *Wnt3* signaling from the visceral endoderm partially rescues the absence of *Wnt3* in the epiblast. To determine whether *Wnt3* signals through the canonical Wnt pathway, we generated *Wnt3* null embryos carrying a copy of the *BatGal* transgene, a reporter line for the canonical Wnt pathway. *Wnt3* null/ *BatGal* heterozygous embryos fail to express beta-galactosidase, indicating that *Wnt3* acts through the canonical Wnt pathway. These data suggest that the PVE acting through *Wnt3* functions as a signaling center for the formation of primitive streak in mouse embryos, in a similar manner to the Nieuwkoop center of amphibians.

22.) NaV-mediated sodium transport acts to initiate appendage regeneration.

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Mammals have limited ability to regenerate organs. In contrast, amphibians such as frogs can restore lost structures, including the tadpole tail and limb. Thus understanding natural regeneration is key to designing therapeutics for restoring lost tissues. While patterned ion currents are important regulators of neural activity, their roles in non-excitable systems are not understood. We identify a novel requirement for the ion transport function mediated by voltage-gated sodium channel (Na_V) in initiating regeneration. Expressed in the *Xenopus* tail regeneration bud early, Na_V induces an increase in intracellular sodium. Inhibition of sodium flux blocks *Xenopus* tail regeneration by decreasing proliferation, altering axonal patterning, and down-regulating downstream genes involved in driving regenerative outgrowth without affecting overall development. Na_V is absent under non-regenerative conditions but ectopic expression of human $Na_V1.5$ can rescue regeneration during these states. Remarkably, a transiently induced chemical sodium current can promote regeneration even after the formation of a scar-like wound epidermis, indicating that non-regenerative states may have regenerative potential and can be reprogrammed. In summary, our data identify a novel endogenous role for sodium currents in regeneration and more importantly, reveal that non-regenerative tissues may retain an intrinsic regenerative program. Furthermore, our findings show that modulation of sodium transport represents an exciting new approach to organ repair.

23.) *Wdr74* is required for blastocyst formation in the mouse.

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Preimplantation is a period of dramatic changes in gene expression and developmental potential. During early cleavage, a combination of maternal and zygotic factors program the embryonic genome. These dynamic events result in critical differentiation steps including the first lineage specification (ICM and TE), which is required for implantation. Using a reverse genetic RNAi-based screen, we identified WD Repeat Domain 74 (Wdr74) as being required for blastocyst formation. Knockdown of Wdr74 results in embryos that develop normally to the morula stage, but fail to form a blastocoel cavity and do not properly specify inner cell mass and trophectodermal lineages. Lack of Wdr74 also results an increase of Trp53-dependent apoptosis as well as global reduction of RNA transcripts. Blocking Trp53 (p53) function in Wdr74-deficient embryos partially rescues blastocyst formation and lineage specification, but does not rescue the overall reduction of RNA. Our findings offer the following conclusions about this poorly understood gene: 1) *Wdr74* is expressed from the zygotic genome and is required for morula to blastocyst transition. 2) In the absence of *Wdr74* function there is a global reduction of RNA transcripts from RNA polymerase I, II and III. 3) Loss of *Wdr74* activates Trp53 dependent apoptosis and when Trp53 function is blocked, dsWdr74 embryos are capable of lineage specification and blastocyst formation. Our results identify Wdr74 as an essential gene in the mouse, with a critical requirement during preimplantation.

24.) An *in vivo* method to study regulatory elements of the medium neurofilament gene in *Xenopus laevis*

Chen Wang*, Ben G. Szaro
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Building an axon requires highly regulated changes in the expression of key structural genes. For example, the developmental onset of the expression of the medium neurofilament (NF-M) subunit is tightly coordinated with neuronal differentiation; subsequent modulation of this expression accompanies axon initiation, pathfinding, synaptogenesis, and myelination. This tight regulation, operating at both the transcriptional and post-transcriptional levels, is mediated through the binding of specific proteins to elements within the gene and its transcribed mRNA. The identification of these elements and the proteins that bind them requires an in vivo model system in which the activity of genes bearing mutated prospective control regions can be rapidly screened within the context of the developing nervous system. We modified the□-C31 integrase-mediated *Xenopus* transgenesis protocol (Allen and Weeks, 2005, Nature Methods 2: 975-79) to generate transgenic *Xenopus laevis* using direct plasmid injection into 2-cell stage embryos. This plasmid contained an *attB* site to facilitate its integration into the *Xenopus* genome and a *DsRed2* reporter gene driven by the *NF-M* promoter. To reduce position effects, the transgene was flanked by two tandem repeats of chicken *HS4* insulator sequences. Injection of 100 pg of this plasmid into embryos yielded strong DsRed2 expression in differentiated neurons at the onset of endogenous NF-M expression. This method provides a useful tool for the study of regulatory elements of the *NF-M* gene in the developing vertebrate nervous system. *Funded by NSF IOS 951043.*

25.) Aurora A kinase is essential for epiblast growth and survival in mouse embryos

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Aurora A is a member of the mitotic serine/threonine kinase family, involved in centrosome maturation and spindle assembly during cell division cycle. Mutations of *Aurora A* (*AurA*) are known to result in mitotic arrest due to defects in centrosome separation and chromosome segregation. A recent report showed that a knockout of *AurA* leads to embryonic lethality at the blastocyst stage with cell proliferation failure, mitotic arrest, and monopolar spindle formation. To examine the function of *AurA* gene in post-implantation mouse embryos, we generated epiblast-specific *AurA* knockout embryos using *Sox2Cre*-mediated genetic recombination and examined their embryonic development process. We observed that an epiblast-specific *AurA* knockout causes epiblast growth inhibition and epiblast-specific cellular death through apoptosis in gastrula stage embryos and leads to resorption of embryos at around E9.5. We also found that an epiblast-specific *AurA* knockout does not affect the axial polarity of mutant embryos, which showed normal anterior visceral endoderm (AVE) movement during post-implantation stages, and expression of *Brachyury*, a primitive streak marker in the posterior region. The fact that epiblast cells are completely ablated in epiblast-specific *AurA* knockout embryos at around E7.5 demonstrates that AurA is essential for epiblast survival and further embryogenesis and provides an alternative way to induce tissue-specific cellular ablation.

Dining and Meeting Room Locations

Friday 3/25

3:45pm – 4:15pm	Coffee Break	Lillie Lobby
6:00pm - 7:15pm	Dinner	Dining Hall
8:45pm – 10:45pm	Beer & Wine Mixer	Meigs Room

Saturday 3/26

8:00am – 9:00am	Breakfast	Dining Hall
10:00am – 10:30am	Coffee Break	Lillie Lobby
12:00pm – 1:30pm	Lunch	Dining Hall
2:30pm – 3:00pm	Coffee Break	Lillie Lobby
6:00pm – 7:15pm	Dinner	Dining Hall
8:45pm – 10:45pm	Beer & Wine Mixer	Café Swope

Sunday 3/27

8:00am – 9:00am	Breakfast	Dining Hall
10:15am – 10:45am	Coffee Break	Lillie Lobby
12:15pm	**BOXED** Lunch	Dining Hall

Meeting Rooms

Fri 3/25	2:00pm – 9:00pm	Lillie Auditorium
Sat 3/26	9:00am – 9:00pm	Lillie Auditorium
Sun 3/27	9:00am – 12:15pm	Lillie Auditorium

Parking

Swope & Stoney Beach Lots

Sponsors

We are grateful to the following sponsors for helping to supports the NESDB Meeting 2011.

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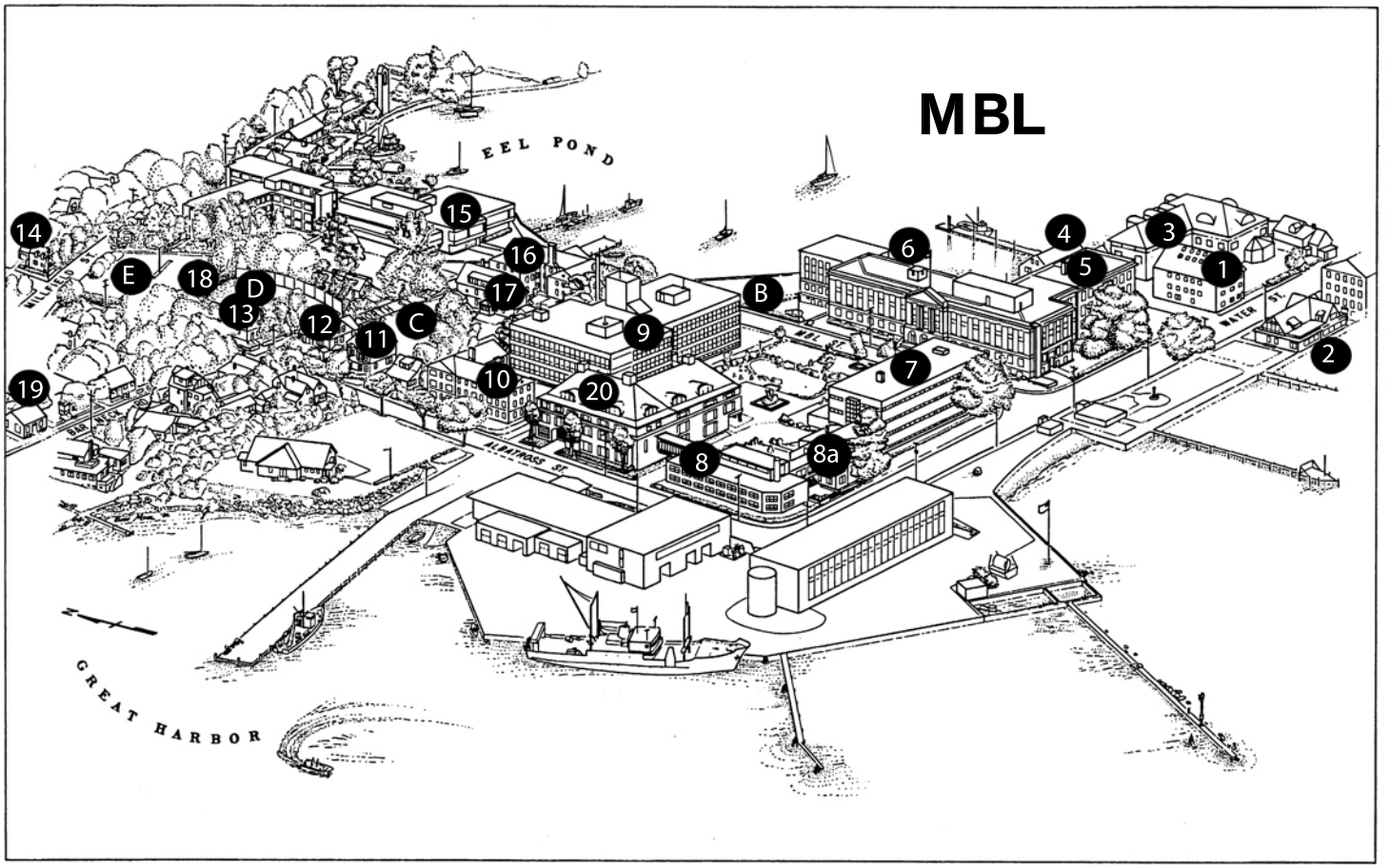

Campus Map Key

1. Candle House (Administration, Lecture Rooms)
2. 100 Water Street (Pierce Visitors Center, Associates Gift Shop, Satellite Club, MBL Club)
3. Marine Resources Center
4. Collection Support Facility
5. Crane Wing, Lillie Laboratory (Labs, Receiving)
6. Lillie Laboratory (Labs, Library, Auditorium)
7. Rowe Laboratory, Speck Auditorium
8. ESL (Environmental Sciences Laboratories
8a. Homestead (Administration)
9. Loeb Teaching Laboratory (Research and Teaching Labs, Lecture Rooms)
10. Apartment House
11. Veeder House Dormitory
12. David House Dormitory
13. Broderick House (Facilities, Services, & Projects Office, Encyclopedia of Life)
14. Crane House
15. Swope Conference Center (Registration, Café Swope, Cafeteria, Meigs Room)
16. Ebert Hall (Dormitory)
17. Drew House Dormitory
18. 15 North Street
19. Smith Cottage and Bar Neck Road property
20. C. V. Starr Environmental Sciences Building (Starr Building)

B, C, D, E - Parking