The Genetics Society of America Conferences

Mouse Molecular Genetics

October 2-6, 2012 • Asilomar Conference Grounds • Pacific Grove, CA

Meeting Organizers

Allan Bradley, Wellcome Trust Sanger Institute, UK
Kat Hadjantonakis, Memorial Sloan-Kettering Institute, USA
Haruhiko Koseki, RIKEN Research Center for Allergy and Immunology, Japan
Michael Shen, Columbia University Medical Center, USA

Invited Speakers

Keynote Lecture: Shinichi Nishikawa (Kobe)
Beddington Lecture: Robb Krumlauf (Stowers Institute)
Patterning: Hiroshi Sasaki (Kumamoto); Mark Lewandoski (NCI Frederick)
Stem Cells and Germ Cells: Fred de Sauvage (Genentech); Phil Beachy (Stanford)
Epigenetics: Atsuo Ogura (Tsukuba); Guoliang Xu (Shanghai)
Organogenesis: Lori Sussel (Columbia); Sylvia Evans (UC San Diego)
Models of Human Disease: Lee Niswander (Colorado); Alea Mills (Cold Spring Harbor)
Genetics and Genomics: Haydn Prosser (Sanger Centre); Kent Lloyd (UC Davis)
Technology: Hiromitsu Nakauchi (Tokyo); Anton Wutz (Cambridge)
Imaging: Takaharu Okada (Yokohama); Mary Dickinson (Baylor)

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Genetics Society of America

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Schedule of Events

TUESDAY, October 2

4:00 pm-9:00 pm	**Registration**	**Merrill Hall**
6:00 pm - 7:00 pm	**Dinner**	**Crocker Dining Hall**
7:00 pm - 10:40 pm	**Plenary/Platform Session 1 - Models of Human Disease**	**Merrill Hall**

WEDNESDAY, October 3

7:00 am-2:00 pm	**Registration**	**Merrill Hall**
7:30 am - 8:30 am	**Breakfast**	**Crocker Dining Hall**
8:30 am - 12:00 noon	**Plenary/Platform Session 2 - Patterning**	**Merrill Hall**
8:30 am - 12:00 noon	**Exhibits**	**Merrill Hall**
12:00 noon - 1:00 pm	**Lunch**	**Crocker Dining Hall**
1:00 pm - 5:00 pm	**Exhibits**	**Merrill Hall**
2:00 pm - 3:00 pm	**Beddington Lecture**	**Merrill Hall**
3:00 pm - 5:00 pm	**Poster Session 1** *Even Poster Number Presentations*	**Fred Farr/Klin**
5:00 pm - 6:00 pm	**Wine and Cheese Reception**	**Seascape**
6:00 pm - 7:00 pm	**Dinner**	**Crocker Dining Hall**
7:00 pm - 10:40 pm	**Plenary/Platform Session 3 - Stem Cells and Germ Cells**	**Merrill Hall**

THURSDAY, October 4

7:30 am - 8:30 am	**Breakfast**	**Crocker Dining Hall**
8:30 am - 12:00 noon	**Plenary/Platform Session 4 - Epigenetics**	**Merrill Hall**
8:30 am - 12:00 noon	**Exhibits**	**Merrill Hall**
12:00 noon - 1:00 pm	**Lunch**	**Crocker Dining Hall**
1:00 pm - 5:00 pm	**Exhibits**	**Merrill Hall**
2:00 pm - 3:00 pm	**Keynote Address**	**Merrill Hall**

THURSDAY, October 4 (continued)

3:00 pm - 5:00 pm	**Poster Session 2** *Odd Poster Number Presentations*	**Fred Farr/Klin**
6:00 pm - 7:00 pm	**Dinner**	**Crocker Dining Hall**
7:00 pm - 10:20 pm	**Plenary/Platform Session 5 - Technology**	**Merrill Hall**

FRIDAY, October 5

7:30 am - 8:30 am	**Breakfast**	**Crocker Dining Hall**
8:30 am - 12:00 noon	**Plenary/Platform Session 6 - Organogenesis**	**Merrill Hall**
9:00 am - 12:00 noon	**Exhibits**	**Merrill Hall**
12:00 noon - 1:00 pm	**Lunch**	**Crocker Dining Hall**
1:00 pm - 6:00 pm	**Exhibits**	**Merrill Hall**
2:00 pm - 3:40 pm	**Plenary/Platform Session 7 - Imaging**	**Merrill Hall**
6:00 pm - 7:15 pm	**Dinner/Banquet**	**Crocker Dining Hall**

SATURDAY, October 6

7:30 am - 8:30 am	**Breakfast**	**Crocker Dining Hall**
8:30 am - 12:00 noon	**Plenary/Platform Session 8** **Genetics and Genomics**	**Merrill Hall**
12:00 noon - 1:00 pm	**Lunch** *Those who have pre-ordered box lunches should pick them up after breakfast in Crocker Dining Hall*	**Crocker Dining Hall**

Notes

LODGING

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OTHER

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As exhibitors at the Mouse Molecular Genetics Conference, the following companies have contributed to the support of this meeting. Registrants are encouraged to visit the exhibits during coffee breaks and other designated times in Merrill Hall to see the new products, publications and services available from these companies.

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Tuesday, October 2 7:00 pm–10:40 pm
Merrill Hall

Plenary/Platform Session 1 - Models of Human Disease

Chair: Lee Niswander, University of Colorado School of Medicine

1 - 7:00
Exploring Neural Tube Closure via Genes, Environment, and Imaging. Lee Niswander. Pediatrics, Univ of Colorado School of Medicine, Aurora, CO.

2 - 7:30
A gene within the modifier locus, *Tgfbm3*, regulates signal transduction *via* TGF-β type I receptor to modify vascular phenotypes in mice and in HHT patients. Kyoko Kawasaki[1], Sylvia Espejel[1], Tom Letteboer[1,2], Michael Benzinou[1], Marie Lee[1], Ritu Roy[1], Hans Kristian Ploos van Amstel[2], Cornelius Westermann[3], **Rosemary Akhurst**[1]. 1) HDFCCC, UCSF, San Francisco, CA; 2) Department of Medical Genetics, University Medical Centre, Utrecht, The Netherlands; 3) St. Antonius Ziekenhuis, Nieuwegein The Netherlands.

3 - 7:50
Phenotypic and Molecular Analyses of Different *vangl2* Mutants Demonstrates Dominant Effects of the *Looptail* Mutation during Hair Cell Development. Haifeng Yin, Catherine Copley, **Michael Deans**. Dept. of Otolaryngology/HNS, Johns Hopkins University School of Medicine, Baltimore, MD.

4 - 8:10
Host Resistance To A Gastrointestinal Parasite Regulated By A SNP Modulating SMAD3 Binding To A TGFβ-Responsive Transcriptional Enhancer Of Mina. Mark Bix[1,3], Meenu Pillai[1,3], Shangli Lian[1], Peter Vogel[1], Mathew Coleman[2], Peter Ratcliffe[2]. 1) Immunology, St. Jude Children's Research Hospital, Memphis, TN; 2) Oxford University, Oxford, UK; 3) Equal contribution.

5 - 8:30
Discovering genes and pathways involved in naevus and melanoma development using the Collaborative Cross. Graeme Walker[1], Ramesh Ram[2], Blake Ferguson[1], Herlina Handoko[1], Peter Soyer[3], Grant Morahan[2]. 1) Skin Carcinogenesis Lab, QIMR, Brisbane, Qld, Australia; 2) Centre for Diabetes Research, WAIMR, The University of Western Australia, Perth, Australia; 3) Dermatology Research Centre, UQ School of Medicine, Brisbane QLD.

8:50 pm - **Break**

6 - 9:10
The (uro)chordate-specific *Gumby* gene governs angiogenesis and modulates Wnt signaling. Elena Rivkin[1,2], Stephanie M. Almeida[1,2], Teresa A. MacLean[1,2], Gang Xie[1], **Sabine P. Cordes**[1,2]. 1) Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada; 2) Department of Molecular Genetics, University of Toronto, 1 King's Crescent, Toronto, ON Canada.

7 - 9:30
Molecular pathogenesis of Joubert Syndrome and related disorders. Tamara Caspary[1], Holden Higginbotham[2], Laura E. Mariani[1,3], Tae-Yeon Eom[2], Miao Sun[1], Vanessa L. Horner[1], Alyssa B. Long[1], Eva S. Anton[2]. 1) Dept. of Human Genetics, Emory University School of Medicine, Atlanta, GA; 2) The UNC Neuroscience Center, The University of North Carolina School of Medicine, Chapel Hill, NC; 3) Graduate Program in Neuroscience, Emory University, Atlanta, GA.

8 - 9:50
Genetic analysis of Down syndrome in mice. Chunhong Liu[1], Masae Morishima[2], Li Zhang[1], Xiaoling Jiang[1], Kai Meng[1], Annie Pao[1], Michael Parmacek[3], Ping Ye[4], William Mobley[5], Allan Bradlley[6], **Yuejin E. Yu**[1]. 1) Genetics Program, Roswell Park Cancer Institute, Buffalo, NY; 2) Department of Anatomy and Developmental Biology, Tokyo Women's Medical University, Tokyo, Japan; 3) Cardiovascular Institute, University of Pennsylvania, Philadelphia, PA; 4) School of Molecular Bioscinces, Washington State University, Pullman, WA; 5) Department of Neuroscinces, University of California, San Diego, La Jolla, CA; 6) Wellcome Trust Sanger Institute, Hixton, Cambridge, UK.

9 - 10:10
CHD5: Chromosome engineering, chromatin dynamics, and cancer. Alea A. Mills. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Notes

Wednesday, October 3 8:30 am–12:00 noon
Merrill Hall

Plenary/Platform Session 2 - Patterning

Chair: Hiroshi Sasaki, Kumamoto University

10 - 8:30
Mechanisms of position-dependent specification of cell fates in preimplantation embryos. Hiroshi Sasaki. IMEG, Kumamoto University, Kumamoto, Japan.

11 - 9:00
FGF4 is required for lineage restriction and salt-and-pepper distribution of primitive endoderm program but not its initiation in the mouse. Minjung Kang[1,2], Ania Piliszek[3], Jérôme Artus[4], Anna-Katerina Hadjantonakis[1]. 1) Developmental Biology, Sloan-Kettering Institute, New York, NY; 2) Biochemistry, Cell and Molecular Biology Program, Weill Graduate School of Medical Sciences of Cornell University, New York, NY 10065, USA; 3) Department of Experimental Embryology, Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, 05-552 Wólka Kosowska, Poland; 4) Institut Pasteur, CNRS URA2578, Mouse Functional Genetics Unit, Paris, France.

12 - 9:20
Cell fate decisions regulating stem cell origins during preimplantation mouse development. Amy Ralston[1], Tristan Trum[1], Stephanie Blij[1], Chaoyang Wang[2], Paul Robson[2], Aytekin Akyol[3,4], Eric Fearon[3], Michael A. Halbisen[1], Yoshikazu Hirate[5], Hiroshi Sasaki[5], Tony Parenti[1], Eryn Wicklow[1]. 1) Molecular, Cell, and Developmental Biology, UCSC, Santa Cruz, CA; 2) Genome Institute of Singapore, Singapore; 3) Division of Molecular Medicine and Genetics, University of Michigan Medical School; 4) Department of Pathology, Hacettepe University School of Medicine, Ankara, Turkey; 5) Department of Cell Fate Control, Institute of Molecular Embryology and Genetics, Kumamoto University, Japan.

13 - 9:40
The T-box factor Eomesodermin is required in the visceral endoderm for proper axis specification in the early mouse embryo. Sonja Nowotschin[1], Gloria Kwon[1], Evan Weiner[1], Anna Piliszek[1], Chai-an Mao[2], Kat Hadjantonakis[1]. 1) Developmental Biology, Sloan-Kettering, New York, NY 10065, USA; 2) Department of Biochemistry and Molecular Biology, University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA.

10:00 am - **Break**

14 - 10:20
How does retinoic acid regulate progenitor cell maintenance and differentiation in the nervous system and sensory organs? Pascal Dolle[1], Monika Rataj Baniowska[1], Muriel Rhinn[1], Wojciech Krezel[1], Karen Niederreither[2], Raj Ladher[3], Marie Paschaki[1]. 1) IGBMC, Strasbourg, France; 2) Dell Pediatric Research Institute, Austin, TX, USA; 3) RIKEN Center for Developmental Biology, Kobe, Japan.

15 - 10:40
The Wnt3a/β-catenin target Mesogenin1 (Msgn1) is a master regulator of presomitic mesoderm differentiation. Ravi B. Chalamalasetty[1], William C. Dunty Jr.[2], Wuhong Pei[3], Kristin K. Biris[1], Rieko Ajima[1], Benjamin Feldman[3], Terry P. Yamaguchi[1]. 1) Cancer and Developmental Biology Laboratory, Frederick National Laboratory, NIH, Frederick, MD; 2) Division of Metabolism and Health Effects, NIAAA, NIH, Bethesda, MD; 3) Medical Genetics Branch, Vertebrate Embryology Section, NHGRI, NIH, Bethesda, MD.

16 - 11:00
ENU-induced disruption of murine ESCRTII complex results in enhancement of the Fgf-Shh signaling loop with polydactyly. Karen Handschuh[1], Matthew Koss[1], Elisabetta Ferretti[1], Michael Depew[2], John Manak[3], Kathryn Anderson[4], Elizabeth Lacy[4], **Licia Selleri**[1]. 1) Dept of Cell and Developmental Biology, Weill Cornell Medical College, NY, NY, USA; 2) Dept of Craniofacial Development, King's College, London, UK; 3) Roy J. Carver Center for Genomics, University of Iowa, Iowa City, IA, USA; 4) Program of Developmental Biology, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, NY, NY, USA.

17 - 11:20
"Diverse roles of Fibroblast Growth Factor signaling during somitogenesis and axis extension". Mark Lewandoski. Frederick National Lab for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick, MD 21702, USA.

Notes

Wednesday, October 3 2:00 pm–3:00 pm
Merrill Hall

Beddington Lecture

18 - 2:00
Hox genes and hindbrain patterning: A story in segments. Robb Krumlauf[1,2], Youngwook Ahn[1], Christof Nolte[1], Hugo Parker[1], Marina Yurieva[1], Bony DeKumar[1]. 1) Stowers Institute, Kansas City, MO; 2) Department of Anatomy and Cell Biology, Kansas University Medical School, Kansas City, KS.

Wednesday, October 3 7:00 pm–10:40 pm
Merrill Hall

Plenary/Platform Session 3 - Stem Cells and Germ Cells

Chair: Frederic de Sauvage, Genentech, Inc.

19 - 7:00
Targeting developmental pathways in cancer cells and stem cells. Frederic J. de Sauvage. Genentech Inc., So. San Francisco, CA.

20 - 7:30
Lineage analysis of basal epithelial cells reveals their unexpected plasticity and supports a cell of origin model for prostate cancer heterogeneity. Zhu Wang[1,4], Antonina Mitrofanova[2,4], Sarah Bergren[1,4], Cory Abate-Shen[3,4], Andrea Califano[2,4], Michael Shen[1,4]. 1) Depts of Medicine and Genetics and Development, Columbia U Med Center, New York; 2) Department of Biomedical Informatics and Center for Computational Biology and Bioinformatics, Columbia University Medical Center, New York, NY; 3) Departments of Urology and Pathology and Cell Biology, Columbia University Medical Center, New York, NY; 4) Herbert Irving Comprehensive Cancer Ctr, Columbia U Med Ctr, NY.

21 - 7:50
Wnt/beta-catenin, BMP and HGF in cancer stem cells. Walter Birchmeier[1], Peter Wend[1,2], Ulrike Ziebold[1], Jane Holland[1]. 1) Max-Delbrueck-Center for Molecular Medicine (MDC), 13125 Berlin, Germany; 2) David Geffen School of Medicine, UCLA.

22 - 8:10
Discovery of a novel, developmentally restricted hematopoietic stem cell. Anna Beaudin, Scott Boyer, **Camilla Forsberg**. Inst for the Biology of Stem Cells, Dept of Biomolecular Engineering, University of California Santa Cruz.

23 - 8:30
A defensive role of Sirt3 against oxidative stress and aging in preimplantation embryos. Yumiko Kawamura[1,2,3], Yasunobu Uchijima[1], Koichi Nishiyama[1], Yukiko Kurihara[1], Kiyoshi Kita[2], Hiroki Kurihara[1]. 1) Dept. of Phys. Chem. and Meta., Grad. Sch. of Med., the Univ. of Tokyo, Tokyo, Japan; 2) Dept. of Biom. Chem., Grad. Sch. of Med., the Univ. of Tokyo, Tokyo, Japan; 3) JSPS Research Fellow.

8:50 pm - **Break**

24 - 9:10
The mouse Y-encoded transcription factor ZFY promotes the second meiotic division and is essential for sperm tail development and head remodelling Nadege Vernet, Fanny Decarpentrie, Shantha Mahadevaiah, Paul Burgoyne. Stem cell biology and developmental genetics, NIMR, Medical Research Council, UK, London.

25 - 9:30
FGFR2IIIc Is Required for Testis Determination but Is Dispensable for Subsequent Testis Cord Differentiation and Spermatogenesis. Stefan Bagheri-Fam[1], Meiyun Yong[1], Anja Dietrich[1], Terje Svingen[2], Peter Koopman[2], Veraragavan Eswarakumar[3], **Vincent R. Harley**[1]. 1) Molecular Genetics & Development, Prince Henry's Inst, Clayton, VIC, Australia; 2) Institute for Molecular Bioscience, University of Queensland, QLD, Australia; 3) School of Medicine, Yale.

26 - 9:50
System-level analysis of sperm maturation in the epididymis. Timothy Karr[1], Sheri Skerget[1], Konstantino Petritis[2], Ashoka Polpitiya[2]. 1) Biodesign Inst, Arizona State Univ, Tempe, AZ; 2) Center for Proteomics, Translational Genomics Research Institute, Phoenix, AZ.

27 - 10:10
Sonic hedgehog-expressing basal stem cells are the cell-of-origin for bladder cancer. Philip A. Beachy, Kunyoo Shin, Agnes Lim, Sally Kawano. Stanford Univ Sch Medicine, Stanford, CA.

Notes

Thursday, October 4 8:30 am–12:00 noon
Merrill Hall

Plenary/Platform Session 4 - Epigenetics

Chair: Guo-Liang Xu, Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences

28 - 8:30
DNA Oxidation towards Totipotency in Mammalian Development. Guo-Liang Xu. Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China.

29 - 9:00
DNA dioxygenases regulate cardiac progenitor pool and their differentiation in heart development. Qing-Yan Cui[1], Guo-Liang Xu[1], Bin Zhou[2]. 1) Group of DNA Metabolism, The State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China; 2) Key laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Graduate School of the Chinese Academy of Sciences, Shanghai 200031, China.

30 - 9:20
Dissection of the molecular mechanism that regulates activation of murine IAP (intracisternal A particles) retrotransposons upon DNA demethylation in ES cells. Jafar Sharif[1], Takaho A. Endo[2], Kayoko Katsuyama[1], Yoko Mizutani-Koseki[1], Yoko Kuroki[1], Tomoyuki Ishikura[1], Takanori Hasegawa[1], Osamu Ohara[1], Tetsuro Toyoda[2], Yoichi Shinkai[3], Haruhiko Koseki[1]. 1) RIKEN Center for Allergy & Immunology (RCAI), Japan; 2) RIKEN Bioinformatics and Systems Engineering Division (BASE), Japan; 3) RIKEN Advanced Science Institute (ASI), Japan.

31 - 9:40
Alterations in genomically imprinted noncoding RNA clusters in a mouse model of Fetal Alcohol Spectrum Disorders (FASD). Benjamin Laufer, Janus Katherine, Kleiber Morgan, Eric Diehl, Sean Addison, Shiva Singh. Biology, Western University, London, Ontario, Canada.

10:00 am - **Break**

32 - 10:20
MeCP2 Interacting Partners in Development and Rett Syndrome. Mary Donohoe[1,2,3], Siva Muthuswamy[1,2,3], Tao Wu[1,2,3]. 1) Burke Medical Research Institute, White Plains, NY; 2) Dept of Neuroscience Weill Cornell Medical College New York, NY; 3) Dept of Cell and Developmental Biology Weill Cornell Medical College New York, NY.

33 - 10:40
The histone H2B ubiquitin ligase Rnf20 is required for self-renewal and pluripotency in mouse ES cells. Kit Wan Ma, Takaho A. Endo, Jafar Sharif, Haruhiko Koseki. RCAI, RIKEN Yokohama Institute, Yokohama, Kanagawa, Japan.

34 - 11:00
Upregulation of the mammalian X chromosome is associated with enhanced transcription initiation and epigenetic modifications. Xinxian Deng[1], Joel Berletch[1], Joseph Hiatt[2], Di Kim Nguyen[1], Jay Shendure[2], Christine Disteche[1,3]. 1) Dept Pathology, Univ Washington, Seattle, WA; 2) Dept Genome Sciences, Univ Washington, Seattle, WA; 3) Dept Medicine, Univ Washington, Seattle, WA.

35 - 11:20
Nuclear transfer for the study of X chromosome inactivation in mice. Atsuo Ogura[1,2], Shogo Matoba[1], Mami Oikawa[1,3], Kimiko Inoue[1,2], Fumitoshi Ishino[3]. 1) BioResource Center, RIKEN, Tsukuba, Ibaraki, Japan; 2) Graduate School of Life and Environmental Science, University of Tsukuba, Ibaraki, Japan; 3) Department of Epigenetics, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan.

Notes

Thursday, October 4 2:00 pm–3:00 pm
Merrill Hall

Keynote Address

36 - 2:00
Development of Hematopoietic Stem Cell: A final scenario. Shinichi Nishikawa. Riken Center for Developmental Biology, Kobe, Kobe, Japan.

Thursday, October 4 7:00 pm–10:20 pm
Merrill Hall

Plenary/Platform Session 5 - Technology

Chair: Anton Wutz, Wellcome Trust Centre for Stem Cell Research

37 - 7:00
Characterization of haploid embryonic stem cells from mouse embryos. Anton Wutz, Martin Leeb. Wellcome Trust Centre for Stem Cell Research, Cambridge, Cambridgeshire, United Kingdom.

38 - 7:30
MFA: A Novel Method For The Generation Of "All-In-One" Null And Conditional Alleles. Christopher Schoenherr, Evangelos Pefanis, Peter Lengyel, Darshi Persaud, YuHong Zhang, Ronald Deckelbaum, Julie Kalter, Dimitrios Skokos, Peter Yang, Andrew J. Murphy, **Aris N. Economides**. Regeneron Pharmaceuticals, Tarrytown, NY.

39 - 7:50
Efficient generation of a conditional knock-out allele in mice by zinc finger nuclease-mediated gene targeting. Keith Anderson[1], Jeremy Burton[1], Tuija Alcantar[2], Jinjie Li[2], Robert Schwingendorf[2], Tim Soukup[1], Merone Roose-Girma[1], J. Colin Cox[2], Xin Rairdan[2], Weilan Ye[1], **Soren Warming**[1]. 1) Department of Molecular Biology, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080; 2) Department of Mouse Genetics, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080.

40 - 8:10
Next generation RNAi mouse models for drug discovery and toxicology assessment. Prem Premsrirut[1], Christof Fellmann[1], Lukas Dow[2], Johannes Zuber[3], Gregory Hannon[4], Scott Lowe[2]. 1) Research & Development, Mirimus Inc., Cold Spring Harbor, NY; 2) Cancer Biology, Memorial Sloan Kettering Cancer Center, New York, NY; 3) Differentiation and Disease, Institute of Molecular Pathology, Vienna, Austria; 4) Bioinformatics and Genomics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

8:30 pm - **Break**

41 - 8:50
Constructing functional genetic networks in mammalian cells. Christopher Kemp, Russell Moser, Michael Kao, Chang Xu, Carla Grandori, Eddie Mendez. Fred Hutchinson Cancer Research Center, Seattle, WA.

42 - 9:10
Hot or Not?: Leveraging mouse genome diversity to identify hotspots of copy number variants. Kathleen A. Hill[1], M. Elizabeth O. Locke[2], Andrea E. Wishart[3], Susan T. Eitutis[3], Jenna Butler[2], Mark Daley[1]. 1) Department of Biology and Computer Science, The University of Western Ontario, London, ON, Canada; 2) Department of Computer Science, The University of Western Ontario, London, ON, Canada; 3) Department of Biology, The University of Western Ontario, London, ON, Canada.

43 - 9:30
PRISM.stanford.edu: 2,500 Human and Mouse transcription factor developmental function predictions. Aaron Wenger[1], Shoa Clarke[2], Jenny Chen[3], Cory McLean[1], **Gill Bejerano**[1,4]. 1) Computer Science, Stanford Univ, Stanford, CA; 2) Genetics, Stanford Univ, Stanford, CA; 3) Biomedical Informatics, Stanford Univ, Stanford, CA; 4) Developmental Biology, Stanford Univ, Stanford, CA.

44 - 9:50
Generation of rat-mouse interspecific chimeras for study of organogenesis and elucidation of xenogenic barrier. Hiromitsu Nakauchi[1,2]. 1) Division of Stem Cell Therapy, Center for Stem Cell Biology and Regeneration Medicine, Institute of Medical Science, University of Tokyo, Japan; 2) Japan Science Technology Agency, ERATO, Nakauchi Stem Cell and Organ Regeneration Project.

Notes

Friday, October 5 8:30 am–12:00 noon
Merrill Hall

Plenary/Platform Session 6 - Organogenesis

Chair: Lori Sussel, Columbia University Medical Center

45 - 8:30
Regulation of pancreatic islet lineage decisions. Lori Sussel, James Papizan, Josh Levine. Genetics and Development Department, Columbia University, New York, NY.

46 - 9:00
Regulation of thymus and parathyroid organ fate specification by Shh and Tbx1. Nancy R. Manley[1], Virginia Bain[1], Julie Gordon[1], Kaitlin Gutierrez[2], Kim Cardenas[2], Ellen R. Richie[2]. 1) Department of Genetics, University of Georgia, Athens, GA; 2) Department of Carcinogenesis, University of Texas, M.D. Anderson Cancer Center, Science Park Research Division, Smithville, TX.

47 - 9:20
Growth Factor Signaling Pathways in Lung Development and Cancer. David M. Ornitz[1], Yongjun Yin[1], Ashley Hill[2]. 1) Developmental Biology, Washington University, St. Louis, MO; 2) Children's National Medical Center, Washington DC.

48 - 9:40
Genome-wide microRNA and mRNA profiling in mouse liver development implicates mir302b and mir20a in repressing TGFβ signaling. Wei Wei[1], Juan Hou[1], Olivia Alder[1], Xin Ye[3], Sam Lee[1,4], Rebecca Cullum[1], Andy Chu[2], Yongjun Zhao[2], Stephanie Warner[3,4], Darryl Knight[3,4], Decheng Yang[3,4], Steven Jones[2,4], Marco Marra[2,4], **Pamela Hoodless**[1,4]. 1) Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada; 2) Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, Canada; 3) The Institute for Heart and Lung Health, St. Paul's Hospital, Vancouver, BC, Canada; 4) University of British Columbia, Vancouver, BC, Canada.

10:00 am - **Break**

49 - 10:20
Taking a different pathway: Notchless plays with p53 and Wnt during embryogenesis. Amy C. Lossie[1,2], Chiao-Ling Lo[1,3], Jeremy Sherrill[4]. 1) Dept Animal Sci, Purdue Univ, West Lafayette, IN; 2) Dept of Medicine, Indiana University School of Medicine, Indianapolis, IN; 3) PULSe Interdisciplinary Graduate Program, Purdue University, West Lafayette, IN; 4) Department of Biological Sciences, Purdue University, West Lafayette, IN.

50 - 10:40
Gadd45 functions in male sex determination by promoting p38 signaling and *Sry* expression. Wolfram H. Gruhn[1,2], Mathias S. Gierl[1], Annika von Seggern[1], Nicole Maltry[3], Christof Niehrs[1,2]. 1) Institute of Molecular Biology, 55128 Mainz, Germany; 2) DKFZ-ZMBH Alliance, Division of Molecular Embryology, DKFZ, 69120 Heidelberg, Germany; 3) Division of Cellular Immunology, DKFZ, 69120 Heidelberg, Germany.

51 - 11:00
***Tbx20* and *Tbx3* act independently of each other in the developing heart. Virginia E. Papaioannou**[1], Svetlana Gavrilov[1,2]. 1) Genetics and Development, Columbia University, New York, NY; 2) Developmental Biology Program, Sloan-Kettering Institute, New York, NY.

52 - 11:20
Genetic pathways required for maintenance of vascular smooth muscle phenotype. Sylvia M. Evans[1], Nuno Guimaraes-Camboa[1,2], Chase Bolt[3], Lisa Stubbs[3]. 1) Skaggs School of Pharmacy, Department of Medicine, University of California San Diego, La Jolla, CA; 2) Biomedical Sciences Institute Abel de Salazar, Graduate Program in Areas of Basic and Applied Biology, University of Porto, Portugal; 3) Institute for Genomic Biology, Department of Cell and Developmental Biology, University of Illinois, Urbana, IL.

Notes

Friday, October 5 2:00 pm–3:40 pm
Merrill Hall

Plenary/Platform Session 7 - Imaging

Chair: Mary Dickinson, Baylor College of Medicine

53 - 2:00
Optical Imaging of the Embryonic Mammalian Cardiovascular System. Mary E. Dickinson. Molecular Physiology & Biophysics, Baylor College of Medicine, Houston, TX.

54 - 2:30
Imaging techniques in *Drosophila* to delineate candidate roles of calcium in murine lung and neural development. Danielle V. Bower[1], Scott E. Fraser[1], Edwin C. Jesudason[2]. 1) Biology and the Biological Imaging Center, California Institute of Technology, Pasadena, CA; 2) Department of Surgery, Children's Hospital Los Angeles, Los Angeles, CA.

55 - 2:50
Live imaging the pluripotent state *in vitro* and *in vivo*. Panagiotis Xenopoulos[1], Minjung Kang[1,2], Anna-Katerina Hadjantonakis[1]. 1) Developmental Biology Program, Sloan-Kettering Institute, New York, NY; 2) Biochemistry, Cell and Molecular Biology Program, Weill Graduate School of Medical Sciences of Cornell University, New York, NY.

56 - 3:10
Imaging of cellular dynamics underlying the immune tissue organization. Takaharu Okada[1,2]. 1) Research Unit for Immunodynamics, RCAI, RIKEN, Yokohama, Kanagawa, Japan; 2) PRESTO, Japan Science and Technology Agency, Tokyo, Japan.

Saturday, October 6 8:30 am–12:00 noon
Merrill Hall

Plenary/Platform Session 8 - Genetics and Genomics

Chair: Kent Lloyd, University of California

57 - 8:30
KOMP2...the next phase of the Knockout Mouse Project. Kent Lloyd. Mouse Biology Program, Univ California Davis, Davis, CA.

58 - 9:00
High-throughput mutant mouse phenotyping is a powerful tool to generate novel hypotheses. Chris Lelliott, Jeanne Estabel, Anna Karin Gerdin, Antonella Galli, Anneliese Speak, Richard Houghton, Joanna Bottomley, Edward Ryder, Ramiro Ramirez-Solis, David Adams, Jacqueline White. The Sanger Mouse Genetics Project, Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, United Kingdom.

59 - 9:20
Recent specialization of the human and mouse X chromosomes for the male germline. Jacob L. Mueller[1], Helen Skaletsky[1], Laura G. Brown[1], Sara Zaghlul[1], Susan Rock[2], Tina Graves[2], Katherine Auger[3], Wesley C. Warren[2], Richard K. Wilson[2], David C. Page[1]. 1) 1Howard Hughes Medical Institute, Whitehead Institute, MIT, Cambridge, MA; 2) The Genome Institute, Washington University School of Medicine, St. Louis, MO; 3) The Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

60 - 9:40
Expression QTL mapping in murine macrophages identifies novel genes and gene networks mediating resistance to *Toxoplasma gondii* and responsiveness to IFNγ/TNF*α*. Musa Hassan, Kirk Jensen, Jeroen Saeij. Dept. of Biology, MIT, Cambridge, MA.

10:00 am - **Break**

61 - 10:20
The Gene Expression Database for Mouse Development (GXD). Martin Ringwald, Constance Smith, Jacqueline Finger, Terry Hayamizu, Jingxia Xu, Ingeborg McCright, Janan Eppig, James Kadin, Joel Richardson. The Jackson Laboratory, Bar Harbor, ME.

62 - 10:40
RNAseq Analysis of 32 KOMP Mutant Mouse Lines. David West[1,2], Andreana Cipollone[2], Michael Adkisson[1], Jared Rapp[2], Eric Engelhard[2], Pieter de Jong[1], Kent Lloyd[1]. 1) Childrens Hospital of Oakland Research Institute, Oakland, CA; 2) University of California, Davis, CA.

63 - 11:00
Coordinated activation of multiple tumor suppressor pathways in benign skin tumors identified by correlation analysis of *Pten* gene expression. David Quigley, Ihn Young Song, Allan Balmain. Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA.

64 - 11:20
A resource of vectors and ES cells for targeted deletion of microRNAs in mice. Haydn M. Prosser, Hiroko Koike-Yusa, Chukwuma A. Agu, James D. Cooper, Frances C. Law, Allan Bradley. Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

Notes

Epigenetics

65

An evaluation of parent-of-origin bias and individual variation in the midgestation mouse placenta. Elizabeth H. Finn, Cheryl Smith, Arend Sidow, Julie C. Baker. Department of Genetics, Stanford University, Stanford, CA.

66

Gene silencing by the *in vivo* dynamics of Polycomb repressive complex PRC1. Kyoichi Isono[1,2], Haruhiko Koseki[1]. 1) RIKEN RCAI, Yokohama, Japan; 2) JST, PREST, Yokohama, Japan.

67

Concerted alterations of promoter-enhancer-PRE association in transcription status transition of *Meis2* during midbrain development. Takashi Kondo, Haruhiko Koseki. Department of Developmental Genetics, RIKEN-RCAI, Yokohama, Kanagawa, Japan.

68

The Histone Acetyltransferase MOF is a Key Regulator of the Embryonic Stem Cell Core Transcriptional Network. Xiangzhi Li[1], Li Li[2], Yali Dou[3]. 1) Institute of Cell Biology, School of Medicine, Shandong University, Jinan, China; 2) Rollins School of Public Health, Emory University, Atlanta, GA 30322; 3) Department of Pathology, University of Michigan, Ann Arbor, MI 48109.

Genetics and Genomics

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Construction of reciprocal chromosome substitution strains from 129P3/J and C57BL/6ByJ mice. Alexander A. Bachmanov, Cailu Lin, Natalia P. Bosak, Theodore M. Nelson, Maria L. Theodorides, Zakiyyah H. Smith, Matthew T. Kirkey, Mauricio Avigdor, Brian R. Gantick, M. Amin Khoshnevisan, Anna Lysenko, Danielle R. Reed. Monell Chemical Senses Center, Philadelphia, PA.

70

Generation of CreERT2 transgenic mouse lines for time and cell specific conditional gene inactivation Validation of 3 pancreas specific lines : Insulin1, Glucagon et Elastase-CreER^{T2}. Marie-Christine Birling[1], Lydie Venteo[1], Olivia Wendling[1,2], Nathalie Chartoire[1], Marie-France Champy[1], Elodie Bedu[1], Tania Sorg[1], Yann Hérault[1,2], Guillaume Pavlovic[1]. 1) Genetic Engineering, Institut Clinique de la Souris, 1, rue Laurent Fries, 67400 Illkirch, France; 2) IGBMC, 1, rue Laurent Fries, 67400 Illkirch, France.

71

Developmental dynamics of the Tbx18 locus and its downstream transcriptional targets. C. Chase Bolt[1], Xiaochen Lu[1], Laura Chittenden[1], Nuno Camboa[2], Sylvia Evans[3], Lisa J. Stubbs[1]. 1) Dept. of Cellular and Developmental Biology, Institute for Genomic Biology, University of Illinois - Urbana/Champaign, Urbana, IL; 2) Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, CA, GABBA Graduate Program and ICBAS, University of Porto, Portugal; 3) Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, CA.

72

Somatic mosaicism detected using the Mouse Diversity Genotyping Array reveals tissue-specific mutation patterns associated with the *harlequin* phenotype. Susan T. Eitutis, Andrea E. Wishart, Kathleen A. Hill. Department of Biology, The University of Western Ontario, London, ON, Canada.

73

CRY1-PHR(313-426): A Key Domain for Negative Feedback Repression and Circadian Clock Function in Mice. Sanjoy Kumar Khan[1], Haiyan Xu[1], Maki Ukai-Tadenuma[2], Hiroki Ueda[2], Andrew Liu[1]. 1) Biological Sciences, University of Memphis, Memphis, TN; 2) RIKEN Center for Developmental Biology, Kobe, Japan.

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Evaluation of Genetic And Genomic Similarity of Inbred Strains of Mice Employing Microsatellite Markers. Mahadeo Kumar[1], Sharad Kumar[1], Akshay Dwarakanath[1], Dinesh Purohit[1], Daya

Shankar Upadhayay[2]. 1) Animal Facility, CSIR-Indian Institute of Toxicology Research, M. G. Marg. Lucknow, India; 2) Animal Facility, Central Drug Research Institute, Lucknow.

75

Use of closely related mouse substrains to clone a QTL for psychostimulant response. Vivek Kumar[1,4], Gary Churchill[2], Fernando Pardo-Manuel de Villena[3], Joseph Takahashi[1,4]. 1) Dept of Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX; 2) The Jackson Laboratory, Bar Harbor, ME, USA; 3) Dept of Genetics, UNC-Chapel Hill, Chapel Hill, NC, USA; 4) Howard Hughes Medial Institute.

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The regulation of gene expression in a cell via DNA- lipids complexes formation. Vasily V. Kuvichkin. Mechanisms of Reception, Institute of Cell Biophysics, Russian Ac. Sci., Pushchino, Moscow oblast, Russian Federation.

77

Demonstrating Resistance-Mitigrating Effect of Artemisia Annua Phytochemical Blend with in-Vitro Cultures of Plasmodium Falciparum and in-vivo with Plasmodium Berghei Anka in Mice. Kangethe N. Lucy[1,3,4], Ahmed Hassanali[2], Sabah Omar[3], Nganga Joseph[4], Kinyua Johnson[5]. 1) LUCY N KANGETHE KENYA POLYTECHTECHNIC UNIVERSITY COLLEGE, NAIROBI, Kenya P.O BOX 52428--00200; 2) AHMED HASSANALI International Centre of Insect Physiology and Ecology P.O BOX 30179 Nairobi, Kenya; 3) SABAH OMARKenya Medical Research Institute P.O BOX 54840-00200 Nairobi, Kenya; 4) JOSEPH K NGANGA Jommo Kenyatta University of Agriculture and Technology P.O BOX 62000 Nairobi,Kenya; 5) JOHNSON KINYUA Jommo Kenyatta University of Agriculture and Technology P.O BOX 62000 Nairobi,Kenya.

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Subtelomere recombination is frequent in B-cell lymphomas lacking telomerase. Tammy A. Morrish[1], Joshua Budman[2], Stephen Dria[2], Vivek Behera[1], Margaret Strong[1], Sarah Wheelan[3], Carol Greider[1]. 1) Molecular Biology and Genetics, Johns Hopkins University, Baltimore, MD; 2) Biomedical Engineering; 3) Oncology and Biostatistics.

79

Evolution of Dosage Compensation of the Mammalian Active X Chromosome. Di Kim Nguyen, Xinxian Deng, Christine Disteche. Pathology, University of Washington, Seattle, WA.

80

C57BL/6NTac double and single albino mice generation for efficient germline transmission of Chimera. Ana V. Perez[1], Gunther Kauselmann[2], Maria R. Da Silva[2], Heidrun Kern[2], Nathalie Uyttersprot[2], Gerald Bothe[3], Branko Zevnik[2]. 1) Genetic Sciences & Compliance, Taconic, Hudson, NY; 2) Applied Genetics, Taconic Artemis GmbH, Cologne, Germany; 3) R&D, Taconic, Rensselaer, NY.

81

Disentangling the genetic architecture of nest building: a fitness-related trait. Andrea Cristina Peripato[1,2], Bruno Sauce[1], Reinaldo Alves de Brito[1]. 1) Genetics and Evolution, University of Sao Carlos, Sao Carlos, SP, Brazil; 2) Biosciences, University of Sao Paulo, Santos, SP, Brazil.

82

Genetic Studies of Inflammatory Responses in Wild-Derived Mice. Alexander N. Poltorak. Tufts University, Boston, MA.

83

Comprehensive molecular characterization of mutant mouse strains generated from the EUCOMM / KOMP ES cell resource. Ed Ryder, Diane Gleeson, Debarati Sethi, Sapna Vyas, Evelina Miklejewska, Priya Dalvi, Bishoy Habib, Ross Cook, Matthew Hardy, Joanna Bottomley, David Adams, Ramiro Ramirez-Solis, Sanger Mouse Genetics Project. Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

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An albino C57BL/6N strain for EUCOMM/KOMP mouse generation in a pure genetic background. Ed Ryder, Diane Gleeson, Thomas Keane, Debarati Sethi, Sapna Vyas, Hannah Wardle-Jones, James Bussell, Richard Houghton,

Jennifer Salisbury, David Adams, Ramiro Ramirez-Solis, Sanger Mouse Genetics Project. Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

85

The transcription factors *Ets1* and *Sox10* interact during murine melanocyte development. Amy Saldana-Caboverde, Lidia Kos. Biological Sciences, Florida International University, Miami, FL.

86

Essential benchmarks for use of Cre-lox mouse genetic tools. Ramkumar Sambasivan[1,2], Glenda Comai[1], Shahragim Tajbakhsh[1]. 1) Institut Pasteur, Stem Cells and Development, CNRS URA 2578, 25 rue du Dr. Roux, 75724 Paris Cedex 15; 2) Institute for Stem Cell Biology and Regenerative medicine, NCBS, GKVK PO, Bellary Road, Bangalore 560065.

87

Genome-wide mapping of gene-microbiota interactions in susceptibility to autoimmune skin blistering. Girish Srinivas[1,2], Steffen Möller[2], Sven Künzel[1], Jun Wang[1,3], Detlef Zillikens[2], John Baines[1,3,4], Saleh Ibrahim[2,4]. 1) Max Planck Institute for Evolutionary Biology, Plön, Germany; 2) Department of Dermatology, University of Lübeck, Lübeck, Germany; 3) Institute for Experimental Medicine, University of Kiel, Kiel, Germany; 4) These authors contributed equally to this work.

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The chromatin remodeler Chd5 modulates neuronal morphology and behavior. Assaf Vestin[1,2], Guy Horev[1], Wangzhi Li[1,2], Alea Mills[1]. 1) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 2) Stony Brook University, Stony Brook, NY.

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Patterns of recurrent and tissue-specific copy number variants in the mouse genome. Andrea E. Wishart[1], M. Elizabeth O. Locke[2], Susan T. Eitutis[1], Kathleen A. Hill[1]. 1) Department of Biology, The University of Western Ontario, London, Ontario, Canada; 2) Department of Computer Science, The University of Western Ontario, London, Ontario, Canada.

Imaging

90

Live cell imaging of random X-inactivation. Osamu Masui[1,2], Isabelle Bonnet[2], Patricia Le Baccon[2], Isabel Brito[2], Tim Pollex[2], Niall Murphy[2], Philippe Hupé[2], Emmanuel Barillot[2], Andrew Belmont[3], Haruhiko Koseki[1], Edith Heard[2]. 1) RIKEN, Yokohama, Japan; 2) Curie Institute, Paris, France; 3) University of Illinois, Urbana, IL, USA.

91

Sphingosine-1-phosphate receptor 2 expression retains follicular helper T cells in germinal centers. Saya Moriyama[1], Noriko Takahashi[1], Jesse A. Green[2], Masato Kubo[3], Jason G. Cyster[2,4], Takaharu Okada[1]. 1) Research Unit for Immunodynamics, RCAI, RIKEN, Yokohama, Kanagawa, Japan; 2) Department of Microbiology and Immunology, University of California, San Francisco, CA, USA; 3) Laboratory for Signal Network, RCAI, RIKEN, Yokohama, Kanagawa, Japan; 4) Howard Hughes Medical Institute, University of California, San Francisco, CA, USA.

92

A single-cell resolution Notch signaling reporter strain of mice. Sonja Nowotschin, Panos Xenopoulos, Evan Weiner, Kat Hadjantonakis. Developmental Biology, Sloan-Kettering, New York, NY 10065, USA.

Models of Human Disease

93

Genetic deletion of p66^{Shc} adaptor protein leads to increased myocardial infarction size. Alexander Akhmedov[1], Vincent Braunersreuther[2], Fabrizio Montecucco[2], Philip Jakob[1], Giovanni G. Camici[1], Francois Mach[2], Thomas F. Luescher[1]. 1) Institute of Physiology, University of Zurich, Zurich, Switzerland; 2) Division of Cardiology, Faculty of Medicine, Foundation for Medical Researches, Geneva University Hospitals.

94

Endothelial overexpression of LOX-1 protects from *in vivo* arterial thrombosis and modulates TF expression. Alexander Akhmedov[1], Giovani G. Camici[1], Simona Stivala[1], Erik W. Holy[1], Alexander Breitenstein[1], Christine Lohmann[1], Juerg-Hans Beer[2], Felix C. Tanner[1], Christian M. Matter[1], Thomas F. Luescher[1]. 1) Institute of Physiology, University of Zurich, Zurich, Zurich, Switzerland; 2) Division of Internal Medicine, Kanton Hospital Baden, Switzerland.

95

Investigation of hippocampal dendritic complexity in a potential XLMR mouse model. C. Y. Chen[1], M. S. Tsai[1], C. Y. Lin[1], I. S. Yu[1], Y. C. Hsu[1], C. Y. Kao[1], S. W. Lin[1], L. J. Lee[2,3]. 1) Department of Clinical Laboratory Sciences and Medical Biotechnology National Taiwan University; 2) Graduate Institute of Anatomy and Cell Biology National Taiwan University; 3) Graduate Institute of Brain and Mind Sciences National Taiwan University.

96

Maged1 deficiency results in increased anxiety-like behaviors, disturbed sexual behaviors and late onset obesity associated with reduced levels of hypothalamic oxytocin. Carlos Dombret[1], Tuan Nguyen[1], Mathieu Bertrand[2], Jacques Michaud[3], Hélène Hardin-Pouzet[4], Olivier Schakman[5],**Olivier RY De Backer**[1]. 1) URPHYM, NARILIS, FUNDP school of Medicine, University of Namur,5000 Namur, Belgium; 2) Molecular Signaling and Cell Death Unit, Department for Molecular Biomedical Research, VIB, 9052 Ghent, Belgium; 3) Université de Montréal, Sainte-Justine Hospital Research Centre, Montréal H3T1C5, Canada; 4) UPMC - Paris 6 University, PMSNC lab, CNRS UMR 7224, INSERM UMRS 952, 75005 Paris, France; 5) Laboratory of Cell Physiology, Institute of Neuroscience IoNS, Université Catholique de Louvain, Brussels B-1200, Belgium.

97

The role of Keratin 76 in epidermal homeostasis and barrier function maintenance. Tia M. DiTommaso[1], Helen Pearson[2], Holger Schluter[2], Pritinder Kaur[2], Patrick Humbert[2], Ian Smyth[1,3]. 1) Biochemistry & Molecular Biology, Monash University, Melbourne, Australia; 2) The Peter MacCallum Cancer Institute, Melbourne, Australia; 3) Department of Anatomy and Developmental Biology, Monash University, Melbourne, Australia.

98

BXD mouse lines as a genetic reference population for the metabolic syndrome. Evan Williams[1], Pénélope Andreux[1], Charles Thomas[2], **Raphael Doenlen**[2], Philippe Cettour-Rose[2], Xavier Warot[2], Robert W. Williams[3], Johan Auwerx[1]. 1) Laboratory of Integrative Systems Physiology, School of Life Sciences, EPFL, Lausanne, Switzerland; 2) Phenotyping Unit, Center of PhenoGenomics, School of Life Sciences, EPFL, Lausanne, Switzerland; 3) Department of Anatomy and Neurobiology, University of Tennessee, Memphis, USA.

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The European Mouse Mutant Archive - EMMA. Sabine Fessele[6], Glauco Tocchini-Valentini[1], Yann Hérault[2,9], Steve Brown[3], Urban Lendahl[4], Jocelyne Demengeot[5], Martin Hrabé de Angelis[6], Paul Flicek[7], Ramiro Ramirez-Solis[8], Lluís Montoliu[10], George Kollias[11], Radislav Sedlacek[12], Raija Soininen[13], Thomas Rülicke[14]. 1) CNR-Monterotondo, CNR Campus "A. Buzzati-Traverso", Monterotondo, Italy (core structure); 2) CNRS - Centre de Distribution, de Typage et d'Archivage Animal (CDTA), Orléans, France; 3) Medical Research Council, MRC-Harwell, Harwell, UK; 4) Karolinska Institutet, Department of Cell and Molecular Biology (KI-CMB), Stockholm, Sweden; 5) Fundaçao Calouste Gulbenkian, Instituto Gulbenkian de Ciencia (FCG-IGC), Oeiras, Portugal; 6) Helmholtz Zentrum München, Institute of Experimental Genetics (HMGU-IEG), Neuherberg, Germany; 7) European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Hinxton, UK; 8) Genome Research Limited, Wellcome Trust Sanger Institute (WTSI), Hinxton, UK; 9) GIE-Centre Européen de Recherche en Biologie et en Médecine, Institut Clinique de la Souris (GIE-CERBM-ICS), Illkirch, France; 10) Consejo Superior de Investigaciones Científicas, Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain; 11) B.S.R.C. Alexander Fleming, Vari/Athens, Greece; 12) Institute of Molecular

Genetics (IMG), Prague, Czech Republic; 13) University of Oulu - Biocenter Oulu, Oulu, Finland; 14) University of Veterinary Medicine - Biomodels Austria (BIAT), Vienna, Austria.

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Occult clonal expansion as a mechanism of intestinal tumor initiation and prevention. Jared Michael Fischer[1], Darryl Shibata[2], Michael Liskay[1]. 1) Molecular and Medical Genetics, Oregon Health and Science University, Portland, OR; 2) Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, CA.

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Neural Tube Defects and Epigenetics of Gene Expression in Non-obese Diabetic (NOD) Mice. Claudia Kappen[1], Carly Oetker[2], Claudia Kruger[1], J. Michael Salbaum[2]. 1) Developmental Biology, Pennington Biomedical Research Center, Baton Rouge, LA; 2) Laboratory of Gene Regulation, Pennington Biomedical Research Center, Baton Rouge, LA.

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Impaired Maternal Cardiovascular Adaptation to Pregnancy in HcB-8 Mice. Jasmin Kristianto, Jacqueline Fisher, Shannon Phillips, Michael Johnson, Suzanne Litscher, Robert Blank. University of Wisconsin-Madison, Madison, WI.

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A mouse model of human Branchio-Oculo-Facial Syndrome. Hong Li, Trevor Williams. Department of Craniofacial Biology, University of Colorado Denver AMC, Aurora, CO.

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Heterogeneous behavioral manifestations in a mouse model of fetal alcohol spectrum disorders (FASD): Assessing the effect of gestational time and gene expression. Katarzyna Mantha, Morgan Kleiber, Benjamin Laufer, Shiva Singh. Western University, London, Canada.

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"Secreted protein coding" genes identified by mining the NOD mouse pancreatic lymph node (PLN) transcriptome, before any clinically detectable pancreatic damages. Evie Melanitou[1], Fredj Tekaia[2], Edouard Yeramian[3]. 1) Immunophysiology & Parasitism laboratory, Department of Parasitology and Mycology; 2) Yeast Molecular Genetics Unit, Department of Genomics & Genetics; 3) Structural Bioinformatics Unit, Department of Structural Biology & Chemistry, Institut Pasteur, Paris, France.

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The Jackson Laboratory Repository: Resources for Addressing Human Disease. Darcy Pomerleau, S. Rockwood, C. Lutz, M. Sasner, LR Donahue, The Repository Team. Genetic Resource Science, The Jackson Laboratory, Bar Harbor, ME.

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Infrafrontier - the European infrastructure for the phenotyping, archiving and distribution of model mammalian genomes. Michael Raess, Martin Hrabé de Angelis, the Infrafrontier Consortium. Inst Experimental Genetics, Helmholtz Zentrum Muenchen, Neuherberg, Germany.

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Disruption of a novel mouse gene, *Jhy*, causes juvenile communicating hydrocephalus. Oliver K. Appelbe[1], Bryan Bollman[1], Ali Attarwala[1], Lindy A. Triebes[1], Daniel J. Curry[2], **Jennifer V. Schmidt**[1]. 1) Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL; 2) Department of Neurosurgery, Baylor College of Medicine, Houston, TX.

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Role of miR-155 in BRCA1-mediated tumorigenesis. Suhwan Chang, **Shyam K. Sharan**. Mouse Cancer Genetics Program, Frederick National Lab. NCI, Frederick, MD. USA.

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Conditional deletion of βA3/A1-crystallin in RPE shows decreased lysosomal activity and drusen-like deposits in mouse eyes. Debasish Sinha. Ophthalmology, The Johns Hopkins University School of Medicine, Baltimore, MD.

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Conditional-invertible genetic strategy to understand the role of NIPBL deficiency in the etiology of developmental defects in Cornelia de

Lange Syndrome. Rosaysela Santos[1,3], **Xu Wang**[1,3], Shimako Kawauchi[1,3], Russell Jacobs[4], Martha Lopez-Burks[2,3], Akihiko Muto[2,3], Mona Yazdi[1], Salvador Deniz[1], Samir Qurashi[1], Scott Fraser[4], Thomas Schilling[2,3], Arthur Lander[2,3], Anne Calof[1,2,3]. 1) Dept. of Anatomy & Neurobiology, University of California, Irvine, Irvine, CA; 2) Dept. of Developmental & Cell Biology, University of California, Irvine, Irvine, CA; 3) Center for Complex Biological System, University of California, Irvine, Irvine, CA; 4) Beckman Insititute, California Institute of Technology, Pasadena, CA.

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Production and Characterization of Transgenic Mice Systemically Expressing Endo-β-galactosidase. Satoshi Watanabe[1], Takayuki Sakurai[2], Masako Misawa[3], Takashi Matsuzaki[3], Takashi Muramatsu[4], Masahiro Sato[5]. 1) Animal Genome Reseach Unit, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan; 2) Department of Cardiovascular Research Graduate school of Medicine, Shinshu University, Matsumoto, Nagano, Japan; 3) Department of Biological Science, Faculty of life and Environmental Science, Shimane University, Matsue, Shimane, Japan; 4) Department of Health Science, Faculty of Psychological and Physical Sciences, Aichi Gakuin University, Nisshin, Aich, Japan; 5) Frontier Science Research Center, Kagoshima University, Kagoshima, Japan.

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Studies of cell behaviors regulated by HH-GLI2 signaling during medulloblastomas tumor progression. Alexandre Wojcinski, Alexandra Joyner. developmental biology, Memorial Sloan-Kettering Cancer Center, new york, NY.

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Pancreas-specific Deletion of mouse Gata4 and Gata6 Causes Pancreatic Agenesis. Shouhong Xuan[1], Matthew Borok[1], Kimberly Decker[2], Michele Battle[3], Stephen Duncan[3], Michael Hale[4], Raymond MacDonald[4], Lori Sussel[1]. 1) Department of Genetics & Development, Columbia University, New York, NY 10032; 2) Department of Biochemistry, University of Colorado Medical School, Aurora, CO 80045, USA; 3) Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226, USA; 4) Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, Texas 75390, USA.

Organogenesis

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Essential role of the Runx2-Cbfβ transcription complex for endochondral bone formation. Na-Rae Park[1], Kyung-Eun Lim[1], Jeong-Eun Kim[2], Ichiro Taniuchi[3], Suk-Chul Bae[4], Jae-Hwan Jeong[1], **Je-Yong Choi**[1]. 1) Biochemistry and Cell Biology, Kyungpook National University, Daegu; 2) Molecular Medicine, Kypook National University, Daegu; 3) Laboratory for Transcriptional Regulation, RIKEN Research Center for Allergy and Immunology, Kanagawa; 4) Biochemistry, Chungbuk National University, Cheongju, Republic of Korea.

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Dual embryonic origin of the mouse inner ear. Laina Freyer[1], Vimla Aggarwal[2], Bernice Morrow[1]. 1) Genetics, Einstein College of Medicine, Bronx, NY; 2) Pediatrics, Columbia University Medical Center, New York, NY.

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Identification of novel mouse testis-determining genes in the MAP3K4 pathway. Nick Warr, Gwenn Carre, Pam Siggers, Rachel Brixey, Madeleine Pope, Sara Wells, **Andy Greenfield**. Mammalian Genetics Unit, Harwell, Oxfordshire, United Kingdom.

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Core mesodermal functions of Tbx1 in branchiomeric muscle formation. Ping Kong[1], **Stephania Macchiarulo**[1], Courtney Carpenter[2], Tingwei Guo[1], Silvia Racedo[1], Steven Ola[1], Vimla Aggarwal[1], Deyou Zheng[1,3], Bernice Morrow[1]. 1) Genetics, Einstein College of Medicine, 1301 Morris Park Avenue, Bronx, NY 10461, USA; 2) Department of Surgery, Montefiore Medical Center, 111 East 210th Street, Bronx, NY 10467, USA; 3) Department of Neurology and Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA.

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Additional sex com-like 1 is essential for normal alveolar development in mice. Seung-Tae Moon, Myengmo Kang, Soo-Jong Um. Department of Bioscience & Biotechnology, Institute of Bioscience, BK21 Graduate Program, Sejong university, Seoul, South Korea.

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The Role of BMPs in Regulating Digit Number and Identity. Jacqueline L. Norrie[1], Qiang Li[1], Cortney Bouldin[2], Brian Harfe[2], Steven A. Vokes[1]. 1) The Section for Molecular, Cellular, and Developmental Biology, The University of Texas at Austin, Austin, TX; 2) The Department of Molecular Genetics and Microbiology, The University of Florida, Gainesville, Florida.

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FULL ABSTRACTS

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Models of Human Disease

1
Exploring Neural Tube Closure via Genes, Environment, and Imaging. Lee Niswander. Pediatrics, Univ of Colorado School of Medicine, Aurora, CO.

Neural tube closure requires the spatial and temporal coordination of multiple tissues (neural ectoderm, the neighboring mesenchyme and overlying ectoderm) and multiple developmental processes (proliferation, patterning, etc) to direct the morphogenetic movements that bring the neural folds together to form the primordia of the brain and spinal cord. Defects in any of these processes can lead to neural tube defects (NTDs). To identify novel genes involved in neural tube closure we have undertaken ENU mutagenesis and forward genetic screens in mice. From this we have identified a large number of unexplored genes and determined their mechanisms of action. Recent examples include roles in spatial and temporal regulation of proliferation and the transcriptional regulation of a set of genes involved in epithelial adhesion and apical junction formation suggesting a coordinated regulation of protein expression needed for epithelial-mediated morphogenesis. The etiology of NTDs is complex and involves both genetic and environmental factors. The most effective environmental influence in decreasing the risk for NTDs in humans is folic acid (FA) fortification. However, only a few mouse models have been tested for FA responsiveness and very little is known of the mechanisms underlying FA action or the extent to which molecular pathway information can be applied to determine which mutations may be environmentally sensitive. Although FA supplementation is clearly efficacious in reducing human NTD incidence, contrary to expectations, we found that some mouse genetic mutants respond in a surprisingly negative way to FA supplementation, showing an increased incidence of NTDs in homozygous mutants, occurrence of NTDs in heterozygous embryos and embryonic lethality prior to NT closure. Our unexpected findings highlight the need to understand how FA influences NT closure and the mechanisms and genetics underlying the response to FA supplementation. Neural tube morphogenesis is highly dynamic, yet has largely been studied in static images. Our lab has coupled the ability to culture the mouse embryo with confocal imaging technology and fluorescent lines to provide a dynamic view of NT closure in a living mammalian embryo. This allows a real-time visualization of cell movements and behaviors during NT morphogenesis in the mouse. We are now coupling live imaging with our genetic mutants to visualize how these genetic changes affect the cell behaviors of NT closure.

2
A gene within the modifier locus, *Tgfbm3*, regulates signal transduction *via* TGF-b type I receptor to modify vascular phenotypes in mice and in HHT patients. Kyoko Kawasaki[1], Sylvia Espejel[1], Tom Letteboer[1,2], Michael Benzinou[1], Marie Lee[1], Ritu Roy[1], Hans Kristian Ploos van Amstel[2], Cornelius Westermann[3], **Rosemary Akhurst**[1]. 1) HDFCCC, UCSF, San Francisco, CA; 2) Department of Medical Genetics, University Medical Centre, Utrecht, The Netherlands; 3) St. Antonius Ziekenhuis, Nieuwegein The Netherlands.

TGF-1, encoded by *Tgfb1*, plays a central role in many diseases, including cancer, cardiovascular disease, autoimmunity and fibrosis. To interrogate biological mechanisms that regulate TGF-1 biology *in vivo*, we previously undertook genetic modifier screens in order to genetically map and characterize variant loci that can suppress prenatal lethality of *Tgfb1-/-* mouse embryos caused by vascular dysgenesis. We show that one of these loci, *Tgfbm3*, is complex and can be genetically dissected into three regions with positive *versus* negative activities in suppressing *Tgfb1-/-* prenatal lethality. Utilizing genetic or pharmacological inhibition of candidate genes in *in vivo* and *in vitro* assays, we have identified a gene that directly interacts with the TGF type I receptor to modulate down-stream signaling pathways and vascular phenotypes. In humans, this gene shows genetic association with disease severity in the human TGF-vasculopathy, Hereditary Hemorrhagic Telangiectasia (HHT), which is caused by mutations in E*NG*, a type III receptor for TGF-/BMP. In a mouse model, these genes interact to cause disorganized retinal vascular development *in vivo*, reminiscent of the telangiectases and arteriovenous malformations seen in some HHT patients.

3
Phenotypic and Molecular Analyses of Different *vangl2* Mutants Demonstrates Dominant Effects of the *Looptail* Mutation during Hair Cell Development. Haifeng Yin, Catherine Copley, **Michael Deans**. Dept. of Otolaryngology/HNS, Johns Hopkins University School of Medicine, Baltimore, MD.

Experiments utilizing the *Looptail* mutant mouse, which have craniorachischisis due to a missense mutation in *vangl2*, are widely used as a developmental model for human neural tube defects. In this context, *vangl2* directs convergent extension movements of the neuroectoderm via planar cell polarity signaling.*Looptail* has also been an important model for studying the planar polarity of sensory receptor hair cells in the mouse inner ear where it directs the polarized morphogenesis of stereocilia bundles in both the auditory and vestibular systems. As the name indicates, *Looptail* heterozygotes have distinctive looped tails suggesting that the mutation has a semi-dominant phenotype. However it is unclear how mutant Vangl2 protein could exert a dominant effect in these mice because the mutant protein is unstable and is not delivered to the cell surface like normal Vangl2. We addressed this by comparing vestibular hair cell development in the inner ears of *Looptail* mice and *vangl2* knockout mice missing a large portion of the *vangl2* gene, and by assaying molecular interactions between mutant Vangl2 and normal proteins *in vitro* and *in vivo*. Overall the *vangl2* knockout phenotype is milder than compound mutants with both the*Looptail* and *vangl2* knockout alleles. In compound mutants, more hair cells are affected and individual hair cells show greater

changes in orientation when quantified. In addition, the protein encoded by the *Looptail* mutation (Vangl2^{S464N}) disrupts Vangl1 and Vangl2 delivery to the surface of cells in vitro due to oligomer formation between Vangl1 and Vangl2 coupled with the intracellular retention of Vangl2^{S464N}. As a result, Vangl1 protein is missing from the apical cell surface of vestibular hair cells in *Looptail* mutants, but is retained at the cell surface in *vangl2* knockouts. Similarly the distribution of Prickle-like2, a putative Vangl2 interacting protein, is differentially affected in the two lines. We propose that altered Vangl2^{S464N} trafficking prevents the delivery of multiple polarity proteins to the cell surface and that this net effect underlies the dominant phenotypic traits associated with the *Looptail* mutation. One interpretation is that prior genetic interactions with *Looptail* may be indirect and reflect a permissive enhancement of this semi-dominant phenotype.

4

Host Resistance To A Gastrointestinal Parasite Regulated By A SNP Modulating SMAD3 Binding To A TGFb-Responsive Transcriptional Enhancer Of Mina. Mark Bix[1,3], Meenu Pillai[1,3], Shangli Lian[1], Peter Vogel[1], Mathew Coleman[2], Peter Ratcliffe[2]. 1) Immunology, St. Jude Children's Research Hospital, Memphis, TN; 2) Oxford University, Oxford, UK; 3) Equal contribution.

Th1 immune responses, characterized by IFN production, promote clearance of intracellular microbes while Th2 responses, characterized by IL4 production, promote control of helminthic parasites. The genetic predisposition of certain inbred mouse strains toward developing T helper 2 (Th2) as opposed to Th1 type immune responses (Th2-bias) has long been associated with differential susceptibility to a wide range of diseases, ranging from cancer, autoimmunity and allergy/asthma to chronic microbial infection. However, the molecular genetic basis of Th2-bias and thus causal relationship to specific disease states has remained poorly understood. We initiated an investigation into the nature of this relationship by mapping a Th2-bias-regulatory quantitative trait locus (*Dice*) to distal chromosome 16. Subsequent interval-specific congenic fine-mapping and gene expression analysis led to the physical isolation of the *Dice1.2* locus and the identification of *Mina*, a *Dice1.2*-resident gene with apparent Th2-bias regulatory activity. Th2-bias phenotype across a representative panel of inbred mouse strains correlated inversely with Mina expression level that in turn was in perfect concordance with two distinct *Mina* haplotypes defined by 21 promoter/exon 1/intron 1-spanning SNPs. These data suggested that a naturally occurring regulatory polymorphism controlling Mina expression level conferred dose-dependent negative regulation of Th2-bias. To study how natural variation at the *Mina* locus affects susceptibility to chronic infectious disease, we selected the gastrointestinal whipworm parasite *Trichuris muris* (Tm, a close relative of the human pathogen *Trichuris trichiura*) because chronic versus acute infection outcomes in this model, respectively, require Th1 versus Th2 host responses. Using a KO mouse model, we found that Mina promoted expression of the resistin-like molecule Relm in intestinal epithelial cells that in turn promoted Th1 development and the secretion of IFN that exacerbated helminthiasis. Further, the allele of an intronic SNP carried by BALB/c but not C57BL/6 mice prevented the binding of SMAD3 to a TGF activated *Mina*enhancer, providing an explanation for the differential susceptibility of BALB/c and C57BL/6 mice to Tm disease.

5

Discovering genes and pathways involved in naevus and melanoma development using the Collaborative Cross. Graeme Walker[1], Ramesh Ram[2], Blake Ferguson[1], Herlina Handoko[1], Peter Soyer[3], Grant Morahan[2]. 1) Skin Carcinogenesis Lab, QIMR, Brisbane, Qld, Australia; 2) Centre for Diabetes Research, WAIMR, The University of Western Australia, Perth, Australia; 3) Dermatology Research Centre, UQ School of Medicine, Brisbane QLD.

Upon metastatic conversion malignant melanoma (MM) is very difficult to treat. The most successful drug treatments target somatic BRAF mutation, but resistance soon develops and new drugs are needed. Genes are critical determinants of how MMs develop and grow, and are arguably more important than sun exposure. The presence of above average numbers of nevi is the strongest phenotypic risk factor. GWAS have revealed many MM susceptibility genes that have improved risk prediction, but none have yet been functionally validated as potential drug targets. We have developed genetically modified mice (Cdk4R24C::Tyr-Nras) which model MM progression via a benign nevus phase, evaluated the evolution of lesions, and developed a tumor staging schema for murine MM. We have combined this model with the Collaborative Cross (CC) as a novel approach to find genes which strongly influence MM development. We find major differences in various MM-related phenotypes when crossed onto the CC strains. We have mapped these phenotypes to small chromosomal regions, containing either just one or a small number of genes, and are testing more strains to enable fine mapping. We have used these data to define a molecular pathway for MM progression, as follows: 1. Nevus histological phenotype (scattered subepidermal vs pagetoid dermal): controlled by a chr 7 locus. 2. Nevus age of onset: one locus is on chr 2, which contains a candidate gene that is frequently mutated in human MM and associated with MM risk. We also found loci on chr 1 (which is associated with MM in published GWAS), chr 8, and chr 14. 3. MM age of onset: controlled by loci that map to chromosomes 1 and 19. The latter is associated with human MM in our cohort, but was not reported previously, hence is a novel finding. 4. Time for conversion of nevus to MM: loci map to regions containing genes known to be involved in MM biology. The strong congruence of our data with human MM genetics highlights the power of our approach. Our findings confirm that MM progression appears to be controlled not only by stochastic events occurring over time, but also by different inherited factors at each stage. The discovery of genes that explain why nevi develop so frequently and rapidly, and often convert to MM, will lead to a much fuller understanding of MM progression. Especially attractive as potential therapeutic targets are protective genes that are dominant over the oncogenic mutations known to generate MM in both mice and humans.

6

The (uro)chordate-specific *Gumby* gene governs angiogenesis and modulates Wnt signaling. Elena Rivkin[1,2], Stephanie M Almeida[1,2], Teresa A MacLean[1,2], Gang Xie[1], **Sabine P Cordes**[1,2]. 1) Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada; 2) Department of Molecular Genetics, University of Toronto, 1 King's Crescent, Toronto, ON Canada.

A complex interplay of signaling pathways regulates the sprouting of blood vessels from preexisting vasculature during angiogenesis, and produces vessels uniquely adapted to organ physiology and function. Here we describe an angiogenic phenotype in embryos homozygous for the mouse *gumby* mutation. We show that a point mutation in the novel (uro)chordate-specific *Gumby* gene is responsible for this angiogenic phenotype. Furthermore we show that Gumby protein interacts with Dishevelled 2 (Dvl2), a member of Wnt signaling pathways, and can stimulate canonical Wnt signaling in culture. In the mouse, Gumby protein is localized to endothelial cells with active canonical Wnt signaling. Mutant gumby protein can still stimulate canonical Wnt signaling in culture and canonical Wnt signaling is active in endothelial cells of *gumby/gumby* embryos, as detected in TOPGAL reporter mice. However, expression of some known Wnt target genes is decreased in *gumby/gumby* embryos. Because *Gumby* is deleted in patients manifesting mental retardation, craniofacial and cardiac deficits of Cri du Chat Syndrome (CdCS), our findings for the first time suggest that Wnt signaling could be affected in CdCS. Thus, understanding how Gumby acts to coordinate Wnt signaling in endothelial cells may provide broader insights into the cell biology of CdCS.

7

Molecular pathogenesis of Joubert Syndrome and related disorders. **Tamara Caspary**[1], Holden Higginbotham[2], Laura E. Mariani[1,3], Tae-Yeon Eom[2], Miao Sun[1], Vanessa L. Horner[1], Alyssa B. Long[1], Eva S. Anton[2]. 1) Dept. of Human Genetics, Emory University School of Medicine, Atlanta, GA; 2) The UNC Neuroscience Center, The University of North Carolina School of Medicine, Chapel Hill, NC; 3) Graduate Program in Neuroscience, Emory University, Atlanta, GA.

Patients with Joubert Syndrome and related disorders (JSRD) suffer from a wide array of symptoms with variable clinical presentation, including developmental delay, intellectual disability, abnormal respiratory rhythms, ataxia, oculomotor apraxia, polydactyly, craniofacial defects, retinal dystrophy and nephronophthisis. The diagnosis of JSRD requires an MRI showing the molar tooth sign. This hindbrain malformation arises from hypoplasia of the cerebellar vermis and thickened, elongated superior cerebellar peduncles that fail to cross the midline. While JSRD is a rare, autosomal recessive, congenital disorder, causative mutations for JSRD have been identified in the small GTPase, *ARL13B*, the inositol phosphatase, *INPP5E* and 16 additional genes, all of which code for proteins related to primary cilia - thus, JSRD are members of the emerging class of diseases known as ciliopathies. Primary cilia are essential for Sonic hedgehog (Shh) signaling, and we previously showed that Shh signaling is regulated by Arl13b in mouse.

Here we investigate the pathogenesis of JSRD in mouse models using a conditional *Arl13b* allele and an ENU-induced *Inpp5e* allele. We found Arl13b is critical for the localization of Inpp5e to cilia. Inpp5e loss misregulated Shh signaling in a similar manner to Arl13b loss. Together these data are consistent with Inpp5e acting as a specific Arl13b effector. In order to better understand the neurological defects in Joubert patients, we deleted *Arl13b* in specific cortical neurons or interneurons. Defects in the placement or migration of these cells alter connectivity, which underlies the cognitive defects in a spectrum of neurological disorders. In the absence of *Arl13b*, we observed defects in the migration and placement of postmitotic interneurons in the developing cerebral cortex. We found guidance cue receptors known to be important for interneuron migration normally localize to interneuronal cilia, but their concentration and dynamics were abnormal in the absence of *Arl13b*. Wild type Arl13b rescued these defects whereas neither ARL13B variants from Joubert patients nor non-ciliary variants of Arl13b did. These data suggest that defects in cilia-dependent interneuron migration underlie the neurological deficits in JSRD patients.

8

Genetic analysis of Down syndrome in mice. Chunhong Liu[1], Masae Morishima[2], Li Zhang[1], Xiaoling Jiang[1], Kai Meng[1], Annie Pao[1], Michael Parmacek[3], Ping Ye[4], William Mobley[5], Allan Bradlley[6], **Yuejin E. Yu**[1*]. 1) Genetics Program, Roswell Park Cancer Institute, Buffalo, NY; 2) Department of Anatomy and Developmental Biology, Tokyo Women's Medical University, Tokyo, Japan; 3) Cardiovascular Institute, University of Pennsylvania, Philadelphia, PA; 4) School of Molecular Bioscinces, Washington State University, Pullman, WA; 5) Department of Neuroscinces, University of California, San Diego, La Jolla, CA; 6) Wellcome Trust Sanger Institute, Hixton, Cambridge, UK.

Trisomy 21, the most common live-born aneuploidy in humans, causes Down syndrome (DS). This genetic disorder is associated with several major phenotypes, including heart defects, hematopoietic abnormalities, intellectual disabilities and suppression of solid tumor growth. The mouse is a premier model organism for DS because the genomic regions on human chromosome 21 are syntenically conserved with three regions in the mouse genome, located on mouse chromosomes 10, 16 and 17. To expedite genetic analysis of DS, we have generated 12 mouse mutants with different genetic rearrangements in the human chromosome 21 syntenic regions, using embryonic stem cell technology. Using these mutants, we have developed a complete genetic model for DS, which is trisomic for all three human chromosome 21 orthologous regions. Generation and characterization of the smaller chromosomal duplications and deletions have facilitated the establishment of the critical genomic regions associated with the mutant phenotypes. These efforts should lead to the identification of the dosage-sensitive genes underlying the phenotypes, which will serve as the entry point to the mechanistic details of the altered developmental processes.

9
CHD5: Chromosome engineering, chromatin dynamics, and cancer. Alea A. Mills. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Although 1p36 loss has been documented for decades and a variety of malignancies have this lesion, the tumor suppressor mapping to this genomic interval had not been identified. Using chromosome engineering to create models with gain and loss of the region of the mouse genome corresponding to human 1p36, we pinpointed a potent tumor suppressive interval, identified Chromodomain Helicase DNA-binding domain protein 5 (CHD5) as the causative gene in the region, elucidated molecular pathways induced by CHD5, and demonstrated that CHD5 is frequently deleted in human cancer. The discovery of CHD5 as a tumor suppressor has had a major impact in the cancer field. Indeed, it is now appreciated that CHD5 is mutated in cancers of the breast, ovary, colon, and prostate, as well as in melanoma, glioma, and neuroblastoma. Furthermore, CHD5 status is a prognostic indicator of patient survival following anti-cancer therapy. Still, the mechanism by which CHD5 exerts its tumor suppressive role is not well understood. CHD5 belongs to the CHD family of SWI-SNF-like ATP dependent-chromatin remodeling proteins; however, the role of CHD5 in modulating chromatin dynamics has not been reported. Here, we show that CHD5s tumor suppressive function is dependent on its tandem plant homeodomains (PHDs)modules that in several other chromatin-remodeling proteins regulate transcription by binding to specific covalent modifications on histone tails. The PHDs of CHD5 preferentially bind unmethylated H3K4 (H3K4me0), an interaction abrogated by methylation of H3K4 but unaffected by modification of H3K9. Both loss- and gain-of-function studies reveal that CHD5 modulates transcription, and furthermore, that CHD5 is a potent inhibitor of cellular proliferation. Mutation of residues within the PHDs of CHD5 that abolish H3 binding effectively abrogate CHD5s ability to modulate expression of target genes as well as its ability to inhibit proliferation, leading to tumorigenesis in vivo. These findings identify CHD5 as a member of a newly appreciated class of H3K4me0-binding PHD containing proteins and reveal a critical role for this interaction in facilitating CHD5s cellular function, providing new insight into the molecular mechanism responsible for the tumor suppressive role of CHD5.

Patterning

10
Mechanisms of position-dependent specification of cell fates in preimplantation embryos. Hiroshi Sasaki. IMEG, Kumamoto University, Kumamoto, Japan.

In preimplantation mouse embryos, specification of cell fates into the trophectoderm (TE) or inner cell mass (ICM) depends on the positions of the cell within the embryos. The transcription factor Tead4 is required for specification of the TE fate. Tead4 is present in the nuclei of all blastomeres. Cell-position-dependent Hippo signaling controls nuclear localization of the coactivator protein Yap as well as the activity of Tead4: inactive Hippo signaling in the outer cells increases nuclear Yap levels, activates Tead4, and promotes TE development; on the other hand, active Hippo signaling in the inner cells suppresses nuclear Yap levels, inactivates Tead4, and promotes ICM development. In this study, we show that cell polarity and cell-cell adhesion together establish position-dependent Hippo signaling at approximately the 32-cell stage. Cell-cell adhesion activates Hippo signaling, but polarity in the outer cells suppresses it. The junction-associated protein Angiomotin (Amot) is essential for activation of the Hippo pathway. In the inner cells, Amot localizes to the adherens junctions and activates the Hippo pathway, whereas in the outer cells, cell polarity sequesters Amot from basolateral membranes to apical domains, thus suppressing the Hippo signal. We propose that cell polarity uncouples cell-cell adhesion and the Hippo pathway by sequestering Amot from the adherens junctions. This mechanism converts positional information into determination of the cell-fate through differential Hippo signaling.

11
FGF4 is required for lineage restriction and salt-and-pepper distribution of primitive endoderm program but not its initiation in the mouse. Minjung Kang[1,2], Ania Piliszek[3], Jérôme Artus[4], Anna-Katerina Hadjantonakis[1]. 1) Developmental Biology, Sloan-Kettering Institute, New York, NY; 2) Biochemistry, Cell and Molecular Biology Program, Weill Graduate School of Medical Sciences of Cornell University, New York, NY 10065, USA; 3) Department of Experimental Embryology, Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, 05-552 Wólka Kosowska, Poland; 4) Institut Pasteur, CNRS URA2578, Mouse Functional Genetics Unit, Paris, France.

The emergence of pluripotent epiblast (EPI) and primitive endoderm (PrE) lineages within the inner cell mass (ICM) of the mammalian blastocyst involves initial co-expression of lineage-associated markers followed by their mutual-exclusion and the salt-and-pepper distribution of lineage-biased cells. Thereafter lineage segregation is completed by the sorting of cells into adjacent tissue layers. Precisely how EPI and PrE cell fate commitment occurs is not entirely clear; however in the mouse FGF/ERK signaling is required. To gain insight into the role of FGF signaling, we investigated the phenotype resulting from zygotic and maternal/zygotic inactivation of Fgf4. Fgf4 heterozygous blastocysts exhibited increased numbers of NANOG-positive EPI cells and reduced numbers of GATA6-positive PrE cells, suggesting that FGF signaling must be tightly regulated to ensure appropriate numbers of cells for each lineage. Fgf4 mutants lacked PrE entirely with their ICM comprising exclusively of NANOG-expressing cells. Notably, an initial period of widespread EPI and PrE marker co-expression was established, even in

the absence of FGF4. Thus, Fgf4 mutant embryos initiated the PrE program but exhibited defects in its restriction phase, where lineage bias is acquired. Consistent with this, XEN cells could be derived from Fgf4 mutant embryos in which PrE had been restored and these cells appeared indistinguishable from wild-type cells. Interestingly, sustained exogenous FGF failed to rescue the mutant phenotype. Instead, depending on concentration, we noted no effect, or conversion of all ICM cells to GATA6-positive PrE. We therefore propose that heterogeneities in the availability of FGF produce the salt-and-pepper distribution of lineage-biased cells.

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Cell fate decisions regulating stem cell origins during preimplantation mouse development. Amy Ralston[1], Tristan Trum[1], Stephanie Blij[1], Chaoyang Wang[2], Paul Robson[2], Aytekin Akyol[3,4], Eric Fearon[3], Michael A. Halbisen[1], Yoshikazu Hirate[5], Hiroshi Sasaki[5], Tony Parenti[1], Eryn Wicklow[1]. 1) Molecular, Cell, and Developmental Biology, UCSC, Santa Cruz, CA; 2) Genome Institute of Singapore, Singapore; 3) Division of Molecular Medicine and Genetics, University of Michigan Medical School; 4) Department of Pathology, Hacettepe University School of Medicine, Ankara, Turkey; 5) Department of Cell Fate Control, Institute of Molecular Embryology and Genetics, Kumamoto University, Japan.

By studying mouse embryogenesis, we aim to discover how mammalian development is regulated and how embryonic stem cells are created. Our approach is to examine the roles of stem cell factors in cell fate decisions in the mouse early embryo. Oct4 and Sox2 are essential regulators of pluripotency in embryonic stem cells, where they regulate each others expression. However, we show that in the mouse blastocyst, these genes do not regulate each other, neither from the zygotic nor the maternal genome. Rather, Oct4 and Sox2 regulate formation of the primitive endoderm, an extraembryonic tissue. Primitive endoderm cell fate is thought to be induced by FGF signaling from the epiblast. We show that primitive endoderm development depends on Sox2, acting upstream of FGF signaling from the epiblast, and Oct4, acting downstream of this signaling. In other words, Sox2 regulates primitive endoderm cell fate non cell-autonomously, while Oct4 regulates primitive endoderm fate cell-autonomously. Finally, we examine stem cell factors thought to be required for trophectoderm, another extraembryonic lineage of the blastocyst. Maternal Cdx2 and Sox2 are thought to be required for trophectoderm development. We use a genetic approach to delete Cdx2 in the female germline and show that maternal Cdx2 is not required for mouse development. We also show that maternal Sox2 is not required for TE fate. Rather, Sox2 expression is regulated by trophectoderm-specifying pathway upstream of Cdx2. Ultimately, our data support a regulative, rather than deterministic, model of early mammalian development.

13

The T-box factor Eomesodermin is required in the visceral endoderm for proper axis specification in the early mouse embryo. Sonja Nowotschin[1], Gloria Kwon[1], Evan Weiner[1], Anna Piliszek[1], Chai-an Mao[2], Kat Hadjantonakis[1]. 1) Developmental Biology, Sloan-Kettering, New York, NY 10065, USA; 2) Department of Biochemistry and Molecular Biology, University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA.

Reciprocal inductive interactions between the epiblast and overlying visceral endoderm are critical for establishing the anterior-posterior (AP) axis of the early mouse embryo. A first step in AP axis formation is the specification of a group of cells at the distal tip of the egg cylinder, the distal visceral endoderm (DVE). The anterior transposition of this distal population of visceral endoderm cells leads to the establishment of the AP axis.

Here we have examined the role of Eomesodermin (Eomes), a member of the T-box family of transcriptional regulators, specifically within the visceral endoderm of the early embryo prior to AP axis formation. Our data demonstrate that Eomes is required in the visceral endoderm for DVE specification, and that visceral endoderm tissue-specific ablation of Eomes results in failure to establish the AP axis. Moreover, loss of Eomes in the visceral endoderm resulted in the upregulation of Nodal signaling pathway components in the epiblast. AP patterning was restored when Nodal levels were reduced. These observations reveal a pivotal role for Eomes, acting in the visceral endoderm to regulate the balance of Nodal signaling within the early post-implantation mouse embryo.

14

How does retinoic acid regulate progenitor cell maintenance and differentiation in the nervous system and sensory organs? Pascal Dolle[1*], Monika Rataj Baniowska[1], Muriel Rhinn[1], Wojciech Krezel[1], Karen Niederreither[2], Raj Ladher[3], Marie Paschaki[1]. 1) IGBMC, Strasbourg, France; 2) Dell Pediatric Research Institute, Austin, TX, USA; 3) RIKEN Center for Developmental Biology, Kobe, Japan.

Retinoic acid (RA) has long been known to be a potent inducer of neuronal differentiation of various cell lines (including P19 or ES cells) or neural precursor cells during in vitro culture. Despite this fact, the in vivo function(s) of RA in cell populations undergoing neurogenesis during development (the embryonic neuroepithelium giving rise to the brain and spinal cord, the placodal epithelia giving rise to sensory neurons, etc) or adult life (e.g. the hippocampal neurons) remain largely debated. The developing olfactory placode offers an interesting paradigm to study these events, as it gives rise to a population of sensory neurons (the olfactory sensory neurons) that differentiate and become functional at perinatal stages, and have the capacity to regenerate through life. Our studies using loss of function approaches in chicken and mouse indicate that RA is not required for the initial inductive steps generating the olfactory placode. Instead, RA appears to promote generation of olfactory basal cells, and prevent their progression into committed neuronal precursors. RA depletion results in a failure of progenitor maintenance and consequently, due to this depletion, differentiation of olfactory neurons is not sustained. Furthermore, the regenerative capacity of the olfactory epithelium in mutant mice deficient for RA synthesis is impaired. Our data suggest a mechanism by which local

modulation of RA signaling may trigger olfactory repair and renewal in adults. Additional experiments have also performed to better characterize the role of RA during neurogenesis in the early embryonic forebrain (telencephalon), the later differentiating spinal cord, and in the generation of neuronal populations such as the dopaminergic nuclei of the substantia nigra and its target output structure, the striatum.

15

The Wnt3a/b-catenin target Mesogenin1 (Msgn1) is a master regulator of presomitic mesoderm differentiation. Ravi B. Chalamalasetty[1], William C. Dunty Jr.[2], Wuhong Pei[3], Kristin K. Biris[1], Rieko Ajima[1], Benjamin Feldman[3], Terry P. Yamaguchi[1]. 1) Cancer and Developmental Biology Laboratory, Frederick National Laboratory, NIH, Frederick, MD; 2) Division of Metabolism and Health Effects, NIAAA, NIH, Bethesda, MD; 3) Medical Genetics Branch, Vertebrate Embryology Section, NHGRI, NIH, Bethesda, MD.

Wnts are secreted signaling molecules that function as important regulators of stem cell fates during development and disease. A Wnt3a signaling gradient in the posterior embryo balances the self-renewal of paraxial mesoderm progenitors with their differentiation into the presomitic mesoderm (PSM) in order to extend the embryonic body axis during gastrulation and segmentation stages, however the underlying molecular mechanisms remain poorly understood. To understand the gene regulatory networks regulated by Wnt3a, we performed a comparative microarray screen of wildtype and Wnt3a mutant embryos to assess the differentially expressed genes. This approach led to the identification of the bHLH transcription factor, Mesogenin1 (Msgn1), as a direct target gene of the Wnt3a/-catenin signaling pathway. Msgn1 null embryos lack posterior somites and PSM, and display grossly enlarged tail buds filled with immature mesodermal progenitors indicative of a block in PSM differentiation or migration. We show that Wnt3a initiates PSM differentiation via Msgn1. Overexpression of Msgn1 in mesodermal progenitors in vivo leads to a dramatic expansion of the PSM and suppresses somitogenesis, while ectopic expression in axial mesendoderm suppresses notochord fates. Transcriptional profiling and genomic ChIP-seq analysis show that Msgn1 overexpression in embryonic stem cells rapidly induces PSM differentiation due to the direct activation of gene expression programs that determine PSM identity, EMT, and the segmentation clock. Genetic epistasis analysis suggests that Msgn1 also suppresses Wnt3a in the mesoderm, thereby establishing a feedback suppressor loop to ensure that mesodermal progenitors commit to a PSM fate. We propose that counteracting positive and negative feedback loops initiated by Wnt3a control the balance between paraxial mesoderm progenitor self-renewal and PSM differentiation and morphogenesis.

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ENU-induced disruption of murine ESCRTII complex results in enhancement of the Fgf-Shh signaling loop with polydactyly. Karen Handschuh[1], Matthew Koss[1], Elisabetta Ferretti[1], Michael Depew[2], John Manak[3], Kathryn Anderson[4], Elizabeth Lacy[4], **Licia Selleri**[1]. 1) Dept of Cell and Developmental Biology, Weill Cornell Medical College, NY, NY, USA; 2) Dept of Craniofacial Development, King's College, London, UK; 3) Roy J. Carver Center for Genomics, University of Iowa, Iowa City, IA, USA; 4) Program of Developmental Biology, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, NY, NY, USA.

Our laboratory uses genetic and developmental approaches, using the mouse as a model, to study morphogenesis and its perturbations during embryonic development. We performed phenotype-based forward genetic screens by ENU mutagenesis to uncover novel genes required for limb and craniofacial development. Mutant line 04/014 was selected based on striking limb and craniofacial abnormalities. Mutant embryos die in utero at E16.5 with marked edema and display pre-axial polydactyly (PPD), a common birth defect. Using high-resolution mapping and microarrays, we identified the gene responsible for the phenotype as one of the ESCRTII complex subunits. Here, we show that receptor accumulation in ECSRTII mutants upregulates signaling. In particular, the Ff-Shh cross-regulatory loop, which is critical for limb patterning and morphogenesis, is spatially enhanced and temporally dilated in mutant limbs. We demonstrate that endosomal trafficking is affected in mutant cells in culture, with engorgement of the multivesicular bodies (MVBs) and trapping of signaling molecule receptors in enlarged MBVs. Fgf4 is spatially expanded and temporally dilated in mutant limbs, as is Shh, which, in addition, is ectopically expressed in limb bud anterior domains. Enhancement of the Fgf-Shh cross-regulatory signaling loop underlies the PPD phenotype. Over-expression of wild type and mutant proteins in transfection assays shows that they co-localize and co-immunoprecipitate. Genetic complementation unequivocally demonstrates that the identified molecular lesion in ESCRTII is responsible for the observed phenotype. These studies elucidate how endosomal trafficking of signaling receptors is critical for unique morphogenetic processes, such as limb patterning and the establishment of digit number. They also suggest a causal link between impaired degradation of signaling factors due to perturbed endosomal trafficking and developmental aberrations.

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"Diverse roles of Fibroblast Growth Factor signaling during somitogenesis and axis extension". Mark Lewandoski. Frederick National Lab for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick, MD 21702, USA.

Normal extension and patterning of the vertebrate axis relies on molecular signals that coordinate various processes such as somitogenesis, neurulation and notochord formation. Amongst the signaling families essential to this process are Bone Morphogentic Proteins (BMPs), WNTs and Fibroblast Growth Factors (FGFs). To understand the how FGFs act in axis extension we have begun a comprehensive study to understand FGF genetic redundancy in this process. I will review our most recent work that defines the relationship between FGF and WNT signals to control the molecular clock that drives the periodicity of

somitogenesis. I also will provide a progress report on unpublished work, including characterization of the FGF3 axis defect and our work on determining the FGF responsive elements in downstream target genes.

Beddington Lecture

18
Hox genes and hindbrain patterning: A story in segments. Robb Krumlauf[1,2*], Youngwook Ahn[1], Christof Nolte[1], Hugo Parker[1], Marina Yurieva[1], Bony DeKumar[1]. 1) Stowers Institute, Kansas City, MO; 2) Department of Anatomy and Cell Biology, Kansas University Medical School, Kansas City, KS.

The hindbrain is a highly conserved and complex co-ordination center in the vertebrate CNS. It forms the medulla, pons and cerebellum which play crucial roles in regulating functions such as sleep, respiration and heart rate. During development regional diversity in hindbrain is established through a process of segmentation which divides an anterior region of the neural tube into seven morphological discrete domains, termed rhombomeres. This segmental organization is also critical for patterning of the cranial neural crest which generates most of the bone and connective tissues of head and facial structures. The Hox family of transcription factors is coupled to this process and genetic studies have demonstrated that they provide a molecular framework for specifying the unique identities of hindbrain segments and facial structures. A goal of our group is to use genetic and genomic approaches in the mouse and comparative studies in other vertebrates to understand the regulatory cascades associated with Hox activity, which control hindbrain segmentation and early head development. We employ functional and regulatory studies in mice, using transgenic assays in BACs containing the HoxB complex; targeted mutagenesis in the endogenous Hox clusters; transcriptional profiling of individual rhombomeres and the genome-wide identification of Hox binding sites and target genes by ChIP-seq to investigate the signaling pathways and transcriptional mechanisms that control Hox expression in developing hindbrain segments. Our results reveal that retinoic acid signaling is directly implicated in early positioning of the anterior boundaries of Hox expression and in modulating later dynamic changes in neural expression. Auto and cross-regulatory interactions between Hox genes actively maintain segmental domains of Hox expression. We find that sharing of retinoid responsive enhancers, long-range regulatory interactions and insulators govern how retinoids establish and modulate ordered domains of Hox activity. Hox genes and retinoid signaling also play key roles in defining specific groups of neurons that underlie the neural circuitry controlled by the hindbrain. Our genomic analyses have revealed that following the initial induction of Hox genes by retinoids, they in turn modulate multiple aspects of retinoid metabolism and catabolism. These feedback loops are important for controlling neurogenesis during hindbrain patterning.

Stem Cells and Germ Cells

19
Targeting developmental pathways in cancer cells and stem cells. Frederic J de Sauvage. Genentech Inc., So. San Francisco, CA.

The Notch, Wnt and Hedgehog signaling pathways play critical roles during embryonic development. These factors modulate proliferation or differentiation of numerous cell types and are also involved in the regulation of the self-renewal and/or differentiation of embryonic and adult stem cells. It is however becoming increasingly clear that these pathways are involved in tumorigenesis when reactivated in adult tissues through mutations or overexpression of pathway components. Aberrant reactivation of the Hedgehog (Hh) pathway in adult tissues leads to the development of cancers such as basal cell carcinoma (BCC) and medulloblastoma is often the result of inactivating mutations in PATCHED (PTCH) or activating SMOOTHENED (SMO) mutations. NOTCH1 mutations are found in most T-cell acute lymphoblastic leukemias and activation of the WNT pathway, often through APC (adenomatous polyposis coli) gene mutations, is observed in a majority of colon adenocarcinomas. However all 3 pathways play a role in normal gut homeostasis and may be involved in some aspect of colon tumorigenesis. The development of inhibitors targeting these pathways is therefore of the highest interest as illustrated by the recent approval of vismodegib, a Hh pathway inhibitor for the treatment of advanced BCC. While targeting of the Wnt pathway has been more challenging, new opportunities have emerged with the identification of Lgr5 as a stem cell marker overexpressed in colorectal cancer.

20
Lineage analysis of basal epithelial cells reveals their unexpected plasticity and supports a cell of origin model for prostate cancer heterogeneity. Zhu Wang[1,4], Antonina Mitrofanova[2,4], Sarah Bergren[1,4], Cory Abate-Shen[3,4], Andrea Califano[2,4], Michael Shen[1,4]. 1) Departments of Medicine and Genetics and Development, Columbia University Medical Center, New York, NY; 2) Department of Biomedical Informatics and Center for Computational Biology and Bioinformatics, Columbia University

Medical Center, New York, NY; 3) Departments of Urology and Pathology and Cell Biology, Columbia University Medical Center, New York, NY; 4) Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY.

A key issue in cancer biology is whether oncogenic transformation of different cell types of origin within an adult tissue gives rise to distinct tumor subtypes that differ in their prognosis and/or treatment response. In the case of prostate cancer, the identity of the normal cell type(s) of origin is currently under investigation. Several studies have shown that the prostate basal epithelial compartment contains stem cells that can be transformed to initiate tumors in tissue reconstitution assays. However, previous lineage-tracing studies from our laboratory have identified a luminal stem cell population in mouse that can serve as a cell of origin for prostate cancer in vivo. Here, we have undertaken a comprehensive ex vivo and in vivo analysis using genetic lineage-marking to examine the properties of the identical basal cell population in multiple assays for stem cell function, and to compare prostate tumors originating from basal cells and luminal cells at the phenotypic and molecular levels. Specifically, we have utilized CK5-CreERT2 transgenic mice for inducible lineage-marking of basal cells, followed by analysis of their properties in prostate sphere and tissue reconstitution assays, as well as in androgen-mediated prostate regeneration and tissue homeostasis. Our results reveal that normal basal cells display considerable plasticity in different stem cell assays. While a relatively large subpopulation of basal cells (around 4%) display stem cell properties in sphere formation and tissue reconstitution assays, the percentage of basal stem cells identified by their ability to generate luminal cells in vivo is much lower (0.05%). Furthermore, using cell-type-specific deletion of Pten using CK5-CreERT2 or Nkx3.1-CreERT2, we find that both basal and luminal cells can serve as cells of origin for prostate cancer, giving rise to tumors with similar histological phenotypes that resemble human prostate adenocarcinoma. However, our cross-species molecular and bioinformatic analysis of basal versus luminal origin tumors shows that the luminal origin tumors are more aggressive, and identifies a 68-gene molecular signature that has predictive value for human patient survival. These results reveal the inherent plasticity of basal cells, and support the cell of origin model as a basis for distinct prostate cancer subtypes.

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Wnt/beta-catenin, BMP and HGF in cancer stem cells. Walter Birchmeier[1], Peter Wend[1,2], Ulrike Ziebold[1], Jane Holland[1]. 1) Max-Delbrueck-Center for Molecular Medicine (MDC), 13125 Berlin, Germany; 2) David Geffen School of Medicine, University of California at Los Angeles, CA 90095, USA.

We show that the combination of activated Wnt/-catenin and blocked Bmp signaling in salivary glands created cancer stem cells and rapidly growing tumors. Cancer stem cells were promoted into a selfrenewing state, and the activities of -catenin, CBP and trithorax-related MLL1 were critical to maintain proliferation and heightened H3K4 trimethylation. Blocking -catenin-CBP with the small molecule inhibitor ICG-001 and by -catenin, CBP or MLL1 siRNA abrogated selfrenewal and forced cancer stem cells to differentiate into organotypic structures. ICG-001 treatment decreased H3K4me3 at promoters of stem cell signature genes and resulted in tumor remission following transplantation. Blocking Bmp signaling reduced apoptosis. We also show that compound gain-of-function mutations of -catenin and HGF using the WAP promotor resulted in the expansion of mammary gland cancer stem cells that rapidly formed basal-like tumors. In cultured cancer stem cells, Wnt signaling stimulated self-renewal, while Met signaling suppressed differentiation. We identified a set of genes whose expression is regulated by Wnt and Met in cancer stem cells, among these CXCL12, the ligand for the chemokine receptor CXCR4. Ablation and inhibition of CXCR4, which acts in the Met pathway, resulted in a signifcant delay in tumor onset and induced differentiation. Furthermore, the expression of the Wnt/Met signature could predict for poor survival in patients with Basal subtypes of breast cancer. Our findings encourage the use of signaling inhibitors for therapy that targets cancer stem cells.

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Discovery of a novel, developmentally restricted hematopoietic stem cell. Anna Beaudin, Scott Boyer, **Camilla Forsberg**. Institute for the Biology of Stem Cells, Department of Biomolecular Engineering, University of California Santa Cruz, Santa Cruz, CA.

We want to understand the cellular and molecular mechanisms of hematopoietic stem cell (HSC) specification and differentiation. We recently established a lineage tracing mouse model where differentiation stage-specific expression of Cre recombinase results in the irreversible switching of a ubiquitous dual-color reporter from Tomato to GFP expression. Using this model, we have demonstrated that, in adult mice, all lineages differentiate through a Flk2-positive stage; that Flk2+ progenitors contribute equally to granulocyte/monocyte and megakaryocyte/erythroid lineages in vivo; and that Flk2+ cells do not dedifferentiate into HSC. In addition, the fact that all functional, adult HSC remain Tomato+ conclusively demonstrates that all developmental precursors of adult HSC lack Flk2 expression. Surprisingly, we found that GFP+ HSC coexist with Tomato+ HSC during fetal development. These GFP+ HSC support long-term, multilineage hematopoiesis when transplanted into adult recipients. Like Tomato+ adult and fetal HSC, the GFP+ HSC retain long-term reconstitution potential in serial transplantation assays, but display differential lineage bias and give rise to distinct subsets of mature immune cells. Thus, we have identified a novel, fetal population of HSC that are capable of reconstituting adult recipients, but do not persist in adulthood or give rise to adult HSC. This finding has important implications for developmental hematopoietic disorders, pediatric leukemias, and derivation of engraftable HSC from pluripotent precursors.

23

A defensive role of Sirt3 against oxidative stress and aging in preimplantation embryos. Yumiko Kawamura[1,2,3], Yasunobu Uchijima[1], Koichi Nishiyama[1], Yukiko Kurihara[1], Kiyoshi Kita[2], Hiroki Kurihara[1]. 1) Dept. of Phys. Chem. and Meta., Grad.

Sch. of Med., the Univ. of Tokyo, Tokyo, Japan; 2) Dept. of Biom. Chem., Grad. Sch. of Med., the Univ. of Tokyo, Tokyo, Japan; 3) JSPS Research Fellow.

In mammals, the reproductive processes are carried on under low-oxygen conditions. In case embryos are subjected to high-oxygen environments, excessive reactive oxygen species (ROS) are produced, causing embryo injury. We have focused on Sirtuins, a family of phylogenetically conserved NAD+-dependent protein deacetylases/ADP-ribosyltransferases, are implicated in diverse biological processes, such as aging and metabolism, as molecules involved in stress defense mechanisms coupled with cellular metabolic status. We, firstly, have characterized the role of sirtuins in mouse preimplantation development under in vitro conditions. All sirtuin members were expressed in eggs, and their expression gradually decreased until the blastocyst stage. Among them, the expression of Sirt3, which localizes to mitochondria and is involved in energy metabolism, was upregulated by H_2O_2-induced oxidative stress, indicating an important role for Sirt3 in mitochondrial functions, which is a crucial factor in determining the developmental potential of embryos. Indeed, siRNA-induced knockdown and genetic deletion of *Sirt3* resulted in increased intracellular ROS originated from mitochondria and decreased blastocyst formation. The antioxidant N-acetyl-L-cysteine and low oxygen conditions rescued these adverse effects. We also found that ROS-induced upregulation of p53 was responsible for this developmental arrest. When Sirt3-knockdown embryos were transferred to pseudopregnant mice after long-term culture, implantation and fetal growth rates were decreased, indicating that Sirt3-knockdown embryos were sensitive to in vitro aerobic conditions and that the effect was long lasting. Furthermore, microinjection of Sirt3 mRNA could improve the developmental outcome of preimplantation embryos exposed to H_2O_2. Thus, Sirt3 protects preimplantation embryos against oxidative stresses under aerobic conditions through regulating mitochondrial functions. Recently, we are pursuing the possibility that Sirt3 may improve the outcome of assisted reproductive technologies (ART), because of previous findings that ROS is critical for ageing of germ cells. Since ART is widely used for the treatment of infertility in clinical practice, many issues such as the low birth rate remain to be solved. We have obtained some preliminary evidences for a negative relation between Sirt3 levels and aging-associated loss of egg quality; thus Sirt3 may work advantageously to improve the ART efficiency.

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The mouse Y-encoded transcription factor ZFY promotes the second meiotic division and is essential for sperm tail development and head remodelling. Nadege Vernet, Fanny Decarpentrie, Shantha Mahadevaiah, Paul Burgoyne. Stem cell biology and developmental genetics, NIMR, Medical Research Council, UK, London.

Human ZFY and mouse *Zfy1* and *Zfy2* encode zinc finger transcription factors and were identified in the 1980s as promising candidates for the mammalian Y-encoded testis determinant. The first functions were ascribed to these genes in 2010 and 2011 when they were shown to have important meiotic quality control functions in sex chromosomally aberrant mice; thus *Zfy1* and *Zfy2* trigger the apoptotic elimination of XYY spermatocytes at the pachytene stage, and *Zfy2* the apoptotic elimination of spermatocytes with a univalent X chromosome at the first meiotic metaphase. *Zfy1* and *Zfy2* are located on the mouse Y short arm (Yp) and for the present study we created three mouse models with a limited Y gene content, and diminishing *Zfy* gene complements: (1) an almost complete Yp gene complement - ***Zfy2***, ***Zfy1***, *Rbmy1a1, H2al2y, Sry, Usp9y, Ddx3y, Uty, Eif2s3y, Kdm5d, Ube1y1*; (2) a highly deleted Yp retaining*Rbmy1a1, H2al2y, Sry, Eif2sy* and creating a fusion gene ***Zfy2/1*** by an ectopic recombination event between *Zfy1* and *Zfy2*; and (3) a limited Y gene content restricted to *Sry* and *Eif2s3y*. All three models lack the Y long arm that normally mediates pairing and exchange with the X via their pseudo autosomal regions (PAR), so we added a minute PAR-bearing X chromosome derivative to each thus enabling the formation of a sex bivalent to avoid any apoptosis triggered by a univalent X chromosome. These three models were first used to substantiate previous findings suggesting a role for a Yp gene(s) in promoting the second meiotic division (**Vernet et al. 2011**), and also to confirm that a Yp gene(s) is required for the initiation of sperm morphogenesis (specifically the round spermatid to elongating spermatid transition; **Vernet et al. 2012**). We have then used a Y transgene addition strategy to show that it is *Zfy1* and *Zfy2* that promote the second meiotic division and that sperm morphogenesis requires *Zfy2* but not *Zfy1*. Thus these Y-encoded zinc finger transcription factors, in addition to meiotic quality control functions, have important roles in normal spermatogenesis. *References*: **Vernet, N**, et al. (2011) The Y-encoded gene Zfy2 acts to remove cells with unpaired chromosomes at the first meiotic metaphase in male mice. Current Biology 21 787-793. **Vernet, N**, et al. (2012) Spermatid development in XO male mice with varying Y chromosome short arm gene content: evidence for a Y gene controlling the initiation of sperm morphogenesis. Reproduction (Submitted).

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FGFR2IIIc Is Required for Testis Determination but Is Dispensable for Subsequent Testis Cord Differentiation and Spermatogenesis. Stefan Bagheri-Fam[1], Meiyun Yong[1], Anja Dietrich[1], Terje Svingen[2], Peter Koopman[2], Veraragavan Eswarakumar[3], **Vincent R. Harley**[1]. 1) Molecular Genetics & Development, Prince Henry's Inst, Clayton, VIC, Australia; 2) Institute for Molecular Bioscience, University of Queensland, QLD, Australia; 3) School of Medicine, Yale.

Loss of Fibroblast Growth Factor Receptor 2 (FGFR2) in mice leads to XY gonadal sex reversal, indicating that FGFR2 acts as the receptor for FGF9 during testis development. FGFR2 exists in two isoforms, FGFR2IIIb and FGFR2IIIc which differ in their FGF ligand-binding specificity. FGFR2IIIb is a low affinity receptor for FGF9 and is not normally expressed in Sertoli cells, and XY Fgfr2IIIb knockout mice do not show abnormalities in testis development. FGFR2IIIc is a high affinity receptor for FGF9 and is expressed in Sertoli cells. However, Fgfr2IIIc knockout male mice were reportedly fertile suggestive of normal testis development. It was speculated that over-expression of the FGFR2IIIb isoform could compensate for the loss of FGFR2IIIc. Alternatively, Fgfr2IIIc knockout male mice might show testicular defects at fetal stages that resolve postnatally. To investigate

these possibilities, we examined gonadal development in XY Fgfr2IIIc knockout mice at embryonic days (E) 13.5 and 15.5. Instead of normal testes, XY Fgfr2IIIcKO mice developed ovotestes or ovaries, phenocopying the gonadal defects of Fgf9KO and Fgfr2KO mice (on a mixed genetic background). The ovarian regions of XY Fgfr2IIIcKO gonads lacked expression of the Sertoli cell marker AMH and expressed the female somatic cell marker FOXL2. XY sex reversal extended to germ cells which expressed SCP3, a marker of meiotic prophase. Subsequent analysis of adult XY Fgfr2IIIc knockout mice revealed that around 20% were phenotypic females. These data suggest that the XY Fgfr2IIIc knockout embryos with ovotestes develop into the reported fertile males, while the XY Fgfr2IIIc knockout embryos with ovaries develop into infertile females. Genes such as Wt1 and Sox9 that are critical for gonadal development or sex determination often have subsequent roles in testicular development and function such as in the maintenance of Sertoli cell identity or in nursing germ cells through spermatogenesis. To examine the role of FGFR2 in Sertoli cells after the sex determination phase, we conditionally inactivated Fgfr2 (both isoforms) in Sertoli cells at E13.5 using the AMH-Cre mouse line. These mice did not show obvious defects in testis cord differentiation and spermatogenesis. Moreover, expression of selected male and female markers was unchanged when compared to control mice. In summary, FGFR2IIIc is required for testis determination, but is dispensable for subsequent testis cord differentiation.

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System-level analysis of sperm maturation in the epididymis. Timothy Karr[1], Sheri Skerget[1], Konstantino Petritis[2], Ashoka Polpitiya[2]. 1) Biodesign Inst, PO Box 875001, Arizona State Univ, Tempe, AZ; 2) Center for Proteomics, Translational Genomics Research Institute, Phoenix, AZ 85004.

The molecular components of complex cellular networks are being identified at a rapidly increasing rate. A key aspect of this effort is to obtain an understanding of interrelated functional elements and pathways using systems biology approaches. Systems levels analyses of transcriptomic and proteomic data is central to this effort, but the complexity of these datasets presents a variety of barriers to achieving a functionally meaningful understanding of system level properties. Our approach has been to leverage existing knowledge of a tractable system, the sperm proteome, to allow the integration of transcriptomic and proteomic landscapes within the epididymus, a tissue through which sperm must traverse to properly mature. This approach is based on our recent studies characterizing the Drosophila, mouse, macaque and other sperm proteomes, coupled with our understanding of transcriptome dynamics during spermatogenesis. We describe our recent results that have identified a surprising number of protein changes occurring in the epididymis during transit and correlate these changes with primary gene/protein networks and mechanisms operating during sperm transit which are functionally necessary for the acquisition of fertilization competency. *Surprisingly, analysis of the three major morphological domains of the epididymis (caput, corpus and cauda) identified over* **600** protein additions to- and over **900** *protein removals from- the sperm proteome during transit*. The overarching goal of our studies is to unify epididymal transcriptomic and sperm proteomic analyses to reveal how sperm sequentially interact within different epididymal regions as part of their acquisition of motility and capacitation. The direct relevance of this approach to humans is highlighted by high levels of functional homology (35%) between the Drosophila and mammalian sperm proteomes, an approximately 50% overlap between mouse and human sperm proteomes, and a 10% orthology of mouse sperm proteins with human associated disease genes. This research will therefore assist in obtaining a more complete molecular understanding of disease gene biology as well as pioneer systems level integration of transcriptomic and proteomic dynamics within a tractable cellular development process essential to sexual reproduction.

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Sonic hedgehog-expressing basal stem cells are the cell-of-origin for bladder cancer. Philip A. Beachy, Kunyoo Shin, Agnes Lim, Sally Kawano. Stanford Univ Sch Medicine, Stanford, CA.

The idea that human malignancies may originate from adult tissue stem cells derives from the intrinsic ability of stem cells to self-renew, from their longevity and consequent ability to accrue multiple mutations, and from the phenotypic resemblance of tumor-propagating cells to stem cells. Experimental tests of this hypothesis have relied on cell-specific genetic manipulation to induce cancer in murine models, and such studies have produced varying conclusions regarding the cancer cell-of-origin. We have recently identified the urothelial stem cell through lineage tracing and organoid culture, and have identified some of the signals that control stem cell proliferative activity. Based on these findings, we examine here the origin of bladder cancer by prospectively marking and tracking these stem cells in a murine model, selected for its similarity to human carcinogenesis, in which invasive bladder carcinomas are induced by prolonged exposure to nitrosamine. In this model, we find that Shh-expressing stem cells in the basal urothelium give rise to the carcinoma, although Shh expression within tumor cells is lost by the time carcinomas develop. This malignant transformation progresses through epithelial hyperplasia, a precursor lesion consisting of a thickened urothelium comprising many layers of cytokeratin 5 (CK5)-expressing basal-like cells. Shh expression occurs in basal but not lumenal layers of this hyperplastic lesion, and these basal cells give rise to the Shh-negative tumor-propagating cells of the full-fledged carcinoma. Confirming that tumor-propagating cells derive from basal stem cells but not more differentiated lumenal cells, we showed that prior ablation of Shh-expressing cells rendered the bladder resistant to nitrosamine-induced formation of epithelial hyperplasia or carcinoma. Our findings thus demonstrate that invasive carcinoma is initiated from the basal urothelial stem cell and typically progresses through a hyperplastic lesion, thus confirming experimentally the view that such lesions are not only associated with but actually give rise to epithelial cancers. Our findings further suggest that the phenotypic properties of mature tumor cells, such as absence of Shh expression, may not provide a sound basis for inferring the identity of the cancer cell-of-origin.

Epigenetics

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DNA Oxidation towards Totipotency in Mammalian Development. Guo-Liang Xu. Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China.

Sperm and eggs carry distinctive epigenetic modifications that are adjusted by reprogramming following fertilization. The paternal genome undergoes active DNA demethylation before the first mitotic division. The biological significance and mechanisms of paternal epigenome remodeling are unclear. We find that, within mouse zygotes, oxidation of occurs in the paternal genome, changing 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC). In Tet3 oxidase-deficient zygotes derived from conditional knockout mice, the conversion of 5mC into 5hmC fails to occur. Thus, the loss of 5mC in the paternal genome in zygotes is caused by its conversion to 5hmC mediated by Tet3. Deficiency of Tet3 also impedes demethylation at the paternal copy of genes such as Oct4 and Nanog and delays the subsequent reactivation of Oct4 in early embryos. Heterozygous mutant embryos lacking maternal Tet3 suffer increased developmental failures. Importantly, oocytes lacking Tet3 also show impaired reprogramming of injected somatic cell nuclei. We conclude that Tet-mediated oxidative DNA demethylation is essential for epigenetic reprogramming in the early embryo following natural fertilization, as well as for the reprogramming of somatic cell nuclei during animal cloning.

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DNA dioxygenases regulate cardiac progenitor pool and their differentiation in heart development. Qing-Yan Cui[1], Guo-Liang Xu[1], Bin Zhou[2]. 1) Group of DNA Metabolism, The State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China; 2) Key laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Graduate School of the Chinese Academy of Sciences, Shanghai 200031, China.

The Tet family functions as dioxygenases to oxidize 5-methylcytosine (5mC), which is thought to play an important role in DNA demethylation and gene activation. However, little is known about the biological significance of this modification in development. To reveal the role of Tet family in vivo, we generated Tet1 and Tet3 mutant mice. Single ablation of Tet1 or Tet3 does not lead to any embryonic developmental defect. However, depletion of both genes leads to embryonic death at mid-gestation due to severe heart defects, including thin compact myocardium and hypoplastic trabeculae at E11.5. Consistent with this phenotype, we found that cardiomyocyte proliferation was significantly decreased in double mutant hearts. Surprisingly, embryos lacking both Tet1 and Tet3 displayed an expansion of the Isl1-positive progenitor cell population at E9.5. Consistently, the Tet1/Tet3 depleted ES cells displayed defective differentiation ability towards cardiomyocytes in embryonic body (EB) differentiation assays, with reduced percentage of spontaneously beating foci within an individual EB, and down-regulated mature cardiomyocyte markers such as MHC, MHC and MLC2a, but up-regulation of cardiac progenitor marker Isl1. Collectively, our data shows an unexpected redundant function of Tet1 and Tet3 in vivo and in vitro, indicating the significance of oxidation-dependent demethylation during early mouse heart development.

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Dissection of the molecular mechanism that regulates activation of murine IAP (intracisternal A particles) retrotransposons upon DNA demethylation in ES cells. Jafar Sharif[1], Takaho A. Endo[2], Kayoko Katsuyama[1], Yoko Mizutani-Koseki[1], Yoko Kuroki[1], Tomoyuki Ishikura[1], Takanori Hasegawa[1], Osamu Ohara[1], Tetsuro Toyoda[2], Yoichi Shinkai[3], Haruhiko Koseki[1]. 1) RIKEN Center for Allergy & Immunology (RCAI), Japan; 2) RIKEN Bioinformatics and Systems Engineering Division (BASE), Japan; 3) RIKEN Advanced Science Institute (ASI), Japan.

IAPs (intracisternal A particles) are a class of endogenous retroviruses (ERVs) that are presently highly active in the rodent genome. In murine embryonic stem (ES) cells, IAPs are rigorously silenced by repressive epigenetic modifications such as DNA and H3K9 methylation. The SRA protein Np95 is the only protein known today that can recognize both DNA and H3K9 methylation marks in mammals, suggesting the possibility that Np95 could be a key mediator for IAP regulation. In this study, by using various conditional knockout ES cells for Np95, Dnmt1 (maintenance DNA methyltransferase) and Eset/Setdb1 (H3K9 trimethylase), we report that Np95 might have a role for DNA demethylation induced IAP activation and that this could be mediated by at least two pathways, namely, reduction of H3K9 trimethylation, and, recruitment of positive transcription factors such PTEF-b at the IAP loci. Moreover, our results implicate that this function of Np95 could be dependent on the recognition of hemi-methylated DNA generated by passive DNA demethylation. Collectively, these results provide the first mechanism to elucidate how retrotransposons could be activated upon loss of DNA methylation and show that Np95 could regulate the dynamic interplay between DNA and H3K9 methylation by recognizing both these modifications to control IAP transcription.

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Alterations in genomically imprinted noncoding RNA clusters in a mouse model of Fetal Alcohol Spectrum Disorders (FASD). Benjamin Laufer, Janus Katherine, Kleiber Morgan, Eric Diehl, Sean Addison, Shiva Singh. Biology, Western

University, London, Ontario, Canada.

Maternal drinking during pregnancy causes Fetal Alcohol Spectrum Disorders (FASD). Previous research on FASD has shown behavioural, neuro-structural, and more recently, gene expression changes. The mechanism(s) underlying these changes however, are not known and form the focus of this research. Specifically, this research examines any involvement of epigenetic mechanisms in a mouse model of FASD using four ethanol treatment protocols in C57BL/6J mice. Total brain from resulting mature males (postnatal day 70) from ethanol exposed and matched controls were used to assess noncoding RNA expression using mouse miRNA arrays, mouse gene expression arrays and qPCR. The results have revealed that a large number of microRNAs and snoRNAs are altered, both up and down, depending on treatment paradigm. Some of the observed changes are unique to a specific treatment protocol, while others overlap across treatments. Strikingly, approximately 20% of the altered noncoding RNA genes map to three imprinted regions of the mouse chromosome. The first two, *Snrpn-Ube3a* (Murine 7qC/Human 15q11-q13) and *Dlk1-Dio3* (Murine 12qF1/Human 14q32.2), are associated with processes involved in neuronal plasticity and several neurodevelopmental disorders. The third cluster contains *Sfmbt2* (Murine 2qA1) and an overlapping antisense transcript that is unique to mice and rats. We followed these results with the assessment of DNA methylation using methylated DNA immunoprecipitation followed by hybridization to DNA arrays (MeDIP-Chip). The results show that fetal alcohol exposure has a genome-wide effect on DNA methylation with imprinted regions of the genome appearing to be particularly sensitive. Ultimately, our results suggest that imprinted noncoding RNAs, many of which are known to play a critical role in neurodevelopment and brain function, may also have a role in the long-term maintenance of cognitive deficit associated altered gene expression in the mouse model of FASD.

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MeCP2 Interacting Partners in Development and Rett Syndrome. Mary Donohoe[1,2,3], Siva Muthuswamy[1,2,3], Tao Wu[1,2,3]. 1) Burke Medical Research Institute, White Plains, NY; 2) Dept of Neuroscience Weill Cornell Medical College New York, NY; 3) Dept of Cell and Developmental Biology Weill Cornell Medical College New York, NY.

Rett Syndrome (RTT) is a neurodevelopmental disorder that is one of the leading causes of mental retardation and autistic behavior in girls. RTT is caused by mutations in the X-linked Methyl CpG-binding protein 2 (MeCP2) gene, which accounts for approximately 80% of sporadic and 45% familial RTT cases. MeCP2 can function as a transcriptional modifier that represses developmental silencing through binding to methylated DNA and complexing with co-repressors. In addition, MeCP2 may operate as a transcriptional activator, splicing factor, and in long-range chromatin looping. MeCP2 is expressed in many mammalian tissues but disruption of this regulator in RTT has a profound effect in mature neurons. Consistent with this, MeCP2 is a key regulator of neural activity-dependent gene expression controlling learning and memory and cocaine drug addiction. MeCP2 shares many characteristics with histones. It is highly enriched with amino acids that may be regulated by post-translational modifications (PTMs). Phosphorylation of MeCP2 can regulate its DNA binding in a neural activity dependent-manner. Other PTMs have not been described for MeCP2. To understand the action of MeCP2, our lab is studying its protein interacting partners as well as PTMs in a mouse model. We show that SUMO1 and SUMO3 proteins bind and modify MeCP2. SUMOylation of MeCP2 is greatly enhanced by neural activity. The transcriptional activity of MeCP2 is modified by SUMO modification. Using sequential Chromatin Immunoprecipitation we show that MeCP2 is SUMOylated at specific promoters in vivo. We have identified an interacting partner for MeCP2. Loss of this MeCP2 co-factor results in an alteration of SUMOylation suggesting that this co-factor serves as a SUMO E3 ligase for MeCP2. We will present our ongoing studies to define the neuroepigenetic regulatory circuit of MeCP2 in normal development and in RTT.

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The histone H2B ubiquitin ligase Rnf20 is required for self-renewal and pluripotency in mouse ES cells. Kit Wan Ma, Takaho A. Endo, Jafar Sharif, Haruhiko Koseki. RCAI, RIKEN Yokohama Institute, Yokohama, Kanagawa, Japan.

Self-renewal and pluripotency are two main features of embryonic stem (ES) cells, giving rise to unlimited expansion ability and differentiation potential into any cell types in the body. They are attained by retaining the cells at the S phase of the cell cycle, activation of pluripotency genes and suppression of differentiation regulator genes. Therefore, a molecular mechanism that shapes the chromatin landscape for DNA replication and gene transcription is a master regulator of the ES cell features. However, such mechanism has not yet been identified. H2B monoubiquitination at lysine 120 (H2BK120ub1) mediated by the Rnf20/Rnf40 complex plays a key role in the regulation of chromatin landscape by manipulating histone modifications and chromatin structure. Previous studies showed that H2BK120ub1 is a prerequisite of H3K4 trimethylation and is responsible for regulating the dynamics of chromatin higher-order folding. Our preliminary results demonstrated that Rnf20, possibly via H2B ubiquitination, is indispensible for maintaining the pluripotency and self-renewal. In mouse Rnf20 knockout ES cells, the global H2BK120ub1 decreases accompanied by growth defect and cell differentiation. Cell cycle analysis by accessing the EdU (thymidine analog) incorporation ability showed that DNA replication activity is severely impaired in Rnf20 knockout cells, resulting in a loss of S phase cells concomitant with G1 phase cell cycle arrest. The blockage of G1/S transition may be due to reduced binding of replication factors to the chromatin. We purified nascent chromatin from Rnf20 depleted cells and found that the binding of PCNA, a loading platform for various DNA replication factors, is completely abolished. It suggests that Rnf20 is essential for the binding of replication factors, leading to efficient DNA replication. Moreover, Rnf20 mediated H2B ubiquitination may be involved in the regulation of the core pluripotency network. Using chromatin immunoprecipitation (ChIP) assay, we found that several core pluripotency genes including Sox2, Nanog and Oct4 are enriched in H2BK120ub1. If Rnf20 is

depleted, these genes are downregulated with decreased H2BK120ub1. Collectively, our results suggest that Rnf20 is a potential master epigenetic control of DNA replication and gene transcription in ES cells, maintaining the stable undifferentiated state.

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Upregulation of the mammalian X chromosome is associated with enhanced transcription initiation and epigenetic modifications. Xinxian Deng[1], Joel Berletch[1], Joseph Hiatt[2], Di Kim Nguyen[1], Jay Shendure[2], Christine Disteche[1,3]. 1) Dept Pathology, Univ Washington, Seattle, WA; 2) Dept Genome Sciences, Univ Washington, Seattle, WA; 3) Dept Medicine, Univ Washington, Seattle, WA.

X upregulation in mammals increases expression of X-linked genes to compensate for bi-allelic expression of autosomal genes. Here we present the first evidence of a molecular mechanism that enhances X transcription based on differential enrichment in specific marks on the active X chromosome. Histone H4 acetylated at lysine16 and the corresponding acetyltransferase MOF known to mediate upregulation of the Drosophila X chromosome, were specifically enriched at the 5 end of active mouse X-linked genes. In addition, H2AZ and the transcriptional initiation form of RNA polymerase II (PolII-S5p) were also specifically enriched at the 5' end of X-linked genes. Importantly, depletion of MOF or of MSL1, two conserved components of the Drosophila upregulation complex, caused a decrease in PolII-S5p and in expression of mouse X-linked genes. Our results suggest that the MSL complex plays a role in mammalian X upregulation via enhanced transcription initiation.

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Nuclear transfer for the study of X chromosome inactivation in mice. Atsuo Ogura[1,2], Shogo Matoba[1], Mami Oikawa[1,3], Kimiko Inoue[1,2], Fumitoshi Ishino[3]. 1) BioResource Center, RIKEN, Tsukuba, Ibaraki, Japan; 2) Graduate School of Life and Environmental Science, University of Tsukuba, Ibaraki, Japan; 3) Department of Epigenetics, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan.

Cloning by nuclear transfer into enucleated oocytes is the sole reproductive engineering technology that endows the donor cells genome with totipotency. Besides its practical applications in production of cloned animals, the nuclear transfer technique can provide invaluable experimental models for the study of mammalian epigenetics. Comparisons of the epigenetic patterns of mouse embryos cloned from donor cells at different times of the life cycle can determine the nature of the epigenetic changes during development and their underlying mechanisms. These include DNA methylation, histone methylation, genomic imprinting, and X chromosome inactivation (XCI). XCI normally triggers inactivation of one of the two X chromosomes in female embryos so that the gene dosage can be compensated with that in males. XCI is established by *Xist* RNA coating in *cis*. In somatically cloned embryos of both sexes, *Xist* was ectopically expressed from the active X chromosome and this perturbation critically affected development of cloned embryos, as revealed by knockout and knockdown experiments. We then extended our XCI analysis to nuclear transfer using different types of germ cells of both sexes. The results suggested that*Xist* is expressed at zygotic gene activation in the default mode and only the genome which has passed through oogenesis can repress this expression. This is the most probable reason why somatically cloned mouse embryos ectopically express *Xist* from the active X chromosome. This finding strongly supports the idea of the existence of a maternal imprint resistance to XCI. Furthermore, we also found that shortly after implantation the aberrant XCI status in cloned embryos had been corrected autonomously in both embryonic and extraembryonic tissues, indicating the presence of a newly established XCI control for postimplantation embryos of the both lineage.

Keynote Address

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Development of Hematopoietic Stem Cell: A final scenario. Shinichi Nishikawa. Riken Center for Developmental Biology, Kobe, Kobe, Japan.

A large gap has existed in our understanding of the course of differentiation from mesoderm to definitive haematopoietic stem cells (HSCs). Recently, Flk1+ mesoderm that expresses Etv2 was determined to be the source from which the progenitors of all endothelial (EC) and blood cells are segregated from other lineages. This result justifies to use Flk1+Etv2+ mesoderm (Etv2-induced mesoderm, ETM) as the starting point of prospective analysis of differentiation course of HSC and EC. With this rationale in mind, we analyzed the progression of HSC differentiation from this Etv2-induced mesoderm (ETM). ETM diversifies into Runx1-Gata1-, Runx1+Gata1- and Runx1+Gata1+ populations in the extra-embryonic region at E7.5. The fate of GATA1+ cells are almost exclusively primitive erythrocytes. Hemogenic EC and HSCs are derived from the Runx1+Gata1- population, which moves from the extra-embryonic space to the embryo proper during E7.5 to E8.0 and contributes to the vasculature. This migration occurs before the heartbeat is initiated and is independent of circulation. This proposed differentiation course with circulation-independent hemogenic angioblasts would unite extra- and intra-embryonic origins of blood cells and settle the long-running dispute about the orgin of blood cells. In this symposium, we would like to propose a new terminology of intermediate stages toward HSC.

Technology

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Characterization of haploid embryonic stem cells from mouse embryos. Anton Wutz, Martin Leeb. Wellcome Trust Centre for Stem Cell Research, Cambridge, Cambridgeshire, United Kingdom.

Haploid embryonic stem cells (ESCs) have recently been derived from mouse parthenogenetic embryos and applied for genetic screens in culture. These cells maintain key properties of mouse ESCs and might overcome existing limitations in developmental genetic approaches in mice. The ability of haploid ESCs to give rise to a wide range of differentiated cell types in the embryo and in vitro has been demonstrated and is paralleled with the gain of a diploid karyotype. It has remained unclear if haploid ESCs can contribute to the germline. Here we show that parthenogenetic haploid ESCs have robust germline competence enabling the production of transgenic mouse strains from genetically modified haploid ESCs. We further show that differentiation of haploid ESCs in the embryo correlates with an efficient gain of a diploid karyotype and that diploidization is likely the result of endoreduplication and not cell fusion. In contrast to the embryo a haploid karyotype is maintained under certain conditions during in vitro differentiation.

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MFA: A Novel Method For The Generation Of "All-In-One" Null And Conditional Alleles. Christopher Schoenherr, Evangelos Pefanis, Peter Lengyel, Darshi Persaud, YuHong Zhang, Ronald Deckelbaum, Julie Kalter, Dimitrios Skokos, Peter Yang, Andrew J Murphy, **Aris N. Economides**. Regeneron Pharmaceuticals, Tarrytown, NY.

The engineering of conditional alleles has evolved from simple floxing of regions of genes to more elaborate methods. We developed a method - Conditional by Inversion (COIN) - that utilizes an exon-splitting intron and an invertible genetrap-like module (COIN module) to create null alleles upon Cre-mediated inversion. While COINs provide unprecedented flexibility in engineering conditional alleles, we have extended COINs by generating a new Multifunctional Allele (MFA), which utilizes a single gene-targeting step and three site-specific recombination systems, to generate four distinct allelic states: (1) Null with reporter (plus drug selection cassette): MFAs start by replacing the gene of interest with a modified version of the gene that is inactivated by both inversion of a critical part of the coding sequence and simultaneous insertion of a reporter gene and selection cassette. This is accomplished by gene targeting. (2) Null with reporter (minus drug selection cassette): MFAs allow the option of subsequent deletion of both the selection cassette and the inverted gene segment using site-specific recombination (Dre). This gives the best reporter gene expression while retaining gene inactivation. (3) COIN-based conditional-null: MFAs can be converted to a phenotypically wild-type, yet conditional allele, via removal of the selection cassette and reporter and simultaneous re-inversion of the critical part of the gene through the action of a second site-specific recombinase (FLP). This step also acts as a built-in complementation test that addresses the possibility of artefacts introduced during the targeting process. (4) Recombinase-induced null: MFAs allow re-inactivation of the conditional allele created in step 3, by re-inversion of the critical gene segment using a third site-specific recombinase (Cre), and concomitant induction of a second reporter (that embedded in the COIN module). Using *Hprt1* as a test locus, we have determined that MFA works robustly to generate all four functionalities. The results obtained with $Hprt1^{MFA}$ are being extended to a set of genes with well-characterized knockout alleles, such as *Il2rg*, in order to provide a stringent comparison with their MFA counterparts. Lastly, given that MFAs rely on standard genetic engineering, gene targeting, and site-specific recombinase systems, they could be easily adopted by laboratories skilled in generating genetically modified mice without any changes in current workflow.

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Efficient generation of a conditional knock-out allele in mice by zinc finger nuclease-mediated gene targeting. Keith Anderson[1], Jeremy Burton[1], Tuija Alcantar[2], Jinjie Li[2], Robert Schwingendorf[2], Tim Soukup[1], Merone Roose-Girma[1], J. Colin Cox[2], Xin Rairdan[2], Weilan Ye[1], **Soren Warming**[1]. 1) Department of Molecular Biology, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080; 2) Department of Mouse Genetics, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080.

The use of sequence-specific zinc finger DNA-binding domains fused to an endonuclease domain (zinc finger nucleases, or ZFNs) has enabled efficient and direct generation of both gene knock-out and knock-in alleles by microinjection into fertilized eggs from either mice or rats. While knock-out alleles result from error-prone repair of a ZFN-mediated double-strand break (DSB), knock-in alleles are obtained by co-microinjection of ZFN mRNA and a donor plasmid that serves as a template for repair. However, existing methods are not suitable for generating conditional knock-out alleles due to extensive homology between host and donor sequences and the presence of the ZFN target site in the donor plasmid. We now describe the successful generation of mice carrying a conditional knock-out allele of the Lrp5 gene using ZFN technology. This was achieved by microinjection of mRNA encoding a highly efficient ZFN pair along with a donor plasmid containing a loxP-flanked (floxed) exon sequence with a number of silent mutations to reduce unintended cross-over and ZFN cleavage in the region between the two loxP sites. When using a donor plasmid with a high degree of homology to the target exon, we instead obtained mice with alleles missing either one or both loxP sites. This conditional knock-out strategy and the results have important ramifications not only for mouse genome engineering using ZFN technology, but also for genetic engineering of other mammalian species.

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Next generation RNAi mouse models for drug discovery and toxicology assessment. Prem Premsrirut[1], Christof Fellmann[1], Lukas Dow[2], Johannes Zuber[3], Gregory Hannon[4], Scott Lowe[2]. 1) Research & Development, Mirimus Inc., Cold Spring Harbor, NY; 2) Cancer Biology, Memorial Sloan Kettering Cancer Center, New York, NY; 3) Differentiation and Disease, Institute of Molecular Pathology, Vienna, Austria; 4) Bioinformatics and Genomics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

RNA interference is a powerful tool for studying gene function, however, the reproducible generation of transgenic RNAi mice remains a significant limitation. One main hurdle is the identification of potent RNAi triggers, or short hairpin RNAs (shRNAs), that will induce stable and regulated gene silencing. Due to the lack of understanding of the requirements for shRNA biogenesis and target suppression, many predicted shRNAs fail to efficiently induce gene suppression. We have developed a Sensor assay that enables the biological identification of effective shRNAs at large scale and show that our assay reliably identifies potent shRNAs that are surprisingly rare and predominantly missed by existing algorithms. By combining our sensored miR30-based shRNAs with high efficiency embryonic stem cell targeting, we developed a fast, scalable pipeline for the production of transgenic RNAi mice. We show that RNAi can cause sufficient knockdown to recapitulate the phenotypes of knockout mice, particularly in cancer models. More importantly, unlike traditional knockout models, RNAi has the powerful advantage of reversibility, since the endogenous gene remains intact. Using this system, we generated a number of inducible RNAi transgenic lines and demonstrate how this approach can identify predicted phenotypes and also unknown functions for well-studied genes. In addition, through regulated gene silencing we validate several tumor suppressor genes as potential therapeutic targets in T cell acute lymphoblastic leukemia/lymphoma and lung adenocarcinoma, respectively. This system provides a cost-effective and scalable platform for the production of RNAi transgenic mice targeting any mammalian gene that will be valuable tools for performing preclinical target identification, validation and toxicity assessment in vivo.

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Constructing functional genetic networks in mammalian cells. Christopher Kemp, Russell Moser, Michael Kao, Chang Xu, Carla Grandori, Eddie Mendez. Fred Hutchinson Cancer Research Center, Seattle, WA.

The majority of biomedical research using model organisms such as mice is performed on a uniform genetic background. However, as phenotype is the product of myriad genetic interactions, this approach limits our view of the underlying genetic basis of phenotype. For example, genome scale analysis of synthetic lethal interactions has not been possible in mammalian cells. Traditional methods to study such interactions in mice have relied on time consuming breeding experiments and are inefficient at identifying functional genetic networks. Using such an approach, we identified synthetic lethality between the DNA damage kinases Atm and DNA-PK (Current Biology 11:191, 2001) and DNA damage-conditional synthetic lethality between DNA-PK and the tumor suppressor p53 (EMBO Reports, 10:87, 2009). However the lack of functional genetic tools limited our ability to further dissect these pathways. To overcome this, we have developed a method to identify synthetic lethal interactions on a genome scale in mammalian cells that utilizes high throughput screening (HTS) with arrayed siRNAs. Using viability as an endpoint, we performed kinome wide siRNA screens with a series of mouse cells that carry defined mutations in p53 pathway genes (Atm, DNA-PK, p19Arf and p53) and identified novel synthetic lethal and synthetic sick interactions with each of these genes. To establish proof of principle that this approach can identify meaningful drug targets for cancer therapy, we focused on the cell cycle kinase WEE-1 that when knocked down was selectively lethal to both mouse and human p53 mutant cancer cells. Relative to p53 wild type cells, p53 mutant cancer cells were ~50 fold more sensitive to the WEE-1 inhibitor MK-1775 providing independent confirmation of our siRNA screen result. This sensitivity of p53 mutant cells to WEE1 inhibition likely results from a greater dependence on the S/G2 checkpoint to repair DNA and avoid mitotic catastrophe. Oral gavage of MK-1775 inhibited growth of p53 mutant xenografts and augmented cisplatin response indicating the therapeutic potential of MK-1775 in p53 mutant tumors. In summary, using high throughput screening with well-based siRNAs identified a genome scale menu of potential drug targets for cancer cells that carry defined oncogenic mutations. More generally, this functional genetic approach enables, for the first time, the study of genetic interactions on a genome scale in mammalian cells.

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Hot or Not?: Leveraging mouse genome diversity to identify hotspots of copy number variants. Kathleen A. Hill[1], M. Elizabeth O Locke[2], Andrea E. Wishart[3], Susan T. Eitutis[3], Jenna Butler[2], Mark Daley[1]. 1) Department of Biology and Computer Science, The University of Western Ontario, London, ON, Canada; 2) Department of Computer Science, The University of Western Ontario, London, ON, Canada; 3) Department of Biology, The University of Western Ontario, London, ON, Canada.

The Mouse Diversity Genotyping Array (MDGA) currently provides a high resolution means for detection of copy number variants using both SNP and CNV probe sets. Also, publicly available MDGA data exist for 306 different mouse genetic backgrounds encompassing feral mice and the broad history and derivation of inbred mouse strains. PennCNV is currently among the best platforms for microarray data analysis not yet applied to a comprehensive analysis of mouse data. We report the first application of PennCNV including genomic wave adjustment to publicly available MDGA data representing 351 individuals, achieving the most comprehensive CNV analysis to date. The data were prepared and converted using the MouseDivGeno package as well as in-house scripts. The resultant 2067 CNV calls at least 500 nucleotides in length were analyzed using our developed and separately reported software, Hotspot Detector for Copy Number Variants (HD-CNV), to identify recurrent CNV regions detected across multiple samples. These regions are either commonly inherited or hotspots of recurrent mutational events. This dataset serves as a reference for comparison with independently reported mouse CNVs associated with different genotypes

and complex phenotypes including diseases, as well as comparison with syntenic regions of the human genome. The CNV data also provide insight into the mutational mechanisms underlying evolution of genome structure. Knowledge of structural variation and instabilities in the mouse genome is pivotal in both the study of the evolutionary history of the mouse genome and identification of de novo copy number variants.

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PRISM.stanford.edu: 2,500 Human and Mouse transcription factor developmental function predictions. Aaron Wenger[1], Shoa Clarke[2], Jenny Chen[3], Cory McLean[1], **Gill Bejerano**[1,4]. 1) Computer Science, Stanford Univ, Stanford, CA; 2) Genetics, Stanford Univ, Stanford, CA; 3) Biomedical Informatics, Stanford Univ, Stanford, CA; 4) Developmental Biology, Stanford Univ, Stanford, CA.

Recently we developed GREAT.stanford.edu to derive insights into transcription factor (TF) function from ChIP-seq peaks. GREAT works remarkably well on a variety of sets. It does so because the majority of TFs bind directly (and often multiple times) next to a large (10-500) number of contextual target genes. However, the same analysis also shows that when a TF has multiple functions, as most of them do, only the subset of functions relevant to the experiment at hand are observed. Thus for example, ChIP-seq of SRF in immune cells reveals its role in regulating the actin cytoskeleton, but not its roles in muscle development. In GREAT we have collected a vast body of knowledge on all human and mouse genes in many different contexts. Meanwhile, using ChIP-seq and other technologies we have learned the DNA binding preferences of hundreds of TFs. This led us to ask the following question: If we take the binding site preferences of a TF, make stringent genome wide binding site predictions for it, and subject these to GREAT, could we accurately predict additional TF functions not directly observed in ChIP-seq performed to date? For example, when we predict binding sites for SRF and analyze them with GREAT we rediscover SRFs role in regulating the actin cytoskeleton. We also learn that SRF regulates muscle, and that its misregulation may result in dilated heart ventricles, and other true SRF functions not seen (and not relevant) in immune cell ChIP-seq. Starting from a high quality non-redundant library of 300 transcription factors, and using very stringent cut-offs we obtain 2,500 transcription factor role predictions (of the form SRF regulates 142 target genes, all annotated for actin cytoskeleton, using these 356 predicted binding sites, p1E-57), at an average TF function false discovery rate (FDR) of 15% and per binding site FDR of 50%. Conservative computational analysis immediately confirms over 10% of our function predictions in a variety of contexts, implicating many novel target genes and binding sites for known TF functions, and predicting exciting novel TF functions. cDNA co-transfections in primary cells matching our TF function predictions shows that our predictions are highly enriched for active enhancers that responds to the predicted TF. A portal (http://PRISM.stanford.edu) will provide searchable access to this unique resource, which we hope will be of great hypothesis generation value to the developmental biology community.

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Generation of rat-mouse interspecific chimeras for study of organogenesis and elucidation of xenogenic barrier. **Hiromitsu Nakauchi**[1,2]. 1) Division of Stem Cell Therapy, Center for Stem Cell Biology and Regeneration Medicine, Institute of Medical Science, University of Tokyo, Japan; 2) Japan Science Technology Agency, ERATO, Nakauchi Stem Cell and Organ Regeneration Project.

Recent development of a culture system using small molecule inhibitors of glycogen synthase kinase 3 (Gsk3) and the Fgf-MAPK signaling cascade has enabled efficient derivation of pluripotent stem cells (PSCs) from not only all mouse strains but also a non-mouse species, Rattus norvegicus. Using rat ES cells and iPSCs we established, we recently demonstrated the generation of mouse-rat interspecific chimeras. These interspecific chimeras were generated in two ways; injecting rat PSCs to mouse blastocysts and injecting mouse PSCs to rat blastocysts. However, both embryonic survival rate and the degree of chimerism were lower in interspecific chimeras than in intraspecific chimeras, implying that there is a xenogeneic barrier for the development of interspecific chimeras. Interestingly, the size of interspecific chimeras grown into adulthood seemed to conform with that in the species from which the blastocyst originated. The origins of placenta and uterus may have a key role in body size determination. We also demonstrated in mouse the generation of functionally normal rat pancreas by injecting rat PSCs into Pdx1-/- (pancreatogenesis-disabled) mouse embryos, providing proof of principle for organogenesis from xenogenic PSCs in an embryo unable to form a specific organ. Although the pancreas thus generated were composed of almost entirely by rat PSC-derived cells, the size was that of mouse indicating that the size of an organ is determined not by cell intrinsic manner but by environmental factors. The results indicate that interspecific chimeras and generation of organs in vivo using PSC-complemented blastocysts provides a new strategy for elucidation of xenogenic barriers and understanding organogenesis.

Organogenesis

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Regulation of pancreatic islet lineage decisions. Lori Sussel, James Papizan, Josh Levine. Genetics and Development Department, Columbia University, New York, NY.

Pancreatic islet cell development and differentiation is coordinately regulated by many well-characterized transcription factors. Nkx2.2 is a homeodomain-containing regulatory factor required for the appropriate differentiation of pancreatic endocrine cells. Nkx2.2 null mice lack all insulin-producing beta cells and have reduced numbers of glucagon-producing alpha cells and pancreatic polypeptide-producing PP cells. In place of these cell types, the mutant islet is populated with cells that produce the hormone ghrelin. To understand how Nkx2.2 regulates islet cell fate decisions we are exploring the molecular activities of Nkx2.2 in the developing pancreas. We have generated a series of modified Nkx2.2 alleles that have allowed us to dissect out the functions of Nkx2.2 during pancreatic development and in the adult. Phenotypic analysis of mice containing different Nkx2.2 mutant alleles demonstrates that Nkx2.2 depends on differential functions and protein interactions to regulate islet cell lineage specification. Furthermore, Nkx2.2 has unique cell- and time-specific functions to regulate several aspects of beta cell formation, identity and function. These studies are allowing us to define the complex regulatory activities of Nkx2.2 that are necessary for specifying and maintaining functional beta cells in the pancreatic islet. Grant support: NIH R01 DK082590 and NIH U01 DK089523.

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Regulation of thymus and parathyroid organ fate specification by Shh and Tbx1. Nancy R. Manley[1], Virginia Bain[1], Julie Gordon[1], Kaitlin Gutierrez[2], Kim Cardenas[2], Ellen R. Richie[2]. 1) Department of Genetics, University of Georgia, Athens, GA; 2) Department of Carcinogenesis, University of Texas, M.D. Anderson Cancer Center, Science Park Research Division, Smithville, TX.

The thymus and parathyroids perform essential roles in adaptive immunity and calcium homeostasis, respectively. Despite their discrete functions, both organs develop from shared organ primordia originating from the third pharyngeal pouch endoderm in mice. These primodria are patterned into two organ domains, indicated by the expression of Foxn1 (thymus) and Gcm2 (parathyroid). Although the molecular mechanisms that establish each cell fate are not understood, both the pouch endoderm itself and the surrounding neural crest cells have been implicated in this process. Our previous studies showed that the Sonic hedgehog (Shh) null mutation results in loss of parathyroid fate and an expanded thymus domain. As Shh signaling is active in both the dorsal 3rd pharyngeal pouch endoderm and neighboring neural crest mesenchyme, we ectopically activated or deleted the Shh signal transducer Smoothened (Smo) in either cell type to determine which cellular target of Shh signaling is the basis for these patterning defects. Surprisingly, no individual loss or gain of function manipulation recapitulated the Shh null mutant phenotype, indicating that Shh signaling to either cell type is sufficient to allow establishment of parathyroid fate. Ectopic and early expression throughout the pouch endoderm of an activated allele of the Shh signal transducer Smoothened (Smo) activated ectopic Tbx1 expression, but failed to expand Gcm2 expression and parathyroid cell fate. Furthermore, although both Bmp4 and Foxn1 expression were suppressed in the mid-ventral pouch, these proteins were expressed normally in the most distal pouch, indicating that these cells were resistant to ectopic expression of activated Smo. Ectopic transgenic expression of Tbx1 itself further indicated that Tbx1 can suppress the differentiation of thymus-fated cells, but is not sufficient to specify ectopic parathyroid fate. These data indicate multiple direct and indirect roles for Shh signaling during thymus and parathyroid fate specification and organogenesis, and identify specific roles for Tbx1 in establishing organ fates within the third pharyngeal pouch.

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Growth Factor Signaling Pathways in Lung Development and Cancer. David M. Ornitz[1], Yongjun Yin[1], Ashley Hill[2]. 1) Developmental Biology, Washington University, St. Louis, MO; 2) Children's National Medical Center, Washington DC.

The origins of lung disease often begin during development. Unraveling the complex mechanisms that regulate development is essential for understanding the pathogenesis of developmental, genetic and acquired lung disease. Lung mesenchyme is a critical determinant of the shape and size of the lung, the extent and patterning of epithelial branching, the formation of the pulmonary vasculature and mesenchymal components of the adult lung. Fibroblast Growth Factor 9 (FGF9) is expressed in developing lung epithelium and mesothelium and has an essential primary role in regulating mesenchymal growth and differentiation through signaling to mesenchymal FGF receptors (FGFRs) 1 and 2. We have identified a feed-forward regulatory network that involves mesenchymal FGFRs and Wnt/-catenin signaling. We show that both FGF and Wnt/-catenin function in vivo to regulate mesenchymal growth and differentiation through a mechanism that involves suppression of Noggin. This finding couples mesenchymal FGF-Wnt/-catenin signaling with Bmp pathways that regulate epithelial growth and differentiation. Another means to gain insight into developmental mechanisms is to study the pathogenesis of cancer, a disease which often co-opts embryonic regulatory mechanisms. A potential link between FGF9 signaling in lung mesenchyme and human lung disease involves the heritable pediatric lung cancer syndrome, pleuropulmonary blastoma (PPB). PPB is interesting because it arises from embryonic

uncommitted lung mesenchymal cells. Mouse embryonic lung that was induced to overexpress FGF9 develops mesenchymal hyperplasia with histology that mimics that of type I PPB, suggesting that FGF9 might be involved in the pathogenesis of PPB. The genetic origins of PPB were mapped to loss-of-function mutations in the microRNA (miRNA) processing gene, DICER1. We find that Dicer1 ablation in developing lung epithelium mimics the early cystic stage of PPB. Immunohistochemical studies of human PPB tumors often show decreased DICER1 in lung epithelium. These observations suggest a model to explain the pathogenesis of PPB in which decreased epithelial miRNAs result in overexpression of an epithelial gene(s) and production of a factor(s) that stimulates the proliferation of adjacent mesenchyme, predisposing the mesenchyme to neoplastic transformation. Preliminary studies show that Fgf9 is directly regulated by DICER1-mediated miRNA pathways and could initiate early pathogenic events that lead to PPB.

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Genome-wide microRNA and mRNA profiling in mouse liver development implicates mir302b and mir20a in repressing TGFb signaling. Wei Wei[1], Juan Hou[1], Olivia Alder[1], Xin Ye[3], Sam Lee[1,4], Rebecca Cullum[1], Andy Chu[2], Yongjun Zhao[2], Stephanie Warner[3,4], Darryl Knight[3,4], Decheng Yang[3,4], Steven Jones[2,4], Marco Marra[2,4], **Pamela Hoodless**[1,4]. 1) Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada; 2) Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, Canada; 3) The Institute for Heart and Lung Health, St. Paul's Hospital, Vancouver, BC, Canada; 4) University of British Columbia, Vancouver, BC, Canada.

During mouse embryonic development, liver progenitor cells are specified from definitive endoderm at the 7-8 somite stage (embryonic day (E) 8.5). At E9.5, cells in the liver domain, known as hepatoblasts, undergo epithelial-to-mesenchymal transition (EMT), invade the surrounding septum transversum, form the liver bud and rapidly proliferate. Around E14.5-15.5, hepatoblasts start to differentiate into hepatocytes or cholangiocytes, which mature into the major functional cells of the liver. MicroRNAs (miRNAs) are small, non-coding RNA molecules that can regulate gene expression by binding to complementary sequences within mRNAs. MicroRNAs have been shown to regulate developmental processes, such as proliferation, differentiation and apoptosis; however, the identity of miRNAs and their functions during liver development are largely unknown. We investigated the miRNA and gene expression profiles for E8.5 endoderm, E14.5 DLK1+ liver cells (hepatoblasts), and adult liver by employing Illumina sequencing. miRNAs were found to be abundantly expressed at all three stages. Using K-means clustering analysis, thirteen miRNA clusters with distinct temporal expression patterns were identified. mir302b, an endoderm-enriched miRNA, was identified as a miRNA whose predicted targets are expressed highly in E14.5 hepatoblasts but low in the endoderm. We validated the expression of mir302b in the endoderm by whole-mount in situ hybridization. Interestingly, mir20a, the most highly expressed miRNA in the endoderm library, was also predicted to regulate some of the same targets as mir302b. By using luciferase and cell-based assays, we show that mir302b and mir20a are able to inhibit TGF-mediated epithelial-to-mesenchymal transition (EMT)through targeting Tgfbr2, suggesting their potential roles in maintaining the epithelial status of endoderm. Moreover, TGF1 can repress liver specification in an embryonic stem cell differentiation model. Collectively, we have identified dynamic patterns of individual miRNAs during liver development, as well as miRNA networks that could be essential for the specification and differentiation of liver progenitors.

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Taking a different pathway: Notchless plays with p53 and Wnt during embryogenesis. Amy C. Lossie[1,2], Chiao-Ling Lo[1,3], Jeremy Sherrill[4]. 1) Dept Animal Sci, Purdue Univ, West Lafayette, IN; 2) Dept of Medicine, Indiana University School of Medicine, Indianapolis, IN; 3) PULSe Interdisciplinary Graduate Program, Purdue University, West Lafayette, IN; 4) Department of Biological Sciences, Purdue University, West Lafayette, IN.

Our interests lie in determining the genes and genetic pathways that are important for establishing and maintaining maternal-fetal interactions during pregnancy. Through a positional cloning strategy, we discovered that mutations in *Notchless* (*Nle1*) lead to embryonic lethality during peri-implantation in mice. Nle1 is a member of the WD40-repeat protein family, and is thought to signal via the canonical Notch pathway. In invertebrates and lower vertebrates, the Notch pathway is critical for directing cell fate prior to gastrulation. However, the role of Notch signaling during the earliest stages of mammalian development is unclear, as gene targeting studies of Notch family members and factors that are necessary for Notch signal transduction, demonstrate that Notch signaling is dispensable for gastrulation in mice. The phenotype of *Nle1* mutant embryos is much more severe than single *Notch* receptor mutations or even in animals where Notch signaling is blocked. To test the hypothesis that *Nle1* functions in multiple signaling pathways during pre-implantation development, we examined expression of multiple *Notch* downstream target genes, as well as select members of the *Wnt* and *Trp53* pathways in wild-type and mutant embryos. Surprisingly, we saw no indication that the *Notch* pathway is disrupted in mutant embryos; *Notch* receptors, ligands and downstream targets showed normal expression levels. Instead, we found that members of the *Wnt* pathway are downregulated in *Nle1* mutants, while *Trp53* and *Cdkn1a* were upregulated in a stage-specific manner. Induction of *Cdkn1a* was highest in morulae and full blastocysts, while *Trp53* overexpression was confined to hatched blastocysts. Our results refute the possibility that Nle1 is a negative regulator of Notch signaling during mammalian pre-implantation development, as mutation of *Nle1* does not lead to increased expression of downstream target genes. Instead, our data implicate Nle1 in Wnt signaling, cell cycle arrest via Cdkn1a, and p53-mediated apoptosis. Although Notch signaling is dispensable in mice prior to gastrulation, Wnt signaling is not. Deletion of *Wnt3* leads to failure prior to primitive streak formation, and multiple ligands and receptors are detected in blastocysts and the uterus during peri-implantation. As p53 inhibits self-renewal and promotes differentiation in ES cells, Nle1 and its co-opted Wnt and p53 pathways could provide novel targets for the design of therapeutic interventions for infertility.

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Gadd45 functions in male sex determination by promoting p38 signaling and *Sry* expression. Wolfram H. Gruhn[1,2], Mathias S. Gierl[1], Annika von Seggern[1], Nicole Maltry[3], Christof Niehrs[1,2]. 1) Institute of Molecular Biology, 55128 Mainz, Germany; 2) DKFZ-ZMBH Alliance, Division of Molecular Embryology, DKFZ, 69120 Heidelberg, Germany; 3) Division of Cellular Immunology, DKFZ, 69120 Heidelberg, Germany.

Sex determination in mammals is tightly controlled by a complex gene regulatory network. In most mammals, male sex development is induced in embryogenesis by the transcription factor Sex-determining region Y protein (Sry). Mice that do not induce *Sry* expression within a critical time window during gonadogenesis develop into phenotypic females. Despite its pivotal role in male sex determination, regulatory events mediating *Sry* transcriptional activation are poorly understood. In this study, we show that male mice mutant for the stress-response gene *Gadd45* display complete male-to-female sex-reversal. In order to shed light on the molecular mechanism of *Sry* transactivation and Gadd45 function in male sex determination, we employed gene expression analysis, MAPK signaling activity investigation, bisulfite sequencing and chromatin immunoprecipitation (ChIP) on the *Sry* promoter. *Gadd45* and *Sry* have a strikingly similar expression pattern in the genital ridge and they are co-expressed in gonadal somatic cells. Notably, *Gadd45* expression is induced in gonadal cells shortly before *Sry* is expressed. In *Gadd45* mutant embryos *Sry* expression is reduced and delayed while other factors known to regulate *Sry* are expressed normally. Although *Gadd45* genes are implicated in active DNA demethylation, the *Sry* promoter undergoes normal DNA demethylation in *Gadd45* mutant embryos. Instead, p38 MAPK signaling is reduced in *Gadd45* mutant gonads. Inhibition of p38 MAPK signaling in gonadal cells strongly decreases *Sry* expression whereas pharmacological induction of p38 MAPK signaling rescues *Sry* expression in *Gadd45* mutant gonads. Furthermore, the transcription factor Gata4, which is required for *Sry* expression, binds to the *Sry* promoter *in vivo* in a p38 MAPK-dependent manner. Based on these findings, we suggest that Gadd45 is a novel regulator of male sex determination in mice, which promotes *Sry* expression by inducing p38 MAPK-dependent Gata4 binding to the *Sry* promoter.

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***Tbx20* and *Tbx3* act independently of each other in the developing heart. Virginia E Papaioannou**[1], Svetlana Gavrilov[1,2]. 1) Genetics and Development, Columbia University, New York, NY; 2) Developmental Biology Program, Sloan-Kettering Institute, New York, NY.

Tbx20 and *Tbx3*, members of the T-box gene family, play important roles in the developing embryo and are critical for proper heart development. Individually *Tbx20* and *Tbx3* genes, when mutated, cause distinct congenital heart abnormalities, but their overlapping expression in the developing heart raises the question of whether they act independently or through transcriptional regulation of common target genes. *Tbx20* gene expression is induced ectopically in the *Tbx3* mutant heart but the potential genetic interaction between these two genes has not been investigated. In this study, we investigated the genetic interaction of *Tbx20* and *Tbx3* in the developing heart. Mice heterozygous for both $Tbx20^{-}$ and $Tbx3^{-}$ locus were intercrossed to generate compound heterozygotes ($Tbx20^{+/-}$; $Tbx3^{+/-}$). Compound heterozygotes were viable and fertile, present at expected Mendelian ratios at weaning. No apparent abnormalities were observed. Double null embryos were recovered at expected frequency from compound heterozygote matings and there were no significant deviations from expected Mendelian ratios. $Tbx20^{-/-}$; $Tbx3^{-/-}$ double null mice are embryonic lethal by midgestation. They display looping defects similar to $Tbx20^{-/-}$ embryos, with small hypoplastic hearts and disturbed chamber morphology. Cardiac marker analysis for *Tbx2*, *Tbx5*, *Nppa* and *Pitx2* confirmed this $Tbx20^{-/-}$ like phenotype in double nulls mutants. In addition, we observed overall developmental delay in double null mutants. An embryo was considered delayed if its somite number was lower than the mean somite number for the litter. In order to compare developmental delay between litters we have calculated the ratio for each embryo (number of somites in an embryo/mean number of somites in the litter) and used notched box plots to compare double null embryos to wild type and single mutants. Using statistical analysis we determined that overall developmental delay in double null mutants is similar to developmental delay in $Tbx3^{-/-}$ mutants. In conclusion, there is no evidence for genetic interaction between *Tbx20* and *Tbx3* and the effects of *Tbx20* are epistatic to *Tbx3*.

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Genetic pathways required for maintenance of vascular smooth muscle phenotype. Sylvia M Evans[1], Nuno Guimaraes-Camboa[1,2], Chase Bolt[3], Lisa Stubbs[3]. 1) Skaggs School of Pharmacy, Department of Medicine, University of California San Diego, La Jolla, CA; 2) Biomedical Sciences Institute Abel de Salazar, Graduate Program in Areas of Basic and Applied Biology, University of Porto, Portugal; 3) Institute for Genomic Biology, Department of Cell and Developmental Biology, University of Illinois, Urbana, IL.

Our lab has been interested in studying genetic pathways required for epicardial cell development. One of the cell lineages derived from epicardium is vascular smooth muscle of the coronary arteries. Vascular smooth muscle plays an important role in coronary vascular disease and is also important for maintaining integrity of the aorta. We have performed gene ablation studies in mouse which have identified a novel pathway required for maintenance and function of vascular smooth muscle. This genetic pathway intersects with pathways affected in human patients with vascular smooth muscle disorders. Results of these studies will be discussed.

Imaging

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Optical Imaging of the Embryonic Mammalian Cardiovascular System. Mary E. Dickinson. Molecular Physiology & Biophysics, Baylor College of Medicine, Houston, TX.

The cardiovascular system is the first functional organ system to develop in mammals. Not long after the heart begins to beat, the cardiovascular system becomes essential for the normal development of the embryo and vitality. Interestingly, the heart, vessels and early hematopoietic cells are all needed for continued normal development and defects in any of these three cell types can lead to lethality as well as an array of secondary defects that may mask which cell type is primarily affected. In the past few years, we have developed live imaging strategies using confocal and multi-photon microscopy as well as Optical Coherence Tomography (OCT). These studies have provided new insights into early cardiovascular development and OCT is emerging as a robust tool for evaluating early lethal mutations in large-scale gene knock-out screens. These results will be discussed.

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Imaging techniques in *Drosophila* to delineate candidate roles of calcium in murine lung and neural development. Danielle V. Bower[1], Scott E. Fraser[1], Edwin C. Jesudason[2]. 1) Biology and the Biological Imaging Center, California Institute of Technology, Pasadena, CA; 2) Department of Surgery, Children's Hospital Los Angeles, Los Angeles, CA.

The *Drosophila* tracheal system and mammalian lungs form in a stereotyped manner via defined patterns of cell migrations and branching. However, what regulates the branching patterns and timing of branch formation is incompletely understood. We investigated the role of calcium signaling in tracheal branching in *Drosophila* embryos by permeablizing the embryos and applying small molecule inhibitors to perturb calcium dynamics. Disrupting calcium dynamics affects the patterning of the *Drosophila* tracheal system as well as neural tracks in the central nervous system. We are now extending these techniques to the mammalian lung to study the role of calcium signaling in the regulation of branching.

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Live imaging the pluripotent state *in vitro* and *in vivo*. Panagiotis Xenopoulos[1*], Minjung Kang[1,2], Anna-Katerina Hadjantonakis[1]. 1) Developmental Biology Program, Sloan-Kettering Institute, New York, NY; 2) Biochemistry, Cell and Molecular Biology Program, Weill Graduate School of Medical Sciences of Cornell University, New York, NY.

Pluripotent stem cell-based therapies hold enormous promise for patients with degenerative and other diseases. However, a deeper understanding of the mechanisms driving pluripotent cells to self-renew or differentiate into specific lineages *in vitro* and *in vivo* is needed in order to use these cells for clinical applications in a safe and effective manner. To that end, it was recently demonstrated that pluripotent stem cell populations of the same genetic background exhibit heterogeneous expression of pluripotency-associated genes. Remarkably, these heterogeneities have been shown to correlate with dynamic fluctuations in the expression of certain pluripotency-associated factors, suggesting the existence of inter-convertible substates within pluripotent stem cell compartments. It is believed that such heterogeneity and fluctuating substates may represent a hallmark of all pluripotent cell types, reflecting a propensity for the decision to self-renew or differentiate. Interestingly, these heterogeneities have not been observed yet in the early mammalian embryo, from where pluripotent cells are derived. An essential tool for investigating the relationship between heterogeneous gene expression and cell fate decisions is live cell imaging. By using BAC recombineering, we have developed a series of novel, universal, high-resolution live imaging reporters of the pluripotent state. We show that these pluripotency-associated imaging reporters are nuclear localized in cultures of mouse embryonic stem (ES) cells and provide excellent single-cell resolution and tracking of cells over time. We are using these imaging tools in order to probe heterogeneities and characterize fluctuating substates in cultures of pluripotent stem cells *in vitro*. Moreover, these reporters have allowed us to visualize the emergence of the pluripotent epiblast lineage in mouse blastocyst stage embryos, as well as to examine whether the heterogeneities observed in culture exist *in vivo*. These studies should help elucidate how cells differentiate or decide to remain in a pluripotent state *in vitro* and *in vivo*.

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Imaging of cellular dynamics underlying the immune tissue organization. Takaharu Okada[1,2]. 1) Research Unit for Immunodynamics, RCAI, RIKEN, Yokohama, Kanagawa, Japan; 2) PRESTO, Japan Science and Technology Agency, Tokyo, Japan.

The immune tissues such as lymph nodes are structurally compartmentalized so that relevant cell types for each mode of immune responses are co-localized and able to access each other. During the last decade, live tissue imaging, in particular using two-photon laser microscopy, has advanced our understanding of immune cell trafficking mechanisms. Studies using this technique have contributed to reveal molecular requirements for leukocyte migration and interaction in native tissue environments. In this talk, I will present data from live imaging experiments to show dynamics of B cells, T cells, and dendritic cells during adaptive immune responses. It will be discussed how dynamics of activated B cells, which is regulated by their differential responsiveness to multiple trafficking cues, contributes to remodeling of B cell follicles to form germinal centers for

long term humoral immunity. I will also discuss diversity of helper T cells specialized in humoral immunity, and show that a subpopulation of these cells are localized in germinal centers by sharing trafficking molecules with germinal center B cells.

Genetics and Genomics

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KOMP2...the next phase of the Knockout Mouse Project. Kent Lloyd. Mouse Biology Program, Univ California Davis, Davis, CA.

As part of KOMP2, an NIH Common Fund project to produce and phenotype The Knockout Mouse Production and Phenotyping (KOMP2) project is an NIH Common Fund program intended to conduct broad based, comprehensive, high throughput phenotyping on homozygous mutant mouse lines produced from gene targeted embryonic stem (ES) cells generated during the last 5 years of KOMP1. Three KOMP2 production and phenotyping centers were awarded to 3 groups: the DTCC Consortium led by Kent Lloyd at UC Davis and including the Toronto Center for Phenogenomics, Childrens Hospital Oakland Research Institute, and Charles River Laboratory; the BaSH Consortium led by Monica Justice at Baylor College of Medicine and including The Sanger Institute and Harwell; and The Jackson Laboratory led by Robert Braun and Leah Donohue. In addition, a Knockout Mouse Phenotyping Project Database was awarded to Dr. Paul Flicek at the European Bioinformatics Institute and will create a data coordination center to collect, archive, and provide online access via a web portal phenotyping data and information generated from the 3 KOMP2 award recipients, as well as complex queries and statistical analyses of the data. Over the next 5 years, for 2500, mostly unannotated genes, live mice, cryopreserved germplasm, and an abundance of data will be generated from a phenotyping pipeline covering all major body systems and most areas of human disease. KOMP2 will provide a unique and valuable scientific resource for the biomedical research community, and will inform our understanding of the pathophysiology of human and animal diseases. If successful, another 5000 genes will be analyzed over an additional 5 years. The KOMP2 efforts are being coordinated with complementary international efforts. The KOMP2 project awarded to the DTCC is supported by NIH grants U42 OD011175 and U54HG006364.

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High-throughput mutant mouse phenotyping is a powerful tool to generate novel hypotheses. Chris Lelliott, Jeanne Estabel, Anna Karin Gerdin, Antonella Galli, Anneliese Speak, Richard Houghton, Joanna Bottomley, Edward Ryder, Ramiro Ramirez-Solis, David Adams, Jacqueline White. The Sanger Mouse Genetics Project, Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, United Kingdom.

As part of the Sanger Mouse Genetics Project we have generated over 600 knockout mouse lines. Viability and fertility of each line are assessed during colony expansion; 28% of lines have been found to be embryonic lethal at postnatal day 14, with a further 12% classed as sub-viable, and 5.7% of lines homozygous for the targeted allele present with fertility issues. Following expansion, dedicated groups of mice are phenotyped through an extensive and standardised battery of tests assessing development, physiology and metabolism, and yielding data on 280 parameters for every line. For homozygous lethal lines, heterozygous animals are phenotyped. Whilst lines homozygous for the targeted alleles present with a higher hit rate (64% of lines assessed in the homozygous state presented with 1 hit and averaged 3.9 hits per line), lines assessed in the heterozygous state were still remarkably informative (42% of lines assessed in the heterozygous state were classed as pheno-deviant with an average of 1 hit per line) and represent a rich source of information, which is openly available on the Sanger Mouse Portal (http://www.sanger.ac.uk/mouseportal/). A significant proportion of genes have been selected based on the absence of available mutant mouse phenotypic information. We will present some of the hypothesis-generating mouse lines that have resulted, including mice with metabolic abnormalities (*Kptn*, *Dusp3*), skeletal and behavioural changes (*Zc3hc1*), haematological alterations (*Crlf3*) and developmental defects (*Psat1*). Our data shows that high-throughput phenotyping is a powerful tool to generate novel hypotheses and that most knockouts have robust phenotypes, few of which could be predicted a *priori*.

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Recent specialization of the human and mouse X chromosomes for the male germline. Jacob L. Mueller[1], Helen Skaletsky[1], Laura G. Brown[1], Sara Zaghlul[1], Susan Rock[2], Tina Graves[2], Katherine Auger[3], Wesley C. Warren[2], Richard K. Wilson[2], David C. Page[1]. 1) 1Howard Hughes Medical Institute, Whitehead Institute, MIT, Cambridge, MA; 2) The Genome Institute, Washington University School of Medicine, St. Louis, MO; 3) The Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

In 1967, Susumu Ohno posited that the gene content of X chromosomes would be conserved across placental mammals, a statement often referred to as Ohnos law. This widely accepted law has not yet been tested systematically and rigorously. We decided to test Ohnos law by comparing the human X chromosome to the single-haplotype, clone-based, mouse X-chromosome sequence. This required that we refine portions of the current human X-chromosome reference sequence with single-haplotype sequence to match the single-haplotype mouse sequence. Single-haplotype, clone-based sequence was selectively generated on large, nearly identical, segmental duplications, termed amplicons. We resolved gaps in the current reference sequence,

reconstructed previously misassembled regions, and identified new palindromic amplicons. Comparing this revised sequence to the mouse X chromosome, we found that only 30% of human X-ampliconic genes and 21% of mouse X-ampliconic genes share orthologs. This is in striking contrast to single-copy genes, which tend to follow Ohnos law of conservation: ~95% are orthologous between humans and mice. Ampliconic genes thus represent the majority of newly acquired genes in both lineages and are the primary violators of Ohnos law. Furthermore, we found that newly acquired genes are expressed almost exclusively in testicular germ cells, suggesting specific roles in the male germline. We conclude that during mammalian X chromosome evolution two processes were at work: single-copy genes, which comprise the oldest genes, generally follow Ohnos law, but ampliconic genes, acquired more recently, tend to violate it.

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Expression QTL mapping in murine macrophages identifies novel genes and gene networks mediating resistance to *Toxoplasma gondii* and responsiveness to IFNg/TNFa. Musa Hassan, Kirk Jensen, Jeroen Saeij. Dept. of Biology, MIT, Cambridge, MA.

Effective response to infection with intracellular parasites is often dependent on the synergistic activation of macrophages by interferon gamma (IFN) and tumor necrosis factor alpha (TNF). Activated murine macrophages inhibit parasite growth directly through the production of nitric oxide (NO) and up-regulation of immunity-related GTPases (IRGs), which are involved in the disruption of the vacuoles many intracellular parasites live in and subsequently the parasite itself. Macrophages can also influence parasite growth indirectly by secreting cytokines, such as interleukin (IL)-10 and IL-12, which can further modulate the immune response. Our hypothesis is that many genetic differences in disease resistance are due to variations in the macrophage response to the pathogen and/or to IFN/TNF. Our goal therefore, was to elucidate the molecular mechanisms underlying variation in murine macrophage response to IFN/TNF, the main mediators of resistance to many pathogens. As a proof of concept we are using the intracellular parasite *Toxoplasma gondii*, which is a common parasite in humans. We have used high throughput RNA-sequencing to analyze the transcriptome of IFN/TNF stimulated or *T. gondii* infected bone marrow derived macrophages obtained from AxB and BxA recombinant inbred mice. Next we used linkage analysis to map the genomic loci controlling parasite growth (cQTL), mouse gene expression (eQTL),and parasite gene expression (pQTL) in these macrophages. Indeed activated macrophages from the resistant AJ mice produce more NO and inhibit *Toxoplasma* growth better than macrophages from the susceptible C57BL/6 mice, and were mapped to chr 4, and chr 13 and 17, respectively. Additionally,, these strains differ significantly in the production of IL-10 and IL-12p70, two cytokines that play a major role in modulating the immune response. Interestingly, unlike other studies, the majority of our eQTLs are trans mapping. We have identified *trans*-acting eQTLs on chrs 10 and 11 following infection, *trans*-acting eQTLs on chrs 12 and 15 in response to IFN/TNF, and *trans*-acting pQTL on chr 2. Majority of these *tran*-acting loci are enriched in cell cycle pathways, Toll-like receptor (TLR) and interferon responsive factor (IRF) signaling, and lipid metabolism. Furthermore, we have identified *cis*-acting eQTLs for long non-coding RNAs on chr 7, and 17 following infection and on chr 3 in response to stimulation.

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The Gene Expression Database for Mouse Development (GXD). Martin Ringwald, Constance Smith, Jacqueline Finger, Terry Hayamizu, Jingxia Xu, Ingeborg McCright, Janan Eppig, James Kadin, Joel Richardson. The Jackson Laboratory, Bar Harbor, ME.

The Gene Expression Database (GXD) is an extensive and freely available community resource of mouse developmental expression information. It integrates data from RNA in situ hybridization, immunohistochemistry, in-situ reporter (knock-in), Northern blot, Western blot, and RT-PCR experiments, covering all developmental stages and data from wild-type and mutant mice. Data are collected through curation of the literature, via electronic data submissions from conventional laboratories, and by collaborations with large-scale data generators such as GenePaint, EurExpress, the Brain Gene Expression Map (BGEM) project, and the GenitoUrinary Developmental Anatomy Project (GUDMAP) project. Accession numbers are provided to researchers so that electronic data submissions can be cited in publications. GXD currently holds more than 1.3 million expression results from almost 60,000 expression assays for more than13,600 genes, including expression data from about 1,700 mouse mutants. In addition, the database holds over 237,000 images of expression data. GXD database records associate these images with extensive metadata such as 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. Further, 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 variety of biologically and biomedically relevant parameters. Recent interface enhancements include the capability to search for expression data of genes that are associated with specific phenotypes and/or human diseases, and improved and more interactive data summaries, with the option to download these data and to export them to other application. In addition, we have significantly boosted query performance so that even large sets of data can be returned quickly. GXD is freely available through the MGI web site (www.informatics.jax.org), or directly at www.informatics.jax.org/expression.shtml. GXD is supported by NIH grant HD062499.

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RNAseq Analysis of 32 KOMP Mutant Mouse Lines. David West[1,2], Andreana Cipollone[2], Michael Adkisson[1], Jared Rapp[2], Eric Engelhard[2], Pieter de Jong[1], Kent Lloyd[1]. 1) Childrens Hospital of Oakland Research Institute, Oakland, CA; 2) University

of California, Davis, CA.

As part of KOMP2, an NIH Common Fund project to produce and phenotype 2,500 unique mutant mouse lines over a 5-year period, we are applying RNAseq to characterize transcriptome changes in mutant tissues. We report here the RNAseq results from 34 KOMP mutant lines derived from IKMC targeted stem cells (www.knockoutmouse.org). Male homozygous (HOM) mutant and wild-type (WT) control mice were ~50 3 days at tissue harvest. For each mutation, RNA extracted from ~ 4 RNA-later stabilized or quick-frozen tissues from each of 5 male mice was used to create indexed cDNA libraries for sequencing on an Illumina HiSeq DNA Analyzer. Approximately 4-5 libraries per lane produced ~20-25M ~45nt reads per library passing trimming and QC which were mapped to the NCBI mouse transcriptome. Total read counts, from all alternative transcripts, were compared between HOM and age-matched WT controls. Our RNAseq analysis revealed a high incidence of localized changes in the 500kb region flanking the target gene. These regional changes were found in approximately 40% of libraries from mutants produced from Velocigene ES cell clones, and from ~20% produced from CSD or EUCOMM targeted clones. We observed both significant over- and under-expression of genes flanking the target. Some of these transcriptome changes are likely compensatory and in response to physiological changes produced by the mutation. However, other changes could be due to disruption of chromatin, introduction of foreign DNA causing localized silencing, and the insertion of an exogenous promoter causing activation of neighboring genes. Global transcriptome changes were evaluated using clustering tools such as Gorilla, the NIAID DAVID bioinformatics toolkit, and the Ingenuity package, in order to predict gene function based upon compensatory changes. These data are available at www.kompphenotype.org. Supported by NIH grants U42OD011175 ,U54HG006364 and U01HG004080.

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Coordinated activation of multiple tumor suppressor pathways in benign skin tumors identified by correlation analysis of *Pten* gene expression. David Quigley, Ihn Young Song, Allan Balmain. Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA.

The DMBA/TPA mouse model of skin cancer creates numerous benign tumors in susceptible animals, a small percentage of which progress to become invasive carcinomas. Most of these hyperplastic lesions remain benign after many months, demonstrating that innate tumor suppression is effective even in an inflamed epithelium with an activating *H-ras* mutation. Most studies of gene expression in tumors focus on detection of changes in levels of expression between two states. Here we have used differential correlation analysis of gene expression between normal skin and benign skin tumors to investigate the rewiring of potential tumor suppressor pathways at an early stage of carcinogenesis. Correlation was among the first statistical tools to be applied to microarray data, but few studies have directly examined how changes in correlation after a tissue is perturbed can elucidate changes in how genes work together under different biological conditions. We demonstrate that while the *Pten* gene does not show significant changes in expression levels in benign tumors, it shows substantial changes in correlations with a network of known tumor suppressors including *Tsc2*, *Smad4* and genes in the mTOR pathway which regulate translation and growth. These relationships are not found in normal skin, and are lost during tumor progression from benign to malignant tumors. We show by immunohistochemistry that Pten protein localizes to the cell membrane of keratinocytes in benign tumors but not adjacent normal skin, compatible with an activation of Pten and consequent down-regulation of PI3K signaling. Transient expression of oncogenic *Hras* in normal keratinocytes did not result in an up-regulation of Pten protein. Although we and others have shown that mice heterozygous for *Pten* in their skin have increased susceptibility to skin tumors in this model, DMBA/TPA-induced carcinomas in wild type mice do not lose *Pten* expression due to DNA copy number changes or mutation. This in vivo finding would not be suggested using standard differential expression analysis or mutation screening. This work demonstrates a framework for examining how pathway activation or suppression can be identified through a statistical approach which provides important insights into gene expression network rewiring in tumors.

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A resource of vectors and ES cells for targeted deletion of microRNAs in mice. Haydn M Prosser, Hiroko Koike-Yusa, Chukwuma A Agu, James D Cooper, Frances C Law, Allan Bradley. Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

The 21-23 nucleotide single-stranded RNAs classified as micro RNAs (miRNA) perform fundamental roles in a wide range of cellular and developmental processes. MiRNAs regulate protein expression through sequence-specific base pairing with target messenger RNAs (mRNA) reducing both their stability and the process of protein translation. At least 30% of protein coding genes appear to be conserved targets for miRNAs. The miRNA genes are distributed throughout the genome either singly or in clusters. We have generated a library of highly germ-line transmissible C57BL/6N mouse mutant embryonic stem (ES) cells with targeted deletions within one miRNA allele for the majority of miRNA genes currently annotated within the miRBase registry. These alleles have been designed to be highly adaptable research tools, as a variety of modifications of the targeted loci can be achieved by applying the technique of recombinase mediated cassette exchange (RMCE). We demonstrate the use of this approach for the reporter and conditional alleles. This ES cell resource can be searched electronically and is available from ES cell repositories for distribution to the scientific community.

Epigenetics

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An evaluation of parent-of-origin bias and individual variation in the midgestation mouse placenta. Elizabeth H Finn, Cheryl Smith, Arend Sidow, Julie C Baker. Department of Genetics, Stanford University, Stanford, CA.

The placenta provides a unique epigenetic landscape characterized by increased flexibility of epigenetic marks and remarkable environmental sensitivity. We examined genomic imprinting and parent-of-origin bias in the mouse placenta using 3-end Sequencing for Expression Quantification (3SEQ) of F1 interspecies hybrid tissues. We sequenced 23 individual midgestation placentas, five late stage placentas and two yolk sac samples, and used differential expression of SNPs to determine whether transcripts were preferentially generated from the maternal or paternal allele. We found 103 genes that show significant parent-of-origin bias, of which 78 were novel candidates for imprinting and 25 were previously identified imprinted genes. Most of our novel candidates show a strong maternal bias which, using multiple models for maternal contamination, we demonstrate is not due to decidual contamination. We also show evidence of paternally biased expression of Xist leading to maternally biased expression of most X chromosome loci, with three regions escaping this inactivation. Finally, sequencing individual placentas allowed us to reveal a surprising expression similarity between littermates. These data demonstrate the complexity and dynamic nature of epigenetic pathways in the placenta.

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Gene silencing by the *in vivo* dynamics of Polycomb repressive complex PRC1. Kyoichi Isono[1,2], Haruhiko Koseki[1]. 1) RIKEN RCAI, Yokohama, Japan; 2) JST, PREST, Yokohama, Japan.

During embryogenesis, expressional change of genes and maintenance of its status give rise to cellular identity. Polycomb group (PcG) proteins mediate heritable but reversible silencing of developmental regulatory genes by modifying their chromatin configuration. Accumulating evidence documents a role for PcG proteins in regulating higher order chromatin structures, likely by forming large complexes. However, little is still known about the molecular mechanisms underlying PcG-mediated gene silencing. In this study using imaging approaches of mouse primary cells, we show that (1) Polycomb repressive complexes-1 (PRC1) including Ring1b, Mel18, Cbx2, Phc2 and so on cluster at canonical PcG target genes, which are visualized as nuclear foci such as a so-called PcG body in *Drosophila*; (2) the PRC1-clustering depends on the head-to-tail polymerization property of the SAM domain of Phc2 and also was accompanied by recruitment of Ring1b, chromatin condensation, and gene silencing. Our findings suggest a new model in which SAM polymerization of Phc2 modulates the structural organization of PRC1 to enable robust yet reversible PcG-mediated gene repression during development and in cell fate decision-making.

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Concerted alterations of promoter-enhancer-PRE association in transcription status transition of *Meis2* during midbrain development. Takashi Kondo, Haruhiko Koseki. Department of Developmental Genetics, RIKEN-RCAI, Yokohama, Kanagawa, Japan.

Recent progress of genetics revealed that associations among remote *cis*-regulatory elements are necessary for proper transcription regulation, although mechanisms of these processes remain unknown. We study *Meis2* gene locus, a co-factor of *Hox* genes, as a model system to study the remote DNA association processes. We identified a midbrain specific enhancer element and a PRE (*Polycomb* responsive element) from 200Kb genomic sequence surrounding *Meis2* locus in addition to a promoter region. Combination of 3C (chromatin conformation capture), transgenic mouse, and FISH (and immuno-FISH) on histological sections, we observed differences in associations of these DNA elements (the promoter, the enhance and the PRE) corresponding to transcription statuses. Further analysis in developing midbrain revealed that step-wise associations of these three DNA regions occurs during transition of transcription status.

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The Histone Acetyltransferase MOF is a Key Regulator of the Embryonic Stem Cell Core Transcriptional Network. Xiangzhi Li[1], Li Li[2], Yali Dou[3]. 1) Institute of Cell Biology, School of Medicine, Shandong University, Jinan, China; 2) Rollins School of Public Health, Emory University, Atlanta, GA 30322; 3) Department of Pathology, University of Michigan, Ann Arbor, MI 48109.

Self-renewal and pluripotency are hallmarks of embryonic stem cells (ESCs). Both ESCs features are subject to epigenetic regulation. Here we show that histone acetyltransferase Mof plays an essential role in the maintenance of ESC self-renewal and pluripotency. ESCs with Mof deletion lose characteristic morphology, alkaline phosphatase (AP) staining and differentiation potential. They also have aberrant expression of core transcription factors Nanog, Oct4 and Sox2. Importantly, the phenotypes of Mof null ESCs can be partially suppressed by Nanog overexpression, supporting that Mof functions as an upstream regulator of Nanog in ESCs. Genome-wide ChIP sequencing and transcriptome analyses further demonstrate that Mof is an integral component of ESC core transcription network and Mof primes genes for diverse developmental programs. Mof is also required

for Wdr5 recruitment and H3K4 methylation at key regulatory loci, highlighting complexity and interconnectivity of various chromatin regulators in ESCs.

Genetics and Genomics

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Construction of reciprocal chromosome substitution strains from 129P3/J and C57BL/6ByJ mice. Alexander A. Bachmanov, Cailu Lin, Natalia P. Bosak, Theodore M. Nelson, Maria L. Theodorides, Zakiyyah H. Smith, Matthew T. Kirkey, Mauricio Avigdor, Brian R. Gantick, M. Amin Khoshnevisan, Anna Lysenko, Danielle R. Reed. Monell Chemical Senses Center, Philadelphia, PA.

The 129P3/J and C57BL/6ByJ inbred strains differ in taste responses, ingestive behavior, alcohol consumption, body size and adiposity. Genome scans of crosses between these strains have detected QTLs for these phenotypes that cluster on chromosomes (Chr) 1, 2, 7 and 9 (*Adip2, Adip3, Adip5, Ap7q, Bwq5, Bwq6, Nattq1, Nattq2, Sucq*). Chromosome substitution strains (CSSs) are a useful resource for positional identification of these QTLs, but extant B6 and 129 CSSs involve different substrains (129S1/SvImJ and C57BL/6J), which are genetically and phenotypically distinct from the substrains used in our studies. We therefore initiated construction of reciprocal CSSs for Chr 1, 2, 7 and 9 from the 129P3/J and C57BL/6ByJ strains using a "speed consomics" approach. During the first two backcross generations (N2 and N3) a genome scan was conducted to identify optimal breeders. In subsequent backcrosses, donor chromosomes were genotyped to prevent the loss of fragments due to double-crossovers. To ensure that the QTLs were retained, we phenotyped several incipient strains and conducted linkage analyses; these analyses confirmed the original QTLs on Chr 1 (*Nattq1*), Chr 2 (*Bwq5, Adip2*), Chr 7 (*Ap7q; Adip3*) and Chr 9 (*Natt2q, Bwq6, Adip5, Sucq*). Some CSS strains are now complete (129P3/J-Chr $7^{C57B6/ByJ}$, C57B6/ByJ-Chr $2^{129P3/J}$, C57B6/ByJ-Chr $7^{129P3/J}$, C57B6/ByJ-Chr $9^{129P3/J}$) and others are nearing completion. Supported by National Institutes of Health (NIH) grants R01DC000882, R01AA011028 (A.A.B), R01DK058797, DK094759 and the Center for Inherited Disease Research (CIDR) (D.R.R.).

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Generation of CreERT2 transgenic mouse lines for time and cell specific conditional gene inactivation Validation of 3 pancreas specific lines : Insulin1, Glucagon et Elastase-CreER^{T2}. Marie-Christine Birling[1], Lydie Venteo[1], Olivia Wendling[1,2], Nathalie Chartoire[1], Marie-France Champy[1], Elodie Bedu[1], Tania Sorg[1], Yann Hérault[1,2], Guillaume Pavlovic[1]. 1) Genetic Engineering, Institut Clinique de la Souris, 1, rue Laurent Fries, 67400 Illkirch, France; 2) IGBMC, 1, rue Laurent Fries, 67400 Illkirch, France.

The generation of mouse mutants using conventional knock out approach is a powerful tool to study the role of specific genes. However, this technology shows two major limitations: (i) disruption of many genes result in lethal phenotypes (ii) it does not allow site specific and time controlled inactivation of a gene. The conditional knock-out strategy overcomes these limitations. When such floxed mice are bred with transgenic mice expressing the Cre recombinase in a tissue/cell-specific manner, the gene of interest is knocked out/altered only in this particular tissue or cell type. An added sophistication is the inclusion of temporal control, which can be achieved using a ligand-activated chimeric recombinase, composed of the fusion of the Cre recombinase with the ligand binding domain of a mutated form of the estrogen receptor (ER), which can only be activated by synthetic ER ligands (e.g. tamoxifen, Cre -ER^{T2}, Indra et al. 1999). Large-scale international mouse mutagenesis programs (see IKMP) are providing conditional knock-out of most mouse genes. As these lines are available to the whole scientific community, the need of a large variety of cell specific deleter lines is essential. At the ICS, we have generated about 50 Cre transgenic mouse lines expected to express the tamoxifen inducible CreER^{T2} recombinase in different target tissues and cells. They are available to the research community and are a powerful tool for the study of disease genes function, the creation of disease models and to answer questions on the cell/organ autonomous or not character of various pathological phenotypes. For details, please see http://www.ics-mci.fr/mousecre/. We will give you an example of 3 fully characterized mouse line: Insulin1-, Glucagon- and Elastase-CreERT2. For the Insulin1-CreERT2 line, the Cre expression is observed in the -cells, the translocation in the nucleus is confirmed in the presence of Tamoxifen as expected for an inducible line. By breeding this line with Rosa26 reporter line, a specific LacZ staining is observed in the -islet cells. This line was phenotyped (under chow diet) and no glucose intolerance was observed at the difference of the Rat Insulin Promoter (RIP)-Cre line. A comparative study (Ins1-CreERT2 versus RIP-Cre) was performed and will be detailed. Similarly, the Glucagon-CreERT2 line is specific for alpha-cells and elastase-CreERT2 line is specific from acinar cells. Further phenotyping are under way and will be discussed;.

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Developmental dynamics of the Tbx18 locus and its downstream transcriptional targets. C. Chase Bolt[1], Xiaochen Lu[1], Laura Chittenden[1], Nuno Camboa[2], Sylvia Evans[3], Lisa J. Stubbs[1]. 1) Dept. of Cellular and Developmental Biology, Institute for Genomic Biology, University of Illinois - Urbana/Champaign, Urbana, IL; 2) Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, CA, GABBA Graduate Program and ICBAS, University of Porto, Portugal; 3)

Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, CA.

The highly conserved T-box transcription factor Tbx18 is expressed in a complex pattern during development of the vertebrate embryo. Tbx18-null phenotypes, including loss of somite polarity, decreased proliferation of the cardiac conduction system, and patterning pathologies of the ureter suggest a complex regulatory mechanism in which Tbx18 acts in these different tissues. In concordance with these known phenotypes, here we describe a new Tbx18 mutation, 12Gso, a chromosome translocation located nearly 80kb downstream of the Tbx18 gene that perturbs the spatio-temporal expression pattern of the gene. We show that this translocation physically separates enhancers necessary for proper expression in the somites and urinary system. Using a LacZ reporter system in transgenic mice we identified a urinary enhancer that confers the proper expression of Tbx18 beginning at E9.0 in the urogenital mesenchyme and persisting through birth in a diminishing proximal-distal wave of the ureter smooth muscle layer. To further understand the genetic pathways through which Tbx18 executes its role during development, we performed chromatin immunoprecipitiation using a custom Tbx18 antibody in C2C12 mouse cells, which have properties similar to mesenchymal stem cells, followed by hybridization to extended promoter arrays (ChIP-chip). To complement this ChIP-chip study, we performed siRNA knockdown of Tbx18 mRNA in C2C12 followed by hybridization to expression arrays. We combined the results of these two experiments to identify putative direct targets of Tbx18. We identified significant enrichment in genes involved in cell cycle regulation, tissue patterning, and cell proliferation/apoptosis, among other biological processes. The targets identified in this study support a role for Tbx18 in regulation of cell proliferation/apoptosis as previously reported by others, but also suggest additional roles in regulating components of the cell cycle. These newly discovered targets suggest a potential role for Tbx18 in other types of biology and may hint towards previously undiscovered phenotypes.

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Somatic mosaicism detected using the Mouse Diversity Genotyping Array reveals tissue-specific mutation patterns associated with the *harlequin* phenotype. Susan T. Eitutis, Andrea E. Wishart, Kathleen A. Hill. Department of Biology, The University of Western Ontario, London, ON, Canada.

A genomic perspective to studying somatic mosaicism can be approached using the Mouse Diversity Genotyping Array (MDGA). First, to establish an optimal genome-wide target, an accurate and complete annotation of single nucleotide polymorphism (SNP) probes was completed. This final probe list was established by selecting only probes 25 nucleotides in length that mapped uniquely to the mouse genome. Probe sets included in the final list had to fit these criteria for both the forward and reverse strand probes for each SNP. The selected probes have an average spacing of 4887 nucleotides. Genotypes for the spleen and the cerebellum, tissues that differ in cell type and proliferation rate, were determined using the final list of 526,808 SNP probes for two premature aging *harlequin* (*hq*) mice and two wild-type (WT) mice. Post genotyping, probes were again filtered based on genotype cluster analysis and the possible effects of copy number variation were considered. This generated a relaxed list of 497,333 SNP probes and a stringent list of 470,147 SNP probes. Differences in genotype calls between tissues were interpreted as putative mutations. In WT compared to *hq* mice, there is a greater difference in the number of mutations between tissues (p0.001). Each sample had a unique distribution of mutations across the genome (p0.001). There is an underrepresentation of putative mutations on the X chromosome consistent with the predicted negative selection. Chromosomes 9 and 17 had an overrepresentation of mutations. Chromosomes 1, 8, and 12 had an overrepresentation of mutations in *hq* tissues. Spleen of *hq* mice had an overrepresentation of mutations on chromosomes 12, 13, 14, and 19. The *hq* cerebellum had an overrepresentation of putative mutations on chromosome 13. Two mutations that were specific to the *hq*cerebellum are predicted to affect two genes on chromosomes 8 and 12. The *hq* spleen-specific mutations potentially affect nine genes on seven chromosomes. Identification of putative mutations unique to one tissue and one genotype, and those potentially affecting gene function is the essential advance in understanding origins and mechanisms of mutations during development.

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CRY1-PHR(313-426): A Key Domain for Negative Feedback Repression and Circadian Clock Function in Mice. Sanjoy Kumar Khan[1], Haiyan Xu[1], Maki Ukai-Tadenuma[2], Hiroki Ueda[2], Andrew Liu[1]. 1) Biological Sciences, University of Memphis, Memphis, TN; 2) RIKEN Center for Developmental Biology, Kobe, Japan.

In mammals, daily physiological processes such as sleep/wake cycle, hormone production and metabolism are driven by an endogenous time-keeping system, namely the circadian clock. The suprachiasmatic nuclei (SCN) of the anterior hypothalamus are the master oscillator that regulates circadian behavioral and physiological activities. The circadian clock mechanism is based on an autoregulatory negative feedback loop, in which two transcriptional activators BMAL1 and CLOCK form a heterodimer and activate the rhythmic transcription of genes including period (Per1, Per2 and Per3) and cryptochromes (Cry1 and Cry2); the resultant PER and CRY proteins translocate to the nucleus where CRYs act as repressors to inhibit transcription by directly interacting with the BMAL1/CLOCK heterodimer. Despite similarities in sequence, domain structure and biochemical activity, CRY1 and 2 play distinct roles in clock function. Loss-of-function studies show that single knockout mice have opposing circadian phenotypes; Cry1-/- mice display shorter behavioral period length, whereas Cry2-/- mice display longer period. In cell-autonomous clock models, Cry1 deletion resulted in arrhythmicity or transient rhythms, whereas Cry2 knockout resulted in longer period and higher amplitude. Recently, using a novel genetic complementation assay in Cry1-/-:Cry2-/- mouse fibroblasts, we demonstrated that Cry1 alone is able to maintain cell-autonomous circadian rhythms, while Cry2 cannot (Cell, 2011). We further identified a domain within CRY1s photolyase homology region (PHR), designated as CRY1-PHR(313-426), that is required for clock function and differentiates CRY1 from CRY2 (JBC, 2012). Our studies also revealed that the C-terminal tail domain is critical for regulating period length, which provides a mechanistic basis for the opposing period length phenotypes of

knockout mice. Efforts are underway to employ lentivirus-mediated gene delivery method and test these findings in vivo in arrhythmic Cry1-/-:Cry2-/- mice, at both tissue (SCN) and behavioral levels. Taken together, our results demonstrate that the CRY1-PHR(313-426) domain is critical for clock function and functionally differentiates CRY1 from CRY2. These findings also provide novel insights into functional evolution of photolyase/cryptochrome family of flavoproteins.

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Evaluation of Genetic And Genomic Similarity of Inbred Strains of Mice Employing Microsatellite Markers. Mahadeo Kumar[1], Sharad Kumar[1], Akshay Dwarakanath[1], Dinesh Purohit[1], Daya Shankar Upadhayay[2]. 1) Animal Facility, CSIR-Indian Institute of Toxicology Research, M. G. Marg. Lucknow, India; 2) Animal Facility, Central Drug Research Institute, Lucknow.

The present investigation details an assessment of genetic relationship among five inbred strains of mice i.e. Balb/c, C57BL/6, AKR, NZB and AJ maintained at CDRI for long time since their inception to the country. The genomic DNA was extracted from 25 samples, five from each strain and processed by ten microsatellite markers for genotyping. Polymorphism was detected for all these studied loci. Summed over these ten informative microsatellite loci, the number of detected alleles ranged from 2 to 5 per locus. The microsatellite primers D1Mit17 and D4Mit54 were found to be the most informative, as they showed maximum number of alleles (5) in the studied samples. The strain AKR showed the maximum genetic similarity (52.70%) with NZB, while the Balb/c and AKR strain showed the least genetic similarity (10.21%) to C57BL/6 for the studied loci. A combined dendrogram based on Neis genetic distance using UPGMA method was constructed, which revealed that black coat colour mice C57BL/6 and NZB were clustered with AJ and AKR respectively. These results indicated that microsatellite markers are valuable marker for study of geneology and evolutionary biology of different strains of mice.

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Use of closely related mouse substrains to clone a QTL for psychostimulant response. Vivek Kumar[1,4], Gary Churchill[2], Fernando Pardo-Manuel de Villena[3], Joseph Takahashi[1,4]. 1) Dept of Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX; 2) The Jackson Laboratory, Bar Harbor, ME, USA; 3) Dept of Genetics, UNC-Chapel Hill, Chapel Hill, NC, USA; 4) Howard Hughes Medial Institute.

Identification of QTLs at the single gene or nucleotide level has been difficult due to low mapping resolutions achieved by traditional F2 or N2 mapping approaches. Even when intervals are narrowed, frequently there are confounding numbers of polymorphisms within the region of interest making identification of a single gene or nucleotide difficult. One alternative approach is to use closely related mouse substrains that have high phenotypic but low genotypic variance. Due to the high degree of relatedness between substrains, there should be limited polymorphisms within the QTL interval allowing for the identification of the causal gene or even nucleotide. Here we use two C57BL/6 substrains, C57BL/6J from Jackson Labs and C57BL/6N from NIH, to map and clone a QTL for psychostimulant response. We characterized the parental substrains, F1 and 250 segregating F2 progeny for open field, psychostimulant response and circadian behavior. Using bioinformatic analysis and the Mouse Diversity Array, we developed a SNP marker panel that can be used to map QTLs between several C57BL/6 substrains including C57BL/6Cr, C57BL/6Tac and C57BL/6NJ. This marker panel was used to genotype the F2 cross and map a single locus mediating cocaine response (LOD = 6.4). Using next generation sequencing technology on the ABI SOLiD platform we sequenced the entire genome of C57BL/6N. Surprisingly, there is only one non-synonomous polymorphism between C57BL/6N and C57BL/6J within the 1.5 LOD support interval of the psychostimulant response QTL. Biochemical analysis reveals this polymorphism destabilizes the protein leading to the behavioral difference between the two substrains. Our data will be of interest to the general mouse community because embryonic stem cells from B6N are being used for the Mouse Knockout Project and behavioral and genetic differences with the reference strain have not been thoroughly documented. Our approach will be of interest to the QTL community since there are over 40 documented C57BL/6 substrains, including 20 that are commercially available, and in addition there are large numbers of C3H/He, BALB/c, and DBA/2 substrains, many of which have known phenotypic differences and become amenable to the approach we have piloted here.

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The regulation of gene expression in a cell via DNA- lipids complexes formation. Vasily V. Kuvichkin. Mechanisms of Reception, Institute of Cell Biophysics, Russian Ac. Sci., Pushchino, Moscow oblast, Russian Federation.

We propose new mechanism of regulation of gene expression and consider the reason of many diseases as failure of such regulation. The understanding of the nature of cancer is impossible without an accepting the basic role of DNA-lipids complexes (DLC). In accordance with our model (Kuvichkin, J. Membr. Biol., 241, 2011, 109-16) DLC are formed in the chromatin areas with three-stranded hybrids: DNA- low molecular weight RNA (lmwRNA) at their interactions with the nuclear envelope. The triple helix unwinds during interaction with nuclear membrane forming a classical R-loop: DNA-RNA hybrid and a single-stranded DNA. The temperature of this transition is considerably lower than the temperature of DNA melting that result in the preferential attachment of triple-stranded hybrids to the nuclear envelope.The number of DLC determinate the set of genes attached to nuclear envelope. Single- stranded DNA in DLC is the site of transcription initiation. The result of such interaction is nuclear pores which thereby serve as sites of initiation of transcriptions in a cell. Therefore, the attachments of any gene to a nuclear envelope result in enhanced level of expression of this and neighboring genes. The structure of interphase chromatin cannot be considered without taking into account its interaction with nuclear pores. Current models of nuclear architecture concur that nuclei of multicellular organisms contain chromosome territories (CTs). DNA is attached to the nuclear pores, forming the big intranuclear loops of DNA. As we proposed early, DNA-membrane complexes are site of transcription initiation. This means

that genes located near pore complexes have enhanced expression than genes in nuclear interior. Last data about simultaneously transcribed genes showed that distance between such genes is around 80000 bp.(Goetze et.al.,MBC, 2007, 27, 4475) and support data of Cook (1995) describing chromatin loops with an average contour length of 86 kbp.

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Demonstrating Resistance-Mitigrating Effect of Artemisia Annua Phytochemical Blend with in-Vitro Cultures of Plasmodium Falciparum and in-vivo with Plasmodium Berghei Anka in Mice. Kangethe N Lucy[1,3,4*], Ahmed Hassanali[2], Sabah Omar[3], Nganga Joseph[4], Kinyua Johnson[5]. 1) LUCY N KANGETHE KENYA POLYTECHTECHNIC UNIVERSITY COLLEGE, NAIROBI, Kenya P.O BOX 52428--00200; 2) AHMED HASSANALI International Centre of Insect Physiology and Ecology P.O BOX 30179 Nairobi, Kenya; 3) SABAH OMARKenya Medical Research Institute P.O BOX 54840-00200 Nairobi, Kenya; 4) JOSEPH K NGANGA Jommo Kenyatta University of Agriculture and Technology P.O BOX 62000 Nairobi,Kenya; 5) JOHNSON KINYUA Jommo Kenyatta University of Agriculture and Technology P.O BOX 62000 Nairobi,Kenya.

ABSTRACT Resistance of Plasmodium falciparum to drugs such as chloroquine and sulfadoxine-pyrimethamine is a major problem in malaria control. Artemisinin derivatives, particularly in combination with other drugs, are thus increasingly used to treat malaria, reducing the probability that parasites resistant to the components will emerge. Although stable resistance to artemisinin has yet to be reported from laboratory or field studies, its emergence would be disastrous because of the lack of alternative treatments. The project was designed to demonstrate resistance-mitigating effects of phytochemical blend of Artemisia annua relative to pure artemisinin against the malaria parasite Plasmodium falciparum and on rodent malaria parasite Plasmodium berghei Anka. For the in vitro experiments selection was undertaken on two cultures of P. falciparum D6 (CQ-sensitive strain from Sierra Leone) and W2 (CQ-resistant strain from Indochina), by exposing them to A. annua phytochemical blend and the pure artemisinin over 50 cycles at doses initially required to give 50% mortality (IC50) of the parasites. Dose-response effects of the blend and the pure compound were determined after 10, 20, 30, and 40, cycles and compared to see if significant difference developed in their efficacy in causing mortality of the parasites. The in vivo experiments mice have been done by inoculating the Swiss mice with the P. berghei ANKA parasite and thereafter treated them with the test drugs. After 4 days the mice were passaged and parasitaemia determined to calculate the ED50 and the ED9O. The ED50 and ED90 got for artemisinin with P. berghei ANKA was 1.43 and 7.18 mg/kg.day respectively while the ED50 and ED90 got for the blend with P. berghei ANKA was 34.5 and 118 mg/kg.day.

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Subtelomere recombination is frequent in B-cell lymphomas lacking telomerase. Tammy A. Morrish[1], Joshua Budman[2], Stephen Dria[2], Vivek Behera[1], Margaret Strong[1], Sarah Wheelan[3], Carol Greider[1]. 1) Molecular Biology and Genetics, Johns Hopkins University, Baltimore, MD; 2) Biomedical Engineering; 3) Oncology and Biostatistics.

Telomere maintenance mechanisms are necessary for tumor growth and are typically achieved by the enzyme telomerase. However, some tumors lack telomerase and rely on recombination-based mechanisms for telomere maintenance. When telomerase is deleted in yeast, multiple mechanisms of break-induced replication (BIR) are necessary to maintain the telomeres and depend on either Rad50 or Rad51. BIR may also occur within telomere or subtelomere sequences. To examine whether BIR occurs in mammalian cells, we are using a mouse B-cell lymphoma model, myc+mTR-/-. Tumors from this model were used in a FISH based assay and detected subtelomere recombination at elevated frequencies in tumors lacking telomerase. To specifically focus on whether subtelomere recombination contributes to telomere maintenance, we are utilizing comparative genomic hybridization (aCGH) to determine the frequency and location of copy number changes within the subtelomere. With these arrays we detect an elevated frequency of copy number changes within the subtelomeres of some tumors lacking telomerase when compared to primary tissues, including primary B-cells or when compared to myc+mTR+/+ tumors. Furthermore, we find many of the breakpoints within the subtelomere occur at specific sites within the subtelomere. At some subtelomeres we observe extensive regions of amplification (20kb) while at other subtelomeres we observe small, dispersed regions of amplification. We are currently verifying whether this occurs within specific types of repetitive sequences and whether Rad50 contributes to the frequency of these events. Overall, these studies focus on the mechanisms of subtelomere recombination in tumors lacking telomerase.

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Evolution of Dosage Compensation of the Mammalian Active X Chromosome. Di Kim Nguyen, Xinxian Deng, Christine Disteche. Pathology, University of Washington, Seattle, WA.

In mammals, X-linked gene expression is adjusted by regulatory mechanisms to compensate for the evolutionary loss of Y-linked genes and balance gene expression in both sexes. Based on analyses of RNA-sequencing datasets, we confirmed our previous findings that expressed genes on the X chromosome are upregulated compared to autosomal genes in five tissues in 14 species (12 eutherian and 2 metatherian mammals). We then compared the current X genes in mammals against the proto-X-autosomal genes in chicken and opossum- and arrived at a similar conclusion. Finally, we showed that X-linked genes in female mammals overall have gained more expression than that in males over evolutionary time in a tissue-specific manner such as in the brain, liver, and sex organs. Differential expression between the sexes may be due either to escape from X inactivation in females or to mutations preferentially selected on the X because the X chromosome spends 2/3 of its time in females.

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C57BL/6NTac double and single albino mice generation for efficient germline transmission of Chimera. Ana V. Perez[1], Gunther Kauselmann[2], Maria R. Da Silva[2], Heidrun Kern[2], Nathalie Uyttersprot[2], Gerald Bothe[3], Branko Zevnik[2]. 1) Genetic Sciences & Compliance, Taconic, Hudson, NY; 2) Applied Genetics, Taconic Artemis GmbH, Cologne, Germany; 3) R&D, Taconic, Rensselaer, NY.

Coat color is usually an easy way to visualize contributions of mouse embryonic stem (ES) cells injected into host blastocysts during generation of genetically modified models, both being of different coat colors. Furthermore, coat color becomes important in order to visually determine germline transmission of the ES cell genome. C57BL/6 is the preferred genetic background of use. Therefore, the use of C57BL/6 mouse ES cells that produce black mice have become the ES cells of choice. In this scenario the preferred host blastocysts used are from albino (white) colored mouse strains such as C57BL/6 albino mice, that have arose as spontaneous mutants, or BALB/c mice. BALB/c mice respond poorly to superovulation resulting in low blastocyst yields which in turn results in usage of more mice. The increase in numbers of females for superovulation is counterproductive due to higher expense and animal use in research. Available albino C57BL/6 strains provide a suitable alternative but due to the recessive nature of the Tyr^{c} mutation, coat-color detection of ES cell contribution in germline transmitted G1 offspring requires breeding of chimeras to albino C57BL/6. More importantly, a pure genetic C57BL/6 substrain background may only be maintained by subsequent backcrossing and in addition the need to breed out the Tyr^{c} allele that will be inherited by the progeny if it is not actively screened against and removed from the colony. We decided to address the problems outlined above by generating a genetically modified C57BL/6NTac mouse albino model that carries the reversion of the non-agouti locus to a dominant agouti (A) allele, $A^{tm1.1Arte}$, and the loss-of-function of the tyrosinase locus by a genetically engineered point mutation, the albino allele, $Tyr^{tm1Arte}$. When this double mutant, C57BL/6NTac-$A^{tm1.1Arte}$ $Tyr^{tm1Arte}$ is used as host blastocyst with C57BL/6 ES cells, the resulting black/albino chimeras can be mated directly to C57BL/6 mice and allow recognition of germline transmission in the G1 offspring just by coat color. In addition, our data shows that the superovulation response of the double mutant albino is similar to C57BL/6NTac and that there is effective germline transmission when using these double albino mutants as host blastocysts.

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Disentangling the genetic architecture of nest building: a fitness-related trait. Andrea Cristina Peripato[1,2], Bruno Sauce[1], Reinaldo Alves de Brito[1]. 1) Genetics and Evolution, University of Sao Carlos, Sao Carlos, SP, Brazil; 2) Biosciences, University of Sao Paulo, Santos, SP, Brazil.

Nest building in mammals is set by an important array of maternal behaviors that ensures pups survival and it is directly associated with fitness. Here we investigated the genetic architecture of nest building in F2 mice LG/J x SM/J. We performed a QTL analysis by using 101 microsatellites markers and six related nest phenotypes (Presence and Structure pre- and postpartum, prepartum Material Used and postpartum Temperature). We found 15 single QTLs, mostly with non-additive effect that explain individually from 4 to 13% of the phenotypic variation of nest building phenotypes. We also found 71 regions interacting epistatically which, along with single QTLs, explain from 28.4 to 75.5% of variation for nest building phenotypes. Our results indicate a genetic architecture with small direct effects and a larger number of epistatic interactions as it is suggested to be the case for fitness-related phenotypes when compared to non-fitness-related phenotypes. We conclude that nest building is a complex maternal behavior critical to offsprings survival and development and in which epistasis plays an important role in their adaptive process. Financial Support: FAPESP.

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Genetic Studies of Inflammatory Responses in Wild-Derived Mice. Alexander N. Poltorak. Tufts University, Boston, MA.

Immune response leads to inflammation which, if excessive, can often lead to the programmed death. How do genes control the balance between inflammation and cell death? To answer this question, we have elected to study the immune responses in so called wild-derived mice (WDM), which evolved from common ancestor with classical laboratory mice more than a million years ago and therefore share only 11% of their genome with laboratory mice. Such divergence of WDM results in phenotypic differences between WDM and classical laboratory mice, which we investigate by means of classical genetic analysis. In the last few years, we established several genetic screens in the WDM including hyper- and hypo-responsiveness to TLR-agonists, resistance to TLR-mediated necroptosis, in vitro responses to infectious pathogens, resistance to septic shock in vivo. In our published and ongoing research, we show that forward studies in WDM provide insights into human biology that would not be available without access to this largely untapped reservoir of genetic diversity.

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Comprehensive molecular characterization of mutant mouse strains generated from the EUCOMM / KOMP ES cell resource. Ed Ryder, Diane Gleeson, Debarati Sethi, Sapna Vyas, Evelina Miklejewska, Priya Dalvi, Bishoy Habib, Ross Cook, Matthew Hardy, Joanna Bottomley, David Adams, Ramiro Ramirez-Solis, Sanger Mouse Genetics Project. Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

The EUCOMM/KOMP mouse embryonic stem cell collection, which is based on JM8 agouti or non-agouti C57BL/6N ES cells is currently the biggest single resource of targeted mutations available for the mouse. The Wellcome Trust Sanger Institute Mouse Genetics Project (Sanger MGP) generates the strains from these clones, and characterizes the phenotypic consequences of the modified alleles in a high-throughput primary phenotypic analysis pipeline. Strains are available from the EMMA or KOMP

repositories for the scientific community to build on the information provided by the primary phenotypic screen for a more detailed and specific analysis. To date over 700 lines have been produced by the Sanger MGP. To ensure the quality of this resource, new colonies are subject to a panel of quality control (QC) PCR-based tests to confirm the targeting of the endogenous allele, structure of the cassette, absence of additional insertions and for the presence of the 3 LoxP site. Approximately 86% of lines passed the targeting and cassette structure tests; while 96% had the expected 3 LoxP site. The characteristics of the QC-failed lines are discussed and should act as a guide for researchers generating their own lines on what potential issues may exist and how to detect them. Many of the issues observed in the mouse lines may be derived from mixed clone populations which were not detected using the mainly PCR-based primary screen on the ES cells. Therefore, it is vital to check all heterozygous chimera progeny for each colony with all tests to detect these events and prevent them from propagating through the colony.

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An albino C57BL/6N strain for EUCOMM/KOMP mouse generation in a pure genetic background. Ed Ryder, Diane Gleeson, Thomas Keane, Debarati Sethi, Sapna Vyas, Hannah Wardle-Jones, James Bussell, Richard Houghton, Jennifer Salisbury, David Adams, Ramiro Ramirez-Solis, Sanger Mouse Genetics Project. Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

The Wellcome Trust Sanger Institute Mouse Genetics Project (MGP) generates 160 strains from the international KOMP and EUCOMM targeted mutant ES cell resource per year. The phenotypic consequences of the modified alleles are characterized in a comprehensive primary screen. All mice are now produced on a pure C57BL6/N background, in line with the emerging International Mouse Phenotyping Consortium (IMPC) and associated programmes including the NIH-funded KOMP2 project. Normally, to preserve the purity of the genetic background, all chimeras are mated to black C57BL/6N mice. Therefore colour selection cannot be used to detect germ-line transmission (GLT), and consequently all G1 mice have to be genotyped. Here, we introduce a C57BL/6N albino strain which restores the ability to score GLT by coat colour of the chimera progeny, greatly decreasing costs and effort, and improving animal welfare. The new strain originated from a mutation of the Tyrosinase locus in an ES clone used to generate one of the mutant strains in the MGP. This mutation consists of a 14.3Kb deletion spanning 940bp 5 of the start of exon 1 to 3.75kb 3 of exon 2. Genome sequencing of the two albino founder mice of the colony is now underway to determine whether any large deleterious mutations are present which could limit its use in future downstream applications.

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The transcription factors *Ets1* and *Sox10* interact during murine melanocyte development. Amy Saldana-Caboverde, Lidia Kos. Biological Sciences, Florida International University, Miami, FL.

Melanocytes, the pigment-producing cells, are derived from a population of pluripotent migratory cells known as the neural crest (NC) that delaminate from the dorsal aspect of the neural tube during vertebrate development. Many of the genes required for the specification of melanocytes from the NC have been identified through the study of mouse pigmentation mutants. Mice carrying a deletion of the transcription factor *Ets1* were recently shown to exhibit hypopigmentation; nevertheless, the function of *Ets1* in melanocyte development is unknown. In the mouse embryo, *Ets1* is widely expressed in developing organs and tissues, including the NC, and is required for the endogenous expression of the melanocyte-specific transcription factor *Sox10* in the chick cranial NC. We aim to establish the temporal requirement and role of *Ets1* in melanocyte development and to examine potential genetic interactions between *Ets1* and melanocyte-specific transcription factors, including *Sox10*. To this end, embryos from crosses between $Ets1^{+/-}$ and *Dct-LacZ* transgenic mice, in which *LacZ* expression is driven to melanocyte precursors (melanoblasts), were stained between embryonic days (E)11.5-15.5. $Ets1^{-/-}$ embryos were found to have fewer melanoblasts compared to $Ets1^{+/-}$ and $Ets1^{+/+}$ littermates. However, cell survival and proliferation assays suggest that *Ets1* deletion does not result in increased melanoblast cell death or decreased proliferation at E11.5. To determine if *Ets1* interacts with *Sox10*, $Ets1^{+/-}$ mice were crossed to $Sox10^{+/LacZ}$mice, in which *LacZ* was inserted in the *Sox10* locus, and the hypopigmentation phenotypes of the progeny were compared. The areas of hypopigmentation of $Ets1^{+/-}$::$Sox10^{+/LacZ}$ mice were significantly greater than the sum of the areas of hypopigmentation of $Ets1^{+/-}$ and $Sox10^{+/LacZ}$ mice. The incidence of hypopigmentation in $Ets1^{+/-}$::$Sox10^{+/LacZ}$ mice was also significantly higher than that of $Ets1^{+/-}$ and $Sox10^{+/LacZ}$ mice. Additionally, *Sox10* expression, examined via LacZ staining, was found to be reduced at E12.5 in 50% of $Ets1^{-/-}$ embryos compared to $Ets1^{+/+}$ littermates. Our results suggest that *Ets1* is critical for melanocyte development on or before E11.5, although it does not appear to regulate melanoblast survival or proliferation at this stage. Furthermore, *Ets1* interacts synergistically with *Sox10* and regulates its embryonic expression.

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Essential benchmarks for use of Cre-lox mouse genetic tools. Ramkumar Sambasivan[1,2], Glenda Comai[1], Shahragim Tajbakhsh[1]. 1) Institut Pasteur, Stem Cells and Development, CNRS URA 2578, 25 rue du Dr. Roux, 75724 Paris Cedex 15; 2) Institute for Stem Cell Biology and Regenerative medicine, NCBS, GKVK PO, Bellary Road, Bangalore 560065.

The vertebrate head and the associated head muscles are evolutionarily recent. We had shown earlier that the head muscle gene regulatory network is distinct from that of the trunk, indicating that head muscle developmental program evolved independently (Sambasivan et al., 2009). However, all distinct upstream networks converge on the core muscle fate determining bHLH transcription factors Myf5, Myod and Mrf4. Cell ablation studies using Myf5Cre driver lines in combination with an ubiquitous Cre-responsive cell-death causing diphtheria toxin (DTA) allele (R26RDTA), have reported the presence of distinct muscle progenitor populations with respect to Myf5 expression (Gensch et al., 2007; Haldar et al., 2007). The studies also reported that the Myf5+ population is dispensable for muscle development. To carefully address the presence and role of distinct muscle

progenitor populations in the head, we employed genetic tracing and cell ablation approaches. Surprisingly, the use of Myf5Cre alleles in combination with the Cre-responsive ablator R26RDTA mouse line resulted in different phenotypic outputs in a specific head muscle group, when compared to the loss of function of Myf5/Mrf4. Results will be discussed taking into account i) the possibility of different cell progenitor populations for head muscles versus trunk muscles; ii) the specificity and strength of Cre drivers and iii) the use of ubiquitous (ROSA26) versus lineage specific (Pax7nGFP/nlacZ) Cre-responsive reporters. More generally, we will highlight the limitations of some commonly used genetic tools, and how this can impact on the biological interpretation of gene deletions, genetic tracing, and cell ablation results. We also propose benchmarks for performing rigorous studies with Cre-lox based mouse genetic tools. References: Gensch, N., Borchardt, T., Schneider, A., Riethmacher, D. and Braun, T. (2008) 'Different autonomous myogenic cell populations revealed by ablation of Myf5-expressing cells during mouse embryogenesis', Development 135(9): 1597-604. Haldar, M., Karan, G., Tvrdik, P. and Capecchi, M. R. (2008) 'Two cell lineages, myf5 and myf5-independent, participate in mouse skeletal myogenesis', Dev Cell 14(3): 437-45. Sambasivan, R., Gayraud-Morel, B., Dumas, G., Cimper, C., Paisant, S., Kelly, R. G. and Tajbakhsh, S. (2009) 'Distinct regulatory cascades govern extraocular and pharyngeal arch muscle progenitor cell fates', Dev Cell 16(6): 810-21.

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Genome-wide mapping of gene-microbiota interactions in susceptibility to autoimmune skin blistering. Girish Srinivas[1,2], Steffen Möller[2], Sven Künzel[1], Jun Wang[1,3], Detlef Zillikens[2], John Baines[1,3,4], Saleh Ibrahim[2,4]. 1) Max Planck Institute for Evolutionary Biology, Plön, Germany; 2) Department of Dermatology, University of Lübeck, Lübeck, Germany; 3) Institute for Experimental Medicine, University of Kiel, Kiel, Germany; 4) These authors contributed equally to this work.

Susceptibility to chronic inflammatory diseases is determined by the interaction of immunogenetic and environmental risk factors. In particular, resident microbial communities as environmental factors are the subject of intense scrutiny due to numerous observations of differences between healthy and diseased states. However, whether differences in community composition or structure are of primary etiological importance or secondary to the altered inflammatory environment remains largely unknown. Here we provide direct experimental evidence for host gene-microbiota interactions contributing to disease risk in a mouse model of epidermolysis bullosa acquisita (EBA), a chronic autoantibody-induced inflammatory disease afflicting the skin. By using an advanced intercross population, we simultaneously identified genetic loci contributing to variability in the skin microbiota, susceptibility to autoimmune skin blistering and their overlap. Furthermore, treating the abundances of individual bacterial species as covariates with disease lead to the discovery of novel disease loci. Specifically, a reduction in the abundance of numerous individual species is associated with increased disease risk. This demonstrates a primary role for resident microbes in disease etiology and underscores the importance of the skin microbiota in protection from disease. We anticipate our results to further motivate the identification and characterization of individual beneficial members of host-associated microbial communities and their interactions, in particular with respect to their potential for immunomodulatory drug development.

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The chromatin remodeler Chd5 modulates neuronal morphology and behavior. Assaf Vestin[1,2], Guy Horev[1], Wangzhi Li[1,2], Alea Mills[1]. 1) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 2) Stony Brook University, Stony Brook, NY.

Chd5 is a predicted chromatin remodeling protein of the SWI/SNF superfamily that is robustly expressed in neuronal tissues such as the brain. Our laboratory previously identified *Chd5* as a tumor suppressor mapping to human 1p36, a region of the genome that is frequently deleted in neuronal malignancies. Although a number of chromatin remodeling proteins have been shown to modulate brain function, whether Chd5 performs a similar role is unknown. Here we show that Chd5 plays a fundamental role in the brain. Chd5 expression initiates in differentiating neurons during embryogenesis and is maintained in post-mitotic neurons in the adult. Chd5-compromised mice are viable and have unique behavioral phenotypes such as hyperactivity and repetitive behaviors. In addition, analysis of Chd5-compromised brain indicates that cortical and CA1 hippocampal neurons have abnormal dendritic morphology. These findings reveal a role for Chd5 in neuronal function in vivo and will help to elucidate how regulation of chromatin, and in turn regulation of gene expression, affects brain function.

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Patterns of recurrent and tissue-specific copy number variants in the mouse genome. Andrea E. Wishart[1], M. Elizabeth O. Locke[2], Susan T. Eitutis[1], Kathleen A. Hill[1]. 1) Department of Biology, The University of Western Ontario, London, Ontario, Canada; 2) Department of Computer Science, The University of Western Ontario, London, Ontario, Canada.

Identifying regions of recurrent copy number variants (CNVs) gives insight into the mechanisms by which CNVs are formed in the mammalian genome, as well as into the potential for functional impact on the expression of genes in and around these regions. Here, we apply the Mouse Diversity Genotyping Array (MDGA), a high-resolution array available for mice querying approximately 530 000 highly reliable SNP positions to detect copy number across autosomes. Genomic DNA was isolated from tissue samples taken from related mice on a mixed C57BL/6J and CBA/CaJ background: spleen and cerebellum of two prematurely aged *harlequin* (*hq*) brothers and two wild-type (WT) brothers, cerebellum only of two additional *hq* brothers, and one repetition of each tissue from one WT mouse. Additionally, spleen and cerebellum from one C57BL/6J inbred mouse was included for a total of 14 samples. CNV calls were made using a Hidden Markov Model within Partek Genomics Suite using stringent parameters, and then filtered to exclude regions called with a low marker density. Using custom tracks in the UCSC Genome Browser constructed from published CNV datasets, we identified CNV regions previously detected in a publicly available 351-mouse MDGA dataset, as well as variants unique to our samples. Of the 62 CNV events called, none were detected

in our C57BL/6J mouse, as expected. Modified Partek output was run through our newly developed Hotspot Detector for CNV (HD-CNV) software to identify 16 recurrent regions (CNV events overlapping each other by a minimum of 40 percent; between samples). CNVs that were detected in only one tissue of a given individual, termed copy number changes, or CNCs, occurred in both genotypes and tissues. The number of amplification CNCs was three times that of deletion CNCs. Six recurrent CNVs on chromosome 8 overlapped with a previously observed CBA/CaJ CNV from the 351 dataset. This workflow, using two different somatic tissues of the same mouse and novel software (HD-CNV) to detect and visualize recurrent CNVs, highlights patterns in the dynamic murine genome that will give great insight into mechanisms underlying CNV formation and their phenotypic relevance.

Imaging

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Live cell imaging of random X-inactivation. Osamu Masui[1,2], Isabelle Bonnet[2], Patricia Le Baccon[2], Isabel Brito[2], Tim Pollex[2], Niall Murphy[2], Philippe Hupé[2], Emmanuel Barillot[2], Andrew Belmont[3], Haruhiko Koseki[1], Edith Heard[2]. 1) RIKEN, Yokohama, Japan; 2) Curie Institute, Paris, France; 3) University of Illinois, Urbana, IL, USA.

Random X inactivation represents a paradigm for monoallelic gene regulation and epigenetic changes during early ES cell differentiation. The choice of X chromosome to inactivate in XX cells is ensured by monoallelic regulation of Xist RNA via its antisense transcription unit Tsix/Xite. Homologous pairing events have been proposed to underlie asymmetric Tsix expression but this has never been addressed experimentally, owing to the dynamic and transient nature of such early developmental events. Here we investigate the live cell dynamics and the outcome of Tsix pairing in differentiating mouse ES cells. We find an overall increase in genome dynamics, including the Xics, during early ES cell differentiation. When they become paired however, Xic loci show markedly reduced movements. Upon separation, Tsix expression becomes transiently monoallelic, thus providing a window of opportunity for Xist up-regulation in cis to the silent Tsix allele. Our findings reveal the spatio-temporal choreography of the two X chromosomes during early differentiation, and point to a direct role for pairing events in facilitating symmetry breaking and monoallelic regulation of Xist during random X inactivation. Our recent live-cell imaging results will be also presented and discussed.

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Sphingosine-1-phosphate receptor 2 expression retains follicular helper T cells in germinal centers. Saya Moriyama[1], Noriko Takahashi[1], Jesse A. Green[2], Masato Kubo[3], Jason G. Cyster[2,4], Takaharu Okada[1]. 1) Research Unit for Immunodynamics, RCAI, RIKEN, Yokohama, Kanagawa, Japan; 2) Department of Microbiology and Immunology, University of California, San Francisco, CA, USA; 3) Laboratory for Signal Network, RCAI, RIKEN, Yokohama, Kanagawa, Japan; 4) Howard Hughes Medical Institute, University of California, San Francisco, CA, USA.

Follicular helper T (Tfh) cells are the helper T cell subset specialized in providing help to cognate antigen-specific B cells in the secondary lymphoid organs, and thus play critical roles in humoral immune responses.Tfh cells are particularly important for the germinal center (GC) reaction that is essential for long-term, high affinity antibody production. However, the molecular mechanisms of how Tfh cells are physically associated with GCs are incompletely understood. Here we report that sphingosine-1-phosphate receptor 2 (S1PR2), which has recently been shown to play a role in GC B cell localization, is also important for GC-association of Tfh cells. Analysis using an S1pr2-reporter mouse strain suggested that S1PR2 is expressed at varied levels in Tfh cells, and that S1PR2-high Tfh cells are localized in GCs whereas S1PR2-low Tfh cells are scattered throughout the B cell follicle. S1PR2-deficient Tfh cells exhibited reduced accumulation in GCs, which was attributed to impaired retention in GCs based on two-photon imaging analysis. Expression of Bcl6, Il4, and Il21 in Tfh cells was found to be positively correlated with S1pr2 expression. These results suggest that S1PR2-high Tfh cells are GC-resident Tfh cells bearing the advanced capability to promote long-term B cell responses.

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A single-cell resolution Notch signaling reporter strain of mice. Sonja Nowotschin, Panos Xenopoulos, Evan Weiner, Kat Hadjantonakis. Developmental Biology, Sloan-Kettering, New York, NY 10065, USA.

Live cell imaging is an essential tool for understanding the highly dynamic and coordinated events that drive cell lineage specification and morphogenesis during mammalian development. To elucidate the critical role of signaling pathways and begin to assay signal responsiveness, signaling reporter strains can be engineered by placing signaling responsive elements to direct the expression of reporter genes. To date, several transgenic Notch reporter strains have been generated marking the sites of active Notch signaling during development.

Here we report the construction of a fluorescent protein-based single-cell resolution Notch signaling reporter designed for live visualization and tracking of individual cells *in vivo* in mouse embryos and adults and *ex vivo* in stem cells such as ES cells. We have placed a CBF (also called RBP-Jk and CSL) responsive element (CBFRE) containing 4 copies of the mouse CBF1 binding sites and an SV40 minimal promoter in front of a fluorescent protein fusion comprising human histone H2B linked to the Venus

yellow fluorescent protein. We have used the *CBFRE::H2B-Venus* construct to generate transgenic ES cells and a strain of transgenic mice designed to report a transcriptional readout of Notch activity.

Characterization of embryonic and adult stages of the resulting *CBFRE::H2B-Venus* strain reveal discrete and specific expression of the transgene at previously characterized and uncharacterized sites of Notch signaling. Our current validation of the *CBFRE::H2B-Venus* strain will be presented and should allow us to determine whether it faithfully reports Notch activity at single cell resolution *in vivo*.

Models of Human Disease

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Genetic deletion of p66^{Shc} adaptor protein leads to increased myocardial infarction size. Alexander Akhmedov[1], Vincent Braunersreuther[2], Fabrizio Montecucco[2], Philip Jakob[1], Giovanni G. Camici[1], Francois Mach[2], Thomas F. Luescher[1]. 1) Institute of Physiology, University of Zurich, Zurich, Switzerland; 2) Division of Cardiology, Faculty of Medicine, Foundation for Medical Researches, Geneva University Hospitals.

Background - Formation of reactive oxygen species (ROS) contributes to many pathophysiological processes. Although ROS production is also involved in some physiological processes, the imbalance between their generation and removal, i.e. oxidative stress, plays a major role in particular in myocardial injury caused by ischemia-reperfusion (I/R). The mammalian Shc locus encodes three Shc isoforms: p46^{Shc}, p52^{Shc} and p66^{Shc}. The p66^{Shc} is not involved in mitogenic signals as p46^{Shc}/p52^{Shc}, but it functions as a critical mediator of intracellular oxidative signal transduction. Various studies relate p66^{Shc} to cardiovascular disease; however, few data are available on the role of p66^{Shc} in myocardial I/R.

Methods and Results - 8-12-week-old male p66^{Shc} deficient (*p66$^{Shc-/-}$*) mice on C57Bl/6 pure background together with corresponding C57Bl/6 wild-type (WT) control mice were subjected in vivo to different durations of ischemia (up to 60 min) followed by 24h of reperfusion. Infarct size was assessed morphologically and by MRI. After 30 min of ischemia, *P66$^{Shc-/-}$* mice developed markedly larger infarcts as compared to WT (infarct size [I]/area at risk [AAR]: 20.465.02 % vs. 7.721.31%, n=12-14, p 0.05). This effect was confirmed by measurement of serum cardiac troponin I (cTnI). *P66$^{Shc-/-}$* mice showed elevated serum levels of cTnI as compared to WT controls at 24 h of reperfusion (27.235.51 ng/ml vs. 10.051.3 ng/ml, n=13, p 0.05). However, by increasing ischemia duration to either 45 or 60 min infarct size did no longer differ between *p66$^{Shc-/-}$* and WT mice. Moreover, differently from WT, infarct size in *p66$^{Shc-/-}$* was not significantly larger with increasing duration of ischemia (from 30 to 60 min).

Conclusions - Our data suggest that genetic deletion of p66^{Shc} leads to an increased sensitivity to myocardial infarction with larger infarcts with shorter, but not prolonged ischemia. Therefore, activation of p66^{Shc} may provide resistance to ischemia and represent a novel therapeutic target in the early phase of myocardial infarction.

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Endothelial overexpression of LOX-1 protects from *in vivo* arterial thrombosis and modulates TF expression. Alexander Akhmedov[1], Giovani G. Camici[1], Simona Stivala[1], Erik W. Holy[1], Alexander Breitenstein[1], Christine Lohmann[1], Juerg-Hans Beer[2], Felix C. Tanner[1], Christian M. Matter[1], Thomas F. Luescher[1]. 1) Institute of Physiology, University of Zurich, Zurich, Zurich, Switzerland; 2) Division of Internal Medicine, Kanton Hospital Baden, 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-B, 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*). 12-week-old male *LOX-1TG* and wild-type (WT) mice were applied for carotid artery thrombosis model. *LOX-1TG* mice developed carotid artery thrombosis within a mean occlusion time of 36.964.83 min, while WT control mice occluded within a mean time period of 22.753.87 min (n=10, P 0.05). Initial blood flow in carotid artery did not differ between both groups of mice. Decreased occlusion time in *LOX-1TG* mice was further associated with decreased tissue factor expression and surface activity as shown by RT PCR and ELISA. Furthermore, *LOX-1TG* mice showed increased mRNA expression of histone deacetylase SIRT1 in carotid artery, pointing out that SIRT1 may be involved in the observed downregulation of tissue factor through its known target transcription factor NF-B.

Conclusions - Thus, our data suggest that LOX-1 plays a protective role in the arterial thrombosis and that SIRT1 may be involved. Hence, modulation of LOX-1 may represent novel therapeutic options for targeting arterial thrombosis.

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Investigation of hippocampal dendritic complexity in a potential XLMR mouse model. C. Y. Chen[1*], M. S. Tsai[1], C. Y. Lin[1], I. S. Yu[1], Y. C. Hsu[1], C. Y. Kao[1], S. W. Lin[1], L. J. Lee[2,3]. 1) Department of Clinical Laboratory Sciences and Medical Biotechnology National Taiwan University; 2) Graduate Institute of Anatomy and Cell Biology National Taiwan University; 3) Graduate Institute of Brain and Mind Sciences National Taiwan University.

Early deletion of Cul4b, encoding a scaffold protein of the E3 ubiquitin ligase complex and responsible for X-linked mental retardation (XLMR), causes prenatal lethality in mice that has frustrated attempts to characterize the phenotypes in vivo. Here, we generated Cul4b conditional knock-out mice and successfully rescued Cul4b mutant mice by deleted Cul4b in epiblast stage using Sox2-Cre transgenic mice. The structure and organization of the cortex, striatum, and hippocampus in Cul4b/Y mice showed no difference. The CUL4B expression in hippocampus was dominant in CA1 and CA3 region. Because the memory circuit is highly related with CA1 pyramidal cells, the apical and basilar dendrites of CA1 pyramidal cells were examined by Golgi impregnation. Although the number of intersections between the dendritic arbors was comparable in Cul4b/Y and Cul4blox/Y mice, the number of segments in apical dendrites was significantly reduced in Cul4b/Y mice. Moreover, we found that deletion of Cul4b lead to increased epileptic susceptibility and spatial learning deficits. Our results suggested Cul4b/Y mice may be as a potential XLMR model that is further valuable for the development of a therapeutic strategy of MR.

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Maged1 deficiency results in increased anxiety-like behaviors, disturbed sexual behaviors and late onset obesity associated with reduced levels of hypothalamic oxytocin. Carlos Dombret[1], Tuan Nguyen[1], Mathieu Bertrand[2], Jacques Michaud[3], Hélène Hardin-Pouzet[4], Olivier Schakman[5],**Olivier RY De Backer**[1]. 1) URPHYM, NARILIS, FUNDP school of Medicine, University of Namur,5000 Namur, Belgium; 2) Molecular Signaling and Cell Death Unit, Department for Molecular Biomedical Research, VIB, 9052 Ghent, Belgium; 3) Université de Montréal, Sainte-Justine Hospital Research Centre, Montréal H3T1C5, Canada; 4) UPMC - Paris 6 University, PMSNC lab, CNRS UMR 7224, INSERM UMRS 952, 75005 Paris, France; 5) Laboratory of Cell Physiology, Institute of Neuroscience IoNS, Université Catholique de Louvain, Brussels B-1200, Belgium.

MAGED1 is a member of the MAGE gene family that also includes NECDIN and MAGEL2, two genes defective in patients with the neurobehavioural Prader-Willi Syndrome. Oxytocin is produced by hypothalamic neurons and can act as a neurotransmitter that promotes social behaviors such as social exploration and recognition, pair bonding and parental care. Growing evidences indicate that oxytocin could be involved in autism spectrum disorders. It has been shown previously that, in mice, Maged1 regulates developmental neuron apoptosis and circadian activity. Here we show that Maged1-deficient mice develop progressive obesity associated with hyperphagia and reduced motor activity. Loss of Maged1 also results in a behavioral syndrome that associates impairment in social interactions, increased anxiety-like behaviors, and infertility of the males caused by a lack of sexual interest. These behavioral traits, which evocate autistic symptoms in humans, are associated with a severe reduction of brain oxytocin level. These observations show that Maged1 plays important and pleiotropic functions in the control of social and sexual behaviors, motor activity and energy balance.

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The role of Keratin 76 in epidermal homeostasis and barrier function maintenance. Tia M. DiTommaso[1], Helen Pearson[2], Holger Schluter[2], Pritinder Kaur[2], Patrick Humbert[2], Ian Smyth[1,3]. 1) Biochemistry & Molecular Biology, Monash University, Melbourne, Australia; 2) The Peter MacCallum Cancer Institute, Melbourne, Australia; 3) Department of Anatomy and Developmental Biology, Monash University, Melbourne, Australia.

In collaboration with the Wellcome Trust Sanger Institute, we have implemented a rigorous reverse genetic screen in mice to identify genes involved in the development and maintenance of the epidermis. The Keratin76 (Krt76) gene is a prime example of new insights into the biology of the skin that have been identified as a result of this screen. The phenotype of the Krt76 knockouts *(Krt76$^{tm1a/tm1a}$)* includes hyperpigmentation, hyperkeratosis as well as defects in homeostasis and differentiation. Transepidermal water loss (TEWL) assays indicate that the epidermal barrier is affected, although the layered organization of the epidermis appears to be normal. Furthermore, loss of Krt76 leads to a significant decrease in lifespan with no mice surviving beyond 12 weeks of age. Lifespan can be significantly increased in the *Krt76$^{tm1a/tm1a}$* mice with the administration of a broad spectrum antibiotic, although this appears to only prolong lifespan by 5-6 weeks. Taken together, these data suggest that a tight junction specific barrier function defect leads to dehydration, bacterial infection, and subsequent lethal epidermal pathology. Closer inspection into the organization of the epidermis with an anti-claudin 1 antibody, a tight junction specific marker, has revealed mislocalization of claudin-1 in *Krt76$^{tm1a/tm1a}$* mice as compared to wild type littermates. The functionality of the tight junction complexes in these mice is being further investigated by comparing the diffusion of a subcutaneously injected biotin tracer toward the skin surface. Our data indicate that the Krt76 knockout mouse may provide insight into the role of intermediate filaments in the molecular architecture and stability of tight junction complexes in mammalian stratified epithelia. Our model may support other emerging research to identify roles of intermediate filaments outside of mechanical and structural cytoprotection. This study highlights an approach to characterize gene expression by targeted mutagenesis, but also furthers the investigation of cell biology using the skin as model system.

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BXD mouse lines as a genetic reference population for the metabolic syndrome. Evan Williams[1], Pénélope Andreux[1], Charles Thomas[2], **Raphael Doenlen**[2], Philippe Cettour-Rose[2], Xavier Warot[2], Robert W. Williams[3], Johan Auwerx[1]. 1)

Laboratory of Integrative Systems Physiology, School of Life Sciences, EPFL, Lausanne, Switzerland; 2) Phenotyping Unit, Center of PhenoGenomics, School of Life Sciences, EPFL, Lausanne, Switzerland; 3) Department of Anatomy and Neurobiology, University of Tennessee, Memphis, USA.

Chronic multifactorial diseases, such as type 2 diabetes and obesity, result from the interaction of genes and environmental factors. In order to better understand this interaction, a mouse genetic reference population allowing control of both genetic and environmental conditions was used in a pipeline of standardized metabolic tests. A panel of 42 BXD recombinant inbred mouse lines, descending from C57BL/6J and DBA2/J strains, was used to identify quantitative trait loci (QTL) and gene networks that modulate metabolic activity. This characterization included exercise endurance, adaptive thermogenesis by means of resistance to a cold stress, and a glucose tolerance test in a diet-induced obesity paradigm with chow diet (CD) groups versus high-fat diet (HFD) groups over 29 weeks. Preliminary results showed a body weight increase during the diet treatment ranging from 16% to 45% in the CD groups versus 45% to 110% in the HFD groups. Similarly, we found significant differences in the body weight loss after two weeks of voluntary exercise, with a variation range of -1% to 12% in the CD groups versus 1% to 23% in the HFD groups. These results reinforce the heterogeneity in the VO2 max, the cold resistance and the glucose tolerance that could be observed between lines and groups. After completion of the same phenotyping pipeline on about 50 BXD lines, the broad data set generated with this method will enable QTL mapping strategies through the GeneNetwork resource (www.genenetwork.org). We presume that the outcome of this work will provide a better understanding of the metabolic syndrome with the identification of signaling pathways and network regulations. These results will initiate new strategies for diagnosis, prevention and therapy complex diseases associated with aging, such as obesity and type 2 diabetes.

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The European Mouse Mutant Archive - EMMA. Sabine Fessele[6], Glauco Tocchini-Valentini[1], Yann Hérault[2,9], Steve Brown[3], Urban Lendahl[4], Jocelyne Demengeot[5], Martin Hrabé de Angelis[6], Paul Flicek[7], Ramiro Ramirez-Solis[8], Lluís Montoliu[10], George Kollias[11], Radislav Sedlacek[12], Raija Soininen[13], Thomas Rülicke[14]. 1) CNR-Monterotondo, CNR Campus "A. Buzzati-Traverso", Monterotondo, Italy (core structure); 2) CNRS - Centre de Distribution, de Typage et d'Archivage Animal (CDTA), Orléans, France; 3) Medical Research Council, MRC-Harwell, Harwell, UK; 4) Karolinska Institutet, Department of Cell and Molecular Biology (KI-CMB), Stockholm, Sweden; 5) Fundaçao Calouste Gulbenkian, Instituto Gulbenkian de Ciencia (FCG-IGC), Oeiras, Portugal; 6) Helmholtz Zentrum München, Institute of Experimental Genetics (HMGU-IEG), Neuherberg, Germany; 7) European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Hinxton, UK; 8) Genome Research Limited, Wellcome Trust Sanger Institute (WTSI), Hinxton, UK; 9) GIE-Centre Européen de Recherche en Biologie et en Médecine, Institut Clinique de la Souris (GIE-CERBM-ICS), Illkirch, France; 10) Consejo Superior de Investigaciones Científicas, Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain; 11) B.S.R.C. Alexander Fleming, Vari/Athens, Greece; 12) Institute of Molecular Genetics (IMG), Prague, Czech Republic; 13) University of Oulu - Biocenter Oulu, Oulu, Finland; 14) University of Veterinary Medicine - Biomodels Austria (BIAT), Vienna, Austria.

The European Mouse Mutant Archive (EMMA) offers the worldwide scientific community a free archiving service for its mutant mouse lines and access to a wide range of disease models and other research tools. A full description of these services can be viewed on the EMMA website at http://www.emmanet.org. At present EMMA holds more than 2700 mouse strains, corresponding to transgenic mice, induced mutants, gene-traps, knock-ins, knock-outs and also including targeted alleles from Deltagen, Lexicon and strains produced from the EUCOMM ES cell resource. The EMMA network is comprised of 14 partners from 11 different countries who operate as the primary mouse repository in Europe. EMMA is funded by the partner institutions, national research programmes and by the European Commission's FP7 Capacities Specific Programme. EMMA's primary objectives are to establish and manage a unified repository for maintaining mouse mutations and to make them available to the scientific community. In addition to these core services, the consortium can generate germ-free (axenic) mice for its customers and also hosts cryopreservation courses, to promote the use and dissemination of frozen embryos and spermatozoa. Dissemination of knowledge is further fostered by a dedicated resource database (Wilkinson et al. Nucleic Acids Res. 2010, 38:D570-6). EMMAs technology development programme focuses on improving sperm cryopreservation methods and the implementation of laser-assisted IVF and ICSI protocols. All applications for archiving and requests for mutant mouse strains are submitted through the EMMA website. Mouse strains submitted for archiving are evaluated by EMMA's external scientific committee. Once approval has been granted depositors are asked to send mice of breeding age to one of the EMMA partners for embryo or spermatozoa cryopreservation. Strains held under the EMMA umbrella can be provided as frozen materials or re-derived and shipped as live mice depending on the customer's needs. However, certain strains that are in high demand are maintained as breeding colonies to facilitate their rapid delivery. All animals supplied by EMMA are classified as SPF in accordance with the FELASA recommendations. EMMA is a founding member of FIMRe (International Federation of Mouse Resources) and actively cooperates with other leading repositories like The Jackson Laboratory and the MMRRC in the US and BRC RIKEN from Japan.

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Occult clonal expansion as a mechanism of intestinal tumor initiation and prevention. Jared Michael Fischer[1], Darryl Shibata[2], Michael Liskay[1]. 1) Molecular and Medical Genetics, Oregon Health and Science University, Portland, OR; 2) Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, CA.

The prevailing dogma of solid tumor progression is that cancer results from the stepwise accumulation of multiple mutations beginning within a single cell. However, determining how and when driver mutations occur is difficult when examining tumors

prospectively. We have utilized a novel mouse model that involves an out-of-frame Cre allele that sporadically becomes activated by frameshift reversion in single, isolated cells. Upon cre activation target genes are altered and marked by beta-galactosidase expression. Thus, by combining our mouse with floxed target genes and a Cre-reporter gene, we can follow the fate of mutant intestinal stem cells even in the absence of any visible phenotypic changes. Our previous studies demonstrated phenotypic plasticity following Apc loss in that, while adenoma formation could ensue, the majority of Apc-deficient intestinal crypts exhibited a normal phenotype. Interestingly, a significant fraction of the normal, Apc-deficient crypts exhibited a growth advantage raising the possibility of crypt fission as an important, albeit occult, intermediate in tumorigenesis. Here we show that mutations occurring during intestinal growth/development are more likely to spread via crypt fission resulting in a field of mutant crypts and hence an altered microenvironment, which is more conducive to transformation. In contrast, later occurring mutant crypts are more likely to remain isolated, resulting in a microenvironment that is not conducive to transformation. Specifically, we show that either early Apc loss or Kras activation in intestinal stem cells results in increased crypt fission, but inefficient transformation. Not surprisingly, the combination of early Apc loss and Kras activation is highly conducive to transformation. Unexpectedly, late Apc loss and Kras activation was inefficient at altering either crypt fission or transformation. Finally, our results show that the NSAID, sulindac, can act as a chemopreventive, not only through increasing apoptosis, but also by inhibiting the increased crypt fission associated with Apc loss. Based on these findings, we suggest that 1) isolated mutant crypts are not sufficient for tumorigenesis, whereas a field of Apc-deficient crypts creates a rich microenvironment that is highly conducive to transformation and 2) limiting Apc-deficient field size is a previously unappreciated mechanism of chemoprevention.

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Neural Tube Defects and Epigenetics of Gene Expression in Non-obese Diabetic (NOD) Mice. Claudia Kappen[1], Carly Oetker[2], Claudia Kruger[1], J. Michael Salbaum[2]. 1) Developmental Biology, Pennington Biomedical Research Center, Baton Rouge, LA; 2) Laboratory of Gene Regulation, Pennington Biomedical Research Center, Baton Rouge, LA.

Neural tube defects (NTDs) and heart defects are the most prominent congenital defects in pregnancies affected by maternal diabetes. Using a chemical induction model of diabetes by administration of Streptozotocin (STZ) to FVB mice, we found NTDs with a frequency of 5.6% in diabetic dams fed regular chow, while a diet specifically formulated for pregnancy elevated the rate of NTDs to 21.6%. These results indicated not only that NTDs occur with incomplete penetrance, but also that NTD frequency is epigenetically regulated. Genome-wide expression profiling demonstrated altered gene expression in diabetes-exposed embryos, which was also accompanied by an increase in variability of gene expression levels, and by altered chromatin marks. To investigate whether elevated variability of gene expression is a general epigenetic feature associated with maternal diabetes, we employed a model in which diabetes occurs spontaneously, the non-obese diabetic (NOD) strain of mice. We observed normal embryonic development in normoglycemic NOD dams, while in diabetic dams, 39% of the embryos had NTDs, confirming incomplete penetrance. Microarrays and next-generation sequencing revealed deregulation of gene expression in diabetes-exposed embryos at embryonic day 10.5, and at day 8.5, during neural tube closure. Similar to the STZ model, transcription and chromatin-binding factors were enriched among differentially expressed genes, again implicating epigenetic mechanisms. A global increase in variation of gene expression was again evident within the group of diabetes-exposed embryos. Intriguingly, while embryos from normal pregnancies were distinguishable from those of diabetic dams by virtue of their differential expression repertoires, the diabetes-exposed embryos with NTDs displayed distinct patterns only when clustering approaches were based on the characteristic of variability. We therefore developed a variation-based model that can explain the incomplete penetrance of the NTD phenotype in embryos of diabetic FVB and NOD mice, and by inference, the phenomenon of incomplete penetrance in inbred strains in general. This model provides a new paradigm for discovery of genes that are involved, causally or as modifiers, in the pathogenesis of neural tube defects and other pathologies.

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Impaired Maternal Cardiovascular Adaptation to Pregnancy in HcB-8 Mice. Jasmin Kristianto, Jacqueline Fisher, Shannon Phillips, Michael Johnson, Suzanne Litscher, Robert Blank. University of Wisconsin-Madison, Madison, WI.

Preeclampsia (~8% of all pregnancies), fetal loss (~15% of all pregnancies) and intrauterine growth restriction (IUGR) (~10% of newborns) are common pregnancy complications. Our laboratory has isolated a pleiotropic quantitative trait locus (QTL) in mouse chromosome 4 in recombinant congenic mice (HcB-8 and HcB-23) mice that harbors differentially expressed Ece1 (the gene encoding Endothelin Converting Enzyme 1) alleles resulting in differences in BP, litter size, birth weight, and frequency of fetal loss. Reduced Ece1 expression in HcB-8 male mice is associated with increase in blood pressure (BP), larger heart size, less compliant and smaller carotid arteries diameter than HcB-23. The non pregnant HcB-8 females also have higher BP than HcB-23 (116 7/90 4 v 103 3/75 3, p =0.016 (systolic)/p= 0.007 (diastolic)). Furthermore, HcB-8 litters are smaller than HcB-23 litters (4.4 + 1.8 v 5.8 + 2.0, p 10-3) and HcB-8 pups are lighter at birth (1.2 + 0.2 g v 1.5 + 0.2 g, p 10-20). We hypothesized that chromosome 4 QTL mediates vascular adaptation in response to pregnancy and that differential Ece1 expression manifests in differences in reproductive phenotypes. HcB-8 females experience higher rate of pregnancy loss (PL) (5 PL/7 dams v 1 PL/8 dams, p= 0.04) based on the finding of vaginal plugs and the failure to produce litter after successful copulation. Placental insufficiency may be the contributing factor. Pregnant HcB-8 females harvested at 17.5 dpc have smaller placental weight than HcB-23 17.5 dpc females (0.085 0.006 v 0.10 0.010, p =0.010). However, pregnant HcB-8 females have larger fetal to placental ratio than HcB-23 (9 1 v 6 0.5, p = 0.009). Pregnant HcB-8 females also have larger fractional heart sizes than HcB-23 (0.006 0.0007 v 0.004 0.0003, p=0.001). Non-pregnant HcB-8 females expressed nearly ~ 5 fold higher brain natriuretic peptide,

a marker for heart failure, at the mRNA level than non-pregnant HcB-23 (p0.001). The findings suggest that the impaired reproductive performance in HcB-8 mice reflects failure of maternal vascular adaptations to pregnancy. Preliminary data in congenic mice in which the QTL has been isolated are consistent with those obtained in HcB-8 and HcB-23.

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A mouse model of human Branchio-Oculo-Facial Syndrome. Hong Li, Trevor Williams. Department of Craniofacial Biology, University of Colorado Denver AMC, Aurora, CO.

Human Branchio-Oculo-Facial syndrome (BOFS) is a dominantly inherited birth defect, which has a range of craniofacial malformations including skull deformity, cleft lip, abnormal external ear morphology, malformed nasal tip, nasolacrimal duct obstruction, and small teeth. Associated problems are eye defects, hearing deficits, branchial clefts and melanocyte dysfunction. The phenotypes show variability. Recent data have indicated that this condition is caused by missense mutations in AP-2 (TFAP2A) that alter conserved amino acid residues, particularly within the DNA binding domain. One of the outstanding questions about human BOFS is whether the condition is caused by haploinsufficiency of AP-2, or if the mutant proteins have a dominant negative effect on the remaining wild-type allele. We have generated mouse models to distinguish between these possibilities. Mice carrying an AP-2 BOFS allele in association with a wild-type allele show more severe defects than mice lacking one copy of AP-2. These findings indicate that the BOFS alleles are working in a dominant negative manner, and the syndrome is not caused by simple haploinsufficiency. Phenotypes in the BOFS mice have many overlaps with those seen in affected humans, including facial clefting and defects in skin, eye, and ear morphology. The mouse model shows variable expressivity, similar to the variability seen in humans. Other phenotypes we observe, including neural tube and limb defects, may help inform the analysis of the affected human population. The phenotype of BOFS homozygous mice indicates that the mutations are acting more broadly than on AP-2 alone - possibly affecting the function of other AP-2 gene family members or co-activators. In conclusion, we have developed a mouse model of human BOFS. This model can be used to probe embryonic development and underling molecular mechanisms of BOFS.

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Heterogeneous behavioral manifestations in a mouse model of fetal alcohol spectrum disorders (FASD): Assessing the effect of gestational time and gene expression. Katarzyna Mantha, Morgan Kleiber, Benjamin Laufer, Shiva Singh. Western University, London, Canada.

Considerable attention has been given to the effect of prenatal alcohol abuse, resulting in well-known cognitive and behavioral deficits termed fetal alcohol spectrum disorders (FASD). Measures to prevent this disorder have been ineffective, primarily due to poor understanding. Towards an explanation of its complexity, we have used an animal model and numerous exposure paradigms to assess its impact on the heterogeneity of behavioral manifestations. The results are assessed in the context of underlying changes in the transcriptome. We have modeled acute alcohol exposure using ethanol injections in C57BL/6J mice on gestational days (G) 8 and 11 (trimester 1), G14 and 16 (trimester 2) and postnatal days (P) 4 and 7 (trimester 3). The resulting offspring are followed from birth until early adulthood using a battery of behavior tests. At maturity (P70), mice are sacrificed and whole brains are used for genome-wide expression analysis and assessment of epigenetic features using microarrays. The nature of genes affected by ethanol is considered using bioinformatic tools. Results of this study show that behavioral heterogeneity of FASD can be partly explained by timing of exposure. Ethanol treatment, independent of trimester, led to motor skill delays and spatial learning deficits (p0.05). Treatment in trimesters 1 and 2 increased activity levels in juveniles (trimester 1, ethanol=2835.6136.8, control=2332.1142.9 beam breaks; trimester 2, ethanol=3429.193.8, control=2291.598.6 beam breaks). Thigmotaxis was increased (p0.01) for trimester 3 ethanol mice (51.46.1 sec in centre) compared to controls (78.16.9 sec in centre). Analysis of the transcriptomes results in relevant pathways such as interleukin and cell signaling networks. We have validated many of the genes including *Tnfrsf19* (p=0.02) in trimester 1, *Cdkn1a* (p=0.02) in trimester 2 and *Cnr1* (p=0.004) in trimester 3. Our results argue that such effects are brought about by epigenetic changes that downregulate a set of imprinted non-coding RNAs. The genes affected explain most of the phenotypic consequences. These results represent a comprehensive comparison of behaviors representing a range of behavioral alterations in a mouse model of FASD. Further, FASD-related abnormalities are correlated with brain gene expression changes. We conclude that common epigenetic modifications play a critical role in regulating developmental pathways in specific brain regions, contributing to FASD.

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“Secreted protein coding” genes identified by mining the NOD mouse pancreatic lymph node (PLN) transcriptome, before any clinically detectable pancreatic damages. Evie Melanitou[1], Fredj Tekaia[2], Edouard Yeramian[3]. 1) Immunophysiology & Parasitism laboratory, Department of Parasitology and Mycology; 2) Yeast Molecular Genetics Unit, Department of Genomics & Genetics; 3) Structural Bioinformatics Unit, Department of Structural Biology & Chemistry, Institut Pasteur, Paris, France.

Statement of purpose: Our aim is to delineate and characterize the early clinically silent mechanisms operating at the onset of the auto-reactive processes resulting in overt Type 1 Diabetes (T1D). This could serve two purposes: i) identify early diagnostic markers and ii) generate data sets to refine the impact of the environment (in particular of hosting microbes) in either NOD mouse sterile tissues or in microbiota-containing tissues. **Methods used:** We used microarray analysis of the PLN of NOD mice (GEO series accession number GSE15582), selected for an early T1D subphenotype (E-Insulin AutoAntibodies) (Melanitou *et al*, 2004; Regnault *et al*, 2009). Novel data mining is presented according to in silico analyses with online tools for functional,

cellular, chromosomal assignment and SNP polymorphisms. To facilitate data visualization a multivariate statistical method (Correspondence Analysis) was applied. **Summary of results:** We identified in the PLN of young NOD mice, 71 differentially expressed transcripts coding for secreted proteins and regulated at the early preinflammatory stages of T1D. By using a multivariate statistical method we identified a clear-cut segregation of the individual samples according to the subphenotype used and the gene expression patterns, rendering these 71 transcripts a candidate-set of predictive biomarkers for T1D. As the majority of these genes have human orthologs, they represent potential candidate biomarkers for the human disease. Three chromosomes contain 47% of the identified genes delineating possibly expression variants hotspots influencing the genetics/epigenetics of T1D. Two chromosomal regions, showed high density of SNPs polymorphisms between the NOD and other non diabetic laboratory strains. Several Idd and human IDDM, diabetes susceptibility loci, lay nearby or within the chromosomal regions of the identified transcripts rendering these genes potential candidates for the corresponding genetic loci. **Conclusions**: i) Subphenotypic evaluation (E-IAA in our study) of complex genetic disorders allows identifying genes otherwise not detected by classical genetic analysis studies. ii) 71 potential early biomarkers for T1D are identified and constitute candidate genes for genetic loci in the NOD mouse and in human through their orthologs. iii) A genetical genomics image of these transcripts together with their functional annotations generate new hypothesis that can be experimentally tested via the identified genes.

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The Jackson Laboratory Repository: Resources for Addressing Human Disease. Darcy Pomerleau, S Rockwood, C Lutz, M Sasner, LR Donahue, The Repository Team. Genetic Resource Science, The Jackson Laboratory, Bar Harbor, ME.

The mouse continues to be the most utilitarian of model organisms, evidenced by the ever increasing number and variety of models generated. To ensure ready access of these research tools to the scientific community The Jackson Laboratory Repository serves as a centralized facility for the purpose of developing, rederiving, cryopreserving and distributing mouse models to the international biomedical research community. Many new strains are added each year to the thousands of unique mouse strains that comprise one of the largest collections of characterized mouse strains available. Models newly imported and/or developed for distribution have applications across multiple therapeutic areas; some selected sets include Spinal Muscular Atrophy (SMA), Parkinsons disease and Autism spectrum disorders. Numerous mutant lines offer a means for researchers to examine the regulation of immunologically-related pathways and their potential for developing translational approaches to human disease therapies. An essential infrastructural component to the Repository is a robust quality control program that surveys allele identity, genetic background and exclusion of unwanted alleles. A new initiative at the Jackson Laboratory is the Rare and Orphan Disease Center. The Center focuses on partnering with scientists, foundations, and other experts around the world to enable the development, standardization, optimization, and rapid distribution of preclinical models for drug discovery. Being able to offer the resources and expertise to enable the design, construction and management of preclinical mouse models of disease, in combination with a global delivery system, expertise in technical transfer issues and genetic quality control, uniquely positions the Center to rapidly put new tools into the hands of scientists for the purpose of accelerating drug discovery. An on-line resource (www.jax.org) allows researchers to retrieve information related to strains in the Repository. Donating a strain to the Repository fulfills the requirements for sharing of mice required by NIHs policy for the sharing of research reagents. Researchers wishing to have strains considered for inclusion in the Repository may use the submission form available at: http://www.jax.org/grc/index.html The Jackson Laboratory Repository is supported by the NIH, The Howard Hughes Medical Institute, Michael J. Fox Foundation for Parkinsons Research and other private charitable foundations.

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Infrafrontier - the European infrastructure for the phenotyping, archiving and distribution of model mammalian genomes. Michael Raess, Martin Hrabé de Angelis, the Infrafrontier Consortium. Inst Experimental Genetics, Helmholtz Zentrum Muenchen, Neuherberg, Germany.

Medically related life sciences use mouse models to understand the functional basis of human disease. However, the existing capacities for the systemic phenotyping, archiving and distribution of mouse models in Europe do not match the increasing demand by the community. Moreover, sustainable funding solutions are not always in place. These issues are being addressed by **Infrafrontier**, which is part of the Roadmap of the European Strategy Forum on Research Infrastructures (ESFRI) since the year 2006:

1) **Capacity building:** Infrafrontier provides access to scientific platforms and services for individual research projects (bottom-up) and for large-scale top-down programs such as the International Mouse Phenotyping Consortium (IMPC);
2) **Securing sustainable funding** for the mouse productions centres, mouse repositories and primary phenotyping centres that contribute to the Infrafrontier Research Infrastructure, both through national funding and the funding instruments for research infrastructures within Horizon 2020;
3) **Providing a single point-of entry** for the users of the scientific platforms and services offered by the Infrafrontier Research Infrastructure; underpinned by common quality standards and operation procedures, pan-European capacity and risk management, and user training.

The Infrafrontier consortium consists of 29 partners, representing the leading mouse clinics, and the major archiving and distribution nodes organized in the European Mouse Mutant Archive (**EMMA**), as well as the related ministries and major funding bodies from 12 European countries and Canada. The Infrafrontier Research Infrastructure will shape the European

Research Area in the field of biomedical research and contribute to our understanding of the role of gene function in human health and disease.

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Disruption of a novel mouse gene, *Jhy*, causes juvenile communicating hydrocephalus. Oliver K. Appelbe[1], Bryan Bollman[1], Ali Attarwala[1], Lindy A. Triebes[1], Daniel J. Curry[2], **Jennifer V. Schmidt**[1*]. 1) Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL; 2) Department of Neurosurgery, Baylor College of Medicine, Houston, TX.

Juvenile hydrocephalus, the accumulation of cerebrospinal fluid (CSF) in the ventricles of the brain, affects 1 in 500 newborns, causing significant morbidity among human children. Hydrocephalus may result from overproduction, decreased absorption, or restricted flow of CSF. Few genetic causes of this disease are known in humans, and animal models can therefore aid in identifying candidate genes and studying relevant pathways. The Juvenile hydrocephalus (*Jhy*) mouse line carries a transgenic insertional mutation on chromosome 9 that disrupts a previously unstudied gene. The integrated *lacZ* reporter gene is expressed in the pineal gland, hypothalamus, ventricular ependyma and choroid plexus. The mutation appears to act as a loss of function, with homozygous *Jhy* mice exhibiting externally detectable hydrocephalus by two weeks of age and few animals surviving beyond six weeks. Histological analysis of *Jhy* mice shows a patent aqueduct, indicating communicating hydrocephalus, with no overt brain morphological changes. Scanning electron microscopy found reduced density and loss of orientation of ventricular ependymal cilia, which suggests loss of ciliary-mediated CSF flow. Current research is directed at characterizing the ciliary phenotype of *Jhy* mice, as well as determining the normal function of the *Jhy* gene.

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Role of miR-155 in BRCA1-mediated tumorigenesis. Suhwan Chang, **Shyam K. Sharan**. Mouse Cancer Genetics Program, Frederick National Lab. NCI, Frederick, MD. USA.

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. We have developed a mouse embryonic stem cell based assay to examine the functional consequences of variants identified in these genes. The assay is based on the ability of human BRCA1 or BRCA2 to complement the loss of endogenous genes in mouse embryonic stem cells. 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 a novel role for BRCA1 in regulation of an oncogenic microRNA, miR-155. Our studies revealed a strong correlation between BRCA1 deficiency and miR-155 up-regulation in murine as well as human BRCA1-deficient tumors. We also found that over-expression of miR-155 augmented tumor growth whereas the knockdown of miR-155 attenuated the growth of BRCA1-deficient tumor cell lines in mice. These findings suggest that mir-155 plays an oncogenic role in BRCA1-mediated mammary tumorigenesis. To test this, we generated a cohort of Brca1-mutant mice that are deficient in miR-155. Mice lacking miR-155 are viable and fertile but have severe defects in the T cells, B cells and dendritic cell function. To examine the effect of mir-155 loss on Brca1-mediated mammary tumorigenesis, we have characterized Brca1; Trp53 conditional knockout mice with a K14-Cre transgene to delete the conditional allele in the epithelial cells. These mice have been shown to develop mammary tumors that are histopathologically similar to human BRCA1-mutant breast tumors. Surprisingly, loss of miR-155 did not reveal any effect on the tumor latency or tumor free survival of the mouse. We are now examining why knockdown of miR-155 in tumor cells resulted in suppression of tumor growth in an allograft model but germline loss of miR-155 function has no effect on spontaneous mammary tumorigenesis. We will describe our findings that suggest a role for miR-155 in the tumor microenvironment. These findings can have important implications on the use of anti-miR-155 agents for cancer therapy.

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Conditional deletion of bA3/A1-crystallin in RPE shows decreased lysosomal activity and drusen-like deposits in mouse eyes. **Debasish Sinha**. Ophthalmology, The Johns Hopkins University School of Medicine, Baltimore, MD.

The Retinal Pigmented Epithelium (RPE) serves many physiological roles that are crucial for maintaining homeostasis of the retina. The RPE is one of the most active phagocytic cell types in the body, phagocytosing 10% of total photoreceptor volume daily. Autophagy, a process by which cellular constituents are degraded and recycled as part of normal cellular remodeling, is likely to be of particular importance in post-mitotic cells with high metabolic demand, such as the RPE. Therefore, proper functioning of the RPE requires that both phagocytosis and autophagy be in balance, as perturbation of either process can lead to some manifestations of age-related macular degeneration (AMD). AMD is the leading cause of legal blindness in developed countries. We recently reported that A3/A1-crystallin, a lens structural protein, is expressed in RPE cells and trafficked to lysosomes, where it is involved in degradation of ingested photoreceptor outer segments (OS) and also in autophagy. We have recently generated a conditional knockout (cKO) mouse whereA3/A1-crystallin has been deleted from the RPE. The expression levels of proteins known to be involved in movement of autophagosomes along microtubules for fusion with lysosomes remain unchanged. However, autophagosome-lysosome fusion studies, in which RPE cells were labeled with mCherry-LC3II (autophagosome) and LAMP1-YFP (lysosome) suggest a possible defect in the fusion process in cells lacking functional A3/A1-crystallin. Moreover, Rab7 GTPase, Rab-interacting lysosomal protein, and lysosome-associated membrane proteins 1 and 2, are decreased in A3/A1-crystallin deficient RPE cells. Our data also show that the activity of Cathepsin D is decreased in RPE cells that lack functional A3/A1-crystallin and have drusen-like deposits. Lysosomal dysfunction is assumed to play an essential role in the formation of drusen, which trigger neovascular and atrophic reactions in AMD. Autophagy and phagocytosis may be

interconnected, particularly since the latter stages of both pathways require fusion with lysosomes. It is tempting to speculate that perturbation of normal phagocytosis and/or autophagy may be a factor in AMD, because A3/A1-crystallin has been reported to be present in human drusen, and a possible role of lysosomes in AMD has recently been suggested.

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Conditional-invertible genetic strategy to understand the role of NIPBL deficiency in the etiology of developmental defects in Cornelia de Lange Syndrome. Rosaysela Santos[1,3], **Xu Wang**[1,3], Shimako Kawauchi[1,3], Russell Jacobs[4], Martha Lopez-Burks[2,3], Akihiko Muto[2,3], Mona Yazdi[1], Salvador Deniz[1], Samir Qurashi[1], Scott Fraser[4], Thomas Schilling[2,3], Arthur Lander[2,3], Anne Calof[1,2,3]. 1) Dept. of Anatomy & Neurobiology, University of California, Irvine, Irvine, CA; 2) Dept. of Developmental & Cell Biology, University of California, Irvine, Irvine, CA; 3) Center for Complex Biological System, University of California, Irvine, Irvine, CA; 4) Beckman Insititute, California Institute of Technology, Pasadena, CA.

Cornelia de Lange Syndrome (CdLS) is a multi-systems birth defects disorder caused, in most cases, by haploinsufficiency for Nipped-B-like (NIPBL), a highly-conserved protein with roles in cohesin loading and transcriptional regulation. Data from multiple systems suggest that developmental defects in CdLS result from the collective action of many otherwise innocuous, small changes in gene expression. To investigate these developmental defects, we have developed mouse and zebrafish models of Nipbl deficiency (Kawauchi et al., 2009; Muto et al., 2011). Both Nipbl+/- mice and nipbl morphants exhibit a spectrum of developmental defects with strong parallels to those observed in human CdLS, and initial studies indicate that many structural defects - such as heart defects - originate during early stages of development. Nipbl-deficient embryos in both systems also show alterations in expression of genes with known roles in L-R patterning and other aspects of heart development. To understand the stage(s) and tissue(s) in which Nipbl deficiency is critical for development of heart defects in CdLS, we have used the EUCE313f02 ES cell line, containing a FLEX (conditional-invertible) gene-trap vector in intron 1 of Nipbl, to develop a line of NipblFLEX/+ mice. We will discuss data showing that this allele can be toggled successfully between mutant and wildtype conformations; and that NipblFLEX/+ mice exhibit cardiac defects at a frequency similar to Nipbl+/- mice. Currently our studies are focused on analyzing hearts of mouse embryos in which Nipbl has been inactivated (or rescued) in cardiogenic mesoderm and/or endoderm, using 3D magnetic resonance microscopy (MRM) to analyze heart morphology. Results of these studies, and their implications for understanding the origins of structural birth defects in CdLS, will be discussed. Supported by NIH grant P01-HD052860.

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Production and Characterization of Transgenic Mice Systemically Expressing Endo-b-galactosidase. Satoshi Watanabe[1], Takayuki Sakurai[2], Masako Misawa[3], Takashi Matsuzaki[3], Takashi Muramatsu[4], Masahiro Sato[5]. 1) Animal Genome Reseach Unit, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan; 2) Department of Cardiovascular Research Graduate school of Medicine, Shinshu University, Matsumoto, Nagano, Japan; 3) Department of Biological Science, Faculty of life and Environmental Science, Shimane University, Matsue, Shimane, Japan; 4) Department of Health Science, Faculty of Psychological and Physical Sciences, Aichi Gakuin University, Nisshin, Aich, Japan; 5) Frontier Science Research Center, Kagoshima University, Kagoshima, Japan.

TheGal epitope (Gal1-3Gal) is a sugar structure expressed on the cell surface of almost all organisms except humans and old-world-monkeys, which express natural anti-Gal antibodies. The presence of these antibodies elicits a hyper acute rejection (HAR) upon xenotransplantation of cellular materials, such as from pigs to human beings. Endo--galactosidase C (EndoGalC), an enzyme isolated from Clostridium perfringens, removes the Gal epitope by cleaving the Gal1-4GlcNAc linkage in the Gal1-3Gal1-4GlcNAc sequence. To explore the possibility that cells or organs from transgenic pigs systemically expressing EndoGalC might be suitable for xenotransplantation, we first introduced the EndoGalC transgene into the mouse genome via pronuclear injection. The progeny of the resulting transgenics expressed EndoGalC mRNA and protein. Flow cytometry and histochemical analyses revealed a dramatic reduction in the expression of the Gal epitope in these mice. They also exhibited abnormal phenotypes such as occasional death immediately after birth, growth retardation and transient skin lesions. Interestingly, the phenotypic abnormalities seen in these transgenics were similar to those observed in 1,4-galactosyltransferase 1 (4GalT-1) knockout mice. Most probably, these phenotypes were caused by exposure of the internal N-acetylglucosamine residue at the end of the sugar chain on the cell surface. The present findings also provide some basis for evaluating possible application of the transgenic approach for xenotranplantation.

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Studies of cell behaviors regulated by HH-GLI2 signaling during medulloblastomas tumor progression. Alexandre Wojcinski, Alexandra Joyner. developmental biology, Memorial Sloan-Kettering Cancer Center, new york, NY.

The quality of life for survivors of Medulloblastoma (MB), the most common developmental brain tumor, is greatly compromised due to treatment. MBs are divided into subtypes based on expression of key developmental genes. Tumors arise from at least two embryonic cell types (cells of origin), and hedgehog (HH) signaling is elevated in ~25% of tumors that arise from cerebellar granule cell progenitors (GCPs). Sonic HH (SHH) functions through GLI2 to stimulate expansion of GCPs in mice (Corrales, 2006). As inhibition of HH signaling can not be used to treat children due to severe developmental defects, a more detailed understanding of the GCP behaviors and critical genes regulated by HH signaling are needed to improve survival of MB patients. Several mouse genetic models of the HH subtype of MB have been generated and have been critical tools for identifying the cell of origin of MBs. However, the induction of mutations in most GCPs in the highly penetrant murine models

does not model sporadic tumors and the relative contribution of cells of the tumor and microenvironment has not been well characterized. We found that expression of a constitutively active form of SMO (SmoM2) in many GCPs at Postnatal (P) day 2 leads to rapid tumor formation before 2 months. Interestingly, while at P8 GCPs throughout the External Granule cell Layer (EGL) exhibited over-proliferation, all tumors formed only in the posterior part of the cerebellum. In addition, the tumors had different cytoarchitectures depending on whether they were located in the hemispheres or the vermis (lateral or medial cerebellum), suggesting that there are regional differences within the cerebellum with respect to susceptibility to tumor formation and progression. In order to address the cellular and molecular basis of these observations we are using a new technique called Mosaic Analysis with Spatial and Temporal control of Recombination (MASTR; Lao, 2012) to alter GLI2 and SMO function in rare GFP marked GCPs and study the impact on GCP behaviors. In addition, sporadic tumors will be produced in different areas of the cerebellum for molecular analysis of GFP+ tumor cells. Our studies should provide new insights into the cellular and genetic mechanisms underlying tumor progression and heterogeneity and to the identification of novel genes (drug targets) to devise new therapies for HH subtype MBs.

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Pancreas-specific Deletion of mouse Gata4 and Gata6 Causes Pancreatic Agenesis. Shouhong Xuan[1], Matthew Borok[1], Kimberly Decker[2], Michele Battle[3], Stephen Duncan[3], Michael Hale[4], Raymond MacDonald[4], Lori Sussel[1]. 1) Department of Genetics & Development, Columbia University, New York, NY 10032; 2) Department of Biochemistry, University of Colorado Medical School, Aurora, CO 80045, USA; 3) Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226, USA; 4) Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, Texas 75390, USA.

Pancreatic agenesis is a human disorder caused by defects in pancreas development. To date, only a few genes have been linked to pancreatic agenesis in humans, with mutations in PDX1 and PTF1A reported in only five families with described cases. Recently, mutations in GATA6 have been identified in a large percentage of human cases and a GATA4 mutant allele has been implicated in a single case. In the mouse, Gata4 and Gata6 are expressed in several endoderm-derived tissues, including the pancreas. To analyze the functions of Gata4 and/or Gata6 during mouse pancreatic development, we generated pancreas-specific deletions of Gata4 and Gata6. Surprisingly, loss of either Gata4 or Gata6 in the pancreas results in only mild pancreatic defects that are resolved postnatally. However, simultaneous deletion of both Gata4 and Gata6 in the pancreas causes severe pancreatic agenesis due to the disruption of pancreatic progenitor cell proliferation, defects in branching morphogenesis, and a subsequent failure to induce the differentiation of progenitor cells expressing Cpa1 and Neurog3. These studies address the conserved and non-conserved mechanisms underlying Gata4 and Gata6 function during pancreas development and provide a new mouse model to characterize the underlying developmental defects associated with pancreatic agenesis. We are currently dissecting out the downstream pathways regulated by Gata factors that mediate pancreatic progenitor cell specification.

Organogenesis

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Essential role of the Runx2-Cbfb transcription complex for endochondral bone formation. Na-Rae Park[1], Kyung-Eun Lim[1], Jeong-Eun Kim[2], Ichiro Taniuchi[3], Suk-Chul Bae[4], Jae-Hwan Jeong[1], **Je-Yong Choi**[1]. 1) Biochemistry and Cell Biology, Kyungpook National University, Daegu; 2) Molecular Medicine, Kypook National University, Daegu; 3) Laboratory for Transcriptional Regulation, RIKEN Research Center for Allergy and Immunology, Kanagawa; 4) Biochemistry, Chungbuk National University, Cheongju, Republic of Korea.

Core binding factor (Cbf), a partner protein of Runx family transcription factor, has not been determined its function in cartilage during endochondral bone formation. To explore in vivo function of Cbf in cartilage, we generated cartilage-specifically deleted Cbf mice ($Cbf^{ca/ca}$) crossing with the type II collagen promoter driven Cre mice. $Cbf^{ca/+}$ mice showed no difference in longevity and skeletal tissues compared with wild type mice. $Cbf^{ca/ca}$ mice resulted in delayed primary ossification center formation, shorter appendicular skeletons, and immediately death after birth. This phenotype resembled that of Smad1 and Smad5 double deficient mice. BMP signaling was compromised in Cbf-deficient chondrocytes as evidenced by reduced expression of BMP target genes like Runx2, Osterix, Osteopontin, and BMP2 as well as reduced phosphorylation of Smad1/5/8. Deficiency of Cbf in chondrocytes caused rapid proteosomal degradation of Runx2 and reduced chondrocyte maturation with almost disappearance of hypertrophic chondrocytes. Rescue of Cbf in Cbf-deficient chondrocytes increased Runx2 expression. Endogenous Runx2-Cbf complex formation and its functional significance provided the first *in vivo* evidence of the essential role of Cbf during early skeletogenesis. Collectively, these results indicate that Cbf is required for Runx2 stability as a partner protein in cartilage and the Cbf-Runx2 complex formation plays an important role for endochondral bone formation.

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Dual embryonic origin of the mouse inner ear. Laina Freyer[1], Vimla Aggarwal[2], Bernice Morrow[1]. 1) Genetics, Einstein College of Medicine, Bronx, NY; 2) Pediatrics, Columbia University Medical Center, New York, NY.

The inner ear and cochleovestibular ganglion (CVG) derive from a specialized region of head ectoderm termed the otic placode. During embryogenesis, the otic placode invaginates into the head to form the otic vesicle (OV), the primordium of the inner ear and CVG. To date, it is widely accepted that the otic placode ectoderm is the only source for the inner ear labyrinth and neurons of the CVG. Using *Wnt1-Cre*, *Pax3*$^{Cre/+}$ and *Hoxb1*$^{Cre/+}$ mice to label and fate map cranial neuroepithelial cells (NECs), including neural crest cells (NCCs), we show that their cellular derivatives contribute directly to the OV from the neural tube. NEC derivatives constitute a significant population of the OV and moreover are regionalized specifically to proneurosensory domains. Descendents of *Pax3*$^{Cre/+}$ and *Wnt1-Cre* labeled cells are localized within sensory epithelia of the saccule, utricle, and cochlea throughout development and into adulthood where they differentiate into hair cells and supporting cells. Some NEC derivatives give rise to neuroblasts in the OV and CVG in addition to their known contribution to glial cells. This finding defines a dual cellular origin of the inner ear from sensory placode ectoderm and NECs and changes the current paradigm of inner ear neurosensory development. Our current goals are to identify signaling pathways that regulate the contribution and localization of NEC derivatives in the inner ear. We also seek to understand the functional significance of this dual embryonic origin. To further investigate, we are performing FACS and comparative gene expression analysis of NEC- versus placode-derived populations. We are also utilizing the putkflox allele to induce conditional cellular ablation of NEC derivatives in a manner that we hope will bypass the requirement for non-autonomous cell signaling from the hindbrain to the OV.

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Identification of novel mouse testis-determining genes in the MAP3K4 pathway. Nick Warr, Gwenn Carre, Pam Siggers, Rachel Brixey, Madeleine Pope, Sara Wells, **Andy Greenfield**. Mammalian Genetics Unit, Harwell, Oxfordshire, United Kingdom.

The mammalian testis develops from an initially bipotential primordium when the Y-linked SRY gene is activated in gonadal somatic cells during a critical period. The MAPK signalling cascade functions in human testis determination (1) and we have previously reported male-to-female gonadal sex reversal in mice lacking the kinase MAP3K4 (2). Map3k4-deficient mutant gonads are characterised by dramatic loss of the key testis-determining gene Sox9, caused by reduced Sry expression. However, the widespread expression of MAP3K4 meant that determining the stage- and cell-type dependency of MAP3K4 function in the developing gonad was difficult. In an attempt to identify novel regulators of MAPK signalling in testis development, and thereby shed light on the spatiotemporal specificity of this pathway, we screened genes encoding MAP3K4-interacting proteins for expression in the developing mouse gonad. One of these genes, encoding a protein associated with the cellular stress response, exhibited strong expression in the gonadal soma in a spatiotemporal profile reminiscent of Sry itself. We show that knockout mice lacking this gene exhibit fully penetrant XY gonadal sex reversal on a C57BL/6J genetic background. We will describe the molecular genetic analysis of this novel sex-reversing mutant, including genetic interaction with Map3k4 and biochemical data suggesting that the disrupted gene acts to regulate MAPK signalling required for normal Sry expression in the developing XY gonad. In addition, we will present novel genetic data identifying a MAP3K4 target protein required for testis determination. (1) Pearlman et al (2010) Am.J.Hum.Genet. 87:898-904 (2) Bogani et al (2009) PLoS Biology e1000196.

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Core mesodermal functions of Tbx1 in branchiomeric muscle formation. Ping Kong[1], **Stephania Macchiarulo**[1], Courtney Carpenter[2], Tingwei Guo[1], Silvia Racedo[1], Steven Ola[1], Vimla Aggarwal[1], Deyou Zheng[1,3], Bernice Morrow[1]. 1) Genetics, Einstein College of Medicine, 1301 Morris Park Avenue, Bronx, NY 10461, USA; 2) Department of Surgery, Montefiore Medical Center, 111 East 210th Street, Bronx, NY 10467, USA; 3) Department of Neurology and Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA.

Haploinsufficiency of TBX1, a T-box transcription factor, is believed to be responsible for most of the physical defects in patients with Velo-cardio-facial syndrome/DiGeorge syndrome/22q11.2 deletion syndrome (22q11DS), including craniofacial, which is of interest to us in this project. These craniofacial anomalies include velo-pharyngeal insufficiency, facial muscle hypotonia and feeding difficulties, in part due to hypoplasia of branchiomeric muscles. Inactivation of both alleles of mouse Tbx1 results in overt cleft palate and reduction or loss of branchiomeric muscles, suggesting loss of tissue at some point during embryogenesis. To determine the precise developmental stage when Tbx1 is required and this tissue is lost, we performed lineage tracing using both pan-mesodermal drivers, Mesp1-Cre and T-Cre, in combination. We found that the core mesoderm cluster within MdPA1, which is present at E9.5, is missing by E10.5 in Tbx1-/- embryos, suggesting that cells are lost earlier than previously thought. Additionally, we performed gene profiling in Tbx1+/+ and Tbx1-/- mouse embryos and identified genes that function downstream of Tbx1 in MdPA1 at these two critical time points, including Lhx2, Chrdl1 and Lrrn1, which were all downregulated in null mutants. Based upon these results, we also sought to answer whether Tbx1 was required tissue-specifically within the core mesoderm for branchiomeric muscle formation. To do this we inactivated Tbx1 with Mesp1-Cre and T-Cre together because neither one alone resulted in complete Tbx1 inactivation. We found that Tbx1 is in fact required in the core mesoderm for proper branchiomeric muscle development.

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Additional sex com-like 1 is essential for normal alveolar development in mice. Seung-Tae Moon, Myengmo Kang, Soo-Jong Um. Department of Bioscience & Biotechnology, Institute of Bioscience, BK21 Graduate Program, Sejong university, Seoul, South Korea.

Retinoic acid (RA) plays a pivotal role in during mouse development and organ morphogenesis. Additional sex com-like 1

(Asxl1) is indentified as an interacting protein of RA receptor (RAR) and functions as either activator or repressor in a cell type-specific manner. However, the Asxl1 function in vivo remains largely unclear. To determine the physiological function of Asxl1, we generated Asxl1 heterozygous mice from gene-trap Asxl1 ES cell line. Heterozygotes were viable and fertile. However, most homozygous Asxl1-null embryos showed severe growth retardation and partial lethality. Even some Asxl1-/- mice were born, all died shortly after birth with cyanosis, reduced alveolus in the lungs, and respiratory failure. To investigate the cause of neonatal death, we isolated lung from Asxl1-/- mice and analyzed using physical and histological methods. Asxl1-null lung, compared to wild-type lung, was floating in PBS. Further histological analysis revealed that Asxl1-/- mice exhibited markedly reduced alveolar space and thickened alveolar walls associated with undifferentiated alveolar epithelia type 1 cells. Overall, our data suggest that the Asxl1 is essential for the differentiation of alveolar epithelial cells in mouse lung, which may be critical for air breathing at birth.

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The Role of BMPs in Regulating Digit Number and Identity. Jacqueline L Norrie[1], Qiang Li[1], Cortney Bouldin[2], Brian Harfe[2], Steven A Vokes[1]. 1) The Section for Molecular, Cellular, and Developmental Biology, The University of Texas at Austin, Austin, TX; 2) The Department of Molecular Genetics and Microbiology, The University of Florida, Gainesville, Florida.

Bone morphogenetic proteins (BMPs) are essential for bone and cartilage formation. In the vertebrate limb there are 3 partially redundant BMPs, making it difficult to define their roles in the regulation of limb development. Collective studies suggest that BMPs play key roles in two phases of limb development. Early in limb development BMPs negatively regulate digit number, resulting in a pentedactylous limb, and at later timepoints BMPs have been proposed to regulate digit identity. We generated a mouse model system containing an inducible BMP inhibitor, Gremlin, which we have used to test the role of BMP regulated processes in the limb. Here we show that in early limb development BMPs negatively digit number. By inhibiting BMPs at various timepoints we detect a spectrum of polydactylies. In contrast to the prevailing model, our preliminary data suggests that BMPs inhibition at later timepoints does not lead to a change in digit identity.

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The influence of endothelial expression of an Apert syndrome mutation on craniofacial bone development. Christopher Percival[1], Kazuhiko Kawasaki[1], Talia Pankratz[1], Ethylin Jabs[2], Kenneth Weiss[1], Joan Richtsmeier[1]. 1) Anthropology Dept, Penn State University, University Park, PA; 2) Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY.

A Pro253Arg missense mutation of fibroblast growth factor receptor 2 (FGFR2) is associated with 35% of Apert syndrome cases. Mice with this mutation display craniofacial dysmorphology similar to affected humans; including coronal suture fusion, midfacial hypoplasia, and abnormal cranial vault shape. Because tissue interactions are critical for craniofacial development and FGFR2 is highly pleiotropic, pinpointing the developmental pathways modified to produce this craniofacial dysmorphology is difficult. Given that Fgfr2 can promote angiogenesis, which is critical for bone development, we crossed Fgfr2(+/P253R) mice with Tek-cre mice to identify the influence of the P253R mutation when expressed only by endothelial cells. Tek-cre(+/-); Fgfr2(+/P253R) mice and their littermate controls were sacrificed at P0, P2, and P8. Landmark based morphometric and individual bone volume based analyses were completed on high resolution computed tomography images of the heads of these mice in order to quantify cranial bone shape, size, and relative density. Comparisons indicated that Tek-cre(+/-); Fgfr2(+/P253R) mouse skulls are reduced in overall scale at P0 and P8 with more serious reductions in length and height of the face, vault, and palate. However, these mice lack the midfacial hypoplasia, coronal craniosynostosis, and extreme rounded vault form of the Fgfr2+/P253R Apert syndrome mice. Although mean volumes of individual craniofacial bones are generally smaller for the Tek-cre(+/-); Fgfr2(+/P253R) mice than their littermates, these differences are not significant in two-sample Wilcoxon-tests. Mean relative densities of individual bones of these mice are slightly lower for some bones at P8, although there is substantial overlap with the littermates. The results of this study suggest that FGFR2 expression in endothelial cells plays a role in regulating craniofacial bone dimensions and that expression of the Fgfr2 P253R mutation in endothelial cells contributes to the abnormal cranial dimensions associated with Apert syndrome. This work was supported in part by a grant from the NSF to CJP (BCS-1061554) and from NIDCR, American Recovery and Reinvestment Act (R01DE018500; 3R01DE018500-02S1) to JTR.

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The Planar Cell Polarity Pathway is required in the Second Heart Field lineage for Outflow Tract Morphogenesis. Tanvi Sinha, Bing Wang, Jianbo Wang. Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham, Birmingham, AL.

Outflow tract (OFT) malformations underlie a majority of human congenital heart defects. The OFT arises in part from the Second Heart Field (SHF) progenitors in the pharyngeal and splanchnic mesoderm (SpM), outside of the initial heart. While SHF proliferation and differentiation have been extensively studied; how these progenitors are deployed to the OFT remains unclear. Using a set of mouse *Dishevelled2 (Dvl2)* alleles, we demonstrate that the planar cell polarity (PCP) pathway, a branch of the -catenin independent non-canonical Wnt pathway that regulates cellular polarity and polarized cell behavior during tissue morphogenesis, is required specifically in the SHF for early OFT morphogenesis and may play a key role in SHF deployment. We find that mutations in mouse core PCP genes *Dvl1/2* and *Vangl2* as well as non-canonical Wnt gene *Wnt5a* result in aberrant cardiac looping. The looping defect is correlated with severe OFT shortening at embryonic day 9.5, characteristic of comprised contribution from the SHF. Consistent with our genetic interaction studies suggesting that Wnt5a signals through the PCP

pathway, *Dvl1/2* and *Wnt5a* mutants display aberrant cell packing along with diminished actin polymerization and filopodia formation in SHF progenitors in the caudal SpM, where Wnt5a and Dvl2 are co-expressed specifically. Based on these results, we propose a novel model in which a Wnt5aDvl PCP signaling cascade promotes SHF deployment by promoting actin polymerization and protrusive cell behavior to continuously recruit SHF progenitors into a cohesive sheet in the caudal SpM, thereby pushing the SpM rostrally into the OFT.

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Transcriptional Mechanisms Underlying Sonic Hedgehog Mediated Regulation. Qiang Li, Jordan P. Lewandowski, Marian Powell, **Steven A Vokes**. Molecular Cell & Developmental Biology, University of Texas at Austin, Austin, TX.

The Sonic hedgehog (Shh) pathway plays critical roles in development and cancer. All Shh activity is mediated by Gli proteins, which act as context-dependent transcriptional activators or repressors in the presence or absence of Shh pathway stimulation. According to current models, the ratio of Gli-activator to repressor forms gives rise to a quantitative transcriptional output. While several studies have persuasively demonstrated a quantitative role for Gli-activator activity, the role of Gli-repressors remains poorly understood. We identified a long-range Gli binding site in the Gremlin locus that has Gli-dependent enhancer activity in transgenic embryos, and exhibits a spatiotemporal pattern consistent with control by Shh. To determine how this domain is regulated by Gli activator and repressor forms, we examined its activity in a number of different Shh pathway mutants. Our results suggest that the enhancer domain requires Gli-activator activity but is not repressed anteriorly by Gli-repressor forms. In light of these results, we were surprised to find that Gli repressor binding to the enhancer does play a direct role in preventing ectopic Gremlin transcription by repressing activity from other cis-regulatory domains. Together, our results suggest a model for Shh-directed transcription where the Gli-bound cis-regulatory domain acts a toggle switch to impose Gli-dependent control over genes with multiple cis-regulatory domains.

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Engrailed genes regulate regional cerebellum growth by modulation of hedgehog signaling. Ryan Willett, Alexandra Joyner. Developmental Biology, Memorial Sloan-Kettering Cancer Center, New York, NY.

The cerebellum (Cb) is a foliated posterior brain structure involved in coordination of motor movements, motor learning, balance, and cognition. Although morphologically and functionally complex, the simple layered cytoarchitecture of the cortex makes the Cb an ideal model for studying neurodevelopmental processes. During the first 2 postnatal weeks, the Cb undergoes rapid growth driven by proliferation of granule cell precursors (GCPs) in the external granule cell layer (EGL). GCPs then differentiate into granule cell (GC) neurons and migrate to form the inner granule cell layer (IGL). The engrailed family of homeobox transcription factors (referred to collectively as EN1/2), are key regulators of mouse cerebellum development including regionalization, patterning, morphogenesis, growth, and assembly of neural circuitry. En1 null mutants do not form a midbrain or Cb, whereas loss of En2 results in a small Cb with altered foliation. We recently found that conditional deletion of En1 and En2 in GCPs (Atoh1-Cre/+; En1fx/fx; En2fx/fx or RLd-Cre; En1/2) results in a Cb with pronounced hypoplasia but normal cytoarchitecture and only a mild foliation defect (Orvis, 2012). Analysis of cell death in these mutants has revealed a cell non-autonomous decrease in survival and delay in maturation of Purkinje cells (PCs). Since SHH, produced by PCs, is a major mitogen for GCPs we examined whether this pathway is altered during postnatal Cb growth in RLd-Cre; En1/2 mutants. Interestingly, we found that En1/2-deficient EGL cells exhibit altered SHH signaling, which had cell autonomous and non-autonomous components. Gli1 expression, a readout of SHH pathway activation, was elevated in the EGL of the anterior zone of the Cb but markedly reduced throughout the central zone in the mutant Cb. Interestingly, central zone morphology was most affected in the RLd-Cre; En1/2 Cb as the appearance of three fissures that surround the folia was delayed and the ultimate depth was reduced in the region of reduced Gli1 expression. Strikingly, GCPs isolated from RLd-Cre; En1/2 Cb showed a Smo-dependent enhancement of proliferation in the absence of SHH despite a normal proliferative response to recombinant SHH in culture. This result suggests that En1/2-deficient GCPs are sensitized to a trace amount of SHH in the medium or have SHH-independent growth activity. Our study indicates that EN1/2 function in RL-derived cells to coordinate SHH pathway activity during Cb growth.

Patterning

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An Lmx1b-miR135a2 Regulatory Circuit Modulates Wnt1/Wnt Signaling and Determines the Boundaries of the Midbrain Dopaminergic Progenitor Pool. Angela Anderegg[1], Hsin Pin Lin[1], Jun An Chen[2], Natalya Cherepanova[1], Milan Joksimovic[1], Randy Johnson[3], Jason Rock[4], Brian Harfe[4], **Raj Awatramani**[1]. 1) Northwestern University, Chicago, IL; 2) Academia Sinica, Taipei, Taiwan; 3) MD Anderson, Houston, TX; 4) U of Florida, Gainsville, FL.

MicroRNAs function to regulate gene expression in diverse physiological scenarios. Their role in the control of morphogen related signaling pathways has been less studied, particularly in the context of embryonic CNS development. Here, we uncover a role for microRNAs in limiting the spatiotemporal range of morphogen function. Wnt1 is a key morphogen in the embryonic

midbrain, and directs proliferation, survival, patterning and neurogenesis. We reveal an autoregulatory negative feedback loop between the transcription factor Lmx1b and a newly characterized microRNA, miR135a2, that controls the extent of Wnt1/Wnt signaling. Conditional gain of function studies reveal that Lmx1b promotes Wnt1/Wnt signaling, and thereby increases midbrain size and increases dopamine progenitor allocation. Conversely, conditional removal of Lmx1b has the opposite effect. Next, we provide evidence that microRNAs are involved in restricting dopamine progenitor allocation. Conditional loss of Dicer1 in ES cells results in expanded Lmx1a/b+ progenitors. In contrast, forced elevation of microRNA135a2 during an early window in vivo phenocopies the Lmx1b conditional knockout, in that the proportion of Lmx1a/b+ progenitors is selectively reduced. Midbrain dimensions are also reduced in these mutants. We demonstrate that this mutant displays reductions in Wnt1/ canonical Wnt signaling, which underpins these phenotypes. MicroRNA modulation of Lmx1b/Wnt1 dosage thus determines midbrain size and allocation of dopamine progenitors. Since canonical Wnt activity has recently been recognized as a key ingredient for programming ES cells towards a dopaminergic fate in vitro, and since microRNA135a2 modulates Wnt1/Wnt signaling, these studies could impact the rational design of such protocols.

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Dissection of the role of Fgf10 after limb bud initiation. Anne M. Boulet, Mario R. Capecchi. Human Genetics, HHMI/University of Utah, Salt Lake City, UT.

Fgf10 is known to be required for limb bud initiation. Knockout of Fgf10 results in the complete absence of limb bud development. Although loss of Fgf10 expression during limb bud growth has been proposed to account for the limb phenotype in other mutants, the role of Fgf10 in further development of the limb has not been studied. It is assumed that continued Fgf10 expression is required to maintain AER function, including AER FGF expression. Expression of FGFs in the AER is in turn thought to be required to maintain Shh expression in the posterior limb bud and Fgf10 expression in the mesenchyme. Using an Fgf10 conditional allele, the role of Fgf10 in post-initiation limb bud development has been investigated. The RARcre driver was used to knock out Fgf10 at approximately E9. It appears that limb buds are initiated in these mutants, but fail to grow, and an increase in the number of apoptotic cells is seen. This result would suggests a requirement for Fgf10 in maintenance of cell survival immediately after limb buds form, perhaps indirectly through an effect on formation of the AER and initiation of Fgf8 gene expression. The AP2cre driver is expressed in distal limb bud mesenchyme by E10.5. Mice in which Fgf10 was inactivated using AP2cre show digit malformations, with 100% penetrance but variable expressivity. Digits do not separate properly, and some digits are truncated or curved. When the Shhcre driver was used to inactivate Fgf10 in the posterior limb bud, a defect in the development of digit 5 could be detected at least as early as E13.5. Newborn skeleton preps revealed subtle defects in the condensations of digit 5 in both forelimbs and hindlimbs. Additional Cre drivers will be utilized to inactivate Fgf10 at different developmental time points in order to elucidate the role of this gene in limb bud growth and patterning.

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Early forebrain patterning of the mouse embryo: a role for the non-neural ectoderm. Marieke I. Cajal, Jerome Collignon, Anne Camus. Université Paris Diderot, Sorbonne Paris Cité, Institut Jacques Monod, UMR 7592, CNRS, F-75013 Paris, France.

The central nervous system of the mouse develops from a single epithelial layer. Between the end of gastrulation and the beginning of somitogenesis, this layer is specified and regionalized in three regions, the forebrain, midbrain, and hindbrain, mostly under the influence of signals emanating from the axial mesendoderm. Our results highlight a critical role of another embryonic region in the formation of the forebrain. When the anterior proximal region, that comprises the non-neural precursors which give rise to surface and buccal ectoderm, is surgically removed at 7.5 days post-coitum, the development of the forebrain is highly affected. In particular, after 30 hours of in vitro culture, the prospective telencephalon is missing. We are interested in understanding the interactions between these non-neural ectoderm cells and neural precursors during neural plate formation. We found that in ablated embryos, all the tissues composing the head, the neuroectoderm, the surface ectoderm, and the neural crest, are specified. The axial mesendoderm, anterior neural ridge (ANR) and isthmus organizer are present. The neuroectoderm appears correctly regionalized as assessed by the exhaustive analysis of anterior regional markers. The morphological defects of ablated embryos are associated with an increase in cell death whereas no change in cell proliferation is detected. Several signaling pathways involved in brain specification and development are disturbed and may account directly or indirectly for the loss of the telencephalon. In particular, the Bone Morphogenetic Protein (BMP) signaling pathway is disrupted, and the Nodal signaling pathway is ectopically activated in the neuroectoderm. We are currently pursuing our investigation of the role of non-neural ectoderm in forebrain development and regionalization using reporter mice strains and pharmacological inhibitors of major signaling pathways.

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The Dimple Mutation Uncovers a Link Between Mouse Gastrulation and Mitochondrial Function. Ivan Duran, Maria J. Garcia-Garcia. Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

During mouse gastrulation, a group of cells in the primitive streak, an area located at the posterior side of the embryo, delaminate from the embryonic epithelia and migrate to form mesodermal and endodermal lineages. To identify novel genes regulating these processes, we performed a forward mutagenesis screen and found dimple, a recessive mutation that causes early embryonic lethality and severe gastrulation defects. In dimple embryos, cells delaminate to form mesoderm, but are unable to migrate and accumulate close to the primitive streak area. Analysis of molecular markers revealed that several signaling pathways required for gastrulation are up-regulated in dimple mutants, including NODAL, WNT3 and FGF. Interestingly, Fgf8 is

up-regulated in dimple embryos before morphological phenotypes are observed, suggesting that increased FGF signaling is primarily responsible for the developmental defects of dimple mutants. Positional cloning revealed that dimple disrupts SLC25A26, a transmembrane protein located in the inner mitochondrial membrane and responsible for the transport of S-Adenosylmethionine (SAM). Preliminary characterization of mitochondrial function in dimple embryos indicates absence of mitochondrial stress and normal rates of proliferation/apoptosis. Together, these results indicate that the gastrulation defects of dimple mutants are not caused by an energetic imbalance, but rather to a role of mitochondria in modulating the signaling pathways that control mouse gastrulation.

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Wnt3a/b-catenin signalling maintains mesoderm progenitors through the Sp1-like zinc finger transcription factors Sp5 and Sp8. Mark Kennedy[1], William Dunty Jr[1], Kristin Biris[1], Kenneth Campbell[2], Terry Yamaguchi[1]. 1) Cell and Developmental Biology Laboratory, CCR, Frederick National Lab, Frederick, MD; 2) Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio.

During gastrulation and axis extension, bipotential epiblast stem cells residing near the primitive streak give rise to the neural and paraxial mesoderm progenitors, the latter of which ultimately generate the axial skeleton, muscle, cartilage and dermis of the trunk and tail. Wnt3a is expressed in the primitive streak where it is required for both the maintenance and differentiation of these paraxial mesoderm progenitors, however the mechanisms by which Wnt3a/-catenin signaling regulates their fate remain unclear. We transcriptionally profiled Wnt3a-/- mouse embryos to identify the gene regulatory network controlled by Wnt3a. This approach identified the Sp1-like zinc finger transcription factors Sp5 and Sp8 as putative Wnt3a target genes. Analysis of Sp5/8 expression in Wnt3a null and mesoderm-specific T-Cre;-catenin conditional loss and gain-of-function embryos, as well as the response of ES cells to recombinant Wnt3a in vitro, validated Sp5/8 as Wnt3a target genes. Surprisingly, Sp5 null mice are viable without any overt mutant phenotype and Sp8-/- embryos have relatively mild paraxial mesoderm defects compared to Wnt3a-/- embryos. Therefore, we hypothesized that these 2 highly related transcription factors may function redundantly in paraxial mesoderm development. In support of this hypothesis, both Sp5/8 were determined to be expressed in paraxial mesoderm progenitors. To test for redundancy between Sp5 and Sp8, we generated mesoderm-specific conditional Sp5/8 double mutants using the T-cre driver. Interestingly, the loss of Sp5/8 activity in mesoderm progenitors led to severe posterior body truncations that closely resemble Wnt3a-/- embryo phenotypes. Examination of E18.5 mutant skeletons indicates that only the anterior most ~15 vertebrae formed. In situ hybridization analysis revealed that the mesodermal stem cell (MSC) markers Brachyury and Fgf8 were down-regulated with the apparent loss of the MSC population before E9.5 in Tcre;Sp5/8 mutants. Together, these results provide strong genetic evidence that Sp5 and Sp8 function as novel redundant effectors of the Wnt3a/ -catenin pathway during paraxial mesoderm development. We are currently addressing the target genes of Sp5/8 in stem cells through ChIP-seq and transcriptional profiling studies.

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Dickkopf1, an antagonist for canonical Wnt signaling, regulates determination between neuroectoderm and surface ectoderm from ectodermal cell lineage. Chiharu Kimura-Yoshida, Isao Matsuo. Dept. of Moelcular Embryology, Osaka Medical Center for MCH, Izumi, Osaka, Japan.

Canonical Wnt signaling plays important roles for the forebrain development throughout vertebrates. Especially, the forebrain induction of the vertebrates was essential to be free from canonical Wnt signaling. Dickkopf1 (Dkk1) is one of antagonists for canonical Wnt signaling and Dkk1-deficient embryos show head-less phonotypes (Glinka et al., 1997; Mukhopadhyay et al., 2001). In order to know more precise function in forebrain development by Wnt and Dkk1, we have generated the Dkk1 over-expressed transgenic mouse (CAG-Dkk1) and analyzed its phenotypes. Previous Xenopus experiments indicated that microinjection of Xenopus Dkk1 gene caused the dorsalization and induced big-head (Glinka et al., 1997). Our Dkk1-expressing mouse displayed larger forebrain and lacked fore/hindlimb. These phenotypes resemble that of Xenopus experiments and showed opposite phenotypes of the Dkk1-deficient mouse (Mukhopadhyay et al., 2001). Notably, in Dkk1-expressing transgenic embryos, molecular maker studies revealed that neural markers were ectopically localized in the surface ectoderm, demonstrating that canonical Wnt antagonist induced the neuroectoderm dominantly, instead of surface ectoderm (epidermis). In contrast by overexpressing Wnt or reducing Dkk1, surface ectoderm expanded, while neuroectodermal cells had decreased. These above findings demonstrated canonical Wnt and its antagonist regulate determination of cell fate between surface ectoderm and neuroectoderm in the mouse embryo. Now we are trying to identify target genes of canonical Wnt signaling, which controls the commitment of surface ectoderm dominantly by means of DNA microarray.

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Guts and gastrulation: dynamic cell behaviors driving the morphogenesis of the early mouse embryo. Manuel Viotti, **Tara Sharma**, Maria Pulina, Gloria Kwon, Kat Hadjantonakis. Developmental Biology, Sloan-Kettering Institute, New York, NY.

During gastrulation changes in cell shape, movement and organization direct the formation of ectoderm, mesoderm, gut endoderm and, the three germ layers of the embryo. In the mouse, gastrulation transforms a cup-shaped bilaminar epithelial structure, comprising the epiblast and visceral endoderm into one containing three tissue layers, two epithelia (ectoderm and gut endoderm) and intervening mesenchyme (the mesoderm). Live imaging combined with genetic labeling experiments have revealed that the gut endoderm forms by a novel morphogenetic mechanism of widespread intercalation. Cells of the definitive endoderm (DE) cell lineage originate in the epiblast, ingress through the primitive streak, migrate within the wings of mesoderm,

and then egress into the overlying visceral endoderm (VE) resulting in formation of an epithelium on the surface of the embryo containing cells from both embryonic (DE) and extra-embryonic (VE) lineages. In this way widespread egression of DE cells leads to VE cell dispersal. This suggests that the segregation of embryonic and extra-embryonic tissue is not as strict as previously believed and that a lineage previously believed to be exclusively extra-embryonic may contribute cellular descendants to somatic tissues. Our studies further reveal that the egression of DE cells into the VE epithelium occurs concomitant with the de novo assembly of a basement membrane at the gut endoderm/mesoderm interface. We propose that this basement membrane may function to: (1) provide the traction force to facilitate DE cell egression into the VE, (2) prevent ingression of DE cells out of the VE, and (3) segregate and compartmentalize the mesoderm and gut endoderm germ layers. The HMG domain containing protein Sox17 is an evolutionarily conserved key regulator of gut endoderm formation. Embryos lacking Sox17 exhibit a failure in DE cell specification and thus egression, and consequently a failure in VE cell dispersal. Notably, a basement membrane is never assembled at the mesoderm/gut endoderm interface of Sox17 mutants. Our studies highlight a previously overlooked feature of gastrulation: the transformation of a single BM (two tissue layer) containing pre-gastrula stage embryo to a two BM (three tissue layer) containing embryo. Our observations reveal a link between de novo basement membrane assembly and germ layer segregation with gut endoderm morphogenesis.

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***Lhx1* promotes prechordal plate formation in a cell non-autonomous manner. William Shawlot**[1], Kenichiro Taniguchi[2]. 1) The Dell Pediatric Research Institute, The University of Texas at Austin, Austin, TX; 2) Department of Cell and Developmental Biology, The University of Michigan Medical School, Ann Arbor, MI.

The prechordal plate is an anterior midline endodermal structure that underlies the presumptive forebrain during vertebrate embryogenesis. Perturbations that affect prechordal plate development cause holoprosencephaly and cyclopia in model vertebrate organisms. In humans, holoprosencephaly is the most common defect in forebrain development with an incidence as high as 1 in 250 during embryogenesis. The molecular and cellular processes that mediate prechordal plate development in mammalian embryos are not well understood. *Lhx1* encodes a homeobox transcription factor that is transiently expressed in the primitive streak and the anterior mesendoderm of gastrulation-stage mouse embryos. We previously generated *Lhx1*-deficient mice and showed that *Lhx1* is absolutely required for anterior head formation and that chimeric embryos with reduced *Lhx1* activity display holoprosencephalic-like phenotypes. Here to determine the short-term fate of *Lhx1*-expressing cells in the anterior mesendoderm, we used the perdurance of -galactosidase produced from the $Lhx1^{lacZ}$ knock-in allele in which the *lacZ* gene is under the control of *Lhx1* regulatory elements. We found that *Lhx1*-expressing cells in the anterior endoderm contribute to the prechordal plate and the rostral-most foregut endoderm. Molecular analysis of genes expressed in the prechordal plate and the rostral foregut endoderm revealed that *Goosecoid* and *Dkk1* were not expressed in embryonic (E) 7.75 $Lhx1^{-/-}$ embryos and that the most anterior domain of *FoxA2*expression in the midline was absent. Analysis of *lacZ* expression from the *Lhx1* locus in null embryos revealed that X-gal staining was increased in the anterior mesendoderm suggesting that *Lhx1* directly or indirectly regulates its own expression. Developmental analysis indicated that *Hhex*, a marker for the newly emerging midline endoderm, was expressed in a smaller domain in the anterior primitive streak region of E6.75 $Lhx1^{-/-}$ embryos. Chimera studies revealed that *lacZ*-marked $Lhx1^{-/-}$ cells were able to contribute to the prechordal plate and the foregut endoderm in the presence of wild-type cells. Together these results suggest that *Lhx1* is necessary to correctly specify prechordal plate precursors in the anterior primitive streak and that *Lhx1* acts in a cell non-autonomous manner. These results identify a gene pathway critical for prechordal plate development that may aid in understanding the pathogenesis of holoprosencephaly in humans.

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Conditional *Aurora A* deficiency differentially affects early mouse embryo patterning. Yeonsoo Yoon[1], Dale O. Cowley[2], Terry Van Dyke[3], Jaime A. Rivera-Pérez[1]. 1) Department of Cell and Developmental Biology, University of Massachusetts Medical School, Worcester, MA 01655, USA; 2) TransViragen, Inc., Research Triangle Park, NC 27709, USA; 3) Mouse Cancer Genetics Program, National Cancer Institute, Frederick, MD 20892, USA.

Aurora A is a mitotic serine/threonine kinase, involved in centrosome maturation, spindle assembly and chromosome segregation during the cell division cycle. Ablation of *Aurora A* in mice results in mitotic arrest and pre-implantation lethality, preventing further investigation on the function of *Aurora A* at later stages of development. Here, we report the effects of *Aurora A* ablation on embryo patterning at early post-implantation stages by tissue-specific ablation of *Aurora A*. Conditional knockout of *Aurora A* in the epiblast or visceral endoderm layers of the conceptus leads to apoptosis and embryo growth inhibition, causing lethality and resorption at approximately E9.5. The effects on embryo patterning, however, depend on the tissue affected by the mutation. Embryos with an epiblast ablation of *Aurora A* are able to properly establish the anteroposterior axis but do not proceed through the gastrulation. In contrast, mutation of *Aurora A* in the visceral endoderm leads to posteriorization of the conceptus or failure to elongate the anteroposterior axis. These results show that Aurora A is essential for proper embryo patterning in post-implantation embryo and suggests that abnormal development of mutant embryos is linked to abnormal growth brought about by a paucity of epiblast or visceral endoderm cells.

Stem Cells and Germ Cells

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Differential regulation of Sox10 during the maintenance of the melanocyte lineage and establishment of the melanocyte stem cell. Melissa L Harris, William J Pavan. Genetic Disease Research Branch, National Human Genome Research Institute, NIH, Bethesda, MD.

During embryogenesis SOX10 functions within neural crest cells to upregulate master switch transcription factors needed for specifying different neural crest sublineages. Melanocytes, being a neural crest derivative, use SOX10 to initiate the expression of the transcription factor, Mitf. SOX10 and MITF together then drive the survival and differentiation of the melanocyte lineage embryonically. Postnatally, melanocytes are incorporated into the hair follicle and those that reside in a region called the hair bulge give rise to the melanocyte stem cells (McSCs). These McSCs replenish the melanocyte system of the hair follicle throughout adult hair cycling, however it is unknown how this population is established. We hypothesize that through the differential regulation of Sox10, postnatal melanocytes can maintain their lineage specification while also allowing a portion of them to acquire the role of a McSC. In support of this idea, we find that McSCs express SOX10 and MITF but remain undifferentiated. By knocking out Sox10 in the melanocyte lineage postnatally (Sox10fl; Tg(Tyr::CreER)), we also show that hair follicle melanocytes need Sox10 for their survival. However, by gain-of-function analysis (Tg(DctSox10)) we demonstrate that overexpression of Sox10 results in premature differentiation of the McSC, their eventual loss, and consequently leads to early hair graying. This suggests that Sox10 must be downregulated in order for the McSC to be established. In an attempt to dissect whether SOX10s role in McSCs is simply to regulate Mitf we asked whether haploinsufficiency for Mitf (Mitfvga9) can rescue hair graying in Tg(DctSox10) animals. Surprisingly, the combination of Mitfvga9 and Tg(DctSox10) exacerbates hair graying and suggests that MITF negatively regulates Sox10 in McSCs. Together these data suggest a mechanism where SOX10 can be present to support the maintenance of the melanocyte lineage while also be inhibited from driving differentiation in the McSC population. These data illustrate how tissue-specific stem cells can arise from lineage-specified precursors, and how this can occur through the regulation of the very transcription factors important in defining that lineage.

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Systems-level analysis of embryonic pluripotency and lineage-specific differentiation. David Emlyn Parfitt[1,2,5], Hui Zhao[1,2,5], Mariano Alvarez[3,4,5], Celine Lefebvre[3,4,5], Andrea Califano[3,4,5], Michael Shen[1,2,5]. 1) Herbert Irving Comprehensive Cancer Center, Columbia University, New York, NY; 2) Department of Genetics and Development; 3) Joint Centers for Systems Biology; 4) Department of Biomedical Informatics; 5) Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY.

The molecular mechanisms of pluripotency maintenance in cell culture and its loss during embryonic development in vivo are of central importance in stem cell biology. To understand these mechanisms, it is essential to perform a complete analysis of their master regulators, both known as well as novel. For this purpose, we have constructed the first genome-wide molecular interaction networks (interactomes) for mouse epiblast stem cells (EpiSC) and embryonic stem cells (ESC). To generate these interactomes, we applied the experimentally validated reverse-engineering algorithms ARACNe and MINDy to expression data gathered from EpiSCs and ESCs undergoing a broad range of differentiation events. This methodology enables the unbiased de novo inference of transcriptional and post-translational gene interactions from large datasets of gene expression profiles. To determine which genes in these networks are most critical for regulating specific ES and EpiSC properties, thus corresponding to master regulators, we are conducting analyses of gene expression signatures using the MARINa algorithm. Using these signatures to interrogate the EpiSC and ESC interactomes, we can identify master regulators of in vivo and in vitro cellular processes of interest. In particular, we have generated gene expression signatures that capture the cascade of molecular changes associated with transitions from pluripotent states towards specific lineage-restricted cell types. For example, we have generated time course signatures from EpiSCs treated with the Nodal inhibitor SB431542, in order to understand how Nodal/Activin signaling is involved in ESC and EpiSC maintenance. Similarly, we have generated signatures corresponding to post-implantation embryos to investigate the mechanisms regulating the different states of pluripotency that exist before and after the onset of gastrulation. We anticipate that these analyses will highlight known and/or novel genes that are most capable of faithfully recapitulating the transcriptional and epigenetic signatures of pluripotency, either individually or in combination, and thereby provide molecular insights into somatic cell reprogramming and transdifferentiation.

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Isolation of a Genetically Stable ES Cell Subline from Chromosomally Instable C57BL/6 ES Cells. Thomas L Saunders[1,2], Virginia Zawistowski[1], Keith Childs[1], Elizabeth Hughes[1]. 1) Transgenic Animal Model Core, University of Michigan, Ann Arbor, MI; 2) Department of Internal Medicine, Division of Molecular Medicine and Genetics, University of Michigan Medical School, Ann Arbor, MI.

Embryonic Stem (ES) cells derived from the mouse are a powerful tool to manipulate the mouse genome and generate new mouse models of human disease. Gene targeting in C57BL/6 mouse ES cells produces genetically engineered mice on a C57BL/6

genetic background. In contrast to 129 mouse ES cell lines, the use of a standard inbred C57BL/6 mouse background reduces variation in experimental measurements and permits comparison of results to a wealth of published data. The C57BL/6 Bruce4 ES cell line determined to be genetically instable during a series of gene-targeting projects. Gene-targeted ES cell clones were expanded, characterized by chromosome counting, and tested against a functional definition of euploidy to assess the genetic stability. For each ES cell clone, 20 chromosome spread were counted. If twelve or more spreads contained the normal chromosome number for mouse (40 chromosomes) the clone was scored as euploid. The functional definition of euploidy was predicated on the likelihood of the formation of germline ES cell-mouse chimeras. The results showed that only 35% of correctly targeted ES cell clones were euploid and suitable for the preparation of chimeras. In an attempt to isolate a robust C57BL/6 ES cell line for gene targeting, a series of Bruce4 sublines were developed and characterized by chromosome counting and formation of germline chimeras. One subline, Bruce4.G9, contained 90% euploid chromosome spreads and formed germline ES cell-mouse chimeras. A series of gene targeting experiments were then conducted to compare Bruce4 and Bruce4.G9 subline ES cells in parallel. Consistent with prior observations, only 26% of gene-targeted Bruce4 clones were euploid. However, 100% of gene-targeted Bruce4.G9 clones were euploid. A sample of euploid gene-targeted clones was used to prepare ES cell-mouse chimeras and tested for germline transmission. Bruce4 clones were less efficient (40% of clones were germline) than Bruce4.G9 clones (80% of clones were germline). Genet targeting efficiency in the Bruce4.G9 subline is superior compared to the parental Bruce4 ES cell line. A genetically stable ES cell subline with high germline potential was established from a parental ES cell line characterized by genetic instability and weak germline potential. These results indicate that mouse ES cell lines with suboptimal performance can be rescued by establishing sublines with robust gene-targeting characteristics.

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Forward genetics identifies Edf1 as a novel regulator of epidermal development and stem cell quiescence. Scott D Weatherbee[1], Sunjin Lee-Wölfel[1], Yong Kong[2,3]. 1) Genetics, Yale University, New Haven, CT; 2) Molecular Biophysics and Biochemistry, New Haven, CT; 3) W.M. Keck Foundation Biotechnology Resource Laboratory, New Haven, CT.

The outermost layer of the skin, the epidermis, plays a key role in animal survival by acting as a barrier to prevent infection and desiccation. Stem cells in the interfollicular epidermis undergo a series of cell fate choices during the differentiation program to form a stratified epidermis. The appropriate balance between proliferation and differentiation is crucial for epidermis function, and alterations in this process can cause human diseases, such as psoriasis and skin cancer. However, the factors that regulate cell fate choices of stem cells in the epidermis are not well understood. To identify new mediators involved in these processes, we performed a forward genetics screen in mice and identified a novel regulator of skin development, the *Epidermal differentiation factor 1* (*Edf1*) gene. Mice carrying a homozygous mutation in *Edf1* develop a hyperproliferative, poorly differentiated epidermis. We have shown that *Edf1* function is essential to curb stem cell proliferation and for normal differentiation of their progeny. We further demonstrate that *Edf1* and the cell cycle regulator *Stratifin* (*Sfn; 14-3-3*) act together to regulate keratinocyte differentiation and epidermal barrier formation. The transcription factor p63 is a master regulator of epidermal development and strongly expressed in the stem cell compartment. *Edf1* mutants, however, exhibit increased levels of p63 throughout the epidermis and reduction of p63 dosage in *Edf1* mutants rescues many aspects of the phenotype, indicating that Edf1 modulates p63 levels. Together, our findings identify Edf1 as a novel regulator of epidermal stem cell proliferation and differentiation that regulates p63 expression and acts with Sfn to balance these processes.

Technology

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Does gene targeting for the generation of mouse conditional alleles still require selection markers and Gateway technology? Luca Brunelli[1], George Lezin[1], Yasuhiro Kosaka[1], H. Joseph Yost[1], Michael R. Kuehn[2]. 1) The University of Utah, Salt Lake City, UT; 2) Laboratory of Protein Dynamics and Signaling, NCI-Frederick, Frederick, MD.

Targeting vector construction for mouse conditional mutagenesis currently involves inserting the selection cassettes necessary for isolating various intermediate recombinant constructs. The Gateway technology is also commonly required at multiple intermediary steps and/or for the final assembly of the targeting vector. As these approaches remain fairly complex, time consuming and expensive, we investigated whether recombinants could be isolated without using selection markers, as well as whether we could develop a low-cost in vivo cloning method to replace the Gateway technology. First, we determined whether the founder principle, a population genetics concept, could be used to isolate markerless recombinants. After inserting the coding sequences of the fluorescent proteins mStrawberry or eYFP into a mouse Nodal bacterial artificial chromosome (BAC), we isolated markerless and seamless mStrawberry and eYFP Nodal BAC recombinants using the founder principle and PCR screening in liquid cultures. Derived transgenic mice expressed mStrawberry and eYFP in a pattern consistent with Nodal. Secondly, using gap repair cloning, a recombineering application using DNA repair to clone DNA into a retrieving vector, we developed a simple and highly efficient in vivo cloning system that is low-cost because it does not require enzymes. This system is based on a new P1 phage-based retrieving vector constructed in our laboratory and which contains four key structural and functional improvements: 1) similar cloning capacity to BACs (~300-kb); 2) copy number modulability; 3) expected

compatibility with other episomal elements; and 4) high cloning efficiency with no multimerization. To test our approach, we used the mini- recombineering system to insert homology arms into our retrieving vector, and then efficiently subcloned a 65 kb BAC region into our retrieving vector without a significant percentage of aberrant recombinants. We believe that the combined benefits of our new approaches may decrease costs and simplify mouse conditional mutagenesis by eliminating the need for using selection cassettes and the Gateway technology.

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Crucial elements of contemporary genotyping assay design criteria and screening procedures in the context of a very high-throughput genetic analysis laboratory. Colin Cox, Deborah Siler, Carol Cain-Hom, Ryan Pabalate, Rhonda Wiler. Mouse Genetics, Genentech, South San Francisco, CA.

The ability to rapidly genotype genetic mouse models with an extremely high degree of accuracy is a critical function of a genetic analysis laboratory or animal research facility. We present three arenas which are topically important for current genetic screening and validation needs. First, we present a resilient methodology for design of TaqMan genetic screening reactions and show how this results in a greater than 50% reduction of failed reactions in comparison with PCR. Traditional screening methods like PCR are no longer considered high-throughput and moreover, PCR itself is not as sensitive or selective as TaqMan. However, while it may be technically straightforward to develop TaqMan assays for simple knock-out, knock-in, and transgene models, it can be difficult to design robust assays capable of resolutely interrogating the three allelic states of a conditional knock-out or knock-in model and we detail how to overcome this challenge. Second, we show how it is essential to monitor for all three allelic states in order to prevent the inadvertent germ-line loss of a conditional gene owning to leaky expression of what should be specific promoter-driven of a recombinase (Cre, Flp, and Dre). In order to easily validate tissue-specific knock-out detection from simple tail or ear clippings, we have developed a simple in vitro assay capable of generating (and detecting) the null allele. Third, we demonstrate the need for implementation of quality assurance validation procedures, both for in-house model creation as well as for acquisition of models from collaborators. Models can be incorrectly created, especially mature models made over a decade ago and/or models using older transgenic creation technology such as simple cloning and restriction-site based assemblage. Quality assurance validation can be especially important for purchased models which may harbor unintentional genes such as a hidden recombinase or genetic selection marker. Finally, we describe other quality and validation opportunities such as single-plex corroboration, DNA sequence analysis verification, matching primer and probe Tms during the design process, etc. In the limit, we show how all these functions serve to design and execute assays that fully meet the investigational needs of a research group in the context of a very high-throughput genetic analysis laboratory performing 750,000 genotyping reactions annually across 1,000 distinct mouse models.

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Whole Body Inducible Knockouts in Mice Using a Novel Tet-Inducible Cre-LoxP System. Joseph E Dinchuk, Fanny Myers, Amy Weiss, Christopher Miller. Functional Genomics, Bristol-Myers Squibb R & D, Princeton, NJ.

There are currently no genetic systems available that allow for a whole body adult robust induction of gene expression that includes the CNS. We devised and describe genetically engineered mice, based upon the tetracycline inducible system, that allows for non-leaky, whole-body inducible and reversible gene expression in the mouse and potentially other mammals. Our first application of this system is the production of a mouse with non-leaky and whole-body induction of Cre recombinase. The availability of such a mouse and adaptation of the Cre-Lox system to it allows the production of mice with the capability of a whole body induction of adult KOs in all organs including those in the CNS. The first component of this system consists of a genetic element that ubiquitously co-expresses a reverse tetracycline transactivator and forward tetracycline transcriptional inhibitor from the Rosa26 locus. This unique combination of elements assures that the responder gene should be both non-leaky and cleanly inducible upon application of tetracycline or tetracycline derivatives to such mice. The second component of this system is a tet-operator genetic element that co-expresses both a Cre recombinase and a reverse tetracycline transactivator from a favorable locus on the X-chromosome. This second system essentially adds a turbo-drive to the induction system in order to allow for high levels of the target gene expression (in this case Cre recombinase). When we combined mice containing these 2 genetic elements with a floxed transgene known to express highly in the CNS (and elsewhere) and fed the mice doxycycline, we were able to demonstrate virtually a complete knockout of the gene in kidney tissue along with a 95% knockout of the same gene in the cerebral cortex. We are currently repeating these experiments using different doses of doxycycline and targeting different floxed genes. We expect this system to be a useful for adult induction of gene expression and especially useful for gene knockout systems when the gene that is regulated is Cre recombinase.

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A method for the conversion of PCR-based fragment analysis genotyping assays to 5' nuclease assay (TaqMan™) platform with validation. Ryan Pabalate, Robert Schwingendorf, Deborah Siler, Gregg Sy, Colin Cox. Genentech, Inc, South San Francisco, CA.

The ability to quickly and robustly genotype genetic mouse models is crucial in an animal care facility in order to rapidly segregate genetically distinct pups in a given litter. Currently, many facilities utilize PCR for genetic screening by either employing agarose gels or capillary electrophoresis-based PCR amplicon detection. While functional, this method is inferior in comparison to other technologies such as the 5 nuclease assay (TaqMan). First, automated technologies like capillary electrophoresis machines may be capable of only processing 96 samples per hour. In comparison, TaqMan instrumentation can

interrogate 20-fold more samples in an equivalent time in a simple read-mode configuration. Second, TaqMan has proven to be more sensitive and specific for genetic screening than PCR alone. Because of these large improvements in using TaqMan for genetic screening, we have developed a method to convert PCR-based genotyping assays into TaqMan assays. One critical component in this process is the validation required to ensure data fidelity during assay conversion. We also describe the elements of various validation procedures as well as ensuring assay concordance.

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Class Switch Recombination in RNA Exosome Deficient Mouse Models. Evangelos Pefanis[1,2], Aris Economides[2], Uttiya Basu[1]. 1) Columbia University, New York, NY; 2) Regeneron Pharmaceuticals, Tarrytown, NY.

Class switch recombination (CSR) and somatic hypermutation (SHM) are critical steps in the diversification of the immunoglobulin heavy chain locus (IgH) in B lymphocytes. CSR involves a somatic rearrangement/deletion event replacing the heavy chain constant region with that of a different isotype, whereas SHM introduces somatic mutations in the variable regions of Ig exons. Both CSR and SHM are initiated by the cytidine deaminase AID. We have previously shown using biochemical assays and CH12F3 B lymphoma cells that the RNA exosome complex provides AID with access to both strands of transcribed duplex DNA. As a continuation of this work we have targeted the Exosc3 and Exosc10 subunits of the RNA exosome complex for conditional mutagenesis in mice. Here we describe a novel Cre mediated conditional inversion (COIN) mutagenesis approach, whereby inversion leads to simultaneous inactivation of the targeted allele and induction of a fluorescent protein reporter. Exosc3-COIN/COIN homozygous B cells have reduced CSR efficiency despite normal expression of AID. These findings further support the role of nuclear RNA surveillance pathways in the generation of immunoglobulin diversification.

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A pipeline of bioinformatics tools for whole-genome analysis in mouse models. Matthew A Richardson[1], Clinton L Cario[1], Matthew Hsieh[4], George D Leikauf[3], Steven D Shapiro[1,2], Annerose Berndt[1]. 1) Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA; 2) University of Pittsburgh Medical Center, Pittsburgh, PA; 3) Department of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, United States; 4) Information Technology, Carnagie Mellon University, Pittsburgh, PA.

Purpose: Genome analysis in mouse models has entered a high demand era as a result of generating high-density and most recently whole-genome single nucleotide polymorphism (SNP) panels for many laboratory mouse strains. Additionally, an increasing number of investigators determine disease phenotypes across multiple mouse strains, which in combination with dense genotype panels can be used in genome-wide association (GWA) analyses to identify novel biomarkers. While several GWA algorithms are publicly available (e.g., EMMA, EMMAX, GEMMA), most often they are computationally intensive and often require skilled personnel for their operation. Additional bioinformatics approaches subsequently to GWA analysis are necessary for SNP annotation, which frequently vary among investigators. Here, we present the development of an automated, user-friendly bioinformatics workflow to increase accuracy and reproducibility in genome analyses that lead to biomarker identification. Methods: We implemented a bioinformatics pipeline through developing three computationally integrated layers: a presentation, middle, and processing layer. The presentation layer was designed using jQuery, Javascript, HTML5, and CSS3 and the middle ware was implemented with Django and the Python programming language. The processing layer operates with custom C and C++ programs and awk, R, Perl, and Python scripts. Results: We generated an open-access web interface (freely available at http://www.berndtlab.pitt.edu/) hosting bioinformatics tools to streamline genome analysis from GWA to biomarker identification. The functionality of the new interface is two-fold: variant identification (tools: Local EMMA/EMMAX/GEMMA Server, the Manhattan Plot Generator, and the Genome Region Search Tool) and gene/variant annotation (tools: SNP Annotator, PolyPhen-2 Submitter, and Microarray Data Explorer). GWA analysis can be performed with EMMA, EMMAX, or GEMMA using one of several high-density and whole-genome SNP panels (incl., 65 million Sanger SNPs) and computation ranges between 3 and 10 minutes per analysis depending on algorithm and SNP panel. SNP annotations are performed using Ensembls Variant Effect Predictor and Harvards PolyPhen-2 algorithms. Conclusions: The work of this project enables high-throughput genome-analysis and biomarker identification to a broad audience of investigators with multi-strain phenotype data.

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Assessment of cryopreserved samples of mutant mice. Johannes Schenkel. Cryopreservation W430, German Cancer Research Center (DKFZ), Heidelberg, Germany.

Genetically modified animals are unique mutants with an enormous scientific potential. Cryopreservation of pre-implantation embryos or of spermatozoa is a common approach to save those lines. Following sufficient cryopreservation and assessment, a mutant line can be taken out of a breeding nucleus. The quality of different donors of the same line may be heterogeneous and the procedure error-prone, too. To make sure that the quality of the frozen material is satisfying, several assessments are available, resource saving approaches should be preferred:

Re-genotyping of the donor animals is helpful, but not ever applicable.

Embryos: An *in vitro* over-night culture can demonstrate the capacity of further development. At least one statistically selected sample must be revitalized and transferred into pseudo-pregnant foster mothers. The litter must be genotyped, if the outcome of genetically modified pups is smaller than to be expected, the reason has to be found out, additional embryos are to be cryopreserved.

Spermatozoa: The *in vitro*-fertilization (IVF) capacity of each line must be shown; it can be reduced or lost due to the

mutation or the genetic background. Each donor animal should be assessed; due to many parallel samples of a donor, the loss of material remains acceptable. Several strategies are published, the gold standard might be an (resource and animal consuming) IVF. To improve the monitoring and to save animals and resources for assessment we developed a fluorescence microscopy based technique analyzing the membrane integrity. This parameter is significantly correlated with the outcome of paralleled IVF.

Physical and hygienic stability: Samples should be protected against an unexpected loss, e.g. by storing parallel samples of a line in different freezers at several locations. If not stored in the liquid phase of LN_2, the temperature stability must be monitored. A powerful data management is important. Samples and receivers of revitalized embryos must be protected against infections during handling and storage. Data of 400 cryopreserved lines are presented.

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Design strategies for creation of robust TaqMan™ based assays for high-throughput genotyping of conditional knock-out/knock-in constructs in mice. Deborah Siler, Carol Cain-Hom, Robert Schwingendorf, J. Colin Cox. Mouse Genetics Department, Genentech, Inc., 1 DNA Way, South San Francisco, CA.

Site-specific genomic recombinases such as Cre (recognizing loxP sites) and Flp (distinguishing frt sites) have been widely used to generate conditional (inducible) knock-out mutations in mice and other models. The basic construct of a conditional genetic model consists of a pair of recombination sites that flank a gene of interest. Recombinase is delivered or activated under an inducible or tissue-specific promoter resulting in the excision of the target DNA sequence in a spatially and/or temporally directed manner. Large-scale breeding strategies rely on highly accurate and rapid genotyping. Traditionally, genotyping has been performed using PCR with agarose analysis or capillary electrophoresis. Here, we describe the design rationale of robust, high-throughput TaqMan-based genotyping assays for Cre/loxP and Flp/frt-driven conditional mutations which often prove challenging on the TaqMan platform in comparison to amplicon length-based PCR methods. Additionally, we provide strategies for ensuring mice do not carry additional or inadvertent Cre or Flp recombinase genes which could interfere with breeding goals. Moreover, Cre recombinase genes are often introduced with a promoter that is not expressed in a completely tissue-specific manner, and as such, an experimentalist is highly encouraged to design to recognize all three allelic breeding states: wildtype, conditional, and knock-out. These so-called leaky Cre promoter sets can result in the unintentional excision of target DNA sequences and loss of the germ-line conditional construct. Finally, we describe a method for generating knock-out DNA from tail samples using a simple *in vitro* method.

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The Sanger Mouse Genetics Project: High Throughput Recessive Lethality Screen. Antonella Galli, Jeanne Estabel, Elizabeth Tuck, Yvette Hooks, Ed Ryder, Jacqueline K. White, Ramiro Ramirez-Solis, Tim Mohun, David Adams on behalf of the Mouse Genetics Project.

The Sanger Institute Mouse Genetics Project (MGP) is committed to participate in the worldwide effort to develop mouse models to understand human genetic diseases. Using the growing knockout-first conditional ready targeted embryonic stem (ES) cell EUCOMM/KOMP resource, the MGP generates and archives over 160 lines of knockout mice per year. A standardised battery of primary phenotyping tests is performed on all lines without any prior assumptions about gene function. The phenotypic data and knockout lines generated provide valuable insight into novel gene functions and new mouse models of human disease. To date, 28% of lines analysed are lethal at postnatal day 14 and a further 14% are classified as sub-viable due to reduced homozygous viability. To explore potential defects during embryogenesis, we are collecting and assessing embryos from heterozygous intercrosses at embryonic day 14.5 (E14.5). Any dysmorphology including growth retardation, oedema, craniofacial, skeletal and neural tube defects are recorded and annotated. Up to now, we have analysed 162 lines and in 55% homozygote embryos are recovered at E14.5 and a subset are further analysed using a relatively new imaging technique named HREM (high resolution episcopic microscopy). Such technique provides highly remarkable detailed 3D models of imaged samples, currently unachievable by alternative imaging modalities. The HREM data will be annotated and publically displayed in the near future. Interestingly, no homozygote embryos are recovered in 45% of the lines assessed suggesting that these mutations are embryonic lethal at an early developmental stage. Our future aim is to investigate further such mutations in order to gain new insights into aspects of cell biology central to mouse development. Viability and anatomical abnormalities will be recorded, annotated and analysed and available to the scientific community. Here we report a summary of recessive lethality data available to date and examples of novel findings for a subset of interesting mutant lines.

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This index includes names, in alphabetical order, of all invited and selected authors and co-authors published in this book. The number following the name refers to the abstract's program number. **Platform presentations 1-64, poster presentations 65-145**

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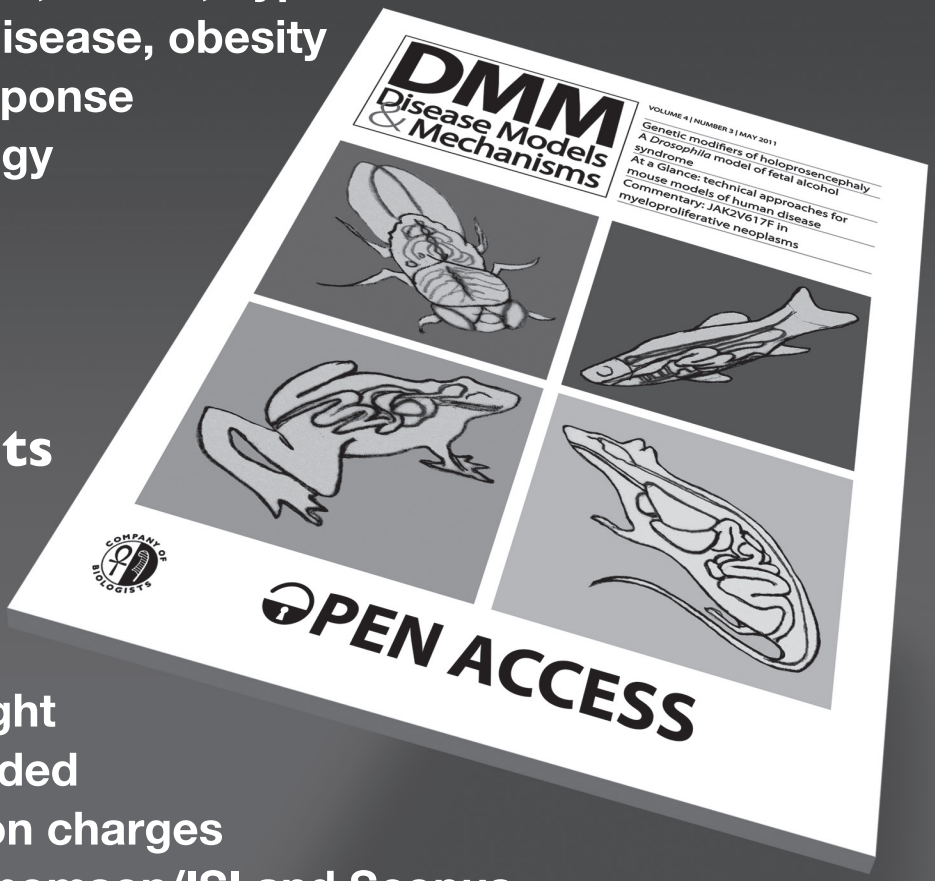

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