2015 Developmental & Stem Cell Biology Symposium

Abstract Book

Poster presentations:

May 6th, 2015
11:30 am - 12:30 am

GSB Lobby & Genomic Cafe

1 Vincent Boudreau, UNC

A genetic view of metazoan mitotic exit through Protein Phosphatase 2A

Vincent Boudreau, Vincent Archambault, Paul S. Maddox

*Abstract not available online

2 Kevin Byrd, UNC

LGN/GPSM2 Controls Cellular Division Orientation in Developing Murine Oral Epithelia

Kevin M. Byrd, T. Anthony Curtis, Kendall Lough, Scott E. Williams

OBJECTIVES: Basal cells of the developing epidermis build stratified tissue and maintain progenitors by balancing planar and perpendicular divisions. Our aim was to assess the timeline of development in diverse murine oral epithelia and to characterize the expression and function of *LGN/GPSM2*, an important regulator of mitotic spindle orientation.

METHODS: Embryos were injected at E9.5 with a high-titer lentivirus against *LGN* containing an H2B-RFP1 reporter, utilizing advances of *in utero* shRNA-mediated knockdown. E14.5–E18.5 littermate control and knockdown heads were embedded in OCT or fixed for whole tissue immunostaining. Tissue was processed for immunofluorescence microscopy and imaged using a confocal microscope. Sections were analyzed using FIJI/ImageJ, and 3D-image reconstruction and analysis was accomplished using Imaris. Phophohistone-H3 marked mitotic cells when assessing LGN polarization. Tissue thickness and cellular division angles were obtained using β4-integrin as a basement membrane marker, and characteristic cleavage furrow protein survivin was used to identify recently divided cells.

RESULTS: Unlike other epithelia, where LGN localizes to the apical cortex of mitotic progenitor cells and promotes perpendicular divisions, dorsal tongue exhibited a high proportion of non-polarized, bipolar and basal LGN crescents between E14.5-E17.5. Buccal, palatal, and ventral tongue epithelia displayed apical LGN and about half of divisions were planar (45.6%, 43%.1 and 47.6%, respectively, n≥50 cells), demonstrating a balance with perpendicular divisions. In contrast, most divisions in dorsal tongue epithelia (85.4%, n≥86) were planar. In buccal, palatal, and ventral tongue epithelia, RNAi-mediated knockdown of *LGN* increased the proportion of oblique/planar divisions relative to wild-type and RFP^- cells (n=≥50 per tissue), leading to stratification defects. Strikingly, *LGN* loss in dorsal tongue has the opposite effect, causing predominately perpendicular divisions (78.2%, n=91), which leads to defective morphogenesis of the pre-papillary placodes.

CONCLUSIONS: The balance of divisions, mediated by LGN, is important for building stratified and specialized epithelia during oral development.

3 Rita Meganck, UNC

Development of snRNA reporter genes to investigate the role of PHAX in snRNP biogenesis

Rita M. Meganck, Casey A. Schmidt, A. Gregory Matera

Sm-class small nuclear RNAs (snRNAs) are major components of the spliceosome, which is required for the splicing of introns in eukaryotic pre-mRNAs. To become biologically active, the snRNAs must assemble into small nuclear ribonucleoprotein (snRNP) complexes. Assembly first requires nuclear export of snRNAs into the cytoplasm, where they undergo several modification steps and acquire protein partners; subsequently, they are re-imported back into the nucleus. Proper snRNP formation is important in development, as mutations in certain snRNP biogenesis factors are lethal. Accordingly, mutations in PHAX, the snRNA export adaptor, would be expected to be lethal. However, about one quarter of flies with a homozygous null mutation in PHAX survive to adulthood. To investigate this effect, we created reporter genes of the snRNAs U1, U2, U4, U6, and Like-U by replacing part of the coding region from each gene with fluorescent RNA aptamer tags. Currently, we are testing the fluorescent properties of the constructs and planning experiments to determine if the reporters can assemble into snRNPs in vivo. Transgenic flies will be created that contain the reporter gene crossed into the PHAX mutant background. Cells from these flies will be imaged to investigate snRNA localization in the absence of PHAX. Additionally, we will use the reporter to explore the dynamics of U-bodies (cytoplasmic aggregates of snRNPs) during Drosophila ovarian development. As most eukaryotic species export their snRNAs into the cytoplasm for snRNP assembly, the results should provide general insight into the mechanisms of snRNA transport and assembly.

4 Leslie Kennedy, UNC

A Novel Tbx20/Casz1 Transcriptional Complex is Essential for Cardiac Function

Leslie Kennedy, Erin Kaltenbrun, Kerry Dorr, Todd Greco, Ileana Cristea, Frank Conlon

Clinical and genetic studies have provided direct evidence for the role of mutations in T-box transcription factors in Congenital Heart Diseases (CHDs). While the T-box transcription factor Tbx20 is an essential transcription factor for heart development and its link to CHD is well established, there are many critical questions unanswered about the mechanism of how Tbx20 functions. The cardiovascular field has yet to identify what proteins complex with Tbx20 during cardiac development and homeostasis, how these interactions regulate Tbx20's choice of distinct transcriptional targets at different times, or how these interactions function to activate and/or repress target gene transcription. To this end, our lab has coupled a novel allele of Tbx20 generated in our lab, $Tbx20^{AVI}$, with a directed proteomic-based approach to identify proteins that function in association with Tbx20. From these studies, we identified Castor (Casz1), a zinc-finger transcription factor essential for cardiac development. We have gone on to demonstrate a biochemical interaction between Tbx20 and Casz1, and to test for genetic interaction, we generated a conditional allele of Casz1 and conducted genetic complementation analyses. From these studies we have shown that mice double heterozygous for both Tbx20 and Casz1, $Tbx20^{+/-}$, $Casz1^{+/-}$, display cardiomyopathy and a subset die within the first 8 weeks of life displaying systolic dysfunction, enlarged cardiomyocytes and interstitial fibrosis. Collectively, these studies define a requirement for the Tbx20-Casz1 interaction in the mammalian myocardium.

5 Jinhu Wang, Duke

Epicardial regeneration is guided by cardiac outflow tract and Hh signaling.

In response to cardiac damage, the epicardium is activated to proliferate and accumulate at the injury site. Recent studies have implicated the epicardium in multiple aspects of cardiac repair: a source of paracrine signals for cardiomyocyte survival or proliferation; a supply of perivascular cells and possibly other cell types like cardiomyocytes; and, a mediator of inflammation. Yet, the biology and dynamism of the adult epicardium is poorly understood. Here, we created a transgenic line to ablate this cell population in adult zebrafish. We find that genetic depletion of epicardium after myocardial loss inhibits cardiomyocyte proliferation and delays muscle regeneration. The epicardium vigorously regenerates after its ablation, through proliferation and migration of spared epicardial cells as a sheet to cover the exposed ventricular surface in a wave from the chamber base toward its apex. By reconstituting epicardial regeneration ex vivo, we show that extirpation of the bulbous arteriosus (BA), prevents epicardial regeneration. Conversely, experimental repositioning of the BA by tissue recombination initiates epicardial regeneration and can govern its direction. Hedgehog (Hh) ligand is expressed in the BA, and treatment with Hh signaling antagonist arrests epicardial regeneration and blunts the epicardial response to muscle injury. Transplantation of Shh-soaked beads at the ventricular base stimulates epicardial regeneration after BA removal, indicating that Hh signaling can substitute for the BA influence. Thus, the ventricular epicardium has pronounced regenerative capacity, regulated by the neighboring cardiac outflow tract and Hh signaling.

6 Jessica Nesmith, UNC

FLT1 Regulation of Blood Vessel Anastomosis

Jessica Nesmith, Julia Cluceru, John Chappell, Feilim Mac Gabhann, Victoria Bautch

Angiogenesis is the main process that generates new blood vessels in physiological and pathological tissue growth. Angiogenesis is implicated in disease progression and treatment in many of the major cardiac and lung diseases, as well as in nearly all solid tumors. Anti-angiogenic therapy targeted to vascular endothelial growth factor (VEGF) is of limited efficacy, potentially due to incomplete understanding of VEGF signaling, the major growth factor in angiogenesis. Thus further investigation of VEGF signaling during angiogenesis is warranted. Anastomosis, wherein two blood vessels form a new conduit in the vessel network, is a critical aspect of angiogenesis about which the regulatory signaling is not well understood.

VEGF-A and its receptor VEGFR1/FLT1 are of particular interest during anastomosis as manipulation of FLT1 prevents effective connection. Active selection of connection sites after sampling by the growing vessel indicates a FLT1/VEGF signaling dependent selectivity for anastomosis. Additional data demonstrate that connection sites are biased away from strong FLT1 expression and that without FLT1 expression new connections are not maintained in the vessel network. Therefore, FLT1 plays a role in regulating anastomosis through restricting potential connection sites and maintaining new connections during angiogenesis. These data not only also progress our understanding of the regulatory signaling involved in anastomosis but are the first to report a pre-anastomotic sampling behavior. Elucidating VEGF/FLT1 signaling during anastomosis could provide a new focus for therapies that selectively inhibit new vessel sprouting while sparing stable vascular beds.

7 Michael O'Connell, NC State

Background subtraction via nuclear Cactus increases the signal-to-noise ratio of the Dorsal gradient

Michael D. O'Connell, Gregory T. Reeves

Dorsoventral (DV) axis patterning in the early *Drosophila* embryo is controlled by transcription factor Dorsal (dl). A homologue of the mammalian transcription factor NF-kB, dl is inhibited from regulating gene expression by the inhibitor protein Cactus (Cact). Signaling through the Toll receptor along the ventral side of the embryo causes Cact degradation and nuclear uptake of unbound dl protein. Once dl becomes localized to the nucleus, it can promote or inhibit its target genes in a concentration-dependent fashion, including *Snail (sna)*, *Ventral Neuroblasts Deffective (vnd), Short Gastrulation (sog)*, and *Decapentaplegic (dpp)*.

The working model of dl gradient formation has changed very little since its discovery in the late 1980s, despite tremendous advances in our quantitative understanding of the dynamics involved. We recently published a new model of dl/Cact dynamics that incorporates the trapping of both dl and Cact by the reforming, post-mitotic nuclear envelopes that shows excellent agreement with the results of live imaging published by Reeves et al. (2012). More importantly, our model suggested that Cact's presence in the nuclei increases the range of dl activity from that measured by fluorescence microscopy, explaining how dl can pattern genes on the lateral side of the embryo such as *sog* and *dpp*.

Based on our analysis of noise in the model, we hypothesize that nuclear Cact acts as a background subtractor to increase the dl gradient's signal-to-noise ratio. This is especially important in regions where the gradient is seemingly too shallow for nuclei to reliably determine their proper location along the DV axis. Using our *in silico* model of gene expression, we can show how Cact minimizes the negative effects of extrinsic noise by increasing the relative difference between neighboring nuclei. Our modeling results suggest that performing a background subtraction operation may be one mechanism by which organisms maintain low-level control over concentration-dependent gene expression.

8 Melissa Pickett, NC State

A Novel Non-Neuronal Role of Acetylcholinesterase in Intestinal Development

Melissa A. Pickett, Nanette Nascone-Yoder

Acetylcholinesterase (AChE) is a highly conserved protein well studied for its role in terminating nervous signaling through degradation of the neurotransmitter, acetylcholine. However, a growing body of evidence suggests AChE also has non-neuronal activities associated with cell adhesion and polarized cell migration, cell behaviors that underlie much of embryogenesis. Consistent with a non-neuronal role in embryonic development, we detected AChE within the non-innervated endoderm cells of the Xenopus embryo that rearrange to lengthen the embryonic gut and form the epithelial lining of the intestine. Exposing embryos to chemical AChE inhibitors, or knocking down endoderm AChE via antisense agents (morpholino), resulted in shortened guts with disrupted cell polarity, decreased expression of adhesion molecules, and failure of the endoderm to differentiate and rearrange into a single epithelial layer. To better define the mechanism(s) by which AChE promotes adhesion and migration during development, we isolated gut endoderm cells and performed ex vivo cell-cell and cell-substrate adhesion assays. Cell-cell and cell-substrate adhesion were unaffected by loss of AChE activity. However, cell protrusive activity and movement were reduced when AChE was inhibited. This suggests that AChE may be required for cell polarization, but not adhesion during gut development. This work reveals a previously unrecognized role for a neurotransmitter hydrolase in coordinating polarized cell rearrangement during organ morphogenesis.

9 Anne-Marie Ladouceur, UNC

Chromosome length scaling to cell size

During mitosis, the longest chromosome arm must be shorter than half of the mitotic spindle for proper chromosome segregation. Multicellular development requires that cells reduce in size due to consecutive cell divisions without increase in embryo volume. To maintain cellular integrity, organelles adapt to cell size throughout development. Using high-resolution time-lapse microscopy of living C. elegans embryos, we previously showed that chromosome length predictably scaled through cell size and nuclear size. In order to identify regulators of chromosome scaling, we performed a large-scale screen in C. elegans using a RNAi sub-library containing 438 genes known or predicted to be chromatin binding and/or modifying enzymes. We used animals with a telomeric fusion between the 2 longest chromosomes resulting in an abnormally long chromosome without changing total amount of DNA. We hypothesized those worms would be more susceptible to any defect in chromosome scaling compared to control. The screen identified known protein required for assembling and/or regulating the mitotic chromosome scaffold. We are currently testing if the scaffold acts as a limiting component where elevated amounts of scaffold protein restricts axial chromosome compaction (more inhibitor in larger cells) or if the scaffold contains condensation factors required to generate smaller chromosomes (more condensation factor in the smaller cells). In summary, we are using large-scale RNAi depletion and high resolution imaging to determine the mechanisms of mitotic chromosome size regulation. Our research exploits the normal developmental context to study the cell biological problem of chromosome condensation.

10 Jingli Cao, Duke

Ex vivo approaches to study epicardial regeneration in zebrafish

Jingli Cao, Jinhu Wang, Amy Dickson, Kenneth Poss

By understanding heart regeneration in zebrafish, we intend to learn how to stimulate a similar process in mammalian species like humans with poor cardiac regeneration. The epicardium is a multifunctional cell layer covering the heart that is critical for cardiac development and repair. Following cardiac injury in zebrafish, the epicardium proliferates and contributes key perivascular cells and paracrine signals to enable new muscle regeneration. Despite its importance in cardiac repair and its potential as a therapeutic target for heart disease, there is virtually nothing known about the regenerative capacity of the epicardium itself. In this study, we developed an ex vivo explant culture system, in which the dissected heart contracted for weeks. Together with a transgenic line to ablate epicardial cells in adult zebrafish, we could monitor epicardial regeneration in real-time through live imaging, and found a directed regeneration of the epicardial cell sheet from ventricle base to apex. We also manipulated epicardial regeneration ex vivo by extirpating or graft a chamber or tissue in heart explant, or transplantation of epicardial cells from another heart. These manipulations confirmed that ventricular epicardial regeneration was regulated by the neighboring tissue-bulbous arteriosus (BA). The ex vivo approach enables high-resolution phenotyping of epicardial regeneration in molecular lever. Through chemical screen, we found several signling pathways that could possibly regulate epicardial regeneration and Hedgehog (Hh) signaling pathway is one of the key players. The ex vivo system deepened our understanding of epicardial regeneration in both cellular and molecular level, will lead to more findings.

11 Erin Sparks, Duke

A Root-Enriched Transcriptional Network Uncover Novel Regulation of SHORTROOT and SCARECROW Expression in Arabidopsis

Erin E. Sparks, Colleen E. Drapek, Allison Gaudinier, Song Li, Mitra Ansariola, Ning Shen, Yongjian Qiu, Musoki Mwimba, Gina Turco, Jalean J. Petricka, Jessica Foret, Xinnian Dong, Meng Chen, Raluca Gordân, Molly MeGraw, Siobhan M. Brady, and Philip N. Benfey

Tissue-specific expression of transcription factors is central to the regulation of cell fate and function in all multicellular organisms. In the *Arabidopsis thaliana* root two transcription factors (TFs), *SHORTROOT* (*SHR*) and *SCARECROW* (*SCR*), are required for endodermal development. Both TFs show tissue-specific expression patterns, with *SHR* transcribed in the central vasculature and *SCR* transcribed in the endodermis. SHR protein moves from the vasculature into the endodermis to interact with SCR and initiate a feedback loop on SCR expression and promote division of the cortex-endodermal stem cell daughter into cortex and endodermal cell fates. This signaling module is one of the best studied, however outstanding questions remain. Specifically, it is unknown how the low-levels of SCR, required for feedback initiation, are established. Further, no upstream regulators of SHR expression have been identified. To address these questions, we generated a transcriptional gene regulatory network using enhanced Yeast-1-Hybrid assays to screen the promoters of 111 TFs against a library of 555 TFs. The resulting gene regulatory network of 871 interactions between 267 TFs provides a resource through which we can address questions of transcriptional regulation.

We validated sub-networks from the broader gene regulatory network to ask how the expression patterns of *SHR* and *SCR* are generated. Interactions were validated *in planta* by assaying *SHR* and *SCR* expression in mutant or over-expression lines of upstream TFs by whole root qRT-PCR. Our results suggest that the tissue-specific expression patterns of *SHR* and *SCR* are established through two different regulatory mechanisms. For *SCR*, several activators are expressed at low levels across multiple cells types. We propose that these lowly expressed TFs provide the platform to initiate feedback up-regulation in conjunction with SHR. In contrast, *SHR* expression is established through a combination of broadly expressed activators and specifically expressed repressors. These results highlight two different mechanisms by which combinatorial TF regulation is utilized to generate tissue specific expression patterns.

12 Nicholas Gomez, UNC

The chromatin landscape of stem cells confers a permissive environment for cancer development

Nick Gomez, Ian Davis

*Abstract not available online

13 Colleen Drapek, Duke

Defining Novel Differentiation Networks in the Arabidopsis Root

Colleen Drapek, Erin Sparks, Louisa Liberman, Philip Benfey

A cell's trajectory from stem cell to differentiation, while often portrayed as a linear progression, is best described as a network that produces a mature state through several pathways acting together. There are few examples that describe gene regulatory network changes during the entire trajectory of cell differentiation. The goal of my project is to define a gene regulatory network containing molecular components required for a stem cell to become a differentiated cell in the *Arabidopsis thaliana* root. The root is a powerful model for identifying basic principles of differentiation. Plant cells do not migrate therefore entire lineages from stem cell to mature progeny are spatially confined. Furthermore, the root displays indeterminate growth, facilitating the study of many different developmental stages at a single time. One cell type of the root, the endodermis, is particularly suitable for generating a regulatory network because the molecular components required for its formation and differentiation are established. The transcription factors SHORTROOT (SHR) and SCARECROW (SCR) have important roles in endodermal specification and development. SHR and SCR act to promote development of a cell wall structure called the Casparian strip, which is the hallmark of endodermal differentiation. Here, I present my work defining the regulatory network between SHR, SCR and a MYB-related transcription factor in promoting endodermal differentiation. The arrangement of the identified gene regulatory network will provide mechanistic information of genetic interactions that drive differentiation.

14 Chen-Hui Chen, Duke

Large-scale surveillance of epithelial cell dynamics during tissue regeneration

Chen-Hui Chen, Alberto Puliafito, Luca Primo, Yi Fang, Stefano Di Talia, Kenneth D. Poss

*Abstract not available online

15 Mandy Womble, NC State

Pitx2c mediates asymmetrical development of the hepatobiliary system

Mandy Womble, Nanette Nascone-Yoder

The hepatobiliary system is left-right asymmetric; however, the mechanisms of left-right asymmetrical hepatobiliary development are unknown. Pitx2c, a homeobox transcription factor, is expressed on the left side of the developing heart, lungs and gastrointestinal tract, and is required for proper asymmetrical morphology in these systems. Nevertheless, the role of Pitx2c in the left-right development of the hepatobiliary system remains unexplored. We found in Xenopus laevis that Pitx2c is expressed in the left mesoderm surrounding the left hepatic diverticulum and that ectopic, right-sided expression of Pitx2c perturbed liver asymmetry. We also discovered that the liver specific homeobox transcription factor, Hhex, has a broader expression domain on the right side of the animal and ectopic, right-sided expression of Pitx2c disrupted this expression. At the cellular level, we identified novel asymmetries in protein expression and epithelial cell shape between the left and right sides of the developing hepatobiliary diverticulum. Additionally, the left diverticulum has higher membrane expression of the cell-cell adhesion protein Î2-catenin. Furthermore, the left diverticulum remains a columnar epithelium while the right diverticulum undergoes pseudostratification. These left-sided differences in the epithelium occur in regions directly adjacent to Pitx2c expressing mesoderm cells. Taken together, these results show that asymmetrical Pitx2c expression is required for hepatobiliary asymmetry and suggests that Pitx2c orchestrates this asymmetry by regulating the expression of liver specification genes like Hhex and altering cellular level epithelial architecture. Our results advance our understanding of how asymmetries develop in all left-right asymmetric organs and give us insight into birth defects involving laterality.

16 Stefanie Denning, NC State

Dual Strategy for Characterizing foxq1b

Stefanie Denning, Antonio Planchart

Foxq1 is a transcription factor shown to be involved in gastric acid secretion, hair follicle formation, and craniofacial development in mammalian models. Foxq1 is overexpressed in cancer cells and contributes to the progression and metastasis of cancer. Since much is still unknown about Foxq1, the zebrafish homolog foxq1b is being analyzed. In order to gain a better understanding of its role in development and cancer, a myc-tag epitope has been engineered at the C-terminus of *foxq1b* to discover genes directly regulated by foxq1b through ChIP-Seq. To complement the knowledge we will gain through the use of myc-tag *foxq1b*, we will utilize CRISPR-Cas9 technology to observe phenotypic abnormalities in zebrafish due to *foxq1b* gene deletion at a critical time point in development.

17 Lara Linden, Duke

Germ cell-somatic cell interactions: a role for the germ cells in inducing niche and ectopic cellular enwrapment

Lara M. Linden, Qiuyi Chi, David R. Sherwood

The stem cell niche is a specialized microenvironment composed of support cells and extracellular matrix. Little is known about how stem cells influence the niche environment. In C. elegans, the germ stem cell niche is located at the distal end of the basement membrane-encased gonad and is composed of a single somatic cell, the distal tip cell (DTC), which extends a dense network of cytoplasmic processes that enwrap adjacent germ cells. Interestingly, somatic support cells also surround germ cells in fly and mouse gonads, suggesting this is a conserved specialized behavior of the niche environment. This enwrapping behavior is thought to anchor stem cells within the niche and possibly regulate niche signaling. How cellular enwrapping behavior is regulated is poorly understood. Through laser ablation studies, we have observed that DTC niche processes are reduced in germline-ablated animals, suggesting that the germ cells may actively signal to the niche. Utilizing live-cell imaging approaches, we have also found that following disruption of the basement membrane encasing the gonad, germ cells escape the gonad. Strikingly, we have observed that muscle cells extend dynamic processes that find and encircle ectopic germ cells, thus enwrapping the escaped germ cells in the body cavity in a manner similar to the normal niche environment. This muscle cell-germ cell interaction further supports the idea that a germline-expressed cue induces enwrapping behavior by somatic cells. To elucidate components of this germ cell to somatic cell signaling, we are conducting an RNAi-based screen to identify genes required for enwrapping behavior.

18 Sophia Tintori, UNC

Generating a transcriptional lineage of *C. elegans* development to identify regulators of morphogenesis

Sophia Tintori, Jason D Lieb, Bob Goldstein

We are creating a transcriptional lineage of early *C. elegans* development using single-cell RNA-seq on individual blastomeres over the course of the first several cell cycles of embryogenesis. We hope that information provided by our transcriptional lineage will be a powerful complement to the invariant cell lineage of *C. elegans* development. Knowing the full complement of RNA molecules in each cell during development will provide a window into the mechanism by which RNA molecules and transcriptional regulatory programs are inherited from cell to cell during development. We alsohope to use this transcriptional lineage to uncover genes that correlate with and are required for fundamental processes of development but that have not been identified through genetic approaches. Of particular interest are genes that regulate morphogenesis, in part because they have been particularly difficult to identify through genetic screens. Our transcriptional lineage will provide another avenue to finding these genes, which we will pursue by comparing transcriptional profiles over time in several different cell lineages that each engage in a similar motility behavior.

19 John Runge, UNC

Epigenetic Regulation by ATP-Dependent Chromatin Remodeling Enzymes: Snf-ing Out Remodeler Crosstalk

John S. Runge, Jesse Raab, Terry Magnuson

*Abstract not available online

20 Kendall Lough, UNC

The Role of Mllt4/Afadin in Establishing Polarity in Mammalian Epidermal Progenitors

Kendall Lough, Anthony Curtis, Kevin M. Byrd, Scott Williams

OBJECTIVE: Balancing asymmetric and symmetric divisions is an essential element of epidermal development and stratification. This process is regulated by the apical-cortical spindle orientation protein complex consisting of LGN-mInsc-Gai3, which ensures proper alignment of the mitotic spindle in asymmetric divisions. Additionally, the apical orientation of this complex is dependent on the polarity gene, Par3. In *Drosophila m.*, the adherens junction protein Canoe (*Cno*) has been demonstrated to participate in regulating this conserved process. The aim of this study is to determine the role of the mammalian *Cno* homolog, *Mllt4*, in regulating spindle orientation in the developing epidermis.

METHODS: Embryos were injected *in utero* at embryonic day 9.5 (E9.5) with a lentivirus containing RNAi constructs against *Mllt4* with a fluorescent H2B-RFP reporter. Backskins were harvested at E16.5, embedded in OCT and sectioned. Sections were labeled with a variety of fluorescent probes and imaged using a confocal or ground state depletion super-resolution microscope.

RESULTS: Knockdown of Mllt4/Afadin significantly reduced epidermal stratification and differentiation in E16.5 epidermis as determined by involucrin and K10 staining. Stratification defects correlated with errors in both spindle orientation and mislocalization of the spindle orientation protein, LGN. Additionally, loss of Mllt4 resulted in reduced cortical accumulation of the polarity gene, Par3.

CONCLUSIONS: Mammalian Mllt4 regulates stratification, differentiation, and spindle orientation of the developing murine epidermis, likely through its modulation of the polarity protein Par3.

21 Matthew Foglia, Duke

Clonal expansion and multi-chamber incorporation of atrial-specified cardiomyocytes in the developing zebrafish heart

Matthew Foglia, Ken Poss

During cardiac morphogenesis, the zebrafish heart changes in structure from a linear tube to a multi-chambered pump composed of one atrium and one ventricle. Embryonic cardiomyocytes whose progeny will contribute to the either chamber are specified early in development. How these populations change over time to produce chambers of distinct size and function is not well understood. To define the patterns of cardiomyocyte proliferation during atrial morphogenesis, we have traced the clonal progeny of embryonic atrial cardiomyocytes from embryogenesis to maturity using multicolor genetic labeling. Unlike ventricular cardiomyocytes, atrial cardiomyocytes undergo a morphologic change during juvenile development that generates a discontinuous, mesh-like structure that persists through maturity. Concomitantly, clonal populations of cardiomyocytes in the atrium expand unequally and, like the ventricle, dominant clones frequently emerge that comprise a disproportionate share of the wall surface. These dominant clones arise, however, through a different mechanism than those of the ventricular compact muscle. Interestingly, we find that interior pectinate muscle fibers, in contrast to the trabeculae of the ventricle, share a clonal relationship with the adjacent wall cardiomyocytes. We also find evidence that a subpopulation of transiently amhc+ cardiomyocytes ultimately contribute to ventricle, providing evidence of re-programming of cardiomyocyte chamber specification in the absence of injury. These experiments stand to shed light on a poorly understood aspect of chamber morphogenesis and provide a foundation for interpreting molecular mechanisms of heart development.

22 Elizabeth Cook, NC State

Leflunomide, an Anti-Rheumatic Drug, Interferes with the Dopamine Synthesis Pathway of Developing Zebrafish

Elizabeth Cook, Carson Lunsford, Carolyn Mattingly, Antonio Planchart

Leflunomide is a drug most commonly used to treat rheumatoid arthritis (RA). Leflunomide™s metabolite, teriflunomide, produces a therapeutic effect by inhibiting dihydroorotate dehydrogenase (DHODH), an enzyme involved in de novo pyrimidine biosynthesis. Leflunomide has also been shown to activate the aryl hydrocarbon receptor (AhR), a receptor that is involved in drug detoxification. Studies have shown that when exposed to leflunomide, zebrafish lack normal levels of melanocyte-derived pigment, which is made from tyrosine. Dopamine, an important neurotransmitter, is also derived from tyrosine. With this knowledge, we hypothesized that leflunomide targets dopamine biosynthesis. To test this, zebrafish were exposed at 4 hours post fertilization to between 250nM and 2.5ÂµM leflunomide and the transcript levels of enzymes involved in the dopamine synthesis pathway were measured. Tyrosine hydroxylase (th), the enzyme catalyzing the initial step of dopamine synthesis, was downregulated after exposure to leflunomide. Tyrosinase, an enzyme that links the melanin and dopamine biosynthesis pathways, was also downregulated. The swimming patterns of the larvae were analyzed after continuous exposure at 5 days post fertilization and it was found that larvae exposed to leflunomide were less active. These data offer evidence that leflunomide affects dopamine synthesis and signaling. Further work will be done to investigate leflunomide™s mechanism of action and possible long-term effects of developmental alterations in dopaminergic biology.

23 Debashish Menon, UNC

Germ Cell Epigenetics: Functions of Chromatin Remodeling in Spermatogenesis

Debashish U. Menon, Terry R. Magnuson

*Abstract not available online

24 Daniel Serber, UNC

INO80 Chromatin Remodeling Activity is Required for Meiotic Progression

Daniel Serber, Terry Magnuson

The ability to faithfully transmit genetic material across generations via the germ line is a critical aspect of mammalian reproduction. Gametogenesis requires a number of large-scale modulations of the chromatin dynamic within the nucleus. One such occasion arises during meiotic recombination, when hundreds of DNA double strand breaks are induced and subsequently repaired, enabling the transfer of genetic information between homologous chromosomes. The inability to properly repair DNA damage is known to lead to an arrest in the developing germ cells and sterility within the animal. Chromatin remodeling activity, and in particular the BRG1 subunit of the SWI/SNF complex, has been shown to be required for successful completion of meiosis. Little is known, however, regarding the contribution of other families of chromatin remodeling complexes. One particularly interesting candidate is the INO80 complex, which has well described functions during DNA double strand break repair. Here we show that INO80 is present in developing spermatocytes during the early stages of meiotic prophase I. Based on this information, we used a conditional allele to delete the INO80 core ATPase subunit, eliminating INO80 chromatin remodeling activity in this lineage. INO80 loss results in an arrest during meiosis associated with a failure to repair the DNA damages associated with meiotic recombination. These data provide further evidence for the importance of chromatin remodeling activity during meiotic recombination and implicate the INO80 complex as essential to this process.

25 Tracy Clement, NIEHS

Actl7b is Associated with the Golgi Derived Developing Acrosome and Required for Acrosome Attachment, Spermatid Morphogenesis, and Fertility

Tracy Clement, Chris Geyer, Garrett Warren, William Willis, Eugenia Goulding, Mitch Eddy

Human male infertility is associated with a high incidence of abnormally shaped sperm heads, suggesting that cytoskeletal regulation may be important for male fertility. Involvement of F-actin has been suggested for several aspects of spermatid differentiation, including acrosome formation and attachment to the nucleus. Although structural components and morphological changes associated with spermiogenesis have been described in detail, relatively little is known about mechanisms that drive structural changes. Actin-like 7b (Actl7b) is an orphan actin related protein family member that we have shown is required for spermatid development and fertility. ACTL7B co-localizes with F-actin in or around the forming acrosome in mouse and human spermatids. Actl7b knockout male mice are infertile due to severe and oligoasthenoteratozoospermia. Electron microscopy observations reveal that the primary defects are disruption of acrosome matrix localization and acrosomal attachment in early spermiogenesis. To determine the mechanism of action of ACTL7B, protein structure, overexpression, and protein associations are being investigated. ACTL7B has a conserved actin domain with striking sequence and predicted structural similarity to beta-actin. ACTL7B also has a unique N-terminal 60aa sequence. Overexpressed GFP-ACTL7B fusion protein is localized to the Golgi in COS-7 cells. Together our data indicate 1. Actl7b is required for spermatid morphogenesis and male fertility, 2. ACTL7B is localized to the Golgi-derived acrosome, likely by its unique N-terminal domain, and 3. ACTL7B is either an acrosomal F-actin or required for proper spermatid F-actin assembly. This research was supported by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences.

26 Lydia Smith, UNC

Centromeric epigenetic regulation post terminal, non-proliferative, differentiation

*Abstract not available online

27 Adam Gracz, UNC

In vitro interrogation of the intestinal stem cell niche with microaft arrays

Gracz AD, Williamson IA, Roche KR, Johnston MH, Wang F, Wang Y, Attayek PJ, Balowski J, Liu XF, Laurenza RJ, Gaynor LT, Sims CE, Galanko JA, Li L, Allbritton NL, Magness ST

Intestinal stem cells (ISCs) undergo rapid rates of proliferation and differentiation in order to maintain the intestinal epithelium, which is renewed approximately every 5-7 days in adult mammals. ISCs respond to local environmental cues from the stem cell niche, which is composed of multiple cell types that support and direct stemness in the intestine. While the development of elegant genetic mouse models has advanced our understanding of ISC-niche interactions, the ability to reconstruct and interrogate niches *in vitro* offers a powerful complementary approach to *in vivo* niche studies. Here we utilize a cell culture microarray platform, termed microraft arrays (MRAs), to functionally and genetically probe interactions between ISCs and niche cells *in vitro*. MRAs facilitate high-throughput screening of niches reconstituted *in vitro*, and allow us to examine the impact of Paneth cell number and cell-cell contact on single ISCs. MRAs also allow for retrieval of *in vitro* niches for downstream gene expression analysis, combining functional and genetic screening capabilities in a single platform with cross-compatibility between many cell and tissue types.

28 Abubakr Ziaullah, UNC

The Tbx20-Casz1 Interaction is Required for Normal Cardiac Function

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T-box transcription factors are a highly conserved family of genes with vital morphogenic roles in heart development. Mutations in a number of T-box genes lead to human disorders such as *TBX3*, which leads to ulnar-mammary syndrome, *TBX5*, which leads to Holt-Oram syndrome and *TBX20*, which leads to Tetralogy of Fallot. Specifically, loss of *Tbx20* has also been shown to be crucial for heart development in several models in which *Tbx20* is ablated. To identify transcriptional complexes required for normal heart development, and to understand how these complexes accomplish their functions in the heart, we performed liquid chromatography and mass spectrometry analysis on isolated Tbx20 complexes. Using this method, we identified an association between Tbx20 and the cardiac transcription factor Casz1. Similar to the defects observed in Tbx20 mutant mice, cardiomyocyte-specific loss of Casz1 leads to cardiomyocyte proliferation defects and embryonic lethality. To identify a genetic requirement for the interaction between *Tbx20* and *Casz1*, we generated compound heterozygous floxed *Tbx20* and *Casz1* mice (*Tbx20*$^{flox/+}$; *Casz1*$^{flox/+}$), with cardiomyocyte-specific depletion at both loci mediated by Nkx2-5^{Cre}. Cardiomyocyte-specific loss of this interaction leads systolic dysfunction and lethality. These mice also exhibited dilated cardiomyopathy, interstitial myocardial fibrosis, and cardiomyocyte hypertrophy. Combined, heterozygous loss of both *Tbx20* and *Casz1* leads to defects that establish a critical role for the interaction between these two cardiac transcription factors in the heart.

29 Reema Davis, UNC

Adrenomedullin Signaling in Lymphatics

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30 Stephen Klusza, UNC Chapel Hill

Engineering a Drosophila histone mutation reveals distinct roles for PR-Set7 and H4K20 methylation

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In mammals, perturbation of function of the H4K20 mono-methyltransferase PR-Set7 produce defects in DNA replication, cell-cycle progression, and chromosome condensation, which results in lethality and is thought to be a direct result of loss of H4K20 methylation. In Drosophila, PR-Set7-null flies do not complete development into adulthood and loss of H4K20 methylation is also seen at the stage of lethality, suggesting that H4K20 methylation is essential for viability in Drosophila as well. Surprisingly, we found that engineered flies in which H4K20 mono-methylation is abolished (H4K20A) can develop to adulthood, indicating that H4K20 methylation is not essential for the completion of Drosophila development. In the absence of detectable H4K20me1, cells undergo cell-cycle progression and actively replicate DNA, as observed with a cytological marker of Cyclin E/Cdk2 activity during S-phase and robust incorporation of the nucleotide analog EdU in Drosophila egg chambers. These results suggest that PR-Set7 may have additional non-histone substrate targets that confer lethality to Drosophila, which is consistent with the finding of multiple PR-Set7 non-histone substrates in mammalian systems. This is further supported by the finding that a viable, hypomorphic combination of Drosophila PR-Set7 alleles retain H4K20 mono-methylation by immunofluorescence analysis but display defects in eye development that are not seen in H4K20A mutant flies. These data suggest that the essential role for PR-Set7 during Drosophila development is not H4K20 methylation, and we are continuing our comparison of PR-Set7 and H4K20A mutant phenotypes in order to further explore this hypothesis.

31 Casey Schmidt, UNC

Investigation of tRNA intronic circular (tric)RNA biogenesis

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Non-coding (nc)RNAs comprise nearly three quarters of the human genome. These molecules perform a myriad of functions in the cell, including regulation of gene expression. Of particular importance are the transfer (t)RNAs, which assist with translation of a nucleic acid message into a polypeptide. Mature tRNAs are generated from several processing steps, which can include removal of introns. Recently, our lab discovered a new class of ncRNA formed from the circularization of excised tRNA introns in Drosophila melanogaster. We term these molecules tRNA intronic circular (tric)RNAs. We have found tricRNAs to be conserved among insects, including Drosophilids, worms, and mosquitos. This conservation suggests that tricRNAs may have a function in the cell. Understanding how and where tricRNAs are made may provide a springboard for future functional studies. To study tricRNA biogenesis, we have generated a reporter that uses the Broccoli fluorescent RNA aptamer system. We replaced the majority of the intron sequence of two Drosophila tRNA genes with the Broccoli sequence while retaining the intron sequences necessary to form a bulge-helix-bulge motif, which is required for proper tRNA splicing. We will use this reporter to study the cis elements and trans factors necessary for proper tricRNA formation. For the cis elements, we will carry out in vitro tRNA splicing experiments using altered versions of the reporter to determine which sequence and structural elements result in proper or optimized production of tricRNAs. For the trans factors, we will identify fly orthologs of the tRNA splicing machinery, knock down these factors in Drosophila cell lines, and use these cell extracts for in vitro splicing assays with the Broccoli reporter. These studies will provide insight into a new field and will inform future investigations into tricRNA function.

32 Joseph Pearson, UNC

Chromatin accessibility in Drosophila CNS midline identifies spatially and temporally-specific enhancers

Drosophila embryogenesis occurs in less than 24 hours, requiring precise, tissue-specific expression that changes rapidly. This expression is controlled by one or more enhancers near each gene to control expression. We used the Drosophila CNS midline as a system to study how chromatin accessibility reflects tissue-specific enhancer and gene expression activity. Nucleosome-poor chromatin isolated from FACS-isolated midline cells primarily reflected accessibility from whole-embryo samples, but many regions were selectively accessible. By comparing these regions to large collections of embryonic enhancer annotation projects, we found that selectively open chromatin reliably indicated midline-specific enhancer activity. Despite the relative simplicity of the midline tissue, multiple enhancers were routinely concurrently active at a given midline-expressed gene, but were not simply acting as "shadow enhancers". A large number of identified midline-active enhancers did not correlate with chromatin accessibility, but these tended to be isolated from genomic regions with no obvious midline-specific gene target. This raises the possibility that genomic sequences with potential enhancer activity are scattered throughout the genome, but are kept from exerting gene regulatory influence by genome or chromatin context.

33 Junsu Kang, Duke

Modulation of tissue repair by regeneration enhancer elements

Junsu Kang, Ravi Karra, Amy Dickson, Kenneth Poss

Although regeneration has been studied for centuries, how regeneration programs are triggered by injury remains poorly understood. To address this, we examined whether dedicated enhancer regulatory elements are engaged in regenerating tissue. By comparing transcriptomes from adult zebrafish fins and heart, we found that the leptin orthologue lepb is strongly induced during regeneration. Chromatin profiling identified a 1.3 kb region distantly localized from lepb that shows an open conformation features during regeneration. We found that this element can drive regeneration-dependent expression from minimal promoters. Extensive transgenic assays demonstrate that this regulatory sequence is divisible into distinct heart and fin regeneration enhancer modules. This lepb-linked enhancer (LEN) placed upstream of fgf20a enables normal development and is sufficient to rescue fin regeneration defects caused by fgf20a mutations. Conversely, fin regeneration is potently blocked in animals harboring a LEN upstream of a dominant-negative Fgf receptor. A LEN can also boost levels of the cardiomyocyte mitogen Neuregulin1 in cardiac injury sites, improving heart regeneration. Thus we have identified bona fide tissue regeneration-enhancer elements (TREEs) that trigger gene expression upon injury and can be engineered to modulate the regenerative potential of vertebrate tissues.

34 Jaime Brozowski, UNC

Regulation of hematopoietic and mesenchymal stem cell receptor signaling via G protein-coupled receptor kinase 3 (GRK3) impacts stem cell functions and transplantation

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35 Joy Meserve, UNC

The *Drosophila* retina: A model for cell cycle control during development and regeneration

Joy H. Meserve, Robert J. Duronio

The developing *Drosophila* retina has been used for decades as an exquisite experimental model for cell cycle regulation. The larval eye imaginal disc contains cell populations in various stages of the cell cycle, including both undifferentiated and differentiated quiescent cells. These cells are considered quiescent as they do not undergo DNA replication or mitosis, but it is unclear how reversible this quiescence is and how this might relate to development and disease. Since undifferentiated cells in the eye disc enter a synchronous round of S-phase prior to becoming quiescent, researchers have long assumed these cells all undergo division and arrest in G_1. Surprisingly, we have recently discovered that many of these undifferentiated cells do not undergo mitosis following S-phase and actually arrest in G_2, based on cell cycle markers and flow cytometric analysis. We are currently testing the hypothesis that this newly discovered G_2-arrested cell population may behave similarly to a G_2-arrested population in the developing wing, which contributes to the bristle cell lineage. We are also investigating how undifferentiated cells re-enter the cell cycle and undergo compensatory proliferation in response to tissue damage. A genetic screen from our lab has identified the transcription factor Scalloped (Sd) as an essential regulator of compensatory proliferation in the quiescent eye disc. We have found that Sd and Yorkie (Yki), the transcriptional co-activator of the Hippo signaling pathway, drive induction of Cyclin E following tissue damage. Additional work from our lab has led to a model in which basal extrusion of apoptotic cells increases cellular tension in surrounding cells, leading to inhibition of the Hippo pathway through the LIM protein Ajuba. Inhibition of Hippo signaling leads to activation of Yki, induction of Cyclin E, and S-phase entry. Initial results suggest that only G1-arrested undifferentiated cells, and not those arrested in G2, re-enter S-phase. Together, our work indicates that undifferentiated cells are not as homogenous as previously assumed and that differences in cell cycle state likely lead to differing potentials for cell cycle entry and differentiation.

36 Max Boeck, UNC

Competition Chip of CBF-1 in yeast reveals transcription factor dynamics

Cells must be able to respond to their environment both at the single cell level and across a whole organism. During development these changes can be dictated as responses to external cell-to-cell signaling, leading to distinct cell-fate decisions. The ability to respond to these environmental signals can lead to whole-cell changes in RNA and protein levels. How these changes manifest themselves are in part dictated by genome-wide changes in gene regulation and protein-DNA binding. If we can better understand genome-wide reprograming of these interactions, we can better predict developmental trajectories. We used competition ChIP-seq (cChIP-seq) of the yeast transcription factor CBF1 in rich media and phosphate depleted media as a model of how protein binding changes genome-wide in response to environmental stimuli. cChIP-seq is a novel method for measuring the dynamics of genome-wide protein binding (residency) as compared to static measurements (occupancy). CBF1 is known to play a role in regulating phosphate metabolism through its regulation of dependent genes. We combined this analysis with comparisons to previously generated measurements of PHO4 binding, a known competitor for binding sites of CBF1. These measurements demonstrate the utility of cChIP-seq in understanding the changes of genome-wide DNA-protein binding dynamics.