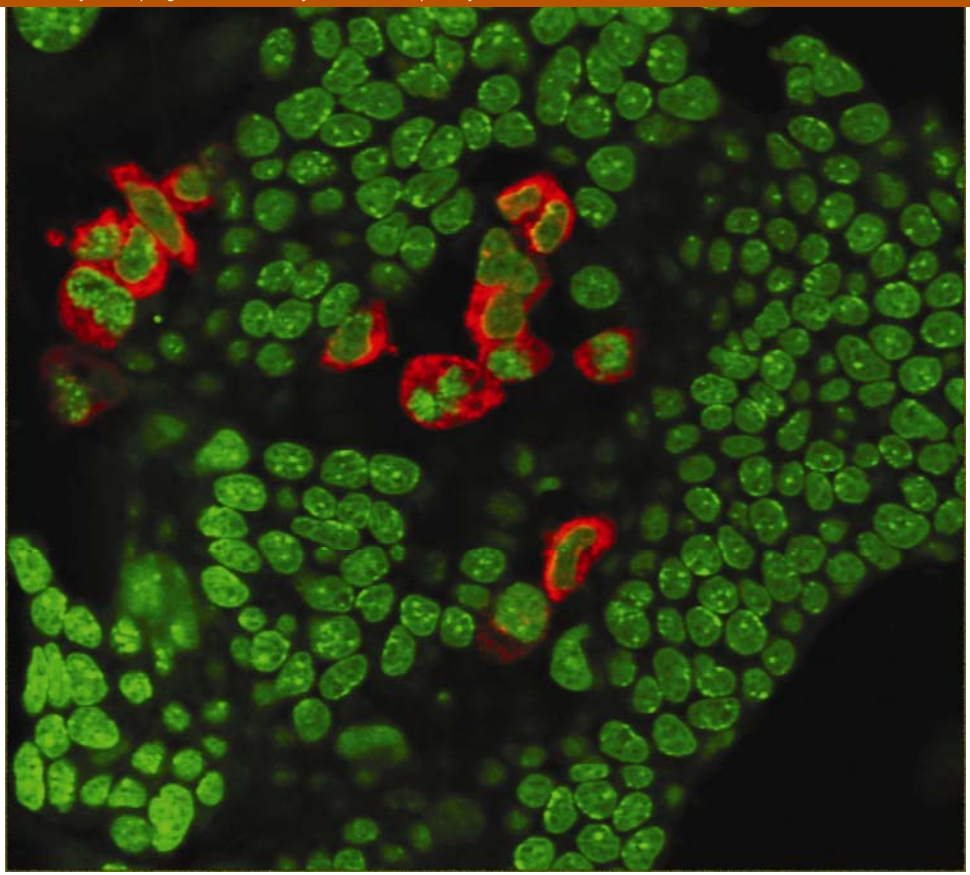
Abstracts of papers presented
at the 2010 meeting on

MOUSE DEVELOPMENT, GENETICS & GENOMICS

October 26–October 30, 2010



Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

Abstracts of papers presented
at the 2010 meeting on

MOUSE DEVELOPMENT, GENETICS & GENOMICS

October 26–October 30, 2010

Arranged by

Kathryn Anderson, *Sloan-Kettering Institute*
Haruhiko Koseki, *RIKEN Center for Allergy & Immunology, Japan*
William Skarnes, *Wellcome Trust Sanger Institute, UK*
Michael Shen, *Columbia University Medical Center*

Cold Spring Harbor Laboratory
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Front Cover: Immunostaining for Cerberus-like in clusters of spontaneously differentiating EpiSC cells. Jianhua Chu (Michael Shen's lab), Columbia University.

Back Cover: A section of an e7.5 embryo stained for E-cadherin (red) and DAPI (blue). Jeff Lee (Kathryn Anderson's lab), Sloan-Kettering Institute.

MOUSE DEVELOPMENT, GENETICS & GENOMICS
Tuesday, October 26 – Saturday, October 30, 2010

Tuesday	7:30 pm	**1** Human Disease and Cancer
Wednesday	9:00 am	**2** Organogenesis
Wednesday	2:00 pm	**3** Poster Session I
Wednesday	4:30 pm	Wine and Cheese Party *
Wednesday	7:30 pm	**4** Technology
Thursday	9:00 am	**5** Patterning
Thursday	2:00 pm	**6** Poster Session II
Thursday	4:30 pm	**Keynote Speaker**
Thursday	7:30 pm	**7** Neurobiology
Friday	9:00 am	**8** Epigenetics
Friday	2:00 pm	**9** Genetics and Genomics
Friday	4:30 pm	**Rosa Beddington Lecture**
Friday	6:00 pm	Banquet
Saturday	9:00 am	**10** Germ Cells and Stem Cells

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, October 26—7:30 PM

SESSION 1 HUMAN DISEASE AND CANCER

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

WEDNESDAY, October 27—9:00 AM

SESSION 2 ORGANOGENESIS

Chairperson: **K. Kaestner,** University of Pennsylvania Medical School, Philadelphia

WEDNESDAY, October 27—2:00 PM

SESSION 3 POSTER SESSION I

WEDNESDAY, October 27—4:30 PM

Wine and Cheese Party

WEDNESDAY, October 27—7:30 PM

SESSION 4 TECHNOLOGY

Chairperson: **A. Nagy,** Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, Toronto, Canada

THURSDAY, October 28—9:00 AM

SESSION 5 PATTERNING

Chairperson: **P. Soriano,** Mount Sinai School of Medicine, New York, New York

SESSION 6 POSTER SESSION II

THURSDAY, October 28—4:30 PM

KEYNOTE SPEAKER

Mario Capecchi
University of Utah School of Medicine

THURSDAY, October 28—7:30 PM

SESSION 7 NEUROBIOLOGY

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

FRIDAY, October 29—9:00 AM

SESSION 8 EPIGENETICS

Chairperson: **T. Bestor,** Columbia University College of Physicians & Surgeons, New York, New York

Timothy Bestor
Presenter affiliation: Columbia University College of Physicians & Surgeons, New York, New York.

Epigenetic regulation of stem cell differentiation in testes
Takayuki Shirakawa, Ruken Yaman-Deveci, Yoshito Kamizato, Jafar Sharif, Masahiro Muto, Shosei Yoshida, Kiyoe Ura, Haruhiko Koseki, Kazuyuki Ohbo.
Presenter affiliation: Yokohama City University, School of Medicine, Yokohama, Japan. 122

Genic transcription precedes loss of Xist coating and depletion of H3K27me3 during X-chromosome reactivation in the mouse inner cell mass
Lucy A. Williams, Sundeep Kalantry, Joshua Starmer, Terry R. Magnuson.
Presenter affiliation: University of North Carolina, Chapel Hill, North Carolina. 123

Aberrant stem cell regulation and differentiation in deer mouse (*Peromyscus*) hybrid extra-embryonic development
Paul B. Vrana, Amanda R. Duselis.
Presenter affiliation: University of South Carolina, Columbia, South Carolina. 124

Mouse and zebrafish Hoxa3 orthologs have non-equivalent in vivo protein function during mouse embryogenesis
Nancy R. Manley, Lizhen Chen, Peng Zhao, Lance Wells, Chris T. Amemiya, Brian G. Condie.
Presenter affiliation: University of Georgia, Athens, Georgia. 125

Anne Ferguson-Smith
Presenter affiliation: University of Cambridge, Cambridge, United Kingdom.

FRIDAY, October 29—2:00 PM

SESSION 9 GENETICS AND GENOMICS

Chairperson: **J. Baker,** Stanford University, California

Chromatin and transcriptional signatures for the SMAD2/3 complex
Se Jin Yoon, Andrea Wills, Si Wan KIm, Edward Chuong, Julie Baker.
Presenter affiliation: Stanford University, Stanford, California. 126

FRIDAY, October 29—4:30 PM

ROSA BEDDINGTON LECTURE

FRIDAY, October 29—6:00 PM

CONCERT
Grace Auditorium

Aaron Goldberg Trio

For the Boston-born, New York-based Aaron Goldberg, Worlds is an encyclopedic circumnavigation of his ever-evolving musicality, which began with piano lessons at the age of seven. In high school Aaron got hooked on jazz by Bob Sinicrope of Milton Academy and continued his pursuit with saxophonist Jerry Bergonzi, two master educators. "At first improvisation was a mystery and a puzzle, but soon it became a profound inner and outer journey as life and music entwined." After receiving awards from Berklee School of Music and DownBeat, Aaron left at age 17 for NYC. At the New School for Jazz and Contemporary Music in 1991 he had his first taste of jazz in the big city, and at school he met many of his current contemporaries and friends, including Omer Avital, Brad Mehldau, Roy Hargrove, Ali Jackson and others.

In 1992 he returned to Boston and enrolled at Harvard College. While at Harvard, Aaron worked with a wide variety of artists from nearby Berklee and beyond, and won the International Association of Jazz Educators' prestigious Clifford Brown/Stan Getz Fellowship award as well as first place in National Foundation for Advancement in the Arts Recognition and Talent Search in 1993. Soon he was discovered by vocalist and first lady of jazz Betty Carter and was a founding member of her historic Jazz Ahead program. He continued to perform at clubs around both New York and Boston, often commuting in the wee hours, and it was not long before he met Rogers and Harland. Aaron graduated magna cum laude from Harvard in 1996 with a degree in History and Science and a concentration in Mind, Brain and Behavior. On the weekends he held a long-time residence at Wally's Cafe in Boston, and the fall after graduation he moved to Brooklyn. Aaron wasted no time in the Big Apple. He quickly established himself as a stellar sideman, performing with a vast array of leaders including Al Foster, Nicholas Payton, Stefon Harris, Tom Harrell, Freddie Hubbard, Mark Turner, and others. In 1998 he joined the band of Joshua Redman, with whom he toured for 4 years and recorded two albums (Beyond, 2001 and Passage of Time, 2002).

Most recently, in addition to leading his telepathic trio Aaron has been touring and recording with young guitar guru Kurt Rosenwinkel. In 2005 he also toured South America with Madeleine Peyroux and spent 6 months performing with Wynton Marsalis in his quartet as well as with the Lincoln Center Jazz Orchestra. Aaron's long and impressive list of recorded credits includes work with a diverse spectrum of artists ranging from Guillermo Klein to Terry Gibbs/Buddy DeFranco, as well as with fellow leaders of the next generation including John Ellis, Jimmy Greene and Eli Degibri. In 2004, Aaron produced and performed in Jazz for America's Future, a fundraising concert for John Kerry's presidential campaign that also featured Savion Glover, Brad Mehldau, Michael Brecker, Dee Dee Bridgewater, Christian McBride and others. He is currently the musical director of All Souls at Sundown, a jazz and poetry series at Manhattan's All Souls Church. His first recording as a leader, Turning Point, was released on the J Curve imprint in 1999, followed by Unfolding in 2002. He's also a member of the OAM Trio, which recorded Trilingual (1999) and Flow (2002) for the Fresh Sound/New Talent label, as well as two collaborations with saxophonist Mark Turner: an upcoming studio project and the acclaimed Live in Sevilla (2003) on Lola Records.

FRIDAY, October 29

BANQUET

Cocktails 7:00 PM Dinner 7:45 PM

SATURDAY, October 30—9:00 AM

SESSION 10 GERM CELLS AND STEM CELLS

Chairperson: **K. Hochedlinger,** Massachusetts General Hospital, Boston

Functions of NANOS2 in the maintenance and differentiation of male germ lineage in mice
Yumiko Saga.
Presenter affiliation: National Institute of Genetics, Mishima, Japan. 133

Successive roles of PDGF signaling in the extraembryonic endoderm of the mouse embryo
Jerome Artus, Kat Hadjantonakis.
Presenter affiliation: Sloan-Kettering Institute, New York, New York. 134

Cdx2 mRNA transport in mouse embryo depends on actin and microtubule network and a zip code identified in the coding sequence
Maria Skamagki, Krzysztof B. Wicher, Sujoy Ganguly, Alexander W. Bruce, Magdalena Zernicka-Goetz.
Presenter affiliation: Wellcome Trust/Cancer Research UK Gurdon Institute, Cambridge, United Kingdom. 135

Requirement for FGF4 in primitive endoderm lineage commitment in the mouse blastocyst
Anna Piliszek, Jerome Artus, Minjung Kang, Robert Aho, Kat Hadjantonakis.
Presenter affiliation: Sloan-Kettering Institute, New York, New York. 136

Satellite cells require Notch signals to recognize their 'niche'
Dominique Broehl, Elena Vasyutina, Carmen Birchmeier.
Presenter affiliation: Max Delbrück Centrum, Berlin, Germany. 137

GENETIC AND MOLECULAR DIRECTION OF SEROTONIN SIGNALING IN THE HYPOTHALAMUS

Gerard Karsenty

Columbia University, Genetics & Development, New York, NY, 10032

Serotonin is a bioamine synthesized in the brain where it acts as a neurotransmitter and the gut where it acts as a hormone. Serotonin does not cross the blood brain barrier hence each pool of this molecule may have different functions. We have shown earlier that brain-derived serotonin favors bone mass accrual and appetite by signaling through distinct receptors in the ventromedial (VMH) and arcuate neurons of the hypothalamus respectively. These two functions of serotonin are inhibited by leptin which signals in serotonergic neurons of the brainstem to inhibit serotonin synthesis and release. To better understand how leptin and serotonin coordinate the regulation of bone mass and energy metabolism we studied the signal transduction and transcriptional cascades elicited by serotonin in VMH and arcuate neurons of the hypothalamus. We show that in both nuclei serotonin uses CREB as a transcriptional mediator of its function and identified genes expressed in these neurons and whose expression is under the positive and negative control of serotonin and leptin respectively. Lastly, we will present proof of principle evidence that modulating the serotonin pathway can treat obesity.

THRESHOLD LEVELS OF TBX5 ENSURE SYMMETRICAL FORMATION OF THE LEFT AND RIGHT FORELIMB

Fatima A Sulaiman, Malcolm P Logan

Developmental Biology, National Institute for Medical Research, London, NW7 1AA, United Kingdom

Although there is no obvious communication between the left and right limb buds they develop to form bilaterally symmetrical structures of equal size (Summerbell & Wolpert, 1973). The underlying mechanisms that ensure symmetrical limb formation are unknown. We present the results of a study using the mouse model as a genetic tool showing a role for the transcription factor *Tbx5* in ensuring the symmetrical formation of the left and right forelimb.

Holt-Oram Syndrome (HOS) [OMIM 142900] is a congenital syndrome associated with mutations in *TBX5* that lead to heart and upper limb defects. Strikingly, over 70% of HOS patients have left-biased upper limb defects. We show that hypomorphic levels of Tbx5 in both the left and right forelimb buds produces forelimb defects that are consistently more severe in the left limb than the right, thus phenocopying the left-biased upper limb defects seen in HOS patients. Using the *INV/INV* mutant background in which the left-right axis is reversed, we show that the laterality of these defects is reversed in Tbx5 hypomorphic mutants with situs inversus. Additionally, we also show that transgenic expression of equal levels of *Fgf10* in the forelimb buds of these Tbx5 hypomorphs can rescue outgrowth defects but not the left-bias asymmetry of their presentation. Together, our data suggest that *Tbx5* has a role in ensuring symmetrical forelimb formation and that this is independent of its transcriptional regulation of *Fgf10*.

AN UNEXPECTED ROLE FOR TBX5 IN THE SECOND HEART FIELD FOR CARDIAC SEPTATION

Linglin Xie, Joshua Friedland-Little, Andrew Hoffmann, Ivan Moskowitz

The University of Chicago, Pediatrics and Pathology, Chicago, IL, 60637

Cardiac septation is an essential specialization of tetrapod cardiac morphogenesis; congenital heart disease (CHD) results when cardiac septation is compromised. Our understanding of the molecular mechanisms driving atrioventricular septation has undergone recent revision, including a novel paradigm relying on the extra-cardiac specification of cardiac progenitors for the atrial septum (e.g. Hoffmann et al., 2009). While Tbx5 is a well studied cardiogenic transcription factor implicated in Holt–Oram syndrome with atrioventricular septal defects in humans, the basis for its requirement in atrioventricular septation remains unknown. We found that Tbx5 is required in cardiac progenitors but not in the heart for atrioventricular septation. Germline Tbx5 haploinsufficiency caused atrioventricular septal defects in 40% of late gestation mouse embryos. Yet Tbx5 haploinsufficiency in myocardial cells, using Tnt:Cre, or in endocardial cells, using Tie2:Cre, resulted in normal septation, suggesting that the Tbx5 activity which required cardiac septation lay outside the heart. We observed Tbx5 expression in progenitors for the atrial septum outside of the heart in the posterior second heart field. Conditional Tbx5 haploinsufficiency in the posterior second heart field using the Hedgehog (Hh) signaling-responsive Gli1:Cre caused atrioventricular septal defects in 40% of embryos, demonstrating a requirement for Tbx5 outside the heart in the second heart field. We found that Tbx5 and Hh-signaling components genetically interact: decreasing Hh signaling caused increased penetrance of atrioventricular septal defects in Tbx5 mutant mice. Furthermore, constitutive activation of Hh-signaling rescued atrioventricular septation in Tbx5 mutant mice, placing Hh signaling downstream of Tbx5 in a pathway required for atrial septation. Finally, using Genetic Inducible Fate Mapping, we demonstrated that Tbx5 is specifically required for proliferation of atrial septum progenitor cells in the posterior second heart field. We present a molecular pathway including Tbx5 and Hh signaling required for cardiac progenitor cell specification and morphogenesis of cardiac septation that informs the ontogeny of atrial septal defects.

IDENTIFICATION OF SOX3/SOX3 AS A XX MALE SEX REVERSAL GENE IN MICE AND HUMANS.

Edwina Sutton[1], James Hughes[1], Stefan White[2], Ryohei Sekido[3], Jacqueline Tan[2], Valerie Arboleda[4], Vincent Harley[5], Eric Vilain[4], Andrew Sinclair[2], Robin Lovell-Badge[3], Paul Thomas[1]

[1]University of Adelaide, School of Molecular & Biomedical Science, Adelaide, 5005, Australia, [2]Murdoch Childrens Research Institute , Early Development and Disease Theme, Melbourne, 3052, Australia, [3]MRC National Institute for Medical Research, Division of Developmental Genetics, London, NW7 1AA, United Kingdom, [4]UCLA, Department of Human Genetics, Los Angeles, CA, 90095-7088, [5]Prince Henry's Institute of Medical Research, Molecular Genetics and Development , Melbourne, 5152, Australia

Therian mammals utilise an XX/XY system of sex determination in which the Y-linked gene *Sry* (Sex-determining region Y) exerts a dominant masculinising influence on sexual development. Sex chromosome homology and comparative sequence studies suggest that *Sry* evolved from the related *Sox3* gene on the X chromosome, although there is no direct functional evidence to support this hypothesis. Indeed, loss of function mutations show that SOX3 is not required for sex determination in mice or humans, but it is critical for normal CNS and pituitary development. To investigate the developmental consequences of overexpression, we created *Sox3* transgenic mice. One of these lines expressed *Sox3* ectopically in the bipotential gonad due to a position effect and frequently exhibited complete XX male sex reversal. Morphological, gene expression and cell co-transfection studies indicate that SOX3 induces testis differentiation in this particular line of mice by activation of *Sox9* via a similar mechanism to SRY. In addition, array-Comparative Genomic Hybridisation analysis of sixteen *SRY*-negative XX males revealed three patients with rearrangements of the SOX3 regulatory region, indicating that *SOX3* gain-of-function in humans also causes XX male sex reversal. Together, these data suggest that SOX3 and SRY proteins are functionally interchangeable in sex determination and support the notion that *Sry* evolved from *Sox3* via a regulatory mutation that led to its de novo expression in the early gonad. These data also provide novel insight into the mechanism that underpins XX male sex reversal in humans in the absence of *SRY*.

ERK1 AND ERK2 HAVE A REDUNDANT ROLE IN PROMOTING EMBRYONIC FIBROBLASTS PROLIFERATION.

Laure Voisin*, Marc K Saba-El-Leil*, Catherine Julien, Christophe Frémin, Sylvain Meloche

Institut de Recherche en Immunologie et Cancérologie, Université de Montréal, Montréal, H3C3J7, Canada

The ERK1/2 mitogen-activated protein (MAP) kinase signaling pathway plays a critical role in the proliferative response of mammalian cells to mitogenic factors. However, the individual contribution of the isoforms ERK1 and ERK2 to cell proliferation control remains unclear. The two proteins have similar biochemical properties, recognize the same primary sequence determinants on substrates and are ubiquitously expressed in cell lines and tissues, although their relative abundance is variable. However, analysis of mice lacking individual ERK genes suggests that ERK1 and ERK2 may have evolved unique functions. Mice homozygous for *Erk2* mutation die *in utero* around day e6.5 due to a defect in trophoblast development. On the other hand, ERK1-deficient mice are viable, fertile and of normal size. Finally, it has been recently proposed that ERK1 and ERK2 exert antagonistic effects on cell proliferation.

In this study, we have used a genetic approach to analyze the individual roles of ERK1 and ERK2 in cell proliferation control using genetically matched primary embryonic fibroblasts. We show that loss of ERK1 reduces mitogenic signaling and significantly slows down the proliferation rate of fibroblasts. To circumvent the early embryonic lethality associated with a null mutation of *Erk2*, we have generated ERK2-deficient embryos using two approaches: tetraploid aggregation with *Erk2* null embryos and the generation of mice carrying a conditional *Erk2* allele. We found that $Erk2^{-/-}$ fibroblasts are also impaired in the activation of signaling effectors and proliferate slower. The decline in proliferation is more important in ERK2-deficient cells, consistent with the higher level of expression of ERK2 in these cells. To further analyze the specific contribution of ERK1 and ERK2, we evaluated the impact of silencing the expression of ERK1 or ERK2 by RNA interference in cells genetically disrupted for the other isoform. Lowering the level of either remaining ERK isoform similarly resulted in a marked decrease in cell proliferation. Finally, we generated fibroblasts genetically deficient in both *Erk1* and *Erk2* by infecting $Erk1^{-/-}$; $Erk2^{f/f}$ primary MEFs with a retroviral vector expressing the Cre recombinase. Combined loss of ERK1 and ERK2 function resulted in a complete arrest of cell proliferation.

Altogether, these findings provide compelling genetic evidence for a redundant role of ERK1 and ERK2 in promoting cell proliferation.

* L.V. and M.K.S.-E.-L. contributed equally to this work.

THE *P63*^{R279H} MOUSE MODEL MIMICS THE HUMAN ECTRODACTYLY-ECTODERMAL DYSPLASIA-CLEFTING (EEC) SYNDROME

Emma Vernersson-Lindahl [1], Elvin Garcia[1], Guy Horev[1], Hans van Bokhoven [2], Alea A Mills[1]

[1]Cold Spring Harbor Laboratory, Cancer Research Center, Cold Spring Harbor, NY, 11743, [2]Radboud University , Nijmegen Medical Centre, GD Nijmegen, 6525, Netherlands

The *p63* gene has a complex structure with two promoters giving rise to the TAp63 and ΔNp63 transcripts. Alternative splicing further diversifies the TAp63 and ΔNp63 isoform classes into α, β and γ isoforms. The prominent phenotypes observed in the p63 deficient mouse models have clearly established p63 as a master regulator of epidermal development. Further, p63 deficient mice provided clues that led to the discovery that p63 mutations are the cause of several different human developmental syndromes. We have established a mouse model for one of these human syndromes – Ectrodactyly-Ectodermal dysplasia-Clefting (EEC) syndrome – by knocking in the R279H mutation causative of human EEC. This model, referred to as *p63*^{R279H}, has severe phenotypes comparable to those found in human EEC patients, including craniofacial clefting and defects in skin and skin related appendages.

We have found that primary keratinocytes from *p63*$^{R279H/+}$ mice exhibit decreased proliferation and enhanced senescence. This phenotype is not present in the p63 deficient model (*p63*^{Brdm3}) indicating a unique role for the R279H point mutation in modulating keratinocyte biology. Further, *p63*^{R279H} homozygous mice, although lacking a mature skin layer at birth, still express markers of keratinocyte differentiation that are not detectable in the *p63*^{Brdm3} homozygous mutants, indicating that the R279H allele is distinct from the null allele. We used cDNA microarray analyses to identify gene expression profiles within primary *p63*$^{R279H/+}$ keratinocytes. Furthermore, by integrating a microarray analysis performed on human EEC patients carrying the R279H mutation, we have identified a number of potential candidate genes that link the mouse *p63*^{R279H} model with the human EEC syndrome. By analyzing expression of these candidates in the EEC mouse model, we are currently identifying molecular signatures responsible for the pathogenesis of human EEC syndrome.

MOUSE MODELS OF SKIN CANCER

Fiona M Watt

CRUK, Cambridge Research Institute, Robinson Way, Cambridge, CB2 0RE, United Kingdom

Mice have been used to model skin cancer for many decades. However, as we learn more about stem cells in the epidermis and about how environmental signals regulate their behaviour, the question we face is: which is the most appropriate model? Transgenic mice that develop all the major types of epidermal cancer are available and have the advantage that tumour formation can be monitored and manipulated within the context of a fully functional immune system. Nevertheless, tumours can also be built from human cells via xenografts in immunocompromised animals. Unlike autochthonous mouse models, xenografts allow quantitative evaluation of the relative tumour-initiating capacity of different stem cell populations. I will discuss the different types of information that are obtained from the different models and will suggest that it is important to compare data obtained from multiple models in order to obtain clinically relevant data.

ENFORCED TBX1 EXPRESSION IN FETAL THYMUS IS INCOMPATIBLE WITH TEC DIFFERENTIATION

Kim T Cardenas[1], Zhijie Liu[2], Micheline Laurent[1], Carla Carter[1], Nancy Manley[2], Ellen Richie[1]

[1]MD Anderson Cancer Center, Molecular Carcinogenesis, Smithville, TX, 78957, [2]University of Georgia, Department of Genetics, Athens, GA, 30602

The thymus and parathyroid glands originate from organ-specific domains of 3rd pharyngeal pouch (PP) endoderm. At embryonic day 11.5 (E11.5), the ventral thymus and dorsal parathyroid domains can be identified by Foxn1 and Gcm2 expression respectively. We have shown that neural crest cells (NCCs) play a role in regulating patterning of 3rd PP endoderm. In addition, pharyngeal endoderm influences fate determination via secretion of Sonic hedgehog (Shh), a morphogen required for Gcm2 expression and generation of the parathyroid domain. Gcm2 is a downstream target of the transcription factor Tbx1, which in turn is positively regulated by Shh. Although initially expressed throughout pharyngeal pouch endoderm, Tbx1 expression is excluded from the thymus-specific domain of the 3rd PP by E10.5, but persists in the parathyroid domain. Based on these observations, we hypothesized that Tbx1 expression is non-permissive for thymus fate specification and that enforced expression of Tbx1 in the fetal thymus would impair thymus development.

To test this hypothesis, we generated knock-in mice containing a Cre-inducible allele that allows temporal and spatial control of Tbx1 expression. Expression of the R26iTbx1 allele in fetal and adult thymus using Foxn1-Cre resulted in severe thymus hypoplasia throughout ontogeny that persisted in the adult. Thymic epithelial cell (TEC) development was impaired as determined by immunohistochemical and FACS analysis of various differentiation markers. The relative level of Foxn1 expression in fetal TECs was significantly reduced. TECs in R26i$^{Tbx1/+}$ thymi assumed an almost universal expression of Plet-1, a marker associated with a TEC stem/progenitor cell fate. In addition, embryonic R26i$^{Tbx1/+}$ mice develop a perithymic mesechymal capsule that appears expanded compared to control littermates. Interestingly, thymi from neonatal and adult R26$^{iTbx1/+}$ but not R26$^{+/+}$ mice were encased in adipose tissue. This thymic phenotype also correlated with a decrease in thymocyte cellularity and aberrant thymocyte differentiation. The results to date support the conclusion that enforced expression of Tbx1 in TECs antagonizes their differentiation and prevents normal organogenesis via both direct and indirect effects. Supported by NIH HD056315 to ER and HD035920 to NM.

γ-GLUTAMYL CARBOXYLASE REGULATES WHOLE BODY ENERGY METABOLISM THROUGH ITS EXPRESSION IN OSTEOBLASTS

Mathieu Ferron, Gerard Karsenty

Columbia University, Genetics and Development, New York, NY, 10032

Osteocalcin is a hormone produced by osteoblast, the bone-forming cell, and regulating energy metabolism. While osteocalcin can exist in two forms, γ-carboxylated and undercarboxylated, only the undercarboxylated form of this molecule functions as a hormone favouring glucose handling and increasing energy expenditure. As a result, the absence of osteocalcin leads to glucose intolerance in mice, and infusions of uncarboxylated osteocalcin in wild type mice protect them from type 2 diabetes and obesity. More recently, we have shown that it is the osteoclast, the bone-resorbing cell, that decarboxylates and activates the osteocalcin trapped in the extracellular matrix (ECM). Surprisingly, it is the low pH generated during bone resorption that allows the osteocalcin to be decarboxylated. Thus, the results obtained so far suggest that γ-carboxylation of osteocalcin have an inhibitory effect on the bioavailability of this hormone and, indirectly, on whole body glucose homeostasis. To formally test this contention we generated osteoblast-specific γ-glutamyl carboxylase (Ggcx)-deficient mice ($Ggcx_{osb}$-/-). In contrast to the *Ggcx* null mice, which died shortly after birth from hemorrhage, the $Ggcx_{osb}$-/- mice were viable and obtained at the expected Mendelian ratio. These mutant mice however, displayed a 5-fold increase in their serum levels of uncarboxylated osteocalcin. In agreement with our working hypothesis glucose handling was significantly improved in $Ggcx_{osb}$-/- as measured by a glucose tolerance test (GTT). We also observed an increase in insulin sensitivity in these mice when we subjected them to an insulin tolerance test (ITT). Fasting insulin was increased in $Ggcx_{osb}$-/- mice, suggesting that pancreatic β-cell function was improved in these animals. Lastly, $Ggcx_{osb}$-/- mice presented a 50% decrease in their fat mass, which results in a lower body weight at 5 month of age. Importantly, when fed a high fat diet (HFD), the $Ggcx_{osb}$-/- mice gained significantly less weight than control mice and were protected against the glucose intolerance induced by HFD. Taken together, these results establish that γ-glutamyl carboxylase through it expression in osteoblasts negatively regulates glucose homeostasis. Thus they further support the hypothesis that it is the undercarboxylated form of osteocalcin that acts as a hormone. This work also suggests that Vitamin K, an essential cofactor of γ-glutamyl carboxylase, may play a role in the control of energy metabolism by bone.

COOPERATIVE ACTION OF *WNT4* AND GERM CELLS IN MAINTAINING OVARIAN CELL FATE DURING MOUSE EMBRYOGENESIS.

Danielle Maatouk, Blanche Capel

Duke University, Department of Cell Biology, Durham, NC, 27713

Primary sex determination in mammals occurs during embryogenesis with the formation of the gonads. In XY embryos, *Sry* shifts the bipotential gonad towards a testicular fate, establishing the expression of *Sox9*, which carries out all known downstream functions of *Sry*. To date, no single gene has been identified that drives female sex determination. However, in humans, the existence of XX males lacking *Sry* predicts that the mutation of female genes could disrupt the female pathway and derepress the male pathway. We are interested in understanding the pathways that divert the bipotential gonad towards an ovarian fate. Two female-specific genes involved in canonical Wnt signaling have been identified that antagonize the male pathway (*Wnt4* and *R-spondin*) and result in XX masculinization in mice and humans when disrupted. We previously showed that activation of β-catenin is sufficient to disrupt the expression of SOX9 and male sex determination, leading to sex reversal. We now show that loss of β-catenin in XX gonads (using a ubiquitously expressed tamoxifen inducible Cre) results in only a partial female-to-male sex reversal, suggesting that other pathways are necessary for ovarian development. We have investigated the role of germ cells in ovarian development and found that a loss of germ cells does not alter the fate of ovarian somatic cells. However, when germ cells are depleted from XX gonads lacking *Wnt4*, a more severe sex-reversal phenotype is observed, including derepression of SOX9 and morphological changes resembling testis cords. This supports previous work from our lab that found an anti-testis role for XX germ cells in vitro and suggests that multiple pathways promote ovarian development.

ANTAGONISM BETWEEN WNT/β-CATENIN SIGNALING AND SIX2 IN THE CELL FATE DECISION OF NEPHRON PROGENITORS

Joo-Seop Park[1], Wenxiu Ma[2], Wing H Wong[2], Andrew P McMahon[1]

[1]Harvard University, Department of Stem Cell and Regenerative Biology, Cambridge, MA, 02138, [2]Stanford University, Department of Statistics, Stanford, CA, 94305

Nephron progenitor cells act as stem cells during kidney development. They are replenished by self-renewal and differentiate into epithelial cells, which eventually form the entire epithelial structure of a nephron. As the neighboring cells called the collecting duct provide signals for the differentiation of nephron progenitors, the fate decision of nephron progenitors is coordinated with the branching of the collecting duct. Nephron progenitors located at the ventral side of the tips of the collecting duct become differentiated by undergoing mesenchymal-to-epithelial transition while nephron progenitors located at the dorsal side of the collecting duct remain undifferentiated. It is critical to maintain a population of these undifferentiated cells and to allow their self-renewal in order to ensure nephrogenesis at each round of branching. Previous genetic studies have shown that Six2, a transcription factor expressed in undifferentiated nephron progenitors, is required for their self-renewal and that Wnt/β-catenin signaling provided by the collecting duct is responsible for their differentiation. In order to better understand how these two pathways regulate nephron progenitors in opposing ways, We performed genomic mapping of Six2 binding sites in freshly FAC-sorted nephron progenitor cells and mapping of β-catenin binding sites in Wnt/β-catenin signal-activated cells by chromatin immunoprecipitation and deep sequencing (ChIP-seq). Interestingly, Six2 and β-catenin share 129 common binding sites. Combining genomic mapping data with gene expression profiles allowed prediction of putative common target genes of Six2 and β-catenin in nephron progenitors. Transgenic analysis of a subset of the common target sites of Six2 and β-catenin revealed that Wnt/β-catenin signaling activates multiple signaling pathways by inducing expression of *Wnt4*, *Fgf8*, and *Bmp7* and that Six2 represses expression of these Wnt target genes by forming a complex with Tcf. In addition, we show that both β-catenin and Six2 bind to an enhancer of *Six2* where Six2 acts as an activator and β-catenin most likely acts as a repressor. Our findings suggest that Six2 and β-catenin antagonize each other in regulating expression of key genes during nephrogenesis.

SIX1 INITIATES BRANCHING MORPHOGENESIS BY REGULATING GREMLIN-BMP4 SIGNALING

Pin-Xian Xu[2]

[1]Mount Sinai School of Medicine, Genetics and Genomic Sciences, New York, NY, 10029, [2]Mount Sinai School of Medicine, Developmental and Regenerative Biology, New York, NY, 10029

Urinary tract morphogenesis requires subdivision of the ureteric bud (UB) into tip- and trunk-specific domains, which will differentiate into intra-renal collecting system and the extra-renal ureter, respectively, by responding to signals in their surrounding mesenchyme. The homeodomain transcription factor SIX1 is transiently expressed in the metanephric mesenchyme (MM) surrounding the UB tip at E10.5 and its expression disappears from E11.5. In *Six1*$^{-/-}$ mouse embryos, the collecting duct system fails to form but the ureter is present. We investigated the molecular and cellular basis of the phenotype displayed by *Six1*$^{-/-}$ embryos and report here that the tip domain of the UB is initially formed in the mutant but the tip cells fail to form an ampulla for branching morphogenesis. Instead, the mutant UB elongates within *Tbx18*- and *Bmp4*-expressing mesenchyme for its differentiation into the ureter. We find that the expression of the extra-cellular BMP antagonist *gremlin 1* (*Grem1*) in the MM is *Six1*-dependent and that SIX1 directly binds to the Grem1 promoter in the MM and can upregulate its expression in transient transfection studies. Treatment of mutant kidney rudiments with recombinant GREM1 protein restores ampulla formation and its subsequent branching morphogenesis. Since GREM1 acts as an antagonist of BMP4 to induce UB branching morphogenesis, we tested whether genetically lowering BMP4 activity could restore kidney organogenesis. Indeed, genetic reduction of BMP4 levels in S*ix1*$^{-/-}$ (*Six1*$^{-/-}$;*Bmp4*$^{+/-}$) embryos restores urinary tract morphogenesis and kidney formation. This study provides the first evidence that *Six1* acts by directly upregulating *Grem1* in the MM to locally antagonizing BMP4 activity to ensure that the UB tip cells are induced for ampulla formation and its subsequent branching. This finding uncovers an essential function for SIX1 as a critical upstream regulator of Gremlin-mediated BMP4 signaling during urinary tract morphogenesis.

THE ROLE OF HETEROCHROMATIN PROTEIN 1 (HP1) Γ IN PRIMORDIAL GERM CELLS

Kanae Abe[1], Chie Naruse[1], Tomoaki Kato[2], Takumi Nishiuchi[2], Mitinori Saitou[3], Masahide Asano[1]

[1]Kanazawa Univ., Div. of Transgenic Animal Sci., Adv. Sci. Res. Ctr., Kanazawa, 920-8640, Japan, [2]Kanazawa Univ., Div. of Functional Genomics, Adv. Sci. Res. Ctr., Kanazawa, 920-0934, Japan, [3]Kyoto Univ., Dep. Anatomy and Cell Biol., Glad. Sch. Med., Kyoto, 606-8501, Japan

Germ cell fate is determined among proximal epiblast cells by the signal from extraembryonic tissues in mice. After specification of germ cell fate, approximately 40 primordial germ cells (PGCs) appear at the base of allantoic bud, proliferate and migrate to genital ridges, reaching around 25,000 cells until E13.5. Heterochromatin protein 1 (HP1) family members, HP1α, β, Γ, are thought to repress gene expression epigenetically by binding the methylated histone H3 lysine 9 and to induce the formation of heterochromatin structure. We generated HP1Γ mutant mice and found that the number of HP1Γ-/- germ cells was dramatically reduced before meiosis. HP1Γ was expressed strongly in PGCs among HP1 family members, while all HP1s were expressed in somatic cells. The number of HP1Γ-/- PGCs was reduced from early bud (EB) stage (E7.25). The ratio of HP1Γ-/- PGCs in S-phase decreased and that in G1-phase increased conversely. Moreover, the ratio of p21 (Cip1)-positive HP1Γ-/- PGCs increased, suggesting G1/S-phase transition was inhibited. On the other hand, we could not detect any difference in apoptosis, PGC differentiation and histone modification between control PGCs and HP1Γ-/- PGCs. These results suggest that reduced number of PGCs was caused by impaired cell cycle and HP1Γ plays a key role in proliferation of PGCs during early embryogenesis.

ENDOTHELIAL OVEREXPRESSION OF LOX-1 INCREASES AORTIC LDL UPTAKE, INDUCES ENDOTHELIAL DYSFUNCTION AND PLAQUE FORMATION *IN VIVO*. ROLE OF TRANSCRIPTION FACTORS NFKB AND OCT-1

Alexander Akhmedov, Izabela Rozenberg, Yi Shi, Carola Doerries, Pavani Mocharla, Alexander Breitenstein, Christine Lohmann, Sokrates Stein

University of Zurich, Switzerland.

Background- The hallmark of the initiation of atherosclerotic lesion is foam cell formation, and oxidized LDL (OxLDL) is believed to play a key role in the initiation of the atherosclerotic process. OxLDL is internalized by several receptors, such as SR-AI/II, SR-BI, CD36, and CD68. OxLDL is also internalized by endothelial cells, but this uptake depends on receptors other than the classic scavenger receptors. In 1997, a lectin-like oxidized LDL receptor-1 (LOX-1, OLR1) was identified in bovine aortic endothelial cells. LOX-1 is a type II membrane glycoprotein with an apparent molecular weight of 50 kDa. It has a C-terminal extracellular C-type lectin-like domain. This lectin-like domain is essential for binding to OxLDL. Binding of OxLDL to LOX-1 induces several cellular events in endothelial cells, such as activation of transcription factor NF-κB, upregulation of MCP-1, and reduction in intracellular NO, which may trigger the onset of cardiovascular events or accelerate the development of atherosclerosis. ***Methods and Results***- We generated endothelial-specific *LOX-1* transgenic mice using the Tie2 promoter (*LOX-1TG*). In endothelial cells, but not macrophages, *LOX-1TG* mice exhibited enhanced oxLDL uptake. 6-week-old male *LOX-1TG* and wild-type (WT) mice were fed a high cholesterol diet for 30 weeks. In *LOX-1TG* vascular cell adhesion molecule-1 (VCAM-1) and E-selectin expression, macrophage accumulation and aortic fatty streaks were increased, while eNOS phosphorylation and endothelial function were reduced. In endothelial cells of *LOX-1TG* mice, reactive oxygen species were increased and the transcription factors NF-κB and Oct-1 activated. In atherosclerotic *LOX-1TG/ApoE*$^{-/-}$ mice, high cholesterol diet increased VCAM-1 expression, number of macrophages, T-cells as well as plaque size. ***Conclusions***-Thus, our data suggest that LOX-1 plays a critical role in the development of atherosclerosis when expressed at unphysiological levels. Therefore, LOX-1 might represent a novel therapeutic target for atherosclerosis.

ROLE OF KERATIN14 IN THE DEVELOPMENT OF STRATIFIED EPITHELIA: A PROSPECT FOR IN VIVO STUDIES.

Hunain Alam, Samrat Kundu, Sorab N Dalal, Milind M Vaidya

ACTREC, Vaidya Lab, Navi Mumbai, 410210, India

Keratins are cytoplasmic intermediate filament proteins preferentially expressed by epithelial tissues in a site-specific and differentiation dependent manner. The complex network of keratin filaments in stratified epithelia is tightly regulated during squamous cell differentiation. Keratin14 (K14) is expressed in mitotically active basal layer cells along with its partner Keratin5 (K5) and their expression is downregulated as cells differentiate. Apart from cytoprotective functions of K14, very little is known about K14 regulatory functions as the K14 knockout mice show embryonic lethality. This suggests that it performs unique tissue specific functions. K14 expression was inhibited using RNA interference in cell lines derived from stratified epithelia, to study the K14 functions in cell proliferation and differentiation. The K14 knockdown clones demonstrated significant reduction in cell proliferation and concomitant reduction in phosphorylated Akt levels. K14 knockdown cells also exhibit enhanced activated Notch1 levels, which were accompanied by increased involucrin and K1 levels. Our in vitro results suggest that K14 maintains proliferation potential in the basal layer of stratified epithelia by modulating PI-3K/Akt dependent cell proliferation and Notch1 dependent cell differentiation processes. The future studies using in vivo system will give us insights into the exact function of K14 in differentiation program and development of stratified epithelia.

FGF3 IS REQUIRED IN THE NORMAL TERMINATION OF EMBRYONIC AXIS EXTENSION

Matthew J Anderson[1], Thomas Schimmang[2], Mark Lewandoski[1]

[1]NCI , Cancer and Developmental Biology Laboratory, Frederick, MD, 21702, [2]University of Valladolid, Institute For Biology and Molecular Genetics, Valladolid, 47002, Spain

Elucidating mechanisms that control segmentation, a common motif by which embryos generate pattern, is essential to understand embryological development. In vertebrates, segmentation of the anterior-posterior (AP) axis is evident in the repeated pattern of the adult vertebrae, which are derived from embryonic segments called somites. Somites also give rise to skeletal muscle, tendons, and dorsal dermis of the torso. Somitogenesis occurs in a progressive AP fashion as new somites reiteratively segment from a posterior region termed the presomitic mesoderm (PSM). Relative to our knowledge on how somitogenesis and axis extension are initiated and maintained little is known about how these processes cease.

Fibroblast growth factors (FGFs) are key players in the regulation of axis extension. Previously we've shown that several *Fgfs* appear to play partially redundant roles during somitogenesis. However, *Fgf3* plays a non-redundant role in the cessation of axis extension as mice lacking this gene have a variably kinked and shortened tail. Consistent with this role for *Fgf3*, its expression in the PSM is downregulated as normal axis extension ceases.

Loss of *Fgf3* leads to an increase in cell death in the distal PSM during early midgestation, diminishing the PSM such that it is depleted prematurely at embryonic day 12.5 instead of 13.5, resulting in fewer somites and AP axis truncation. Normally, molecular signals that determine somite size are modulated during the gradual loss of PSM so that smaller somites form. In mutants, this occurs ahead of schedule causing abnormally shaped somites and a tail that tapers faster than normal.

Our data suggests a mechanism by which somite formation and cessation of the AP axis is directly influenced by the amount of PSM. In *Fgf3* mutants, premature PSM loss leads to an adjustment in timing of somitogenesis cessation, causing abnormal somite and notochord morphogenesis, leading to the kinked and shortened tails in the adult *Fgf3* mutant. This work sheds light on the mechanisms that control the final stages of axis extension and illuminates the role of *Fgf3* in this process.

A MOUSE MODEL FOR JUVENILE HYDROCEPHALUS

Oliver K Appelbe, Elena A Glick, Jenniffer R Ramalie, Ekaterina Y Steshina, Jennifer V Schmidt

University of Illinois at Chicago, Biological Sciences, Chicago, IL, 60607

Juvenile hydrocephalus, the accumulation of cerebrospinal fluid (CSF) in the ventricles of the brain, causes significant morbidity among human children affecting roughly 1 in 500 newborns. The disease manifests due to overproduction, decreased absorption, or restricted flow of CSF. Few genetic causes of this disease are known, and therefore animal models can prove beneficial in identifying candidate genes.
The Juvenile hydrocephalus (*Jh*) mouse line contains a transgenic integration on mouse chromosome 9. Homozygous *Jh* mice exhibit hydrocephalus by two weeks of age and few survive beyond eight weeks. This phenotype represents a novel cause of the disease since no known hydrocephalus mutations map to the region. Analysis of the integration site showed disruption of an uncharacterized gene, *I11*. Preliminary data suggests that a transgene carrying a functional copy of *I11* can rescue the *Jh* phenotype. However, the predicted protein product of *I11* has no recognizable functional domains and its role in CSF maintenance is unknown. Future research will focus on definitively establishing the role of *I11* in hydrocephalus and dissecting its function.

FIBRONECTIN AND INTEGRIN ALPHA 5 PLAY ESSENTIAL ROLES IN DEVELOPMENT OF LEFT-RIGHT ASYMMETRY IN MICE.

Maria Pulina[1], Ashok Mittal[2], Shuan-Yu Hou[3], Dorthe Julich[4], Scott Holley[4], Sophie Astrof[3]

[1]Memorial Sloan-Kettering Cancer Center, Program in Developmental Biology, New York, NY, 10065, [2]Jacobi Medical Center, Albert Einstein University, Medicine, Bronx, NY, 10461, [3]Yale University, Molecular, Cellular and Developmental Biology, New Haven, CT, 06511, [4]Thomas Jefferson University, Medicine, Philadelphia, PA, 19107

Early studies in Xenopus laevis suggested that cell-extracellular matrix (ECM) interactions regulate the development of the left-right axis of asymmetry; however, the identity of ECM components and their receptors important for this process have remained unknown. We have analyzed left-right patterning in mouse embryos lacking fibronectin (FN) and discovered that FN is required for the establishment and maintenance of asymmetric gene expression by regulating morphogenesis of the node, expression of Lefty1 at the embryonic midline and signaling by TGF beta family of proteins. Furthermore, our experiments indicate that a major cell surface receptor for FN, integrin α5, is also required for the development of left-right asymmetry and that this requirement is evolutionarily conserved in vertebrates. Taken together, our studies demonstrate the requisite role for a structural ECM protein and its integrin receptor in the development of the left-right axis of asymmetry in vertebrates.

THE CRE PORTAL - A NEW INFORMATION RESOURCE FOR CONDITIONAL MUTAGENESIS IN THE MOUSE (WWW.CREPORTAL.ORG)

Randy Babiuk[1], Steven Murray[2], Caleb Heffner[2], Martin Ringwald[1], Michael Sasner[2], Janan T Eppig[1]

[1]The Jackson Laboratory, Mouse Genome Informatics, Bar Harbor, ME, 04609, [2]The Jackson Laboratory, Genetic Resource Science, Bar Harbor, ME, 04609

Conditional mutagenesis is a powerful tool for examining effects of stage-specific gene function or for use in cases where gene deletion results in developmental lethality in knockout mice. Large-scale efforts are underway to produce 'conditional-ready' (floxed) ES cells for most mouse genes. Mice produced from these ES cells will have great value for examining gene function and regulation in different tissues or at different developmental ages. However, to achieve this potential, it will be critical to know which recombinase (cre) allele/driver combination will provide correct activity and specificity to fulfill the research aims. A significant number of mouse strains expressing cre recombinase, including inducible cre alleles driven by different drivers, have been generated. To maximize the utility of these cre strains in generation of conditionally-deleted mutations, tissue specificity of cre activity (or sites of ectopic function) must be known.

The cre portal is a new, comprehensive web resource (www.creportal.org) for researchers using conditional mutagenesis in mice. Querying by a specific driver or anatomical system can retrieve curated annotations of cre expression/activity and specificity data. Each allele in the cre portal has a link for phenotypic information at Mouse Genome Informatics (MGI, www.informatics.jax.org). Phenotypes can be found here for progeny of many of the cre-bearing strains that have been crossed to conditional-ready mice. This integration of cre specificity data with phenotypic information is a powerful tool that can assist researchers in designing experiments or identifying important reagents. For some cre alleles, specificity or phenotype data may not currently be available, but molecular details of the cre construct, inducibility (and inducing agent) if applicable, publications in which a particular cre has been used, and the availability of strains carrying cre alleles in public repositories can be accessed for all of the cre alleles.

At the present time, >1200 cre transgenic or knock-in alleles and >500 unique drivers are represented. In addition to cre, data for other recombinases such as Flp, dre, and phiC31 are available.

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SONIC HEDGEHOG'S ROLE AS A REGULATOR OF THIRD PHARYNGEAL POUCH FATE.

Virginia Bain, Nancy Manley

University of Georgia, Genetics, Athens, GA, 30602

The thymus, an organ essential for T-cell development, originates from third pouch endoderm. Thymus fate is ascertained by the presence of transcription factor *Foxn1* which is first expressed at E11.25 in mice. Previous work from the Manley lab has shown that in the absence of *Sonic hedgehog* (*Shh*), *Foxn1* is expressed throughout the third pharyngeal pouch and in neighboring pharynx while the parathyroid marker *Gcm2* is absent from the *Shh* null pouch. Collaboration from the Richie lab and Manley lab has shown that in the absence of neural crest mesenchyme (*Splotch* mice) the third pouch has an increased *Foxn1* positive thymus domain and a decreased *Gcm2* positive parathyroid domain at E11.5. However, initial pouch patterning is normal at E10.5. Previous work from our lab has shown evidence for *Shh* signaling both within the dorsal-anterior 3rd pouch endoderm and in the adjacent NCC mesenchyme. Similarities between the *Splotch* mutant and the *Shh* mutant led us to ask where is *Shh* signaling required to negatively regulate thymus fate and positively regulate parathyroid fate.
We generated tissue specific knockouts of the *Shh* signal transducer *Smoothened* in neural crest mesenchyme or pouch endoderm to determine where and how *Shh* signaling regulates the fates of the thymus and parathyroid. In the absence of *Shh* signaling in NCC mesenchyme, the phenotype at least partially mimics the neural crest mesenchyme deletion in that the *Foxn1* and *Gcm2* domains are altered within the pouch at E11.5 while initial *Gcm2* expression at E10.5 is normal. This result suggests that *Shh* from NCC mesenchyme is upstream of a signal that negatively regulates thymus fate while *Shh* from pouch endoderm positively regulates parathyroid fate.

MOUSE MODELS FOR TRANSLATIONAL CONTROL: STUDY OF THE RACK1-EIF6 AXIS IN DEVELOPMENT AND CANCER

Stefano Biffo[1,2], Simone Gallo[1,2], Anne Beugnet[1], Viviana Volta[1], Annarita Miluzio[1]

[1]San Raffaele Scientific Institute, DIBIT, Milano, 20132, Italy, [2]University of Easetrn Piedmont, DISAV, Alessandria, 15100, Italy

Studies on cellular models have shown that RACK1 is an intracellular receptor for PKC and a ribosomal protein (1). We have shown that RACK1 can lead to translational activation by dissociating eIF6 from ribosomal subunits (2), and that eIF6 itself is rate-limiting in translational control (3). The physiological relevance of RACK1 in vivo, and whether it acts primarily as a ribosomal protein or a PKC receptor is unknown. We therefore produced RACK1 mutant mice. Here we show that RACK1 depletion results in embryonic lethality. At heterozygosity RACK1 hypomorphic mice show defects in neural crest migration and postnatal growth. The molecular mechanism by which RACK1 causes lethality is under investigation.

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VARIABLE CRANIOFACIAL DEFECTS IN *TWSG1* MUTANT MICE CORRELATE WITH TRANSCRIPTOME CHANGES IN ASSOCIATION WITH REPETITIVE DNA SEQUENCE ENVIRONMENT

Charles J Billington[1,2], Wuming Gong[2], Brandon C Ng[1], Cynthia L Forsman[1,2], Brian A Schmidt[1], Rajaram Gopalakrishnan[3], Aaron L Sarver[4], York Marahrens[2], Anna Petryk[1,2]

[1]University of Minnesota, Pediatrics, Minneapolis, MN, 55455, [2]University of Minnesota, Genetics Cell Biol. & Dev., Minneapolis, MN, 55455, [3]University of Minnesota, Dentistry Diag. and Biol Sci., Minneapolis, MN, 55455, [4]University of Minnesota, Masonic Cancer Center, Minneapolis, MN, 55455

Severity of craniofacial defects may vary widely even when the underlying cause is the same, but the sources of this variation are poorly understood. Mice with disruption of *Twisted gastrulation (Twsg1)* develop craniofacial malformations with a wide range of severity despite being an isogenic C57/BL6 background. The goal of this study was to understand the basis of craniofacial defects and their variability in *Twsg1-/-* mice. We used expression arrays to analyze the transcriptome of the mandibular component of the first branchial arch at E10.5 from three classes of *Twsg1*$^{-/-}$ embryos: phenotypically normal, mildly affected, and severely affected and compared to wild type (WT) mice. Transcriptional alterations correlated with phenotypic severity. Remarkably, unaffected mutants also differed transcriptionally from WT embryos. Additional effectors of the *Twsg1* mutant phenotype included *Alx1, Dkk1, Isl1, Pitx2* and *Sox2*. Giving insight into compensatory mechanisms that may support normal development, several genes that regulate craniofacial development (*Eya4, Lhx8, Prrx2, Satb2, Tbx22*) showed opposing expression changes with up-regulation in unaffected mutants but down-regulation in affected mutants. Since the expression levels of genes may be influenced by nearby repetitive DNA elements, we attempted to associate repetitive sequence environment with the phenotype. Partial least squares discrimination analysis was performed using either gene expression values alone, or when taking into account both gene expression and repetitive sequence data. This analysis clearly separated all four groups of mouse embryos from each other into tight clusters when B2, B4, LINE-1, and ERV elements were taken into account. Severely affected *Twsg1*$^{-/-}$ samples were classified with 95% accuracy when only LINE-1 was taken into account. Gene expression alone delivered only 63% accuracy. Our study suggests repetitive sequence environment can affect the expression of neighboring genes involved in craniofacial development and account for some of the observed phenotypic variability and disease susceptibility.
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INTEGRATION OF PATHWAYS MEDIATING CHONDROCYTE ADAPTATION TO ER STRESS *IN VIVO*

Kathryn S Cheah[1], Zhijia Tan[1], Ben Niu[1], Maggie W Cheng[1], Kwok Yeung Tsang[1], Michael Q. Zhang[2], David Ron[3], Danny Chan[1]

[1]University of Hong Kong, Biochemistry, Hong Kong[2]The University of Texas at Dallas, Molecular and Cell Biology, Center for Systems Biology, Richardson, TX, 75080, [3]Institute of Metabolic Science, Metabolic Research Laboratories, University of Cambridge, CB2 0QQ, UK

The assembly and folding of secreted proteins in the ER is regulated by ER stress signaling (ERSS) which responds to the load of folded and unfolded protein to maintain an equilibrium. Perturbation of this homeostasis induces ER stress and may seriously affect the viability of cells, which if not alleviated, triggers apoptosis. In humans, mutations in *COL10A1* trigger ER stress and result in metaphyseal chondrodysplasia type Schmid (MCDS). Through studying the impact of expressing a mouse equivalent of a MCDS mutation, a 13bp deletion in *Col10a1* (13del), in transgenic mice we had previously identified a new mechanism of adaptation to ER stress. In hypertrophic chondrocytes (HCs) expressing mutant collagen X 13del proteins which cannot fold properly in the endoplasmic reticulum (ER), ERSS is triggered. These 13del HCs successfully adapt to ER stress and survive by reverting to an earlier differentiation state. We compared the transcriptomes of 13del HCs in different phases of adaptation to ER stress with those of wild type HCs undergoing terminal differentiation. Bioinformatics analyses were applied to identify the factors and regulatory circuitries that underlie the adaptive response of 13del HCs. We found major perturbation of metabolic pathways, especially affecting amino acid biosynthesis and mitochondria function. Our results also implicate the oxidative stress and hypoxic stress pathways in the adaptation of 13del HCs providing insight into the integration and cross-talk of these stress pathways in vivo. We also probed genetically the relationship between ERSS and the mechanisms of adaptation by 13del HCs. Up-regulation of CHOP and its downstream target GADD34 mark activation of the PERK pathway upon ER stress. CHOP is a transcription factor involved in ER stress-induced apoptosis and translational control. Up-regulation or recovery of protein synthesis upon alleviation of ER stress requires GADD34, which is also a downstream target of CHOP. We found that the degree of altered differentiation of 13del HCs and adaptation to ER stress is controlled by both CHOP and GADD34. This is the first evidence for a direct link between the ERSS and the changed differentiation programme of 13del HCs. Our findings have broad implications for adaptive mechanisms to ER stress in vivo and pathophysiology underlying chondrodysplasias caused by mutations that impact on protein assembly and secretion.

INTERACTION OF SPINDLIN AND SERBP1 SUGGESTS A TRANSLATIONAL ROLE OF SPINDLIN DURING THE MOUSE OOCYTE-TO-EMBRYO TRANSITION

TingGang Chew, Chanchao Lorthongpanich, Davor Solter, Barbara Knowles

Institute of Medical Biology, A-STAR, One-North, S138648, Singapore

Transcription is silenced in the fully grown oocyte and newly formed embryo, consequently they depend on the regulated translation of mRNAs that have been transcribed and stabilized during oocyte growth to complete meiosis and activate embryonic genome. SPINDLIN is an abundant cytoplasmic protein, which also localizes to the meiotic spindle, and is translated from stored maternal mRNA.

SPINDLIN-interacting proteins were isolated from a mouse ovary expression library by the yeast two-hybrid method and the hyarulonan/mRNA-binding protein family members, SERPINE1 RNA Binding Protein (SERBP1) and Hyarulonan Binding Protein 4 (HABP4), were identified. SERBP1 has previously been implicated in regulating mRNA stability.

We find ectopically expressed SPINDLIN binds to SERBP1 in HEK293T cells and that an endogenous protein complex is also co-immunoprecipitated from mouse embryonic fibroblasts. SPINDLIN contains three TUDOR-like domains which have been suggested to bind methylated protein. Indeed, a point-mutation in one of the three TUDOR decreases SPINDLIN binding to SERBP1. Both Spindlin and Serbp1 transcripts and proteins are found in full-grown and mature oocytes suggesting a role in the oocyte-to-embryo transition. Furthermore, both proteins localize to the cytoplasm in full-grown oocytes and also to the meiotic spindle in mature oocytes. We suggest that SPINDLIN modulates SERBP1 activity to regulate mRNA stability during the oocyte-to-embryo transition.

A NEW ALLELE OF *ZBTB16* SEPARATING SKELETAL PATTERNING AND SPERMATOGONIAL RENEWAL FUNCTIONS

Yung-Hao Ching[1], Lawriston A Wilson[2], John C Schimenti[3]

[1]National LaboratoryAnimal Center, Research and Development, Taipei, 115, Taiwan, [2]The Jackson Laboratory, R&D, Bar Harbor, ME, 04609, [3]Cornell University , Department of Biomedical Sciences College of Veterinary Medicine , Ithaca, NY, 14853

The zinc finger and BTB domain containing 16 *Zbtb16* (also called *Plzf*, *Zfp145* or Green's *luxoid*) belongs to the POZ/zinc-finger family of transcription factors. It contains a BTB/POZ domain that mediates epigenetic transcriptional repression. ZBTB16 is essential for proper skeleton patterning and male germ cell renewal. Two alleles have been reported that display similar phenotypes: a targeted knock-out, and the spontaneous nonsense mutation *luxoid*. We describe a new ENU induced mis-sense allele of *Zbtb16* called *seven toes* (*Zbtb16* ^{7t}). Homozygous animals exhibit hindlimb and axial skeleton abnormalities. Whereas the skeletal abnormalities are similar to those of the other alleles, *Zbtb16* ^{7t} differs in that it does not cause spermatogonial depletion and male infertility. Positional cloning revealed a point mutation changing the evolutionarily conserved amino acid Glu44 to Gly, possibly altering the BTB domain's activity. Therefore, *Zbtb16* ^{7t} is a separation-of-function allele that reveals differential requirements for domains of ZBTB16 in different developmental milieus.

EXPRESSION OF NA+-K+-2CL- COTRANSPORTER 1 IS EPIGENETICALLY REGULATED DURING POSTNATAL DEVELOPMENT OF HYPERTENSION.

Hyun-Min Cho, In Kyeom Kim

Kyungpook National University School of Medicine, Pharmacology, Daegu, 700-422, South Korea, [2]Brain Korea 21, , Daegu, 700-422, South Korea

The expression of Na+-K+-2Cl- cotransporter 1 (NKCC1) is upregulated in various models of hypertension such as spontaneously hypertensive rat (SHR) and rats with coarcted aorta. We tested the hypothesis that expression of NKCC1 is epigenetically regulated during postnatal development of hypertension. The mesenteric arteries from 5, 10, and 18 week-old Wistar Kyoto (WKY) and SHR were excised and subjected to vascular contraction. The expression levels of Nkcc1 mRNA and protein in aorta, heart, and kidney from 5, 10, and 18 week-old WKY and SHR were measured by real-time PCR and western blot, respectively. The methylation status and histone modification of Nkcc1 promoter region was analyzed by bisulfite sequencing and chromatin immunoprecipitation (ChIP) assay, respectively.
The inhibition of dose-response curves by bumetanide as well as the expression levels of Nkcc1 mRNA and of NKCC1 protein in aorta, heart, and kidney were comparable between 5 week-old SHR and age-matched WKY, but much greater in 18 week-old SHR than in age-matched WKY. CpG dinucleotides of Nkcc1 promoter region in WKY were getting methylated with age whereas those in SHR mostly remained unmethylated after development of hypertension. DNA methyltransferase 3B (DNMT3B) highly associated to the promoter in WKY, whereas CXXC finger protein 1 (Cfp1) highly bound to the promoter in SHR. Suppressive histone code dm-H3K9 was greater in WKY than SHR. Activating histone code Ac-H3 as well as mRNA levels of histone acetyltransferases were higher in SHR than WKY. In conclusion, expression of NKCC1 is epigenetically regulated during postnatal development of hypertension.

A NOVEL MUTATION IN THE MOUSE DEAD/H-BOX HELICASE DDX11 DISRUPTS CELL CYCLE PROGRESSION AND CAUSES EARLY EMBRYONIC LETHALITY.

Christina D Cota, Maria J Garcia-Garcia

Cornell University, Molecular Biology & Genetics, Ithaca, NY, 14850

DDX11 is a member of the highly conserved DEAD2 sub-family of DEAD/H-box helicases that also includes ERCC2 (Excision Repair Cross-Complementation Group 2), FANCJ (Fanconi Anemia Group J) and RTEL1 (Regulator of Telomere Length 1). Studies with yeast and human *Ddx11* have demonstrated an involvement of these helicases in sister chromatid cohesion, cell cycle progression and genome stability. Recently, mutations in DDX11 have been shown to be the causative genetic defect in Warsaw Breakage Syndrome, a human disorder characterized by hypersensitivity to DNA damaging agents and defects in sister chromatid cohesion. In mouse, loss of DDX11 has been previously shown to result in embryonic lethality. However, the developmental defects that lead to embryonic death in mouse *Ddx11* mutants are still poorly understood.

Here we describe the phenotypic characterization and positional cloning of *Cetus*, a mouse ENU-induced mutation that disrupts *Ddx11*. Homozygous *Cetus* mutant embryos are small, show a reduction in mesodermal cell populations and fail to thrive beyond E8.5. Positional cloning of *Cetus* revealed a non-synonymous point mutation in helicase domain motif V of DDX11. *Cetus* failed to complement a null allele of *Ddx11*, indicating that the point mutation in motif V completely disrupts the activity of *Ddx11*. Our results have revealed that $Ddx11^{Cetus}$ and $Ddx11^{-/-}$ embryos contain a significant number of cells with misaligned chromosomes at metaphase and a corresponding disruption of cell cycle progression. These observations are consistent with a conserved role for DDX11 in proper sister chromatid cohesion in mouse and demonstrate a specific requirement of the DDX11 motif V for helicase function and early mouse morphogenesis.

PRC2-DEPENDENT AND -INDEPENDENT DEPOSITION OF RING1B-MEDIATED UH2A AT DEVELOPMENTAL GENE PROMOTERS IN ES CELLS

Mitsuhiro Endoh[1], Takaho A Endo[2], Tamie Endoh[1], Miguel Vidal[3], Bradley E Bernstein[4], Haruhiko Koseki[1]

[1]RIKEN , Research Center for Allergy and Immunology, Yokohama, 2300045, Japan, [2]RIKEN , Bioinformatics and System Engineering Division, Yokohama, 2300045, Japan, [3]Centro de Investigaciones Biologicas, CSIC, Cell Proliferation and Development, Madrid, 28040, Japan, [4]Massachusetts General Hospital, Harvard Medical School and Broad Institute, Department of Pathology, Boston, MA, 02114

Two distinct Polycomb complexes, PRC1 and PRC2, have been shown to repress many common developmental genes in embryonic stem cells (ESCs). Previous studies suggested that local binding of PRC1 depends on PRC2. While this model is widely assumed, it is still unclear to what extent PRC1 functions are dependent on PRC2. This is partly because the genome-wide location of the PRC1-catalyzed histone modification, mono-ubiquitinated H2A (uH2A), is still not available except for that in MEF cells (Kallin et al. 2009).

In this study, using a ChIP on chip approach, we present genome-wide maps of uH2A deposition in ESCs, and comparative analysis to key PRC1 subunits, Ring1B and Cbx2, and the PRC2-catalyzed histone modification, trimethylated H3 lysine 27 (H3K27me3). uH2A deposition is evident at a distinct subset of H3K27me3 targets gene that show striking enrichment for PRC1 and are particularly dependent on PRC1 components for continued repression. Compared to other H3K27me3 targets, uH2A target genes show de-repression in much more extent by developmental cues or PRC1 loss, higher overrepresentation of gene ontology (GO) terms involved in transcription and/or development, and also correspond to longer hypomethylated CpG islands. Based on these observations, we concluded the genes enriched for uH2A along with H3K27me3 as core targets for Polycomb repression to maintain ES cells in an undifferentiated state.

In this process, we found that the degree of de-repression of the uH2A targets is not so prominent in ESCs deficient for Eed (a PRC2 component) as that in PRC1-deficient ESCs. We also found that these gene promoters retain a considerable level of uH2A in the absence of Eed. Furthermore, using a complementation approach, we demonstrate the presence of PRC2-dependent and -independent mechanisms for uH2A deposition at developmental gene promoters.

THE POLYCOMB COMPLEX IN MAMMALIAN SKIN DEVELOPMENT AND STEM CELLS

Elena Ezhkova, Nicole Stokes, Elaine Fuchs

Howard Hughes Medical Institute, The Rockefeller University, New York, NY, 10065

Identification of molecular mechanisms that control stem cells is fundamentally important to understanding tissue development and the progression of various tissue disorders, including cancer. Skin, the outermost barrier between body and environment, is an excellent model system to uncover these mechanisms. A single layer of embryonic skin stem cells gives rise to all skin lineages, including the epidermis, the hair follicles, and the sebaceous glands. Postnatally, separate pools of adult stem cells located in the interfollicular epidermis and in the bulge region of the hair follicle maintain skin lineages during normal homeostasis and upon wounding.

In vitro studies of embryonic stem cells have identified Polycomb-dependent trimethylation on H3K27 (triMeK27-H3) as a key regulator of stem cell identity however its role in control of tissue-specific stem cells in vivo is largely unknown. The Polycomb complex is a transcriptional repressor that is targeted to gene promoters and prevents recruitment of transcriptional machinery. EZH2, the catalytic subunit of the Polycomb complex, has been previously reported as the sole histone methyltransferase of K27-H3. Recently, EZH1, a homolog of EZH2, has been shown to be physically present in a Polycomb complex and is capable of establishing triMeK27-H3 histone modification and Polycomb-dependent gene repression.

Here we conditionally ablated both Ezh1 and Ezh2 to address the role of Polycomb repression in the control of skin development and regulation of skin stem cells. We have shown that loss of Polycomb repression has a dramatic effect on skin development leading to defective formation of hair follicles and sebaceous glands. Moreover Ezh1/2 2KO skin stem cells loose their self-renewal ability and are unable to sustain skin appendages. These data underscore the importance of the Polycomb complex in control of tissue development and maintenance of stem cells.

DEVELOPMENT OF GENOMIC RESOURCES FOR *PEROMYSCUS* RESEARCH

Adrienne Lewandowski[1], Jane Kenney-Hunt[1], Travis Glenn[2], Chin-Fu Chen[3], Michael J Dewey[1], Gabor Szalai[1], Paul B Vrana[1], Michael R Felder[1]

[1]University of South Carolina, Biological Sciences, Columbia, SC, 29208, [2]University of Georgia, Environmental Health Sciences, Athens, GA, 30602, [3]Greenwood Genetics Center, Molecular Studies, Greenwood, SC, 29646,

Mice of the genus *Peromyscus* (deer mice) are among the most abundant mammals of North America occurring in the wild from Alaska to Central America. They occur in diverse natural habitats. A major advantage of research with *Peromyscus* is their ready adaptability to colony conditions. Research on this genus has been widespread across disciplines. To enhance *Peromyscus* as a research organism genetic, reproductive, and genomic resources are being developed including: (1) a medium density genetic map of nearly 400 loci, (2) ESTs that are sequenced and analyzed from various tissues of *P. maniculatus* (BW stock) and *P. polionotus* (PO stock), (3) whole genome sequencing of 4 Peromyscus species is approved by NIHGR and underway, and (4) embryo manipulation and cryopreservation.
Variation in microsatellites and RFLPs in protein encoding genes have been used as markers for development of a genetic map. A backcross progeny panel was used to follow meiotic events in F1 hybrids produced from crossing BW females with PO males. The backcross panel consisting of 88 members representing 4 families was genotyped for approximately 400 marker loci and almost all families were informative. The End-Join program was used to construct and analyze the map. All linkage groups are associated with specific chromosomes. Comparison of the linkage maps of Peromyscus with mouse and rat suggests a closer relationship between Peromyscus and rat than mouse.
Approximately 50,000 ESTs have been sequenced and analyzed representing 8 tissues (brain, testes, liver, kidney, skin, newborn, fetus and spleen). Between 5,500 and 6,700 unique EST sequences are represented. The EST clones are available from the Clemson University Genomics Center.
Development of genetics, genomics, and reproductive resources will be useful for studies in *Peromyscus*. The *Peromyscus* Stock Center maintains numerous species and mutant stocks with single gene inheritance such as epilepsy, juvenile ataxia and adult ataxia. There are also numerous behavioral differences between the BW and PO interfertile species making genetic analysis feasible. Several phenotypes under the control of single genes will be briefly described.

P63-DEPENDENT TRANSCRIPTIONAL REGULATION AND FUNCTION IS ESSENTIAL FOR PREGNANCY-INDUCED MAMMARY GLAND DEVELOPMENT

Nicole Forster, Lei He, Matthew R Ramsey, Leif W Ellisen

Massachusetts General Hospital and Harvard Medical School, Cancer Center, Boston, MA, 02114

Development and differentiation of mammalian epithelial tissues is regulated by transcriptional programs in which the transcription factor p63, a member of the p53 gene family, has a key role. The constitutive *p63-null* mouse shows a complete lack of stratified squamous epithelium and is deficient in mammary glands, pointing to a critical role for p63 in these tissues. In humans, *p63* is overexpressed in several types of cancer, and germline mutations in *p63* result in a variety of epithelial malformation syndromes, including limb mammary syndrome. The mammary gland is mainly composed of luminal and basal epithelial cells. In the mature mammary gland, *p63* is expressed only within basal cells, which are interposed between luminal cells and the basement membrane to mediate their interactions. During pregnancy the postnatal mammary gland undergoes development including proliferation, differentiation, and cell death. Although *p63* expression seems to be temporally regulated during pregnancy, its function and regulated signals during pregnancy and lactation are poorly understood. Since *p63* expression is associated with breast cancer and early pregnancy protects women from breast cancer, the analysis of pregnancy-induced p63-dependent development may give insights for targeting breast cancer.

To address this we are using a conditional mouse model to delete *p63* in the adult murine mammary gland prior to pregnancy. Subsequently, this induces a dramatic failure of pregnancy-associated lobuloalveolar cell proliferation and differentiation which is associated with a complete absence of lactation and consequently death of all pups. Based on these findings, we hypothesize that p63 regulates non cell-autonomous signals in basal cells that are required for survival, proliferation and/or differentiation of alveolar cells. Since aberrant cell-cell signaling is associated with many diseases including cancer, we plan to identify the signals targeted by p63 that are required for pregnancy-induced mammary gland development. These studies will likely uncover new pathways in p63-dependent cell-cell signaling, and may provide novel therapeutic strategies for treatment of human malformation syndromes and/or cancer. To complement these studies, we have developed biochemical means to identify proteins interacting with p63 and thereby regulating p63-dependent transcription, which might play roles in mammary glands. Furthermore, these proteins may ultimately serve as therapeutic targets for treatment of cancer subtypes which overexpress *p63*.

OVEREXPRESSION OF NUCLEOSOME BINDING PROTEIN HMGN5 IN CARDIOMYOCYTES LEADS TO ALTERED CHROMATIN ORGANIZATION AND CARDIAC MALFUNCTION

Takashi Furusawa, Mark Rochman, Michael Busutin

National Institutes of Health, National Cancer Institute, Bethesda, MD, 20892

High Mobility Group N (HMGN) is a family of non-histone chromosomal proteins that specifically bind to nucleosome, the building block of the chromatin fiber, and alter chromatin structure. Mouse HMGN5 is a unique member of HMGN family which is specifically localized to euchromatin, transcriptionally active region. Cell culture studies revealed that altered expression of mouse HMGN5 significantly affected cellular transcription profile by modulating global chromatin architecture. To test the role of HMGN5-dependent chromatin reorganization *in vivo*, we have generated conditional transgenic mice in which overexpression of HMGN5 is induced by cre-mediated excision of LacZ sequence. Since proper cardiac differentiation and function are known to be impacted by disruption of chromatin structure, we have generated heart specific HMGN5 overexpressing mouse by utilizing *α-myosin heavy chain* (*α-MHC*) promoter-driven cre recombinase. The transgenic mice were smaller than non-transgenic siblings, and mice with highest level of HMGN5 overexpression died around 6 weeks old. Histological analysis revealed that transgenic mice exhibit signs of heart hypertrophy. RT-PCR showed elevated expression of *β-MHC* mRNA, a marker of cardiac hypertrophy, which is normally expressed in embryonic cardiomyocyte. Immunofluorescece revealed that HMGN5 overexpressing cardiomyocytes in transgenic mice exhibited enlarged nuclei with gross abnormalities in chromatin architecture, including decreased number and redistribution of heterochromatic foci. Several histone modifications such as H3K14-ac and H3K4-me3 were also affected by HMGN5 overexpression. MRI analysis demonstrated increased myocardiac mass accompanied by cardiac malfunction, namely reduced right and left ventricle ejection fractions. Overall, our results demonstrate that HMGN5-induced alterations of chromatin organization in cardiomyocytes cause morphological abnormalities in heart and lead to cardiac malfunction. Our results provide the direct link between structural integrity of chromatin fiber, non-histone chromosomal proteins, and functional properties of the entire organ.

THE ATYPICAL CADHERIN *CELSR1* NON-CELL AUTONOMOUSLY REGULATES THE DIRECTIONALITY OF FACIAL BRANCHIOMOTOR NEURON MIGRATION IN THE MOUSE HINDBRAIN

Derrick M Glasco[1], Anagha Sawant[1], Yibo Qu[2], Bernd Fritzsch[3], Jennifer N Murdoch[4], Fadel Tissir[2], Anand Chandrasekhar[1]

[1]University of Missouri, Division of Biological Sciences, Columbia, MO, 65211, [2]University of Louvain, Institute of Neuroscience, Developmental Biology, Brussels, B-1200, Belgium, [3]University of Iowa, Department of Biology, Iowa City, IA, 52242, [4]Medical Research Council, Mammalian Genetics Unit, OX11, 0RD, United Kingdom

Neuronal migration is a developmental process essential to the formation of functional neural networks in the central nervous system. In the vertebrate hindbrain, facial branchiomotor neurons (FBMNs), which control jaw and facial movements, undergo a characteristic caudal migration during development through several hindbrain segments, or rhombomeres (r). In zebrafish, components of the non-canonical wnt/planar cell polarity (PCP) pathway, including the atypical cadherins *celsr1a*, *celsr1b*, and *celsr2*, regulate migration in a non-cell autonomous manner. To examine the extent of the molecular and cellular conservation of these mechanisms, we examined the role of *Celsr1* in mice. In *Celsr1*^{Crash} missense mutants and *Celsr1*^{KO} mutants, a subset of FBMNs exhibited direction reversal, migrating *rostrally* instead of caudally, and was not due to defects in hindbrain patterning, axon guidance, or misspecification of FBMNs into non-migratory neuron types.

Celsr1 is expressed broadly in the developing hindbrain, including the floor plate and ventricular zones, but not in differentiated FBMNs. To investigate the tissue-specific requirement for *Celsr1* in regulating FBMN migration, we used the Cre-Lox system to conditionally inactivate *Celsr1* in the floor plate, r4, and r3/r5. FBMNs fail to migrate caudally in *Gli2* mutants, which lack floor plate cells, indicating a role for the floor plate in this process. However, *Celsr1* function is not required in the floor plate. On the other hand, inactivation of *Celsr1* in r3/r5 phenocopied the *Celsr1*^{KO} migration defect, with inactivation in r4 showing a less severe phenotype. Together, these data suggest that *Celsr1* expression in the ventricular zone of multiple rhombomeres non-autonomously regulates the *directionality* of FBMN migration. We are currently testing whether *Celsr1* regulates the expression of neuronal guidance molecules (*Wnt5a*, *Sema3a*, *VEGF-A*, *Plxna1*, *Cdh8*) which may control FBMN migration.

THE WNT/PCP PROTEIN VANGL2 IS NECESSARY FOR MIGRATION OF FACIAL BRANCHIOMOTOR NEURONS IN MICE, AND FUNCTIONS INDEPENDENT OF DISHEVELLED FUNCTION

Derrick M Glasco[1], Vinoth Sittaramane[1], Mi-Ryoung Song[2], Anagha Sawant[1], Bernd Fritzsch[3], Goncalo C Vilhais-Neto[4], Olivier Pourquie[4], Jennifer N Murdoch[5], Anand Chandrasekhar[1]

[1]University of Missouri, Division of Biological Sciences, Columbia, MO, 65211, [2]Gwangju Institute of Science and Technology, School of Life Sciences, Buk-gu, Gwangju, 500-712, South Korea, [3]University of Iowa, Department of Biology, Iowa City, IA, 52242, [4]Institute of Genetics and Molecular and Cellular Biology, Department of Cell Biology and Development, Strasbourg, B.P. 10142, France, [5]Medical Research Council, Mammalian Genetics Unit, OX11, 0RD, United Kingdom

A fundamental process in brain development involves the migration of facial branchiomotor neurons (FBMNs) in the embryonic brain stem across several rhombomeres (r) to their final locations. Several components of the Wnt/planar cell polarity (PCP) pathway, including the transmembrane protein vangl2, have been shown to regulate FBMN migration in zebrafish. Therefore, we analyzed FBMN migration in the *Vangl2* mouse mutant, *Looptail (Lp)*. In $Vangl2^{Lp/+}$ embryos, FBMNs failed to undergo their characteristic caudal migration from r4 into r6 and instead formed elongated facial motor nuclei spanning r4 and r5. FBMNs in $Vangl2^{Lp/Lp}$ embryos were arrested in r4 and showed a delayed radial migration to the pial surface of the neural tube. However, hindbrain patterning and FBMN progenitor specification were intact, and FBMNs did not transfate into other non-migratory neuron types, indicating a specific effect on migration. While a number of Wnt/PCP genes were expressed normally in $Vangl2^{Lp}$ mutants, the domain of *Wnt5a* expression was shifted anteriorly.

Studies in zebrafish suggest that FBMN migration is regulated independent of the Wnt/PCP pathway, since some, but not all, Wnt/PCP genes regulate FBMN migration. We tested whether this was also the case in mammals. In embryos null for *Ptk7*, a PCP component, FBMN migration was partially blocked. In contrast, FBMNs migrated normally in *Dishevelled (Dvl) 1/2* double mutants, and in zebrafish embryos with disrupted *dvl* signaling, indicating *Dvl* function is largely dispensable for FBMN migration. Consistent with this, loss of *Dvl2* function in $Vangl2^{Lp/+}$ embryos did not exacerbate the $Vangl2^{Lp/+}$ FBMN migration phenotype.

CHARACTERIZATION OF PDCD2 EXPRESSION DURING MOUSE ONTOGENIC DEVELOPMENT

Celine J Granier[1], Katherine Piso[2], Catherine Liu[1], Hatem E Sabaawy[2], Arnold B Rabson[1]

[1]Child Health Institute of New Jersey, Depts of Pediatrics-UMDNJ-RWJMS, New Brunswick, NJ, 08901, [2]Cancer Institute of New Jersey, Dept of Medicine-UMDNJ-RWJMS, New Brunswick, NJ, 08903

PDCD2 (Programmed cell death domain 2) is a zinc finger MYND domain-containing protein which is highly conserved from yeast to humans. Currently, PDCD2 function is unknown. Recent findings in Drosophila (Minakhina *et al*. 2007, 2010) and zebrafish (Kramer *et al*. in preparation) suggest that PDCD2 is a regulator of hematopoiesis and PDCD2 has been shown to be down-regulated by the B-cell lymphoma associated repressor protein Bcl-6 and may play a role in lymphomagenesis (Baron *et al*., 2007). We are employing the mouse model system to study the *in vivo* function of PDCD2. *In silico* promoter analyses of vertebrate PDCD2 homologues demonstrated multiple binding sites for transcription factors controlling early embryogenesis and hematopoiesis. We were therefore interested in examining PDCD2 expression during embryogenesis and hematopoiesis. We detected PDCD2 mRNAs and protein widely in the post-implantation mouse embryo, which were particularly prominent in sites of hematopoiesis and in the developing nervous system. With the progress of ontogenic development, PDCD2 protein continued to be expressed in several tissues but was strongly expressed in a subset of hematopoietic cells in the fetal liver and the bone marrow. Moreover, PDCD2 protein was enriched in the subcapsular zone of the newborn thymus, containing CD4-/CD8- thymocytes. We also detected PDCD2 as early as the preimplantation embryo and in mouse embryonic stem cells, identifying this gene as an early marker of embryogenesis. Thus, our data describe a pattern of PDCD2 expression that commences in the preimplantation embryo, is highly enriched in ESCs and continues to be expressed in a subset of hematopoietic cells during progression of ontogenic development. These initial analyses encourage us to investigate the effect of PDCD2 loss of expression during embryogenesis and hematopoiesis.

CONTROLLING ENDOGENOUS PROMOTER ACTIVITY THROUGH REVERSIBLE EPIGENETIC SILENCING IN VIVO

Anna C Groner[1], Patrick Tschopp[2], Jens E Dietrich[3], Sandra Offner[1], Sonia Verp[1], Isabelle Barde[1], Takashi Hiiragi[3], Didier Trono[1]

[1]Frontiers-in-Genetics National Center of Competence in Research, École Polytechnique Fédérale de Lausanne (EPFL) , School of Life Sciences, Lausanne, 1015, Switzerland, [2]Frontiers-in-Genetics National Center of Competence in Research, University of Geneva , Department of Zoology and Animal Biology, Geneva, 1211, Switzerland, [3]Max-Planck-Institute for Molecular Biomedicine, Mammalian Development Laboratory, Münster, 48149, Germany

The characterization of genes through loss-of-function studies provides a powerful method to understand biological mechanisms. For this reason we are developing a tool, which allows inducible silencing of a specific endogenous promoter in a drug-controllable manner. We use promoter-less lentiviral-based gene traps, which only exhibit YFP reporter expression, if adjacent cellular promoters are “trapped”. By adding Tet operator (TetO) repeats to our gene trap, we additionally have the means to recruit an ectopic repressor, which induces heterochromatin, in a reversible way. More specifically, the tTRKRAB repressor contains the KRAB domain of the human KOX1 zinc finger protein fused to the E. coli tetracycline repressor (tTR), and binds to TetO in a doxycycline (dox)-controllable fashion. In proof-of-principle experiments we make use of lentiviral-mediated transgenesis to engineer mice with trapped promoters. We then cross these mice to a line expressing tTRKRAB and characterize YFP expression in different tissues of the offspring. Preliminary results on lymphocytes show that YFP expression, which is a direct read-out of promoter activity, can be reduced in the presence of tTRKRAB. This reduction is reversible through the addition of dox, which is consistent with reversible silencing of the cellular promoter. We are currently screening more mouse lines with differentially trapped promoters, in addition, to improving our methodological setup. These results will be presented here.

TEMPORAL AND FUNCTIONAL REQUIREMENTS FOR SOX10 DURING THE ESTABLISHMENT AND MAINTENANCE OF THE FOLLICULAR MELANOCYTE SYSTEM.

Melissa L Harris[1], Kristina Buac[2], Arturo Incao[1], William J Pavan[1]

[1]National Human Genome Research Institute, NIH, Genetic Disease Research Branch, Bethesda, MD, 20892, [2]Massachusetts General Hospital, Department of Medicine, Boston, MA, 02114

Melanocyte stem cells (MSCs) originate from the melanoblast lineage of the neural crest. During melanoblast development the transcription factor SOX10 functions to define the melanoblast lineage by upregulating genes critical to melanogenesis. However, the role of SOX10 in the adult organism is unknown. We have found that an increased level of SOX10 affects MSC behavior in adult mice and results in premature hair graying. We have also correlated mutations in human SOX10 with melanoma. The fact that both normal stem cells and tumor cells share the ability to self renew suggests that understanding the role of SOX10 in MSC establishment or maintenance will provide insight into how SOX10 might participate in cancer.

In order to ascertain the temporal and functional requirement for SOX10 in the melanocyte system of the hair follicle, we propose to assess the presence and position of MSCs and melanocyte progenitors in two transgenic mouse lines--one driving the overexpression of Sox10 from a melanocyte-specific promoter (Tg(DctSox10)) and another that will allow us to conditionally induce the knockout of Sox10 in the melanocyte lineage during different timepoints. During hair follicle morphogenesis, SOX10 is downregulated in MSCs and regained by melanocyte progenitors during hair cycling. Preliminary evidence suggests that low/absent levels of SOX10 are required to maintain MSC fate, as Sox10 overexpression in Tg(DctSox10)/+ mice results in ectopic pigmentation in cells residing in the stem cell compartment of the hair (hair bulge). Furthermore, during the first adult hair cycle, Tg(DctSox10)) homozygotes exhibit a reduction or loss of both melanocytes in the hair bulb and pigmentation in the hair that is correlated with an absence of MSCs. Interestingly, this loss of MSCs in Tg(DctSox10))/Tg mice is not associated with an increase in apoptotic cells in the hair bulge.

Complete loss of SOX10 is embryonic lethal due to the requirement of SOX10 for neural crest development, thus our inducible/conditional knockout approach will elucidate the stages of the hair cycle where SOX10 is needed. Once we better understand the biological mechanisms by which SOX10 affects MSC establishment and maintenance, we will determine whether these same processes are disrupted or coopted in human melanomas in which SOX10 is expressed. In addition, MSCs and hair graying may prove to be a highly relevant, in vivo biological system to identify novel pathways involved in cellular self-renewal.

SIZE REGULATION OF EARLY MOUSE EMBRYO *IN VITRO*

Tomoka Hisaki, Ikuma Kawai, Sakiko Kobayashi, Kunihiko Naito, Kiyoshi Kano

Laboratory of Applied Genetics, Graduate school of Agricultural and Life Science, The University of Tokyo, Tokyo, 1138657, Japan

The mouse embryo has a remarkable ability to compensate for a substantial increase or decrease in cell number, and the resulting offspring are normal in size.
This indicates that there are growth control mechanisms during mouse embryo development, but details, including whether or not size is regulated by the embryos or by the maternal environment, are still poorly understood. To determine whether or not the size regulation mechanisms are intrinsic to the embryo, we produced a culture system that enables us to analyze embryos at the post-implantation stage.
We produced embryos having half the normal number of cells (1/2 embryos), which were produced from a blastomere during the two-cell stage, and embryos that were doubly and triply increased in cell number (X2 and X3 embryos) by aggregating two or three eight-cell embryos. To explore whether or not differences in growth exist between differently sized embryos under the culturing condition, we examined the cell number and area of outgrowth during the culture period (3.5 - 8.5 dpc). The rates of increase in both cell number and outgrowth area of the 1/2 embryos were higher than those of the control, which indicated what exactly is causing the difference in growth speed.
Downward size control was not seen in our study of aggregated embryos, while upward size control happened under *in vitro* condition. Furthur exploration of the embryos under the culturing system could be helpful to determine whether the embryos or the maternal environment plays a main roll in regulating the size of mouse embryos.

THE ROLE OF SYSTEM A AMINO ACID TRANSPORTERS SNAT1 & SNAT2 IN PLACENTAL AND FETAL GROWTH AND DEVELOPMENT

Katharina Hoelle[1,3], Ionel Sandovici[1,3], Ilona Zvetkova[2], Miguel Constância[1,3]

[1]University of Cambridge Metabolic Research Laboratories, Department of Obstetrics and Gynaecology, Cambridge, CB2 0SW, United Kingdom, [2]University of Cambridge Metabolic Research Laboratories, Institute of Metabolic Science, Cambridge, CB2 0QQ, United Kingdom, [3]University of Cambridge, Centre for Trophoblast Research, Cambridge, CB2 3EG, United Kingdom

System A amino acid transport is one of the major neutral amino acid transporters and has been hypothesised to play an essential role in providing the growing fetus with nutrients. It directly mediates the uptake of small neutral amino acids and indirectly contributes to uptake of a wide variety of other amino acids through establishing a cellular amino acid gradient. In order to address the long standing question of the role of System A transport in placental and fetal development and whether altered placental amino acid transport is a cause or a response to altered fetal growth, we have created a novel conditional mouse model by using chromosome engineering. We employed MICER vectors to target System A genes *Slc38a1* (SNAT1) and *Slc38a2* (SNAT2) on mouse chromosome 15 and are using a combination of ubiquitous, embryo and placenta specific Cre-expressing strains to investigate the role of *Slc38a1* and *Slc38a2* in placental supply and fetal demand. We have shown that deletion of *Slc38a1/Slc38a2* results in severe growth restriction in both placenta (76%N) and embryo (58%N) at day 18.5 of gestation and that homozygous null embryos suffer from severe anemia due to a reduction in the number of red blood cells (25%N). In addition, in a subset (~20%) of homozygous null embryos we also observe neural tube defects.
Ongoing work is currently focussing on investigating a potential impairment in definitive haematopoiesis in the null embryos, the origins of the neural tube defect and whether placental or embryonal deletion of *Slc38a1/Slc38a2* have independent or additive effects on the growth of the developing fetus.

ALTERED GENE EXPRESSION AND SPONGIOTROPHOBLAST DIFFERENTIATION IN DIABETIC MOUSE PLACENTA

J. Michael Salbaum[1], Claudia Kruger[2], XiaoYing Zhang[3], Gabriela Pavlinkova[4], David H Burk[2], Claudia Kappen[2]

[1]Pennington Biomedical Research Center, Laboratory for Regulation of Gene Expression, Baton Rouge, LA, 70808, [2]Pennington Biomedical Research Center, Developmental Biology, Baton Rouge, LA, 70808, [3]Academy of Sciences of the Czech Republic, Institute of Biotechnology, Prague, 14220, Czech Republic, [4]Pennington Biomedical Research Center, Cell Biology and Biomimaging Core, Baton Rouge, LA, 70808

Pregnancies complicated by diabetes have a higher risk for congenital defects, such as heart and neural tube defects. Diabetes in pregnancy also predisposes mothers and children to disease later in life, such as metabolic syndrome and hypertension.

In a mouse model of diabetic pregnancy, we have previously shown that diabetes-exposed embryos exhibit altered gene expression, with enrichment of transcription factors, chromatin-modifying proteins, and Wnt-pathway genes. Approximately a third of the currently known neural tube defect genes are also misregulated, implicating known pathways as well as epigenetic mechanisms in birth defect risk in diabetic pregnancies.

In this study, we hypothesized that placental abnormalities contribute to adverse outcomes from diabetic pregnancies. By gene expression profiling, we detected significantly altered gene expression in diabetic placenta. This involved genes in the maternal as well as the embryonic compartments, such as Sfrp5 and Ascl2, respectively. We also found several genes for which expression in placenta was previously unknown, such as Ankyrin repeat domain 2, Slc6a4 (Serotonin transporter), Calcitonin related peptide B and Cholecystokinin.

The cellular composition of the decidual compartment was altered and the junctional and labyrinth layers were reduced in diabetic placenta at later stages. This was accompanied by ectopic differentiation of spongiotrophoblast-like cells within the labyrinth. The molecular changes and abnormal differentiation of multiple cell types precede impaired growth of junctional zone and labyrinth, and placenta overall. These alterations are likely to play a role in pregnancy complications, and may have implications for developmental origins of adult disease.

ROLE OF DISCOIDIN DOMAIN RECEPTOR 2 (DDR2) IN MOUSE GROWTH

Ikuma Kawai, Hirokazu Matsumura, Tomoka Hisaki, Sakiko Kobayashi, Kunihiko Naito, Kiyoshi Kano

University of Tokyo, Graduate school of Agricultural and Life Sciences, Bunkyoku, 1138657, Japan

Discoidin domain receptor 2 (DDR2) is a receptor tyrosine kinase. We recently identified homozygous smallie mutant mice (BKS.HRS. *Ddr2*$^{slie/slie}$ /J, *Ddr2*$^{slie/slie}$ mutants), which lack a functional DDR2. *Ddr2*$^{slie/slie}$ mutant mice are dwarfed; they are also infertile due to peripheral dysregulation of the endocrine system.

To understand the role of DDR2 in growth, we produce transgenic mice that ubiquitously overexpress *Ddr2* cDNA harboring a CAG promoter. *Ddr2* transgenic mice gain weight slowly after weaning and do not undergo the growth spurt observed in control littermates, most noticeably in females. Almost all tissues examined, including cartilage and skeleton, appear histologically normal. In contrast to control mice, *Ddr2* transgenic mice do not develop increased adiposity in subcutaneous or visceral fat with aging. *Ddr2* transgenic mice eat in similar amounts per body weight to wild-type mice, which suggests that DDR2 might have an important role in lipid metabolism.

To evaluate DDR2's role in endochondral ossification, we analyzed it using ATDC5 cells, which are known as a chondrogenic cell line. We produced stable *Ddr2* knockdown cell lines by the continuous expression of miRNA for *Ddr2*, and analyzed cell growth and differentiation into chondrocytes. Interestingly, suppressed *Ddr2* caused the activation of cell proliferation and accelerated cell differentiation in stable *Ddr2* knockdown cells. We also quantified the expression of FGF2, which activates the proliferation of mesenchymal cells including chondrogenic cells, and revealed that FGF2 mRNA was reduced in the *Ddr2* knockdown cell line.

Characterization of the DDR2 gene might provide new insights into growth including the control of adiposity and endochondral ossification.

ANALYSIS OF HEART DEFECTS IN THE *NIPBL*+/- MOUSE, A MODEL FOR CORNELIA DE LANGE SYNDROME

S Kawauchi[1], R Santos[1], M Lopez-Burks[2], A D Lander[2,3], A L Calof[1,3]

[1]UCI, Anatomy & Neurobiology, Irvine, CA, 92697, [2]UCI, Developmental & Cell Biology, Irvine, CA, 92697, [3]UCI, Ctr for Complex Biol. Systems, Irvine, CA, 92697

Cornelia de Lange Syndrome (CdLS: OMIM#122470), a birth defects disorder that affects nearly every tissue and organ system in the body, is most often caused by heterozygous mutations in *NIPBL*, which encodes a cohesin-associated protein conserved among all eukaryotes. Recent studies suggest that NIPBL regulates gene expression through mechanisms independent of cohesin's established role in chromatid cohesion. *Nipbl*+/- mice reproduce many features of CdLS, including small body size, craniofacial anomalies, behavioral disturbances and high perinatal mortality [Kawauchi, Calof et al., 2009, PLoS Genetics, 5]. About 34% of individuals with CdLS suffer from heart defects, with ventriculoseptal (VSD) and atrioseptal (ASD) defects common. Similarly, ASDs are seen in 30-60% of late-gestation *Nipbl*+/- mouse embryos; these likely contribute to the high perinatal mortality (~80%) observed in *Nipbl*+/- mice.

To understand the etiology of cardiac defects in CdLS, we are performing morphological and molecular analyses of heart development in *Nipbl*+/- mice. We find that defects are evident much earlier than observed initially. For example, delayed fusion of the ventricular septum with the cardiac cushion is seen in ~75% of *Nipbl*+/- mice at E13.5. Moreover, when we quantify expression of genes known to play a role in cardiac patterning, especially ones associated with septal defects, we see significant upregulation of a subset of these, such as Hand1 and Pitx2, from E10.5-E13.5. At E10.5, Hand1 is normally restricted to the developing left ventricle; this pattern is not altered in *Nipbl*+/- mice, nor is in situ hybridization intensity for Hand1. What is altered, however, is the proportion of left-vs-right ventricular tissue, due largely to decreased right ventricle size. Interestingly, when *Nipbl*+/- hearts are examined at E9.5, both left and right ventricles are smaller than normal, suggesting a defect in cell proliferation. Consistent with this, we find c-myc expression to be decreased by ~25% in the hearts of E10 *Nipbl*+/- mice.

To pinpoint when and in what cell types heart defects originate in *Nipbl*+/- mice, we have begun using conditional-invertible gene-trap alleles. Germline transmission of one such "FLEX" (Flp/Cre) allele has been confirmed, and we find that $Nipbl^{FLEX/+}$ mice exhibit decreased Nipbl mRNA, as well as reduced body size. These data suggest that these animals will show other defects observed in *Nipbl*+/- mice, and will prove useful for analyzing cardiac defects in CdLS. Supported by NIH P01-HD052860.

MOUSE MODELS MADE EASIER: DEVELOPMENT OF NEW C57BL/6J ES CELL LINES FACILITATE TETRAPLOID COMPLEMENTATION

Sang-Yong Kim

Cold Spring Harbor Laboratory, Gene Targeting , Cold Spring Harbor, NY, 11724

The C57BL/6 (B6) mouse strain is the most widely strain used for transgenesis and mutagenesis. However, gene targeting in B6 embryonic stem (ES) cells is not very efficient and therefore is currently done using 129 ES cells, which then require extensive backcrosses to B6 mice to establish a pure genetic background. Typically, ten backcrosses are needed, a process that can take up to two years and is therefore costly and inefficient. To make the generation of mouse models more rapid and high throughput, we have developed B6 ES cell lines. These B6 ES cell lines have a number of advantages over those already available. First, they can be used for gene targeting and have a high percentage of germline transmission (~50-80%). Second, these ES cell lines can be used for tetraploid complementation, producing a high percentage (15-20%) of mice that are entirely B6. Third, these ES cells lines can be used to rapidly generate mice that are homozygous for the gene of interest. These advantages indicate that these B6 ES cells facilitate the rapid generation of mouse models in a cost-effective and efficient manner.

THE HISTONE DEMETHYLASE LSD1/KDM1 REPROGRAMS EPIGENETIC MEMORY TO RE-ESTABLISH TOTIPOTENCY BETWEEN GENERATIONS

Ashley L King[1], William G Kelly[2], David J Katz[1]

[1]Emory University, Cell Biology, Atlanta, GA, 30322, [2]Emory University, Biology, Atlanta, GA, 30322

Extensive epigenetic reprogramming occurs during cloning and in the induction of pluripotent stem cells (IPS). Similarly, during normal germline transmission, the epigenetic state of the genome is known to undergo extensive reprogramming both in the gametes and in the early embryo. This reprogramming may be essential to re-establish a developmental ground state between generations, but the mechanism of these epigenetic reprogramming events remains poorly understood. We have previously shown that mutants in spr-5, the *C. elegans* ortholog of the histone demethylase LSD1/KDM1 exhibit progressive sterility over many generations due to the transgenerational accumulation of the histone modification dimethylation of histone H3 on lysine 4 (H3K4me2). This suggests that H3K4me2 can serve as a stable epigenetic memory and that erasure of H3K4me2 by KDM1 in the germline prevents the inappropriate transmission of this epigenetic memory from one generation to the next. To pursue this role further, we generated mice with germline mutations in KDM1. Progeny from mice that lack maternal KDM1 exhibit significant embryonic and perinatal lethality. These progeny are genotypically normal, suggesting that the defects are due to the stable transmission of inappropriate histone methylation from the parents to the offspring. Based on this data, we propose that KDM1 demethylation may be required for somatic cell nuclear transfer (SCNT) and for the somatic reprogramming that is induced during the generation of IPS cells.

COMPREHENSIVE ANALYSIS OF ICSI INDUCED TRANSCRIPTOME PERTURBATION IN THE MOUSE

Takashi Kohda[1], Narumi Ogonuki[2], Kimiko Inoue[2], Tamio Furuse[3], Hideki Kaneda[3], Tomohiro Suzuki[3], Tomoko Kaneko-Ishino[4], Teruhiko Wakayama[5], Shigeharu Wakana[3], Atsuo Ogura[2,6,7], Fumitoshi Ishino[1]

[1]Tokyo Medical and Dental University, Department of Epigenetics, Medical Research Institute, Tokyo, 113-8510, Japan, [2]RIKEN, Bioresource Engineering Division, Bioresource Center, Tsukuba, 305-0074, Japan, [3]RIKEN, Technology and Development Team for Mouse Phenotype Analysis, Bioresource Center, Tsukuba, 305-0074, Japan, [4]Tokai University, School of Health Sciences, Isehara, 259-1193, Japan, [5]RIKEN, Center for Developmental Biology, Kobe, 650-0047, Japan, [6]The University of Tokyo, Center for Disease Biology and Integrated Medicine, Tokyo, 113-0033, Japan, [7]University of Tsukuba, Graduate School of Life and Environmental Science, Tsukuba, 305-8572, Japan

Faithful transcriptome regulation is crucial for reproductive and regenerative medical applications, because it changes dynamically in each cell lineage with the epigenetic/transcription factor regulation cascades during the course of development and growth. Intracytoplasmic sperm injection (ICSI), one of the human assisted reproductive technologies (ART), has long raised concerns about its influence on development. Therefore, it is important to determine its pre- and postnatal effects as well as possible transmission to the next generation. By conducting comprehensive transcriptome and phenotypic analyses in mice under strict conditions, we demonstrate that ICSI induces distinct long-lasting transcriptome change (epigenetic shift) not only at preimplantation but also remains at the neonatal stage. However we observe no remarkable differences in the ICSI adults in either the gene expression or phenotypic profile, and there was no indication of transmission to the next generation via natural mating. Our results suggest there are no lifelong or transgenerational effects of ICSI, but there is a need for intense follow-up of ICSI children during infancy and early childhood in assisted reproductive technologies.

DIFFERENTIAL FUNCTIONS OF MAPK AND PI3K PATHWAYS IN THE DEVELOPMENT OF THE SCHWANN CELL LINEAGE

Maria E Kolanczyk[1], Katja S Grossmann[3], Walter Birchmeier[2], Carmen Birchmeier[1]

[1]Max Delbruck Center For Molecular Medicine, Developmental Biology/Signal Transduction, Berlin, 13125, Germany, [2]Max Delbruck Center For Molecular Medicine, Signal Transduction, Invasion and Metastasis of Epithelial Cells, Berlin, 13125, Germany, [3]The Salk Institute for Biological Studies, Molecular Neurobiology, La Jolla, CA, 92037

The nonreceptor tyrosine phosphatase Shp2 (PTPN11) has been implicated in tyrosine kinase, cytokine, and integrin receptor signaling. We have recently shown that conditional mutation of Shp2 in neural crest cells and in myelinating Schwann cells resulted in deficits in glial development that are remarkably similar to those observed in mice mutant for Neuregulin-1 (Nrg1) or the Nrg1 receptors, ErbB2 and ErbB3. Our genetic and biochemical analyses demonstrated that Shp2 is an essential component in the transduction of Nrg1/ErbB signals (Grossmann et al., 2009). Here, we investigate the importance of two known downstream signaling cascades, MAPK and PI3K, both of which can be affected by the loss of Shp2. We attempt to rescue the loss of Shp2, Nrg1 or ErbB3 in neural crest and in Schwann cells by introducing gain-of-function alleles that express either constitutive active form of MEK1 or p110α (catalytic subunit of PI3K). Preliminary results indicate that the activation of MAPK pathway in neural crest is sufficient to rescue the phenotypes in early Schwann cell development observed in Shp2 mutant mice, whereas activating PI3K pathway fails to do so. We intend to address the role of both MAPK and PI3K also in immature and in myelinating Schwann cells and to elucidate by genetic means the mechanism of Nrg1/ErbB signal transduction at several distinct stages of Schwann cell lineage development.

Grossmann, K.S., Wende, H., Paul, F.E., Cheret, C., Garratt, A.N., Zurborg, S., Feinberg, K., Besser, D., Schulz, H., Peles, E., et al. (2009). The tyrosine phosphatase Shp2 (PTPN11) directs Neuregulin-1/ErbB signaling throughout Schwann cell development. Proceedings of the National Academy of Sciences of the United States of America 106, 16704-16709.

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

Matthew Koss*[1], Andrea Brendolan*[1], Matilde Saggese[1], Terence Capellini[1], Richard Harvey[2], Licia Selleri[1]

[1]Weill Cornell Medical College, Cell and Developmental Biology, New York, NY, 10065, [2]Victor Chang Cardiac Research Institute, Developmental Biology Division, Darlinghurst, NSW 2010, Australia

* These authors contributed equally to this work.

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, the roles of Pbx homeoproteins are discussed in the regulation of mammalian growth and organ size. We use the spleen as a model system, exploiting mouse models deficient for the transcription factors Pbx1, Hox11, Nkx2.5, Nkx3.2, and Pod1, 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 splenic anlagen of the mouse, 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* is dramatically up-regulated in the hypoplastic spleens of $Pbx1^{\Delta ex3/\Delta ex3}$;*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, $Pbx1^{\Delta ex3/\Delta ex3}$;*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. Stereology on spleen size, counts of cell density, and in vivo proliferation assays have been conducted on spleens from $Pbx1^{\Delta ex3/\Delta ex3}$;*Nkx2.5-Cre*+ and $Pbx1^{\Delta ex3/\Delta ex3}$;*Nkx2.5-Cre*+;$p15ink4b^{+/-}$ as well as $Pbx1^{\Delta ex3/\Delta ex3}$;*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.

ULTRASONIC-RANGE VIBRATORY MICROINJECTION RAISES EFFICIENCY OF PRONUCLEAR MICROINJECTION

Dilidaer Kudereti, Fujio Miyawaki

Tokyo Denki University, Life Science and Engineering, Saitama, 350-0394, Japan

Aim: We have been developing the vibratory microinjection system (VMS), which is completely different from the Piezo Impact Micro Manipulator. The current version of VMS provides a micropipette with longitudinal vibration up to 100 kHz, the amplitude of which is controlled by voltage applied to a vibrator composed of three multilayer piezoelectric actuators and the housing. In this study, we evaluated 35-kHz vibratory microinjection (VM), compared with the ordinary microinjection (OM).
Methods: 1) The voltages controlling the amplitude of vibration ranged from 5 to 10 V, which also made lateral vibration of the pipette tip slightly visible. 2) Fertilized eggs obtained from BDF1 mice were alternately injected with and without vibration using a single micropipette in M2 medium at 26ºC and at an injection pressure of 30 hPa. The quota for one micropipette was 30 eggs (15 eggs for each group). Even when the micropipette pulled materials from pronucleus, it was being used until the quota was fulfilled. Each procedure of microinjection was taken on a CCD video camera for subsequent image analysis. 3) After microinjection, all the eggs (210 eggs for each group) were cultured in M16 medium in a CO_2 incubator. Embryonic development was assessed for 4 days.
Results: 1) VM shortened the injection time by 25%: 3.39 ± 1.87 sec (average ± standard deviation) in VM group and 4.55 ± 2.78 sec in OM group ($P < 0.0001$, Student's t test). 2) Degrees of pronuclear swelling and times spent swelling the pronucleus were measured. The pronuclei were swollen identically: 114.8 ± 7.68% and 114.9 ± 7.04% in VM and OM groups, respectively. However, VM shortened the time spent swelling the pronucleus, or DNA injection time, by 30%: 1.87 ± 0.94 sec in VM group and 2.65 ± 1.17 in OM group ($P < 0.0001$), indicating that VM increased the injection speed of DNA by 42%. 3) A total of 420 eggs were manipulated with 14 micropipettes. Materials inside pronucleus were pulled out in 11 of 210 eggs in VM group whereas the "pulling-out" event was observed in 24 of 210 eggs in OM group ($P = 0.02$, χ^2 test). 4) After 4-day culture, 98 eggs (46.7%) in VM and 92 eggs (43.8%) in OM group developed to blastocyst stage. Seventy-nine eggs (37.6%) in VM and 82 eggs (39.1%) in OM group were dead.
Conclusions: These results indicate that the VMS is an efficient option in gene transfer. In addition, the higher speed of DNA injection suggests that the VMS is capable of injecting larger DNA fragments and/or more viscous DNA solutions. Much less frequent "pulling-out" events suggest that ultrasonic-vibration may remove materials already stuck to a micropipette.

BMP2 EXPRESSION IN THE VISCERAL ENDODERM DIRECTS ANTERIOR MORPHOGENESIS DURING GASTRULATION

Elizabeth Lacy, Mary Madabhushi

Sloan-Kettering Institute, Developmental Biology, New York, NY, 10065

In pre and early gastrulation mouse embryos reciprocal interactions between the visceral endoderm (VE), epiblast, and extraembryonic ectoderm direct anterior-posterior axis formation and allocation of progenitors to the three primary germ layers. It is unknown whether the VE contributes additional organizing functions as gastrulation proceeds. Through the generation and analysis of tissue-specific *Bmp2* mutants we find that VE-expressed BMP2 plays a central role in ventral folding morphogenesis, a fundamental but poorly understood developmental process, which when impaired, can result in human birth defects affecting the heart and body wall. Our studies show that *Bmp2* exhibits a dynamic pattern of transcription in the VE during pre-streak and gastrulation stages. Contrary to previous studies, *Bmp2* expression localizes to a discrete region of the anterior VE in mid-to-late streak embryos. While loss of BMP2 in epiblast-derivatives perturbs amnion formation, absence of BMP2 in VE blocks foregut invagination, an integral step in ventral morphogenesis, leading to abnormal positioning of head and heart. Embryos lacking VE-expressed BMP2 display phenotypic defects strikingly similar to those observed in an epiblast-specific knockout of *Bmpr1a*. Thus these findings potentially identify a signaling cascade in which BMP2 supplied by the anterior VE signals to its receptor on epiblast cells to direct anterior morphogenesis during mouse gastrulation.

A GENETIC INDUCIBLE MOSAIC ANALYSIS (GIMA) TO MARK AND MUTATE CELLS IN MICE WITH CONDITIONAL FLOXED ALLELES

Zhimin Lao[1], Praveen G Raju[1], Brian Bai[2], Alexandra L Joyner[1]

[1]MSKCC, Developmental Biology Program, New York, NY, 10065, [2]Case Western Reserve University School of Medicine, 1Department of Genetics, Cleveland, OH, 44106

Although conditional mutagenesis in mice using gene targeting and site specific recombination technology has revolutionized our ability to study the in vivo function of genes, there remain a number of severe limitations to the existing techniques. We have developed a new genetic approach for mosaic mutant analysis in mouse that simultaneously conditionally inactivates a gene and permanently marks the cell and its descendents with GFP. In this technique termed Genetic Inducible Mosaic Analysis (GIMA) inducible Flp based transgenes (FlpeER or FLPoER) are combined with a new targeted allele (R26FNGC) that expresses an eGFPcre fusion protein from the ROSA26 locus following Flp-mediated recombination of an frt flanked STOP sequence. Within 48 hr of administering tamoxifen to mice carrying a FlpER transgene, R26FNGC, and a floxed endogenous allele, up to 97% of the GFP marked cells also carry a mutant allele, with eventual 100% concordance of GFP and a mutant allele. GIMA was used to study the fate of small cohorts of β-Catenin mutant cells in the developing spinal cord with single cell resolution, and to provide further evidence that Wnt signaling can dorsalize cells in the ventral spinal cord. The GIMA approach is a broadly applicable tool for conditional mosaic mutant analysis in mouse using the growing number of engineered floxed alleles, including developmental studies, tumor analysis, and studies of gene function in adult mice. Furthermore, R26FNGC can be used to convert cell type specific Flp-expressing strains into effective Cre deleter mice in which mutant cells are labeled with GFP.

THE *DLX5/DLX6* LOCUS : A NEW CANDIDATE FOR NON-SYNDROMIC FAMILIAL PREMATURE OVARIAN FAILURE

Kamal Bouhali[1], Sandrine Caburet[2], Aurelie Dipietromaria[1], Anastasia Fontaine[1], Marc Fellous[3], Giovanni Levi[1]

[1]CNRS/MNHN UMR7221, Evolution des Régulations Endocriniennes, Paris, 75005, France, [2]Université Denis Diderot, CNRS UMR7592, Institut Jacques Monod, Paris, 75013, France, [3]Université Paris Descartes, CNRS UMR 8104, Inserm, U567, Paris, 75006, France

The human condition known as Premature Ovarian Failure (POF) is characterized by loss of ovarian function before the age of 40. Most cases of POF are idiopathic, but 10-15% of POF cases are familial, underlying a genetic origin of the disease. The homeobox transcription factors Dlx5 and Dlx6 are involved in the control of steroidogenesis and of male genital development. We show that allelic reduction of *Dlx5* and *Dlx6* in the mouse is associated with a POF-like phenotype, characterized by reduced fertility and premature follicular depletion. We also provide evidence supporting the notion of a reciprocal regulation between *Dlx5;Dlx6* and *Foxl2*, another POF-associated transcription factor. Furthermore, we report the linkage and homozygosity analysis of a large POF family presenting a recessive pattern of inheritance. We identify a region of high LOD score on chromosome 7q21.3 which includes *DLX5* and *DLX6*.
Our findings suggest that the *DLX5;DLX6* locus is a new POF candidate which might be part of a steroidogenic regulatory pathway including *FOXL2*.

DISRUPTION OF CHROMODOMAIN HELICASE DNA BINDING PROTEIN 5 (CHD5) IMPAIRS SPERMATOGENESIS

Wangzhi Li, Sang Yong Kim, Alea Mills

Cold Spring Harbor Laboratory, Cancer Center, Cold Spring Harbor, NY, 11724

Mouse spermatogenesis is a complex process through which diploid spermatogonia develop into haploid spermatozoa. Extensive chromatin remodeling takes place during this process, transforming the mouse genome into a highly compacted form with unique features. Chromatin remodeling proteins are expected to play critical roles in epigenetic reprogramming during spermatogenesis. Indeed, targeted disruption of several chromatin remodelers in mice causes defects in spermatogenesis and impaired fertility. However, it remains poorly understood that which chromatin remodelers are essential for spermatogenesis and how they function during the process. Here, we discovered that chromodomain helicase DNA binding protein 5 (Chd5), a member of the CHD chromatin remodeling protein family, plays important roles during mouse spermatogenesis. We found Chd5 is highly expressed in mouse testis and is especially enriched in spermatids where the most dramatic chromatin remodeling occurs. We developed a mouse model with Chd5 specifically disrupted by gene targeting and found that disruption of Chd5 results in impaired fertility in male mice. Detailed analyses revealed reduced sperm number, impaired sperm motility and increased sperm morphologic abnormality in Chd5 mutant mice. Out data suggest for the first time that Chd5, which we previously identified as a novel tumor suppressor, also plays essential roles in spermatogenesis.

SPECIFIC EXPRESSION OF HEPARAN SULFATE GLYCOSAMINOGLYCAN CHAINS IS CRUCIAL FOR LOCAL DISTRIBUTION OF FGF SIGNALING DURING EARLY MAMMALIAN EMBRYOGENESIS

Kayo Shimokawa, Chiharu Kimura-Yoshida, Kyoko Mochida, Isao Matsuo

Osaka Medical Center and Research Institute for Maternal and Child Health, Department of Molecular Embryology, Osaka , 594-1101, Japan

Heparan sulfate proteoglycans comprising heparan sulfate glycosaminoglycan chains (HS chains) and core proteins have been functionally implicated as co-receptors for multiple growth factors in the cell surface and extracellular matrix. However, the precise mechanisms by which HS chains regulate the activity of growth factors in complex mammalian morphogenetic processes remain to be elucidated. Here, we identified the transgene insertion allele of *Ext2*, a gene causative of multiple hereditary exostoses that catalyzes the elongation of HS chains. Marker expression analyses of *Ext2*-deficient embryos revealed that HS chains are essential for the response to Fibroblast growth factor (FGF) signaling and, in particular, for the local distribution of FGF ligands in the extraembryonic ectoderm. Moreover, the expression of HS chains attached to the cell surface is specific to those areas in which FGF signaling is potentially active. Additional fine mosaic studies with single-cell resolution involving chimeras suggested that the expression of cell surface-attached HS chains is crucial for early embryonic development. Given that 22 FGF ligands, 4 FGF receptors, and 12 membrane-associated core proteins are redundantly expressed, we propose that the spatially and temporally localized expression of HS chains attached to cell surfaces also contributes to FGF signaling distribution during mammalian morphogenesis.

LINEAGE MAPPING THE PRE-IMPLANTATION MOUSE EMBRYO BY TWO-PHOTON MICROSCOPY, NEW INSIGHTS INTO THE SEGREGATION OF CELL FATES

Katie McDole*[1,3], Yuan Xiong*[2], Pablo A Iglesias[2], Yixian Zheng[1,3]

[1]Johns Hopkins University, Department of Biology, Baltimore, MD, 21218, [2]Johns Hopkins University, Department of Electrical and Computer Engineering, Baltimore, MD, 21218, [3]Carnegie Institution for Science, Department of Embryology, Baltimore, MD, 21218

The first lineage segregation in the pre-implantation mouse embryo gives rise to cells of the inner cell mass and the trophectoderm. Segregation into these two lineages during the 8-cell to 32-cell stages is accompanied by a significant amount of cell movement, and as such it has been difficult to accurately track cellular behavior while maintaining embryo viability using conventional imaging techniques. Consequently, it is unclear whether dividing cells that give rise to the same (symmetric division) or different (asymmetric division) cell fates exhibit different behaviors. To achieve the high spatial and temporal resolution necessary for tracking individual cell lineages, we utilized two-photon light-scanning microcopy (TPLSM) to visualize and follow every cell in the embryo using fluorescent markers. We found that cells undergoing asymmetric cell fate divisions originate from a distinct population of cells that have been previously classified ambiguously as outer or inner cells. This imaging technique, coupled with a tracking algorithm we developed, allows us to show that these cells exhibit different dynamic behaviors and occupy a unique position in the mouse embryo between the fourth and fifth cleavages. We also provide an accurate description of the correlation between cell division order and cell fate, as well as demonstrating the lack of a strong correlation between cell division angle and cell fate. Our studies demonstrate the utility of two-photon imaging in answering questions in the pre-implantation field that have previously been
difficult or impossible to address. This system and tracking ability further provides a framework for the use of specific markers to track live cell fate molecularly and with high accuracy.

IMPRINTING ANALYSIS IN THE *ACRODYSPLASIA* REGION OF MOUSE CHROMOSOME 12

Erin N McMurray, Eric D Rogers, Jennifer V Schmidt

University of Illinois at Chicago, Biological Sciences, Chicago, IL, 60607

The insertional mouse mutation *Acrodysplasia* (*Adp*) confers a parent-of-origin developmental phenotype, with animals inheriting the mutation from their father showing skeletal abnormalities, while those inheriting the mutation from their mother are normal. This parental-specific phenotype, along with mapping of the insertion to a region of chromosome 12 proposed to contain imprinted genes, suggested that disruption of genomic imprinting might underlie the *Adp* phenotype. Genomic imprinting is the process by which autosomal genes are epigenetically silenced on one of the two parental alleles; imprinting mutation phenotypes manifest after inheritance from one parent but not the other. Imprinted genes typically occur in dense clusters that contain few nonimprinted genes, and therefore assaying representative genes from the *Adp* critical region might identify any imprinted domains. Fourteen genes spaced across the *Adp* region were analyzed for imprinting, but all were found to be biallelically expressed. Other explanations must therefore be considered for the parent-of-origin *Adp* phenotype.

A BIOLUMINESCENT TRANSGENIC MOUSE MODEL TO MONITOR CREB TRANSCRIPTIONAL ACTIVATION

Kenneth Miller[1], Susan Bove[2], Robin Kleiman[2], Wenning Qin[1]

[1]Pfizer Research, Genetically Modified Models, Groton, CT, 06340, [2]Pfizer Research, Neuroscience Department, Groton, CT, 06340

We describe a transgenic reporter mouse model in which luciferase expression is dependent upon activation of cAMP Response Element Binding protein (CREB), a well characterized transcription factor downstream of multiple signaling pathways. CREB binding sites (CREs) are found in the promoter region of many genes involved in a wide variety of cellular functions. CREB is activated by phosphorylation of a conserved serine (Ser 133) in response to, among other things, cellular cAMP levels. In addition to its phosphorylation state, transcriptional induction by CREB is regulated by its binding to accessory proteins and the methylation state of the CREs, thus providing cellular context and tissue specificity.

In vivo bioluminescent imaging (BLI) in small animals is an important technology enabling the evaluation of potential drug candidates, delivery systems, cell migration, tumor progression and metastases, and a variety of other pharmaceutically relevant biological events. BLI has the advantage of being noninvasive and highly sensitive. In addition, the ability to monitor cellular processes in living animals allows for longitudinal studies that both reduce the number of animals needed and provide insights not achievable from conventional endpoint studies.

We have begun to characterize this model for its utility in monitoring phosphodiesterase (PDE) activity in the brain. PDEs hydrolyze cyclic nucleotides in cells, and inhibition results in the accumulation of cyclic nucleotides, activation of Protein Kinase A and CREB mediated transcription, resulting in light production in our model. Current protocols to monitor cAMP levels in rodent brain are labor intensive and terminal, requiring large animal numbers while providing only a one time snapshot of the cellular signaling environment. This model provides noninvasive imaging of CRE-mediated transcription downstream of cAMP accumulation and is useful for evaluating potential drug candidate efficacy, specificity, blood brain barrier penetration, and PK characteristics.

Given the ubiquity of CREB transcriptional activation, we have also begun to evaluate the utility of this model as a readout for other physiological processes. Data will be presented in which the model was used to monitor gluconeogenic gene upregulation, as well as signaling in brown adipose tissue.

INDIVIDUAL EFFECTS OF INGREDIENTS OF DNA SOLUTION ON EMBRYONIC DEVELOPMENT IN CULTURE AFTER PRONUCLEAR MICROINJECTION

Fujio Miyawaki, Dilidaer Kudereti, Risa Shimizu, Hiroki Haremaki, Tomohiro Yoshizawa

Tokyo Denki University, Life Science and Enginerring, Saitama, 350-0394, Japan

Aim: Some cellular damages are inevitable in pronuclear microinjection, many factors of which are considered to relate to the damages. We focused on some ingredients of DNA solution.
Methods: 1) The DNA solution used in this study was made of GFP gene (1.67 kb), Tris/EDTA (TE) buffer containing 0.1 mM EDTA and ultrapure water. Then, we tested three liquids as injection liquid: TE buffer alone (TE group), ultrapure water alone (Water group) and a 2.0 ng/μl GFP gene solution (GFP group). In addition, 150 mM KCl solution was tested for the sake of interest (KCl group). 2) Fertilized eggs were collected from 13 BDF1 mice, and divided into the 4 groups. Pronuclear injection was performed in M2 medium at 26ºC and at an injection pressure of 30 hPa. The volume of liquid injected was controlled so that pronuclei swelled equally. Eggs that had undergone improper microinjection were excluded from assessment. Each injection procedure was taken on a CCD video camera for subsequent image analysis. 3) After injection, all the eggs were cultured in M16 medium in a CO_2 incubator. Embryonic development was assessed for 4 days.
Results: 1) The rates of pronuclear swelling were almost identical among 4 groups: 114.0 ± 12.5% (average ± standard deviation) in GFP group (N = 40), 117.4 ± 11.6% in TE group (N = 28), 116.6 ± 11.5% in Water group (N = 30) and 115.1 ± 10.2% in KCl group (N =30). 2) One day after injection, 90% of eggs in GFP group reached 2-cell stage, 92.9% in TE group, 93.3% in Water group and 60.0% in KCl group. On 4th day, 57.5% in GFP group developed to blastocyst stage, 67.9% in TE group, 50% in Water group and 16.7% in KCl group. Cumulative death rates after 4 days were 7.5% in GFP group, 10.7% in TE group, 6.7% in Water group and 33.3% in KCl group. Both embryonic development and survival curve in KCl group were significantly worse than those in the other three groups, wheres those in the other three groups were similar (Not significant).
Conclusions: Because the GFP solution and TE buffer are isotonic and have almost the same pH, only difference may be presence of GFP gene, suggesting that its concentration of 2.0 ng/μl is not so influential. Because the main differences between the TE buffer and ultrapure water are presence of 0.1 mM EDTA, pH and osmolarity, the detrimental effect of 0.1 mM EDTA may be equal to the combined harmful effects of lower pH and zero osmolarity in pure water. The results in KCl group suggest that increase in intracellular chloride concentration may be significantly detrimental to cell survival and development.

TRANSCRIPTIONAL REGULATION OF MYF5 EXPRESSION IN THE BRANCHIAL ARCHES.

Natalia Moncaut, Christine Siligan, Ricardo Ribas, Kevin Taylor, Jaime J Carvajal, Peter W Rigby

The Institute of Cancer Research, Gene Function and Regulation, London, SW3 6JB, United Kingdom

In vertebrates, skeletal myogenesis is controlled by four myogenic regulatory factors: Myf5, Mrf4, Myogenin and MyoD. Myf5 is the first to be expressed and initiates the myogenic cascade. Our group and others identified several Myf5 and Mrf4 enhancers responsible for the expression of both genes during development.
Myf5 expression in the branchial arches (BA) is controlled by at least five separate elements. An element located in the intergenic region between Myf5 and Mrf4, the proximal arch element (PAE), is able to drive early expression in the second BA and later on maintain the expression of Myf5 until 12.5 dpc in both BAs. Using a bioinformatic approach we identified an evolutionary conserved peak in the PAE. We have cloned this region, referred to as small proximal arch element (sPAE), in our standard Myf5-promoter-nlacZ reporter construct and analyzed its ability to drive expression during embryogenesis. Further sequence analysis revealed two highly conserved E-boxes located in the middle of the sPAE. Using site-directed mutagenesis we tested the relevance of these binding sites to drive LacZ expression in vivo. Previous studies shown that the bHLH factors, MyoR and Capsulin are expressed in the BA and mice lacking these factors were missing some craniofacial muscles. In this context, using chromatin immunoprecipitation we confirmed the binding of MyoR to this highly conserved element in vivo.
These findings suggest that MyoR is the main transcription factor controlling the expression of Myf5 through binding to this specific enhancer. Further identification of conserved transcriptional binding sites on other BA enhancers will reveal the complex regulatory scenario controlling the myogenic program during craniofacial muscle development.

THE HISTONE DEMETHYLASE LSD1/KDM1 REPROGRAMS EPIGENETIC MEMORY DURING THE SPECIFICATION OF NEW CELL FATES

Dexter A Myrick[1], William G Kelly[2], David J Katz[1]

[1]Emory University, Cell Biology, Atlanta, GA, 30322, [2]Emory University, Biology, Atlanta, GA, 30322

Extensive epigenetic reprogramming occurs during cloning and in the induction of pluripotent stem cells (IPS). Similarly, during normal germline transmission, the epigenetic state of the genome is known to undergo extensive reprogramming both in the gametes and in the early embryo. This reprogramming may be essential to re-establish a developmental ground state between generations, but the mechanism of these epigenetic reprogramming events remains poorly understood. We have previously shown that mutants in spr-5, the *C. elegans* ortholog of the histone demethylase LSD1/KDM1 exhibit progressive sterility over many generations due to the transgenerational accumulation of the histone modification dimethylation of histone H3 on lysine 4 (H3K4me2). This suggests that H3K4me2 can serve as a stable epigenetic memory and that erasure of H3K4me2 by KDM1 in the germline prevents the inappropriate transmission of this epigenetic memory from one generation to the next. To pursue this role further, we generated mice with germline mutations in KDM1. Progeny from mice that lack maternal KDM1 exhibit significant embryonic lethality, suggesting that KDM1 is also required to reprogram epigenetic memory between generations in the mouse. Furthermore, deletion of KDM1 in the mouse testis results in sterility, with a complete failure to maintain the testis stem cell population. Consistent with this, Wang *et al.* previously demonstrated that ES cells can be derived from KDM1 mutant embryos, but undergo massive cell death when induced to differentiate. Based on these data, we propose that KDM1 could be required to reprogram epigenetic transcriptional memory during the specification of new cell fates. We are currently testing this model by conditionally deleting KDM1 in neural crest cells and in neural stem cells.

LARGE-SCALE *IN VIVO* ANALYSIS OF THE REGULATORY ARCHITECTURE OF THE MOUSE GENOME WITH A TRANSPOSON-ASSOCIATED REGULATORY SENSOR SYSTEM

Sandra Ruf[1], Orsolya Symmons[1], Chloe Hot[1], Dirk Dolle[2], Laurence Ettwiller[2], Francois Spitz[1]

[1]EMBL, Developmental Biology Unit, Heidelberg, 69117, Germany,
[2]Heidelberg University, Institute of Zoology, Heidelberg, 69120, Germany

Many recent findings underline the critical role of sequences located far from protein-coding genes in regulating their transcriptional activities. However, our understanding of the mechanisms governing the range and specificity of action of such remote regulatory elements is still rather limited.
To get insight into these mechanisms and explore the regulatory organization of the genome, we have developed GROMIT (Genome Regulatory Organisation Mapping with Inserted Transposons). GROMIT is based on an *in vivo* transposition system that distributes a regulatory sensor gene and a loxP site throughout the mouse genome. We used GROMIT to generate and characterize about 150 mouse lines, each with an insertion of the reporter transposon at a different genomic position. Strikingly, most of these insertions - close to or several hundreds of kilobases away from genes, linked or not to developmental genes - showed highly tissue-specific expression of the reporter gene, contrasting with the rather widespread expression of most genes. This pervasive presence of regulatory potentials throughout the genome reveals the complex and modular organization of the genome in distinct but interlaced regulatory domains with restricted tissue-specific activities.
Importantly, each insertion can be remobilised to produce new mice with insertions around the selected starting point. With GROMIT, the remobilisation rate of single copy transposon is very high (from 20% to 50%), and due to the local hopping bias of *Sleeping Beauty*, a large proportion of the new insertions lands within 1 to 2 Mb of their initial position. This property facilitates the fine mapping of the regulatory organization of specific loci, and reveals the range of action of enhancers, the presence of silencers and regulatory boundaries, complementing other genome-wide approaches. Thus, by the virtue of its simplicity and efficiency, requiring only breeding and genotyping, GROMIT opens an unprecedented level of access to functional investigations of the mouse genome. Furthermore, combined with *in vivo* CRE-mediated recombination, this dynamic and versatile resource enables to generate series of overlapping deletions and duplications over regions of interests, notably to model human aneuploidies and copy-number/structural variants. I will illustrate the general use of our approach and resource and discuss the new insights it provides into the regulatory organization of mammalian chromosomes.

DUAL RMCE PERMITS EFFICIENT RE-ENGINEERING OF MUTANT ALLELES IN MOUSE ES CELLS

Javier Lopez-Rios[1], Marco Osterwalder[1], Antonella Galli[1,3], Barry Rosen[2], William C Skarnes[2], Rolf Zeller[1]

[1] University of Basel, Developmental Genetics, Department of Biomedicine, , Basel, 4058, Switzerland, [2]The Welcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB101HH, United Kingdom, [3]Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, Department of Medicine and Genetics & Development, New York, NY, 10032

Gene targeting by homologous recombination (HR) in mouse embryonic stem (ES) cells represents a powerful tool for tailored manipulation of the mouse genome. However, the efficiency of this technology is limited by great variation in targeting frequencies among different loci. In light of the increasing need to re-engineer more subtle mutations at the same locus, Recombinase-Mediated Cassette Exchange (RMCE) was developed. RMCE takes advantage of pairs of heterotypic, non-interacting recombination sites for a particular site-specific recombinase (usually Cre or Flp) that need to be first introduced into the locus of interest by conventional HR and only few compatible alleles are currently available. Anticipating the release of thousands of conditional mouse alleles by the International Knockout Mouse Consortium, we developed an approach to efficiently re-engineer these alleles with the aim to extend the long-term value and use of these vast ES cell resources. This novel approach, called dual RMCE (dRMCE) takes advantage of the wild-type *loxP* and *FRT* sites already present in the majority of all conventional conditional alleles and many gene traps and permits re-engineering of the corresponding loci with frequencies of up to ~70% correct replacement. Minimally, this represents a 5- to 65-fold increase in efficiency in comparison to HR. For example, dRMCE can be used to express mutant cDNAs, epitope or fluorescent tags and heterologous genes under control of any endogenous locus of choice. Due to its efficiency and simplicity, the dRMCE technology is well suited for high-throughput approaches such as functional screening of disease-causing mutations in particular pathways and/or genes of interest directly in mouse ES cells (e.g. upon induced differentiation into specific cell-types) or in mice generated from engineered clones. Finally, the dRMCE technology provides non-specialist researchers with access to advanced genetic engineering of a large fraction of the mouse genome and enhances the long-term value of the existing large collections of mutant mouse ES cell lines.

HIGH EFFICIENCY TARGETING OF AN INDUCIBLE EXPRESSION SYSTEM TO THE ROSA26 LOCUS IN EMBRYONIC STEM CELLS

Samuel Wormald, Daniel Turner, Mahalia Page, Shih-pei Shen, Wensheng Zhang, Peri Tate, William C Skarnes

The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, CB10 1SA, United Kingdom

We have developed a modular, inducible expression system that targets with very high efficiency to the Rosa26 locus of mouse embryonic stem cells (ESCs). Our system combines Gateway modularity with the Tet-On expression system in a highly efficient Rosa26 targeting vector (pGATOR). This technology enables the rapid generation of inducible RNAi and cDNA expression alleles at the Rosa26 locus in ESCs, either clonally or as a pooled library. To illustrate the utility of our system, we generated ESCs harboring a tetracycline-inducible library of 94 shRNAs, and used massively parallel sequencing to identify “dropouts” during a long-term screen for self-renewal phenotypes. Of 12 shRNAs targeting Oct4, 9 were depleted from the library by more than 8-fold after 4 weeks. Hits from the screen were then validated by targeting individual tetracycline-inducible pGATOR-shRNA clones to the Rosa26 locus. The pGATOR system thus provides a simple and scalable platform for conducting functional studies in ESCs.

COIN (CONDITIONAL BY INVERSION): A BETTER TYPE OF CONDITIONAL KNOCK-OUT ALLELE THAT IS UNIVERSALLY APPLICABLE REGARDLESS OF GENE STRUCTURE

Aris N Economides[1], David Frendewey[2], Peter Yang[1], David M Valenzuela[2], Andrew J Murphy[1], George D Yancopoulos[1]

[1]Regeneron Pharmaceuticals, Inc., Genome Engineering Technologies, Tarrytown, NY, 10591, [2]Regeneron Pharmaceuticals, Inc., VelociGene, Tarrytown, NY, 10591

Conditional mutagenesis is rapidly becoming the method of choice for the study of gene function, but the types of alleles that can be engineered are limited both by vector design and target gene structure. We therefore developed a new technology — Conditional-by-Inversion (COIN) — that utilizes an optimized invertible gene trap-like cassette, the COIN module. The COIN module is placed in the antisense strand of the target gene, where it is effectively inert until activated by a recombinase-mediated inversion event that flips the COIN module into the sense strand, thereby disrupting transcription of downstream exons while simultaneously providing a reporter for tracking the mutation. The COIN module can either be inserted into one of the target gene's natural introns (an intronic COIN) or directly into a coding exon as part of an artificial intron (an exonic COIN), greatly increasing allele design flexibility over existing conditional knockout approaches. The unique artificial intron feature enables the introduction of the COIN module (or other elements) in nearly any location within the target gene, freeing up design choices and increasing the types of alleles that can be designed.

We have constructed COIN alleles for a large number of genes and performed detailed phenotypic analysis on ES cells and mice before and after activation of the COIN modules. Beyond establishing the robustness, reliability, and broad applicability irrespective of exon-intron structure of this method, our study uncovered rare cases of post-inversion hypomorphic alleles, observed only with intronic COINs, that were caused by 'skipping' of the inverted COIN module, resulting in expression of a mixture of both wild type and COIN-encoding mRNA. We showed that re-engineering the intronic COIN allele as an exonic COIN by placing the COIN module into the immediate neighboring exon could rectify this problem. These results not only inform future design decisions, but may explain the hypomorphism occasionally observed with traditional gene trap alleles, which are by nature intronic. Finally, the exon-splitting and reporter features of COINs open up new engineering modalities for the generation of multifunctional alleles that go beyond conditional-nulls, ranging from simple splitting of exons to introduce novel intronic elements to complex multifunctional alleles with more than two functionalities. Examples of these allele designs will be presented.

ENGINEERING ES CELLS FOR EPIGENETIC STUDIES

Maria Rostovskaya[1], Andrea Kranz[2], Konstantinos Anastassiadis[1], Francis Stewart[2]

[1]Technische Universitaet Dresden, Center for Regenerative Therapy, Dresden, 01307, Germany, [2]Technische Universitaet Dresden, BioInnovationsZentrum, Dresden, 01307, Germany

We are developing new options for genome engineering with particular emphasis on engineering stem cell genomes. Recent progress with site specific recombinases includes further applications with Cre, FLPo and Dre recombinases. Using recombineering, we have developed a variety of allele designs for gene targeting and BAC transgenes, including high throughput methods for protein tagging. We have applied these methods to explore several issues relevant to the epigenetic status of ES cells, including the molecular basis of bivalency. Bivalency refers to the dual presence of histone 3 lysine 4 trimethylation (H3K4me3) and H3K27me3 over the same promoter region. Genes displaying bivalency in ES cells are typically expressed in specific lineages and not, or very lowly, expressed in ES cells, presumably due to repression by H3K27me3 and polycomb-group action. We are examining these issues in mouse ES cells cultured in normal conditions (serum + LIF) or in 2i conditions (GSK3β and MEK inhibitors + LIF) using tamoxifen-inducible conditional mutagenesis and tagged protein expression from BAC transgenes. As a further point, we have developed a robust method for BAC transgenesis in human ES cells based on assisted integration using piggyBac transposase. These methods extend the utility of ES cells to serve as experimental models for the study of pluripotency and regenerative therapy.

EPHRIN-B SIGNALING IN DEVELOPMENT

Philippe Soriano, Jeffrey O Bush

Mt. Sinai School of Medicine, Developmental and Regenerative Biology, New York, NY, 10029

Eph-ephrin signaling regulates multiple processes during development and in adult physiology and disease, and can function both by forward signaling to the Eph receptor or by reverse signaling into the cell which expresses the ephrin. We have taken an approach integrating genetic analysis, proteomic, and transcription profiling methods to identify how ephrin-B1 controls craniofacial, skeletal, and neural development. By generating mice harboring point mutations that specifically ablate reverse signaling while maintaining forward signaling, we find that reverse signaling is critical for axon guidance whereas forward signaling controls skeletal and craniofacial development. I will discuss the mechanisms by which Eph-ephrin signaling regulates these diverse processes.

AN INTERPLAY BETWEEN ANTERIOR AND POSTERIOR VISCERAL ENDODERM CONTROLS ANTERIORPOSTERIOR FORMATION IN MOUSE

Tingting Huang[1], Maki Wakamiya[2], Richard Behringer[3], Jaime Rivera[1]

[1]University of Massachusetts Medical School, Cell Biology, Worcester, MA, 01604, [2]University of Texas Medical Branch, Sealy Center For Cancer Biology, Galveston, TX, 77555, [3]University of Texas, M.D. Anderson Cancer center, Genetics, Houston, TX, 77030

During early mouse embryogenesis, temporal and spatial regulation of gene expression and cell signaling influences lineage specification and embryonic polarity. The visceral endoderm, plays a central role not only in nutrient uptake and delivery, but also in embryonic patterning. Two groups of visceral endoderm cells, located on the opposite sides of the anteroposterior axis, the anterior visceral endoderm (AVE) cells and posterior visceral endoderm (PVE) cells, are thought to establish this primary body axis.
Wnt3 is expressed in the PVE opposite to the AVE, suggesting that AVE cells block *Wnt3* expression in the anterior side of the embryo. To address this question, we analyzed the expression of *Wnt3* in *Cripto* mutants, in which, the AVE fails to move to the anterior side of the embryo. Our results show that the removal of AVE cells from the anterior side of the embryo allows *Wnt3* expression to expand to the AVE.
Previous data showed that several primitive streak markers, are radialy expressed in the proximal epiblast region of *Cripto* mutant embryos. To test the hypothesis that the expansion of *Wnt3* expression in these mutants is responsible for this ectopic expression, we inactivated *Wnt3* in *Cripto* mutants through the generation of double *Cripto/Wnt3* mutant embros. Our results show that the ablation of *Wnt3* signaling in *Cripto* mutants completely abolishes the expression of primitive streak markers such as *Brachyury*. These results support a model in which the AVE restricts the expression of *Wnt3* to the PVE, preventing it from expanding to the anterior region of the embryo and leading to the formation of the primitive streak opposite to the AVE. It also suggests that the radialized *Wnt3* signaling in *Cripto* mutants is responsible for radialization of primitive streak markers in a non-cell antonomous manner.

NON-CELL AUTONOMOUS ROLE FOR SOX17 TRANSCRIPTION FACTOR IN THE MORPHOGENESIS AND IDENTITY OF GUT ENDODERM IN THE MOUSE GASTRULA

Manuel Viotti[1,2], Kat Hadjantonakis[1]

[1]Sloan-Kettering Institute, Developmental Biology, New York, NY, 10065, [2]Weill Graduate School of Cornell University, BCMB, New York, NY, 10065

Using a combination of live imaging and genetic labeling we have previously shown that the gut endoderm tissue layer forms by a coordinated multifocal egression of epiblast-derived definitive endoderm (DE) cells into the preexisting visceral endoderm (VE) epithelial layer. VE cells are dispersed by egressing DE cells, and even though the resulting gut endoderm tissue exhibits an apparent molecular homogeneity, it comprises cells of two different (extraembryonic and embryonic) origins.
Various studies have implicated the Sry-related HMG-box transcription factor 17 (Sox17) in the formation of endoderm in vertebrates. Using live imaging reporter strains we note that Sox17 mutants fail to fully disperse the VE, resulting in a series of endoderm-specific defects including failure in: the emergence of the node onto the surface of the embryo, left-right asymmetry establishment, and gut tube formation.
Furthermore, tissue-specific (VE vs. DE) ablation of Sox17 reveals that the requirement for this transcription factor is non-cell autonomous within the gut endoderm, suggesting that a novel mechanism of cellular cross-talk may operate in the acquisition of molecular equivalence in cells of the gut endoderm tissue. Sox17 is required in both embryonic (DE) and extraembryonic (VE) tissues during their intercalation, where it appears to non-cell autonomously regulate its own expression, which may be critical for achieving molecular equivalence between DE and VE derivatives.

FGF4 AND *FGF8* COMPRISE THE WAVEFRONT ACTIVITY THAT CONTROLS SOMITOGENESIS

L. A. Naiche, Nakisha Holder, Mark Lewandoski

National Cancer Institute, Cancer and Developmental Biology Lab, Frederick, MD, 21702

Somites form along the embryonic axis by progressive segmentation from the anterior border of the presomitic mesoderm (PSM) and later differentiate into segmented body structures, such as vertebrate and ribs, as well as unsegmented skeletal muscle, tendons, and dermis. Somites are thought to form via the intersection of two signaling activities, known as the clock and the wavefront. Previous work in cultured embryos has suggested that Fibroblast Growth Factor (FGF) activity encodes the wavefront signal, which maintains the PSM in its undifferentiated state. However, it is unclear which (if any) of the six FGFs expressed in the PSM encode this activity, as removal of any one ligand is insufficient to disrupt early somitogenesis.

Here we show that when both *Fgf4* and *Fgf8* are deleted in the PSM, we observe a highly unusual phenotype whereby the entire PSM apparently engages in a single, simultaneous round of somitogenesis. In these mutants, expression of FGF signaling targets are lost despite ongoing expression of other FGF ligands. This loss of FGF signaling results in early failure of somitogenesis and truncation of the embryonic axis at approximately the 3-somite stage. Expression of most PSM genes is absent in mutants, including cycling genes, markers of undifferentiated PSM, and WNT pathway genes. Instead, at the stage when the anterior PSM would normally start to segment off somites, we observe that somite markers are prematurely expanded throughout the PSM of mutant embryos, demonstrating premature differentiation of the entire PSM into somitic tissue. These data demonstrate that FGF signaling prevents somitic differentiation of the PSM and can therefore be considered the wavefront activity.

We have further investigated the separate requirements for FGF and WNT signaling by restoring WNT activity to the FGF mutant PSM using a gain-of-function β-catenin allele. When WNT signaling is restored, PSM progenitor markers such as Brachyury are partially restored, but premature differentiation of the PSM still occurs, demonstrating that FGF wavefront signaling operates independently of the WNT pathway.

This study provides the first genetic evidence that FGF signaling is the source of the Wavefront and identifies the FGF ligands that encode this activity. Furthermore, these data show that FGF activity maintains WNT signaling, and that both signaling pathways are required in parallel to maintain PSM progenitor tissue.

BRACHYURY REPRESSES *MESP2* EXPRESSION IN MOUSE TAILBUD IN SOMITEGENESIS

Yukuto Yasuhiko[1], Jun Kanno[1], Yumiko Saga[2]

[1]National Institute of Health Sciences, Division of Cellular and Molecular Toxicology, Tokyo, 158-8501, Japan, [2]National Institute of Genetics, Division of Mammalian Development, Mishima, 411-8540, Japan

In vertebrate embryogenesis, *mesp* family genes encoding bHLH transcription factors show cyclic expression in the anterior presomitic mesoderm (PSM) as narrow bands just before somite formation. Mouse *Mesp2* expression is initiated by encountering with the Notch signal oscillation, an output of a segmentation clock, in a Tbx6 (a T-box transcription factor)-dependent manner. Mesp2 then degrades Tbx6 directly or indirectly, forming a sharp anterior boundary of Tbx6. This anterior border of Tbx6 likely defines the anterior limit of *Mesp2* expression domain. However, it is still to be elucidated why *Mesp2* expression is repressed in the posterior PSM, despite Tbx6 expression and oscillating Notch signal are both active in the posterior region. Previously FGF signaling has been implicated as a negative regulator of *Mesp2* transcription. Here we show that another T-box transcription factor Brachyury (Bra) could repress *Mesp2* in the posterior PSM and tailbud. Bra mRNA expression is stronger at the tailbud and the protein expression domain extends to the anterior PSM just like Tbx6. However, unlike Tbx6, Bra did not activate *Mesp2* in synergy with Notch signal. *In vitro* reporter assay indicated that the induction of *Mesp2* expression by Tbx6 and Notch was suppressed when Bra is included in the assay. T-box deleted mutant of Bra failed to repress *Mesp2* activation. Bra can bind weakly to Tbx6 binding sites in *Mesp2 cis* regulatory sequence, and we've not identified any independent *cis*-element in *Mesp2* promoter required for the repression by Bra. Taking together, it is plausible that Bra contributes to maintain *Mesp2* expression silence in the posterior PSM by competing with Tbx6.

SONIC HEDGEHOG SIGNALLING COORDINATES ANTERO-POSTERIOR WITH PROXIMO-DISTAL LIMB BUD DEVELOPMENT BY ENSURING RETINOIC ACID CLEARANCE

Simone Probst[1], Conradin Kraemer[2], Philippe Demougin[3], Rushikesh Sheth[1,4], Gail R Martin[5], Hidetaka Shiratori[6], Hiroshi Hamada[6], Dagmar Iber[2], Rolf Zeller[1], Aimée Zuniga[1]

[1]University of Basel, Developmental Genetics, Department of Biomedicine, Basel, CH-4058, Switzerland, [2]ETH Zurich, Department of Biosystems Science and Engineering (D-BSSE), Basel, CH-4058, Switzerland, [3]University of Basel, Life Sciences Training Facility, Pharmazentrum, Basel, CH-4056, Switzerland, [4]University of Cantabria, Deptartmentof Anatomy and Cell Biology, School of Medicine, Santander, E-39011, Spain, [5]University of California at San Francisco, Department of Anatomy, San Francisco, CA, 94158-2324, [6]Osaka University, Developmental Genetics Group, Graduate School of Frontier Biosciences, Osaka, 565-0871, Japan

Limb bud development is controlled by complex regulatory networks. These networks instruct coordinated patterning and proliferation of mesenchymal progenitors along the dorso-ventral (DV), antero-posterior (AP) and proximo-distal (PD) limb axes. In particular, sonic hedgehog (SHH) is known to specify the AP limb bud axis and regulate its expansion as part of a larger self-regulatory signalling system. Transcriptome analysis now uncovers an unexpected function of SHH in PD limb axis development as *Shh*-deficient mouse limb buds are proximalized. Expression of proximal genes and retinoic acid (RA) pathway activity is up-regulated and distally expanded *Shh*-deficient limb buds. In parallel, the expression of the RA inactivating enzyme *Cyp26b1* is decreased in the distal mesenchyme. We have investigated the possible SHH-RA interactions using a combination of experimental manipulation, genetics and mathematical simulations. Our findings reveal a SHH-dependent signalling module that normally enhances RA clearance by increasing FGF signalling in the apical ectodermal ridge (AER), which in turn up-regulates *Cyp26b1* expression. Disruption or reduction of CYP26b1-mediated RA clearance interferes with distal limb bud development leading to molecular proximalization of *Shh*, *AER-Fgf* and *Cyp26b1* deficient limb buds. In addition we provide molecular evidence for early specification of the PD axis by a mutually inhibitory interaction of RA with AER-FGFs. Subsequently AP and PD limb bud patterning becomes interlinked via SHH mediated regulation of the AER-FGF/CYP26b1/RA signalling module, which enables spatially coordinated progression of limb bud development.

FROM PROGENITOR TO PRODUCT: ASSEMBLY AND REPAIR OF THE MAMMALIAN KIDNEY

Andrew P McMahon[1,2,3]

[1]Harvard University, Stem Cell and Regenerative Biology, 16 Divinity Ave, Cambridge, MA, 02138, [2]Harvard University, Molecular and Cellular Biology, 16 Divinity Ave, Cambridge, MA, 02138, [3]Harvard University, Harvard Stem Cell Institute, 12 Church Street, Cambridge, MA, 02138

The basic functional unit of the kidney is the nephron. In man, the kidney is comprised of many hundreds of thousands of nephrons through which the plasma is filtered several times per hour. In so doing, nitrogenous waste-products and other toxic small molecules are removed and the osmotic balance of interstitial fluids kept within physiological bounds.

We are interested in the mechanisms by which progenitor cell maintenance and commitment are balanced in the process of nephrogenenesis ensuring the formation of a full complement of epithelial nephron precursors and the subsequent mechanisms governing patterning and morphogenesis of the complex tubular network of the functional nephron. The ability to form new nephrons is lost with exhaustion of the nephron progenitor pool. This leaves repair of the existing structures as the only normal option for maintaining kidney function. I will discuss our findings on generation, patterning and repair of the mammalian nephron.

INVOLVEMENT OF THE RECK TUMOR SUPPRESSOR PROTEIN IN MATERNAL AND EMBRYONIC VASCULAR REMODELING IN MICE

Ediriweera P. S. Chandana, Mako Yamamoto, Yoko Yoshida, Hitoshi Kitayama, Tomoko Matsuzaki, Makoto Noda

Kyoto University Graduate School of Medicine, Department of Molecular Oncology, Kyoto, 606-8501, Japan

Developmental angiogenesis proceeds through multiple morphogenetic events including sprouting, intussusception, and pruning. Mice lacking the membrane-anchored metalloproteinase regulator Reck [1] die *in utero* around embryonic day 10.5 with halted vascular development [2] and precocious neuronal differentiation [3]; however, the mechanisms by which the vascular phenotype arises remained unclear.

We found that Reck is abundantly expressed in the cells associated with blood vessels undergoing angiogenesis or remodeling in the uteri of pregnant female mice. Some of the Reck-positive vessels show morphological features consistent with non-sprouting angiogenesis. Treatment with a vector expressing a small hairpin RNA targeting Reck severely disrupts the formation of blood vessels with a compact, round lumen. Similar defects were found in the vasculature of Reck-deficient or Reck conditional knockout embryos.

Our findings implicate Reck in vascular remodeling, probably through non-sprouting mechanisms (i.e., intussusception and pruning), in both maternal and embryonic tissues.

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THE IKMC MOUSE KNOCKOUT PORTAL - SHARING AND INTEGRATING RELATED DATA

Darren Oakley[1], Vivek Iyer[1], Sébastien Briois[1], Damian Smedley[2], Martin Ringwald[3], Jim Kadin[3], Jeremy Mason[3], Bill Skarnes[1]

[1]The Sanger Institute, High Throughput Gene Targeting, Hinxton, Cambridge, CB10 1HH, United Kingdom, [2]European Bioinformatics Institute, Mouse Informatics, Hinxton, Cambridge, CB10 1SA, United Kingdom, [3]The Jackson Laboratory, Mouse Genome Informatics, Bar Harbor, ME, 04609

The International Knockout Mouse Consortium (IKMC) aims to mutate all genes in the mouse using a combination of gene trapping and high-throughput gene targeting technology[1]. The IKMC has established a website (http://www.knockoutmouse.org) that serves as a single point of entry to these extensive mutant resources, providing standardized information about alleles generated by the international programs (EUCOMM, KOMP, NorCOMM, TIGM, MirKO).

Here we focus on the 'MartSearch' component of the portal (http://www.knockoutmouse.org/martsearch) that uses BioMart[2] and search engine technology to integrate IKMC mouse knockout resources with other relevant biological data. Currently, this portal combines information on mouse knockouts with numerous other datasets, including gene information from MGI and Ensembl, gene expression data from EurExpress, phenotype data from Europhenome, mouse distribution information from EMMA, and human disease associations from MGI and OMIM. Work is underway to develop and integrate BioMarts of GXD gene expression data and biochemical pathways. Data representation, integration, and querying capabilities via the BioMart interface will be refined and data from other resources will be added.

The MartSearch portal showcases a novel use of both a search engine and data warehouses, allowing users to make both simple and expert queries and to provide bulk data downloads. In addition, this approach allows other groups to re-present IKMC data through other web interfaces: indeed both the WTSI Mouse Portal (http://www.sanger.ac.uk/mouseportal) and the EUMMCR Repository (http://www.eummcr.org) use these technologies to show different subsets of the same IKMC data with different emphases.

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REDUNDANT CONTROL OF ACTIN DYNAMICS *IN VIVO*: THE CASE OF THE EPS8L FAMILY

Nina Offenhauser, Charlotte B Ekalle Soppo, Pier P Di Fiore

IFOM, Endocytosis and Cancer, Milano, 20146, Italy

Eps8 is the prototype of a family of proteins involved in Actin remodeling. Eps8 is a modular protein that interacts with a number of proteins themselves involved in Actin remodeling, e.g. Abi and Irsp53. In addition, Eps8 binds directly to Actin and acts both as an barbed end Actin capper as well as a F-actin bundler. The specific activity of Eps8 appears to be regulated by its interaction partner and phosphorylation state. Eps8L2 shares the modular organisation of Eps8 and -as far as investigated- the same biochemical functions. *In vivo* the two proteins are co-expressed in the majority of the organs (Offenhäuser et al., 2004). In C.elegans and D. melanogaster in which only one EPS8 gene exists the loss of Eps8 is lethal. Instead, in the mouse the loss of either Eps8 or of Eps8L2 has no effect on viability. Eps8-KO and Eps8L2-KO mice are healthy and fertile. A detailed phentoype analysis of Eps8-KO mice has shown that these mice show a number of defects due to altered actin dynamics: 1) increased NMDA currents in neurons leads to increased resistance to ethanol (Offenhäuser et. al, 2006), 2) shortend microvilli lead to reduced bodyweight, improved metabolism and increased lifespan (Tocchetti et al., 2010). Eps8L2-KO mice instead show normal resistance to ethanol consistent with its lack of expression in the CNS. In addition, Eps8L2-KO show normal insulin sensitivity despite a slight reduction in bodyweight, indicating that also the metabolic phenotype is specific for Eps8. Thus the phenotypes identified in Eps8-KO appear to be specific for Eps8. To unmask redundant roles of the two proteins, we generated Eps8Eps8L2-DKO. Only a fraction of Eps8Eps8L2-DKO mice arrive to weaning with evident growth retardation and survive beyond weaning only if provided with soft food in the cage. Thus, the combined loss of Eps8 and Eps8L2 is incompatible with life also in mammals. Current studies are directed towards understanding the precise defects in Eps8Eps8L2-DKO animals.

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MOUSE TARGETED TRANSGENESIS THROUGH PRONUCLEAR INJECTION

Masato Ohtsuka, Hiromi Miura, Minoru Kimura, Hidetoshi Inoko

Tokai University, School of Medicine, Department of Molecular Life Sciences, Isehara, Kanagawa, 259-1193, Japan

Pronuclear injection (PI) is the most common method used to generate transgenic mice, despite wide variability in the level and pattern of transgene expression because of its dependence on gene copy number, configuration, and integration site. Targeted integration of a single-copy transgene has become recently possible through homologous recombination or site-specific recombination in embryonic stem (ES) cells. Such targeted transgenesis is advantageous for achieving predictable and reproducible transgene expression. However, the targeted system is more technically demanding and time-consuming than PI-based transgenesis.
This study established a PI-based targeted transgenesis (PITT) system based on Cre-loxP site-specific recombination in fertilized eggs, but not in ES cells. Seed mice were initially generated through ES cell targeting to contain loxP-site derivatives at a predetermined locus (e.g. *Rosa26*). A transgene, flanked by two mutant loxP sites, was then integrated into target loci in the seed mice by recombinase-mediated cassette exchange (RMCE) in fertilized eggs. By applying this method, we have established a total of 25 transgenic lines so far, including fluorescent gene transgenic mice showing ubiquitous, strong, and reproducible transgene expression. We also demonstrated that knockdown mice can be readily generated by PITT by taking advantage of our reproducible and highly efficient expression system. The PITT method could therefore provide a strong basis for systematically generating a variety of targeted transgenic lines, without going thorough ES cells, for reliable transgene expression and for gene knockdown.

THE GDNF TARGET - VISININ LIKE 1 IS A NEW URETERIC TIP MARKER UPREGULATED IN THE WD PRIOR TO BUDDING

Roxana Ola[1], Madis Jakobson[1,2], Jouni Kvist[1], Nina Perälä[1], Satu Kuure[2], Karl-Heinz Braunewell[3], Darren Bridgewater[4], Norman Roselund[4], Dmitri Chilov[2], Tiina Immonen[1], Kirsi Sainio[1], Hannu Sariola[1]

[1]University of Helsinki, Biochemistry and Developmental Biology, Institute of Biomedicine , Helsinki, 00014, Finland, [2]University of Helsinki, Institute of Biotechnology , Helsinki, 00014, Finland, [3]Southern Research Institute , Biochemistry & Molecular Biology Department , Birmingham, AL, 35205, [4]University of Toronto, Department of Paediatrics, Toronto, M5G 1X8, Canada

Glial cell line-derived neurotrophic factor (GDNF) is implementary to ureteric budding and branching. If applied exogenously, GDNF promotes ectopic ureteric buds from the Wolffian duct. Microarray screening of GDNF-regulated genes in the Wolffian duct revealed several early response genes to GDNF. Among them was Visinin like 1 (Vsnl1) encoding for a neuronal calcium sensor protein. We now show that Vsnl1 is expressed exclusively in the ureteric epithelium and is lacking in Gdnf-null kidneys. In tissue culture, its expression was restored by exogenous GDNF and by the alternative bud inducers, FGF7/Follistatin. Hence, Vsnl1 characterizes the tip of the ureteric bud epithelium regardless of the inducer, also in the absence of Ret. Vsnl1 shows a mosaic expression pattern in the tips. This pattern of Vsnl1 was mutually exclusive with that of Xgal labeling in the Batgal reporter mice for Wnt activity. In accordance, Vsnl1 was downregulated in β-catenin stabilized kidneys, but also in β-catenin deficient kidneys. Moreover, in mouse collecting duct cells, Vsnl1 compromised β-catenin stability, suggesting that Vsnl1 and β-catenin are counteracting in the ureteric epithelium.

CELL DYNAMICS DIRECTING GUT ENDODERM MORPHOGENESIS IN THE MOUSE EMBRYO

Gloria Kwon, Maria Pulina, Yvonne Pao, Kat Hadjantonakis

[1]Sloan Kettering Institute, Developmental Biology, New York, NY, 10065

The cellular behaviors underlying the morphogenesis of the gut endoderm, the tissue that will give rise to the respiratory and digestive tracts and associated organs, are complex and not well understood. We are combining live imaging and genetic labeling, to visualize the dynamic cell behaviors during endoderm formation in the mouse gastrula.

Our data reveal that the gut endoderm arises by widespread intercalation of embryonic (definitive endoderm) and extraembryonic (visceral endoderm) tissues. We propose that this morphogenetic event facilitates an increase in the surface area of the embryo to accommodate its rapid growth at this stage, and leads to extensive cell mixing which results in the gut endoderm comprising cells of two distinct origins.

Epithelial-to-mesenchymal transition (EMT) and cell delamination at the primitive streak provide both the appropriate morphology and trajectory for definitive endoderm cells. We therefore suggest that definitive endoderm cells egress (i.e. insert) into the pre-existing visceral endoderm epithelium, and in doing so undergo a process of mesenchymal-to-epithelial transition (MET). Acquisition of endoderm-specific marker expression could reveal a commitment for primitive streak-derived cells to egress and subsequently undergo MET to epithelialize. Elevated rates of cell proliferation in the epiblast and its derivatives as compared to the overlying visceral endoderm could provide a driving force for remodeling of visceral endoderm cell junctions, and promote egression of epiblast-derived cells onto the surface of the embryo. Furthermore, active remodeling of the extracellular matrix may initially facilitate cell egression but later on enforce lineage segregation.

To gain mechanistic insight into the process of gut endoderm formation, we have begun to characterize the sequential events driving this event in both wild type embryos and mutants in which different steps are affected.

THE GENE *TEASHIRT1 (TSHZ1)* IS ESSENTIAL FOR THE DEVELOPMENT OF OLFACTORY BULB GRANULAR CELL INTERNEURONS

Daniela Ragancokova*[1], Elena Rocca*[1,2], Thomas Müller[1], Hagen Wende[1], Carmen Birchmeier[1], Alistair Garratt[1]

[1]Max-Delbrück-Center for Molecular Medicine, Developmental Biology/Signal Transduction, Berlin, 10409, Germany, [2]GenØk , Senter for biosikkerhet, Tromsø, 9294, Norway

The mouse *Teashirt1 (Tshz1)* gene is one of three homologues of the Drosophila gene *Teashirt*, which encodes a zinc-finger protein that functions both in the Wnt signaling pathway and as a homeotic determinant of trunk identity. All vertebrate *Teashirt* genes encode zinc-finger homeodomain factors, which are expressed throughout the developing and adult nervous system.
We present here a detailed characterisation of the cell populations within the granule cell layer of the embryonic mouse olfactory bulb, and assign to *Tshz1* an essential role in both the radial migration and the molecular specification of early born, distally generated granule cell neurons. Early-born granule cell neurons arrived within the bulbs of *Tshz1*$^{-/-}$ mutant mice, but distributed aberrantly within the radial dimension, forming closely-packed cell aggregates. Within these clusters, cells of the *Tshz1* lineage failed to express the zinc-finger transcription factors Sp8 and Sall3 and remained in an immature state as defined by the loss of expression of the markers neuN, glutamic acid decarboxylase (GAD)-67, γ-amino butyric acid (GABA), tyrosine hydroxylase and guanine deaminase (cypin). Currently, we are using FACSorting to isolate GFP-positive cells from control and mutant olfactory bulbs in order to compare changes in gene expression.
Tshz1 is the first molecule to be characterised, which functionally distinguishes between the differentiation programs of olfactory bulb granule cell and periglomerular cell neurons. Furthermore, our analyses indicate that soluble and/or cell surface factors produced by the *Tshz1*$^{+}$ outer granule cell lineage regulate the spatial distribution of other neuronal populations within the olfactory bulb.

THE SANGER INSTITUTE MOUSE GENETICS PROGRAMME

Ramiro Ramirez-Solis, Ed Ryder, Jacqui White, Richard Houghton, Joanna Bottomley

Wellcome Trust Sanger Institute, Mouse Genetics Programme, Hinxton, Cambridgeshire, CB10 1SA, United Kingdom

The Sanger Institute Mouse Genetics Programme (MGP) capitalizes on the availability of the mutant ES cell resources generated by the EUCOMM and KOMP projects by generating and phenotyping mutant mouse lines at a large scale. The mutant strains and the phenotypic data are freely available to the scientific community to promote deeper analysis aimed at uncovering the molecular mechanisms involved in the phenotypic alterations resulting from the mutant alleles.
The MGP has produced over 400 mutant strains, of which more than 200 have finished the phenotypic screen. The phenotypic data can be obtained by visiting the Sanger Mouse Portal (http://www.sanger.ac.uk/mouseportal/). The website offers the opportunity to download a weekly updated summary heat map that includes all the strains being examined. Scientists are also encouraged to sign up for a regular phenotypic alert email to receive early warnings on interesting phenotypes. When two (2) heterozygote mice are genotyped for a particular allele, the strain is advertised to the community at the International Knockout Mouse Consortium (IKMC) website (http://www.knockoutmouse.org/about). Although the specific mice to be distributed may not be available immediately, the MGP seeks to receive notifications of interest, and while the mice are on the shelf, a reasonable effort is made to distribute them to interested parties with minimum delay. Although a majority of strains is currently selected because of prior interest on the gene, we expect that increasingly strains will be selected because of the phenotypic data generated from the MGP. The selection of phenotypic tests included in the program is strongly influenced by an active interaction with experts from the community and is aimed at exploring a biological space of medical relevance. We will present an update on the program activities and highlight some interesting and novel phenotypic findings.

KTELC1, A NOVEL PROTEIN GLUCOSYLTRANSFERSE, IS REQUIRED FOR MOUSE EMBRYONIC MORPHOGENESIS AND NOTCH SIGNALLING.

Nitya Ramkumar[1,2], Jeffrey D Lee[1,3], Kathryn V Anderson[1]

[1]Sloan-Kettering Institute, Developmental Biology, New York, NY, 10065, [2]Weill Graduate School of Medical Sciences of Cornell University, Biochemistry, Cell and Molecular Biology Program, New York, NY, 10021, [3]Regeneron, Regeneron, New York, NY, 10591

Glycosylation of proteins is postulated to be important for protein stability, protein folding and quality control, ligand recognition and binding. The importance of glycosylation in development is apparent from the vast number of congenital disorders caused by altered glycosylation of proteins. Through an ENU induced screen to identify genes important in mouse embryonic development, we identified *wsnp (wing shaped neural plate)* as an allele of *Ktelc1*, which encodes a protein glucosyltransferase. The *wsnp* allele harbors a point mutation in a splice donor site resulting in multiple improperly spliced products that are likely to be non-functional. These mutants die before e9.0 and have severe defects in epithelial-to-mesenchymal transition at the primitive streak, and the embryos are deficient for mesoderm-derived structures, as visualized by *Meox1* expression. Additionally, the mutants have an abnormally shaped neural plate that fails to close and form the neural tube. O-glucosylation is a very rare modification found in only a handful of proteins and the functional importance of this modification on proteins remains to be elucidated. The Drosophila homologue of KTELC1, Rumi, was shown to O-glucosylate Notch, a cell surface receptor involved in multiple cell fate decisions. Absence of this O-glucosylation leads to an inability to cleave the Notch intracellular domain therefore altering the Notch signalling. Consistent with the fly data, we find that *wsnp* mutants have defects in processing Notch and therefore are unable to produce the activated form of Notch upon ligand binding. Furthermore, the expression of Notch target genes is decreased in these mutants. However, the phenotype of *wsnp* mutant is more severe than all known mutations that affect the Notch pathway indicating a Notch-independent function of KTELC1. We are currently investigating the importance of glucosylation in the Notch pathway and aiming to identify other potential targets of KTELC1 that play important roles in embryonic development.

MECHANISMS UNDERLYING SPINA BIFIDA IN THE ZIC2 LOSS-OF-FUNCTION MUTANT, KUMBA

Saba R Raza, Valentina Massa, Dawn Savery, Nicholas D Greene, Andrew J Copp

UCL Institute of Child Health, Neural Development Unit, London, WC1N 1EH, United Kingdom

Spina bifida aperta is a common and disabling neural tube defect in humans; it occurs when the caudal progression of neural tube closure from the hindbrain/cervical boundary is interrupted. Spinal neural tube closure requires the formation of 'hinge points', which are localised regions of bending in the neuroepithelium. At upper spinal levels, a single median hinge point (MHP) is formed, which is dispensible for closure. At lower spinal levels, dorsolateral hinge points (DLHPs) become apparent, and are essential for closure between the 16- and 30-somite stages. Mice homozygous for the *Zic2* loss-of-function allele, Kumba (*Zic2*$^{Ku/Ku}$) have a fully penetrant spina bifida that is characterised by the absence of (DLHPs) in the neuroepithelium. Although DLHP formation is known to be regulated by varying inhibition from the Shh and Bmp signalling pathways, little is known about the genetic and molecular targets of *Zic2* that preclude normal neuroepithelial morphogenesis. Using a microarray screen of Kumba posterior neuropore regions, we have identified and validated differentially expressed genes that may contribute to the *Zic2*$^{Ku/Ku}$ spina bifida. Our analysis confirmed that canonical Wnt signalling is down-regulated via *Wnt3a* in the *Zic2*$^{Ku/Ku}$ posterior neuropore at the time DLHPs are expected to form. We have also identified an orphan nuclear receptor, *GCNF/Nr6a1*, that is downregulated in the *Zic2*$^{Ku/Ku}$ neuroepithelium, and is linked to Bmp pathway activation. These findings suggest that an alteration of dorsal Wnt or Bmp signalling may be linked to failure of DLHP formation. Although an overactivation of Shh signalling is expected to inhibit DLHP formation, markers of Shh signalling activity, such as dorso-ventral patterning of the neural tube, and expression of the downstream effectors *Ptc* and *Gli2*, are unaltered in *Zic2*$^{Ku/Ku}$ mutants. This suggests that Shh is not responsible for the neurulation defect in these embryos. In fact, in parallel with our microarray analysis, we have characterised a defect in the *Zic2*$^{Ku/Ku}$ notochord, which is narrow and discontinuous compared to wild-type controls. This might be expected to cause defects in ventral neural plate development in *Zic2*$^{Ku/Ku}$ embryos and, indeed, preliminary findings indicate enhanced mitotic activity in the ventral neural plate, 'blunting' the dorso-ventral proliferation gradient. These findings highlight the need for precise regulation of dorsal and ventral signals for neural plate morphogenesis, prior to the onset of neurogenesis.

INNOVATIVE MOUSE MODELS FOR TARGET DISCOVERY

Gesa Rickheit, Jost Seibler, Nico Scheer, Branko Zevnik, Gunther Kauselmann

TaconicArtemis GmbH, Molecular Biology, Cologne, 51063, Germany

TaconicArtemis together with Taconic Cranbury represents the highly innovative site of Taconic. Together we are able to offer full range services from mouse model generation to phenotyping and compound profiling. Speed, Quality and Innovation have been the driving forces for TaconicArtemis to develop custom-tailored genetically engineered mouse (GEM) models for fast and efficient in vivo drug target validation. We are offering a wide range of mouse models that include constitutive / conditional Knock Out, constitutive / conditional Knock In, humanizations, targeted Transgenesis, and inducible gene Knock Down by RNA interference within competitive timelines and retaining high quality design and production.

We further enlarged our biological platform in order to position our models from the target validation to the pre-clinical phase of the drug discovery pipeline. In order to reduce drug attrition rates we generated innovative transADMET (absorption, distribution, metabolism, excretion, toxicology) mouse models for drug discovery and development. Key murine ADMET genes such as nuclear receptors (e.g. PXR and CAR), Phase I enzymes (e.g. Cyp3A) and drug transporters have either been exchanged for their human counterparts or knocked out for use as controls.

To improve to the drug discovery process further we developed the inducible KinaseSwitch System which permits the selective inhibition of only the desired target kinase. Proven technologies (ASKA) are used to create mutations that allow binding of the inhibitor only by the kinase of interest.

Our major goal is the development and constant improvement of in vivo systems that allow on the one hand a better understanding of the molecular basis of human pathology and fundamental biological processes and on the other hand the fast and predictive discovery of novel and more efficient drugs.

THE GENE EXPRESSION DATABASE FOR MOUSE DEVELOPMENT (GXD)

Constance M Smith, Jacqueline H Finger, Terry F Hayamizu, Ingeborg J McCright, Janan T Eppig, James A Kadin, Joel E Richardson, Martin Ringwald

The Jackson Laboratory, Mouse Genome Informatics, Bar Harbor, ME, 04609

The Gene Expression Database (GXD) collects and integrates different types of mouse developmental expression data from wild-type and mutant mice, including data from RNA in situ, immunohistochemistry, in-situ reporter (knock-in), Northern blot, Western blot, and RT-PCR experiments. The GXD staff reads the scientific literature and enters the expression data from those papers into the database on a daily basis. GXD also acquires expression data directly from researchers, including laboratories performing large-scale expression studies. GXD records expression information in detail using standardized text annotations, and data entries are accompanied by images of the original data whenever possible. GXD currently includes data from more than 38,000 experiments containing over 430,000 expression results, and data from almost 1,500 mouse mutants. In addition, the database holds over 72,000 images of original expression data. These images have been carefully indexed with respect to the genes analyzed, the probes used, the strain and genotype of the specimen, the developmental stages and anatomical structures in which expression was reported to be present or absent, and other parameters. Furthermore, by being an integral part of the larger Mouse Genome Informatics (MGI) resource, GXD combines its expression data with other genetic, functional, phenotypic, and disease-oriented data. Therefore, users can search for expression data and images in many different ways, using a wide variety of biologically and biomedically relevant parameters.

We welcome direct submissions of expression data to GXD. Data submissions receive accession numbers that can be cited in publications and grant applications. Inclusion of expression data in GXD provides integration with all the other data in GXD and MGI and makes it widely accessible to database queries, thus increasing its utility and exposure. Guidelines and tools for electronic data submission are accessible at our web site.

GXD is freely available through the MGI web site (http://www.informatics.jax.org), or directly at http://www.informatics.jax.org/expression.shtml. GXD is supported by NIH grant HD033745.

THE CIRCADIAN RHYTHM RELATED ARYL HYDROCARBON RECEPTOR NUCLEAR TRANSLOCATOR-LIKE 2 PROTECTS AGAINST TYPE 1 DIABETES

Chenxia He[1], Nicolas Prevot[2], Christian Boitard[2], Philip Avner[1], Ute C Rogner[1]

[1]Institut Pasteur, CNRS, URA2578, BIODEV, GMM, Paris, 75015, France, [2]Inserm, U986, Hôpital St. Vincent de Paul, Paris, 75014, France

The locus *Idd6* is involved in type 1 diabetes development in the nonobese diabetic (NOD) mouse through its effect on the immune system and in particular T cell activities. Analysis of congenic strains for *Idd6* revealed the *Aryl hydrocarbon receptor nuclear translocator-like 2* (*Arntl2*) as candidate. Arntl2 belongs to the basic helix-loop-helix/Per-Arnt-Sim (bHLH/PAS) family of transcription factors controlling the circadian rhythm. Our results show that diabetes incidence is increased by *Arntl2* mRNA interference in *Idd6* congenic mice concomittent with an increase in CD4+ T cells and a decrease in regulatory T cells in the peripheral immune system. Our results show further that upregulation of cellular Arntl2 levels correlates with inhibited CD4+ T cell proliferation and their decreased diabetogenic activity. These data suggest that *Arntl2* in the *Idd6* interval may act on CD4+ T cell proliferation and the balance between effector and regulatory T cells to protect against autoimmune type 1 diabetes in the NOD mice. They also reinforce the current findings on key functions of circadian rhythm related genes in controlling metabolic pathways. These genes include the *Arntl2* homologues *Aryl-hydrocarbon receptor* (*AhR*), which controls key regulatory immune functions, *ARNT* and *ARNTL1*, which both have been associated with diabetes in human and in mouse.

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ANALYSIS OF MICE LACKING FOLATE RECEPTOR 4.

J. Michael Salbaum[1], Vishwa D Dixit[2], Claudia Kappen[3]

[1]Pennington Biomedical Research Center, Regulation of Gene Expression, Baton Rouge, LA, 70808, [2]Pennington Biomedical Research Center, Neuroendocrine Immunology, Baton Rouge
, LA, 70808, [3]Pennington Biomedical Research Center, Developmental Biology, Baton Rouge, LA, 70808

Folate is vitamin of the B complex, and constitutes an essential micronutrient. It serves as an enzymatic cofactor in single-carbon transfer reactions in the processes of nucleotide, amino acid and lipid synthesis, and is thought to contribute to the development and maintenance of the epigenome through its role in histone and DNA methylation. Folate deficiency has been linked to risk for birth defects, cardiovascular disease, reduced immune function, and certain types of cancer.

Mammalian cells have developed an elaborate mechanism to harvest folate, involving extracellular folate receptors, several transmembrane carriers, and an intracellular enzymatic 'trapping' strategy to prevent folate from leaving the cell. Despite the essential nature of folate, which would suggest ubiquitous expression, genes for folate receptors show distinct tissue-specific expression patterns.

To gain insights into the biological functions of Folate receptor 4, we have generated mice with a 'floxed' allele of the Folr4 gene, where the first two coding exons of the gene are flanked by loxP sites. For a germline deletion of Folr4, we crossed $Folr4^{fl/fl}$ homozygous mice with mice carrying a Cre recombinase transgene under the control of the MMTV promoter. These mice show high expression of Cre in the oocyte; introduction of a paternal floxed allele lead to recombination at loxP sites before the first cell division, which created a germline deletion in the resulting mouse. Based on FACS analyses of splenocytes, this Cre-mediated deletion resulted in a null allele for the Folr4 gene.

We report that mice homozygous for the Folr4 null mutation are viable, as crosses of heterozygous animals yield all three genotypes in Mendelian fashion. However, while Folr4 homozygous mutant males are fertile, Folr4 homozygous mutant females appear infertile despite seemingly normal ovulation. We find the Folr4 gene expressed in several locations in the female reproductive tract, most notably the ovary and the developing oocyte, but also the uterine endometrium. Lack of Folr4 affects gene expression in the uterus at the time of implantation, whereas periconceptual supplementation with folate can reverse such gene expression changes. We conclude that the Folr4 gene represents an important checkpoint for fertility and reproduction.

GERMLINE POTENCY OF GENE-TARGETED ES CELL CLONES IMPORTED FROM RESOURCE CENTERS.

Keith F Childs[1], Elizabeth D Hughes[1], Debora L Vanheyningen[1], Galina B Gavrilina[1], Margaret L Van Keuren[1], Thom L Saunders[1,2]

[1]University of Michigan, Transgenic Animal Model Core, Ann Arbor, MI, 48109, [2]University of Michigan, Internal Medicine: Division of Molecular Medicine and Genetics, Ann Arbor, MI, 48109

The ability to prepare genetically modified mice from mouse embryonic stem (ES) cells with defined mutations has accelerated the discovery of gene function in whole animal models. The international consortium to knock out every gene in the mouse (KOMP) has generated thousands of gene-targeted ES cell clones that can be used to prepare ES cell-mouse chimeras and gene-targeted mouse strains. This methodology simplifies access to mouse models for investigators, obviating the need to design gene-targeting vectors, to manipulate totipotent ES cells in culture and to screen ES cell clones for desired genetic changes. Gene-targeted and gene-trapped ES cell clones were imported from participating members of KOMP (EUCOMM, NorCOMM, SIGTR, BayGenomics, FHCRC, GGTC, CMHD, Regeneron, and TIGM). ES cell clones were analyzed with respect to euploid chromosome numbers, Mycoplasma contamination, verification of gene targeting event, generation of ES cell-mouse chimeras, and germline transmission of gene trapped alleles. More than 100 clones were characterized. The majority (73%) formed germline chimeras. Rarely, clones were incorrectly targeted or required subcloning to isolate gene-targeted subclones. The most common reason that ES cells did not go germline was because of aneuploid chromosome makeup. Additional clones failed to form germline chimeras for unspecified reasons. The data presented suggest that in order for an investigator to be assured of obtaining a knockout mouse model at least two ES cell clones with mutations in the gene of interest will need to be procured. When it is necessary to achieve germline transmission from two independent ES cell clones so that confounding phenotypes arising from spurious spontaneous mutations during ES cell culture can be eliminated then at least three ES cell clones should be imported for chimera production and germline breeding.

QUALITY ASSESSMENT OF CRYOPRESERVED SPERMATOZOA BY A SUBTRACTIVE STAINING TECHNIQUE

Ann-Kathrin Diercks[1], Heinrich Bürgers[2,4], Hansjoachim Hackbarth[3], Johannes Schenkel[1,4]

[1]German Cancer Research Centre, Cryopreservation, Heidelberg, 69120, Germany, [2]Tissuegnostics, Development, Wien, 1020, Austria, [3]University of Veterinary Medicine, Institute of Animal Welfare and Behaviour, Hannover, 30552, Germany, [4]Heidelberg University, Dept. of Physiology and Pathophysiology, Heidelberg, 69120, Germany

Transgenic animals are unique mutants to study genes, their function, regulatory mechanisms, or (human) diseases. Cryopreservation of pre-implantation embryos or spermatozoa is a common approach to save those lines against loss or to keep them in a repository. Only following the cryopreservation of a sufficient number of samples and a successful quality assessment a mutant line can be taken out of a breeding nucleus.
The quality of spermatozoa of different donors within a mouse line is heterogeneous. Technical problems during the cryopreservation procedure might happen, too. Subsequently, a significant quality assessment of the cryopreserved samples of each donor-animal is mandatory.
To replace the common, but animal consuming in vitro-fertilization and the subsequently needed embryo transfer for quality assessment purposes of cryopreserved spermatozoa, an animal free procedure was developed: A fluorescence microscopy based technique was established and validated. Viability, density, motility, and morphology of spermatozoa were analyzed as parameters of major interest.
Spermatozoa are treated with two dyes staining all or only dead cells, respectively. Several fields of view are acquired fluorescence-microscopically and analyzed with the appropriate software. The data received cover the three parameters viability, density, and morphology to assess the quality of the spermatozoa: In addition to the fluorescence microscopy a video sequence of 200 frames is recorded to analyse the motility.
To determine the reproducibility of viability, density and motility cryopreserved spermatozoa of wildtype and transgenic mice with a different genetic background were investigated. Therefore different samples of the same animal and repeated measurements within the same sample were investigated. The data received indicate that the parameter “viability” is statistically significant most important to assess cryopreserved spermatozoa. Under the prerequisite that each transgenic spermatozoa donor mouse can be re-genotyped and a single IVF is performed to determine the fertilization capacity of a line, this approach facilitates a reliable quality assessment of cryopreserved spermatozoa without additional animal consuming experiments for quality assessment purposes. This contributes to the animal welfare and the 3R principles postulated by Russell and Burch, too.

HANDLING IMPORTANT INFORMATION OF GENETICALLY MODIFIED MICE

Michael Staudt[1], Iris ElHindi[2], Claudia Galuschka[3], Dagmar Sitek[4], Johannes Schenkel[1,5]

[1]German Cancer Research Center, Cryopreservation, Heidelberg, 69120, Germany, [2]German Cancer Research Center, Technology Transfer, Heidelberg, 69120, Germany, [3]German Cancer Research Center, Information Technology, Heidelberg, 69120, Germany, [4]German Cancer Research Center, Central Library, Heidelberg, 69120, Germany, [5]Heidelberg University, Dept. of Physiology and Pathophysiology, Heidelberg, 69120, Germany

Due to their major scientific potential the number of GM mice is rapidly increasing. Several limitations when working with these animals are to be considered resulting in the need for an effective storage system to keep and store also those lines which are out of current use. Therefore cryopreservation of pre-implantation embryos or spermatozoa is a valuable tool.
To gather quickly relevant information about all GM mouse lines available *in-house*, a database was developed enabling optimum knowledge management and easy access to the resources available.
Originally, the database was developed for a repository of cryopreserved GM mice. To offer a complete information, all active GM lines were introduced into this database, too. This is also a support for the Technology Transfer Department handling frequent requests about possibly available animal models and having the duty to find out a possible exploitation.
In a further step a cross link to the institutional publication database was performed, offering the direct link between a publication and a mouse described or mentioned there. In addition, legal restraints ask to gather further information, especially about the mutation itself.
To achieve an easy access the data base is available as a web-based application, no software must be installed locally.

Data organization
Detailed information is provided for each mouse line:
- Description:
Name of the mouse line (short, long, and international version), characterization/description, usage of an animal model, genotype, genetic background, backcross generation, possible phenotype, genetic modification and the generation technique used, origin of the mutation (gene-ID and donor organism), the origin of regulatory elements with their donor and vectors; housing conditions, a possible need of rederivation, or a movement into another facility.
- Possible publications, keywords, use as an animal model:
A registered GM mouse line is linked with key publications and vice versa.
- Legal status:
Techniques used when generating the mouse line, material transfer agreements, or patents. The investigator keeping the rights of the GM line might declare whether and under which conditions she/he wishes to make this line accessible for others.

ETV4 AND ETV5 MEDIATE FGF10 SIGNALING DURING PANCREAS MORPHOGENESIS

Martin Schmerr[1], Sune Kobberup[2], Jan Jensen[1]

[1]Cleveland Clinic , Stem Cell Biology and Regenerative Medicine, Cleveland, OH, 44195, [2]Lund University, Stem Cell and Pancreas Developmental Biology, Lund, SE-22184, Sweden

Mesenchymal-to-epithelial signaling plays a key role in the development of many organs. For example, secretion of Fgf10 from the pancreatic mesenchyme stimulates proliferation of progenitor cells in the neighboring pancreatic epithelium. Although Fgf10 signaling is crucial for expansion of the pancreatic epithelium, few of the downstream genes are known. In an effort to identify cell-intrinsic factors regulated by Fgf10 signaling in the pancreatic epithelium, we found that two members of the ETS-family transcription factors, Etv4 and Etv5, were upregulated by Fgf10 overexpression. In wild type embryos, both factors are expressed almost exclusively within the pancreatic epithelial progenitors. We also observed expression of Etv4 in pancreatic progenitor cells active in MAPK signaling. Moreover, inhibition of the MAPK pathway suppressed the FGF-dependent transcriptional activation of Etv4 and Etv5 *in vitro*. We examined the pancreas of $Etv4^{-/-}$ and $Etv5^{-/-}$ embryos to ascertain if the effects of Fgf10 signaling are mediated through these two factors in vivo. The pancreas of $Etv4^{-/-}$ embryos appeared normal, which suggests that Etv5 may compensate for Etv4 function as the DNA binding domains of these factors are almost identical. We used an outbreeding strategy to circumvent the embryonic lethality of Etv5 null embryos. This approach was successful, and we found that $Etv5^{-/-}$ embryos displayed a profound reduction in the pancreatic progenitor population. In addition, mature endocrine and exocrine cell types were diminished in the Etv5 null embryos, which is suggestive of pancreatic epithelial hypoplasia. Thus, Etv4 and Etv5 are key components of Fgf10 signaling that drive pancreatic progenitor cell proliferation.

IDENTIFICATION OF NOVEL NR2E1-DIRECT-TARGET GENES BY SAGE ANALYSIS

Jean-Francois Schmouth[1], Ximena Corso Diaz[1], David Arenillas[2], Xie Robert[2], Wyeth Wasserman[1,2], Elizabeth M Simpson[1,2,3]

[1]University of British Columbia, Genetics, Vancouver, V5Z 4H4, Canada, [2]University of British Columbia, Medical genetics, Vancouver, V5Z 4H4, Canada, [3]University of British Columbia, Psychiatry, Vancouver, V5Z 4H4, Canada

Background: Nuclear receptor 2e1 gene (Nr2e1) encode for an orphan nuclear receptor acting as a transcription factor that functions in progenitors and neural stem cells to regulate proliferation and cell fate decisions. We evaluated the transcriptome of $Nr2e1^{-/-}$ (fierce) animals to identify genes directly regulated by Nr2e1.
Method: LongSAGE libraries from laser microdissected dorsal ventricular/subventricular zones of the telencephalon of wild-type (Wt) and $Nr2e1^{-/-}$ embryos at E13.5, E15.5 and E17.5 were analyzed using the DiscoverySpace system. Sequence tags exhibiting differential expression patterns between Wt vs $Nr2e1^{-/-}$ were mapped to the corresponding gene. The list of differentially regulated genes was assessed with the oPOSSUM promoter analysis system. Genes containing predicted Nr2e1 binding sites were subjected to a gene ontology (GO) term enrichment analysis using the DAVID service. Expression levels of genes annotated with the most relevant enriched GO terms were studied by quantitative reverse transcriptase PCR (Q-RT-PCR) in Embryonic Stem Cells (ESCs) differentiated using a method of adherent-monoculture neurogenesis (Wt vs $Nr2e1^{-/-}$ ESCs). Wt and $Nr2e1^{-/-}$ ESCs targeted with specific luciferase constructs will be tested in the cell differentiation procedure to assess the function of specific Nr2e1-binding-site predictions.
Results: 572 genes at E13.5, 647 genes at E15.5, and 380 genes at E17.5 displayed significant differences in expression ($p \leq 0.05$). These correspond to 1,275 genes for the three time points, of which 774 contain putative Nr2e1-binding sites. 570 of those genes are annotated with enriched GO terms ($p \leq 0.05$ after correction). The most relevant enriched terms for Nr2e1 function are “Cell division” (33 genes, $p \leq 0.000$), “Cell cycle” (59 genes, $p \leq 0.003$), “Nervous system development” (55 genes, $p \leq 0.004$), and “Neurogenesis” (35 genes, $p \leq 0.031$). Consistent with the literature, nestin tag counts were found to be lower by 7 fold in the $Nr2e1^{-/-}$ library in comparison to the Wt library at E13.5. Amongst the potential new targets discovered, Cntn2 and Hes6 were expressed consistently in both the SAGE data and the Q-RT-PCR of differentiating ESCs.
Conclusion: Bioinformatic analysis of SAGE data may identify novel genes involved in fate specification of neural stem cells.

LENTIVIRAL MEDIATED TRANSGENESIS BY IN-VIVO MANIPULATION OF SPERMATOGONIAL CELLS

Lalit Sehgal, Rahul Thorat, Nileema Khapare, Amitabha Mukhopadhaya, Sorab N Dalal

Sorab Lab, KS-215 ACTREC, Tata Memorial Centre, Navi Mumbai, Maharashtra, 410210, India

The generation of genetically modified mice is a laborious and lengthy process. We have recently developed a new technique to generate transgenic mice with a very high rate of success as compared to conventional transgenic technology. Spermatogonial stem cells (SSCs) in pre-pubescent animals were infected in vivo with recombinant lentiviruses expressing EGFP. Once the mice attained adulthood, they were mated with normal females and the progeny of this cross analyzed for the presence of the EGFP transgene. 60% of the first generation pups showed the presence of the transgene and EGFP was expressed in most tissues tested. The transgene was heritable as shown by mating experiments between two transgenic animals as well as between a normal animal and a transgenic animal. This technique has eliminated several of the low efficiency steps that are involved in the generation of transgenic mice such as implantation of the modified embryo and germline transmission. Further, we have developed vectors to generate knockdown animals as well as inducible constructs for overexpression and knockdown experiments. These results will be presented at the meeting.

THE IMPRINTED PLACENTAL-SPECIFIC *IGF2* ISOFORM IS REQUIRED FOR ADAPTIVE RESPONSES OF THE MOUSE PLACENTA TO UNDERNUTRITION

Amanda N Sferruzzi-Perri, Owen R Vaughan, Phil M Coan, Miguel Constancia, Graham J Burton, Abigail L Fowden

Centre for Trophoblast Research, University of Cambridge , Department of Physiology, Development and Neuroscience, Cambridge, CB2 3EG, United Kingdom

Fetal growth during pregnancy is controlled by maternal nutrient availability and in turn, by placental capacity to deliver these nutrients to the fetus. Recently, the placenta has been shown to adapt morphologically and functionally during maternal undernutrition in the mouse to maintain fetal growth until close to term, despite its small size (Coan *et al*., 2009). The mechanism by which placental phenotype may be modulated in response to nutrient restriction is unknown. However, in part, these adaptations may involve the placental-specific isoform of the paternally expressed *Igf2* gene (*Igf2P0*), a key regulator of placental growth and nutrient transfer capacity (Constancia *et al.,* 2002, 2005). Thus, this study aimed to determine the role of the *Igf2P0* isoform in the placental adaptive response to maternal calorie-restriction. Mice bearing wildtype (WT) or *Igf2P0* deficient litters were fed either ad libitium (CT) or 80% of the CT intake (undernourished, UN) during pregnancy. In WT litters, UN reduced placental weight from D16 but fetal weight only on D19. It also increased amino acid transfer to the fetus per gram of WT placenta. This functional adaptation to UN failed to occur in the small, *Igf2P0* deficient placenta which resulted in earlier onset of fetal growth restriction, evident on D16. The *Igf2P0* fetuses were also more growth restricted by UN on D19 than WT pups. Thus, the imprinted placental-specific *Igf2* isoform is required for placental adaptation to UN in mice, possibly via regulation of amino acid transporter genes.

FAST AND RELIABLE DETECTION OF GENOMEWIDE CPG ISLAND METHYLATION BY MBD-MEDIATED ENRICHMENT OF METHYL DNA FOLLOWED BY MICROARRAY ANALYSIS

Jafar Sharif[1], Takaho A Endo[2], Tajima Shoji[3], Tetsuro Toyoda[2], Haruhiko Koseki[1]

[1]RIKEN, Research Center for Allergy & Immunology (RCAI), Kanagawa, 230-0045, Japan, [2]RIKEN, Bioinformatics & System Engineering (BASE), Kanagawa, 230-0045, Japan, [3]Osaka University, Department of Biological Sciences, Osaka, 565-0871, Japan

DNA (5-cytosine) methylation functions as a major epigenetic mechanism that has essential roles for development, regulation of transcription, silencing of retroelements and reprogramming of the genome. Aberrations in DNA methylation are frequently associated with diseases such as cancer. Despite the immense importance of DNA methylation in mammalian cells, at present detection and quantification of this epigenetic mark in a genomewide scale remains difficult and time consuming. To address this problem, here, we have developed a convenient method to examine genomewide DNA methylation by enriching methyl DNA with a recombinant MBD domain (from murine Mbd1 protein), followed by comprehensive tiling array analysis of more than 16,000 CpG islands in the mouse genome (UCSC mm8, 2008). The recombinant MBD domain has high affinity for methylated CpG sites and captures robust amount of DNA that can be directly labeled with fluorescence dyes (Cy5) and applied to the microarray, without requiring laborious LM-PCR or IVT-mediated amplification steps, which could also contribute to reduction of amplification-related bias in the data. In addition, to interpret the raw data, we have developed a novel bioinformatics-based platform that is suitable for analyzing DNA methylation microarrays. Our method can successfully detect and quantify genomewide DNA methylation profiles for different types of cells such as stem (ES), stem-like (iPS: induced pluripotent), germ (sperm) and somatic (MEF, B cell, thymocytes) cells, exhibiting the sensitivity and dynamic range of this technique. Furthermore, our methylation studies are highly compatible with previously reported genomewide bisulfite-sequencing (RRBS) analyses, which demonstrate the reliability of this method. Taken together, we report that MBD domain mediated enrichment of methyl DNA followed by CpG island microarray could be a useful tool to analyze genomewide DNA methylation profiles, with possible future applications for screening and evaluation of genome reprogramming, such as, in iPS cells.

THE ROLE OF FOCAL ADHESION KINASE IN CORNEAL DEVELOPMENT AND WOUND HEALING

Tang-Long Shen[1,2], Hsin-Jung Wu[1], Chung-Ming Wang[1]

[1]National Taiwan University, Plant Pathology and Microbiology, Taipei, 10617, Taiwan, [2]National Taiwan University, Center for Biotechnology, Taipei, 10617, Taiwan

Given the importance in several cellular processes, including cell migration and proliferation, focal adhesion kinase (FAK) has attracted much interest on its role in physical and pathological relevance. The biological role of FAK is emerging to be elucidated due to the availability of genetic engineered mice, in particular the FAK floxed mice, by combining with and varied Cre mice, it allows us to examine the functionality of FAK in physiopathologic conditions which are precluded due to the embryonic lethality of the conventional FAK knockout. Taking advantage of the Cre/loxP driven FAK conditional knockout approach, we generated the K12-rtTA/tetO-Cre/ FAK f/Δ triple transgenic mice to specifically delete FAK in corneal epithelial cells (CE-FAK), which consequently resulted in an impairment of corneal epithelial wound healing compared with the control mice. The impaired wound healing in corneal epithelial cells predominantly occurred in the earlier stage of the healing process in contrast to the prominent delay of wound healing in the late stage in AKT1-/- mice. Furthermore, the defect in the corneal epithelial wound healing of CE-FAK mice was reversed by infection/overexpression with adenovirus-bearing wild type FAK but not Y397F or FRNK mutant. In agreement with the above in vivo result, we also demonstrated several analogous in vitro results using human corneal epithelial cells (HCECs), highlighting that increased FAK expression and activation are concurrently associated with the promotion of cell adhesion and migration toward collagen IV and EGF. These results indicate a pivotal role for FAK and its mediated signaling in response to corneal epithelial wound stimuli to promote its healing. In addition, via applying several pharmacological inhibitors, such as PP2 (an Src inhibitor), SP600125, and SB203580, we also found that Src and p38 are potential downstream effectors of FAK-mediated wound healing in corneal epithelial cells in mice. Taken together, this study provides comprehensive data for an important role of FAK in corneal epithelial wound healing and underscores the need for FAK in physiopathological maintenance.

RRNA-MEDIATED NONRANDOM NONLINEAR EPIGENETIC AND GENETIC INHERITANCE

Yih-Horng Shiao[1], Robert M Leighty[2], Cuiju Wang[1], Xin Ge[3], Erik B Crawford[1], Joshua M Spurrier[1], Sean D McCann[1], Janet R Fields[1], Laura Fornwald[3], Lisa Riffle[3], Craig Driver[3], Octavio A Quiñones[2], Ralph E Wilson[4], Kazimierz S Kasprzak[1], Gregory S Travlos[4], W G Alvord[2], Lucy M Anderson[1]

[1]National Cancer Institute, Laboratory of Comparative Carcinogenesis, Frederick, MD, 21702, [2]Data Management Services Incorporated, Statistics, Frederick, MD, 21702, [3]SAIC-Frederick, Basic Research Program, Frederick, MD, 21702, [4]National Institute of Environmental Health Science, National Toxicology Program, Research Triangle Park, NC, 27709

Stressors or stimulants induce epigenetic and genetic changes in unicellular and multicellular organisms. Some of these changes are inheritable by subsequent generation(s) via germ cells. It has been thought that such transmission is a copying of germline epigenetic and genetic codes to somatic cells during ontogeny, termed linear inheritance here. If so, the linear inheritance should be established in an early stage of ontogeny. To test this, we injected two stressors, $CrCl_3$ or acidic saline, i.p. into male mice and monitored the DNA methylation as well as the homologous recombination of the *rRNA* repeats in sperm and at various developmental stages of their offspring. In contrast to the above assumption, we observed a nonlinear inheritance, evidenced by dissimilar paternally-mediated responses among day-8 embryos and three tissues of 6-week adult offspring. The nonlinear inheritance was resulting in part from unequal DNA methylation level and gene rearrangement among tissues, integrated in normal development and organogenesis. These were demonstrated by increased intralitter variances of the *rRNA* epigenetic and genetic marks during maturation as well as differences by direct comparisons among adult offspring tissues. This ontogeny-driven nonlinear *rRNA* epigenetic and genetic inheritance signifies that the genome is more dynamic than we thought. It may reshape our thinking of organismal evolution and disease processes.

DOES PRONUCLEAR MICROINJECTION ALLOW ENTRY OF CALCIUM INTO CYTOPLASM OF FERTILIZED EGGS?

Risa Shimizu, Fujio Miyawaki, Dilidaer Kudereti, Hiroki Haremaki, Tomohiro Yoshizawa

Tokyo Denki University, Life Science and Engineering, Saitama, 350-0394, Japan

Aim: Some cellular damages are inevitable in pronuclear microinjection. Among many factors causing the damages, we targeted calcium because it may enter cytoplasm through a gap which might be created between the outer surface of a micropipette piercing the cell membrane and the cell membrane surrounding the micropipette. In addition, the ultrasonic-range vibratory microinjection (VM) system, which we developed to facilitate pronuclear microinjection by providing a micropipette with longitudinal vibration, may allow more calcium entry because unintentional lateral vibration of the micropipette tip is inevitable. As the voltage applied to the vibrator becomes higher, the amplitude of lateral vibration as well as longitudinal vibration becomes larger. VM is more effective when the lateral vibration is slightly visible than when it is not. Therefore, effective VM may create a larger gap than the ordinary microinjection (OM).
Methods: 1) Two types of 44-kHz VM were compared: higher-voltage (High VM) and lower-voltage (Low VM) types. The former had visible lateral vibration and the latter did not. 2) Fertilized eggs collected from 27 BDF1 mice were immersed in 5 μM Fura-2 AM solution for 30 minutes. The intensities of fluorescent light (510 nm) emitted by exposing the eggs in HBSS to two excitation lights (340 nm and 380 nm) were measured, and ratios of the respective intensities were calculated. 3) The eggs were divided into 3 groups: OM group (N = 83), Low VM group (N = 59) and High VM group (N = 38). GFP gene was injected into a pronucleus in M2 medium, which contains 1.17 mM $CaCl_2$, at a pressure of 30 hPa and at 26ºC. 4) After injection, the same procedures were repeated again to obtain the ratios. 5) In another experiment, 44-kHz VM at higher voltages and OM (N = 158 each) were compared in *in-vitro* embryonic development.
Results: 1) The ratios of intensities in each group rose significantly after microinjection, but the changes in ratio were 1.116 in OM, 1.142 in Low VM and 1.133 in High VM. No statistically significant difference was observed between the three groups before or after injection. 2) In another experiment, VM resulted in slightly better embryonic development and quite a lower death rate (24.7% = 39/158 vs. 33.5% = 59/158; P = 0.083, χ^2 test).
Conclusions: Pronuclear microinjection with or without vibration does not allow biologically significant calcium entry. The unintentional lateral vibration of the tip of a micropipette produced by the current version of VM system may be restricted when the tip pierces the zona pellucida and cell membrane.

A REGULATORY TOOLBOX OF MINIPROMOTERS TO DRIVE SELECTIVE EXPRESSION IN THE BRAIN

Elodie Portales-Casamar[1], Douglas J Swanson*[1], Li Liu*[2], Charles N de LeeuwΨ[1,3], Kathleen G BanksΨ[1], Shannan J Ho Sui[1,4], Debra L Fulton[1,4], Steven J Jones[3,5,6], Robert A Holt[5,6,7], Daniel Goldowitz[1,3], Wyeth W Wasserman[1,3], Elizabeth M Simpson[1,3,7]

[1]Centre for Molecular Medicine and Therapeutics at the Child & Family Research Institute, University of British Columbia, Vancouver, V5Z 4H4, Canada, [2]Department of Anatomy and Neurobiology, University of Tennessee, Memphis, TN, 38163, [3]Department of Medical Genetics, University of British Columbia, Vancouver, V6T 1Z3, Canada, [4]Genetics Graduate Program, UBC, Vancouver, V6T 1Z3, Canada, [5]Canada's Michael Smith Genome Sciences Centre, BC Cancer Agency, Vancouver, V5Z 1L3, Canada, [6]Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, V5A 1S6 , Canada, [7]Department of Psychiatry, UBC, Vancouver, V6T 2A1, Canada

*ΨBoth authors contributed equally to the work

The Pleiades Promoter Project (www.pleiades.org) is an international endeavour that integrates genome-wide bioinformatics, large-scale knock-in mouse production, and histological examination of expression patterns to develop MiniPromoters. These MiniPromoters (≤ 4 kb human regulatory sequence), and related tools, are designed to study and treat the brain by directed gene expression. Methods: Genes with brain expression patterns of interest are subjected to bioinformatic analysis to delineate candidate regulatory regions, which are then incorporated into a panel of compact human MiniPromoters to drive expression in brain regions and cell-types of interest. Using single-copy, homologous-recombination “knock-ins” in embryonic stem cells, each MiniPromoter reporter is integrated immediately 5' of the *Hprt* locus in the mouse genome. MiniPromoter expression profiles are characterized using *in vitro* differentiation assays of the transgenic embryonic stem cells and by *in vivo* analysis following transgenic mouse production. Histological examination of adult brains, eyes, and spinal cords for reporter gene activity is coupled to co-staining with cell-type specific markers to define expression. Results: The Pleiades Promoter Project uses a high-throughput bioinformatically-driven approach to produce brain-specific MiniPromoters. To date, 27 novel MiniPromoters have demonstrated positive brain expression (32% of constructs tested), greatly increasing the availability of brain promoters for new research initiatives. New promoters for the blood brain barrier (e.g. Ple34), proliferating neurons (e.g. Ple131), and glia (e.g. Ple185) may be particularly impactful. Conclusion: The publicly available Pleiades MiniPromoters are a key resource to facilitate research on brain function, development, and therapies.

LEARNING FOXN1 ROLES FROM LOXP-CRE/CREER^{T} MOUSE MODELS

Zhijie Zhang, Liguang Sun, Jianfei Guo, Moshiur Rahman, Jiangyan Xia, Dong-Ming Su

University of Texas Health Science Center at Tyler, Dept. of Biomedical Research, Tyler, TX, 75708

Using the gene targeting approach, we generated a *loxP*-floxed-*FoxN1* (termed: fx) mouse model, in which the exons 5&6 (DNA binding domain) are flanked by *loxP*s. Crossing fx mice with ubiquitous- or K14-Cre transgenic (Tg) mice, we found that FoxN1 is required not only for prenatal (previous knowledge) but also crucially for postnatal epithelial homeostasis, because deletion of *FoxN1* in prenatal (mediated by Cre from parents) alone could not, but deletion in prenatal and postnatal (via offspring-self Cre) could induce thymus and skin phenotypes. Crossing fx mice with ubiquitous-CreER^{T2} Tg mice, we found that decline of FoxN1 expression mediated by spontaneous leakage of CreER^{T2} causally speeded up thymic aging, and FoxN1 expression has an age-related haploinsufficient feature, because deletion of *FoxN1* in one copy of *FoxN1* gene also caused accelerated thymic involution with age. Deleting *FoxN1* by different keratin promoter-driven Cre/CreER^{T}, we found that the mice developed phenotypes in the skin and thymus with different degrees of severity. For example, deletion of *FoxN1* mediated by K5-CreER^{T2} in the postnatal thymus (tamoxifen-induction) caused severe phenotype mainly in thymic medulla but also involving cortex, and deletion via K14-Cre, even in the prenatal thymus (at gemeline level) caused only a certain degree of phenotype, such as generation of thymic cysts, while the deletion via tamoxifen-induced K18-CreER^{T2} in the postnatal thymus did not cause any obvious defect. The results imply that FoxN1 role is associated with epithelial stem/progenitor cells because these keratin promoter types, such as K5 and K14, are active in epithelial stem/progenitor cells. Together, *loxP*-Cre/CreER^{T} system lets us gain insights into new roles of *FoxN1* gene in mouse model.

THE POSTERIOR VISCERAL ENDODERM ACTING THROUGH *WNT3* SIGNALING FUNCTIONS AS A NIEUWKOOP CENTER EQUIVALENT IN MICE

Giovane G Tortelote[1], Maki Wakamiya[2], Sakthi Sundararajan[1], Gloria Kwon[3], Anna K Hadjantonakis[3], Richard R Behringer[4], Jaime A Rivera-Perez[1]

[1]University of Massachusetts, Department of Cell Biology, Worcester, MA, 01655, [2]University of Texas, Medical Branch, Sealy Center for Cancer Cell Biology, Galveston, TX, 77555, [3]Sloan-Kattering Institute, Developmental Biology Program, New York, NY, 10065, [4]University of Texas, MD Anderson Cancer Center, Houston, TX, 77030

Mouse embryos lacking *Wnt3* fail to gastrulate, lack a primitive streak, and in consequence, mesoderm and endoderm. *Wnt3* is first expressed in the posterior visceral endoderm of E5.5 embryos and subsequently in the adjacent epiblast at E6.0. These observations led us to hypothesize that *Wnt3* emanating from the posterior visceral endoderm is responsible for primitive streak formation and gastrulation in mouse embryos. To test this hypothesis we used *TtrCre* mice to generate mouse embryos lacking *Wnt3* specifically in the visceral endoderm, but retaining *Wnt3* expression in the epiblast. Embryos with a visceral endoderm-specific knockout of *Wnt3* phenocopy the *Wnt3* null mouse phenotype. They fail to gastrulate or express primitive streak markers. These experiments indicate that *Wnt3* function in the posterior visceral endoderm is essential for gastrulation in mice. To determine whether visceral endoderm-derived *Wnt3* is sufficient to drive gastrulation in mouse embryos we generated mouse embryos lacking *Wnt3* specifically in the epiblast, but retaining the expression of *Wnt3* in the visceral endoderm. These embryos display delayed expression of primitive streak markers, show abnormal gastrulation and are resorbed at around E9.5, suggesting that *Wnt3* signaling from the visceral endoderm partially rescues the absence of *Wnt3* in the epiblast. Finally, to investigate whether *Wnt3* signals through the canonical Wnt pathway we generated *Wnt3* null embryos carrying a copy of the *BatGal* transgene, a canonical Wnt pathway marker. *Wnt3* null/ *BatGal* heterozygous embryos fail to express β-galactosidase, indicating that *Wnt3* acts through the canonical Wnt cascade. Taken together these data support the hypothesis that the posterior visceral endoderm acting trough *Wnt3* functions as a signaling center for the formation of primitive streak in mouse embryos, in a similar manner to the Nieuwkoop center of amphibians.

UNDERSTANDING THE ROLE OF HOXA3 IN PHARYNGEAL DEVELOPMENT: TISSUE-SPECIFIC AND TEMPORAL-GLOBAL DELETIONS

Heidi Trau[1], Kyoko Masuda[1], Kirk Thomas[2], Mario Capecchi[2], Nancy Manley[1]

[1]University of Georgia, Genetics, Athens, GA, 30602, [2]University of Utah, Human Genetics and HHMI, Salt Lake City, UT, 84112

Homeobox (Hox) genes encode an evolutionarily conserved family of transcription factors involved in numerous developmental pathways. Mouse Hoxa3 is expressed in neural crest derived mesenchymal cells of the pharyngeal pouches, and in the pharyngeal endoderm. Hoxa3 is required for the development of the third and fourth pharyngeal pouch-derived organs, formation of the cartilages of the throat and the hyoid bone, formation of the ninth and tenth cranial nerves, and in development of the soft palate.
To determine when Hoxa3 is required in the formation of each of these structures, we are using a globally expressed inducible Cre recombinase to temporally knock out Hoxa3 during development. Twelve hours after recombination was induced, the Hoxa3 gene was efficiently deleted, while the mRNA was significantly reduced by 36 hours post-injection. Recombination was then induced in embryos at various time points between E9.5 and E11.5, and inspection at E13.5 indicated that Hoxa3 is required initially for the formation of the third pouch derived organs (E9.5), then for their separation from the pharynx (E10.5), and finally for their migration (E11). The thyroid and ultimobranchial bodies formed and migrated normally. Skeleton preparations of E18.5 embryos in which recombination was induced at time points between E8.5 and E12.5 indicated that Hoxa3 plays a very early role in the formation of the hyoid bone and thyroid cartilage.
In mice in which Hoxa3 is knocked out in the neural crest cells using Wnt1-Cre, the posterior throat cartilages, cranial nerves, and secondary palate are similar to the Hoxa3 null phenotype. These structures are neural crest cell derived, indicating that the phenotypes result from a cell-autonomous neural crest cell defect. However, the lesser horn of the hyoid is not deleted as in the Hoxa3 null, but is reduced, indicating that its formation may also depend on another cell type, such as the endoderm. The endoderm-derived thymus and parathyroid organs are also affected in the NCC-specific Hoxa3 deleted embryo, indicating that the neural crest cells have a specific role in their formation. The thymus was ectopic, and remained persistently attached to the pharynx, while the parathyroids showed delayed separation from the thymus.
We also tested whether Hoxa3 is required in the thymus and parathyroid after their initial organogenesis. Deletion in the thymus using Foxn1-Cre resulted in mild hypoplasia but no other detectable defects. Deletion specifically in the parathyroid gland using PTH-Cre revealed a role for Hoxa3 in maintaining parathyroid development.
Together, these experiments show that Hoxa3 function is required in different cell types and at multiple times during pharyngeal region development.

THE FIRST MOUSE THAT MODELS PROLIFERATIVE DIABETIC RETINOPATHY

Stephen H Tsang[1], Matthew C Naumann[1], Yao Li[1], Joaquin Tosi[1], Vinit B Mahajan[2]

[1]Columbia University, Ophthalmology, New York, NY, 10032, [2]University of Iowa, Ophthalmology, Iowa City, IA, 52242

Retinal ischemia and subsequent neovascularization is a common pathogenic process in several leading causes of disabilities including diabetic retinopathy, retinopathy of prematurity, vascular occlusive disease, macular degeneration, and ocular cancer. Yet, the lack of an animal model with proliferative retinal angiogenesis has prevented advances in therapy and identification of the underlying molecular mechanisms. Currently available retinopathy models including non-human primates do not develop preretinal neovascularization and only display mild non-proliferative disease within the lifespan of diabetic animals. Hypoxia inducible factor (HIF) is a transcription factor and universal sensor for tissue ischemia, but over expression of HIF in transgenic models have thus far failed to recapitulate human retinal diseases. Using a novel strategy to regulate HIF signaling, we reproduced retinal neovascularization in a model with broad relevance and translational potential for a number of blinding diseases. Methods and Findings. In this study, we identified HIF expression in neovascular retinal tissue of humans with mutation of the tumor suppressor Von Hippel Lindau (VHL) gene. This suggested that similar regulation of HIF in transgenic mice might better reproduce the key features of human ischemic retinal disease. A Vhl knockout mouse was created and found to stably activate HIF protein, while HIF was not stably expressed in control littermates. The Vhl -/- mouse also showed expression of various HIF transcriptional targets, such as nitric oxide synthase (NOS). The most striking feature of the Vhl-/- mutants was the development of proliferative angiogenic disease in a similar sequence to humans with ischemic retinopathy. Over the course of a week, there was formation of ischemic retinal areas, progressive retinal capillary loss, retinal neovascularization, vitreous hemorrhage, iris neovascularization, and retinal detachment. Conclusions. We have developed a new mouse model for the study of human ischemic retinal diseases by constitutive down regulation of HIF1 regulator, VHL. Rapid progression of retinopathy in these mutants will expedite the evaluation of therapeutic agents for this group of blinding disorders.

ANNOTATION AND HIGH THROUGHPUT KNOCKOUT MOUSE DESIGN FOR KOMP

Amy C Horton[1], Andrew Van Brunt[1], Alejandro O Mujica[3], Mark D Thomas[2], Jennifer L Harrow[2], William C Skarnes[3]

[1]Washington University School of Medicine, Genetics, St. Louis, MO, 63108, [2]Wellcome Trust Sanger Institute, HAVANNA, Hinxton, Cambridge, CB10 1SA, United Kingdom, [3]Wellcome Trust Sanger Institute, ES Cell mutagenesis (TEAM 87), Hinxton, Cambridge, CB10 1SA, United Kingdom

Several large-scale gene targeting and gene trapping projects are participating in the International Knockout Mouse Consortium to mutate each protein coding gene. Major ongoing pipelines include the EUropean COnditional Mouse Mutagenesis program (EUCOMM) and the KnockOut Mouse Project (KOMP). The goal of the KOMP project is to generate over 5000 targeted conditional and over 3500 targeted deletion alleles in ES cells or mice. The Genome Center at Washington University in St. Louis is a core contributor to KOMP, providing annotation of targeted genes, construct design, and validation. We present here project methods, interfaces, and progress notes. Genes are manually annotated according to HAVANA (Wellcome Trust Sanger Institute) standards using the Otterlace software package. Target exons are selected based on exon phase, exon and intron size, protein domain structure, distribution of repetitive elements, and any overlapping gene structure. Since manual gene annotation is more complex and limits the rate of knockout design, an initial process has been devised to hasten designs into the pipeline based on detailed examination of transcript data supporting target genes via the EnsEMBL and UCSC genome browsers. For these loci, final verification via manual annotation is prioritized depending on successful pipeline status. Since beginning the project, Washington University scientists have annotated 1500 genes, completed 600 manual designs, and validated 900 designs, with a further 2300 annotations, 1500 designs, and 4000 validations remaining. While the wet lab phase of KOMP is due to end in late 2011, we anticipate completion of the validation process one year later.

CHD5 FUNCTION IN THE BRAIN

Assaf Vestin, Wangzhi Li, Guy Horev, Alea A Mills

Cold Spring Harbor Laboratory, Cancer Center, Cold Spring Harbor, NY, 11724

CHD5 is a predicted chromatin remodeling protein of the SWI/SNF superfamily that is robustly expressed in neuronal tissues such as the brain. We previously identified CHD5 as a tumor suppressor mapping to human 1p36, a region of the genome that is frequently deleted in neuroblastoma. Although a number of chromatin remodeling proteins have been shown to modulate brain function, whether CHD5 performs a similar role is unknown. Here we investigate the hypothesis that CHD5 functions in the brain. We found that Chd5 is expressed in the nucleus starting at embryonic day 13, and maintains its expression postnatally and into adulthood. The expression of CHD5 starts during neuronal differentiation and is particularly robust in post-mitotic differentiated neurons. Generation of Chd5 deficient mice revealed that while homozygotes are viable, they have unique behavioral phenotypes such as compulsive-repetitive behavior. We are currently looking for altered neuronal pathways in the Chd5 deficient brain and to date we have determine that expression of components in serotonergic neurons is altered in Chd5 deficient mice. We are currently conducting genome-wide gene expression microarray analysis to thoroughly examine what other neuronal pathways are altered in the Chd5 deficient brain. These findings reveal a role for CHD5 in neuronal function *in vivo*.

DELIVERY OF ENDOSOMES TO LYSOSOMES VIA MICROAUTOPHAGY IN THE VISCERAL ENDODERM OF MOUSE EMBRYOS

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

[1]Division of Biological Sciences, Institute of Scientific and Industrial Research, Osaka University, Ibaraki, 567-0047, Japan, [2]Department of Biochemistry, Faculty of Pharmaceutical Sciences, Doshisha Womens College, Kyotanabe, 610-0395, Japan

The visceral endoderm, an epithelial tissue, appears at a very early stage of embryogenesis. This single cell-layered tissue sustains embryonic growth through nutrients/gas exchange, and participates in the axis determination of pregastrulation embryos by orchestrating various signaling cascades. Both nutritional and signaling functions rely upon the endocytosis of various molecules from the cell surface through the endocytic pathway. Here, we focused on the endocytic dynamics in the visceral endoderm of mouse embryos. The apical vacuoles, whose limiting membranes were positive for lamp2 and syntaxin-7, accumulated cathepsin B and immunoglobulins, indicating that apical vacuoles are equivalent to lysosomes.
The assembly of this large digestive compartment requires the function of phosphoinosityde 3-phosphate signaling, and the small GTP binding protein rab7. Loss of these functions resulted in severe alteration in the morphology, dysfunction of endocytic pathway and simultaneously, caused defective embryogenesis at the peri-gastrulation stages.
Delivery of endosomes to the apical vacuoles involves unique, microautophagy-like process. Fluorescent endocytic markers were internalized from the cell surface and delivered to endosomes, which were then engulfed by the apical vacuoles. The mixing of endosomal and lysosomal contents required lipase activity, indicating that the limiting membranes needed to be disintegrated during this process. These results revealed a unique membrane dynamics in the visceral endoderm, i. e., endosomes are delivered to lysosome-like apical vacuoles through a microautophagic process.

LINEAGE-TRACING OF BASAL EPITHELIAL CELLS DURING PROSTATE REGENERATION

Zhu A Wang, Michael M Shen

Columbia University Medical Center, Departments of Medicine and Genetics & Development, Herbert Irving Comprehensive Cancer Center, New York, NY, 10032

The mouse prostate gland can undergo serial rounds of regression and regeneration in response to deprivation and restoration of testicular androgens. Therefore, the prostate epithelium of castrated mice is likely to contain a resident pool of castration-resistant stem cells that is responsible for driving prostate regeneration. Previous literature has suggested that this stem cell pool is likely to reside within the basal epithelial layer, based on tissue reconstitution analyses in renal grafts. However, lineage-marking studies from our laboratory have identified a luminal stem cell population that we identified as castration-resistant Nkx3.1-expressing cells (CARNs) (X. Wang et al. (2009) Nature 461: 495-500). Therefore, we have been interested in using analogous lineage-marking approaches to explore the potential stem cell properties of prostate basal cells.

In our studies, we have utilized *CK5-Cre*^{ERT2} transgenic mice to perform lineage analysis by inducible marking of basal cells in the regressed prostate epithelium with YFP, followed by analysis of their behaviors during androgen-mediated prostate regeneration. Our preliminary results suggests that basal cells may not behave as stem cells that drive prostate regeneration *in vivo*, since they are extremely inefficient in generating luminal cells. Moreover, basal cells do not proliferate extensively during prostate regeneration relative to luminal cells. We will present our ongoing lineage-marking studies of basal cells in the hormonally-intact and regenerating prostate epithelium, and their possible implications for the cell of origin for prostate cancer.

TRANSPLANTATION OF REPROGRAMMED EMBRYONIC STEM CELLS IMPROVES VISUAL FUNCTION IN A MOUSE MODEL FOR RETINITIS PIGMENTOSA

Katherine J Wert[1,2], Nan-Kai Wang[1,2], Chyuan-Sheng Lin[1,2], Stephen H Tsang[1,2]

[1]Bernard & Shirlee Brown Glaucoma Laboratory, Department of Pathology & Cell Biology, New York, NY, 10032, [2]Edward S. Harkness Eye Institute, Columbia University, New York, NY, 10032

INTRODUCTION: Specialized retinal cells called the retinal pigment epithelium (RPE) maintain vision. Death of these cells lead to degeneration of the retina, vision loss, inability to perform daily tasks, and even depression in many individuals with retinitis pigmentosa (RP) and macular degenerations. Nearly 20% of Americans between the ages of 65 and 75 years are expected to experience RPE loss due to macular degenerations, and its incidence is expected to double by year 2020. A major obstacle to restoring vision by cell therapy is the availability of cadaver RPE donors for transplantation. Our central hypothesis is that RPE cells derived from embryonic stem (ES) cells can be used to successfully replace diseased counterparts.

METHODS: The mouse C57BL/6J-Tyr$^{c\text{-}2j}$/J (C2J) ES cells were labeled with a yellow fluorescent protein (YFP) and made into the RPE cells of the eye in culture. To prove that these were RPE-like cells, specific gene markers for the development of RPE were shown to be present in these cells. After becoming RPE-like cells, the cells were transplanted into the eyes of the retinitis pigmentosa mouse model at 5 days after birth of the mouse. Imaging of live mice after transplantation by looking for the yellow fluorescence determined that the cells survived the transplantation process. Electroretinogram tests that determine visual function were performed on the mice to evaluate their vision and see if it had been restored.

RESULTS: The ES cells that have become RPE-like cells expressed the correct markers for being RPE cells. After transplantation, the stem cells were imaged in the eyes of the mice for as long as 7 months. The mice that underwent the transplantation procedure showed a significant gain in vision over the 7-month period, while those that were injected with saline and other control groups did not show any restoration of visual function.

CONCLUSIONS: For the first time, congenic embryonic stem (ES) cells were successfully used to replace diseased retinal cells and restore nervous system function in a mouse model of retinitis pigmentosa; a strategy to treat millions of humans with age-related macular degeneration and other forms of retinal disease, such as retinitis pigmentosa.

MMP14 HAPLOINSUFFICIENCY IMPROVES BODY COMPOSITION IN MOUSE MODEL OF DIET-INDUCED OBESITY

Thaddeus J Wolfram, Sergey Filippov, Linyan He, Diane Nadeau, Sandra J Engle, Jeffrey L Stock

Pfizer, Inc., Genetically Modified Models, Groton, CT, 06340

Rising rates of obesity and the obesity-related conditions that make up metabolic syndrome are a major detriment to global health. As more and more work is done to better clarify and understand the complex combination of factors affecting obesity and metabolic syndrome, proteins are characterized, the elucidation of which may lead to the discovery of therapies for these conditions. Matrix metalloproteinases (MMPs) make up a family of more than 25 enzymes involved in the remodeling of the extracellular environment. In an effort to determine the relevance of MMP14 on body composition and metabolism as related to the development of obesity, we characterized the phenotype of C57BL/6 mice with a targeted mutation in *Mmp14* (*Mmp14*$^{+/-}$) compared with C57BL/6 wild-type control (*Mmp14*$^{+/+}$) mice. Heterozygous mice exhibited resistance to diet-induced weight-gain, demonstrated by comparatively lower percent body-fat, total tissue mass, and fat mass following a high fat diet. Additionally the heterozygous mice simultaneously demonstrated similar tissue area and percent lean mass to the wild-type mice, corresponding with an overall resistance to increase in body-mass composition. Therefore loss of one *Mmp14* allele is shown not to affect lean mass or tissue area, and thus due to a concurrent increase in fat we demonstrate here that heterozygous mice exhibit a change in overall body mass composition when fed a high fat diet.

TRANSCRIPTIONAL REGULATION OF C-MAF IN MOUSE EMBRYONIC LENS DEVELOPMENT

Qing Xie[1,2], Louise Wolf[1,2], Ying Yang[1,2], Ales Cvekl[1,2]

[1]Albert Einstein College of Medicine, Genetics, Bronx, NY, 10461, [2]Albert Einstein College of Medicine, Ophthalmology and Visual Sciences, Bronx, NY, 10461

Lens development has been an attractive model for studies of cell lineage specification and terminal differentiation. Lens fiber cell differentiation is characterized by high levels of expression and accumulation of water-soluble proteins, crystallins. Lens development is controlled by a relatively small group of transcription factors including AP-2α, c-Maf, Foxe3, Hsf4, Pax6, Prox1, Six3, Sox1 and Sox11. Studies of crystallin gene regulation in mouse revealed key roles of Pax6 and c-Maf. In addition, genetic and molecular biology data suggest cross-regulation between these genes. Herein, ChIP-on-chip studies in lens chromatin identified c-Maf locus as a potential direct target of Pax6. We have now identified several evolutionary conserved regions in c-Maf locus as potential regulatory regions. A 1.3 kb promoter was not sufficient for expression of the EGFP reporter in transgenic mouse lens. In contrast, a 1.6 kb 5'-upstream region, CR1 (conserved regions 1), in combination with the c-Maf promoter was capable to elicit expression of EGFP reporter in lens as early as E10.5. Using EMSAs, at least five Pax6-binding sites were identified in c-Maf locus. Interestingly, two c-Maf BAC transgenic reporter did not support the EGFP expression in developing lens, suggesting additional levels of complexity of gene regulation of c-Maf locus. The ongoing studies are aimed to identify additional enhancers and to determine their properties in regulation of c-Maf expression in lens development. Finally, we are also testing a hypothesis that FGF signaling directly regulates expression of c-Maf during the onset of primary lens fiber cell differentiation.

MOUSE PHENOTYPES AND HUMAN DISEASE MODELS: THE MOUSE GENOME INFORMATICS (MGI) RESOURCE

Jingxia Xu, Howard Dene, Anna Anagnostopoulos, Randy Babiuk, Susan M Bello, Jason Bubier, Donna L Burkart, Michelle N Knowlton, Hiroaki Onda, Beverly Richards-Smith, Cynthia L Smith, Monika Tomczuk, Linda L Washburn, Janan T Eppig

The Jackson Laboratory, Mouse Genome Informatics, Bar Harbor, ME, 04609

The Mouse Genome Informatics Resource (http://www.informatics.jax.org/) provides integrated genetic, genomic and phenotypic information about the laboratory mouse to facilitate the use of mouse models for studying human disease. Users can navigate the web site using query and browsing methods or access data using our web services and batch querying tools. MGI phenotypic data describe effects of spontaneous, induced, and genetically engineered mutations (targeted knockouts and conditionals, transgenics, Cre-constructs, targeted reporters, gene traps, transposon-induced mutations, etc.) in the context of their strain-specific genetic background. Over 189,000 phenotype annotations have been made using the Mammalian Phenotype Ontology to describe genotypes carrying over 36,100 different mutant alleles in nearly 13,000 genes. More than 3,100 mouse genotypes are annotated as models for over 1,000 human diseases found in OMIM (Online Mendelian Inheritance in Man, http://www.ncbi.nlm.nih.gov/omim). Allele and phenotype data are integrated in MGI with data on gene characteristics, expression, function (GO), genomic sequences, and strain characteristics, including SNPs and trait polymorphisms (QTL). Sophisticated query functions take advantage of this high level of integration to permit users to (1) locate mutants in a particular gene and identify any associated disease models, (2) find mutations in all genes that show a specific phenotype, (3) locate genotypes that show multiple phenotypes or multiple diseases, (4) locate relevant mouse models for a specific human disease/syndrome, and (5) find mutant alleles and phenotypes in a specific genome region of interest.

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INVOLVEMENT OF THE TUMOR SUPPRESSOR GENE *RECK* IN LIMB PATTERNING

Mako Yamamoto[1], Tomoko Matsuzaki[1], Rei Takahashi[2], Eijirou Adachi[3], Hiroshi Kiyonari[4], Naoko Oshima[4], Sachiyo Yamaguchi[1], Hitoshi Kitayama[1], Yoko Morioka[1], Shigeyoshi Itohara[5], Takashi Nakamura[6], Haruhiko Akiyama[6], Makoto Noda[1]

[1]Kyoto University Graduate School of Medicine, Department of Molecular Oncology, Kyoto, 606-8501, Japan, [2]Faculty of Pharmaceutical Sciences, Doshisha Women's College of Liberal Arts, Department of Pharmacotherapeutics, Kyotanabe, Kyoto, 610-0395, Japan, [3]Kitasato University Graduate School of Medical Science, Department of Matrix Biology and Regenerative Medicine, Sagamihara, Kanagawa, 252-0373, Japan, [4]RIKEN Center for Developmental Biology, Laboratory for Animal Resources and Genetic Engineering, Kobe, Hyogo, 650-0047, Japan, [5]RIKEN Brain Science Institute, Laboratory for Behavioral Genetics, Wako, Saitama, 351-0198, Japan, [6]Kyoto University Graduate School of Medicine, Department of Orthopaedic and Musculoskeletal Surgery, Kyoto, 606-8501, Japan

The membrane-anchored metalloprotease regulator RECK is down-regulated in various types of human cancers, and forced expression of RECK in cancer cells results in suppression of tumor angiogenesis, invasion, and metastasis, suggesting its role as a tumor suppressor. On the other hand, *Reck*-deficient ($Reck^{-/-}$) mice die around mid-gestation with multiple defects, including arrested vascular development [1] and precocious neuronal differentiation [2], suggesting its importance in mammalian development. In the present study, we analyzed the phenotype of hypomorphic and conditional *Reck* mutant mice to gain more insights into the mechanisms of its actions during mammalian development. The hypomorphic *Reck* mutant mice ($Reck^{low/-}$) are largely intact, but they show some limb abnormalities including cutaneous horns (i.e., hyperkeratosis) on the dorsal side of extremities, round and porous fingertip bones, and the loss of skeletal elements on the posterior side of forelimbs. Most of these abnormalities could be attributed to the reduced *Reck* expression in limb mesenchyme, since the tissue-specific knockout ($Reck^{flox/-}$*; PrxI-Cre*) mice recapitulate the phenotype. Whole-mount *in situ* hybridization indicated the initial formation and subsequent attenuation of the Shh-Fgf positive feed-back loop in the mutant mice, implicating *Reck* in the maintenance of the zone of polarizing activity (ZPA) required for proper anterior-posterior limb patterning.

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AURORA A KINASE (AURA) IS ESSENTIAL FOR EPIBLAST GROWTH AND SURVIVAL

Yeonsoo Yoon[1], Dale O Cowley[2], Terry A Van Dyke[3], Jaime A Rivera-Perez[1]

[1]University of Massachusetts, Department of Cell Biology, Worcester, MA, 01655, [2]TransViragen, Inc, Research Triangle Park, NC, 27709, [3]National Cancer Institute, Mouse Cancer Genetics Program, Frederick, MD, 21702

Aurora A (AurA) is a member of the mitotic serine/threonine kinase family, involved in centrosome maturation and spindle assembly during cell division cycle. Mutations of *AurA* are known to result in mitotic arrest due to defects in centrosome separation and chromosome segregation. A recent report showed that a knockout of *AurA* leads to embryonic lethality at the blastocyst stage with cell proliferation failure, mitotic arrest, and monopolar spindle formation. To examine the function of AurA in post-implantation mouse embryos, we generated epiblast-specific *AurA* knockout embryos using *Sox2Cre*-mediated genetic recombination. We observed that an epiblast-specific *AurA* knockout causes epiblast growth inhibition and epiblast-specific cellular death through apoptosis in gastrula stage embryos and leads to resorption of embryos at around E9.5. We also found that despite a reduction in the size of the epiblast at E6.5 an epiblast-specific *AurA* knockout does not affect the axial polarity of mutant embryos, which showed normal anterior visceral endoderm (AVE) movement and expression of *Brachyury*, a primitive streak marker in the posterior region. The fact that epiblast cells are ablated in epiblast-specific *AurA* knockout embryos demonstrates that AurA is essential for epiblast survival and further embryogenesis and provides an alternative way to induce tissue-specific cellular ablation.

ECTOPIC EXPRESSION OF INTERFERON-GAMMA IN METANEPHRIC MESENCHYME RETARDS KIDNEY DEVELOPMENT

Kangsun Yun[1], Arthur A Hurwitz[2], Alan O Perantoni[1]

[1]National Cancer Institute/NIH, Cancer and Developmental Biology Laboratory, Frederick, MD, 21702, [2]National Cancer Institute/NIH, Laboratory of Molecular Immunoregulation, Frederick, MD, 21702

Wilms tumor (WT) is one of the most common pediatric solid cancers and is believed to arise from the abnormal proliferation and differentiation of the metanephric blastema in the developing kidney. We have shown previously that activation of STAT1 contributes to the pathogenesis of Wilms tumor and induces proliferation while inhibiting differentiation of renal progenitor cells. However, the role of STAT1 in maintaining the undifferentiated state of metanephric mesenchymal progenitor cells remains unclear. Here we present that STAT1 activation through conditional ectopic expression of interferon (IFN)-gamma in metanephric mesenchymal progenitor cells results in renal hypoplasia in the developing mouse embryo. The kidney size of mutant embryos was reduced to about half that of normal control embryos despite comparable rates of proliferation and apoptosis. Kidney development was retarded showing slower outgrowth of the bud and reduced branching in IFN-gamma knock-in embryos. There was no great difference in the expression of molecules such as Six1, Eya1, Pax2, and Pax8, which are involved in early kidney development,while the expression domain for nephron progenitor marker Six2 appeared to be reduced. These data are consistent with our explant culture studies and suggest that in vivo STAT1 activation also inhibits the differentiation of normal renal progenitor cells and thus causes retardation of kidney development.

TRANSCRIPTIONAL INTERACTOMES AND CHROMATIN ORGANIZATION IN MOUSE GENOME

Yubo Zhang, Chia-Lin Wei

Genome Institute of Singapore, Genome Technology & Biology, Singapore, 138672, Singapore

The spatial organization of chromatin in the nucleus is critical for the regulation of gene expression. Distant regulatory elements have been shown to influence gene function via long-range chromatin interactions. However, the global genomic architecture directly associated with transcription network has not yet been revealed. In this study, we depict the dynamic transcription interactomes mediated by RNA polymerase II with the newly developed Chromatin Interaction Analysis by Pair End diTag (ChIA-PET) to define the chromatin organizations pertinent to transcription regulation and epigenetic control in various mouse cells of progressing defined developmental lineages. In ChIA-PET analysis, tethered DNA by factor specific immunoprecipitated chromatins were joined in together by linker ligation. The ligated DNA was analyzed by pair end ditag based sequencing to reveal the long range interactions. With nearly saturated PET sequencing, thousands of cis- and trans- interactions tethered by RNA Pol II are defined and modes of transcription coordinated regulation are revealed. Integrated with global transcription expression changes, promoter activities, gene activities and major epigenomic features, spatial distribution and temporal regulation of transcription networks are defined. Such transcriptional inteactomes in the context of cells of differential developmental potential offer insights on how genes coordinate function as a cluster and uncover the developmental architecture that establishes and maintains the nucleus function of mammalian genomes.

CONSTRUCTION OF A MOUSE EPIBLAST STEM CELL INTERACTOME

Hui Zhao[1,2,3], Mariano Alvarez[1,4,5], Celine Lefebvre[1,4,5], Andrea Califano[1,4,5], Michael Shen[1,2,3]

[1]Columbia University Medical Center, Herbert Irving Comprehensive Cancer Center, New York, NY, 10032, [2]Columbia University Medical Center, Department of Medicine, New York, NY, 10032, [3]Columbia University Medical Center, Department of Genetics and Development, New York, NY, 10032, [4]Columbia University Medical Center, Joint Centers for Systems Biology, New York, NY, 10032, [5]Columbia University Medical Center, Department of Biomedical Informatics, New York, NY, 10032

The molecular mechanisms that govern embryo stem cell pluripotency, self-renewal, and lineage-specific differentiation have only been partially elucidated. Mouse Epiblast Stem Cells (EpiSC) are derived from the early post-implantation embryo and share many similarities with human embryonic stem cells. In our studies, we are seeking to identify novel master regulators of EpiSC pluripotency and self-renewal, which may also provide novel insights into induced pluripotency and the reprogramming process. For this purpose, we are constructing the first genome-wide molecular interaction map (interactome) for mouse EpiSC, to identify the regulatory relationships among gene products.

To assemble a mouse EpiSC interactome, we are using novel bioinformatics algorithms, applying them to expression profiling data gathered from EpiSC undergoing a wide range of differentiation events. Direct interactions among gene products, both transcriptional and post-translational, will be inferred *de novo* using experimentally validated reverse-engineering algorithms, such as ARACNe and MINDy. This makes the approach unbiased, ensuring the fidelity of the interactomes constructed. Master regulators will be inferred by interrogating the interactome using the MARINa algorithm. Our preliminary data shows that whole-genome gene profiling accurately depicts the differential regulatory responses to different chemical inducers of differentiation. We will report on our progress towards the assembly of a draft EpiSC interactome.

GENETIC ANALYSIS OF SIGNALING PATHWAYS CONTROLLING MOTOR NEURON CONNECTIVITY

Samuel L Pfaff, Ge Bai

The Salk Institute for Biological Studies, Howard Hughes Medical Institute, 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.

TRANSCRIPTIONAL CONTROL OF *HOX* GENES IN THE DEVELOPING SPINAL CORD - A KEY TO MOTONEURON ORGANIZATION?

Patrick Tschopp[1,2], Nadine Fraudeau[2], Alix J Christen[1,3], Denis Duboule[1,2,3]

[1]National Center of Competence in Research, NCCR Frontiers in Genetics, Geneva, CH-1211, Switzerland, [2]University of Geneva, Department of Zoology and Animal Biology, Geneva, CH-1211, Switzerland, [3]EPFL, School of Life Sciences, Lausanne, CH-1015, Switzerland

The importance of *Hox* genes in specifying the diverse neuronal fates in the spinal cord has long been recognized. Yet the nature of the transcriptional controls that generate the partially overlapping expression domains required for these specifications remain largely unknown. Here, we report that the correspondence between the physical order of *Hoxd* genes and their rostral limits of expression (spatial colinearity), while respected in the developing spinal cord, does not display a fully progressive, staggered distribution. Instead, two major anterior-posterior boundaries are detected, coinciding with the functional sub-divison of the spinal cord at the positions of the brachial and the lumbo-sacral plexii. Interestingly, tiling array-based transcriptome analysis reveals that these two domains of *Hoxd* gene expression are regulated as part of two distinct blocks of transcription, established independently from one another. Whenever targeted deletions abolish the boundary between the two transcriptional blocks *in vivo*, posterior genes are mis-regulated and become expressed ectopically, at the expense of anterior, non-coding transcripts. We further evaluated the regulatory potential of each gene locus by constructing a series of lacZ-reporter transgenes covering the entire *HoxD* complex. The transcriptional profile of these transgenes is now being investigated on tiling arrays, by crossing them over the respective gene-specific deletions.

DELETION OF GENES AT 16P11.2 CAUSES AUTISM-LIKE PHENOTYPES IN MICE

Guy Horev[1], Jacob Ellegood[2], Ellen Son[1], Julian Taranda[1], Lakshmi Muthuswamy[1,3], Pavel Osten[1], Mark Henkelman[2], Michael Wigler[1], Alea A Mills[1]

[1]Cold Spring Harbor Lab, Cancer Center, Cold Spring Harbor, NY, 11724, [2]Mouse Imaging Centre, Toronto Centre for Phenogenomics, Toronto, Ontario, M5T 3H7, Canada, [3]Ontario Institute for Cancer Research, Cancer Research, Toronto, Ontario, M5G 1L7, Canada

Autism is a neurocognitive disorder characterized by clinical features including repetitive behaviors, restricted interests, language impairment, and altered social interactions. Although chromosome rearrangements affecting specific genomic intervals have been found in patients with autism, the basis for this syndrome is unknown. Here we used chromosome engineering to generate a mouse model with deletion of the region of the genome corresponding to 16p11.2—a genomic lesion associated with autism in humans—as well as a model harboring a duplication of this region. Mice with altered dosage of this 34-gene region have unique phenotypes, with mice heterozygous for its deletion having neuroanatomical, physiological, and behavioral phenotypes reminiscent of clinical features of autism. This provides the first functional evidence that compromised dosage of 16p11.2 is causal in autism, providing new insight into this syndrome.

GENETIC MOSAIC ANALYSIS REVEALS A CENTRAL ROLE OF OLIGODENDROCYTE PRECURSOR CELLS IN GLIOMAGENESIS

Chong Liu[1], Jonathan C Sage[1], Michael R Miller[1], Roel G Verhaak[2], Simon Hippenmeyer[3], Hannes Vogel[4], Oded Foreman[5], Liqun Luo[3], Hui Zong[1]

[1]University of Oregon, Institute of Molecular Biology, Eugene, OR, 97403, [2]MIT and Harvard University, Broad Institute, Cambridge, MA, 02141, [3]Stanford University, HHMI and Dept of Biology, Stanford, CA, 94305, [4]Stanford University, Dept of Neuropathology, Stanford, CA, 94305, [5]The Jackson Laboratory, Pathology Service, Sacramento, CA, 95838

Cancer is a disease of genetic mosaicism. “Mosaicism” as a genetic term describes the phenomenon that cells within a multi-cellular organism harbor distinct genetic makeups. In the case of cancer, it refers to distinct genotypes in cancerous cells from normal ones in the same individual. Cancerous mosaicism in human is often initiated by the loss of tumor suppressor genes in as few as a single cell, which then evolves through further gene mutations and interactions with its stromal niche to develop into malignant tumors.

To study the cancerous process in vivo, a genetic mosaic animal model should be ideal. Previously, Drosophila geneticists have developed a powerful mosaic system to address these problems. Many elegant and otherwise unachievable studies have been carried out with the mosaic system, such as mutant-WT cell competition behavior, recessive genetic screening for tumor suppressor genes or metastasis-promoting genes. Based on a similar design principle to the Drosophila system, we established a mouse genetic mosaic system termed MADM (Mosaic Analysis with Double Markers) [Zong et al 2005 Cell]. Through Cre-loxP mediated inter-chromosomal mitotic recombination, the MADM system can generate homozygous mutant cells that are unequivocally labeled by green fluorescent protein (GFP) and their sibling WT cells labeled by red fluorescent protein (RFP) within a heterozygous colorless mouse. Due to the low probability of the recombination between chromosomes, mutant cells generated by MADM are very sparse (0.1-1% or much lower), closely mimicking clonal initiation of human tumors. With the permanent GFP-labeling, progeny of mutant cells can be studied along each and every lineages for their tumorigenic potentials. Last but not least, the RFP-labeled WT sibling cells provide an excellent internal control for in vivo phenotypic analysis. Here I will present our recent work in glioma modeling with the MADM system, which revealed the involvement of a specific glial cell lineage in gliomagenesis. Finally, it is important to point out that MADM can be applied to study other diseases and normal development for in-depth mechanistic studies.

TARGETOME ANALYSIS REVEALS THE CENTRAL ROLE OF THE TRANSCRIPTION FACTOR ATOH1 IN CEREBELLAR GRANULE PRECURSOR PROLIFERATION AND MEDULLOBLASTOMA FORMATION

Adriano Flora[1], Tiemo J Klisch[1,2], Wei Li[3], Huda Y Zoghbi[1,2]

[1]Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, TX, 77030, [2]Baylor College of Medicine, Howard Hughes Medical Institute, Houston, TX, 77030, [3]Baylor College of Medicine, Department of Molecular and Cellular Biology, Houston, TX, 77030

Many medulloblastomas, the most frequent malignant brain tumors in children, originate from cerebellar granule neuron precursors (GNPs) following mutations in genes that normally regulate pathways involved in neuronal differentiation. One of these pathways is regulated by sonic hedghehog (Shh), a secreted molecule that controls many processes in the developing embryos. In the post-natal cerebellum, Shh stimulates the proliferation of GNPs and inhibits neuronal differentiation. Gain-of function mutations in genes that code for proteins involved in Shh signaling result in a dramatic increase in the proliferation of GNPs and can lead to medulloblastoma formation both in humans and in mice. Due to its central role normal development and in tumorigenesis, it is crucial to understand how the Shh signaling is regulated at the molecular level.
We have recently described that the transcription factor Atonal homolog 1 (Atoh1) regulates the expression of Gli2, the main transcriptional effector of the Shh signaling pathway. Atoh1 is thus required for the transduction of the signaling of Shh and its deletion is sufficient to prevent the formation of Shh-induced medulloblastomas. We have now extended our studies to identify the target genes of Atoh1 in the developing cerebellum. Using a combination of transcriptional profiling and *in vivo* molecular biology, we generated an extensive list of genes that are directly regulated by Atoh1. Strikingly, we observed that Atoh1 activates the expression of numerous genes that are involved in the Shh signaling pathway, suggesting that Atoh1 plays a role of "master regulator" in the control of GNP proliferation.
Our data place Atoh1 at the core of cell proliferation regulation in the developing cerebellum making it an attractive target for the development of therapies aimed at the inhibition of malignant cell proliferation in medulloblastomas.

CONSTRUCTION OF COMPLEX BRAIN CIRCUITS: LESSONS FROM THE ENGRAILED HOMEOBOX GENES

Roy Sillitoe[1], Anamaria Sudarov[1,3], Yulan Cheng[1], Alexandra Joyner[1]

[1]Sloan-Kettering Institute, Developmental Biology Program, New York, NY, 10021
[2]Present address: Albert Einstein College of Medicine, Bronx, NY.
[3]Present address: Weill Medical College of Cornell University, New York.

The neurons that comprise each neural circuit have a specific spatial, or topographical organization that is optimized both for the functioning of individual circuits, as well as to allow diverse circuits to interact with precision. How neurons become organized in 3D during development is a critical unanswered question. The cerebellum, which coordinates fine motor control, is an excellent system for studying this question, as neurons that carry out related functions project afferent axons into the cerebellum to distinct positions along the anterior-posterior (AP) and medial-lateral (ML) axes. Furthermore, the distribution of afferents reflects the intrinsic organization of the cerebellum along the AP axis into morphological lobules and in the ML axis into stripes of gene expression. The Engrailed (En) homeobox transcription factors have provided a genetic entry point for studying the relationship between intrinsic patterns within the cerebellum and afferent organization. By analyzing a series of temporal and cell type specific conditional *En1/2* mutants we uncovered that the two genes play key roles in patterning striped gene expression and foliation, as well as the precise targeting of afferents to distinct lobules and their subsequent resolution into discrete medial-lateral bands. Moreover, each *En* gene coordinately regulates afferent targeting and patterning of gene expression independent of regulating foliation. En1/2 are therefore master regulators of 3D organization of the cerebellum. Furthermore, construction of complex neural circuits involves transcription factors acting at a pivotal point to direct both path finding of afferent projections and developmental processes that generate the correct numbers of each cell type within the target field.

THE HISTONE DEMETHYLASE LSD1/KDM1 REGULATES STEM CELL FATE BY REPROGRAMMING EPIGENETIC MEMORY

David J Katz[1], William G Kelly[2]

[1]Emory University, Cell Biology, Atlanta, GA, 30322, [2]Emory University, Biology, Atlanta, GA, 30322

Extensive epigenetic reprogramming occurs during cloning and in the induction of pluripotent stem cells (IPS). Similarly, during normal germline transmission, the epigenetic state of the genome is known to undergo extensive reprogramming both in the gametes and in the early embryo. This reprogramming may be essential to re-establish a developmental ground state between generations, but the mechanism of these epigenetic reprogramming events remains poorly understood. We show that mutants in *spr-5*, the C. elegans ortholog of the histone demethylase LSD1/KDM1 exhibit progressive sterility over many generations due to the transgenerational accumulation of the histone modification dimethylation of histone H3 on lysine 4 (H3K4me2). This suggests that H3K4me2 can serve as a stable epigenetic memory and that erasure of H3K4me2 by KDM1 in the germline prevents the inappropriate transmission of this epigenetic memory from one generation to the next. To pursue this role further, we generated mice with germline mutations in KDM1. Progeny from mice that lack maternal KDM1 exhibit significant embryonic and perinatal lethality. These progeny are genotypically normal, suggesting that the defects are due to the stable transmission of inappropriate histone methylation in the embryo. Furthermore, deletion of KDM1 in the mouse testis results in sterility, with loss of the testis stem cell population. These data suggest that KDM1 may be required both to maintain stem cell populations, as well as to restore totipotency after fertilization. In addition, KDM1 demethylation may be required for the somatic reprogramming that is induced during the generation of IPS cells.

EPIGENETIC REGULATION OF STEM CELL DIFFERENTIATION IN TESTES

Takayuki Shirakawa[1], Ruken Yaman-Deveci[2], Yoshito Kamizato[1], Jafar Sharif[2], Masahiro Muto[2], Shosei Yoshida[3], Kiyoe Ura[4], Haruhiko Koseki[2], Kazuyuki Ohbo[1]

[1]Yokohama City University, School of Medicine, Department of Histology and Cell Biology, Yokohama, 236-0004, Japan, [2]RIKEN Research Center for Allergy and Immunology, Developmental Genetics group, Yokohama, 230-0045, Japan, [3]National Institute for Bisic Biology, Division of Germ Cell Biology, Okazaki, 444-8585, Japan, [4]Graduate School of Medicine, Osaka University, Department of Molecular Therapeutics, Osaka, 123-8585, Japan

To preserve homeostasis during entire life, stem cells resist premature differentiation and senescence, and maintain self-renewing cycle. The stemness is regulated by two different mechanisms, cell-intrinsic regulation and exogenous signals from microenvironments. In order to gain insights into the cell-intrinsic mechanism, we focused on analysis of epigenetic properties of adult tissue-specific stem cells in testes. Testicular germ cells connect each other after cell division so that the number of chains reflects their cell division history. We initially expected the existence of A single (As)-spermatogonia specific chromatin modification, since it is widely accepted that only As-spermatogonia acts as a stem cell and produces progenies called A paired (Apr) and A aligned (Aal) spermatogonia that are believed to be no longer capable of self-renewal. In this study, we found that spermatogonia from As to Aal-8 showed homogenous epigenetic profiles, lacking the expression of de novo DNA methyltransferases with global low cytosine methylation levels and the repressive histone modification, H3K9me2. These major repressive modifications were simultaneously integrated at the stage of Aal-16 spermatogonia when the cells started expressing Kit and completely lost stem cell activity. The artificial expression of Dnmt3b in Kit-negative spermatogonia induced the expression of progenitor marker, Kit, suggesting that premature differentiation might initiate. On the contrary, NP95-deficiency testes showed sever differentiation defects in testes at the transition from Kit-negative to Kit-positive spermatogonia, suggesting that de novo DNA methylation is crucial for the transition to progenitor cells.

GENIC TRANSCRIPTION PRECEDES LOSS OF XIST COATING AND DEPLETION OF H3K27ME3 DURING X-CHROMOSOME REACTIVATION IN THE MOUSE INNER CELL MASS

Lucy A Williams[1], Sundeep Kalantry[2], Joshua Starmer[1], Terry R Magnuson[1]

[1]University of North Carolina, Department of Genetics, Chapel Hill, NC, 27599-7264, [2]University of Michigan, Department of Human Genetics, Ann Arbor, MI, 48109-5618

Repression of Xist RNA expression is considered a prerequisite to reversing X-chromosome inactivation (XCI) in the mouse inner cell mass (ICM), and reactivation of X-linked genes is thought to follow loss of Xist RNA coating and heterochromatic markers of inactivation, such as H3K27me3. We analyzed X-chromosome activity in developing ICMs and show reactivation of gene expression from the inactive-X initiates in the presence of Xist coating and H3K27me3. Furthermore, forced upregulation of NANOG results in depletion of Xist RNA coating, yet extinction of the Xist coat does not lead to altered reactivation kinetics. Taken together, our observations suggest that X-linked gene transcription and Xist coating are uncoupled. These data fundamentally alter our perception of the reactivation process and support the existence of silencing mechanisms in the ICM that operate independent of Xist and H3K27me3.

ABERRANT STEM CELL REGULATION & DIFFERENTIATION IN DEER MOUSE (*PEROMYSCUS*) HYBRID EXTRA-EMBRYONIC DEVELOPMENT

Paul B Vrana[1], Amanda R Duselis[2]

[1]University of South Carolina, Biological Sciences & Peromyscus Genetic Stock Center, Columbia, SC, 29208, [2]University of California Irvine, Biological Chemistry, Irvine, CA, 92799

Crosses between two North American deer mouse species, *Peromyscus maniculatus* (BW), and *P. polionotus* (PO), produce dramatic parent-of-origin effects on growth and development. BW females mated to PO males (bw x po) results in growth-retarded offspring but apparently healthy offspring. In contrast, PO females mated to BW males (PO x BW) produce overgrown but severely dysmorphic conceptuses. The rare PO x BW litters which reach parturition harbor fatal defects (e.g. hemorrhaging) and/or are unable to pass through the birth canal, resulting in maternal lethality. The placenta is particularly affected in both cases; ~ 10% of PO x BW conceptuses consist only of extra-embryonic tissues. The PO x BW conceptuses share similarities with gestational trophoblast disease and other developmental disorders. BrdU and TUNEL studies show that both proliferation and cell death are altered. Expression of imprinted genes and DNA methylation at associated regulatory regions is perturbed in the PO x BW hybrids. To understand the developmental etiology of the placental phenotypes, we undertook further *in situ* hybridization and gene expression studies. As suggested by morphology, marker assays show a great reduction in the spongiotrophoblast of bw x po placentas. In contrast, the hyperplastic PO x BW placentas are disorganized: markers such as *Tpbp1* and *Cdkn1c* suggest intermingled portions of labyrinth and spongiotrophoblast. Giant cells are thought to derive directly from trophoblast stem cells (TSCs); they are present in at less than expected numbers in PO x BW placentas. However, certain PO x BW cell clusters did not stain with *Tpbp1*, *Cdkn1c*, or *Prl3b1/Pl2*, a marker of mature giant cells. These cells were positive for *Hand1*, thought to determine giant cell fate. Despite the presence of *Hand1*, these cells appeared diploid. Expression of *Cdx2*, a primary TSC marker, drops over time in PO x BW placentas (relative to parental strains and bw x po hybrids). At the same time, the PO x BW placentas re-express *Pou5f1/Oct4* at high levels. Thus both differentiation and stem cell numbers and/or maintenance appear compromised. As *Pou5f1/Oct4* is also regulated by DNA methylation, we hypothesize an epigenetic de-differentiation occurs in these PO x BW tissues.

MOUSE AND ZEBRAFISH HOXA3 ORTHOLOGS HAVE NON-EQUIVALENT IN VIVO PROTEIN FUNCTION DURING MOUSE EMBRYOGENESIS

Nancy R Manley[1], Lizhen Chen[1], Peng Zhao[2,3], Lance Wells[2,3,4], Chris T Amemiya[5,6], Brian G Condie[1]

[1]University of Georgia, Genetics, Athens, GA, 30602, [2]University of Georgia, Center for Complex Carbohydrate Research, Athens, GA, 30602, [3]University of Georgia, Biochemistry and Molecular Biology, Athens, GA, 30602, [4]University of Georgia, Chemistry, Athens, GA, 30602, [5]Virginia Mason, Benaroya Research Insititute, Seattle, WA, 98101, [6]University of Washington, Biology, Seattle, WA, 98195

Hox genes play evolutionarily conserved roles in specifying axial position during metazoan development and are widely implicated in the evolution of body plans. The Hoxa3 null mutant mouse was among the first made using gene targeting, generated nearly 20 years ago. While the Hoxa3 null phenotype has been well described, little is known about the mechanisms by which Hoxa3 (or any other Hox protein) influences development, including the functions of domains outside of the highly conserved homeodomain and cofactor-binding hexapeptide. Many studies have emphasized conservation of Hox function both between species and among paralogs within species, with changes in gene expression being proposed as the major route by which Hox genes influence morphological evolution. We performed a cross-species Hoxa3 gene swap to test whether the functional equivalence of paralogous Hox3 genes extends across vertebrate species, substituting the coding sequence of mouse HOXA3 with that of its zebrafish ortholog Hoxa3a. Zebrafish hoxa3a (zfhoxa3a) expressed from the mouse Hoxa3 locus can substitute for mouse Hoxa3 in some tissues, but has novel or null phenotypes in others. We further show using an allele encoding a chimeric protein that this difference maps primarily to the zfhoxa3a C-terminal domain (CTD). Molecular evolutionary analysis also shows that zebrafish Hoxa3a has undergone rapid evolution relative to other vertebrates. These data are consistent with our functional data, and together suggest that the mouse and zebrafish proteins have diverged considerably since their last common ancestor, and that the major difference between them resides in the CTD. Our data further show that Hox protein function can evolve independently in different cell types or for specific functions, suggesting a mechanism by which 'toolkit' transcription factors can evolve new functions. Our results also support the hypothesis that functional differences between these Hoxa3 proteins are due to divergence in non-homeodomain sequences, specifically in the C-terminal domain. Our data further demonstrate that cross-species gene swaps of highly conserved proteins can identify protein domains that mediate functional divergence between orthologous genes.

CHROMATIN AND TRANSCRIPTIONAL SIGNATURES FOR THE SMAD2/3 COMPLEX

Se Jin Yoon, Andrea Wills, Si Wan KIm, Edward Chuong, Julie Baker

Stanford University, Department of Genetics, Stanford, CA, 94305

Uncovering the network that mediates NODAL signaling is critical toward understanding both self-renewal and commitment to endoderm. To elucidate the NODAL transcriptional network in embryonic stem cells and derived endoderm, we elucidated the genomic targets for SMAD2/3, SMAD3, SMAD4, and FOXH1. We demonstrate that targets for these transcription factors are highly dynamic between the two cell types and are concentrated around promoters in ES cells, but not derived endoderm. Furthermore, using motif analysis, we find that 30% of all SMAD2/3, SMAD4, and FOXH1 binding genome-wide associates with a novel motif (CAGCAGG). We find that this motif is an E-box HLH motif known to associate with the TCF family of proteins (TCF3, 4 and 12; previously E2A, HEB and E2.2). Further, we validate that TCF proteins, SMAD proteins and FOXH1 bind to this CAGCAGG consensus sequence and that FoxH1 interacts with TCF3. Morpholino knock down of TCF3 in Xenopus embryos inhibits gastrulation by inhibiting mesendodermal specification. Together this suggests a role for TCF as a Nodal co-factor.

To elucidate how the SMAD complex functions together with the chromatin state, we performed genome wide mapping of H3K4me3 and H3K27me3 in both ES cells and derived endoderm. Interestingly, we find that bivalent regions, containing both H3K4me3 and H3K27me3, increase substantially during endodermal differentiation, making promoters in endodermal cells extensively bivalent. A few ES cell bivalent promoters do indeed become monovalent in endoderm, having only H3K4me3. These monovalent promoters are both significantly associated with SMAD2/3 binding, are highly transcriptionally active, and are enriched for known endodermal specification genes, including *FOXA2, EOMES, SOX17, GATA4, GATA6* and *GSC* The correlation between SMAD2/3 binding, monovalent formation and transcriptional activation suggests that SMAD2/3 plays an important role in bivalent resolution within regions critical for endodermal specification

USING FORWARD GENETICS TO ADVANCE OUR UNDERSTANDING OF MOUSE LIMB DEVELOPMENT.

Kasey Basch, Emily Mis, Sunjin Lee, Scott Weatherbee

Yale University, Genetics, New Haven, CT, 06510

Despite our knowledge of signaling centers and genes controlling patterning along the primary axes in the developing limb, there are gaps in our understanding of how a limb is built. In an effort to broaden our insight into the genetic mechanisms underlying limb formation and patterning, our lab is currently focused on identifying new genes required during embryonic limb development. Work by multiple labs have verified that forward genetics screens are feasible in the mouse and are an excellent, unbiased method to uncover factors critical for a wide variety of developmental processes. We are currently working with several novel, recessive mouse mutants derived from chemical-based mutagenesis screens. One difficulty of forward genetics in mice has been the time required to identify the affected locus through standard meiotic mapping. However, advances in sequencing technology are streamlining mutant identification, making forward genetics more tractable. We have utilized a genome reduction approach (Sequence Capture; Nimblegen), coupled with massively parallel sequencing (Illumina) to identify the affected gene in three of our mutants. These mutants have led to insights on 1) the role of cilia in limb patterning, 2) the Shh-Gre-Fgf feedback loop and 3) the role of proteoglycans in limb outgrowth.

CONTROL OF ONCOMIR MIR-155 BY TUMOR SUPPRESSOR BRCA1 AND ITS IMPACT ON TUMORIGENESIS

Suhwan K Chang, Shyam K Sharan

National Cancer Institute, NIH, Mouse Cancer Genetics Program, CCR., Frederick, MD, 21702

Breast cancer is the most common malignancy in women. To date, inheritance of a mutant BRCA1 or BRCA2 gene is the best-established indicator of an increased risk of developing breast cancer, the most frequently diagnosed cancer in women. For BRCA1/2 mutation carriers the lifetime risk of developing breast cancer is up to 80%. Therefore, it is not surprising that individuals with a family history of these cancers are now opting to know if they have a mutation in one of these genes. One of the inherent drawbacks of sequencing-based approaches is the determination of the actual risk associated with any variant identified in the gene. This is evident by the fact that more than 800 variants of BRCA1 and 1100 variants of BRCA2 are listed as variants of unknown clinical significance in the breast cancer information core database. We have recently reported a mouse embryonic stem cell based assay to test the functional significance of variants of unknown clinical significance identified in BRCA1 and BRCA2. The assay is based on the ability of human BRCA1/2 to complement the loss of endogenous genes in mouse embryonic stem cells. The procedure involves generation of a desired mutation in BRCA1 or BRCA2 present in a bacterial artificial chromosome (BAC) and introduction of the BAC into ES cells engineered for the assay. Using this assay we have characterized R1699Q, a low risk variant of BRCA1. Interestingly this variant affects ES cell survival but exhibits no defect in genomic stability or cell cycle regulation. We have used this variant to uncover the role of BRCA1 in regulation of an oncogenic micro RNA, miR-155. We show that over expression of this micro RNA contributes to tumorigenesis.

DEVELOPMENTAL STAGE-SPECIFIC BINDING OF HNF4A AND FOXA2 REVEALED BY CHIP-SEQ DEMONSTRATES DYNAMIC TRANSCRIPTION FACTOR BINDING DURING IN VIVO HEPATOBLAST MATURATION

Olivia Alder[1], Sam Lee[1], Rebecca Cullum[1], Gordon Robertson[2], Nina Thiessen[2], Yongjun Zhao[2], Steven Jones[2], Marco A Marra[2], Pamela A Hoodless[1]

[1]BC Cancer Agency, Terry Fox Laboratory, Vancouver, V5Z 1L3, Canada, [2]BC Cancer Agency, Genome Sciences Centre, Vancouver, V5Z 4S6, Canada

Hepatocytes comprise approximately 70% of the adult liver and carry out wide-ranging roles; from lipid homeostasis, hormone production, plasma protein secretion to chemical detoxification. Accomplishing such a plentitude of critical processes exerts extraordinary demands on transcriptional control mechanisms within the liver. Indeed, two decades of gene-by-gene promoter analysis in hepatocytes has revealed a prevalence of combinatorial transcription factor control over gene expression. Here, we explore the dynamic and collaborative behaviour of two well-characterised transcription factors during in vivo cell differentiation and organ formation. Using ChIP-Seq and RNA-Seq analysis of hepatoblasts isolated from 14.5 d.p.c embryos and adult livers we have observed over 5000 peaks occurring exclusively in each developmental stage. We show that differential temporal binding patterns of HNF4a and FOXA2 may regulate distinct transcriptional programs during liver development

To understand the mechanisms underlying developmental stage-specific transcription factor binding, we profiled the chromatin signatures of sites co-occupied by HNF4a and FOXA2 in embryonic and adult liver. Using ChIP-seq analysis of H3K4me1, H4ac and H3K27me3 in adult liver we have shown that sites co-occupied by transcription factors exhibit a clear bimodal distribution indicative of nucleosome displacement. Sites targeted only in embryonic liver show a monomodal distribution of H3K4me1 and H4ac, intriguingly H3K27me3 is also evident at developmentally repressed loci, suggesting that dynamic chromatin remodeling accompanies in vivo cell differentiation and differential transcription factor recruitment. Interestingly, developmentally distinct target genes are implicated in divergent biological process, for example embryonic FOXA2 associates with loci involved in chromatin and nucleosome remodeling while sites bound in the adult associate more significantly with metabolic process. Although transcriptional regulators are known to contribute to divergent processes in different tissue types, this study highlights the versatility of well-characterised transcription factors during tissue maturation. In summary our results suggest that differential binding of HNF4a and FOXA2 to chromatin in embryonic and adult liver may reflect the adaptive nature of these key transcriptional regulators during in vivo hepatoblast differentiation and liver formation.

PTIP IS CRITICAL FOR HISTONE METHYLATION, GERMLINE TRANSCRIPTION, AND ENHANCER-PROMOTER INTERACTIONS DURING IMMUNOGLOBULIN HEAVY CHAIN (IGH) CLASS SWITCH RECOMBINATION (CSR)

Kristopher R Schwab[1], Sanjeevkumar R Patel[2], Inna Levitan[1], Gregory R Dressler[1]

[1]University of Michigan, Pathology, Ann Arbor, MI, 48109, [2]University of Michigan, Medicine, Ann Arbor, MI, 48109

CSR is the mechanism by which B cells replace the constant region (CR) of IgH with one of the other downstream CRs encoding different isotypes. Stimulation of resting splenic B cells by α-CD40 or LPS with IL4 results in CSR of IgH isotypes in which the μ constant region is replaced with a downstream constant region, γ1 through DNA breaks and subsequent DNA repair. Recent studies have identified dual roles for PTIP, a BRCT domain containing protein in both epigenetic regulation of gene expression and DNA damage repair. PTIP interacts with Pax2 during activation of transcription by recruiting a histone 3 lysine 4 (H3K4) methylation complex to a Pax2 DNA binding site. Additionally, PTIP is recruited to sites of DNA damage and interacts with repair complex protein PA1. *ptip* was conditionally deleted in the B cell lineage using a CD19-Cre transgene. Surprisingly, *ptip* conditional knockout (KO) B cells are able to develop and mature normally, but *ptip* KO B cells have significantly reduced CSR efficiency when stimulated *in vitro* to undergo CSR. Analysis of the *ptip* KO phenotype suggests that CSR efficiency is reduced from the loss of the production of germline transcripts of the γ1 downstream constant region. Preceding the induced DNA breaks, germline transcripts are produced upstream of each constant region and are necessary for CSR. Correlating with the loss of transcription, histone 3 lysine 4 trimethylation (H3K4me3) was significantly reduced at the germline transcript promoter of γ1 using ChIP. Pax5, a Pax2 homolog and key regulatory transcription factor in B cells, is enriched at the promoter of γ1 and the IgH 3' enhancer hypersensitive site 4 (HS4) but is reduced in ptip KO stimulated B cells. Additionally, Pax5, Rbbp5 (a H3K4me3 complex member) and H3K4me3 are specifically enriched at the γ1 promoter during CSR stimulation in normal B cells, but enrichment is significantly reduced in *ptip* KO B cells. PTIP-dependent Pax5 enrichment at both the γ1 promoter and HS4 enhancer which are separated by over 100 kB suggests that PTIP may be responsible for long-range enhancer-promoter interactions during CSR. Chromosomal conformation capture (3C) was performed to quantify the physical interactions of γ1 and HS4. In stimulated *ptip* KO B cells, the ligation efficiency measured by PCR of γ1 and HS4 was decreased suggesting PTIP is needed for long range promoter/enhancer interactions during CSR. Our model suggests that PTIP brings Pax5 and the 3' enhancer into close proximity of γ1 to promote H3K4me3 and germline transcription.

PKD1L1 ESTABLISHES LEFT-RIGHT ASYMMETRY AND PHYSICALLY INTERACTS WITH PKD2

Sarah Field*, Kerry-Lyn Riley*, Daniel T Grimes*, Helen Hilton, Michelle Simon, Nicola Powles-Glover, Pam Siggers, Debora Bogani, Andy Greenfield, Dominic P Norris

MRC Harwell, Mammalian Genetics Unit, Oxfordshire, OX11 0RD, United Kingdom

In mammals, left-right (L-R) asymmetry is established by posteriorly oriented cilia driving a leftwards laminar flow in the embryonic node, thereby activating asymmetric gene expression. The two cilia hypothesis argues that immotile cilia detect and respond to this flow through a Pkd2-mediated mechanism. While Pkd2 protein is argued to comprise an ion channel a putative sensory partner protein in L-R development has remained unidentified, raising the question of whether Pkd2 is actually responding to nodal flow. We have identified *Pkd1l1* as a critical component of L-R patterning in mouse. Systematic comparison of *Pkd1l1* and *Pkd2* point mutants reveals strong phenocopying, evidenced by both morphological and molecular markers of sidedness; both mutants fail to activate asymmetric gene expression at either the node or in the lateral plate and exhibit right isomerism of the lungs. Node and cilia morphology were normal in mutants while cilia demonstrated typical motility, consistent with Pkd1l1 and Pkd2 activity downstream of nodal flow. Cell biological analysis reveals that Pkd1l1 and Pkd2 localise to the cilium, while biochemical experiments demonstrate that they can physically interact. Together with co-expression in the node, these data argue that Pkd1l1 is the elusive Pkd2 binding partner required for L-R patterning and support the two cilia hypothesis.

* Authors contributed equally

STRAIGHTENING OUT NOTCH

Gavin Chapman, Duncan B Sparrow, Sally L Dunwoodie

Victor Chang Cardiac Research Institute, Developmental Biology Division, Sydney, 2010, Australia

Notch signalling determines cell fate in a variety of contexts. Signalling instructs developmental processes using different modes of action that include lateral inhibition, lineage segregation, inductive signalling, and boundary formation. Signalling is activated by cell-cell contact with ligand expressed on one cell, activating Notch on another cell. This *trans*-activation of Notch signalling sees Notch-dependent transcriptional activation in the Notch-expressing, signal-receiving cell. DSL ligands of Notch also can inhibit signalling when expressed in the same cell as the Notch receptor. This *cis*-inhibition of Notch signalling is a more recently identified process and the mechanism of inhibition is not well understood. *Trans*-activation and *cis*-inhibition of Notch are features of all DSL ligands except for the divergent ligand Dll3, which inhibits but does not activate Notch signalling. We are using this unique attribute of Dll3 to address the mechanism of *cis*-inhibition.

Somitogenesis represents a model for studying the role of Notch signalling in boundary formation in vertebrates. Somites are reiteratively formed from the presomitic mesoderm and patterned to direct vertebral column formation. Notch1 signalling is critical for somitogenesis, and several pathway components have been identified as being critical for the process in mammals. Moreover in humans the *DLL3* ligand of Notch and three Notch target genes *(MESP2, LFNG, HES7)* are all associated with causing the congenital vertebral malsegmentation defect spondylocostal dysostosis. A specific, cyclical pattern of Notch1 activation is required in the presomitic mesoderm for normal somitogenesis. This Notch1 activation pattern is achieved through the activity of two ligands, Dll1 and Dll3. We have localised these ligands and the receptor in the presomitic mesoderm in order to evaluate how the pattern of Notch1 signalling is achieved.

Elucidating how Dll3 *cis*-inhibits Notch1 will lead to a better understanding of how Notch signalling drives somitogenesis; it will also elucidate the mechanism of *cis*-inhibition of Notch generally, and increase our understanding of other Notch-dependent processes.

FUNCTIONS OF NANOS2 IN THE MAINTENANCE AND DIFFERENTIATION OF MALE GERM LINEAGE IN MICE

Yumiko Saga

National Institute of Genetics, Mammalian Development, Mishima, 411-8540, Japan

The germ cell lineage is discriminated from that of somatic cells via species-specific mechanisms by which germ cells acquire their special cell fate and characteristics. In mammal, germ cells are generated in the place far away from future gonads. Although germ cells have potency to produce both oogonia and spermatogonia, sexual differentiation is induced after their colonization with somatic cells in the embryonic gonads, in which the sex of germ cells is determined by signals derived from somatic cells surrounding germ cells. The expression of *Nanos2*, encoding an RNA binding protein is restricted in the male germ cells after colonization into gonads and plays a key role on the sexual differentiation of germ cells via promoting male fate and suppressing female fate. The NANOS2 protein expression is maintained in all male gonocytes during embryogenesis. However, the expression becomes confined only in a small population of spermatogonia after birth. Genetic evidence indicates that NANOS2 is required for the maintenance of spermatogonnial stem cells. In this meeting, I like to summarize functions of NANOS2 during embryogenesis and after birth. Finally I also show a possible molecular function of NANOS2 based on our recent findings.

SUCCESSIVE ROLES OF PDGF SIGNALING IN THE EXTRAEMBRYONIC ENDODERM OF THE MOUSE EMBRYO

Jerome Artus, Kat Hadjantonakis

Sloan-Kettering Institute, Developmental Biology, New York, NY, 10065

Mammal preimplantation development is mainly devoted to the production of extraembryonic tissues, the trophectoderm and primitive endoderm (PrE), essential for embryo survival *in utero*. While the mechanisms of trophectoderm specification have been extensively studied, little is known about PrE formation. We previously characterized *in vivo* an early marker of PrE lineage, Platelet-derived growth factor receptor alpha (PDGFRα), which is dynamically expressed in PrE and its derivatives, the parietal and visceral endoderm.

By combining live imaging of embryos and embryo-derived stem cells expressing a histone H2B-GFP fusion reporter under the control of *Pdgfra* regulatory elements with the analysis of lineage-specific markers, we have determined that *Pdgfra* expression coincides with GATA6 the earliest expressed transcriptional regulator of the PrE lineage. In addition, our data show that GATA6 is required for the activation of *Pdgfra* expression. Based on these observations, we propose a model whereby initiation of *Pdgfra* expression requires GATA6, and its maintenance requires GATA4 and GATA6.

Using pharmacological inhibition and genetic inactivation, we have addressed the role of the PDGF pathway in the PrE lineage as well as its derivative tissue, the visceral endoderm. Our results reveal a role for PDGF signaling in the establishment and proliferation of XEN cells, isolated from mouse blastocyst stage embryos and representing the PrE lineage. While implanting *Pdgfra* mutant blastocysts exhibit a reduced number of PrE cells, an effect that is exacerbated by delaying implantation. Furthermore, tissue-specific inactivation of *Pdgfra* in the visceral endoderm reveals an unanticipated role for PDGF signaling in early postimplantation development.

Taken together, our data provide new insights into the roles of PDGF signaling in the establishment and propagation of ExEn lineage.

CDX2 MRNA TRANSPORT IN MOUSE EMBRYO DEPENDS ON ACTIN AND MICROTUBULE NETWORK AND A ZIP CODE IDENTIFIED IN THE CODING SEQUENCE.

Maria Skamagki[1], Krzysztof B Wicher[1], Sujoy Ganguly[2], Alexander W Bruce [1,3], Magdalena Zernicka-Goetz[1]

[1]Wellcome Trust/Cancer Research UK Gurdon Institute, The Henry Wellcome Building of Cancer and Developmental Biology, University of Cambridge, Cambridge, CB2 1QN, United Kingdom, [2]Department of Applied Mathematics and Theoretical Physics, University of Cambridge, Cambridge, CB3 0WA, United Kingdom, [3]Dept. of Molecular Biology, Faculty of Science, University of South Bohemia, Ceske Budejovice, 37005, Czech Republic

Caudal type homeobox 2 (Cdx2) transcriptional factor is a major lineage marker and cell fate determinant of trophectoderm during early mouse development. Cdx2 mRNA localizes apically within blastomeres of 8- and 16-cell stage mouse embryos. Mechanism and role of this asymmetric distribution are poorly understood. Here we developed a sensitive assay for studying the dynamics of Cdx2 mRNA localization in vivo using microinjection of fluorescently labeled RNA in one or more blastomeres of living 8-cell stage embryos. We show that the injected Cdx2 transcripts localize at the apical side of compacted blastomeres at 8-cell stage embryos in the same way as endogenous mRNA. This localization requires intact microtubule and actin network. We found that the cis-element responsible for the localization resides in the Cdx2 coding sequence. We also reveal that during transition from 8- to 16-cell stage the domain containing Cdx2 mRNA undergoes dynamic reorganization. Taken together, our experiments, for the first time, present evidences for nonuniform inheritance of mRNA during preimplantation mammalian development, which could result in differential cell fate determination.

REQUIREMENT FOR FGF4 IN PRIMITIVE ENDODERM LINEAGE COMMITMENT IN THE MOUSE BLASTOCYST

Anna Piliszek, Jerome Artus, Minjung Kang, Robert Aho, Kat Hadjantonakis

Sloan-Kettering Institute, Developmental Biology Program, New York, NY, 10065

Preimplantation mouse development involves a critical period of differentiation and lineage commitment. Two lineages arise from the inner cell mass (ICM) of the blastocyst: the pluripotent epiblast (EPI), and extraembryonic primitive endoderm (PrE). Lineage choice is dictated by the expression of lineage-specific transcription factors. These are initially expressed in overlapping manner in all cells of the ICM at the early blastocyst stage. Then, after the 64-cell (mid-blastocyst) stage, these factors become mutually exclusive and specific to lineage-commited cells, and are organized in salt-and-pepper manner within the ICM. The mechanisms responsible for PrE vs. EPI specification are still poorly understood, however ERK signaling has been suggested as involved in this lineage decision. Here we show a critical requirement for FGF4, in the primitive endoderm specification, and in the derivation of embryo-derived stem cells. At the time of implantation (late blastocyst stage) *Fgf4* mutant embryos lack primitive endoderm, and their ICM is comprised of exclusively EPI marker expressing cells, a phenotype that can be rescued by addition of FGF4. However, earlier on, at the early blastocyst stage, *Fgf4* mutant embryos correctly express markers of both EPI and PrE. These data suggest that FGF signaling is not required for the initiation of lineage-specific transcription factor expression, but rather for the choice of specific cell fate.
We also note that Fgf4 deficient ES cells can be derived at increased efficiency than wild type ES cells. By contrast, we failed to establish XEN cells *Fgf4* mutant embryos. Further analysis suggests that the requirement for FGF4 in these embryo-derived stem cells lines appears to be dose-dependent.

SATELLITE CELLS REQUIRE NOTCH SIGNALS TO RECOGNIZE THEIR 'NICHE'.

Broehl Dominique[1], Vasyutina Elena[1,2], Birchmeier Carmen[1]

[1]Max Delbrueck Centrum, Neuroscience, Berlin, 13125, Germany, [2]University of Cologne, Center of Molecular Medicine, Koeln, 50931, Germany

Myogenesis is a tightly regulated process that is essential in muscle development and regeneration. During mammalian development, phases of embryonic and fetal myogenic differentiation lead to the formation and growth of skeletal muscles. In the postnatal and adult organism, skeletal muscle grows and regenerates by the myogenic differentiation of stem cells, the satellite cells. In the developing muscle, a pool of myogenic progenitor cells is formed and maintained. These resident progenitors provide a source of cells for muscle growth in development and generate satellite cells in the perinatal period. In the postnatal muscle, satellite cells are activated after injury of the muscle. Activated satellite cells proliferate, and either self renew or differentiate into myoblasts capable of forming new muscle.

The Notch signaling system is highly conserved in evolution. Notch signaling initiates when the receptor binds to its ligand presented on neighboring cells, which induces a conformational change resulting in cleavage and release of the Notch intracellular domain that moves to the nucleus where it interacts with the major transcriptional mediator of Notch signals, RBPJ. We used mouse genetics to analyze Notch functions in the developing and postnatal muscle. Our data show that Notch signals are essential for self-renewal of muscle progenitors and satellite cells, and that Notch target genes are required for the recognition of the satellite cell niche.

DISTINCT STEM/PROGENITOR CELL POPULATIONS CONTROL SKELETAL MUSCLE DEVELOPMENT

Ramkumar Sambasivan[1], Danielle Gomés[1], Gérard Dumas[1], Clémire Cimper[2], Shahragim Tajbakhsh[1]

[1]Institut Pateur, Stem cells and Development, CNRS URA 2578, Developmental biology, Paris, 75724, France, [2]Institut Pateur, Developmental biology, Paris, 75724, France

All skeletal muscles in vertebrates derive from the mesodermal germ layer in several distinct developmental phases. The stem/progenitor cells controlling these temporally defined phases are different (Biressi et al., 2007; Hutcheson et al., 2009; Tajbakhsh, 2009). Interestingly, in the embryo, temporally co-inciding myogenic cell populations can be sub-categorized based on the expression of specification and commitment genes such as Pax3/Pax7 (hierarchical paired-box/homeodomain transcription factors) and Myf5/Mrf4/Myod (muscle regulatory bHLH transcription factors), respectively. This scenario raises some key questions. What is the functional relevance of these distinguishable founder stem cells? Are they lineage related, to what extent do they contribute to the developmental myogenic waves, and subsequently to adult muscle stem/progenitor cells? In this context, recent findings suggested that the preponderant Myf5+ population is dispensable for muscle development (Gensch et al., 2008; Haldar et al., 2008). However, the significance of this population has not been addressed in detail for head myogenesis as it has been for trunk muscles. This is a key issue given that the embryonic source for the progenitors of the head muscle groups (extraocular (EOM) and jaw muscles) is different compared to that of trunk/limb muscles. Moreover, distinct genetic hierarchies control EOM, jaw and trunk muscle development (Sambasivan et al., 2009). Here, we show that the population that had expressed Mrf4, a weak muscle determination gene and a strong differentiation-promoting gene, acts as a reservoir for all adult muscle stem/progenitor cells. We show also that reflecting their unique genetic program, the EOMs are dependent upon the Myf5+ population in stark contrast to other head/trunk muscles. Therefore, divergent skeletal muscle founder cells in the embryo deploy different genetic networks to achieve myogenesis. The link between these founder stem cells and their adult muscle counterparts can be defined by the specification and determination genes they recruit for their function. Lineage and cell ablation strategies have provided important information on how organogenesis is achieved. During these studies we noted that there is reporter line dependant variability in the readout from the Cre driver lines. Since the Cre/loxP strategy is used extensively for lineage tracing and cell ablation studies, our findings will be of wide interest to the community.

DISSECTING THE MECHANISMS OF CELLULAR REPROGRAMMING

Konrad Hochedlinger

Massachusetts General Hospital, Cancer Center and Center for Regenerative Medicine, Harvard Stem Cell Institute, Boston, MA, 02114

My lab is studying the mechanisms of cellular reprogramming using transcription factor-mediated conversion of somatic cells into induced pluripotent stem (iPS) cells. For example, we have identified biomarkers to track and prospectively isolate intermediate cell populations during the reprogramming process and are currently using these to understand the transcriptional, epigenetic and proteomic changes in cells undergoing reprogramming. In addition, we have shown that terminally differentiated beta cells and lymphocytes can be reprogrammed into iPS cells, thus demonstrating that induced pluripotency is not limited to rare adult stem cells as has been suggested. Interestingly, however, we discovered that immature hematopoietic cells give rise to iPS cells more efficiently than any tested mature cell types, suggesting that the differentiation stage of the starting cell can influence the efficiency of reprogramming. We have further identified the p53 and p16/p19 tumor suppressor pathways as roadblocks during the reprogramming process, pointing out similarities between pluripotent cells and cancer cells. One major roadblock for the therapeutic use of iPS cells is the fact that integrating viruses are used to deliver the reprogramming genes to cells, resulting in genetically altered iPS cells. By using adenoviruses expressing the reprogramming factors transiently in cells, we were able to produce iPS cells devoid of any viral elements and thus any genetic manipulation. More recently, we have developed a "reprogrammable mouse" carrying a single doxycycline-inducible cassette with the four reprogramming genes in all tissues. We are employing this system to perform genetic and chemical screens to identify molecules important during the reprogramming process as well as for comparative studies between iPS cells and embryonic stem cells.

Participant List

Ms. Kanae Abe
Kanazawa University
kana-abe@kiea.m.kanazawa-u.ac.jp

Dr. Alexander Akhmedov
University of Zurich
alexander.akhmedov@access.uzh.ch

Mr. Hunain Alam
ACTREC
hunainalam@gmail.com

Ms. Stephanie Almeida
Mount Sinai Hospital
stephanie.m.almeida@gmail.com

Dr. Kathryn Anderson
Sloan-Kettering Institute
k-anderson@ski.mskcc.org

Dr. Matthew Anderson
National Cancer Institute - Frederick
andersonmj@mail.nih.gov

Mr. Oliver Appelbe
University of Illinois at Chicago
oappel2@uic.edu

Dr. Jerome Artus
Memorial Sloan-Kettering Cancer Center
artusj@mskcc.org

Dr. Sophie Astrof
Thomas Jefferson University
sophie.astrof@gmail.com

Dr. Randy Babiuk
The Jackson Laboratory
rbabiuk@informatics.jax.org

Ms. Virginia Bain
University of Georgia
vbain@uga.edu

Ms. Julie Baker
Stanford University
jbaker@stanford.edu

Dr. Timothy Bestor
College of Physicians and Surgeons of Columbia Uni
thb12@columbia.edu

Prof. Stefano Biffo
Università del Piemonte Orientale
biffo.stefano@hsr.it

Mr. Charles Billington
University of Minnesota
billi020@umn.edu

Dr. Carmen Birchmeier
Max Delbrueck Centrum
cbirch@mdc-berlin.de

Dr. Anne Calof
University of California, Irvine
alcalof@uci.edu

Dr. Mario Capecchi
University of Utah School of Medicine
mario.capecchi@genetics.utah.edu

Ms. Kim Cardenas
The University of Texas MD Anderson Cancer Center
kcardenas@mdanderson.org

Prof. Kathryn Cheah
University of Hong Kong
kathycheah@hku.hk

Dr. TingGang Chew
Institue of Medical Biology
tinggang.chew@imb.a-star.edu.sg

Dr. Yung-Hao Ching
National Laboratory Animal Center
yching@nlac.org.tw

Ms. Hyun-Min Cho
Kyunpook National University
jude7998@hanmail.net

Dr. Gary Churchill
The Jackson Laboratory
garyc@jax.org

Dr. Michel Cohen-Tannoudji
CNRS/Institut Pasteur
m-cohen@pasteur.fr

Dr. Chris Cooper
University of Georgia
ccoop17@uga.edu

Ms. Christina Cota
Cornell University
cdc27@cornell.edu

Dr. Sally Dunwoodie
Victor Chang Cardiac Research Institute
s.dunwoodie@victorchang.edu.au

Dr. Aris Economides
Regeneron Pharmaceuticals, Inc
aris@regeneron.com

Dr. Mitsuhiro Endoh
RIKEN Research Center for Allergy and Immunology
mendoh@rcai.riken.jp

Dr. Elena Ezhkova
The Rockefeller University
eezhkova@mail.rockefeller.edu

Dr. Michael Felder
University of South Carolina
mrfelder@mailbox.sc.edu

Dr. Anne Ferguson-Smith
University of Cambridge
afsmith@mole.bio.cam.ac.uk

Dr. Mathieu Ferron
Columbia Presbyterian Center
mf2456@columbia.edu

Dr. Adriano Flora
Baylor College of Medicine
aflora@bcm.edu

Dr. Nicole Forster
Massachusetts General Hospital
nforster@partners.org

Dr. Christophe FREMIN
Montreal University
christophe.fremin@gmail.com

Dr. Takashi Furusawa
National Institutes of Health
furusawt@mail.nih.gov

Mr. Derrick Glasco
University of Missouri
dmgce3@mail.missouri.edu

Dr. Celine Granier
RWJMS-Child Health Institute of New Jersey
graniecj@umdnj.edu

Ms. Anna Groner
Swiss Federal Institute of Technology (EPFL)
anna.groner@epfl.ch

Dr. Melissa Harris
National Institutes of Health-NHGRI
harrisml2@mail.nih.gov

Dr. Nicholas Hastie
Western General Hospital
katie.browne@hgu.mrc.ac.uk

Mr. David Heslin
Regeneron Pharmaceuticals
david.heslin@regeneron.com

Dr. Ryuji Hiramatsu
Osaka Medical Center for Maternal and Child Health
rhira@mch.pref.osaka.jp

Ms. Tomoka Hisaki
University of Tokyo
aa106291@mail.ecc.u-tokyo.ac.jp

Dr. Konrad Hochedlinger
Massachusetts General Hospital
khochedlinger@helix.mgh.harvard.edu

Ms. Katharina Hoelle
University of Cambridge
kh400@cam.ac.uk

Dr. Pamela Hoodless
BC Cancer Agency
hoodless@bccrc.ca

Ms. Tingting Huang
Umass Medical School
tingting.huang@umassmed.edu

Dr. Johan Jirholt
AstraZeneca R&D
johan.jirholt@astrazeneca.com

Dr. Alexandra Joyner
Sloan Kettering Cancer Center
joynera@mskcc.org

Dr. Klaus Kaestner
University of Pennsylvania Medical School
kaestner@mail.med.upenn.edu

Ms. Maria Kaltcheva
National Cancer Institute - Frederick
kaltchevam@od.nih.gov

Dr. Kiyoshi Kano
The University of Tokyo
akano@mail.ecc.u-tokyo.ac.jp

Dr. Claudia Kappen
Pennington Biomedical Research Center
claudia.kappen@pbrc.edu

Dr. Gerard Karsenty
Columbia University
gk2172@columbia.edu

Dr. David Katz
Emory University
djkatz@emory.edu

Mr. Ikuma Kawai
University of Tokyo
aa096289@mail.ecc.u-tokyo.ac.jp

Dr. Shimako Kawauchi
University of California, Irvine
skawauch@uci.edu

Dr. Sang Yong Kim
Cold Spring Harbor Laboratory
kimsy@cshl.edu

Ms. Ashley King
Emory University
alking2@uga.edu

Dr. Takashi Kohda
Tokyo Medical and Dental University
tkohda.epgn@tmd.ac.jp

Dr. Maria Kolanczyk
Max Delbruck Center Berlin
maria.kolanczyk@mdc-berlin.de

Dr. Haruhiko Koseki
RIKEN Research Center for Allergy and Immunology
koseki@rcai.riken.jp

Dr. Matthew Koss
Weill Cornell Medical College
mjk2007@med.cornell.edu

Ms. Dilidaer Kudereti
Tokyo Denki University
09udq02@ms.dendai.ac.jp

Dr. Elizabeth Lacy
Sloan-Kettering Institute
e-lacy@mskcc.org

Mr. Zhimin Lao
Memorial Sloan-Kettering Cancer Center
Laoz@mskcc.org

Dr. Jeffrey Lee
Regeneron Pharmaceuticals, Inc.
jeffrey.lee@regeneron.com

Dr. Giovanni Levi
CNRS
glevi@mnhn.fr

Dr. Wangzhi Li
CSHL
wali@cshl.edu

Dr. Javier Lopez-Rios
University of Basel
javier.lopez-rios@unibas.ch

Dr. Min Luo
Baylor College of Medicine
mluo@bcm.tmc.edu

Dr. Danielle Maatouk
Duke University
d.maatouk@cellbio.duke.edu

Mr. Vinit Mahajan
Univeristy of Iowa
mahajanlab@gmail.com

Dr. Nancy Manley
University of Georgia
nmanley@uga.edu

Dr. Isao Matsuo
Osaka Medical Center for Maternal and Child Health
imatsuo@mch.pref.osaka.jp

Ms. Katie McDole
HHMI - Carnegie Institution
mcdole@ciwemb.edu

Dr. Andrew McMahon
Harvard University
amcmahon@mcb.harvard.edu

Ms. Erin McMurray
University of Illinois at Chicago
schmidtlab@hotmail.com

Mr. Kenneth Miller
Pfizer, Inc.
kenneth.s.miller@pfizer.com

Dr. Alea Mills
Cold Spring Harbor Laboratory
mills@cshl.org

Prof. Fujio Miyawaki
Tokyo Denki University
miyawaki@mail.dendai.ac.jp

Dr. Natalia Moncaut
The Institute of Cancer Research
Natalia.Moncaut@icr.ac.uk

Dr. Ivan Moskowitz
The University of Chicago
imoskowitz@uchicago.edu

Mr. Dexter Myrick
Emory University
myrickd2@gmail.com

Dr. Andras Nagy
Samuel Lunenfeld Research Institute, Mt. Sinai Hospital
nagy@mshri.on.ca

Dr. L. Naiche
National Cancer Institute
adlernl@mail.nih.gov

Dr. Makoto Noda
Kyoto University Graduate School of Medicine
mnoda@virus.kyoto-u.ac.jp

Dr. Dominic Norris
MRC
d.norris@har.mrc.ac.uk

Mr. Darren Oakley
Wellcome Trust Sanger Institute
do2@sanger.ac.uk

Dr. Nina Offenhaeuser
IFOM FOUNDATION
emanuela.orlando@iofm-ieo-campus.it

Dr. Kazuyuki Ohbo
Yokohama City University, School of Medicine
kohbo@yokohama-cu.ac.jp

Dr. Masato Ohtsuka
Tokai University, School of Medicine
masato@is.icc.u-tokai.ac.jp

Ms. Roxana Ola
Helsinki University
roxana.ola@helsinki.fi

Dr. Joo-Seop Park
Harvard University
jspark@mcb.harvard.edu

Dr. Samuel Pfaff
HHMI/Salk Institute
pfaff@salk.edu

Ms. Cristy Phillips
Arkansas Biosciences Institute
cphillips@astate.edu

Dr. Anna Piliszek
Sloan-Kettering Institute
piliszea@mskcc.org

Ms. Simone Probst
Universität Basel
simone.probst@unibas.ch

Dr. Maria Pulina
Slaon Kettering Institute
mpulina@gmail.com

Dr. Daniela Ragancokova
Max-Delbrück-Center for Molecular Medicine,Berlin
daniela.ragancokova@mdc-berlin.de

Dr. Ramiro Ramirez-Solis
Wellcome Trust Sanger Institute
rrs@sanger.ac.uk

Ms. Nitya Ramkumar
Sloan-Kettering Institute
nir2009@med.cornell.edu

Ms. Saba Raza
UCL Institute of Child Health
s.raza@ich.ucl.ac.uk

Dr. Ellen Richie
MD Anderson Cancer Center
erichie@mdanderson.org

Dr. Gesa Rickheit
TaconicArtemis GmbH
gesa.rickheit@taconicartemis.com

Dr. Martin Ringwald
The Jackson Laboratory
ringwald@informatics.jax.org

Dr. Jaime Rivera
University of Massachusetts Medical School
jaime.rivera@umassmed.edu

Ms. Heidi Roberson-Trau
University of Georgia
robersoh@uga.edu

Dr. Ute Rogner
Institut Pasteur
ute-christine.rogner@pasteur.fr

Dr. Yumiko Saga
National Institute of Genetics
ysaga@lab.nig.ac.jp

Dr. J. Michael Salbaum
Pennington Biomedical Research Center
michael.salbaum@pbrc.edu

Dr. Ramkumar Sambasivan
Institut Pasteur
ramkumar.sambasivan@pasteur.fr

Dr. Thom Saunders
University of Michigan
tsaunder@umich.edu

Dr. Johannes Schenkel
German Cancer Research Centre
dj5@ix.urz.uni-heidelberg.de

Dr. Martin Schmerr
Cleveland Clinic
schmerm@ccf.org

Mr. Jean-Francois Schmouth
UBC/CMMT
jschmouth@cmmt.ubc.ca

Dr. Kristopher Schwab
University of Michigan
krschwab@umich.edu

Mr. LALIT SEHGAL
TATA MEMORIAL CENTRE, ACTREC
LALIT.GENETICS@GMAIL.COM

Dr. Amanda Sferruzzi-Perri
University of Cambridge
ans48@cam.ac.uk

Dr. Shyam Sharan
National Cancer Institute, NIH
sharans@mail.nih.gov

Dr. Jafar Sharif
RCAI, RIKEN, Yokohama
jafarsharif@rcai.riken.jp

Dr. Michael Shen
Columbia University Medical Center
mshen@columbia.edu

Dr. Tang-Long Shen
National Taiwan University
shentl@ntu.edu.tw

Dr. Yih-Horng Shiao
National Cancer Institute
shiaoy@mail.nih.gov

Ms. Risa Shimizu
Tokyo Denki University
09rmb19@ms.dendai.ac.jp

Dr. Elizabeth Simpson
University of British Columbia
simpson@cmmt.ubc.ca

Ms. Maria Skamagki
University of Cambridge
ms739@cam.ac.uk

Dr. William Skarnes
Wellcome Trust Sanger Institute
skarnes@sanger.ac.uk

Dr. Philippe Soriano
Mount Sinai School of Medicine
philippe.soriano@mssm.edu

Dr. Francois Spitz
EMBL
spitz@embl.de

Dr. A. Francis Stewart
Technische Universitaet Dresden
stewart@biotec.tu-dresden.de

Dr. Dong-Ming Su
University of Texas Health Science Center at Tyler
dong-ming.su@uthct.edu

Ms. Fatima Sulaiman
National Institute for Medical Research
fsulaim@nimr.mrc.ac.uk

Dr. Ge-Hong Sun-Wada
Doshisha Women's College
kwada@dwc.doshisha.ac.jp

Ms. Natalie Terry
Children's Hospital of Philadelphia
terryn@email.chop.edu

Dr. Paul Thomas
University of Adelaide
paul.thomas@adelaide.edu.au

Dr. Giovane Tortelote
University of Massachusetts Medical School
giovane.tortelote@umassmed.edu

Dr. Stephen Tsang
Columbia University
gene.targeting@gmail.com

Dr. Patrick Tschopp
University of Geneva
patrick.tschopp@frontiers-in-genetics.org

Dr. Otoya Ueda
Chugai Pharmaceutical Co., Ltd.
uedaoty@chugai-pharm.co.jp

Mr. George Andrew Van Brunt
Washington University School of Medicine
avanbrun@watson.wustl.edu

Dr. Emma Vernersson Lindahl
Cold Spring Harbor Laboratory
vernerss@cshl.edu

Mr. Assaf Vestin
Stony Brook University
vestin@cshl.edu

Mr. Manuel Viotti
Sloan-Kettering Institute
manolov9@hotmail.com

Dr. Paul Vrana
University of South Carolina
VRANAP@MAILBOX.SC.EDU

Dr. Yoh Wada
Division of Biological Scinece
yohwada@sanken.osaka-u.ac.jp

Dr. Daniel Wainstock
Developmental Cell (Elsevier)
dwainstock@cell.com

Mr. Paul Wakenight
Seattle Children's Hospital
paul.wakenight@seattlechildrens.org

Dr. Chengbing Wang
Weill Cornell Medical College
chw2012@med.cornell.edu

Dr. Baolin Wang
Weill Medical College of Cornell University
baw2001@med.cornell.edu

Dr. Zhu Wang
Columbia University
zw2103@columbia.edu

Dr. Soren Warming
Genentech, inc.
sorenw@gene.com

Dr. Fiona Watt
CR UK Cambridge Research Institute
fiona.watt@cancer.org.uk

Dr. Scott Weatherbee
Yale University
scott.weatherbee@yale.edu

Ms. Jamie Weaver
University of Pennsylvania
jamiere@mail.med.upenn.edu

Ms. Katherine Wert
Columbia University
kjw2126@columbia.edu

Dr. Trevor Williams
University of Colorado Denver
trevor.williams@ucdenver.edu

Dr. Robert Williams
University of Tennessee
rwilliams@uthsu.edu

Ms. Lucy Williams
University of North Carolina
haizlip@email.unc.edu

Mr. Thad Wolfram
Pfizer, Inc.
thaddeus.j.wolfram@pfizer.com

Dr. Samuel Wormald
Wellcome Trust Sanger Institute
sw7@sanger.ac.uk

Mr. Qing Xie
Albert Einstein College of Medicine
qing.xie@phd.einstein.yu.edu

Ms. Jingxia Xu
The Jackson Laboratory
jx@informatics.jax.org

Dr. Pin-Xian Xu
Mount Sinai School of Medicine
pinxian.xu@mssm.edu

Ms. Mako Yamamoto
Kyoto University Graduate School of Medicine
mkyamamo@virus.kyoto-u.ac.jp

Dr. Yukuto Yasuhiko
National Institute of Health Sciences
yasuhiko@nihs.go.jp

Mr. Young Soo Yoon
UMass Medical School
Yeonsoo.Yoon@umassmed.edu

Dr. Kangsun Yun
National Cancer Institute - Frederick
yunk@mail.nih.gov

Dr. Yubo ZHANG
Genome Institute of Singapore
zhangy6@gis.a-star.edu.sg

Dr. Hui Zhao
Columbia University
hz2159@columbia.edu

Dr. Hui Zong
University of Oregon
hzong@uoregon.edu

VISITOR INFORMATION

EMERGENCY	CSHL	BANBURY
Fire	**(9) 742-3300**	**(9) 692-4747**
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Emergency Room **Huntington Hospital** 270 Park Avenue, Huntington	**631-351-2300** **(1037)**
Dentists Dr. William Berg Dr. Robert Zeman	 **631-271-2310** **631-271-8090**
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Drugs - 24 hours, 7 days Rite-Aid 391 W. Main Street, Huntington	**631-549-9400** (**1039**)

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Helpful tips - Obtain PIN from Meetings & Courses Office to enter Library after hours. See Library staff for photocopier code.

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Breakfast 7:30–9:00, Lunch 11:30–1:30, Dinner 5:30–7:00
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Helpful tip - If there is a line at the upper dining area, try the lower dining room

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Message Board, Grace, lower level

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Dolan Hall, east wing, lower level
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Pay Phones, House Phones

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.

1-800 Access Numbers

AT&T	**9-1-800-321-0288**
MCI	**9-1-800-674-7000**

Local Interest

Fish Hatchery	631-692-6768
Sagamore Hill	516-922-4447
Whaling Museum	631-367-3418
Heckscher Museum	631-351-3250
CSHL DNA Learning Center	x 5170

New York City

Helpful tip -

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.

TRANSPORTATION

Limo, Taxi

Syosset Limousine	516-364-9681 (**1031**)
Super Shuttle	800-957-4533 (**1033**)
To head west of CSHL - Syosset train station	
Syosset Taxi	516-921-2141 (**1030**)
To head east of CSHL - Huntington Village	
Orange & White Taxi	631-271-3600 (**1032**)
Executive Limo	631-696-8000 (**1047**)

Trains

Long Island Rail Road	822-LIRR
Schedules available from the Meetings & Courses Office.	
Amtrak	800-872-7245
MetroNorth	800-638-7646
New Jersey Transit	201-762-5100

Ferries

Bridgeport / Port Jefferson	631-473-0286 **(1036)**
Orient Point/ New London	631-323-2525 **(1038)**

Car Rentals

Avis	631-271-9300
Enterprise	631-424-8300
Hertz	631-427-6106

Airlines

American	800-433-7300
America West	800-237-9292
British Airways	800-247-9297
Continental	800-525-0280
Delta	800-221-1212
Japan Airlines	800-525-3663
Jet Blue	800-538-2583
KLM	800-374-7747
Lufthansa	800-645-3880
Northwest	800-225-2525
United	800-241-6522
US Airways	800-428-4322