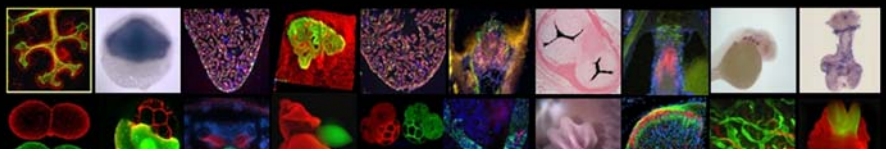
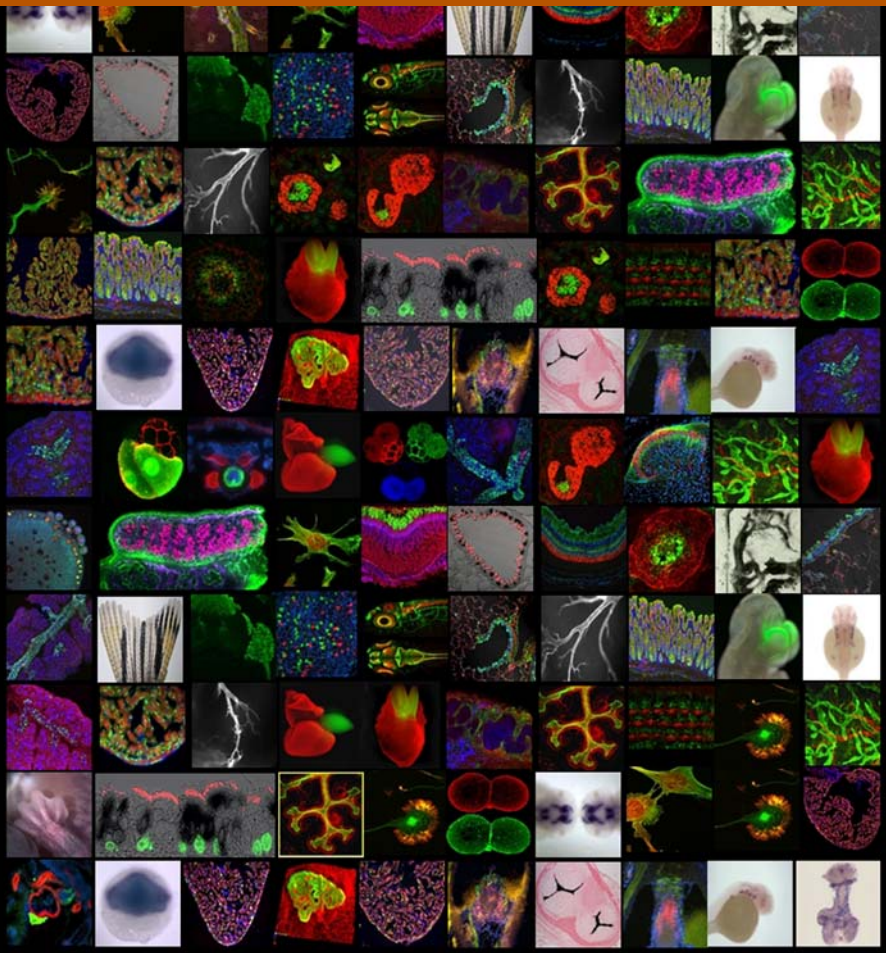
Abstracts of papers presented
at the 2010 meeting on

VERTEBRATE ORGANOGENESIS

April 27–May 1, 2010





Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

Abstracts of papers presented
at the 2010 meeting on

VERTEBRATE ORGANOGENESIS

April 27–May 1, 2010

Arranged by

Jonathan Epstein, *University of Pennsylvania School of Medicine*
Brigid Hogan, *Duke University Medical Center*
Guillermo Oliver, *St. Jude Children's Research Hospital*

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

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Front cover: Montage of images related to the theme of the meeting. Assembled by Dr Tim Oliver, Department of Cell Biology, Duke University Medical Center.

VERTEBRATE ORGANOGENESIS

Tuesday, April 27 – Saturday, May 1, 2010

Tuesday	7:30 pm	**1** Axis Formation
Wednesday	9:00 am	**2** Development of the Nervous System
Wednesday	2:00 pm	**3** Poster Session I
Wednesday	4:30 pm	Wine and Cheese Party*
Wednesday	7:00 pm	**4** The Vascular System in Health and Disease
Thursday	9:00 am	**5** Epithelial Organ Morphogenesis
Thursday	2:00 pm	**6** Poster Session II
Thursday	7:30 pm	**7** Developmental Mechanisms of Heart Development
Friday	9:00 am	**8** Myopathies and Muscloskeletal Formation
Friday	2:00 pm	**9** Developmental Origin of Genito-Urinary Defects
Friday	6:00 pm	Banquet
Saturday	9:00 am	**10** Endodermal-derived Organs, Diabetes and Cancer

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Poster sessions are located in *Bush Lecture Hall*

**Airslie Lawn,* weather permitting

Mealtimes at Blackford Hall are as follows:

Breakfast 7:30 am-9:00 am

Lunch 11:30 am-1:30 pm

Dinner 5:30 pm-7:00 pm

Bar is open from 5:00 pm until late

Printed on 100% recycled paper.

PROGRAM

TUESDAY, April 27—7:30 PM

SESSION 1 AXIS FORMATION

Chairperson: **B. Hogan,** Duke University, Durham, North Carolina

SESSION 2 DEVELOPMENT OF THE NERVOUS SYSTEM

Chairperson: **G. Oliver,** St. Jude Children's Research Hospital, Memphis, Tennessee

SESSION 3 POSTER SESSION I

WEDNESDAY, April 28—4:30 PM

Wine and Cheese Party

WEDNESDAY, April 28—7:00 PM

SESSION 4 THE VASCULAR SYSTEM IN HEALTH AND DISEASE

Chairperson: **L. Iruela-Arispe,** University of California, Los Angeles

THURSDAY, April 29—9:00 AM

SESSION 5 EPITHELIAL ORGAN MORPHOGENESIS

Chairperson: **S. Pfaff,** The Salk Institute, La Jolla, California

THURSDAY, April 29—2:00 PM

SESSION 6 POSTER SESSION II

THURSDAY, April 29—7:30 PM

SESSION 7 DEVELOPMENTAL MECHANISMS OF HEART DEVELOPMENT

Chairperson: **J. Epstein,** University of Pennsylvania School of Medicine, Philadelphia

BMP-mediated inhibition of FGF signaling lies at the heart of differentiation
Eldad Tzahor.
Presenter affiliation: Weizmann Institute of Science, Rehovot, Israel. 104

FRIDAY, April 30—9:00 AM

SESSION 8 MYOPATHIES AND MUSCOSKELETAL FORMATION

Chairperson: **G. Karsenty,** Columbia University, New York, New York

Embryo patterning the vertebrate axis
Olivier O. Pourquie.
Presenter affiliation: IGBMC, Illkirch, France. 105

Sonic hedgehog—A new player in temporal control of somite formation
Tatiana P. Resende, Mónica Ferreira, Marie-Aimée Teillet, Ana T. Tavares, Raquel P. Andrade, Isabel Palmeirim.
Presenter affiliation: Life and Health Sciences Research Institute (ICVS) Braga, Portugal. 106

Regulation of muscle stem cell fate by *Pax* genes
Margaret Buckingham, Mounia Lagha, Sylvia Brunelli, Takahiko Sato, Tsutomu Kume, Frederic Relaix.
Presenter affiliation: Institut Pasteur, Paris, France. 107

Pleiotropic actions of Wnt during muscle development, postnatal myogenesis and aging
Thomas A. Rando.
Presenter affiliation: Stanford University, Stanford, California. 108

Recruitment and maintenance of tendon progenitors by TGFβ signaling are essential for tendon formation
Brian A. Pryce, Spencer S. Watson, Nicole Dunker, Ronen Schweitzer.
Presenter affiliation: Shriners Hospital for Children, Portland, Oregon; Oregon Health and Science University, Portland, Oregon. 109

Skeleton as an endocrine organ
Gerard Karsenty, Paul A. Marks.
Presenter affiliation: Columbia University, New York, New York. 110

SESSION 9 DEVELOPMENTAL ORIGIN OF GENITO-URINARY DEFECTS

Chairperson: **A. McMahon,** Harvard University, Cambridge, Massachusetts

FRIDAY, April 30

BANQUET

Cocktails 6:00 PM Dinner 6:45 PM

SATURDAY, May 1—9:00 AM

SESSION 10 ENDODERMAL-DERIVED ORGANS, DIABETES AND CANCER

Chairperson: **K. Zaret,** University of Pennsylvania School of Medicine, Philadelphia

CILIA AND HEDGEHOG SIGNALING

Kathryn V Anderson, Karel Liem, Miquel Tuson, Polloneal J Ocbina

Sloan-Kettering Institute, Developmental Biology, 1275 York Ave, New York, NY, 10014

Patterning of many different vertebrate organs depends on Hedgehog signaling, and vertebrate Hedgehog signaling, in turn, depends on the primary cilium. To understand why the primary cilium is an appropriate venue for Shh signal transduction, we are investigating the relationships among cilia genes and between cilia genes and the core components of the Sonic hedgehog signal transduction pathway Protein kinase A (PKA) is a conserved negative regulator that acts at that step of the pathway. We find that embryos that lack all PKA catalytic activity show a very strong activation of the pathway, and that PKA activity depends on the presence of cilia. Costal2 is a negative regulator of the Shh pathway, and we find that the mouse homologue of Costal2, Kif7, has complex roles as both a negative and positive regulator of Shh signaling. Kif7 activity also depends on cilia, and the Kif7 protein appears to act as a motor within cilia. Finally, certain combinations of mutations in proteins that affect transport within the cilium partially restore normal Hedgehog signaling, suggesting that the balance of anterograde and retrograde trafficking within the cilium can control the level of signal output.

SPATIAL RESTRICTION OF BMP SIGNALLING IN MOUSE GASTRULA BY THE ENDOCYTIC PATHWAY

Yoh Wada[1], Ge-Hong Sun-Wada[2]

[1]Osaka University, ISIR, Mihogaoka 8-1, Ibaraki, 567-0047, Japan,
[2]Doshisha Woman's College, Faculty of Pharmaceutical Sciences, Kohdo, Kyotanabe, 610-0395, Japan

Embryonic body plan is established by positive and negative controls on various signalling cascades. Late endosomes and lysosomes, the late stages of the endocytic pathway are thought to terminate signal transduction by compartmentalising and degrading the signalling molecules, however, their regulatory roles in embryogenesis remains poorly understood. We focused on the endocytic membrane dynamics in the mouse embryos at the perigastrulation stages. The visceral endoderm, a single cell-layered tissue sustaining embryonic growth through nutrients/gas exchange and participating in the axis determination by orchestrating various signalling cascades, exhibits high activity of endocytosis. The apical vacuoles are large digestive compartments located to the apical cytoplasm, and their limiting membranes were positive for lysosomal and late endosomal marker proteins. We generated mutant mice defective in the assembly of apical vacuoles. The mutant embryos failed to differentiate neural ectoderm or embryonic mesoderm, but developed extraembryonic tissues including the allantois and chorion. The mutant embryos exhibited ectopic activation of BMP signalling whereas FGF- and Nodal-signalling remained regulated normally. These results showed that the late stage of endocytic pathway plays fundamental roles in the early embryogenesis by controlling the spatiotemporal pattern of BMP signalling.

PLANAR CELL POLARITY BREAKS THE BILATERAL SYMMETRY BY CONTROLLING CILIARY POSITIONING

Hai Song, Jianxin Hu, Wen Chen, Gene Elliott, Bo Gao , Yingzi Yang

Developmental Genetics Section, National Human Genome Research Institute, 49 Convent Dr., Bethesda, MD, 20892

Defining the three body axes is a central event of vertebrate morphogenesis. The establishment of left-right (L-R) asymmetry in development follows the determination of dorsal-ventral (D-V) and anterior-posterior (A-P) body axes, though the molecular mechanism by which L-R symmetry breaking is precisely triggered in reference to the other two axes is still poorly understood. Here by removing both Vangl1 and Vangl2, the two mouse homologues of a Drosophila core planar cell polarity (PCP) gene Van Gogh (Vang), we have uncovered a previously unappreciated function for PCP in initial breaking of lateral symmetry. The leftward nodal flow across the posterior notochord (PNC, also referred to as “the node”) has been identified as the earliest event in the de novo formation of L-R asymmetry.We found that PCP is essential in interpreting the A-P patterning information and linking it to L-R asymmetry by controlling posterior ciliary positioning in the PNC, which is a prerequisite for the generation of the nodal flow. In the absence of Vangl1 and Vangl2, cilia are positioned randomly around the center of the PNC cells and nodal flow is turbulent, which results in disrupted L-R asymmetry. Importantly, PCP in mouse, unlike what has been implicated in other vertebrate species, is not required for ciliogenesis, cilium motility, Sonic hedgehog (Shh) signaling or apical docking of basal bodies in ciliated tracheal epithelial cells. Our data suggest that PCP is an event earlier than the unidirectional nodal flow during bilateral symmetry breaking in vertebrates and provide insight into the functional mechanism of PCP in organizing the vertebrate tissues in development.

ORIGIN OF BODY AXES IN THE MOUSE EMBRYO

Hiroshi Hamada

Osaka University, Graduate School of Frontier Biosciences, Osaka, 563, Japan

How and when are the axes of the body determined? The left-right axis of the mouse embryo is generated *de novo* at embryonic day (E) 8.0 by utilizing pre-existing positional cues. One of the pre-existing cues is the anterior-posterior (A-P) information, which is represented by the posterior tilt of node cilia. The basal body, which determines the tilt of node cilia, is initially located centrally in the node cells, but it gradually shifts to the posterior side. Node cells are polarized in that Dvl proteins are located at the posterior side of these cells. Thus, polarization of node cells along the A-P axis is responsible for posterior tilt of node cilia. However, identity of the initial A-P information that polarizes node cells remains unknown. The A-P axis becomes apparent when distally located visceral endoderm (DVE) migrates toward the future anterior side at E5.5. *Lefty1*, one of the markers for DVE, is asymmetrically expressed in the primitive endoderm of the implanting blastocyst, pushing back the origin of the A-P axis to the peri-implantation stage. The fates of Lefty1-positive cells suggest that DVE is specified earlier by Lefty1-positive cells in a blastocyst while AVE is newly generated after DVE migrates. Time-lapse observation of cell movement and cell ablation experiments suggest that DVE may be necessary to initiate cell movement and to guide AVE migration. In all, the origin of the A-P axis can be pushed back to Lefty1-postive cells in blastocyst, but it remains to be seen how a subset of blastomeres are selected to express *Lefty1*.

APOBEC2 IS A SELECTIVE TGFB INHIBITOR REQUIRED FOR LEFT-RIGHT AXIS DETERMINATION IN VERTEBRATES

Alin Vonica, Alessandro Rosa, Brigitte Arduini, Ali H Brivanlou

The Rockefeller University, Vertebrate Embryology, 1230 York Ave., New York, NY, 10065

Specification of left-right asymmetry is a conserved process in vertebrates. Two TGFβ ligands, Derrière/GDF1 and Xnr1/Nodal, together with their inhibitors Lefty and Cerl2/Coco, provide essential signals for establishing laterality in Xenopus and mouse embryos. We identified apobec2 (A2), a conserved member of the cytidine deaminase family of DNA/RNA editing enzymes, as a target of Derrière in Xenopus. Depletion of A2 protein in Xenopus and zebrafish produced a left-right phenotype, caused by the absence of Xnr1 expression. Biochemically, A2 specifically inhibits signaling by Derrière, and not Xnr1, by posttranscriptionally decreasing the nodal coreceptor and Cripto homologue XCR2. The inhibitory effect on TGFβ signaling is conserved, as A2 also blocks TGFβ signaling in mammalian C2C12 cells. The inhibitory activity requires nuclear localization of A2 and an intact putative catalytic site. This is the first report of a cytidine deaminase regulating TGFβ signaling and a major developmental process.

THE ROLE OF MOZ IN REGULATING CHROMATIN STRUCTURE, HOX GENE EXPRESSION AND ESTABLISHMENT OF THE VERTEBRATE BODY PLAN.

Tim Thomas, Anne K Voss
Walter and Eliza Hall Institute of Medical Research , Molecular Medicine, 1G Royal Pde, Melbourne, 3052, Australia

Embryonic development requires the accurate regulation of time-space patterns of gene expression. The regulation of gene expression is intimately associated with the regulation of chromatin structure, which is determined by post-translational modification of amino acids, particularly lysine acetylation in the N-terminal tails of histones. This process is under the control of transcription factors with enzymatic activity, such as histone acetyltransferase activity. We have shown that one family of transcription factors with chromatin modifying activity, MYST, have surprisingly specific functions in regulating key events in embryogenesis.

Monocytic leukaemia zinc finger protein (MOZ) is a member of the MYST family of histone acetyltransferases originally identified in recurrent translocations leading to acute myeloid leukaemia. Since genes associated with translocations in leukaemia, such as MOZ, are typically important regulators of blood formation, we investigated if MOZ has a role in haematopoiesis and showed that Moz is required for the formation and maintenance of haematopoietic stem cells during embryonic development (Thomas et al., Genes & Development 2006). Moz is closely related to Querkopf, a gene we have shown is required for normal development of the cerebral cortex (Thomas et al., Development 2000) and has a function in maintenance and differentiation of adult neural stem cells.

Further analysis of *Moz* mutant mice showed that they have a homeotic transformation of the cervical vertebrae suggesting that MOZ is a global regulator of *Hox* gene expression in mammals. Hox genes 5 prime of the second paralogous group showed a caudal shift in the anterior expression boundary by one body segment. Correspondingly, body segment identity is shifted anteriorily by one segment such that MOZ deficient mice show a complete homeotic transformation of the axial skeleton and the nervous system affecting 19 body segments from the second cervical segment to the 13th thoracic segment. The homeotic transformation caused by lack of MOZ was fully reversed by treatment with retinoic acid. We then investigated the molecular function of MOZ in regulating chromatin structure at *Hox* loci. Our data show that the MOZ is specifically required for normal levels of H3K9 acetylation at *Hox* gene loci and *Hox* gene expression (Voss et al., Developmental Cell, 2009).

Together, our results demonstrate that MOZ and retinoic acid act in parallel to establish specific *Hox* gene expression boundaries and that the primary molecular function of MOZ is to regulate chromatin structure via acetylation of H3K9.

MORPHOGENESIS AND PROXIMODISTAL AXIS SPECIFICATION IN THE EARLY LIMB BUD

Cliff Tabin, Jerome Gros, Kim Cooper, Jimmy Hu

Harvard Medical School, Department of Genetics, 77 Avenue Louis Pasteur, Boston, MA, 02115

The vertebrate limb bud has long been a model for understanding embryonic patterning and organogenesis. Surprisingly however, several major aspects of the early morphogenesis of the limb bud have been largely ignored. The limb bud forms an elongated hemispherical mound of proliferating lateral plate mesenchyme. This is transformed into the familiar flattened paddle-shaped bud with the proximodistal (PD) axis being the longest dimension. However, this happens without differential proliferation at the distal tip. We have used a variety of imaging techniques to understand this process more fully. We find that the changes in the shape of the early limb bud are driven by a combination of oriented cell division and oriented cell movements. Functional studies indicate that these processes are partially governed by Wnt5a signaling. As the limb bud grows out, it becomes patterned along its PD axis to form the various segments of the eventual limb. How this is achieved has remained controversial. Hypotheses for PD patterning can be broadly divided into those based on a cell autonomous mechanism, for example linked to the cell cycle, and those invoking instructive, non-autonomous signaling processes. To decouple cell cycle from patterning and to have a controlled system where the signaling environment cells see can be precisely manipulated, we have developed a system where primary mesenchymal cells can first be cultured for long periods in vitro, and then be pelletted and grafted back onto a host embryo where they develop and form a limb-like structure. Our data indicate that PD patterning of the limb is determined by changes in the dynamic signaling environment cells encounter during limb outgrowth.

BREAKING SYMMETRY IN THE BRAIN – FROM GENES TO CIRCUITS

Stephen Wilson

UCL, Cell and Devel Biol, Gower St, London, WC1E 6BT, United Kingdom

Although superficially symmetrical, the vertebrate brain exhibits many functional asymmetries. The neuronal circuitry underlying these functional asymmetries is unknown but is likely to involve many cell groups in diverse regions of the brain. The epithalamus shows conserved asymmetries in many vertebrates and we have been studying the development of this region of the forebrain in embryonic and larval zebrafish. I will discuss our progress in studying the signalling pathways that influence the generation of asymmetry and the subsequent laterality of the asymmetry of epithalamic nuclei. For instance, we find that asymmetries are largely absent in *fgf8* mutants and that a key role for Fgf signalling is to mediate the migration of cells that contribute to a left-sided nucleus. We are also studying the lateralised projections and terminal morphologies of habenular neurons that convey information from the asymmetric epithalamic nuclei to the ventral midbrain, and the behavioural consequences of brain asymmetries.

NOVEL HUMAN NEURAL STEM CELLS: NEUROGENIC RADIAL GLIA IN THE OUTER SUBVENTRICULAR ZONE OF DEVELOPING NEOCORTEX

David V Hansen, Jan H Lui, Philip R L Parker, Arnold R Kriegstein

Univ. California San Francisco, Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, 513 Parnassus Ave, HSW1201 Box 0525, San Francisco, CA, 94143-0525

Studies of neural stem cells in brain development have relied largely on murine models of human development. However, rodent models may be misleading, especially when considering the neocortex which is uniquely enlarged in the human brain and probably not well modeled by developmental studies in rodents. In rodent brain, cortical neurons are generated from radial glial cells that function as neural stem cells. These epithelial cells line the cerebral ventricles and generate intermediate progenitor cells that migrate into the subventricular zone (SVZ) and further proliferate to increase neuronal number. The developing human SVZ has a massively expanded outer region (OSVZ) thought to contribute to cortical size and complexity. However, OSVZ progenitor cell types and their contribution to neurogenesis are not well understood. Here we show that large numbers of radial glia-like cells and intermediate progenitor cells populate the human OSVZ, similar to the progenitor cell types and proportions found near the ventricle. We find that OSVZ radial glia-like cells (oRG cells) have a long basal process but, surprisingly, are non-epithelial since they lack contact with the ventricular surface. Using real-time imaging and clonal analysis in cultured tissue slices, we demonstrate that these cells can undergo proliferative divisions and self-renewing asymmetric divisions to generate neuronal precursor cells that can further proliferate. We also describe a remarkable behavior of oRG cells that we term ‘mitotic somal translocation’, providing insight for how this novel stem cell population originates from the ventricular epithelium and expands to become the predominant source of cortical neurons. The emergence of non-epithelial, radial glia-like cells may have been a critical evolutionary advance underlying increased cortical size and complexity in the human brain, and suggests new targets for deriving specific subtypes of cortical neurons from human stem cells.

GENETIC ANALYSIS OF SIGNALING PATHWAYS CONTROLLING MOTOR NEURON CONNECTIVITY

Sam Pfaff

Howard Hughes Medical Institute and The Salk Institute for Biological Studies, Gene Expression Laboratory, 10010 North Torrey Pines Road, La Jolla, CA, 92037

The ability to produce coordinated and productive movement is dependent on an orderly flow of neural commands through the spinal cord that ultimately lead to contractions of specific muscle groups. A key part of this circuitry is the connectivity between spinal motor neurons and muscles. During embryonic development distinct subclasses of motor neurons are specified within the ventral spinal cord. Each motor neuron subclass encodes a unique repertoire of receptor systems that are sensitive detectors of environmental cues. These signaling systems modulate the assembly and disassembly of cytoskeletal components involved in axon growth and turning. Motor neurons share guidance receptors such as CXCR4, FgfR, EphA, EphB, DCC, Robo, and Npn with many other neuronal types. Thus, one challenge in the field of axon guidance has been to understand how the vast complexity of brain connections can be established with a relatively small number of factors. In the context of motor guidance, we have taken a genetic approach to dissect the signaling systems required for axon navigation to better understand the temporal and spatial mechanisms used to optimize the fidelity of pathfinding and increase the functional diversity of the signaling proteins. I will describe our findings that show membrane-proteases are critical modulators of the output signals from guidance receptors.

THE DETERMINATION OF CELL FATE IN THE VERTEBRATE RETINA

Connie Cepko[1,2], Karolina Mizeracka[1], Jeff Trimarchi[1], Rahul Kanadia[1,2]

[1]Harvard Medical School, Genetics, 77 Avenue Louis Pasteur, Boston, MA, 02115, [2]Howard Hughes Medical Institute, Genetics, 77 Avenue Louis Pasteur, Boston, MA, 02115

Vertebrate retinal neurons can be classified into >60 cell types, based upon morphology, physiology, distribution, and molecular markers. We are interested in learning how these cell types choose their fate during development. To this end, we have profiled single retinal progenitor cells, and newly postmitotic cells, throughout the course of development using Affymetrix microarrays. The results show an enormous amount of heterogeneity among progenitor cells, even those taken from the same stage in retinal development. In addition, single, newly postmitotic neurons were found to simultaneously express canonical markers of several different cell types. This has prompted us to examine whether newborn retinal neurons are undetermined regarding their final choice of cell fate. We thus manipulated the level of Notch signaling within these newly postmitotic cells, and found that they could change their fates accordingly. We have also examined the downstream consequences of changes in Notch signal levels using microarrays and have identified a network of genes that likely influence the choice of cell fate under the control of Notch.

SOX2 AND PAX6 DOSAGES CONTROL RETINAL CELL FATE

Whitney E Heavner[1,2,3], Danielle Matsushima[1], Larysa H Pevny[1,2]

[1]University of North Carolina, Neuroscience Center, 115 Mason Farm Rd, Chapel Hill, NC, 27599, [2]University of North Carolina, Department of Genetics, 120 Mason Farm Rd, Chapel Hill, NC, 27599, [3]University of North Carolina, Curriculum in Genetics and Molecular Biology, 120 Mason Farm Rd, Chapel Hill, NC, 27599

Two transcription factors that play key roles in directing retinal cell fate decisions are SOX2 and PAX6. Human mutations in either of these genes are associated with eye malformations including aniridia (no iris), anopthalmia (no eye), and micropthalmia (small eye). Using mouse genetics, we demonstrate that SOX2 and PAX6 act antagonistically to regulate neural versus non-neural cell fate in the developing retina. Ablation of SOX2 in multipotent retinal progenitor cells (RPCs) restricts them to a non-neural ciliary epithelial fate coincident with elevated PAX6 signaling. Therefore, in the absence of SOX2, elevated PAX6 expression is not sufficient for neuronal differentiation, suggesting that SOX2 represses PAX6 to maintain neural RPC multipotency. Furthermore, ablation of SOX2 on a Pax6-haploinsufficient genetic background partially rescues the neural-to-non-neural cell fate conversion. This counterintuitive result suggests that there is a direct dosage dependent relationship between SOX2 and PAX6 in RPC specification. Additionally, through the temporal and spatial specific ablation of SOX2 in the retina, we uncover a novel expression profile of a cell in transition between multipotent retinal progenitor and ciliary epithelium.

MODELING HUMAN NEURONAL MIGRATION MUTANTS IN THE MOUSE: INSIGHTS INTO THE ASSEMBLY OF THE BRAIN.

Anthony Wynshaw-Boris[1], Yong-Ha Youn[1], Tiziano Pramparo[1], Hyang-Mi Moon[1], Kazu Toyo-oka[1], Shinji Hirotsune[2]

[1]UCSF School of Medicine, Pediatrics and Institute for Human Genetics, 513 Parnassus Ave, San Francisco, CA, 94143-0794, [2]Osaka City University Graduate School of Medicine, Department of Genetic Disease Research, Asahi-machi 1-4-3 Abeno, Osaka, 545-8586, Japan

Haploinsufficiency of *LIS1* was the first genetic defect identified in human with neuronal migration defects, a finding confirmed in mice with graded reduction of *Lis1*. Complete loss of murine *Lis1* results in peri-implantation lethality, and genetic studies in model organisms suggest a role for *Lis1* in cell division. We found an essential role for *Lis1* in neuroepithelial stem cells using mice using a genetic approach to produce mice with a graded reduction of *Lis1* as well as the complete loss of *Lis1* using a conditional *Lis1* allele and transgenic Cre lines. We have investigated the mechanisms responsible for these defects in vivo and in vitro using neural stem cell and mouse embryonic fibroblasts. We have made a number of mouse mutants with neuronal migration defects and used these mice to further understand the role of the LIS1 complex during development. Our studies suggest a broader role for LIS1 in the regulation of neurogenesis and neuronal migration.

THE *HOXA5* MUTATION PERTURBS KEY SIGNALING PATHWAYS DURING RESPIRATORY TRACT ONTOGENY

Josee Aubin, Olivier Boucherat, Lucie Jeannotte

Centre de recherche en cancérologie de l'Université Laval, Centre de recherche de L'Hôtel-Dieu de Québec, 9 rue McMahon, Québec, QC, G1R 2J6, Canada

HOX transcription factors control embryonic patterning by directing morphogenetic events that give rise to the diverse body forms. We have shown that *Hoxa5* function is essential for proper respiratory tract development. The *Hoxa5* mutation causes trachea and lung anomalies that lead to premature death at birth and to emphysematous lung morphology in surviving *Hoxa5*$^{-/-}$ adult mice. During respiratory tract development, *Hoxa5* expression is restricted to the mesenchyme. However, its loss of function has both stromal and epithelial repercussions. In the stroma, the *Hoxa5* mutation perturbs alveolar myofibroblasts specification, resulting in their mislocalization and abnormal elastin deposition. Consequently, lungs from surviving *Hoxa5* mutant lungs present enlarged air spaces. The *Hoxa5* mutation also affects at least two epithelial cell populations: the goblet and the basal cells. In *Hoxa5* mutants, these two cell types are localized more distally along the respiratory tract compared to wild-type (wt) specimens suggesting defective proximo-distal patterning. Epithelial branching morphogenesis is also diminished, a process relying on mesenchymal-epithelial (m-e) communication. How *Hoxa5* exerts its influence on the patterning of the respiratory tract and on pulmonary cell specification remains unsolved. We have undertaken a comparative expression analysis of signaling molecules (FGF, BMP, Wnt) and their effectors, taking advantage of candidate genes shown to impact on branching morphogenesis and on the specification of the goblet and basal cell lineages. During branching morphogenesis, the ERK/MAPK and the BMP signaling pathways are affected in embryonic *Hoxa5* mutant lungs as revealed by immunostaining and western blot analyses. In parallel, the Wnt canonical pathway, known to be involved in goblet cell hyperplasia, has also been considered as a putative mediator of *Hoxa5* action. The use of *Hoxa5;TCF/Lef-lacZ* animals to monitor ß-galactosidase activity in lungs from embryo and adult specimens reveals that the loss of *Hoxa5* function results in activation of the Wnt canonical pathway. Taken together, these data suggest that *Hoxa5* action in respiratory tract morphogenesis implicates the interplay of multiple signaling pathways.
(Supported by CIHR)

MITOTIC ARREST OF GRANULOSA PRECURSOR CELLS IN THE DEVELOPING MOUSE OVARY

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Granulosa cells surround oocytes to form follicles in the adult ovary. In mice, it has been proposed that granulosa cells and Sertoli cells, the analogous supporting cell type in the testis, derive from a common precursor population in the bipotential gonad. In the presence of a Y-chromosome, these cells express Sry and adopt Sertoli cell fate, whereas they differentiate as granulosa precursors in XX gonads. Foxl2, a gene required for follicle differentiation, is upregulated in a subpopulation of ovarian somatic cells at about E12.0. To determine whether FOXL2 specifically marks 'pre-granulosa' cells, we stained gonads from Sry:EGFP transgenic XX mice with a FOXL2 antibody, and found that all GFP+ cells co-labeled with FOXL2. We assessed the proliferation profile of the FOXL2+ cell population by co-labeling wild-type ovaries with FOXL2 and phospho-Histone H3 (a marker of mitosis) or Ki67 (a marker of active cell cycle). Surprisingly, all FOXL2+ cells were Ki67– and pHH3– at all embryonic stages examined. In contrast, they strongly expressed p27 and appeared to have entered cell cycle arrest. Unlike Sertoli cells in the testis, the entire population of FOXL2+ cells was arrested from E12.0 to postnatal day 6-7, when a few follicles were activated. This result indicates that the population expands via recruitment from non-expressing cells rather than intrinsic proliferation. Lineage-tracing studies demonstrated that new FOXL2+ cells arise mostly by E12.5 from both the coelomic epithelium and other somatic cells. To investigate whether Wnt signaling, critical for female development, regulates mitotic arrest of female somatic cells, we assessed the proliferation status of FOXL2+ cells in Wnt4-/- and Sf1:Cre;β-catenin^{exon3} XX mutant gonads, which respectively represent loss and gain of Wnt function. Although FOXL2+ cells arrested normally in Wnt4 mutants, they reentered cell cycle by E15.5 in the Sf1:Cre;β-catenin^{exon3} gonads. This suggests that the Wnt pathway is unlikely to be the primary signal driving arrest in the wild-type ovary, although accumulation of β-catenin seems to have an oncogenic effect. Likewise, FOXL2+ cells in embryos treated at E10.5 with busulfan, a chemotherapeutic drug that depletes primordial germ cells, remained in cell cycle arrest at E14.5, indicating that the presence of germ cells is not required to maintain arrest. Although the upstream regulators and general significance of this pre-granulosa cell cycle arrest remain unclear, we have identified an intriguing new facet of ovarian morphogenesis that may offer insight into how follicle endowment and organization are regulated in the developing ovary.

DIRECT AND INDIRECT ROLES FOR SHH SIGNALING IN OTIC VESICLE PATTERNING

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In mouse embryos lacking Sonic Hedgehog (Shh), patterned gene expression along the dorsoventral axis of the otic vesicle is greatly disrupted. Consequently, the development of ventral otic derivatives including the cochlear duct, utricle and cochlear-vestibular ganglion (cvg), the sensory nerve that relays sound and positional information to the brain, are greatly impaired. In addition, dorsal otic derivatives including the semicircular canals, endolymphatic duct and utricle are malformed or absent in $Shh^{-/-}$ embryos. Since inner ear patterning and morphogenesis is heavily dependent on extracellular signals derived from surrounding tissues that are also compromised by the loss of Shh, the extent to which Shh signaling directly acts on inner ear development is unclear. To address this question we generated embryos in which *Smoothened (Smo)*, an essential transducer of Hedgehog (Hh) signaling, was conditionally inactivated in the otic vesicle (Smo^{ecko}). While ventral otic derivatives including the cochlear duct and saccule failed to form in Smo^{ecko} embryos, vestibular structures developed properly. These findings indicate that the otic epithelium is directly dependent on Shh signaling for the formation of the cochlea and saccule, but indirectly dependent on Shh for semicircular canal, endolymphatic duct and utricle development. The neurogenic domain of the otic vesicle is defined by the expression of *Ngn1*, which is spatially restricted to the anteroventral region of the otic vesicle, in part, by *Tbx1*. In $Shh^{-/-}$ ears, *Tbx1* expression expanded into the neurogenic domain and *Ngn1* expression was reduced. In contrast, *Tbx1* expression appeared normal in the otic vesicle of Smo^{ecko} embryos, and *Ngn1* was properly localized to the neurogenic domain. Nonetheless, cvg neurons were still reduced in Smo^{ecko} embryos, albeit to a lesser degree than that seen in $Shh^{-/-}$ embryos. The reduction of cvg neurons in Smo^{ecko} embryos correlates with reduced proliferation in the neurogenic domain of the otic vesicle suggesting that cvg proliferation is dependent on direct hedgehog signaling. Interestingly, cvg neurons were partially rescued in $Shh^{-/-};Tbx1^{+/-}$ embryos, suggesting that the additional loss of cvg neurons in $Shh^{-/-}$ versus Smo^{ecko} mutants can be accounted for by the expansion of *Tbx1* in the anteroventral region of the otic vesicle. These results support the idea that Shh promotes cvg formation directly by promoting neuroblast proliferation, and indirectly by limiting the expression of *Tbx1*.

MAMMALIAN LENS PLACODE INVAGINATION REQUIRES THE ACTION OF THE THREE SMALL RHO GTPASES *CDC42*, *RHOA* AND *RAC1*.

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Purpose. The formation of the mammalian lens pit provides an excellent model to study the morphogenetic pathways involved in epithelial invagination. In this study, we attempt to address the importance of three chief small Rho GTPases, Cdc42, RhoA and Rac1, in this process.

Methods. Three conditional mouse mutants of *Cdc42*, *RhoA* and *Rac1* were crossed with the *le-cre* mouse line to generate corresponding deleted alleles only in the induced lens ectoderm. Analysis, including immunofluorescent-staining and quantification, was conducted at specific stages of lens placode invagination.

Results. We show that F-actin-rich filopodia link adjacent presumptive lens and retina. The filopodia, most of which originate in the presumptive lens, form at E9.5 when presumptive lens and retina first come into close contact, and have retracted by E11.5 when invagination is complete. Formation of filopodia is dependent on Cdc42 and its effector IRSp53 (Baiap2). Loss of filopodia results in reduced lens pit invagination. Pharmacological manipulation of the actin-myosin contraction pathway showed that the filopodia can respond rapidly in length to change the inter-epithelial distance.

Next, we show that there are apical actomyosin complexes in the lens placode that respond to pharmalogical manipulation, thereby reducing invagination. We observe that activated myosin and F-actin is reduced in RhoA mutant lens pits, therefore producing shallower pits. In contrast, early invagination of placodes occurs in the Rac1 mutants compared to wild-types. Interestingly, the lens pits are thinner in the Rac1 mutants, but noticeably thicker for the RhoA mutants.

Conclusion. Collectively, our results suggest that basal lens filopodia provide a physical tether that coordinates invagination and correct positioning of the lens in the early eye. The role of RhoA is to regulate the apical actomyosin complexes that drive invagination, and Rac1 is responsible for migration of the lens placode towards the presumptive retina.

SSDP ACTION INDEPENDENT OF LDB IS REQUIRED FOR EARLY LENS DEVELOPMENT

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Transcriptional regulation of embryonic development in invertebrates and vertebrates involves a variety of protein complexes composed of transcription factors and nuclear co-regulators. Among these are the Single-stranded DNA-binding proteins (SSDPs) and the LIM-domain binding (Ldb) proteins. They can physically interact with each other and are recognized as essential co-regulators of embryonic development.
In order to test if both types of regulators are obligatory members of transcription complexes in vivo we generated a conditional transgene that can be activated by Cre action. This transgene encodes the N-terminal end of SSDP1 which is responsible for the binding to Ldb proteins. Activation of the conditional transgene in the developing lens of mouse embryos has Ldb-independent tissue-specific consequences which severely affect early lens development. To identify proteins that interact with SSDP1 and might mediate its Ldb-independent activity, we performed a yeast two hybrid analysis using the SSDP1 N- terminus as bait and detected several potential binding partners. Among these are the products of other members of the SSDP gene family that can interact with each other in the form of homodimers and heterodimers. This suggests the possibility that activation of the N-terminal end of SSDP1 interferes with essential, albeit Ldb-independent, action of SSDP proteins in early lens development. Our findings suggest hitherto unknown functions of SSDP proteins during embryonic development.

A MECHANICS THEORY OF BIOLOGICAL NETWORK FORMATION

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With the justification of accumulating experimental evidence, the direction of migration of an individual invader cell during the invasion of a host cell population is assumed to be controlled by the magnitude of the strains in the host medium (host cells plus extracellular matrix) that surround the invader. Strains arise in the host medium because it deforms to accommodate the intrusion of the invaders.

This single assumption of a strain cue for invader migration is sufficient to generate network structures. The key concept is that the strain induced in the host medium by a short line of invaders is greatest at the extremity of the line. Thus the strain field breaks symmetry, stabilizing branch formation. The same strain cue also triggers sprouting from existing branches, with no further model assumption. Closed networks result by coalescence of branches that impinge on one another.

The density of the network depends primarily on the ratio of two rates: that of the advance of the invaders and that of the shifting of the host cells to relax the strains induced by the invasion. Cell characteristics that influence these two rates are inferred to control network morphology.

The strain field that cues an individual invader is generated by the nearest 100 cells to order of magnitude; the mechanism is therefore a collective response of the combined cell populations. The negligible influence of more remote cells implies that the mechanism does not rely on the pre-existence of the entire host medium prior to invasion. It is sufficient that host cells are recruited fast enough to maintain a layer several cells thick around each invader.

In agreement with recent experiments, networks result only from a strain cue that is based on strain magnitudes; spatial strain gradients do not break symmetry and therefore cannot stabilize branch formation.

This extremely simple theory recreates most of the main features of the nervous network in the mouse gut. With appropriate minor modifications to the details of stress and strain fields, the same strain-cue rule can account for the formation of both nervous and vascular networks.

THE ROLE OF ISLET1 IN ENTEROENDOCRINE CELL DEVELOPMENT

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Endocrine cells in the gut epithelium from the stomach to the colon represent the largest population of hormone producing cells in the body and may constitute up to 1% of the total gastric epithelial cells. We are currently exploring the role of Islet1 *(Isl1)* during the differentiation of enteroendocrine cells in the stomach. *Isl1* belongs to the family of LIM-homeodomain transcription factors. While the role of *Isl1* in the developing pancreas has been well established, its function in the stomach remains unclear. Furthermore, the temporal and spatial expression of *Isl1* has yet to be characterized in the stomach. We have shown that *Isl1* expression is first detected in the ventral gastric mesenchyme at E9.5. As the development proceeds, *Isl1* expressing cells are found in the lateral mesenchyme at E10.5-11.5 and dorsal mesenchyme at E12.5. *Isl1* expression in the gastric epithelium is not detected until E13.5 and is restricted only to the posterior stomach from E14.5 onwards. As the stomach matures, *Isl1* expression is found in subsets of gastrin-expressing cells and most of somatostatin-expressing cells, both of which have a common precursor population. To better understand the role of *Isl1*, we have generated a gain-of-function mouse model to overexpress *Isl1* in the developing gastric epithelium. Initial analysis of this mouse model suggests that *Isl1* is involved during the differentiation of gastrin and somatostatin expressing cells in the gastric epithelium.

SORTING IT OUT: REGULATION OF INTERSTITIAL CELL FATE DURING MAMMALIAN TESTIS MORPHOGENESIS

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Cellular reorganization is a hallmark of organogenesis and is especially critical in the gonad, where sex-specific morphogenesis is necessary for fertility. During the differentiation of the mammalian embryonic testis, the basic process of cellular reorganization defines two compartments: the testis cords and the interstitium. The testis cords will become the seminiferous tubules, where sperm are produced in adult life, whereas the androgen-producing Leydig cells and other less well-characterized cell populations differentiate in the interstitium. Although the process of cord formation is essential for masculinization of the fetus and fertility of the adult, it is not well understood. It has been viewed as a Sertoli-cell-driven process; however, several lines of evidence suggest that interstitial cells play a critical role in shaping the process of testis formation. One gene that is essential for cellular reorganization during gonad morphogenesis in *Drosophila* is *traffic jam* (*tj*). *tj* encodes a member of the Maf transcription factor family, and promotes organogenesis of the *Drosophila* gonad via the regulation of cell adhesion molecules (CAMs). We show that the mammalian orthologs of *tj*, *Mafb* and *cMaf*, are expressed in an uncharacterized population of progenitor cells that sort out from Sertoli cells at the beginning of fetal testis formation and give rise to the interstitium of the testis. *Mafb* and *cMaf* mutants show defects in the formation of testis cords; we are investigating whether this phenotype arises because of a disruption in the control of CAMs or extracellular matrix (ECM) proteins as in the *Drosophila* gonad. We are also testing the hypothesis that Notch signaling controls the specification of *Mafb*/*cMaf*-expressing cells during interstitial cell differentiation. Using cell type-specific fluorescent markers, we have performed live imaging to track the in vivo development of the interstitium and its relationship to vasculature and Sertoli cells. Current experiments are designed to use fluorescence-activated cell sorting to define the transcriptome in *Mafb*-positive cells, and to elucidate the mechanisms of *Mafb* and *cMaf* function and the role of Notch signaling in the interstitium. This study of cell fate specification and cell-cell interactions will further our understanding of organogenesis pathways and provide insight into the developmental basis for infertility and reproductive disorders.

SIGNALING FROM THE DERMAL PAPILLA REGULATES MORPHOGENESIS AND REGENERATION OF THE HAIR FOLLICLE.

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Epithelial-mesenchymal interactions drive hair follicle specification and morphogenesis of this mini-organ. Although keratinocytes comprise the bulk of the follicle and form the hair shaft and its associated root sheaths, their activity is regulated by a specialized mesenchyme embedded in the base of the hair follicle, the dermal papilla. The size of the follicle and the morphology, structure, length and pigmentation of the hair shaft are all influenced by signals from the dermal papilla. Tools to manipulate gene expression in keratinocytes have allowed rapid progress towards characterizing gene function in the epithelial compartment. To complement this approach, mice expressing cre recombinase in the dermal papilla were developed to probe gene function in this compartment in vivo. Keratinocytes adjacent to the dermal papilla act as stem/progenitor cells and generate progeny that give rise to 6 concentrically arranged cell types that form the hair shaft and inner root sheath. Inactivation of the ß-catenin gene within the dermal papilla of fully developed hair follicles results in dramatically reduced proliferation of these matrix stem cells and their immediate progeny and a decrease in hair caliber and growth rate. This, and premature termination of the growth phase of the hair cycle with concomitant loss of this progenitor population result in greatly reduced hair length. Pigmentation of the hair is also altered in these mice. This study reveals that ß-catenin activity in the DP is required to maintain adjacent hair follicle matrix stem cells and provides new insights into a reciprocal signaling loop that employs Wnt/ß-catenin signaling in both the stem cells and their niche to govern and coordinate the interactions that are essential for the function of these two compartments. By systematically altering the capacity of DP cells to respond to different signaling pathways that impinge on them, we have begun to dissect the interactions that guide follicle morphogenesis.

THE HSP90 CO-CHAPERONE, UNC45B, IS REQUIRED FOR SARCOMERIC MYOSIN INTEGRATION AND TIMELY Z-DISC FORMATION IN THE *XENOPUS TROPICALIS* MUTANT *DICKY TICKER*

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Myofibrillogenesis is the differentiation of myocytes into contractile muscle myofibrils comprising many repeating units known as sarcomeres. Within the sarcomere, myosin fibres assemble upon a scaffold of thin and intermediate filaments in between two Z-discs. This ultimately allows muscular contraction and relaxation to take place. The *Xenopus* tadpole provides an outstanding platform on which to study myofibrillogenesis owing to the large amount of muscle derived from the somites in the tail and an easily accessible heart. From a pilot genetic screen we have identified and characterised a chemically-induced *Xenopus tropicalis* mutant in which myofibrillogenesis is disrupted. The mutant *dicky ticker* (*dit*) does not integrate myosin fibres into the sarcomere resulting in paralysis and an absent heartbeat. Positional cloning has identified a thymidine to cytidine nucleotide transition in a gene coding for unc45b, a co-chaperone of hsp90. Recent evidence has shown both unc45b and hsp90 play important roles in skeletal and cardiac muscle formation, binding to, and folding, the complex sarcomeric myosin motor domain. The nucleotide change results in a Cysteine to Arginine amino acid substitution in the C-terminal UCS domain, thought to be critical for unc45b chaperone activity. However, although severely impaired, the program of myofibrillogenesis is still initiated as evidenced by the presence of Z-discs within *dit* tadpole tail muscle. Despite this, while Z-discs appear to form relatively normally, our analysis of early sarcomere development shows a significant delay in the polymerisation of Z-bodies (Z-disc precursors) into the mature disc. This has implications for our understanding of myofibrillogenesis and presents a new role for unc45b in this process.

YAP IS AN IMPORTANT REGULATOR OF CELL DIFFERENTIATION.

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Yes-associated protein 65 (YAP) contains multiple protein-protein interaction domains and functions as both a transcriptional co-activator and a scaffolding protein. It is required for mouse yolk sac vasculogenesis, chorioallantoic fusion, and axis elongation. To better define the role of YAP in early embryonic development, we used MO-mediated YAP loss-of-function and injected mRNA gain-of-function assays in frog and zebrafish. In the absence of YAP, mesoderm induction was delayed and embryos did not progress normally through gastrulation. YAP gain-of-function assays expanded the neural plate (Sox2) and the paraxial mesoderm (Pax3), while inhibiting the induction and/or expansion of the neural crest and the preplacodal ectoderm. While YAP gain-of-function assays maintained and expanded the neural and muscle progenitor fields, neural and muscle differentiation were inhibited. YAP loss-of-function resulted in a complete loss of Pax3 expression, suggesting that Pax3 may be a direct gene target of YAP. Co-expression of YAP with a known interacting transcription factor, TEAD1, enhanced the expansion of Pax3 paraxial mesoderm expression. Chromatin immunoprecipitations for endogenous YAP showed that it localized to the 5' regulatory region of Pax3. Furthermore, structure/function experiments highlighted a region of YAP that may be important for its inhibition of cellular differentiation. Thus, YAP is an important regulator of cellular differentiation, and Pax3 is a gene target of YAP within the paraxial mesoderm.

AUTOCRINE SLIT-ROBO SIGNALING INDUCES WNT/B-CATENIN ACTIVATION DURING INTESTINAL EPITHELIAL CELL HOMEOSTASIS AND CARCINOGENESIS

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The Slit family of guidance cues binds to Roundabout (Robo) receptors for modulation of neuronal, leukocytic and endothelial migration. Wnt/β-catenin signaling maintains homeostasis of intestinal epithelium and dysregulation of Wnt/β-catenin activity induces colorectal carcinoma. Despite its importance, soluble factor(s) regulating the Wnt/β-catenin gradient along the crypt-villi axis remain(s) elusive. We report here that Slit2 binding to Robo1 induced E-cadherin degradation, β-catenin re-distribution, Wnt signaling activation and epithelial-mesenchymal transition (EMT). The expression of pan-Slit and Robo1 was significantly associated with an increased metastatic risk and poorer overall survival. Surprisingly, the pan-Slit and Robo1 antigens were higher in the crypts and lower in the villi of normal intestines and specific blockade of Slit2-Robo1 interaction decreased villi size while Slit2 overexpression increased it. We conclude that autocrine Slit-Robo signaling activates Wnt/β-catenin for physiological homeostasis of intestinal epithelium and for pathological growth and metastasis of colorectal carcinoma.

DLX5 AND *DLX6* EXPRESSION IN THE ANTERIOR NEURAL FOLD IS ESSENTIAL FOR PATTERNING THE DORSAL NASAL CAPSULE.

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Morphogenesis of the facial skeleton depends upon inductive interactions between cephalic neural crest cells (CNCCs) and cephalic epithelia. The nasal capsule, a cartilage surrounding the nasal cavity of vertebrates, is a modular structure build upon chondrogenic differentiation of CNCCs into ventral (mesethmoid) and dorsal (ectethmoid) components. While lineage experiments have unravelled the contribution of CNCCs progenitors (1), the cellular interactions and molecular signals patterning the components of the capsule remain to be fully elucidated (2).
We have previously reported that Shh-*Gli1* signalling from the rostral foregut endoderm is required for mesethmoid specification and patterning (3). However, the formation of the ectethmoid cartilage of remained an unsolved issue.
We show here that *Dlx5/Dlx6* expression in a restricted territory of the anterior neural folds (referred to as NF-ZA) is necessary to instruct CNCCs to pattern the ectethmoid cartilage. *Dlx5/Dlx6* initial expression in the both avian and murine embryos is restricted to NF-ZA, and their targeted inactivation in the mouse (4,5) results in loss of the dorsal, but not of the ventral, component of the nasal capsule.
Further, NF-ZA surgical removal in chick embryos specifically prevents ectethmoid formation, whereas grafting a supernumerary NF-ZA results in an ectopic ectethmoid. Simultaneous ablation and/or grafting of NF-ZA and the rostral foregut endoderm, results in complete ablation or duplication of the nasal capsule, respectively.
Our work suggests that the development of the nasal capsule results from the early integration of ectodermal and endodermal signals to CNCCs, leading to the formation of a sensory and respiratory nose.

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BMP7 PROMOTES SEPTATION OF THE MURINE CLOACA BY INTERACTING WITH THE CANONICAL WNT AND PLANAR CELL POLARITY PATHWAYS

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Development of the mammalian urethra, the rectum and external genitalia are intimately connected with morphogenesis of the embryonic cloaca. We have previously reported that loss of *Bone morphogenetic protein 7 (Bmp7)* results in severe defects in reorganization of the caudal endoderm during cloacal septation and development of the genital urethra. Here, we investigated the roles of BMP7 in cloacal patterning and morphogenesis, and the interactions between the BMP7 and canonical and non-canonical Wnt pathways. We show that in mid-embryogenesis, BMP7 contributes to the patterning of the cloacal endoderm by restricting the domain WNT/ LEF1 signaling to the dorsal cloaca, which subsequently develops into a rectum. We further show that BMP7 promotes cell survival, adhesion and stratification in the cloacal endoderm. BMP7 also promotes apical-basal polarity in the cloacal epithelium by positively regulating expression of the planar cell polarity (PCP) component, *Van Gogh-like 2*, and activity of the c-Jun kinase. In summary, our studies indicate that BMP7 functions to promote cloacal septation by interacting with the canonical Wnt and PCP pathways to regulate reorganization of the cloacal endoderm.

SECRETORY CELLS OF THE MURINE AIRWAYS MAY BE SPECIFIED BY SEVERAL SPATIALLY AND GENETICALLY DISTINCT NOTCH-DEPENDENT MECHANISMS

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The airway epithelium comprises a heterogeneous population of cells that have been broadly classified as secretory, ciliated, basal and neuroendocrine. There is increasing evidence of diversity within these lineages and in the context of secretory cells this diversity manifests in distinct cellular phenotypes along the proximal-distal axis. We have investigated whether the differences among secretory cells arise from distinct developmental programs. Analysis of the putative secretory cell marker Scgb3a2 at early stages of airway differentiation (E11.5-E16.5) reveals two distinct patterns. Clusters of cells expressing Scgb3a2 are localized to emerging foci of Ascl1 expressing cells (E13.5) and these clusters are not detected in Ascl1 KO animals. The other pattern of variegated expression initiates in the proximal airways at an earlier stage (E12.5). We find that these Scgb3a2 expressing cells are juxtaposed with cells expressing the transcription factor Trp63 to create a salt-and-pepper pattern. We, and others, have previously shown that developing airways deficient in Notch signaling have no secretory cells. We now propose that Trp63 and Ascl1 regulate two spatially and genetically distinct programs of Notch-dependent secretory cell development. Evidence from other systems suggests that the transcription factors Trp63 and Ascl1 regulate Notch activation in adjacent cells via transcriptional control of Jagged1/Jagged2 and Dll1 expression respectively. Observed patterns of Jagged 1, Jagged 2 and Dll1 in the developing airways are consistent with this hypothesis.

STEM CELL FACTOR/C-KIT SIGNALING MAINTAINS EPITHELIAL END BUD PROGENITOR CELLS DURING SUBMANDIBULAR GLAND ORGANOGENESIS

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Irradiation damage to adult mouse submandibular glands (SMGs) causes irreversible loss of function that can be regenerated by transplanting c-Kit+ progenitor/stem cells. This therapeutic approach will be used to treat head and neck cancer patients whose salivary glands have been damaged during radiotherapy. We hypothesize that stem cell factor (SCF)/c-Kit signaling also regulates progenitor cell maintenance during the development of salivary glands and other branching organs. SMG branching morphogenesis begins at embryonic day 13 (E13). Analysis of c-Kit during development by microarray, q-PCR, immunostaining, and FACS, show that it increases during development until differentiation begins after E16. c-Kit is expressed in the epithelial end buds, suggesting these structures contain progenitor cells, and SCF is expressed in both the mesenchyme and the end buds. Fgf10/Fgfr2b signaling is essential for epithelial proliferation during SMG development. Reducing Fgfr2b signaling in ex vivo SMG culture by adding soluble recombinant Fgfr2b decreased branching morphogenesis and *c-Kit* expression, suggesting potential crosstalk between Fgfr2b and c-Kit signaling. When Fgf10 was added to isolated SMG epithelia SCF expression increased within 2 hours in a MAPK-dependent manner. At later time points, *c-Kit* expression also increased, likely due to autocrine induction by epithelial SCF. In addition, exogenous recombinant SCF upregulated epithelial *c-Kit* within 2 hours, also demonstrating paracrine induction of epithelial c-Kit expression. Additionally, we performed gain and loss of SCF/c-Kit function in SMG epithelia. Exogenous SCF alone does not support epithelial morphogenesis, growth, or survival. In combination with Fgf10, exogenous SCF did not induce additional morphological changes. Reducing c-Kit expression with siRNA in isolated SMG epithelium cultured in Fgf10 decreased SCF expression. In addition, there was downregulation of *Etv4*, *Etv5*, and *Sox10*, transcription factors involved in stem cell maintenance in other systems. The addition of exogenous SCF restored expression of these transcription factors to control levels, indicating they are downstream of c-Kit signaling. In conclusion, epithelial SCF/c-Kit expression is regulated by Fgf10/FgfR2b signaling. SCF/c-Kit signaling regulates transcription factors that may maintain end bud progenitor cells.

ADDRESSING PROMININS' FUNCTION IN ZEBRAFISH (*D.RERIO*)

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Prominins are evolutionarily conserved five-transmembrane proteins localized in various plasmalemmal protrusions including microvilli and primary cilium of numerous epithelial and non-epithelial cell types. The first member of this family, prominin-1 (CD133) became a widely used cell surface marker for prospective identification of stem- and progenitor cells from various tissue sources. Their physiological function remains elusive. We have identified three prominin-like paralogues in zebrafish. Remarkably, the antisense depletion of one of them (prominin-like 3) leads to laterality defects as revealed by disturbed molecular asymmetry in the lateral plate mesoderm and concomitant anatomical defects in heart tube looping, i.e. either inversion of the left-right asymmetry (situs inversus) or absence of laterality. Beside the randomization of heart position, the morphants show additional pleiotropic defects with signs of curved body axis, severe pathological pericardial edema at later stages of development. Interestingly, antisense depletion of another prominin paralogue (prominin-1a) did not result in gross morphological changes. The phenotype of prominin-like 3 morphants is strikingly reminiscent to vertebrate disease conditions with impaired primary ciliary function. Indeed, the ciliary morphology of the Kupffer's vesicle is compromised in these morphants. Taken together, our study suggests a role for prominin in elaboration/maintenance of ciliary appendages.

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INVESTIGATING THE ROLE OF A WNT RECEPTOR, FRIZZLED2, IN LUNG DEVELOPMENT

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The Wnt signaling pathway plays a fundamental role in many aspects of organogenesis including promoting proliferation, regulating apoptosis, patterning tissues and establishing cell fates. Several Wnt proteins, including Wnt2a, Wnt2b, Wnt5a, Wnt7b, and Wnt11, are expressed in the developing lung and loss of each of these ligands causes specific defects in lung development. Wnt proteins can signal through several intracellular pathways including the canonical β-catenin dependent pathway, the Ca^{2+} dependent pathway, and the planar cell polarity pathway. Although there is evidence that signaling through the canonical β-catenin pathway plays a significant role in lung development very little work has been done to elucidate the role of non-canonical signaling.

Previous work in our lab showed that the transcription factor Gata6 is required for the expression of Fzd2, a Wnt receptor implicated in Ca^{2+}dependent non-canonical signaling, in the developing lung. Fzd2 signaling negatively regulates canonical Wnt signaling in vitro and increased Wnt/β-catenin signaling is observed in lungs lacking Gata6 expression. Furthermore, distal lung progenitors fail to properly differentiate in Gata6 mutant lungs and restoring Fzd2 expression in Gata6 mutant lung explants rescues this phenotype. Based on these results, I hypothesized that non-canonical Wnt signaling acts through the Fzd2 receptor to attenuate canonical Wnt signaling and pattern the developing lung.

In order to investigate the role of Fzd2 in lung development, our laboratory has generated both a global knockout and a conditional floxed-allele of Fzd2. Homozygous Fzd2 null embryos die before the earliest stages of lung development. I therefore used SHH-cre, which expresses cre recombinase in the ventral foregut endoderm prior to lung specification, to excise floxed alleles of Fzd2 thereby eliminating Fzd2 expression in the presumptive lung endoderm. While the lungs are specified in $SHH^{cre/+}$; $Fzd2^{F/F}$ embryos, they are hypoplastic with dilated distal airways similar to those in the Gata6 mutant animals. Appropriate proximal to distal patterning is established, but secondary branching morphogenesis is disrupted in lungs from $SHH^{cre/+}$; $Fzd2^{F/F}$ embryos.

THE GENETIC BASIS FOR EVOLUTIONARY CHANGES IN HUMAN ECCRINE GLAND DEVELOPMENT

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Human skin is strikingly distinct from that of other mammals. This is due to a set of cutaneous transformations that enable the uniquely human mechanism of thermoregulation, sweating. In contrast to non-human primates and other mammals, humans rely exclusively on water secretion by eccrine sweat glands onto the skin surface as the main mechanism for heat loss to the environment. To achieve this, the number and distribution of eccrine sweat glands has dramatically increased in the human lineage. However, despite the critical role of eccrine glands in human evolution and body homeostasis, relatively little is known about their development and patterning. Consequently, the genetic and evolutionary basis for the nature of human skin remains unknown. In light of the conservation of known molecular mechanisms governing the development of cutaneous appendages between mouse and man, we have begun to address these questions by screening for genetic loci that underlie variation in murine eccrine gland density. To this end, we have used a combination of quantitative trait loci (QTL) mapping and comparative haplotype analysis to identify major effect QTL underlying eccrine gland density variation on murine chromosomes 1 and 9. Significantly, while none of the annotated genes in the candidate QTL regions have been implicated previously in cutaneous appendage development, the human syntenic region of the chromosome 1 QTL has been reported to harbor a QTL underlying Ectodermal Dysplasia, a disease characterized by loss of two or more cutaneous appendages. Our work has identified novel genetic elements specifically controlling variation in eccrine gland density and can ultimately provide insight into the molecular mechanisms responsible for critical evolutionary changes in human skin.

BCL6 CANALIZES THE TRANSCRIPTION OF SELECTED NOTCH TARGET GENES DURING LEFT-RIGHT PATTERNING.

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The Notch signaling pathway is an evolutionally conserved intracellular signaling pathway and regulates downstream responses, such as cell-fate specification, progenitor cell maintenance, boundary formation, cell proliferation and apoptosis. To understand how Notch signaling regulates transcription during embryogenesis, we isolated B-cell leukemia/lymphoma 6 (BCL6), a transcriptional repressor, as a Notch-associated factor by an interaction-based screen. BCL6 maintains both the expression of Pitx2, a left-specific gene, in the left lateral plate mesoderm (LPM) and left-right (LR) asymmetry in *Xenopus*. Importantly, while *Xenopus* Notch signaling positively regulates the expression of Xnr1, a master gene for the left-side determination, at the early developmental stage, it can negatively control the expression of Pitx2 at the later stage. To inhibit this Notch activity during normal development, BCL6 competes with MAM1 for the intracellular domain of Notch1 receptor (NICD) and recruits BCL6 co-repressor (BCoR). Interestingly, the BCL6/BCoR complex shuts down the transcription of selected Notch target genes such as enhancer of split related1 (ESR1), which may not be required for normal development of LPM. For this, BCL6 directly binds the ESR1 genomic locus and inhibits Notch signaling by occupying NICD when it is activated. Taken together, our study shows that BCL6 is a regulator of LR asymmetric patterning and a repressor of Notch signaling during this process. Furthermore, this regulatory mechanism can provide a crucial means by how Notch signaling can control specific cell fate determination or differentiation via only one key DNA-binding transcriptional factor, CSL.

TRANSCRIPTIONAL PROFILING OF 2.5-DIMENSIONAL TUBULOGENESIS

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Hepatocyte growth factor-induced three-dimensional tubulogenesis (3D) is a simple and highly controllable system for studying epithelial tubule initiation and maintenance. However, due to its limited efficiency and asynchronous development, isolating genes associated with specific morphological changes observed during tubule formation has been unfeasible until now. Here we report a significantly enhanced in vitro culture method called 2.5-dimensional tubulogenesis (2.5D). Detailed image analysis of 2.5D has revealed five morphologically distinct stages-monolayer, multicellular apical protrusion (MAP), extension, migration, and tubule, and finds that these stages display in a highly synchronized manner. Using time course transcriptional profiling we collected genes whose expression changes specifically associate with extension and tubule stage. From this data set we seek to identify genes responsible for the tubule formation and understand how the morphological changes contribute to tubule formation.

TRANSGENIC STUDIES REVEAL ESSENTIAL ROLES FOR EMBRYONIC PANCREATIC MESENCHYME

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The pancreas consists of functionally distinct cell types that arise from common precursors located in the pancreatic epithelium during development. While cell culture experiments suggest that proper development and differentiation of epithelial cells depends on critical signals provided by the surrounding mesenchyme, direct evidence for its *in vivo* role, especially at later stages of organogenesis, are limited. Here we study the role of the mesenchymal layer and mesenchymal signals in pancreas development by using a Cre line that allows specific manipulation of gene expression in this cell layer. In the first set of experiments, we employed a Diphtheria toxin-based cell ablation system to deplete the pancreatic mesenchyme at various developmental stages. Our results point to a previously unanticipated requirement for epithelial-mesenchymal interactions during later stages of embryonic pancreas development. In addition, we have performed experiments aimed to manipulate Wnt signaling levels in the pancreatic mesenchyme. Results from these studies show that both increase and decrease in Wnt signaling activity perturb pancreas growth, demonstrating a critical role for regulated Wnt activity during pancreas development. In summary, we provide an *in vivo* proof for the crucial roles mesenchymal cells and signals play in supporting proper pancreas development.

DYNAMIC ANALYSIS OF VERTEBRATE HEART FORMATION

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Heart morphogenesis is a complex process that integrates different structures and cell types compelled to interact by genetic and epigenetic factors. In vivo time-lapse imaging of the processes that underlie organ morphogenesis—cell division, migration and differentiation, or the formation of gene expression patterns—is central to reaching a better understanding of organ development in embryos. Over the past decade uninterrupted, long-term time-lapse imaging of organogenesis over multiple hours or even days has now become feasible. However, in many settings such as the developing cardiovascular system, complications from the rapid motions of the tissues or neighboring tissues have made it difficult or impossible to fully exploit this important research tool. We have developed transgenic quail that express fluorescent proteins as a new model system specifically to improve dynamic analyses of embryogenesis and organogenesis using advanced imaging and analysis techniques.

DYNAMIC EXPRESSION OF NUMB IN DIVIDING CELLS OF THE MOUSE DERMOMYOTOME

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During skeletal myogenesis in the mouse, the paired-box/homeodomain proteins Pax3 and Pax7, together, are necessary for the emergence and survival of muscle stem and progenitor cells. In addition, Pax7 expression identifies the emerging future adult satellite cells during development and into adulthood. The aim of the study is to unveil the mechanisms underlying stem/progenitor cell self-renewal and commitment to differentiation during myogenic embryonic development and post-natally. Based on previous studies on Drosophila and vertebrates, we hypothesized that Numb could be a candidate to regulate the fate and/or self-renewal capacity of embryonic myogenic stem/progenitors.

To investigate the function of Numb during early myogenesis, we generated transgenic mouse lines that overexpress the p66 isoform of Numb preferentially in the medial aspect of the mouse somite, and subsequently the dermomyotome. The dermomyotome, a transitory structure composed of epithelial cells, harbours stem/progenitors for at least five lineages, including skeletal muscle and dorsal dermis. Our studies led us to propose that Numb can regulate the self-renewal of dermal and muscle stem/progenitors during a lineage progression (Jory et al., 2009 Stem Cells, 27 : 2769-2780). To gain further insight into the function of Numb we undertook a detailed characterisation of Numb expression in dividing cells of the dermomyotome. Numb is expressed ubiquitously in small vesicles in all cells. In addition, in dividing cells, Numb accumulates on the apical-lateral cell cortex corresponding partially to the aPKC and Par3 expression domain. This overlapping domain of expression appears to be unique to the dermomyotome cells since in the neural tube Numb and aPKC/Par3 are expressed in adjacent domains. In parallel, we performed live videomicroscopy of the dermomyotome to follow the very dynamic expression of Numb during the cell cycle. Importantly, these studies show that Numb can be either segregated symmetrically or asymmetrically in daughter cells.

TRANSCRIPTOME ANALYSIS OF MOUSE FETAL LIVER PRECURSOR CELLS

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Hepatoblasts are bipotential liver cells that give rise to hepatocytes and cholangiocytes, two cell types that are central to adult liver function. While hepatoblasts are first detected around embryonic day (E) 9 in the mouse, they do not begin their differentiation until 4 days later at E13.5. This implies that hepatoblasts are capable of timing their differentiation to occur at a specific developmental timepoint. Although genes that regulate hepatoblast differentiation have been identified, genes that regulate the timing of differentiation remain largely unknown. To identify these genes, we performed a temporal analysis of the hepatoblast transcriptome. Five stages of liver cells (E10.5, E12.5, E14.5, E16.5 hepatoblasts; and adult hepatocytes) were collected, and serial analysis of gene expression (SAGE) libraries were generated from each cell type. K-means clustering analysis of the five SAGE libraries revealed fourteen gene clusters with distinct temporal expression patterns. Each gene cluster is enriched for specific Gene Ontology categories, indicating the clusters to be biologically significant. Specifically, cell cycle genes are found to be highly expressed at E10.5 and E16.5 but lowly expressed at E12.5 and E14.5. In contrast, Lin28b and Igf2r, two key genes that regulate proliferation, are lowly expressed at E10.5 and E16.5 but highly expressed at E12.5 and E14.5. These data show the cell cycle to be regulated in a stage-specific manner, and suggest possible roles for cell cycle genes in regulating the timing of hepatoblast differentiation during mouse development.

JAW MUSCULARIZATION REQUIRES *DLX* EXPRESSION BY CRANIAL NEURAL CREST CELLS

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The origin of active predation in vertebrates is associated with the rise of three major uniquely derived developmental characters of the head: 1) migratory Cranial Neural Crest Cells (CNCCs) giving rise to most skeletal skull elements; 2) expression of Dlx genes by CNCCs(1) in the Hox-free first pharyngeal arch (PA1)(2) and 3) muscularization of PA1 derivatives(3). Here we show that these three innovations are tightly linked. Expression of *Dlx* genes by CNCCs is not only necessary for head skeletogenesis, but also for the determination, differentiation and patterning of cephalic myogenic mesoderm leading to masticatory muscle formation. In particular, inactivation of *Dlx5* and *Dlx6* in the mouse results in loss of jaw muscles. As *Dlx5/6* are not expressed by the myogenic mesoderm, our findings imply an instructive role for Dlx5/6-positive CNCCs in muscle formation. The defect in muscularization does not result from the loss of mandibular identity observed in *Dlx5/6*$^{-/-}$ mice(4, 5), as masticatory muscles are still present in *EdnRA*$^{-/-}$ mutants, which present a similar jaw transformation. The genesis of jaws and their muscularization should, therefore, be seen as an integrated Dlx-dependent developmental process at the origin of the "New Head"(3). The role of *Dlx* genes in defining gnathostome jaw identity (6, 7) is therefore secondary to a more primitive function in the genesis of the oral skeletomuscular system.

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IN VIVO ECTOPIC EXPRESSION OF SOX2 AND PROX1 IN POSTNATAL COCHLEAR OUTER HAIR CELLS BY REACTIVATING NOTCH SIGNALING

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Mouse auditory epithelium, referred as organ of Corti, contains inner hair cells, outer hair cells and supporting cells, all of which are believed to derive from the same fully undifferentiated progenitor cells. These progenitor cells are pretty homogenous prior to their differentiation programming occurs, whereas after which contrast gene expression profiles including Sox2 and Prox1 are present. Sox2 and Prox1 are expressed only transiently in the differentiating hair cells, but persistently in the developing supporting cells. It is unclear how such differences are maintained. Additionally, little is known of the long term effect if expression of Sox2 and Prox1 are somehow prolonged in hair cells and whether it will affect the normal morphogenesis and functions of hair cells. To give an answer to these two questions, we performed in vivo genetic gain-of-function study of Notch signaling in neonatal hair cells where Notch signaling, expression of Sox2 and Prox1 normally are inactive. Following reactivation of Notch signaling, ectopic Sox2 expression was observed in both inner and outer hair cells, whereas ectopic Prox1 expression is exclusively in outer hair cells. Surprisingly, hair cells with ectopic Notch signaling can survive and maintain hair cell markers, Myosin-VI, Myosin-VIIa, and the outer hair cell marker, Prestin. In addition, the overall morphology of the organ of Cort is normal, and their hearing is normal at 6 weeks of age. Taken together, our data suggested that the rapid down-regulation of Sox2 and Prox1 in the normal differentiating hair cells occurred in a Notch signaling-dependent manner, and that postnatal hair cell could tolerate ectopic activation of Notch signaling. Finally, the restriction of ectopic Prox1 in outer hair cells but not inner hair cells highlighted that Prox1 expression is controlled by not only Notch signaling, Sox2 but also other unknown signals or proteins that have differential effects between inner and outer hair cells.

NOTCH SIGNALING IS REQUIRED TO MAINTAIN A NOVEL NEURAL STEM CELL POPULATION IN HUMAN NEOCORTEX

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Cerebrocortical neurons can be broadly categorized into two classes: excitatory neurons that project to other CNS compartments, and inhibitory neurons that act locally to regulate circuit excitability. Whereas excitatory neurons are produced within the dorsal cortex, rodent studies have established that inhibitory neurons are generated ventrally in the ganglionic eminence (GE) and must migrate substantially to populate the cortex. In the developing human however, it has been reported that inhibitory neurons are generated in both the dorsal and ventral telencephalon, raising the question of what progenitor types are responsible. Unlike the rodent, the developing human cortex contains a massively expanded outer subventricular zone (OSVZ) that is thought to account for the bulk of cortical neurogenesis. However, the progenitor cell types that populate the human SVZ have not been defined. Using post-mortem fetal tissue and molecular markers defined in rodent studies, we have begun to characterize the types and locations of progenitor cells in this region. We find that the OSVZ is populated with a large number of undifferentiated radial glia-like stem cells, as well as more committed progenitor cells that express Ascl1 or Tbr2, neuronal fate determinants that have been shown in rodents to specify inhibitory and excitatory neurons, respectively. We also find an inverse correlation between Notch activation and these fate determinants in cortical progenitors, suggesting that Notch restrains the differentiation of OSVZ neural stem cells. Chemical inhibition of Notch in cortical slices results in rapid differentiation of neural stem cells towards Ascl1+ and Tbr2+ neuronal lineages. We are actively exploring whether these two progenitor cell types are lineally related or represent separate classes that give rise to distinct neuronal subtypes.

THE ROLE OF NONMUSCLE MYOSIN II-B IN MOUSE HEART DEVELOPMENT

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We study the roles of the cytoskeletal proteins nonmuscle myosin (NM) II-A, II-B and II-C in cardiac development. Cardiac myocytes express both NM II-A and II-B from the earliest stages of development. Although the expression of II-A terminates at E8.5 except in the area of the outflow tract, II-B expression continues throughout life but is confined to the intercalated discs following birth. NM II-C expression initiates in the cardiac myocytes at approximately E12 and is also confined to the intercalated discs after birth. Cardiac nonmyocytes continuously express NM II-A and II-B. NM II-B null mice die by E14.5 showing both heart and brain defects. The heart defects include a membranous ventricular septal defect (VSD) and double outlet of the right ventricle (DORV) in which the aorta exits from the right ventricle. To further understand the role of NM II-B in mouse heart development, we generated conditionally ablated mice. Mice ablated for NM II-B using the Nkx2.5 promoter to drive Cre recombinase die as embryos and show impaired cytokinesis of the cardiac myocytes as well as a VSD and abnormalities in coronary vessel formation, similar to NM II-B null mice. However, B^{Nkx}/B^{Nkx} hearts show no defects in alignment of the aorta. Since we do not observe obvious deletion of NM II-B in B^{Nkx}/B^{Nkx} epicardial cells, the defects in coronary vessel formation are most likely due to the abnormal development of the cardiac myocytes. To further understand whether NM II-B is directly involved in coronary vessel development, we ablated NM II-B in epicardial cells using WT-1-cre knock-in mice to generate B^{WT-1}/B^{WT-1} mice. Similar to B^{Nkx}/B^{Nkx} hearts, B^{WT-1}/B^{WT-1} hearts show defects in coronary vessel development but also develop a DORV. Consistent with the contribution of epicardial cells to the cardiac myocyte lineage, we also observe the loss of NM II-B in some cardiac myocytes and defects in cytokinesis in B^{WT-1}/B^{WT-1} myocytes. These results show that ablation of NM II-B in epicardial cells impairs coronary vessel formation during mouse heart development, but does not inhibit differentiation of epicardial cells into cardiac myocytes. The presence of the DORV in B^{WT-1}/B^{WT-1} hearts and its absence in B^{Nkx}/B^{Nkx}hearts suggests a possible contribution of the epicardium to the proper orientation of the aorta during mouse heart development. Finally, mice ablated for NM II-B in both epicardial and myocardial cells using SM22-cre mice develop arrhythmogenic right ventricular cardiomyopathy associated with coronary defects. Our studies reveal critical roles of NM II-B in coronary vascular development.

FOXN1-DEPENDENT THYMIC EPITHELIAL CELL DIFFERENTIATION ORCHESTRATES THE VASCULARIZATION OF THE FETAL THYMUS

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Organogenesis requires the assembly and coordination of multiple cell types, as well as their differentiation and proliferation. We are interested in the molecular control of organogenesis and differentiation of the thymus in mice. The thymus is a specialized microenvironment responsible for the development of self-tolerant and self-restricted T cells. Development of a functional thymus is dependent on crosstalk between thymic epithelial cells (TECs) and lymphoid progenitor cells (LPCs). Crosstalk between the developing vasculature and thymic epithelial cells has also been proposed to contribute to structuring of the thymic architecture. The TEC-specific Foxn1 transcription factor is required at multiple stages for both TEC differentiation and proliferation. We used an allelic series of Foxn1 to define normal thymic vascular development and to investigate a potential role for Foxn1-dependent TEC differentiation in the formation of the thymic vasculature. We show that endothelial cells initially enter the wild-type thymus at E13.5, with PDGFR-β+ mesenchymal cells following at E14.5. These events are delayed by 1-2 days in hypomorphic *Foxn1Δ/Δ* mice, and subsequent vascular development is abnormal, with a 'leaky vessel' phenotype in mutants. VEGF-A and PDGF-B expression were reduced at E13.5 in *Foxn1Δ/Δ* mice compared with controls. In *Foxn1Δ/nu* mice, endothelial cells could not be detected in the thymus until E15.5, and never enter in *Foxn1nu/nu* null mutants. In contrast, the intrathymic vasculature is connected to the main vasculature as early as E14.5 in controls, and this mechanism is not delayed in mutants. Together, these data suggest that Foxn1 is required in TECs to orchestrate the cellular and molecular environment needed for normal thymic vascularization, and may mediate a TEC-mesenchyme-endothelial form of crosstalk required for fetal thymus organogenesis.

INTERPLAY OF WNT AND FGF SIGNALING DETERMINES THE MESENCHYMAL STEM CELL FATE IN SKELETAL DEVELOPMENT AND DISEASE.

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Craniosynostosis affecting one in ~2,500 individuals has been attributed to defects in intramembranous ossification, causing premature suture closure. We have previously linked an evolutionary conserved Wnt signal transduction pathway for the first time to synostosis-related syndromes. Axin2 is essential for suture morphogenesis through modulating a dual function of β-catenin during osteoblast development.

We further investigate the crosstalk of Wnt with another evolutionary conserved Fgf pathway intimately involved in skeletal development and disease. Here we show that the interplay of Wnt and Fgf is essential for stem cell fate determination in calvarial morphogenesis. Disruptions of Axin2 and Fgfr1 in mice induce ectopic chondrogenesis, leading to suture abnormality and fusion. Genetic analyses further reveal that activation of β-catenin is essential for cooperation with Fgfr1 to alter the lineage commitment of mesenchymal stem cells. Using several state of the art mouse genetic models, we demonstrate that Wnt signaling controls the stem cell population whereas its fate is determined by Fgf signaling through modulating a dual role of Bmp signaling in skeletogenesis. This study also reveals that endochondral ossification caused by switching the fate of mesenchymal stem cells is a causal link to craniosynostosis. We have proposed a mechanism underlying the interplay of signal transduction pathways orchestrating the lineage commitment and development of skeletogenic mesenchyme.

Our new data also identify quiescent/slow cycling cells at the putative suture niches responsible for calvarial morphogenesis. These potential stem cells are capable of self renewal and differentiate into highly specialized descendants over an extended period of time. Using a newly developed mesenchymal sphere culture study, we found that the calvarial stem cells divide asymmetrically. Congenital skeletal dysplasia maybe attributed to disruptions of calvarial stem cell properties caused by aberrant Wnt/β-catenin signaling. Surgical repairs to separate the prematurely fused suture are currently used for the disease treatment. However, fusion reoccurrence in the surgically repaired suture has a very high frequency, resulting in multiple rounds of surgery necessary for the patient. Therefore, it is important to build the reconstructed suture in which stem cells are present to prevent the synostosis process. Better understanding of calvarial stem cells may provide new concepts for reconstructive suture surgery and potential molecular and cell based therapies in human diseases.

A ROLE FOR MIR-196 IN PATTERNING THE VERTEBRATE AXIAL SKELETON THROUGH HOX GENE REGULATION

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Patterning of the vertebrate axial skeleton requires precise spatial and temporal control of Hox gene expression during embryonic development. MicroRNAs (miRNAs) are recently described modulators of gene activity, and the miR-196 family of miRNAs have been shown to target several Hox genes. To address the *in vivo* relevance of mir-196 activity, we have performed loss-of-function studies in both chick and mouse. In chick, we have developed protocols for introducing modified antisense oligonucleotides (antagomiRs) *in ovo* and have identified a layer of regulatory control provided by the miR-196 family in defining the boundary of Hox gene expression along the anterior-posterior (A-P) embryonic axis. Following knockdown of miR-196, we observe a homeotic transformation of the last cervical vertebrae toward a thoracic identity. This phenotypic alteration is, in part, due to an anterior expansion of *Hoxb8* gene expression. In mouse, we have replaced individual miR-196 genes with GFP, allowing for the first time an assessment of their unique expression patterns during development and importantly, enabling assessment of individual miR-196 gene function. Together, this work consolidates the *in vivo* relevance of posttranscriptional Hox gene regulation provided by miRNAs in the complex hierarchies governing axial pattering.

FUSION OF THE NEPHRIC DUCT WITH THE CLOACA IS A NOVEL RET-DEPENDENT EVENT CRUCIAL FOR FUNCTION OF THE URINARY OUTFLOW TRACT

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Urinary tract function depends on patent connections between the upper and lower urinary tract. Ureters join the bladder at a stereotypical position which is established during ureter maturation, when the ureter detaches from its original insertion site in the nephric duct, the primordium that gives rise to most of the male genital tract, and moves to the bladder neck. Defects in ureter maturation can result in mal-positioned distal ureters, a major cause of hydronephrosis and renal disease in children.
The first connection between the upper and lower urinary tract occurs on E9, when nephric ducts, paired epithelial tubes that extend along the rostro-caudal embryonic axis, fuse with the cloaca, the primitive bladder. One day later, the ureteric bud begins to form at the base of the nephric ducts. The distal ureteric bud will invade kidney mesenchyme and give rise to the renal collecting duct system; the proximal ureteric bud will form the ureter. At this stage the ureter drains into the common nephric duct (CND), the terminal portion of the nephric duct, however by E14, the ureter has separated from the CND, moved to the bladder and fused with the bladder epithelium, its final insertion site. We showed previously that Ret signaling in CND cells is crucial for epithelial remodeling that drives this process.

Ret-Gdnf signaling has been shown to be a major regulator of urinary tract formation. In humans, mutations in the Ret gene lead to renal malformations and vesicoureteral reflux. In mice, loss of Ret results in renal agenesis, hydronephrosis and megaureter, suggesting that Ret is normally important for ureteric bud formation, for branching morphogenesis within the kidney and for ureter maturation. Our recent studies identify a novel Ret dependent process that is also crucial for urinary tract formation. The majority of Ret mutants display renal agenesis, however the population of mutants that form kidneys display severe hydronephrosis and megaureter. We find that Instead of joining the bladder ureters remained joined to the nephric ducts indicating that ureter maturation has not occurred. In addition, nephric ducts either fail to join the urogenital sinus or join at an abnormal position. Analysis at earlier stages reveals that in Ret mutants, nephric ducts elongate and reach the cloaca, but fail to fuse. Interestingly, in wild type animals, cells at the nephric duct tip display numerous filopodia, suggesting that fusion of the nephric duct with the cloaca may normally depend on adhesion or migration. The observation that filopodia are not evident in the nephric duct tip cells of Ret mutants supports this idea. Together these findings suggest that urinary tract obstruction and hydronephrosis can be caused by abnormalities at a much earlier stage than previously thought, during nephric duct insertion. It would not be surprising if similar defects lead to hydronephrosis in humans.

UNCOVERING THE EMBRYONIC MOLECULAR PLAYERS IN CRANIAL MYOGENESIS

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Research on the myogenesis in the head has been increasingly accumulated in the past decade and a growing number of studies, investigating the role of both intrinsic and extrinsic signals that govern proper specification and differentiation of cranial muscles have emerged. It is well accepted that head muscle progenitors are coordinately regulated by distinct regulatory mechanisms: extrinsic signals from the adjacent tissues (e.g, WNTs, BMPs, FGFs) as well as cell autonomous (intrinsic) regulation by a set of transcription factors (e.g. TBX1, PITX2, MyoR (MSC), Capsulin (TCF21) and ISL1). However, how these two signaling mechanisms regulate head myogenesis remains obscure. The goal of this research is to reveal the molecular mechanisms underlying skeletal muscle development in the vertebrate head using the chick embryo as a model system. Specifically we wish to explore how the combinatorial signals from the non-muscle tissues regulate the shift from proliferating progenitors to differentiating myogenic cells. First, we would like to establish an in vitro system (explant culture) to manipulate myogenesis in the head. Second, we wish to examine whether and how FGF signaling pathway as well as the NF-kB/YY1 module regulate cranial myogenesis. We have carried out a detailed analysis of myogenic gene expression of cultured muscle progenitors, as well as in vivo gene expression analysis by in situ hybridization. The preliminary data demonstrate that the endogenous expression of FGFs decreases coordinately with myogenic differentiation, suggesting a regulatory role for these growth factors in myogenesis. Furthermore, myogenesis in the head is efficiently blocked by FGF signals, possibly through NF-kB/YY1/MSX pathway.

KIDNEY DEVELOPMENT IN THE ABSENCE OF GDNF AND SPRY1 REQUIRES FGF10

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GDNF signaling through the Ret receptor tyrosine kinase (RTK) is required for ureteric bud (UB) branching morphogenesis during kidney development in mice and humans. Furthermore, many other mutant genes that cause renal agenesis exert their effects via the GDNF/RET pathway. Therefore, RET signaling is believed to play a central role in renal organogenesis. Here, we re-examine the extent to which the functions of Gdnf and Ret are unique, by seeking conditions in which a kidney can develop in their absence.
We find that in the absence of the negative regulator Spry1, Gdnf, and Ret are no longer required for extensive kidney development. Gdnf-/-;Spry1-/- or Ret-/-;Spry1-/- double mutants develop large kidneys with normal ureters, highly branched collecting ducts, extensive nephrogenesis, and normal histoarchitecture. However, despite extensive branching, the UB displays alterations in branch spacing, angle, and frequency. UB branching in the absence of Gdnf and Spry1 requires Fgf10 (which normally plays a minor role), as removal of even one copy of Fgf10 in Gdnf-/-;Spry1-/- mutants causes a complete failure of ureter and kidney development. In contrast to Gdnf or Ret mutations, renal agenesis caused by concomitant lack of the transcription factors ETV4 and ETV5 is not rescued by removing Spry1, consistent with their role downstream of both RET and FGFRs.
This shows that, for many aspects of renal development, the balance between positive signaling by RTKs and negative regulation of this signaling by SPRY1 is more critical than the specific role of GDNF. Other signals, including FGF10, can perform many of the functions of GDNF, when SPRY1 is absent. But GDNF/RET signaling has an apparently unique function in determining normal branching pattern. In contrast to GDNF or FGF10, Etv4 and Etv5 represent a critical node in the RTK signaling network that cannot by bypassed by reducing the negative regulation of upstream signals.

SLIT/ROBO1 SIGNALING REGULATES MAMMARY BRANCHING MORPHOGENESIS BY REGULATING MYOEPITHELIAL CELL NUMBER

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Branching morphogenesis is a developmental program that imparts functional complexity to higher organisms. The mouse mammary gland begins as a small, embryonic mass of epithelial cells that later undergoes repeated primary and secondary branching during puberty, giving rise to a bi-layered tubular branching system. The master regulator, TGF-β1, is one of few factors identified as a potent branching inhibitor during tissue growth. We have uncovered a critical role for the SLIT/ROBO1 signaling pathway in inhibiting precocious branching morphogenesis by regulating myoepithelial cell number. Previous data has shown that the expression of receptor ROBO1 is restricted to the cap and myoepithelial cell layer, whereas the ligand, SLIT2, is expressed throughout the epithelium during early mammary morphogenesis. To investigate their function, we transplanted *Slit2-/-;Slit3-/-* or *Robo1-/-* epithelium into precleared immunocompromised hosts. After 4 weeks, whole gland analysis revealed an abundance of secondary and tertiary branches in *Robo1-/-* and *Slit2-/-;Slit3-/-* that is not observed in the *wildtype*. This phenotype is recapitulated in 3-D Matrigel cultures. In dissecting the mechanism underlying the hyperbranching phenotype, we show that TGF-β1 upregulates Robo1 specifically in the myoepithelium through a noncanonical signaling pathway. In vitro branching assays demonstrate enhanced inhibition of branching upon addition of both SLIT2 and TGF-β1 compared to addition of either alone. We also find that SLIT2 treatment significantly decreases proliferation of myoepithelial cells by regulating cyclin-D1, with no affect on luminal epithelial cells. Analysis of the *Robo1-/-* gland shows increased cap cell proliferation in the end bud and multiple layers of myoepithelium along the ducts. To investigate whether myoepithelial cell number, alone, regulates the branching state of the gland, we generated organoids by adding different ratios of myoepithelial and luminal cells. We find that we can recapitulate the *Robo1-/-* branching phenotype with *wildtype* cells in 3-D culture by simply increasing myoepithelial cell number. Taken together, these results suggest that SLIT/ROBO1 signaling controls myoepithelial cell number and that this, in turn, influences branch formation.

CRITICAL FUNCTIONS OF *DICER* IN URETERIC BUD MORPHOGENESIS AND DIFFERENTIATION DURING MAMMALIAN KIDNEY DEVELOPMENT

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microRNAs (miRNAs) are important modulators of the development of a variety of organ and tissue systems. To address their roles in mammalian kidney formation, we specifically ablated Dicer, an enzyme required for the processing and thus functions of all miRNAs, from the ureteric bud (UB) epithelium. In the absence of *Dicer* function, branching morphogenesis at the UB tips terminated prematurely resulting in significant reduction in kidney size and nephron numbers in mutants. Reduction in *Wnt11* and *c-Ret* expression at the mutant UB tips with normal levels of *Gdnf* expression in the metanephric mesenchyme suggests that *Dicer*, and presumably miRNAs, regulate the reception of branching signals in the UB epithelium. Consistent with and extended from a previous report (Pastorelli et al, 2009), we found that cystic dilations initiated at embryonic day (E)15.5, and were observed throughout the UB epithelium-derived collecting duct network in *Dicer* deleted UB mutants. We further showed that excessive cell proliferation and disruption of ciliogenesis preceded and accompanied cyst formation, suggesting that they are likely causally associated with the cystic phenotype. Moreover, terminal differentiation of the collecting duct epithelium appeared compromised from *Dicer* removal. Together, our results demonstrate the critical involvement of *Dicer*, and by inference miRNAs, in normal organization and assembly of the collecting duct network and kidney organogenesis. Our work may also have implications on the pathogenesis of renal anomaly such as renal dysplasia and renal cystic disease.

CYCLIC AMP CONTROLS LUMEN FORMATION IN MAMMARY ACINI BY ACCELERATING APOPTOSIS

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Cyclic AMP (cAMP) is an important second messenger playing a major role in regulation of epithelial cell functions. Depending on cell type, cAMP has been shown to regulate proliferation, apoptosis, vesicular trafficking, ion and water transport and hormone secretion. cAMP is considered to be essential for cultured mammary epithelial cells both in monolayer 2-dimensional (2D) culture and in 3D conditions, where cells are embedded into an extracellular matrix and form solid spheroids or acini with central lumen. However, the role of cAMP in epithelial morphogenesis is unclear. We used 3D culture of MCF10A cells to study the role of cAMP in epithelial morphogenesis.

When grown in a laminin-rich extracellular matrix (Matrigel), MCF10A form spheroids or acini with a hollow lumen; lumen formation is due to the death of centrally-located cells during morphogenesis. The standard culture medium for MCF10A contains cholera toxin, a reagent that elevates cellular cAMP level by direct interaction with cAMP-producing enzymes, adenylyl cyclases. Upon omitting cholera toxin from the medium, a hollow lumen is not formed. Since cAMP is known to accelerate proliferation of mammary epithelial cells, we assessed if the effects of reduced cAMP activations were due to reduced cell proliferation. However, in MCF10A acini expressing HPV 16 E7, an oncoprotein that maintains high levels of proliferation of cells, lumen formation still required cAMP. Strikingly, we observed that the presence of cAMP strongly increased luminal apoptosis in MCF10A acini. This was concomitant with elevated expression of the pro-apoptotic proteins BIM and Bad. Because BIM has been proposed as an essential mediator of luminal cell death in MCF10A 3D acini, we hypothesize that cAMP regulates lumen formation via induction of BIM and possibly other apoptotic proteins. Although previous work indicates that the detachment of MCF10A cells from extracellular matrix is the primary regulator of BIM, our results indicate that this induction at least partially depends on signals downstream of cAMP. In summary, cAMP-dependent signaling represents a parallel, detachment-independent pathway for induction of apoptotic proteins in MCF10A spheroids and thus contributes to lumen formation in mammary epithelial 3D culture.

THE TRANSCRIPTIONAL INTERMEDIARY FACTOR TIF1Γ CONTROLS ERYTHROID CELL FATE BY SPECIFICALLY REGULATING TRANSCRIPTIONAL ELONGATION

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Vertebrate organogenesis is regulated by cell-specific transcription factors, RNA polymerase-associated basal machinery and chromatin remodeling factors. One critical chromatin factor is the transcriptional intermediary factor *TIF1γ*. Loss of *TIF1γ* function in zebrafish mutant *moonshine* causes a profound anemia during embryogenesis, associated with a progressive decrease in expression of most erythroid mRNAs such as GATA1 and globin. *TIF1γ* deficiency has also been linked to TGF-β signaling, although the *in vivo* mechanism for the anemia remains unclear. In an effort to find genes that interact with *TIF1γ*, we undertook a genetic suppressor screen in which we sought mutations in another gene that would restore blood to normal levels in the background of *moonshine* deficiency. Few suppressor screens have been done in vertebrate genetic models, and the haploid genetics of zebrafish was a great advantage for this screen. After screening 800 families of fish, two suppressor mutants,*"eclipse"* and *"sunrise"*, were found that could greatly rescue the erythroid defects in *moonshine*. The deficient gene in *sunrise* has been mapped to the locus of *cdc73* (also known as parafibromin/HRPT2), a subunit of the PAF1 complex known to regulate RNA polymerase II (Pol II) elongation and chromatin modification. Furthermore, we have found that knocking down other subunits in the PAF1 complex also rescued the blood defect in *moonshine*, suggesting that PAF1 as a complex antagonizes *TIF1γ* function during erythropoiesis. This strongly suggests that the erythroid defects in *TIF1γ* deficiency is caused by attenuated Pol II elongation. In an effort to understand the cell-specific phenotype of *TIF1γ* deficiency, we introduced a FLAG tagged *TIF1γ* into K562 erythroleukemia cells to pull down interacting proteins. Physical interactions were found among *TIF1γ*, FACT, p-TEFb and surprisingly the SCL hematopoietic transcription complex. The interaction with the SCL complex provides a cell-specific control over transcriptional elongation. In light of the recent discoveries of widespread Pol II stalling in the promoter proximal region in metazoan genomes, we speculate that similar mechanisms will regulate cell fates in other blood lineages as well as non-blood tissues.

CASTOR FUNCTIONS THROUGH THE DIRECT REGULATION OF EGFL7 AND MIR-126 TO REGULATE VASCULAR INTEGRITY

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We have recently demonstrated a crucial role for the transcription factor CASTOR (CST) in vertebrate vascular development: CST-depleted *Xenopus* embryos fail to develop a functional vascular system and display an aberrant branching pattern. To determine the mechanisms by which CST functions in the developing vasculature, we have gone on to identify the direct transcriptional targets of CST by developing CST specific antibodies and conducting chromatin immunoprecipitation (ChIP) from early stage embryos. Results from these studies show that Egfl7 and its intronic microRNA miR-126 are direct CST targets. Consistent with these findings we demonstrate that Egfl7 and microRNA miR-126 are downregulated in CST depleted embryos and that depletion of either Egfl7 or miR-126 phenocopies the vascular phenotype observed in embryos lacking CST. To determine if the regulation of Egfl7/miR-126 is direct, we cloned the Egfl7/miR-126 locus from *Xenopus* and using ChIP on tissue extract from early stage embryos verified that CST binds to a set of small regulatory element within the Egfl7/miR-126 locus. We further demonstrate that Egfl7 and miR-126 are evolutionarily conserved targets of CST by cloning CST from human, demonstrating that CST is expressed in human endothelial cells, and showing and that shRNA-mediated knock-down of hCST results in a dramatic downregulation of human Egfl7 and miR-126. Collectively these studies demonstrate that CST acts to regulate vascular integrity through the direct regulation of Egfl7/miR-126.

INSTRUCTIVE ROLE OF THE VASCULATURE IN BILE DUCT DEVELOPMENT

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Mutations in the human Notch ligand Jagged1 (Jag1) result in a multisystem disorder called Alagille Syndrome (AGS). AGS, chiefly characterized by a paucity of intrahepatic bile ducts (IHBD), also includes an array of cardiac, ocular, skeletal, craniofacial, and renal defects. The disease penetration and severity of the affected organs can vary significantly and the molecular basis for this broad spectrum of pathology remains unclear. Abnormal IHBD formation in several mutant mouse lines have implicated Jag1 and its receptor Notch2 in this process, but the temporal, spatial, and mechanistic aspects of Notch signaling throughout liver development are not fully understood. Using cell-specific deletion in mice, we report that Jag1 inactivation in the perivascular mesenchyme (PVM), but not the endothelium, selectively leads to the hepatic defects associated with AGS. Jag1 expression in vascular smooth muscle precursors of the PVM surrounding the portal vein is required for bile duct morphogenesis and the final stages of biliary differentiation, revealing an instructive role of the vasculature in liver development. These findings uncover the cellular basis for the defining feature of AGS, identify Notch-Jag1 dependent and independent stages of duct development, and provide mechanistic information for the role of Jag1 in IHBD formation.

NOTCH FUNCTIONS TO REGULATE VENOUS/LYMPHATIC ENDOTHELIAL CELL SPECIFICATION BY SUPPRESSING PROX1.

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Notch signaling is an evolutionarily conserved pathway that functions to modulate cellular responses during bi-potential cell-fate decisions. In the developing blood vasculature, Notch regulates arterial/venous specification and sprouting angiogenesis, by regulating the expression of ephrinB2 and VEGF-receptors. We have found that Notch directly up-regulates the lymphatic endothelial VEGF-receptor, VEGFR-3, suggesting a role for Notch in lymphangiogenesis. In mammals, lymphatic endothelial cells differentiate from the venous endothelium and this requires the transcription factor, Prox1. Prox1 functions as the master regulator of lymphatic endothelial specification and is necessary for the maintainence of lymphatic endothelial cell identity. Lymphangiogenesis begins with the asymmetrical expression of Prox1 in the embryonic cardinal vein. This onset of venous Prox1 expression represents a bi-potential cell fate decision that Notch may regulate. Consistent with this hypothesis, we find that Notch1 signal activation suppressed Prox1 expression in cultured endothelial cells. To explore this finding further, we crossed a mouse carrying a floxed allele of a constitutively active form of Notch1 with the lymphatic endothelial cell driver, Prox1CreERT2. Activation of Notch1 signaling in the Prox1-expressing venous endothelial cells, at a time point when venous/lymphatic endothelial specification occurs, resulted in embryonic lethality. Double transgenic embryos in which Notch signaling was activated displayed edema, dilated and blood-filled lymphatics and reduced lymphatic endothelial Prox1 expression. This Notch1 gain-of-function lymphatic endothelial phenotype was similar to that described for embryos with reduced levels of endothelial Prox1. Thus, we propose that Notch signaling functions in early lymphatic endothelial specification to inhibit Prox1 expression and restrict the number of venous cells that differentiated into lymphatic endothelial cells.

ROLE OF DLL4/NOTCH AND SYNECTIN-DEPENDENT SIGNALING IN LYMPHATIC DEVELOPMENT

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Despite its importance in health and disease, the molecular basis of the specification and outgrowth of the lymphatic system remains poorly understood. Lymphatic endothelial cells originate from venous blood endothelial cells and Prox-1 and recently also Sox18, were shown to play a key role in this process. Remarkably, despite their venous origin, formation of lymph vessels also requires a number of molecular players, which are exclusively related to arterial blood vessel development such as the forkhead transcription factors Foxc1 and Foxc2 as well as ephrhinB2. Recently, we have revealed an important role in lymphatic development for two additional 'arterializing' signaling pathways: synectin (GIPC1)-dependent signaling and Dll4/Notch signaling .

The PDZ domain-containing scaffold protein synectin (GIPC1) acts as an important selective regulator of arterial vascular growth. Morpholino knockdown of synectin in zebrafish impaired formation of the first perfused lymph vessel in the trunk, the thoracic duct (TD). In control embryos, the TD arises from parachordal lymphangioblast cells, which in turn derive from secondary lymphangiogenic sprouts from the posterior cardinal vein (PCV). Silencing of synectin impaired the formation of these lymphangiogenic sprouts, while angiogenic sprouting from the PCV was not affected. Correspondingly, knockdown of synectin in Xenopus laevis tadpoles resulted in an under-development of Prox-1^{+} lymphatic ECs and lymphatic morphogenesis defects.

The Notch signaling pathway is well known for its involvement in the differentiation and wiring of the blood vascular network. Recently, we revealed for the first time a role of Notch signaling in the development of the lymphatic system in zebrafish embryos by selecting chemical and genetic silencing strategies to avoid vascular defects. In zebrafish embryos, inhibition of Notch signaling by a gamma-secretase inhibitor or by knockdown of presenilin-1 (PS-1), the Notch ligand Dll4 or the Notch-1b/6 receptors all impaired lymphangiogenesis at two levels. First, Dll4/Notch silencing reduced the fraction of lymphangiogenic sprouts from the PCV; instead, angiogenic sprouts were formed. Also, activation of Notch in venous endothelial cells upregulated lymphatic markers in vitro. Second, silencing of Notch signaling impaired navigation of lymphatic intersomitic vessels along their arterial templates. These studies imply critical roles for Notch signaling and synectin-dependent signaling in the formation and wiring of the early lymphatic network.

TRANSCRIPTIONAL CONTROL OF EPITHELIAL MORPHOGENESIS

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Morphogenesis of many epithelial organs involves the thickening and bending of cell sheets by polarized changes in cell shape. Shroom family proteins have emerged as essential upstream regulators of these processes in vertebrate embryos. Shroom3 is essential for apical constriction and apicobasal cell elongation in many epithelial tissues, including the neural plate, the gut, and the lens of the eye. Surprisingly, Shroom3 is in fact sufficient to induce these subcellular behaviors in naïve cells. Ectopic Shroom3 simultaneously drives actin-based apical constriction and microtubule-based cell elongation. These changes in cell behavior do not require new transcription, so transcriptional control of Shroom3 itself must be a key node for patterning epithelial morphogenesis in vertebrates. We have now identified the Pitx family of transcription factors as direct activators of transcription from the Shroom3 locus. We find that inhibition of Pitx1 disrupts Shroom3 expression, cell shape changes, and morphogenesis in the gut. Moreover, though they do not activate transcription of any known Shroom3 effector, Pitx proteins can induce dramatic Shroom3-dependent cell shape changes. Thus, we have identified a surprisingly simple transcriptional system that is sufficient to initiate epithelial morphogenesis in vertebrate embryos.

WISE/SOSTDC1 MODULATES WNT SIGNALING TO CONTROL TOOTH NUMBER AND PATTERNING

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Mice carrying mutations in *Wise/Sostdc1* display abnormalities in tooth number, size and cusp patterning. We have performed genetic interaction and molecular studies to investigate signaling pathways modulated by Wise in tooth development. We observed that inactivation of *Wise* leads to elevated Wnt signaling and that reducing the dosage of the Wnt co-receptor genes, *Lrp5* and *Lrp6*, rescues the *Wise*-null tooth phenotypes, indicating that Wise inhibits Wnt signaling *in vivo*. In the mutant, rudimentary or vestigial tooth buds in the normally toothless diastema region display increased proliferation and continuous development to form supernumerary teeth. Our gene expression analyses reveal that the FGF and Shh pathways are major downstream targets of the Wise-regulated Wnt signaling and that in turn Shh acts as a negative regulator of Wnt signaling. Gain-of-function studies show that ectopic Wise reduces Wnt signaling and tooth number and demonstrate a temporal requirement for Wise during tooth development. These data provide insight into the mechanisms that control Wnt signaling in tooth development and into how cross-talk among signaling pathways controls tooth number and growth.

UNCOVERING THE CELLULAR ORIGIN OF MERKEL CELLS DURING EMBRYONIC DEVELOPMENT AND ADULT HOMEOSTASIS

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Merkel cells are located in the touch sensitive area of the epidermis and mediate mechanotransduction in the skin. Whether Merkel cells originate from embryonic epidermal or neural crest progenitors has been a matter of intense controversy since their discovery over 130 years ago. In addition, how Merkel cells are maintained during adulthood is currently unknown. Here, using lineage tracing experiments, we show that MCs arise through the differentiation of epidermal progenitors during embryonic development. In adults, MCs undergo slow turnover, and are replaced by cells originating from epidermal stem cells, not through proliferation of differentiated MCs. Conditional deletion of the Atoh1/Math1 transcription factor in epidermal progenitors results in the absence of MCs in all body locations including the whisker region. Our study demonstrates that MCs arise from the epidermis by an Atoh1 dependent mechanism and opens new avenues for study of MC functions in sensory perception, neuroendocrine signaling and Merkel cell carcinoma.

PARASYMPATHETIC INNERVATION MAINTAINS EPITHELIAL PROGENITOR CELLS DURING SUBMANDIBULAR GLAND ORGANOGENESIS

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The parasympathetic ganglion (PSG) is essential for adult submandibular gland (SMG) secretion and regeneration. The embryonic SMG is also innervated by the PSG, with the nerves and the epithelium undergoing parallel development. However, it is unclear whether the PSG is required for glandular development. We report that parasympathetic innervation during early embryonic development (E12-E13) of the submandibular gland epithelium is required for normal morphogenesis through the maintenance of the basal keratin 5-positive progenitor cell population. Using organotypic recombination experiments to remove the parasympathetic ganglion in embryonic SMG explant culture we found epithelial branching was significantly reduced in the absence of the PSG. Inhibition of the acetylcholine muscarinic receptor 1 (M1) expressed by the SMG epithelium using the muscarinic inhibitor 4-DAMP or siRNA knockdown of M1 decreased epithelial morphogenesis. Analysis of gene expression in recombined SMG explants cultured with or without the PSG and intact SMG explants cultured in 4-DAMP showed a significant reduction in several markers of epithelial progenitor cells: keratin 5, keratin 15, and Aquaporin 3, suggesting that acetylcholine/M1 signaling regulated SMG progenitor cells. FACS analysis and immunohistochemistry further confirmed the decrease in keratin 5 protein expression with loss of PSG function. In gain of function studies, addition of the acetylcholine analogue carbachol (CCh) increased epithelial branching and keratin 5 expression in epithelial rudiment cultured in laminin, and importantly, rescued epithelial morphogenesis and keratin-5 expression in recombined PSG-free explants. Rescue of both morphogenesis and keratin 5 expression was dependent upon transactivation of EGFR. Therefore, we conclude that the PSG is essential for SMG development by maintaining the keratin 5 expressing progenitor cell population through an ACh/M1/EGFR signaling pathway in the epithelium.

SCRIBBLE IS REQUIRED FOR LUMEN MAINTENANCE IN THE MAMMALIAN LUNG

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Lung organogenesis involves formation of a network of epithelial tubes with an extensive surface area that can support postnatal respiration. The establishment and maintenance of a central lumen is a key step in tubulogenesis. Each epithelial tube must have a centrally located lumen through which transport of liquids or gases can occur and lumen diameter must be carefully regulated to enable optimal organ function.
Studies have shown that cell polarity, cell-cell adhesion and formation of a uniformly localised apical membrane are required to form and maintain a normal lumen. The PDZ domain protein Scribble has a role in establishing apical-basal polarity and is required for maintenance of tight junctions in Drosophila. Mammalian *Scribble* also plays a role in cell adhesion and directed cell migration and thus we sort to determine the role of *Scribble* in lung development and lumen formation by studying the mouse mutant *Circletail*, which carries a loss of function mutation in *Scribble*.
Scribble^{Crc} lungs appear smaller, with fewer, malformed branches compared to littermates. Quantification revealed a 46% reduction in the number of 'normal' airways that display an organised lumen in *Scribble*^{Crc} compared with controls.
Immunostaining with anti-pan-cytokeratin and DAPI, highlighted severely disorganised airways in *Scribble*^{Crc} homozygotes, with epithelium almost indistinguishable from the surrounding mesenchyme. In agreement with previous studies, both adherens junctions and tight junctions are disrupted in *Scribble*^{Crc} lung epithelial airways, yet apical-basal polarity appears largely unaffected. Moreover, real-time imaging of ex vivo lungs following Morpholino knockdown of Scribble revealed considerable shifting of individual cells in relation to one another in comparison to controls, where cells maintain contact with their neighbours. This data is consistent with the idea that Scribble regulates epithelial cell contacts.
We therefore propose that mutations in *Scribble* lead to defects in lumen formation as a result of disrupted cell adhesion and loss of cell-cell contacts during epithelial tube formation.

GENETIC CONTROL OF AIRWAY MORPHOGENESIS

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The sizes and shapes of the epithelial tubes that comprise organs such as lung, kidney, and vasculature are critical for their function. We investigated the mechanisms that control airway morphogenesis and found that early in lung development, airway shape is a function of the orientation of planar cell division. In normal airways, a large proportion of epithelial cell divisions are oriented parallel to the airway longitudinal axis, whereas this distribution is randomized when RAS-regulated ERK1/2 signaling is increased, leading to shorter and wider airways. We have developed a mathematical model that predicts epithelial tube shape from the distribution of mitotic spindle angles during development, and show that the abnormal shapes of airways in which ERK1/2 signaling is increased can be accounted for by the observed alterations in mitotic spindle orientation. Our data reveal that regulating ERK1/2 signaling is essential to ensure appropriate oriented planar cell division, and demonstrate the importance of the negative regulators of this signaling pathway that are encoded by the Sprouty genes for maintaining the normal airway morphogenesis program.

RTK SIGNALLING REGULATES LIVER BUD MORPHOGENESIS IN ZEBRAFISH

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The formation of a functional organ is the result of multiple coordinated processes, including progenitor specification, differentiation, proliferation and morphogenesis. While the molecular details of specification and differentiation are emerging for various organs, our understanding of their morphogenesis is often very poor. We use zebrafish as a model to study the morphological and molecular aspects of liver bud morphogenesis. Hepatoblasts are initially located ventrally in the intestinal rod, but form subsequently a liver bud in an anterior position on the left side of the rod. Using lineage-labelling, we show that posteriorly located hepatoblasts move anteriorly to contribute to the nascent liver bud. Moreover, differences and changes in cell-shape support this hypothesis. These data further indicate that hepatoblasts exhibit different cellular behaviours depending on their position within the developing bud.
Ephrin/Eph signalling is known to control cell migration, cell affinity and maintenance of boundaries, as well as proliferation in many tissues. *ephrinB1* is one of the first genes specifically expressed in the newly-specified hepatoblasts. Embryos depleted of EphrinB1 exhibit defects in liver bud formation, both the anterior as well as the leftward displacement of cells is defective. These defects result in a dysmorphic liver and extrahepatic duct. Mosaic expression of dominant-negative constructs is used to dissect the specific contributions of EphrinB1 reverse and Eph forward signalling. Furthermore, we are using loss-of-function studies to identify the Eph receptor interacting with EphrinB1 in liver bud formation. We propose that EphrinB1 signalling is required for liver morphogenesis, controlling timely anterior as well as leftward movement of hepatoblasts to form the nascent organ bud.

VISININ LIKE 1 IS A NEW URETERIC TIP-SPECIFIC MOLECULE THAT MAY ACT AS AN INTERPLAYER BETWEEN GDNF, FGF AND CANONICAL WNT SIGNALING DURING KIDNEY BRANCHING MORPHOGENESIS

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Glial-cell-line-derived neurotrophic factor (GDNF) binding to its co-receptor GDNF family receptor alpha-1 (GFRα1) and signaling through Ret receptor tyrosine kinase is adequate to promote ureteric bud outgrowth, and is required for the formation and proliferation of the tip cells needed for the subsequent branching of the ureteric tree.
Here we report the expression of new ureteric tip marker gene visinin-like 1/vilip1 that encodes for a neuronal calcium sensor protein vsnl1/vilip1. Vilip1 is one of the unique proteins with a mosaic cellular expression pattern in the ureteric tip epithelium.
We show that in the absence of GDNF/Ret signaling, vilip1 is missing, but the protein is again activated in Gdnf null ureteric buds rescued by exogenous GDNF or by FGF7/Follistatin. Hence, vilip1 is specific for the induced ureteric epithelium regardless of the inducer. This finding is further supported by the expression of vilip1 in GDNF downstream target Wnt11 deficient mouse kidneys. Furthermore, vilip1 is downregulated in β-catenin stabilized mouse kidneys with ureteric branching morphogenesis defect and may have an antagonistic effect on the β-catenin stabilization during kidney branching morphogenesis.
Based on our results, we suggest that vilip1 is a new ureteric epithelium tip specific marker that could act as an interplayer between GDNF, FGF and WNT signaling in the ureteric epithelium during kidney branching morphogenesis.

CREB MEDIATES BRAIN-DERIVED SEROTONIN REGULATION OF BONE MASS ACCRUAL

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Serotonin is a bioamine synthesized in neurons of the brainstem and in enterochromaffin cells of the duodenum that does not cross the blood brain barrier. In the brain, serotonin is a well characterized neurotransmitter affecting cognitive functions whereas in the periphery, it is thought to act locally in the gut and at a distance as a hormone whose spectrum of functions only begins to be delineated. Besides its well known role in influencing cognitive functions brain-derived serotonin emerged recently as a molecule regulating, centrally, three homeostatic functions, bone remodeling, appetite and energy expenditure. Briefly, following its binding to the Htr2c receptor in neurons of the ventromedial hypothalamus (VMH) nuclei serotonin favors bone mass accrual, while following its binding to the Htr1a and Htr2b receptors in neurons of the arcuate hypothalamus nuclei, serotonin favors appetite and decreases energy expenditure. Recently, gut-derived serotonin was described as an inhibitor of bone formation by acting directly on osteoblasts. Gut-derived serotonin binds to the Htr1b receptor present on osteoblasts and through a PKA-dependent pathway uses cAMP response element binding protein (CREB) as a transcriptional mediator to inhibit osteoblast proliferation.
CREB is a broadly expressed leucine zipper-containing transcription factor implicated in the control of differentiation and proliferation of multiple cell types. Although it acts in many different cellular contexts CREB is a major regulator of multiple aspects of neurobiology such as neuron survival, axon growth and synaptic transmission. In this context it is important to note that CREB activation is a cAMP/PKA-dependent or a Ca2+/Calmodulin-dependent kinase (CaMK) process. These roles of CREB in neurobiology together with the fact that it mediates the function of gut-derived serotonin raised the testable hypothesis that CREB could also mediate some of the homeostatic functions of brain derived-serotonin.
We show here, through cell-based assays and cell-specific gene inactivation studies, that brain derived-serotonin uses calmodulin-dependent kinases that are expressed in VMH neurons and calmodulin as second messenger downstream of the Htr2c receptor. We further show that CREB mediates brain-derived serotonin regulation of bone mass accrual. These data along with the one already gathered identify CREB as the main effector of the regulation of bone mass by serotonin, regardless its site of synthesis and of action. As such they further underscore the role of this transcription factor in regulating bone mass.

TBX4 AND *WNT2* IN ALLANTOIS VASCULAR DEVELOPMENT

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Targeted inactivation of the T-box family gene *Tbx4* results in failure of chorioallantoic fusion and lethality at E10.5. *Tbx4* homozygous mutant allantoises are stunted and apoptotic, have normal endothelial cell fate specification but do not form blood vessels *in vivo* or *in vitro*. The mRNA expression pattern of *Tbx4* in whole mount allantois and lineage trace has shown that endothelial cells which line the fetal vessels of the placenta have never expressed *Tbx4*. To investigate this non-cell autonomous effect of *Tbx4* on endothelial cells we analyzed extracellular matrix and looked for secreted morphogens that could be affected in *Tbx4* mutant allantoises. *Wnt2*, which is a member of the canonical wnt signaling family was absent in *Tbx4* mutant allantoises. Both lithium chloride, an agonist of the canonical wnt signaling pathway and Wnt2 conditioned media rescued endothelial tube formation in the allantois *in vitro*. Versican, an extracellular matrix component, was expressed in the allantois and has been shown to be downstream of canonical wnt signaling in other organs. This molecule was absent in *Tbx4* mutant allantoises. We hypothesize that *Tbx4* acts upstream of canonical wnt signaling which subsequently controls expression of matrix molecules and other molecular pathways to regulate blood vessel formation in the developing allantois.

AN EPITHELIAL-ENDOTHELIAL CROSSTALK REGULATES EXOCRINE DIFFERENTIATION IN DEVELOPING PANCREAS

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Endothelial cells are required to initiate pancreas development from the endoderm. They also control the function of endocrine islets after birth. Here, we investigate in developing pancreas how the endothelial cells become organized during branching morphogenesis and how their development impacts on exocrine cell differentiation. We show that endothelial cells closely surround the epithelium at the pancreatic bud stage. During branching morphogenesis, the endothelial cells become preferentially located near the central (trunk) epithelial cells and remain at a distance from the branch tips where acinar cells differentiate. This correlates with predominant expression of the angiogenic factor Vascular Endothelial Growth Factor-A (VEGF-A) in trunk cells. In vivo ablation of VEGF-A expression by pancreas-specific inactivation of floxed Vegfa alleles results in reduced endothelial development and in excessive exocrine development. Treatment of embryonic day 12.5 explants with VEGF-A or with VEGF receptor antagonists indicates that exocrine development is tightly controlled by endothelial cells. We also provide evidence that endothelial cells repress the expression of Ptf1a, a transcription factor essential for acinar cell differentiation, while not affecting known signaling mechanisms involved in development of exocrine cells. In explants, VEGF-A signaling is also required, but not sufficient, to induce endocrine differentiation. In conclusion, our data suggest that, in developing pancreas, epithelial production of VEGF-A determines the spatial organization of endothelial cells which, in turn, limit exocrine differentiation of the epithelium.

SHROOM3 AND N-CADHERIN COOPERATIVELY FUNCTION TO ASYMMETRICALLY ALTER CELL SHAPE CHANGES DURING GUT MORPHOGENESIS

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The cytoskeletal protein Shroom3 is a potent inducer of epithelial cell shape change and is required for murine embryonic morphogenesis of the lens and neural plate. Analysis of gut morphogenesis of Shroom3 deficient mouse embryos revealed that the direction of gut rotation is also disrupted. In control embryos, the proximal end of the mid-gut rotates in a clockwise direction while the mid-gut of mutant embryos rotates counter-clockwise. This phenotype is comparable to mouse embryos deficient for Pitx2, a transcription factor known to regulate left-right asymmetry during gut morphogenesis. It was recently established that Pitx2-dependent, asymmetrical cellular behaviors in the dorsal mesentery (DM) of the early mid-gut, a structure connecting the gut-tube to the rest of the embryo, contribute to the direction of gut rotation in chicken embryos by influencing the direction of the dorsal mesenteric tilt. Asymmetric cell shapes in the DM epithelium are hypothesized to contribute to the tilt, however, it is unclear what lies downstream of Pitx2 to alter epithelial cell shape. The cells of the left DM epithelium in both Pitx2 and Shroom3 deficient embryos are shorter and wider compared to control embryos and resemble the shape of those on the right, demonstrating that like Pitx2, Shroom3 is required for cell shape asymmetry and the leftward DM tilt. It was also determined that the Shroom3 dependent cell shape change in the left epithelium of the DM is associated with elevated levels of apical F-actin and Myosin II. Shroom3 is expressed throughout the midgut but its expression is not altered in the absence of Pitx2. However, N-cadherin expression is specific to the left side and is Pitx2 dependent. To determine if Shroom3 and N-cadherin function together to regulate cell shape in the left DM epithelium, mouse embryos lacking one copy each of Shroom3 and N-cadherin were generated. Similar to Pitx2 and Shroom3 null embryos doubly heterozygous mice possess thinner and shorter epithelial DM cells on the left side suggesting that these molecules function jointly during morphogenesis. Together these data provide evidence that Shroom3 and N-cadherin cooperatively function downstream of Pitx2 to directly regulate cell shape changes necessary for early gut tube morphogenesis.

SHROOM3 INDUCED APICAL CONSTRICTION IS DEPENDENT ON THE RHOA ACTIVATING DOMAIN OF THE GUANINE EXCHANGE FACTOR TRIO

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Early embryonic lens morphogenesis is, in part, driven by epithelial cell shape changes in the lens placode. The placodal cells change from a columnar to a conical shape causing invagination of the lens pit in a process that is dependent on the cytoskeletal protein Shroom3. This cell shape change, termed apical constriction (AC), occurs when the apical circumference of an epithelial cell is reduced via Rho-kinase (Rock1/2) dependent activation of non-muscle myosins and their contraction of the apical actin-myosin-junctional complexes. Analysis of chicken embryos treated with a Rock1/2 chemical inhibitor revealed that invagination of the lens pit requires Rock1/2 function. Although it has been established that the ability of Shroom3 to induce AC is Rho-kinase dependent, it is unclear how Shroom3 activates Rock1/2. To investigate this, a cell culture-based AC assay was used to determine that Shroom3 induced AC is dependent on RhoA, a Rock1/2 activator, and Trio, a guanine exchange factor (GEF) that activates RhoA. Furthermore, these assays demonstrated that the AC ability of Shroom3 is enhanced by the co-expression of Trio. To determine if Trio is required for AC during lens morphogenesis, a peptide inhibitor of the RhoA GEF domain of Trio (Tripα) ectopically expressed in the lens pit of chicken embryos. Lens pit cells expressing Tripα do not undergo AC, however, AC does occur in the lens pit when they simultaneously express Trio and constitutively active RhoA. Together these data support a role for Trio in mediating the RhoA/Rock1/2 dependent activity of Shroom3 during AC of the cells within the invaginating lens pit.

INTERPLAY BETWEEN WNT2 AND WNT2BB CONTROLS MULTIPLE STEPS OF EARLY FOREGUT-DERIVED ORGAN DEVELOPMENT

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The vertebrate liver, pancreas and lung originate in close proximity from the multipotent foregut endoderm, raising central questions regarding the identity of the signals governing their development and as to how they are coordinated.
Multiple studies, including mosaic analysis, have revealed that interactions between the endoderm and the adjacent lateral plate mesoderm (LPM) are essential for digestive system development. Interestingly, different components of the Wnt signaling pathway are expressed in discrete domains in the LPM. While requirements for Wnt/β-catenin signalling in endodermal organogenesis have been shown, little is known about the respective Wnt ligands and their specific functions. Previous work showed that zebrafish *wnt2bb*, bilaterally expressed in the LPM, is required for liver specification. *wnt2bb/prometheus* mutants form a small liver, suggesting that additional factors act in liver specification.
We demonstrate that *wnt2*, is specifically required for newly-specified hepatoblasts proliferation and can functionally compensate for the loss of *wnt2bb* in liver specification, since depletion of both ligands leads to liver agenesis. In addition, *wnt2bb*, subsequent to its role in specification, cooperates with *wnt2* in promoting hepatoblast proliferation. Consistently, *wnt2* and *wnt2bb* overexpression is sufficient to induce a striking liver expansion at the expense of pancreatic tissue. Hence, precisely localized expression of Wnt ligands in the LPM is important to maintain a balance between liver and pancreas progenitors. Moreover, lack of both ligands causes agenesis of the swim bladder, the structural homolog of the mammalian lung, uncovering two essential developmental regulators.
Our study suggest that specific and partially overlapping functions of *wnt2* and *wnt2bb* provide robustness for the development of vital organs, highlighting the importance of an intricate Wnt signalling network in coordinating endodermal organogenesis.

ABL FAMILY TYROSINE KINASES IN GLIA ARE ESSENTIAL FOR BASEMENT MEMBRANE INTEGRITY AND CORTICAL LAMINATION IN THE CEREBELLUM

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The cortical basement membrane, forming between radial glial endfeet and the meninges, is a network of ECM proteins secreted by meningeal fibroblasts. Accumulating evidence suggests that basement membrane is instrumental in regulating corticogenesis. Mutations in genes encoding basement membrane components (laminin α5 or γ1, perlecan), laminin receptors (α-dystroglycan, integrin β1 or α6), and integrin-associated signaling molecules (FAK or ILK) leads to local breaches of basement membrane and cortical dysplasia. These cortical malformations resemble cobblestone (type II) lissencephaly found in some forms of congenital muscular dystrophy. The genes responsible for these syndromes are several known or putative glycosyltransferases that potentially regulate glycosylation of α-dystroglycan, which is essential for its maturation into a laminin-binding form.

Abl-family non-receptor tyrosine kinases consist of Abl and Arg (Abl-related gene) in vertebrates. Their function in transducing signals from cell surface receptors into cytoskeletal reorganization is well established. To investigate their role in brain development, we generated CNS-specific Abl knockout mice on an Arg null background. Abl/Arg deficiency in the brain results in local disruptions of cerebellar basement membrane at two developmental stages, including at E15 in the anterior/medial cerebellar primordium and after P8 in the rest of the cerebellum. The loss of basement membrane leads to anterior cerebellar patterning defects, and granule cell ectopia and hypoplasia throughout the cerebellum. The fiber networks of cerebellar radial glia and Bergmann glia are both disorganized and their endfeet are protruded beyond the pial surface, coinciding with regions of broken basement membrane. Furthermore, ablation of Abl-family kinases specifically in granule cells does not cause any defect indicating that deletion of Abl/Arg from glia is required for the abnormalities. Consistently, Abl-family kinases are not essential for granule cell proliferation and migration in vitro. These observations establish Abl-family kinases as critical signaling components in the regulation of basement membrane integrity during cerebellum development. Moreover, defective Abl-family kinases-mediated signaling may underlie some aspects of the pathology of cobblestone lissencephaly.

ROLE OF C-MYC AND P21 IN VASCULAR REGRESSION

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The hyaloid vasculature is one of the temporary capillary networks of the developing eye and is an excellent model system to study scheduled vascular regression. We have previously demonstrated that macrophages play a key role in inducing cell death in vascular endothelial cells in a cell cycle dependent manner. Our data suggested that the cell cycle related proteins c-myc and p21might play a role in hyaloid regression. In this study we have analyzed the effects of c-myc and p21 deletion on hyaloid vessel regression. Using the Pdgfcre^{ERT2}, a tamoxifen induced vascular specific Cre, we were able to delete both c-myc and p21 in the vasculature. Given the established roles of the oncoprotein c-myc and tumor suppressor p21, we were surprised to find that deletion of either c-myc or p21 results in a persistent hyaloid vasculature. The combined heterozygotes of c-myc and p21 have a much stronger phenotype then either heterozygotes alone, thus suggesting that these two proteins function in the same pathway that ultimately results in the death of vascular endothelial cells. In the c-myc conditional mutants as expected, loss of c-myc, a transcriptional inhibitor of p21, results in an increase in p21 transcripts. However, in the hyaloid vasculature of the p21 conditional mutants the c-myc transcript levels are higher than wild type suggesting that there exists a feedback loop between these two proteins. We are currently analyzing the functional relationship between c-myc and p21 to understand their role in the death of the vascular endothelial cells. The results from this study will help to further our understanding of the interaction between these two proteins and their role in cell cycle progression and thus help us to design new anti-angiogenic therapies

MIR-200C REGULATES FGF AND REELIN/VLDLR SIGNALING DURING EPITHELIAL BRANCHING MORPHOGENESIS

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Multiple epithelial organs such as salivary glands develop by branching morphogenesis, which requires regulated gene expression that leads to epithelial proliferation and differentiation into end buds and ducts. Non-coding microRNAs (miRNAs) are post-transcriptional regulators of gene expression involved in developmental processes such as proliferation and cell fate determination. Nevertheless, the identity and function of miRNAs in regulating these processes during branching morphogenesis remain unclear. In this study, we detect the miRNAs expressed during embryonic mouse sumbandibular gland (SMG) development, and propose a role for miR-200c in regulating epithelial branching morphogenesis. We screened the expression of miRNAs in intact embryonic SMG, the epithelium separated from the mesenchyme, and the epithelial end buds separated from the primary duct. We detected 36 epithelial-specific and 85 mesenchymal-specific miRNAs, and miR-200c showed a discrete epithelial end bud expression. Screening of miRNA function using antagomirs showed that blocking miR-200c function increased epithelial branching during SMG morphogenesis. We performed miR-200c target prediction studies, and confirmed that Hs3st1 is targeted, and discovered that Vldlr and proliferation-related genes Dusp1, Asap1 and Rlf, are also targeted. Surprisingly, the mesenchymal and miR-200c-targeted gene Reln is upregulated in the epithelium when using a miR-200c antagomir in ex vivo organ cultures. Cdh1 and a progenitor cell marker krt5 were downregulated, whereas FGF- and beta-catenin-related genes were upregulated, and epithelial end bud proliferation increased after miR-200c inhibition. These findings indicate that miR-200c regulates FGF and Reelin/Vldlr signaling, and beta-catenin gene expression downstream to these signaling pathways during SMG morphogenesis.

ROLES OF NEPHRON LINEAGE-DERIVED *DICER* AND MIRNAS IN KIDNEY DEVELOPMENT

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MicroRNAs (miRNAs) have been shown to play critical regulatory roles in the development of several mammalian organ systems. Dicer is an essential enzyme for the biogenesis of all miRNAs. Ablation of its function blocks miRNA maturation and thus their functions. To address the roles of nephron lineage-expressed *Dicer* and miRNAs in kidney development, we specifically ablated *Dicer* function from the nephron tissues (referred to as Dicer nephron mutants) with a *Six2TGC* driver line, which expresses an EGFP:Cre fusion protein from the *Six2* promoter in the nephron progenitors, a population of stem-like cells giving rise to all epithelial components of the nephron. This genetic manipulation led to elevated apoptosis in nephron progenitors and the developing nephron epithelium, and a premature termination of nephrogenesis. Interestingly, the expression of *Six2*, a nephron progenitor gene essential for maintenance of the undifferentiated state and survival of nephron progenitors, was significantly reduced prior to the onset of the loss of nephron progenitors. Our work demonstrates that *Dicer* action, and presumably that of miRNAs, is essential for maintaining the viability of the critical self-renewing nephron progenitor pool and the nephron epithelium, and consequently, development of a normal nephron complement of the kidney.

STAGE-DEPENDENT ROLE FOR BONE MORPHOGENETIC PROTEIN SIGNALING IN THE DEVELOPING MOUSE ESOPHAGUS AND FORESTOMACH

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Bone morphogenetic protein (Bmp) signaling has been implicated in the development of multiple vertebrate organ systems. For example, we and others have shown that Bmp signaling is critical for the separation of the anterior foregut into trachea and esophagus and for the subsequent development of the trachea. By contrast, little is known about its role in the later development of the esophagus and in the formation of the sharp boundary between the forestomach, which like the esophagus has a stratified squamous epithelium, and the glandular hindstomach. To address these issues we first examined the distribution of canonical Bmp signaling in the relevant tissues during development using a Bmp reporter mouse line harboring a *BRE-lacZ* allele. We also used in situ hybridization to localize the temporal and spatial expression of transcripts for Bmp signaling components, including different antagonists. Our initial findings suggested a two-stage Bmp signaling model in the developing esophagus/forestomach from embryonic day (E) 11.5 to postnatal (P) day 5. During the first stage (E11.5~E15.5), suppression of Bmp signaling is required for the conversion of the simple columnar epithelium ($p63^{low}$, cytokeratin 8^{hi}, cytokeratin 14^{neg}) to a multiple-layered but undifferentiated epithelium ($p63^{hi}$, $Krt8^{low}$ and $Krt14^{pos}$). Subsequently, (>E15.5) active Bmp signaling is required for the differentiation and keratinization of the suprabasal cells and restriction of p63 to the basal layer. To test this hypothesis we exploited a *Shh-Cre* allele that drives recombination in the embryonic foregut epithelium to generate either gain-or-loss-of-function models for the Bmpr1a (Alk3) receptor. In *Shh-Cre;Rosa26*$^{CAG\text{-}loxpstoploxp\text{-}caBmpRIa}$ embryos high levels of ectopic Bmp signaling stall the transition from simple columnar to multilayered undifferentiated epithelium, both in the esophagus and forestomach. This results in the persistence of a columnar monolayered epithelium that fails to express p63 and Krt14 and has strong Krt8 expression. In loss-of-function experiments, conditional deletion of Bmp receptor in *Shh-Cre;Bmpr1a*$^{flox/flox}$ embryos allows the formation of a multilayered squamous epithelium but this fails to differentiate, as shown by absence of expression of suprabasal markers, including loricrin and involucrin. Our findings shed new light on the role on Bmp signaling in esophagus and forestomach development and potentially provide new insight into associated human diseases.

UNDERSTANDING THE MECHANISMS DOWNSTREAM OF *WNT7B* ACTION IN RENAL MEDULLA FORMATION

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The renal medulla is essential for urine concentration and thus body salt and water homeostasis. Despite its role in renal physiological function, the mechanisms governing collecting duct elongation and renal medulla formation are not well understood. *Wnt7b* has been shown to mediate renal medulla formation, most likely through directing oriented cell division in the prospective medullary collecting ducts. *Wnt7b* activates the canonical β-catenin pathway in the neighboring interstitial cells, but the mechanism whereby the interstitial cells mediate *Wnt7b* action is unknown. *p57Kip2*, a cyclin-dependent kinase inhibitor expressed in both the medullary interstitium and in podocytes, has also been shown to cause a renal medulla defect when ablated. Whether the interstitial *p57Kip2* is a direct target of canonical Wnt signaling and mediates in part *Wnt7b* function in renal medulla development is under investigation. The results of these studies will be reported.

RETINOIC ACID DEFICIENCY DISRUPTS MORPHOLOGICAL AND MOLECULAR ASPECTS OF HEART FORMATION

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In humans, congenital heart defects occur in approximately 1% of live births. In mice, heart malformations are observed in numerous mutant strains. The high incidence of cardiac defects in mice and humans reflects the extreme sensitivity of the developing mammalian heart to perturbation by genetic and environmental influences. Transcriptional regulation by retinoic acid, the active metabolite of Vitamin A, is one of the key pathways regulating embryonic development. Perturbation of retinoid levels, either excess or insufficiency, results in a variety malformations of the heart, including defects in formation of the outflow tract and atria. The fact that proper retinoid levels are essential for cardiac development is well established, however, to date, the molecular and morphological events regulated by retinoic acid during heart formation remain largely unknown.

We previously identified, from a recessive mutagenesis screen, a mutant mouse line with severely reduced embryonic synthesis of retinoic acid. The retinoid deficiency of this line was caused by a mutation in the gene encoding the short chain reductase enzyme RDH10. Characterization of mutant embryos from the *Rdh10* mutant line demonstrated, for the first time, that RDH10 was the primary enzyme required for the initial step in conversion of Vitamin A into retinoic acid and revealed a new nodal point in synthesis of this important transcriptional regulator.

Here we characterize the molecular and morphological changes associated with abnormal cardiac development in retinoid-deficient *Rdh10* mutant embryos. We show that *Rdh10* mutant embryos have impaired septation of the outflow tract, resulting in persistent truncus arteriosus. We find the retinoid-deficient defects in outflow tract septation are preceded by abnormal cell adhesion and abnormal distribution of the adhesion molecule NCAM1 in the endocardial layer of the conotruncus. Further, we demonstrate that retinoid-deficiency results in disruption of anterior-posterior patterning of heart progenitors, as assessed by altered expression of *Hox* gene family members. Surprisingly, *Hox* expression changes within the mesodermal cardiac progenitor domain are not posterior shifts as expected based on well-established retinoid regulation of *Hox* genes within the neural tube. Instead, within the mesoderm, including the cardiac progenitor mesoderm, retinoid-deficiency results in anterior shifts in expression of *Hox* family members. These data reveal morphological and molecular aspects of heart formation regulated by retinoic acid.

THE AVIAN MESONEPHROS AS A "NEW" MODEL SYSTEM FOR STUDYING KIDNEY ORGANOGENESIS

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The basic functional filtration unit of the vertebrate kidney is the nephron. Three types of kidney tissue are formed during embryogenesis: pronephros, mesonephros and (in amniotes) metanephros. All three kidneys are comprised of nephrons, but they differ significantly in terms of the number of nephrons they contain and in the complexity of their arrangement. Historically, the most utilized systems for studying vertebrate kidney formation have been the mammalian metanephros and the anamniote pronephros. While these continue to yield fundamental information about nephrogenesis, particularly regarding the genetics of kidney formation, each has important limitations. Non-genetic in vivo manipulation of the developing mammalian kidney is difficult, and the pronephroi of zebrafish and Xenopus, because of their simple structure, are less useful for studying the organization of nephrons into complex kidneys.

In order to address some of these limitations, we have begun to develop the avian mesonephros as a complementary system for studying kidney development. The tubules of the chicken mesonephros undergo the same stages of differentiation as the mammalian metanephros, including Mesenchymal to Epithelial Transition (MET), and comma- and S-shaped bodies, and contain well-developed glomeruli and tubules. Mesonephric tubules differentiate in a rostral-caudal sequence, allowing one to examine multiple differentiation stages in the same embryo. Molecularly, we find that the mesonephros expresses, in homologous locations, almost all of the markers that have been used to characterize the mammalian metanephros, including Osr1, Pax2, Lim1, Wt1, Eya1, Wnt4, cRet, Sim1, Wnt9b, Vegf, podocin, and region-specific tubular markers. Taking advantage of the ease of manipulating the avian embryo in vivo, we blocked the posterior migration of the nephric duct, thus preventing it from interacting with mesonephric mesenchyme. Under these conditions, a set of early transcription factors is activated and maintained normally in the mesonephric mesenchyme, including Osr1, Pax2, and Eya1, but activation of tubule differentiation markers, including Wnt4 and Lim1, does not occur. These experiments allow us to distinguish two distinct phases of kidney differentiation: an early duct-independent phase, and a later duct-dependent stage. Ongoing experiments are using cell transplantation and ectopic gene expression to characterize in vivo the precise roles of Wnt and other signaling pathways in mediating the duct-dependent phase of kidney induction. We are also using this system to investigate nephron patterning on the proximal-distal (glomerular-tubular) axis.

REGULATION OF ORGAN SIZE: DUAL ROLE OF PBX IN THE CONTROL OF SPLEEN EXPANSION BY ACTIVATION OF NKX2.5 AND REPRESSION OF P15INK4B.

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A basic question that has long fascinated developmental biologists is how growth is regulated during development to produce organs of specific sizes. Cell proliferation is an essential process that mediates organ growth and morphogenesis during vertebrate development. Here, we discuss the roles of Pbx homeoproteins in the regulation of mammalian growth and organ size, with implications for both development and disease conditions. We use the spleen as a model system, exploiting mouse models deficient for the transcription factors Pbx1, Hox11 (Tlx1), Nkx2.5, Nkx3.2 (Bapx1), and Pod1 (Capsulin), which are either asplenic or exhibit hyposplenia. From our previous studies, Pbx1 has emerged as a central, hierarchical co-regulator of spleen organogenesis. By generating a conditional Pbx1 loss-of-function in the mouse splenic anlage, by using Nkx2.5-, as well as Wt1-Cre deleter lines, we found that Pbx1 removal in the spleen mesenchyme causes dramatic morphological and growth defects of the organ, including marked downregulation of Nkx2.5. Also, by RT-PCR profiler array analyses, we discovered that p15ink4b (of the INK4 family of cell cycle inhibitors) is dramatically up-regulated in the hypoplastic spleens of Pbx1Δex3/Δex3;Nkx2.5-Cre+ mice. Chromatin immunoprecipitations and transcriptional assays indicate that Pbx1 regulates p15Ink4b expression in the spleen mesenchyme. Crosses of p15ink4b-deficient mice with spleen-specific Pbx1-deficient mice demonstrate that the impaired spleen expansion in the latter mice is rescued by removing p15ink4b function in vivo. Indeed, Pbx1Δex3/Δex3; Nkx2.5-Cre+ mice that are also deficient for p15ink4b develop spleens that are significantly larger and more compact than those present in spleen-specific Pbx1 mutants with intact p15ink4b function. The spleens of the latter mice are significantly smaller and present as multiple, minuscule, unjoined splenules of irregular morphology. Stereology on spleen size, counts of cell density, and in vivo proliferation assays have been conducted on spleens from Pbx1Δex3/Δex3;Nkx2.5-Cre+ and Pbx1Δex3/Δex3;Nkx2.5-Cre+; p15ink4b+/- as well as Pbx1Δex3/Δex3;Nkx2.5-Cre+;p15ink4b-/- embryos. “Rescued” spleens from embryos that lack both Pbx1 and p15ink4b exhibit rates of cellular proliferation that are higher than those present in spleens from embryos lacking Pbx1 alone in the spleen mesenchyme, and are similar to those of wild type spleens. These results highlight the essential role of Pbx in mammalian organ expansion in vivo, via direct regulation of the Cdk inhibitor p15ink4b, a validated biological mediator of cell proliferation in the embryonic spleen.

SEAHORSE (*SEH*): A NOVEL *FGFR2* MUTATION DISRUPTING EMBRYONIC XY GONAD DEVELOPMENT.

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In mammals, the gonadal sex of an individual is determined by the presence or absence of the Y chromosome and the associated activity of SRY during early male gonad development. In addition to SRY, a number of autosomal and X-linked gene products are also required for development of testes and ovaries in males and females, respectively. Amongst these are fibroblast growth factor 9 (FGF9) and its gonadal receptor, FGFR2. Mice lacking *Fgf9* die at birth from severe lung defects and exhibit embryonic XY gonadal sex reversal. Mice lacking *Fgfr2* die before sex determination due to extraembryonic defects, but conditional ablation of *Fgfr2* in the developing gonad results in XY gonadal sex reversal. The seahorse (*Seh*) mutation was identified in a forward genetic screen for loci controlling embryonic gonad development in the mouse (Bogani et al 2009 *PLoS Biol* 7(9):e1000196). On a mixed genetic background *Seh/Seh* embryos lack limbs and exhibit rudimentary lungs. XY embryonic gonads show defects in testis development at 14.5 days *post coitum* (dpc), including disrupted or absent testis cords. When backcrossed onto C57BL/6J, XY homozygous mutants exhibit an ovarian morphology at 14.5 dpc. *Seh* maps to distal chromosome 7 and its critical region contains the *Fgfr2* gene. Sequencing of *Fgfr2* revealed a mis-sense mutation in the *Seh* allele. We will describe gonad development in *Seh/Seh* embryos in more detail in an attempt to define the molecular effects of *Seh* allele and potentially shed new light on FGF signalling and its role in testis determination.

PDX1 TRANSCRIPTION FACTOR IS CRITICAL FOR PANCREATIC EPITHELIUM REGENERATION

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Pdx1(pancreatic and duodenal homebox 1) transcription factor is a critical regulator of pancreas development. Genetic ablation of pdx-1 in mouse results in complete blockade of pancreas formation and postnatal lethality. Although Pdx-1 has been characterized as a master regulator of β cell function, its role in exocrine pancreas is largely unknown despite its broad expression. In order to address this central question, we employ a transgenic mouse model that allows the conditional doxycycline (DOX)-dependent suppression of Pdx1 in adult animals. Doxycycline was administrated to 6-8 week old mice in drinking water for 14 consecutive days, and no histological change of exocrine pancreas was observed in the pdx1 off mice in comparison to control (pdx1 on) mice. When the mice were treated with cerulein (cholecystokinin analogue) to induce an acute pancreatitis, no discrepancy was seen between pdx1 off and control mice with regards to edema, cellular necrosis and digestive enzyme releasing. These finding suggests that pdx1 is dispensable for the maintenance of exocrine pancreatic tissue architecture, cell survival and enzyme production. However, in a cerulein induced chronic pancreatitis model, the pdx1 off pancreata exhibited more profound tissue damage and fibrosis with less residual epithelium in comparison to the control pancreata. In order to address whether pdx1 is critical for epithelium regeneration after chronic injury, we replenished pdx1 by withdrawal doxycycline after cerulein treatment. Indeed, the exocrine pancreatic epithelium with re-expressed pdx1 was able to regenerate itself, exhibiting minimal tissue damage, fibrosis and abundant epithelial tissue. In addition, we also found that heterozygous loss of pdx-1 is sufficient to induce more advanced tumors in a pancreatic cancer model (LSL-Kras; p48Cre model). Taken together, this evidence supports a model where pdx-1 primes the exocrine pancreatic epithelial cells for differentiation, thereby promoting adult epithelium regeneration after injury. Conversely, loss of pdx-1 prevents this regeneration and enriches progenitor-cell-like population which is more susceptible to oncogenic Kras induced tumorigenesis.

THE NUCLEAR HORMONE RECEPTOR COUP-TFII IS REQUIRED FOR THE INITIATION AND MAINTENANCE OF PROX1 EXPRESSION IN LYMPHATIC ENDOTHELIAL CELLS

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The homeobox gene *Prox1* is crucial for mammalian lymphatic vascular development. In the absence of Prox1, lymphatic endothelial cells (LECs) are not specified. The maintenance of LEC identity also requires the constant expression of Prox1. However, the mechanisms controlling the expression of this gene in LECs remain poorly understood. The SRY-related gene *Sox18* is required to induce Prox1 expression in venous LEC progenitors. Although Sox18 is also expressed in embryonic arteries, these vessels do not express Prox1, nor do they give rise to LECs. This finding suggests that some venous endothelial cell–specific factor is required for the activation of *Prox1*. Here we demonstrate that the nuclear hormone receptor Coup-TFII is necessary for the activation of *Prox1* in embryonic veins by directly binding a conserved DNA domain in the regulatory region of *Prox1*. In addition, we show that the direct interaction between Coup-TFII and Prox1 is necessary for the maintenance of *Prox1* expression in differentiating LECs.

AN ENU SCREEN REVEALS NOVEL GENES REQUIRED FOR MAMMALIAN FOREBRAIN DEVELOPMENT

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The forebrain is responsible for many higher order cognitive functions. Relatively few genes have been shown to be specifically required for cortical patterning, and we suggest that such genes can be discovered using a phenotype-driven ENU mutagenesis analysis. To assess the most sensitive and efficient method for mutant detection, a recent iteration of this screen employed three components: a traditional phenotypic screen complemented by histological analysis, use of a reporter allele, and use of a sensitizing allele (*Lis1*). Using this forward genetic approach, we have ascertained seven mutations affecting CNS development and have thus far identified most by positional cloning. Mutants obtained in our screen show phenotypes such as cortical hypocellularity, disrupted cortical patterning, hydrocephaly, anterior encephalocele, and craniorachischisis.

The development of rich resources for querying brain development has facilitated the further application of this forward genetic approach. We have recently initiated a new screen employing reporter alleles that enable us to focus on identifying mutants with perturbed cortical lamination and axon guidance. Specifically, we are using *Rgs4-lacZ*, which is expressed in cortical layers 2/3 and 5, to detect defects in both superficial and deep cortical layers. We are also using a *TAG-1-tau-lacZ* reporter to label developing axons. Preliminary analyses suggest we have mutants that affect both forebrain cortical layer formation and axon tract formation.

The most remarkable phenotype uncovered to date is the *rudolph* mutation with severe developmental defects in both the CNS and appendicular skeleton. The organization of the neocortex is profoundly disrupted and contains clustered cell bodies/ neurogenic foci. The causal gene is the cholesterol biosynthesis enzyme *Hsd17b7*, which is notable given the recent implication of a role for oxysterols in mediating intracellular components of Hedgehog signaling. We see decreased induction of known Sonic hedgehog (*Shh*) target genes in the cortex, retina and skeleton. *In vitro*, this mutation results in decreased cellular response to Shh, revealing a requirement for embryonic cholesterol metabolism in both CNS development and normal Shh signaling. Notably, we find that introduction of a mutation which results in *Shh* ligand-independent upregulation of hedgehog signaling (as a result of a mutation in *Ttc21b*) ameliorates the mutant phenotype. This result supports an evolving model in which intracellular cholesterol synthesis mediates activity of *Smoothened*; a hypothesis we are exploring further in the rudolph mutant.

REGULATION OF PANCREATIC ISLET CELL FATES

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Pancreatic islet cell development and differentiation is coordinately regulated by many well-characterized transcription factors. Nkx2.2 is a homeodomain regulatory factor required for the appropriate differentiation of pancreatic endocrine cells. Nkx2.2 null mice lack all insulin-producing beta cells and have reduced numbers of glucagon-producing alpha cells. In place of these cell types, the mutant islet is populated with cells that produce another hormone, ghrelin. To understand how Nkx2.2 regulates islet cell fate decisions during organogenesis, we are exploring the molecular activity of Nkx2.2 in the developing pancreas. We have demonstrated that Nkx2.2 predominantly acts as a repressor to initiate the formation of alpha cells and immature beta cells. Furthermore, we have determined that the TN domain of Nkx2.2 interacts with Grg3, a co-repressor protein expressed in the pancreatic islets. We have now generated mice carrying mutations in the putative Grg3-interaction domain of Nkx2.2 and the SD domain which defines the NK2 family. Phenotypic analysis of these Nkx2.2 mutant mice is allowing us to define the respective functions of Nkx2.2 in specifying lineage choice in the pancreatic islet. These studies have also demonstrated that Nkx2.2 is required for the maintenance of the beta cell fate.

THE ROLE OF FOLLISTATIN-LIKE 1 IN EARLY SKELETAL DEVELOPMENT.

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Follistatin-like 1 (Fstl1) is a secreted protein first identified in an osteoblastic cell line as a factor induced by Tgf-β. In situ hybridization analysis has shown that Fstl1 mRNA is broadly expressed in adult and embryonic tissue. Fstl1 has been shown to play a role in different biological and pathological processes such as dorso-ventral axis establishment in zebrafish, inflammation, angiogenesis, and cardiac regeneration in mice. Based on the presence of a domain homologous to follistatin, Fstl1 was classified as a potential Tgf-β superfamily inhibitor. Although, in DV-axis determination, Fstl1 has proven to play a role redundant to other Tgf-β superfamily inhibitors, a direct inhibitory effect of Fstl1 on any of these family members has not been shown. Recently Fstl1 has been shown to induce PI3 mediated-Akt phosphorylation via the receptor disco-interacting protein2A.
In our lab a Fstl1 knock out (KO) mouse was generated. Although KO embryos were isolated in a mendelian ratio , KO pubs die at birth due to respiratory distress. Histological evaluation of E18.5 embryos revealed hypoplastic and scarce tracheal rings, most-probably underlying the neonatal respiratory distress. Whole-mount Alcian Blue/Alizarin Red staining revealed multiple skeletal defects such as bowing of the long bones, absence of the patella and hypoplastic and bifid cervical vertebrae. Analysis of younger embryos revealed long bone defects as early as E13.5. The expression patterns of Prrx1, Sox9, Col2A1 and Col10A1 (chondrogenic hallmarks) were found to be similar in WT, HET and KO embryos from E12.5 onward. The total amount, proliferation and apoptosis of chondrocyte precursors in E11.5 to E13.5 limb buds is currently evaluated.
In vitro assays, using a Bmp-responsive element reporter, showed no inhibitory effect of Fstl1 on Bmp4 and Bmp7 mediated Smad signaling. Other Tgf-β super-family members are currently investigated. The recent findings that Fstl1 can bind and signal via Dip2a cannot explain the phenotype observed in the KO mice directly since Dip2a is expressed only in some parts of the neuro-ectoderm. The expression patterns of its highly homologous family members, Dip2b and Dip2c, are unknown and investigated by in situ hybridisation and qRT-PCR.
Decades of research have identified several growth factor families involved in skeletal development. Here we demonstrate that a new protein, that is secreted, is involved in early skeletal development, which might represent a novel signaling pathway involved in chondrogenesis.

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SKELETAL MUSCLE STEM CELLS IN DEVELOPMENT AND REGENERATION

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The ontogeny of skeletal muscle occurs in an unusual fashion, with an anlage being established, then future founder stem cells that are Pax7-positive emerge to assure continued embryonic and postnatal growth (1, 2). We showed that multiple stem cell populations defined by anatomical location and genetic requirements characterise this tissue. In the body, *Pax3* acts in a pathway complementary to the core determination genes *Myf5*, and *Mrf4*, upstream of Myod. In contrast, *Tbx1*, but not *Pax3*, complements the *Mrfs* for pharyngeal muscle development (3). Surprisingly, extraocular muscles have dispensed with a complementary pathway as they have an obligate requirement for *Myf5* and *Mrf4*.

To characterise further these regulatory relationships, we have carried out specific genetic ablation and lineage tracing experiments using Cre expressing knock-in lines. We find that different strategies are adopted in different anatomical locations (head vs. body) for the emergence of future muscle stem cell populations. In more general terms, we find that different reporter mice ($R26R^{STOP//lacZ}$, $Pax7^{GFP\text{-}Puro//nlacZ}$), yield distinct read-outs, thus underscoring the importance of exercising caution when interpreting some genetic lineage studies. To investigate the molecular nature of muscle stem/progenitors, we generated a developmental series of microarrays using *Tg:Pax7-nGFP* mice. Our analysis shows that profound changes in molecular signature occur in embryonic, fœtal and postnatal skeletal muscle stem/progenitor cells, including in diverse signalling pathways. As such novel regulators displaying spatiotemporal specifity have been identified.

During perinatal growth emerging satellite cells resolve and give rise to the adult skeletal muscle satellite population. To address the issue of heterogeneity of satellite cells, we compared at the molecular level, satellite cells from different locations as well as satellite cells within a single muscle group. Two types of heterogeneity are observed. The first reflects in part the ontological molecular signature observed during development that was related to anatomical location. The second reflects gene expression differences between subpopulations of satellite cells. These differences will be discussed in the context of heterogeneity arising stocastically, how skeletal muscle is established, and how stem/progenitors behave functionally in transplantation assays during regeneration.

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BILATERAL OVEREXPRESSION OF LEFT-SIDED LEFTOVER DISRUPTS HABENULAR ASYMMETRIES

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The zebrafish epithalamus, composed of the pineal complex and paired habenular nuclei, has emerged as the premier model for the study of asymmetric neural development in vertebrates. The left and right habenulae differ in both morphology (greater density of neuropil and synapses on the left) and gene expression patterns.

leftover (lov/kctd12.1) is expressed asymmetrically, predominantly in the large left habenula, but its molecular role in habenular development remains unclear. Leftover protein consists of a tetramerization domain and a domain of unknown function connected by a disordered loop region. Using the Gal4/UAS sytem, we were able to overexpress Lov protein in both habenulae. To our surprise, bilateral overexpression of normally left-sided Lov inhibited asymmetrical morphological and molecular markers in the left habenula, while the right habenula remained largely unaffected. Simultaneously, yeast-2-hybrid interaction screening identified intracellular trafficking molecule Unc-51-Like Kinase 2 (ULK2) as a potential binding partner of Lov. A direct interaction between Lov and the central proline-serine-rich domain of ULK2 has been confirmed by yeast 2-hybrid assay and in vitro co-immunoprecipitation. Additionally, Lov and ULK2 are coexpressed in habenular neurons and GFP:ULK2 fusion protein colocalizes with Lov in habenular processes. Knockdown of ULK2 levels by morpholino injection can also phenocopy Lov overexpression larvae.

Taken together, our data suggests that Lov can influence habenulogenesis by negative regulation of intracellular protein trafficking ULK2. We plan to test this hypothesis by driving expression of a kinase-dead dominant negative form of ULK2 in developing habenular neurons. Also, we plan to analyze the morphological consequences of disruption of this regulatory system by labeling individual habenular neurons in morphant and Lov overexpression contexts.

QUANTIFYING MEDULLARY ORGANIZATION IN THE DEVELOPING THYMUS

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The postnatal mammalian thymus is responsible for the development and proliferation of T-cells. Although the developmental mechanisms that control thymus organogenesis and postnatal function are poorly understood, several genes have been identified which affect thymic development. Among these is the Foxn1 gene, which is required for thymic epithelial cell (TEC) differentiation, and whose postnatal down regulation in mice has been shown to cause rapid involution and decreased thymic epithelial cell production. Thus, Foxn1 is required both for fetal organ development and to maintain the postnatal thymus, although its exact role is not understood. It has previously been reported, that transgenic mice overexpressing Cyclin D1 (K5CyclinD1) in TECs experience runaway thymic growth and do not undergo age-asociated thymic degeneration, or involution. The crossing of these two lines of mice results in a thymus with neither phenotype predominating, with normalized thymus size and timing of involution, but with stromal organization resembling the K5CyclinD1 transgenics. When analyzing thymic phenotypes, relative organization is a crucial factor in analyzing the development of the organ; immunostaining for region-specific markers has been used to mark and visualize TEC's specific to the medullary or cortical regions of the thymus. However, beyond a superficial qualitative analysis of these images, no method for measuring the organ's organization has been developed. We are using a cross correlation function relating the location of several different TEC subtypes that can successfully quantify the organization of the medullary region of the thymus. This information allows the quantitative analysis of a significant aspect of the developing and aging thymus by filling a statistical void, and therefore allows rigorous analysis of tissue organization.

MYOCARDIAL-ENDOCARDIAL INTERACTIONS DURING HEART TUBE MORPHOGENESIS IN *ZEBRAFISH*.

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The vertebrate heart is of great interest in the field of developmental biology as a model to explore organ formation and function. Moreover, its study yields greater understanding of the molecular nature of hereditary cardiovascular diseases. The assembly of the primitive heart tube requires coordinated asymmetrical cell migration and reorganization of myocardial- and endocardial tissues. Recent studies on cardiac tube formation mainly focused on the role and regulation of myocardial cell movement behavior, but only little is known about the cell biology and contribution of endocardial cells. Using time lapse confocal imaging, we examined the migrational properties of myocardial and endocardial cell layers during cardiac tube morphogenesis. We find that endocardial cell migration is highly dynamic and precedes the migration of myocardial cells. By studying mutants with impaired myocardium or lacking endocardial cells, we observed that both cells types are independently able to respond to asymmetric guidance cues. However, cell movement measurements suggest a mutual interdependency, as deduced from their respective migratory speed and distance. These findings provide new insights into the molecular cross-talk of myocardial and endocardial tissues during heart tube morphogenesis. Since Nodal and BMP pathways are known to play an essential role in controlling heart tube development, future studies will focus on whole-transcriptome microarray expression analyses for Nodal/BMP-regulated effector genes to better understand how collective cardiac motility is controlled on the single cell level.

PATTERNING OF EPITHELIAL ORGAN MORPHOGENESIS BY PITX TRANSCRIPTION FACTORS AND THE SHROOM FAMILY PROTEINS.

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Morphogenesis of many epithelial organs involves the thickening and bending of cell sheets by polarized changes in cell shape. Shroom family proteins have emerged as essential upstream regulators of these processes in vertebrate embryos. Shroom3 is essential for apical constriction and apicobasal cell elongation in many epithelial tissues, including the neural plate and the gut. Surprisingly, Shroom3 is also sufficient to induce these subcellular events in naïve cells. Ectopic Shroom3 simultaneously drives actin-based apical constriction and microtubule-based cell elongation. These changes in cell behavior do not require new transcription, so transcriptional control of Shroom3 itself must be a key node for patterning epithelial morphogenesis in vertebrates. We have now identified the Pitx family of transcription factors as direct activators of transcription from the Shroom3 locus. We find that inhibition of Pitx1 disrupts Shroom3 expression, cell shape changes, and morphogenesis in the gut. Moreover, Pitx proteins can induce dramatic Shroom3-dependent cell shape changes in epithelial cells, even though they do not activate transcription of any of the known Shroom3 effector proteins. Thus, we have identified a surprisingly simple transcriptional system that is sufficient to initiate epithelial morphogenesis in vertebrates.

GENETIC MARKING AND TRACING OF LATERAL AND MEDIAL HEPATIC AND PANCREATIC PROGENITORS

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We found that the liver emerges from two separated domains of endoderm, medial-ventral and lateral (Tremblay & Zaret, 2005), and it has been known that the pancreas emerges from two separated domains of endoderm, medial-dorsal and lateral, but functional difference in the descendants of these different progenitor populations for each tissue have not been explored. Specifically, we recently showed that different signaling induces medial-ventral versus lateral liver progenitors (Wandzioch & Zaret, 2009) and many previous studies showed that different genes and signals induce medial-dorsal and lateral pancreas progenitors. We are interested in developing genetic tools to lineage-trace descendants of these different populations and determine their respective functionality in tissue development and adult regeneration. To address this question we used Laser Capture Microdissection (LCM) followed by microarray analysis, and discovered genes specifically expressed in medial and lateral endoderm. We have generated transgenic mice with BACs of lateral and medial-specific genes driving the expression of tamoxifen inducible CRE; *Pyy*-CRE-MCM and *Gsc*-CRE-MCM, respectively. By crossing these mice to the ROSA26 reporter strain, the descendants of respective progenitor pool can be traced throughout the development. Our results indicate that *Pyy*-CRE labels hepatic and ventral, but not dorsal, pancreatic progenitors, while *Gsc*-CRE labels medial hepatic progenitors and dorsal, but not ventral pancreatic progenitors. Moreover, the labeled descendants in the developing liver appear to be expressed in different cell populations. We are now using this system to selectively ablate medial and lateral liver descendant cells, and ventral and dorsal pancreatic descendant cells, and examine their respective contributions to tissue function and regeneration.

LOSS OF PROX1 ACTIVITY PREDISPOSES MICE TO PANCREATITIS

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The mammalian pancreas consists of two distinct glandular tissue types: endocrine and exocrine, which are responsible for glucose homeostasis and food digestion, respectively. An intricate system of ducts is embedded within the exocrine tissue, and functions to collect and deliver digestive enzymes to the duodenum. In contrast to endocrine and acinar cell development, our current knowledge of pancreatic ductal development remains very limited. Prox1, a homeodomain transcription factor, is widely expressed during mouse embryogenesis and in adult tissues, and is critical for proper development of several organs, including the liver, heart, and lymphatic system. Our studies revealed that in the developing murine pancreas Prox1 is broadly expressed in multipotent progenitors and in the endocrine and ductal lineages, but not in differentiating or mature acinar cells. To investigate the role of Prox1 during pancreas development, we specifically deleted this gene in pancreatic progenitors of $Prox1^{\Delta Panc}$ mice. We found that the pancreatic ducts of $Prox1^{\Delta Panc}$ embryos and newborns had enlarged lumens and were tortuous. In the pancreata of embryonic and postnatal $Prox1^{\Delta Panc}$ mice, the intralobular ducts contained a cuboidal epithelial lining instead low-cuboidal or flattened cells, and their terminal portions harbored unusual cells co-expressing both ductal and exocrine markers. Starting at around P10, numerous acinar cells died by apoptosis in $Prox1^{\Delta Panc}$ pancreata, and in extracts of these tissues we detected intrapancreatic activation of Carboxypeptidase A. In the pancreas of older $Prox1^{\Delta Panc}$ mice we observed accumulation of stromal cells, interstitial fibrosis and immune infiltrates. All these defects indicate that $Prox1^{\Delta Panc}$ mice develop acute pancreatitis. Microarray data identified increased expression of *Claudin2* (a gene encoding a tight junction protein) in $Prox1^{\Delta Panc}$ pancreatic samples. Immunohistochemical analyses confirmed up-regulation of Claudin2 specifically in the ductal epithelium in $Prox1^{\Delta Panc}$ pancreata. Claudin2 has been shown to generate a “leaky” epithelium, and more importantly, elevated levels of Claudin2 positively correlated with increasing severity of inflammatory bowel disease. We hypothesize that impaired function of the pancreatic ducts could be the primary cause of pancreatitis in $Prox1^{\Delta Panc}$ mice. The results of this analysis conclusively demonstrate that Prox1 is a novel, crucial regulator of pancreatic ductal development. We also propose that proper regulation of Claudin2 levels is critical to maintain normal ductal physiology in the pancreas.

A GENE REGULATORY NETWORK FOR PARAXIAL MESODERM FORMATION AND DIFFERENTIATION.

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The formation of the trunk paraxial mesoderm from mesodermal progenitor cells involves a tightly controlled interaction of inductive events and the establishment of cell type specific transcriptional programmes.
Wnt signalling in the caudal end of the extending trunk induces this mesodermal progenitors. The subsequent formation and patterning of these cells to paraxial mesoderm is controlled sequentially by the transcription factors Brachyury (T), Tbx6 and Msgn1. In a next step, the paraxial mesoderm is segmented. This process involves a morphogen gradient of Wnt3a and Fgf8, and the segmentation clock comprised of three interacting oscillators drived by Wnt, FGF and Notch-Delta signalling, followed by somite formation. In order to establish a gene regulatoy network of this process, transcriptional profiles of caudal ends derived from 8 to 12 somite stage mutant embryos (TS12-13) of Wnt3a, T, Tbx6 and Dll1 knock-out lines were analysed in comparison to wildtype embryos. By using a SELEX approach, binding motives of TCF/LEF, Tbx6 and T were isolated and identified by massively parallel sequencing. A genome wide annotation of these binding motives helped to distinguish direct and indirect targets among deregulated genes identified by expression profiling. This enabled us to establish a core gene regulatory network of trunk mesoderm induction, patterning and differentiation into the paraxial mesodermal lineage.

CHICKATLAS: A THREE-DIMENSIONAL ATLAS OF GENE EXPRESSION DURING CHICK DEVELOPMENT.

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The control of gene expression is important in embryonic development. Quantitative assays, such as microarrays, provide gene expression levels for large numbers of genes, but with low spatial resolution. In contrast, in situ methods, such as in situ hybridisation and immunohistochemistry, provide high spatial resolution, but poorer quantification and low genome coverage. Furthermore, crucial 3D data is lost with planar samples. Optical projection tomography (OPT) can capture the full 3D expression pattern in a whole embryo at a reasonably high resolution and at moderately high throughput. A large database containing spatio-temporal patterns of expression for the mouse (EMAGE) has been created and is proving to be a valuable resource. Recently, the chick has become an important model for spatially and temporally controlled gain- and loss-of-function approaches. To date, a well-established gene expression database for the chick does not exist. Thus, the aim of this project is to produce a 3D anatomical atlas and ontology of the chick embryo with a database of gene expression patterns during chick development. This involves a major collaboration between groups in Edinburgh, Dublin, Bath and London. This database will be based on EMAGE and cross-referenced to the mouse through orthologous gene pairs (http://www.emouseatlas.org/testemage/home.php). Throughout this project, the data and framework will be used to identify groups of genes that are co-expressed in important signalling regions. Conservation of these genes will be examined in the chick and mouse. This database will be made publicly available (http://www.echickatlas.org/) and will be a valuable resource to the developmental community.

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GPR107, AN EVOLUTIONARILY ANCIENT SEVEN TRANSMEMBRANE PROTEIN, FUNCTIONS IN THE VISCERAL ENDODERM TO REGULATE DEFINITIVE ENDODERM MORPHOGENESIS

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During early development, both the specification of early tissue lineage progenitors and their regulated migration determine how cells form functional organs. During gastrulation, the three primary germ layers form. Of these three germ layers, the least well understood is definitive endoderm, especially in regards to its interaction with visceral endoderm. Definitive endoderm development is crucial to the formation of the gut epithelium and associated organs such as the liver, pancreas and respiratory system, and also to the formation of organs patterned by endoderm-derived signals, such as the head. To uncover mechanisms involved in endoderm development, we identified a novel gene required for definitive endoderm development and visceral endoderm function, Gpr107. Gpr107 encodes a highly conserved, evolutionarily ancient seven transmembrane protein. Gpr107 mutants display an abnormal retention of visceral endoderm along the axial midline at E7.5, as well as reduction of the Cerberus-expressing sub-population of definitive endoderm. These defects in endoderm development suggest that Gpr107 is participates in the specification or migration of midline definitive endoderm. Other populations of definitive endoderm that express Sox17 and FoxA2 are not affected in mutants. In addition, Gpr107 mutants display abnormal anterior head development and arrest by day E8.5. Chimera analysis reveals that Gpr107 mutant ES cells can contribute normally to endoderm-derived tissues. Injection of Gpr107 mutant ES cells into tetraploid wild-type embryos reveals that the wild-type visceral endoderm rescues the Gpr107 mutant phenotype. Together, these results demonstrate that Gpr107 functions in the visceral endoderm in a non-cell autonomous manner to regulate the development of embryonic definitive endoderm.

PITX2 IS REQUIRED FOR THE SURVIVAL AND SPECIFICATION OF EXTRAOCULAR MUSCLES

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During embryonic development, skeletal muscle is formed from two different populations, segmented somitic mesoderm in the trunk and unsegmented cranial and paraxial mesoderm in the head. While the commitment to the myogenic pathway is controlled by the muscle regulatory factors (MRFs), *Myf5, Myod1, Mrf4*, and *Myog*, in both locations, the signals and transcriptional activators upstream of the MRFs are distinct. *Pax3* activates MRF expression in the somites, and *Tbx1, Musculin*, and *Tcf21* activate the MRFs in the branchial arches, but in the extraocular muscles (EOMs), the upstream activator of the MRFs remains unknown, although the homeodomain transcription factor *Pitx2* has been proposed to play this role. The goal of our experiments was to identify the functions of *Pitx2* during EOM development and evaluate the ability of PITX2 to directly activate MRF expression. We found that EOMs are absent in *Pitx2* knockout embryos because the EOM precursors undergo apoptosis prior to e10.5, before activating the MRFs. To evaluate the role of *Pitx2* in MRF activation, we used the ubiquitously expressed inducible *UBC-CreER^{T2}* to delete *Pitx2* at e10.5, which temporarily rescues EOM precursor survival. Many mutant EOM precursors fail to express MYOD and MYOG protein, and expression of all MRF mRNAs is significantly reduced, suggesting that *Pitx2* is upstream of the MRFs. We found that *Pitx2* continues to be required to prevent EOM precursors from undergoing apoptosis, even if MRFs are expressed, during the period of EOM specification. After this window, *Pitx2* is no longer required for the survival of differentiated EOMs. To determine if PITX2 can directly activate the MRFs, we examined the *Myf5* promoter and *Myod1* promoter and 258 bp enhancer, which have been shown to drive *LacZ* expression in the EOMs. These regions have predicted PITX2 binding sites and we performed chromatin immunoprecipitation to demonstrate that PITX2 is able to bind the *Myf5* and *Myod1* promoters in limb and EOM precursor cells. We also found that PITX2 can activate the *Myf5* and *Myod1* promoters *in vitro*, but this activation does not require the predicted PITX2 binding sites. These data indicate that PITX2 directly activates expression of *Myf5* and *Myod1* by stimulating their promoters through non-canonical sites. In all, our results show that *Pitx2* has a unique role in EOM precursor survival and provide strong genetic and biochemical support for the hypothesis that *Pitx2* is an upstream activator of the MRFs in the EOMs.

BONE RIDGE PATTERNING DURING MUSCULOSKELETAL ASSEMBLY IS MEDIATED THROUGH SCX REGULATION OF BMP4 AT THE TENDON-SKELETON JUNCTION

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During the assembly of the musculoskeletal system, bone ridges provide a stable anchoring point and stress dissipation for the attachment of muscles via tendons to the skeleton. In this study, we investigate the development of the deltoid tuberosity as a model for bone ridge formation. We show that the deltoid tuberosity develops through endochondral ossification in a two-phase process: Initiation is regulated by a signal from the tendons, whereas the subsequent growth phase is muscle-dependent. We then show that the transcription factor scleraxis (SCX) regulates Bmp4 in tendon cells at their insertion site. The inhibition of deltoid tuberosity formation and several other bone ridges in embryos in which Bmp4 expression was blocked specifically in Scx-expressing cells implicates BMP4 as a key mediator of tendon effects on bone ridge formation. This study establishes a mechanistic basis for tendon-skeleton regulatory interactions during musculoskeletal assembly and bone secondary patterning.

MOUSE MODELS OF HUMAN *MYH9*-RELATED DISEASES

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Humans with point mutations in *MYH9*, the gene encoding nonmuscle myosin heavy chain IIA (NMHC IIA), develop a variety of syndromes with defects in their platelets (macrothrombocytopenia), kidneys (glomerulonephritis), eyes (cataracts), hearing (deafness) and granulocytes (inclusion bodies). The purpose of the present study is to gain insight into the pathological mechanism of these abnormalities by generating mouse models for two of these mutations and characterizing the resultant mouse phenotypes. We have produced both Arg702Cys and Asp1424Gln mutant mice by using homologous recombination to replace wild type NMHC IIA with the two point mutant isoforms. We chose these two mutations because they duplicate the human mutations and because the Arg702Cys mutation is present in the amino-terminal motor domain of myosin II and the Asp1424Gln mutation is located in the carboxyl-terminal rod domain, which regulates myosin filament formation. Breeding of heterozygous Arg702Cys mutant mice has not produced homozygous mutant offspring. The homozygotes die between embryonic day (E)8.5 and 10.5, which is considerably later in development than knockout myosin IIA mice (E6.5). On the other hand, breeding of heterozygous Asp1424Gln mutant mice produced homozygous mutant offspring at close to normal ratios, indicating that the motor domain function of NMHC IIA is critically important during mouse embryonic development. Interestingly, giant platelets were found in the blood smears from both Arg702Cys and Asp1424Gln adult heterozygous mice. The homozygous Asp1424Gln adult mice have even larger platelets and an abnormally low platelet count. Kidney function was studied by examining the albumin/creatinine ratio in urine samples. Some but not all adult heterozygotes of both mutant lines have higher albumin/creatinine ratios at 8-9 weeks. Light and transmission electron microscopy studies show the development of focal segmental glomerulosclerosis in the heterozygous Arg702Cys and both the heterozygous and homozygous Asp1424Gln mice. These preliminary results suggest that these mouse models should be useful in understanding the pathophysiology of human *MYH9*-related diseases.

DEVELOPMENT OF CARDIAC FORM AND FUNCTION

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Organ health and function rely on continuous blood flow and pressure generated by well-paced cardiac output. A method to experimentally control heart function in an animal model would be useful to model common pathological conditions. Here we use optogenetic tools, patterned illumination and high-speed selective plane illumination microscopy to localize cardiac pacemaker cells, control the heart rate and modulate cardiac output in embryonic and larval zebrafish. This intervention is local, rapid, reversible, non-invasive, and quantitative. Photostimulation of Halorhodopsin (NpHR) is used to block depolarization, and activation of Channelrhodopsin-2 (ChR2) to facilitate it. By automated sequential illumination
of small areas of the developing heart with a digital micromirror device (DMD), while simultaneously monitoring heart contractions, we identify the regions responsible for initiating and relaying cardiac conduction. Initially encompassing most of the inflow region, the cardiac pacemaker becomes increasingly confined and comprises less than a dozen cells by the time the heart loops. By directly targeting the pacemaker area with well-defined light pulses, the heart rate can be increased with ChR2 stimulation or decreased to a full stop with NpHR stimulation. Manipulating the atrioventricular canal induces atrioventricular blocks. Pulsed activation of the distal ventricle reverses cardiac conduction. These studies
advance our ability to control heart function as well as our understanding of the development, function, and dysfunction of the cardiac conduction system.

SPECIFICATION OF CARDIAC ASYMMETRY BY THE CILIA INVOLVES A TWO-STEP PROCESS

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Some of the most complex congenital heart disease (CHD) is observed in patients with heterotaxy, a condition associated with randomized left-right visceral organ situs. The mechanism by which left-right patterning defects give rise to complex structural heart disease (CHD) is poorly understood. To examinet his question, we analyzed heart and visceral organ situs in mice deficient for Dnai1, a motor dynein gene commonly associated with primary cilia dyskinesia (PCD), a heritable disorder exhibiting sinopulmonary disease caused by mutations affecting motile cilia function. Half of the Dnai1 mutants showed situs solitus or situs inversus totalis, and the other half exhibited heterotaxy. While mutants with situs solitus/situs inversus had no structural heart defects and are postnatal viable, all mutants with heterotaxy died prenatally/neonatally from complex CHD. Analysis of E7.75 Dnai1 KO embryos showed nodal cilia are present but immotile. As Dnai1 KO mice exhibit a high incidence of situs solitus/situs inversus totalis (50%), this would suggest motile cilia are not required for organ situs specification. Examination of E9.5-10.5 embryos showed L-looped hearts out numbered D-looped hearts 2:1, and remodeling of the aortic arch and great arteries was concordant with heart tube looping direction. However, analysis of mutants at E14.5 or later showed the D- and L-looped hearts each had an equal (50%) chance of developing CHD. The CHD observed included a spectrum of anomalies such as malalignment of the great arteries, defects in atrioventricular connection, abnormalities in ventricular arterial alignment, abnormal venous connections, and ventricular and atrial septal defects. These findings suggest two discrete steps in cardiac morphogenesis: first specification of cardiac loop orientation, followed by an independent event specifying left-right asymmetries within the heart. Normal D-looped hearts and L-looped hearts exhibited significantly thicker morphological left ventricles (mLV), whether situated on the anatomic right (D-looped hearts) or left (R-looped hearts). In contrast, heterotaxy mutants with CHD had uniformly thin ventricles regardless of the specific CHD. Aortic stenosis was commonly observed in the heterotaxy mutants, but not in mutants with situs solitus/situs inversus. The mLV chamber wall thickness, was significantly correlated with the circumference of the aorta and ductus arteriosus in the normal D and L-looped hearts, but not in the CHD mutants. Overall, these findings are consistent with the integration of cardiac anatomy with cardiac physiology. The potential role of the cilia in this process will require further investigation.

NEK8 IS REQUIRED FOR THE ESTABLISHMENT OF LEFT-RIGHT ASYMMETRY AND RENAL TUBULE INTEGRITY IN THE MOUSE EMBRYO

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The *Nek8* gene is a highly conserved member of the Nek family of serine/threonine kinases. A missense mutation was identified in *Nek8* in the mouse juvenile cyctic kidneys (*jck*) model of recessive polycystic kidney disease (PKD) in which renal cysts develop within a week after birth, worsen as the mice mature, and cause renal failure and death by six months of age. NEK8 is the only kinase that has been shown to localize to the ciliary axoneme, and the cilia in *jck* cysts are elongated and exhibit mislocalization of the PKD-associated polycystin proteins. *Nek8* is clearly required for the maintenance of renal epithelial integrity, but the function of *Nek8* is otherwise unknown. We generated a *Nek8*-null mouse model and have found that mutants survive gestation but die shortly after birth. Randomization of left-right asymmetry is observed in the *Nek8*-null embryos, which implicates NEK8 in the proper function of the embryonic node and/or the nodal cilia. Approximately 20% of the mutants display *situs inversus totalis* while a majority of mutants exhibit right pulmonary isomerism (RPI), which may occur with or without abdominal situs defects. Mutants with RPI exhibit severe cardiac anomalies including double outlet right ventricle and septal defects. Analysis of laterality marker gene expression and nodal cilia revealed that genes in the Nodal signaling pathway are misexpressed in *Nek8*-null embryos, while the nodal cilia are intact. These data indicate a requirement for NEK8 in ciliary signaling in the node rather than in cilia formation. Interestingly, *Nek8*-null kidneys do not develop tubular cysts like those of *jck* animals. To assess the cyst formation of the *jck* mutation in the context of the null allele, we generated *Nek8*-:jck compound heterozygotes. These animals survive and develop moderate PKD by postnatal week 7, but surprisingly, the disease is not as severe as in *jck* homozygotes. To determine whether the *Nek8*-null kidneys have cystogenic potential, we utilized kidney explant cultures and induced cyst formation with a cyclic AMP analog. *Nek8*-null kidneys do not develop cysts, while the *Nek8*-: *jck* and *jck* homozygous kidneys develop moderate and severe cystic disease, respectively, similar to what we observe in the adults. These data suggest the *jck* mutation results in a gain-of-function, and we are currently addressing this implication. Understanding the consequences of *Nek8*-deficiency is critical for appreciating its role in both embryonic development and the maintenance of renal tubular integrity.

CARDIAC REGENERATION IN ZEBRAFISH

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Recent studies indicate that mammals, including humans, maintain some capacity to renew cardiomyocytes throughout postnatal life. Yet, there is little or no significant cardiac muscle regeneration after an injury like acute myocardial infarction. Unlike mammals, adult zebrafish regenerate lost cardiac muscle, providing a model for understanding how natural heart regeneration may be blocked or enhanced. Here, we have identified responses to injury by the endocardial lining of the zebrafish heart that are important for wound recognition and myocardial regeneration. Within three hours of resection of the ventricular apex, the entire endocardium induces expression of several genes, including the retinoic acid (RA)-synthesizing enzyme raldh2. These responses disappear in areas away from the wound by 1-2 days post-trauma. By contrast, the injury border zone retains endocardial cells enriched with raldh2 and other cardiogenic factors for several days as new cardiac muscle is formed. Transgenic inhibition of RA receptors blocked cardiomyocyte proliferation at the injury site, indicating that RA signaling is critical for regeneration. Thus, heart regeneration in zebrafish is initiated through a dynamic, organ-wide endocardial injury response that targets RA synthesis to areas of damaged cardiac muscle.

DAAM-MEDIATED WNT/RHOA SIGNALING REGULATES THE ADHESION, CYTOSKELETAL ORGANIZATION AND PROLIFERATION OF DIFFERENTIATING CARDIAC MYOCYTES.

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In addition to signaling through a canonical pathway that ends in β-catenin-dependent transcription, Wnt proteins can signal via Rho-family GTPases to regulate the adhesion, cytoskeletal organization and differentiation of responding cells. We have conditionally deleted the Disheveled-associated affector of morphogenesis proteins, Daam1 and Daam2, in cardiac progenitors to determine if Wnt/RhoA signaling is required for their differentiation into mature cardiac myocytes. Daam1 and Daam2 are formin-homology proteins required for Wnt signaling to activate RhoA in Xenopus embryos and cultured cells. Daam1 and Daam2 are co-expressed by second heart field cardiac progenitors in the outflow tract as well as in the differentiating myocardium. Daam1/2 mutant mice are viable but have thick ventricular walls that encroach into the heart lumen. Co-staining for α-cardiac actin and nuclei reveals that the sarcomeres are frequently disorganized or discontinuous in Daam1/2 mutant myocytes. Moreover, wild type myocytes generally have a single elongated nucleus but Daam1/2 deficient myocytes often have two or more spherical nuclei. Furthermore, the epicardium is highly proliferative in Daam1/2 mutants but quiescent in control animals. Epicardial cells give rise to fibroblasts in response to cardiac injury and staining for the fibroblast marker vimentin is highly increased in Daam1/2 mutant hearts relative to control hearts. While cardiac myocytes are not proliferative in either wild type or Daam1/2 mutant adults, myocardial hyperplasia is apparent in Daam1/2 mutant embryos by mid-gestation and is associated with the increased proliferation of embryonic cardiac myocytes relative to controls. Finally, staining for β-catenin reveals that β-catenin is evenly distributed around the periphery of wild type cardiac myocytes but located in sparsely distributed punctate foci in Daam1/2 mutant myocytes. Taken together, these data suggest that Daam-mediated Wnt/RhoA signaling regulates the adhesion, cytoskeletal organization and proliferation of myocardial cells as they differentiate into functionally mature cardiac myocytes.

BMP-MEDIATED INHIBITION OF FGF SIGNALING LIES AT THE HEART OF DIFFERENTIATION

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The transition from progenitors to differentiated cells is critical for successful organogenesis; alterations in this process can lead to serious developmental disorders. The anterior heart field (AHF) encompasses a niche in which cardiac progenitors maintain their multipotent and undifferentiated nature in response to signals from surrounding tissues. Here we investigate the shift from proliferating cardiac progenitors to differentiating cardiomyocytes in chick embryos. Genomic and systems biology approaches as well as perturbations of signaling molecules, in vitro and in vivo, reveal tight crosstalk between the bone morphogenic protein (BMP) and fibroblast growth factor (FGF) signaling pathways within the AHF: BMP4 promotes myofibrillar gene expression and cardiomyocyte contraction, by blocking FGF signaling. Furthermore, inhibition of the FGF-ERK pathway is both sufficient and necessary for these processes, suggesting that FGF signaling blocks premature differentiation of cardiac progenitors in the AHF. Investigating the molecular mechanisms downstream of BMP signaling revealed that BMP4 induced a set of neural crest-related genes; including MSX1. Overexpression of Msx1 was sufficient to repress FGF gene expression and cell proliferation, thereby promoting cardiomyocyte differentiation. Hence, BMP and FGF signaling pathways act via inter- and intra-regulatory loops in multiple tissues, to coordinate the balance between proliferation and differentiation of cardiac progenitors.

EMBRYO PATTERNING THE VERTEBRATE AXIS.

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The vertebrate body can be subdivided along the antero-posterior (AP) axis into repeated structures called segments. This periodic pattern is established during embryogenesis by the somitogenesis process. Somites are generated in a rhythmic fashion from the paraxial mesoderm and subsequently differentiate to give rise to the vertebrae and skeletal muscles of the body. Somite formation involves an oscillator-the segmentation clock-whose periodic signal is converted into the periodic array of somite boundaries. This clock drives the dynamic expression of cyclic genes in the presomitic mesoderm and requires Notch and Wnt signaling. Microarray studies of the mouse presomitic mesoderm transcriptome reveal that the segmentation clock drives the periodic expression of a large network of cyclic genes involved in cell signaling. Mutually exclusive activation of the Notch/FGF and Wnt pathways during each cycle suggests that coordinated regulation of these three pathways underlies the clock oscillator. In humans, mutations in the genes associated to the function of this oscillator such as Dll3 or Lunatic Fringe result in abnormal segmentation of the vertebral column such as those seen in congenital scoliosis. Whereas the segmentation clock is thought to set the pace of vertebrate segmentation, the translation of this pulsation into the reiterated arrangement of segment boundaries along the AP axis involves dynamic gradients of FGF and Wnt signaling. The FGF signaling gradient is established based on an unusual mechanism involving mRNA decay which provides an efficient means to couple the spatio-temporal activation of segmentation to the posterior elongation of the embryo. Another striking aspect of somite production is the strict bilateral symmetry of the process. Retinoic acid was shown to control aspects of this coordination by buffering destabilizing effects from the embryonic left-right machinery. Defects in this embryonic program controlling vertebral symmetry might lead to scoliosis in humans. Finally, the subsequent regional differentiation of the precursors of the vertebrae is controlled by Hox genes, whose collinear expression controls both gastrulation of somite precursors and their subsequent patterning into region-specific types of structures. Therefore somite development provides an outstanding paradigm to study patterning and differentiation in vertebrate embryos.

SONIC HEDGEHOG: A NEW PLAYER IN TEMPORAL CONTROL OF SOMITE FORMATION

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Vertebrate embryo somite formation is temporally controlled by the cyclic expression of somitogenesis clock genes in the presomitic mesoderm (PSM). The somitogenesis clock is believed to be an intrinsic property of this tissue, operating independently of embryonic midline structures and the signaling molecules produced therein, namely Sonic hedgehog (Shh). This work revisits notochord-signaling contribution to temporal control of PSM segmentation by assessing the rate and number of somites formed and somitogenesis molecular clock gene expression oscillations upon notochord ablation. The absence of the notochord causes a delay in somite formation, accompanied by an increase in the period of molecular clock oscillations. Shh is the notochord-derived signal responsible for this effect since these alterations are recapitulated by Shh signaling inhibitors and rescued by an external Shh supply. We have characterized chick *smoothened* expression pattern for the first time and found that the PSM expresses both *patched1* and *smoothened* Shh signal transducers. Upon notochord ablation, *patched1*, *gli1* and *fgf8* are downregulated, while *gli2* and *gli3* are overexpressed. Strikingly, notochord-deprived PSM segmentation rate recovers over time, coupled with *raldh2* overexpression. Accordingly, exogenous RA supplement attenuates notochord-ablation effects on somite formation. A model is presented, where Shh and RA pathways converge to inhibit PSM Gli activity, ensuring timely somite formation. Altogether, our data evidences that a balance between different pathways ensures the robustness of timely somite formation and unveils notochord-derived Shh as a new component of the molecular network regulating the pace of the somitogenesis clock.

REGULATION OF MUSCLE STEM CELL FATE BY *PAX* GENES

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Pax3 and Pax7 mark myogenic progenitor cells and play a critical role in regulating their entry into the myogenic programme. We had shown that in the *Pax3/7-/-* double mutant the myogenic determination genes, *Myf5* and *MyoD* are not activated, leading to a major deficit in skeletal muscle. Pax3 directly activates *Myf5*, thus promoting a myogenic cell fate. However, it is essential to maintain a balance between differentiation and renewal of the progenitor cell population. We show that this can be achieved by Pax3 modulation of FGF signaling via *Sprouty1* and *Fgfr4*, which is a direct Pax3 target. Other Pax3 targets will be discussed, including *Dmrt2* and *Foxc2*, both expressed in the dermomyotome, the part of the somite from which skeletal muscle cells derive. *Foxc2* is negatively regulated by Pax3 and in turn feeds back negatively on *Pax3/7* expression. This negative feedback loop is implicated in cell fate decisions of the multipotent Pax3/7 positive stem cells of the dermomyotome. These cells can form derm, brown fat endothelial and smooth muscle cells of blood vessels as well as skeletal muscle. Taking the latter tissues as an example, we show genetically that up-regulation of Foxc2 promotes endothelial and smooth muscle cell fates whereas Pax3/7 promote myogenesis. This is also demonstrated by manipulation of these factors in somite explants. Signaling from adjacent tissues, such as the dorsal ectoderm, affects the equilibrium between *Pax3/7:Foxc2* and the choice of cell fate of the multipotent cells expressing these genes in the dermomyotome.

PLEIOTROPIC ACTIONS OF WNT DURING MUSCLE DEVELOPMENT, POSTNATAL MYOGENESIS AND AGING

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We study the molecular pathways that regulate the transitions between quiescence, activation, and differentiation of adult muscle stem cells (satellite cells). Interestingly, we have found that Wnt signaling plays an important role in the commitment to differentiation of transit amplifying population of cells, with an increase in Wnt signaling in those cells leading to a more differentiated myogenic progenitor, or myoblast. During development, Wnt signaling has been shown to regulate myogenic differentiation as well. Interestingly, we have found a very different role of Wnt signaling in the functionality of aged muscle stem cells. In the aged environment, Wnt, acting on the quiescent stem cell, induces a subpopulation of cells to lose their myogenic fate and adopt a fibrogenic fate. In contrast to the pro-myogenic roles in development and postnatal myogenesis, this effect in aged animals inhibits myogenesis and promotes fibrosis instead.

Given these pleiotropic actions of Wnt on the progenitors from a single lineage, we have begun to explore the molecular determinants of the transcriptional and cellular responses to activation of the Wnt pathway. In studies of the expression or transcriptional co-regulators, we have found that the differentiation-inducing effects of Wnt are dependent on the expression of the Wnt co-activator, BCL9, in postnatal progenitors. Using a muscle-specific driver of Cre recombinase and mice in which BCL9 is flanked by loxP sites, we found muscle development to be normal but muscle regeneration postnatally to be impaired when BCL9 was deleted in the myogenic lineage. Intriguingly, the effects of Wnt on aged stem and progenitor cells to induce transdifferentiation and differentiation revert to being BCL9-independent. Furthermore, the transcriptional profiling of quiescent aged stem cells compared to quiescent adult stem cells revealed both quantitative and qualitative differences in the expression of putative Wnt target genes. Therefore, the transcriptional output and cellular responses to activation of the Wnt pathway differ among embryonic, adult, and aged myogenic progenitors, some of which are correlated with BCL9-dependence or BCL9-independence of Wnt signaling. Current studies are focusing on the role of epigenetic modifications of Wnt target genes and the role of BCL9 as an “epigenetic decoder” as potential mechanisms regulating the pleiotropic actions of Wnt on myogenesis.

RECRUITMENT AND MAINTENANCE OF TENDON PROGENITORS BY TGFB SIGNALING ARE ESSENTIAL FOR TENDON FORMATION

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Tendons and ligaments mediate attachment of muscle to bone and bone to bone to provide connectivity and structural integrity in the musculoskeletal system. We show that TGFβ signaling plays a major role in the formation of these tissues. TGFβ signaling is a potent inducer of the tendon progenitor markers Scleraxis and Tenascin-C both in organ culture and in cultured cells and disruption of TGFβ signaling in TGFβ2-/-;TGFβ3-/- double mutant embryos or through inactivation of the type II TGFβ receptor (TβRII) results in loss of all tendons and ligaments in the limbs, trunk, tail and in the head. Induction of Scleraxis expressing tendon progenitors is not affected in mutant embryos and the tendon phenotype is first manifested at E12.5, a developmental stage in which the tendon progenitors are first positioned between the differentiating muscles and cartilage. Interestingly, TGFβ2 or TGFβ3 are expressed both in the Scleraxis expressing tendon progenitors and in the differentiating muscles and cartilage and genetic interactions suggest that signals that emanate from the muscles and cartilage at this stage are essential for tendon development. We therefore suggest that at E12.5, TGFβ signaling is essential both for maintenance of the tendon progenitors and for recruitment of new tendon cells by the differentiating muscles and cartilage to establish the connections between tendon primordia and their respective musculoskeletal counterparts leading to the formation of an interconnected and functionally integrated musculoskeletal system.

SKELETON AS AN ENDOCRINE ORGAN

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In the recent years our laboratory has shifted its research interest from the development to the physiology of the skeleton. These physiological studies are conducted under the assumption that there is a common control of bone mass, energy metabolism and reproduction. An implication of this hypothesis is that bone if not the skeleton may be an endocrine organ regulating both energy metabolism and reproduction. Consistent with this hypothesis we have shown that osteocalcin, an osteoblast-specific secreted molecule in a hormone regulating insulin secretion and sensitivity and energy expenditure. This finding raised several important questions. Among them we focused our attention on 1) how is osteocalcin activated since it exists in two forms, carboxylated and inactive and undercarboxylated and metabolically active; 2) how is osteocalcin expression or function regulated; 3) what is the identity of its receptor. In addition to these questions related to the regulation of energy metabolism by the skeleton we also explored the other aspects of our hypothesis namely the possibility that skeleton may regulate reproduction. Results relevant to these various aspects of osteocalcin biology and skeleton biology will be presented at the meeting.

BENEATH THE BATTLE OF THE SEXES: DEFINING THE TRANSCRIPTIONAL ARCHITECTURE UNDERLYING SEX DETERMINATION.

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The gonad is unique among organ primordia as it initially forms with the potential to develop as one of two organs, a testis or an ovary. Recent results suggest that the gonad is initially balanced between these fates by antagonistic signaling networks. In XY embryos, this balance is disrupted by the expression of the Y-linked gene, *Sry*, which activates genes that promote the testis pathway and oppose the ovarian pathway. While the roles of a few genes have been defined by mutation, the interactions of many signaling pathways and transcriptional networks are involved in the establishment of sexual fate. Microarray studies have confirmed a large and complex transcriptome during sex determination, but have failed to establish links between genes. A systems-level approach is needed in the field to assemble genetic networks and weave pathways together. To elucidate the transcriptional network underlying sex determination, we took advantage of two inbred strains, C57BL/6J (B6) and 129S1/SvImJ (129S1), which differ in their susceptibility to sex reversal. We identified significant reproducible strain differences in the XY gonad transcriptome at the critical timepoint of sex determination (E11.5). Importantly, we found that many female-enriched genes were upregulated in XY gonads from the sensitive B6 strain. We then quantified the expression of a subset of sex-associated genes in (B6x129S1)F2 XY gonads. Gene expression was highly variable across the F2 population, suggesting remarkable plasticity in the network. Nonetheless, strong correlations between the expression levels of genes emerged. To identify the regulators of these co-regulated gene clusters, we genotyped each F2 individual at SNPs throughout the genome and used linkage analysis to map areas of the genome that affect gene expression in the E11.5 testis (expression Quantitative Trait Loci = eQTLs). eQTL mapping has been successfully applied in adult mouse tissues, however this study represents the first application of this approach to a developing organ. We mapped previously unknown autosomal regions that control the expression of many important sex determination genes, including *Sry* and *Sox9*. These studies establish links between developmental pathways, yielding a more integrated view of the gene networks that control cell fate decisions and patterning during organogenesis of the gonad.

FOXL2 AND WNT4 SIGNALLING ARE REPRESSING THE TESTIS SPECIFIC ENHANCER OF *SOX9 IN VIVO*

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For normal ovarian development it is essential that *Sox9* becomes down-regulated in the XX gonad around 11.5 dpc and is kept in a suppressed status throughout life. It has been shown that a 1.4 kb enhancer element (TESCO) is sufficient to regulate *Sox9* expression specifically in the testis. FOXL2 is a forkhead transcription factor, which, during gonad development, is exclusively expressed in the female supporting lineage from 12.5 dpc onwards. In mice, homozygous mutations of *Foxl2* lead to infertile females displaying ovarian failure with up-regulation of *Sox9* and other testis-specific genes in the supporting cells of postnatal ovaries. WNT4 signalling has also been shown to be important for proper ovarian development. *Wnt4* is expressed in the indifferent gonad from 9.5 dpc and becomes female specific at 11.5 dpc. Moreover, homozygous mutations of *Wnt4* in mice results in the masculinisation of XX gonads.
Our data shows that FOXL2 can repress TESCO activation *in vitro*. This repression is aggravated by estrogen receptor α. Homozygous loss of *Foxl2* results in re-activation of TESCO activity in the ovary, but not until after birth. This effect is similar to that seen for the endogenous *Sox9* gene, implying that the latter occurs via the TESCO element. Homozygous loss of *Wnt4* leads to re-activation of TESCO activity, but not endogenous *Sox9* expression, during embryonic development. Interestingly, *Sox8* becomes de-repressed in XX gonads concomitant with the TESCO activity. XX gonads with a homozygous double mutation for both *Foxl2* and *Wnt4* show a simultaneous re-activation of TESCO and *Sox9* just after birth. These data suggest that both *Wnt4* and *Foxl2* are involved in repressing TESCO activity in XX gonads: *Wnt4* during embryonic development and *Foxl2* after birth (maybe due to its co-operation with estrogen receptor).

DOWNSTREAM NON-CANONICAL WNT COMPONENTS DAAM1 AND WGEF ARE REQUIRED FOR EPITHELIAL TUBULOGENESIS IN THE *XENOPUS* PRONEPHROS

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Epithelial tubulogenesis is critical for the development and function of organ systems including the cardiovascular, pulmonary, gastrointestinal, reproductive, excretory, and auditory systems. Wnt signaling via either the canonical or non-canonical signaling trajectories has been shown to be required for the induction, specification, proliferation, and morphogenesis involved in tubulogenesis within tissues including the lungs, kidneys, ears, mammary glands, gut, and heart. Numerous Wnt ligands have been shown to be expressed within the developing kidney in both amphibians and mammals. Additionally, both canonical and non-canonical Wnt signaling pathways have been linked to kidney diseases such as Wilms' tumor and cystic kidney diseases like polycystic kidney disease and nephronophthisis. Recent work from both our group and others indicates that canonical/β-catenin signaling is essential to nephric tubulogenesis. Non-canonical pathways contribute to normal processes such as cell polarization and cytoskeletal control, and while Wnt9b involvement in kidney tubulogenesis has recently been shown, the specifics of the pathways involved remain in question. Here, using the amphibian *Xenopus laevis*, we have tested the roles of downstream non-canonical Wnt components in kidney tubule morphogenesis. *Xenopus* offers experimental advantages including the facile introduction of exogenous constructs to block or activate signaling pathways, rapid development and easy visualization of the forming kidney immediately beneath the lateral surface ectoderm. One of the planar cell polarity branches of the non-canonical Wnt pathways, namely the Daam1/ WGEF/ Rho trajectory, is being assessed in the current study. Daam1 and WGEF are both expressed in the developing pronephric anlagen during tubulogenesis. Additionally, knockdown of Daam1 or WGEF expression using antisense morpholino oligonucleotides or inhibition of the pathway using dominant negative inhibitors of Daam1 or Rho results in reduced pronephric tubulogenesis. Daam1, a formin protein, and WGEF, a Rho GEF, may contribute to tubulogenic morphogenesis via regulation of the actin cytoskeleton. Our data thus suggest that the Daam1/ WGEF/ Rho planar cell polarity (PCP) trajectories are necessary for pronephric tubulogenesis.

CONTROL OF BRANCHING MORPHOGENESIS DURING KIDNEY DEVELOPMENT

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Signaling by GDNF through the Ret receptor tyrosine kinase is required for the normal formation, growth and branching of the ureteric bud (UB) during kidney development. However, the precise role of GDNF/Ret signaling in this process, and the specific responses of UB cells to GDNF, remain to be fully elucidated. Recent studies provide new insight into the effects of Ret signaling on cell behavior, the functional overlap between GDNF and other growth factors, and the genes functioning downstream of Ret. Lineage studies show that the UB tip cells, which express Ret, are the progenitors for UB growth, while GDNF-expressing mesenchymal cells are the progenitors of nephron epithelia. Time-lapse studies of chimeric embryos reveal that the earliest role of Ret signaling is in the Wolffian duct, where it promotes cell movements that give rise to the first ureteric bud tip. The requirement for GDNF/Ret signaling can be largely relieved by removing the negative regulator Sprouty1, implicating other growth factors, in particular FGF10, in the support of UB growth and branching. However, the kidneys that develop in the absence of GDNF/Ret and Sprouty1 display branching abnormalities, suggesting a unique role for GDNF in determining UB branching pattern. A number of genes whose expression is induced in the UB by GDNF has been identified, including two ETS transcription factors Etv4 and Etv5. These genes are required downstream of Ret for the Wolffian duct cell movements that form the UB tip domain, as well as for later UB growth and branching.

CERBERUS 1 BINDS BMP4 INDUCING GDNF/RET/WNT11 SIGNALLING LOOP TO COORDINATE URETERIC BUD DEVELOPMENT IN THE CONTROL OF KIDNEY SIZE

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Intrinsic genetic programs are thought to be involved in organ size regulation in mammals but the mediators remain unknown. MCerberus 1 homolog (Cer1) that binds and antagonizes Bmp2/4 but not Gdnf or Wnt11 signalling appears to be involved, since both Cer1 gain and loss of function enlarges the kidney. Time-lapse fate mapping and analysis of the 3D structure of the ureteric bud by optical projection tomography (OPT) suggest that Cer1 enhances kidney organogenesis by promoting formation of trifid and lateral over bifid type of ureteric bud branches leading to changes in the 3D structure of the ureteric tree being the likely primary reason for the Cer1 induced enhancement of kidney size induced by Cer1 gain of function. Closer inspection of the potential molecular mediators involved in the Cer1 induced size regulation suggests that Cer1 operates in the ureteric bud by preventing Bmp mediated repression of Gdnf expression when Cer1 level is enhanced. Lower Bmp levels caused by Cer1 antagonism is expected to lead to induction in Gdnf expression and via the enhanced Gdnf activity consequently induction of Wnt11 expression being signals that cooperatively promote ureteric bud branching. Consistent with this suggestion genetic reduction of Wnt11 or excess BMP4, that normally inhibits Gdnf and Wnt11 expression both reduce ureteric branching and limit the Cer1-induced kidney growth. Besides influencing the ureteric bud signalling events Cer1 may contribute to kidney organ size regulation by controlling functions of mesenchymal signals, namely Wnt-4 that coordinates nephrogenesis. This suggestion is based on the results that similarly to Cer1 gain of function Cer1 knock out enlarges kidney size and that high BMP4 doses convert BMP4 from an inhibitor of Wnt-4 expression to an inducer. Thus besides influencing the regulatory networks that coordinate ureteric bud branching during kidney organogenesis, Cer1 knock out may promote kidney size via the enhanced Wnt-4 expression, a situation that may take place in the case of Cer1 deficiency leading to high BMP4 doses and Wnt-4 induction. Thus the results point to a conclusion that Cer1 functions in the mammalian kidney as part of the intrinsic genetic program that determines the constrains on organ size.

EMBRYONIC SIGNALING PATHWAYS REGULATE PANCREAS DEVELOPMENT, REGENERATION, AND NEOPLASIA

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The pancreas is a heterogeneous organ that consists of acinar cells that secrete digestive enzymes into an elaborate duct system and hormone-producing endocrine cells that function to regulate blood sugar homeostasis. Our work demonstrates that tight control of embryonic signaling pathways, including the Hedgehog and Wnt signaling pathways, is critical for the formation of the organ during embryogenesis. In addition, embryonic signaling pathways play crucial roles in maintaining adult pancreas function and deregulation of pathway activities is involved in pancreatic diseases, including diabetes and pancreatic ductal adenocarcinoma (PDA).

Increasing evidence points to a relationship between tissue regeneration in normal tissues and neoplastic events. We present evidence that canonical ß-catenin signaling is critical for acinar cell regeneration upon injury in wild type adult pancreas. In addition, we show that mutant Kras, the predominant oncogene in PDA, promotes acinar-ductal metaplasia (ADM) by blocking acinar regeneration in the adult pancreas. Furthermore, we find that stabilized ß-catenin antagonizes Kras induced ADM that precedes the development of PDA precursor lesions. Thus, our results point to ß-catenin signaling as a molecular switch between acinar homeostasis and a pathologic state prone to PDA progression.

PROX1 REGULATES MULTIPLE STEPS OF LIVER ORGANOGENESIS

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The basic structure of the liver starts to be established during embryogenesis and is completed at postnatal stages. Elucidating the complex molecular network that governs liver organogenesis is necessary to understand the cause of certain liver pathologies, and to disclose molecular mechanisms involved in liver regeneration. Most of the mature liver is composed of hepatocytes; the remaining cells are bile duct epithelial cells (BECs or cholangiocytes), Kuppfer cells, stellate cells and endothelial cells. Hepatocyte function is paramount as these cells perform numerous metabolic, endocrine and secretory tasks. BECs line the lumina of the intrahepatic and extrahepatic biliary tree, and modify the composition of the bile via secreting and absorbing various compounds. BECs and hepatocytes derive from a common embryonic progenitor, the hepatoblast. The vast majority of parenchymal hepatoblasts differentiate into hepatocytes, whereas those hepatoblasts surrounding the portal vein branches differentiate into BECs. BEC specification requires the local production of a TGFβ/Activin signaling gradient.

The mouse homeodomain transcription factor Prox1 is highly expressed in most embryonic hepatoblasts, in hepatocytes and in some adult BECs. In contrast, developing BECs express very low levels of Prox1 proteins. We previously showed that Prox1 controls a critical step of early mouse liver morphogenesis: the delamination of hepatoblasts. Our most recent results indicate that Prox1 also promotes hepatocyte differentiation in the developing liver via antagonizing TGFβ/Activin signaling. In the absence of Prox1 function, parenchymal hepatoblasts adopt an alternative BEC fate of differentiation, which results in ectopic formation of bile ducts, lack of expression of hepatocyte markers and neonatal death due to liver failure. These results indicate that Prox1 is a crucial regulator of multiple processes during liver organogenesis.

BAOBAB IS A NEGATIVE REGULATOR OF CFTR-DEPENDENT FLUID SECRETION

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To better understand how CFTR functions in vivo, numerous studies have searched for and identified potential interacting partners but to date, there is little evidence linking any of the known CFTR interactors to its activity. During a forward genetic screen in zebrafish, aiming to identify genes regulating endodermal organ development, we identified a mutant, which we named *baobab* (*bao*), after the African tree that accumulates water during the wet season, which exhibits a dramatically enlarged fluid-filled gut tube. This phenotype is accompanied by a stretching of the gut epithelium and a loss of microvilli. Most interestingly, we have shown that various CFTR inhibitors significantly reduce the appearance of enlarged guts in bao mutant embryos. Conversely, pharmachological activation of the CFTR channel leads to an expansion of the gut lumen that closely resembles that of bao mutant embryos. We have positionally cloned the bao locus. Bao is a soluble protein expressed in the gut and other endoderm-derived organs. Loss of function in *bao*^{s866} or knockdown using anti-sense morpholinos leads to fluid accumulation in the gut but does not affect CFTR expression or localization, suggesting that the phenotype results from the uncontrolled activation of the CFTR channel. Moreover, overexpression of bao in MDCK cells abrogates the stimulatory effect of cAMP in CFTR-dependent fluid secretion. Furthermore, depletion of Bao leads to the accumulation of fluid in MDCK cysts that is reminiscent of the phenotype observed in vivo in zebrafish. Co-immunprecipation experiments showed that CFTR interacts with WT bao but not with the mutant version of protein. Altogether our results indicate that Bao is a new negative regulator of CFTR activity and that control of fluid secretion is crucial for organogenesis.
This work establishes a new genetic model system for studying the regulation of the CFTR channel that will contribute to the understanding of the pathophysiology of Cystic Fibrosis (CF) and several intestinal secretory conditions.

SIGNALS ORCHESTRATING EPITHELIAL MORPHOGENESIS AND FATE SPECIFICATION IN THE PANCREAS

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Commitment of endodermal cells to pancreatic fate occurs as a multistep process that eventually leads to mature pancreatic tissue. While much is known about pancreas differentiation and growth, we know little about how epithelial morphogenesis may influence early pancreatic specification. In particular, important gaps still persist in our knowledge of molecular players that might coordinate these two events to achieve proper pancreas formation during embryogenesis. In previous studies in Xenopus we identified a novel RhoGAP signaling factor, which we named Shirin, playing a role in early pancreas development. Very little is known about the biological function of this gene and, in particular, no embryological function has been assigned to it in mammals. Here, we show that Shirin is specifically expressed in the endoderm and pancreatic buds from gastrulation onwards both in frog and mouse embryos. Gain-of-function experiments in Xenopus indicated that Shirin alone is sufficient to induce pancreatic identity in the embryo. In line with this, we observe defects in pancreas formation upon conditional ablation of Shirin gene expression in the mouse pancreatic endoderm. In particular, Shirin conditional knockout mouse exhibits severe pancreatic hypoplasia, possibly due to a reduction in number of specified pancreatic progenitors. Taken together, our functional studies suggest a conserved role for Shirin in the epithelial organization of the pancreas, which in turn would affect pancreatic fate specification.

DIFFERENT STATES OF CHROMATIN COMPETENCE WITHIN MULTIPOTENT ENDODERM CELLS

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The endoderm germ layer is uniquely competent to give rise to multiple gut organs, but the basis by which the endoderm, ectoderm, or mesoderm germ layers selectively become competent to activate a particular set of organogenic programs is not known. Our laboratory is interested in the molecular mechanisms by which transcription factors and chromatin states endow the competence for multipotent progenitors for specific cell fates, using the endoderm' ability to activate the liver or pancreas programs as a model. Understanding these principles will be useful for predicting the developmental competence of stem cells to liver hepatocytes and pancreatic beta cells. We previously found that FoxA transcription factors can function as competence or “pioneer” factors for the endoderm, engaging a silent liver gene and helping to confer the potential to induce transcription, upon hepatic specification. Purified FoxA proteins are capable of engaging their target sites in highly compacted nucleosome arrays in vitro, generating local hypersensitivity and allowing other factors to enter the chromatin. We have now used flourescence-activated cell sorting (FACS) to isolate definitive foregut endoderm cells from mouse embryos at the multipotent stage, prior to differentiation, along with scaled-down chromatin immunoprecipitation, to characterize numerous chromatin modifications at functional regulatory elements of early liver and pancreas genes in the endoderm. We find that silent liver and pancreas genes in the endoderm contain distinct patterns of chromatin marks. This information is being used as a benchmark to compare with the patterns seen in endoderm-like cells generated from embryonic stem cells. Genetic and pharmacologic studies have confirmed that different regulatory factors control the different chromatin states at liver and pancreas genes in native embryonic endoderm, and have allowed us to trace a BMP signal, crucial for the liver vs. pancreas cell type choice, to one of the differentially expressed chromatin marks. Small molecules that modify the relevant histone modifying enzymes can alter the distribution of nascent hepatic or pancreatic cells that is generated from the endoderm. Aspects of these findings and the relevance of the approach to diverse progenitor types and stem cell programming will be discussed

LGR5 STEM CELLS IN SELF-RENEWAL AND CANCER

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The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. Current models state that 4-6 crypt stem cells reside at the +4 position immediately above the Paneth cells in the small intestine; colon stem cells remain undefined. Lgr5/Gpr49 was selected from a panel of intestinal Wnt target genes for its restricted crypt expression. Two knock-in alleles revealed exclusive expression of Lgr5 in cycling, columnar cells at the crypt base. In addition, Lgr5 was expressed in rare cells in several other tissues including the hair follicel and stomach. Using an inducible Cre knock-in allele and the Rosa26-LacZ reporter strain, lineage tracing experiments were performed in adult mice. The Lgr5+ve crypt base columnar cell (CBC) generated all epithelial lineages over a 14 month period, implying that it represents the stem cell of the small intestine and colon. Similar obserations were made in hair follicles and stomach epithelium. The expression pattern of Lgr5 suggests that it marks stem cells in multiple adult tissues and cancers. We have now established long-term culture conditions under which single crypts undergo multiple crypt fission events, whilst simultanously generating villus-like epithelial domains in which all differentiated cell types are present. Single sorted Lgr5+ve stem cells can also initiate these crypt-villus organoids. Tracing experiments indicate that the Lgr5+ve stem cell hierarchy is maintained in organoids. We conclude that intestinal crypt-villus units are self-organizing structures, which can be built from a single stem cell in the absence of a non-epithelial cellular niche. The same technology has now been developed for the Lgr5+ve stomack stem cells. Intestinal cancer is initiated by Wnt pathway-activating mutations in genes such as APC. As in most cancers, the cell of origin has remained elusive. Deletion of APC in in Lgr5+ve stem cells leads to their transformation within days. Transformed stem cells remain located at crypt bottoms, while fueling a growing microadenoma in stomach, small intestine and colon. These microadenomas display unimpeded growth and develop into macroscopic adenomas within 4-6 weeks. When APC is deleted in short-lived Transit Amplifying (TA) cells using a different Cre mouse, the growth of the induced microadenomas rapidly stalls. Even after 30 weeks, large adenomas are very rare in these mice. We conclude that stem cell-specific loss of APC results in progressively growing neoplasia. Moreover, a stem cell/progenitor cell hierarchy is maintained in early stem cell-derived adenomas, lending support to the “cancer stem cell”-concept.

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VISITOR INFORMATION

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Emergency Room **Huntington Hospital** 270 Park Avenue, Huntington	**631-351-2300** **(1037)**
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Dial the four numbers (********) from any **tan house phone** to place a free call.

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Located in Grace Auditorium, lower level.

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Grace, lower level; Cabin Complex; Blackford Hall; Dolan Hall, foyer

CSHL's Green Campus

Cold Spring Harbor Laboratory is pledged to operate in an environmentally responsible fashion wherever possible. In the past, we have removed underground oil tanks, remediated asbestos in historic buildings, and taken substantial measures to ensure the pristine quality of the waters of the harbor. Water used for irrigation comes from natural springs and wells on the property itself. Lawns, trees, and planting beds are managed organically whenever possible. And trees are planted to replace those felled for construction projects.

Two areas in which the Laboratory has focused recent efforts have been those of waste management and energy conservation. The Laboratory currently recycles most waste. Scrap metal, electronics, construction debris, batteries, fluorescent light bulbs, toner cartridges, and waste oil are all recycled. For general waste, the Laboratory uses a "single stream waste management" system, removing recyclable materials and sending the remaining combustible trash to a cogeneration plant where it is burned to provide electricity, an approach considered among the most energy efficient, while providing a high yield of recyclable materials.

Equal attention has been paid to energy conservation. Most lighting fixtures have been replaced with high efficiency fluorescent fixtures, and thousands of incandescent bulbs throughout campus have been replaced with compact fluorescents. The Laboratory has also embarked on a project that will replace all building management systems on campus, reducing heating and cooling costs by as much as twenty-five per cent.

Cold Spring Harbor Laboratory continues to explore new ways in which we can reduce our environmental footprint, including encouraging our visitors and employees to use reusable containers, conserve energy, and suggest areas in which the Laboratory's efforts can be improved. This book, for example, is printed on recycled paper.

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New York City

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Take Syosset Taxi to Syosset Train Station ($8.00 per person, 15 minute ride), then catch Long Island Railroad to Penn Station (33rd Street & 7th Avenue). Train ride about one hour.

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