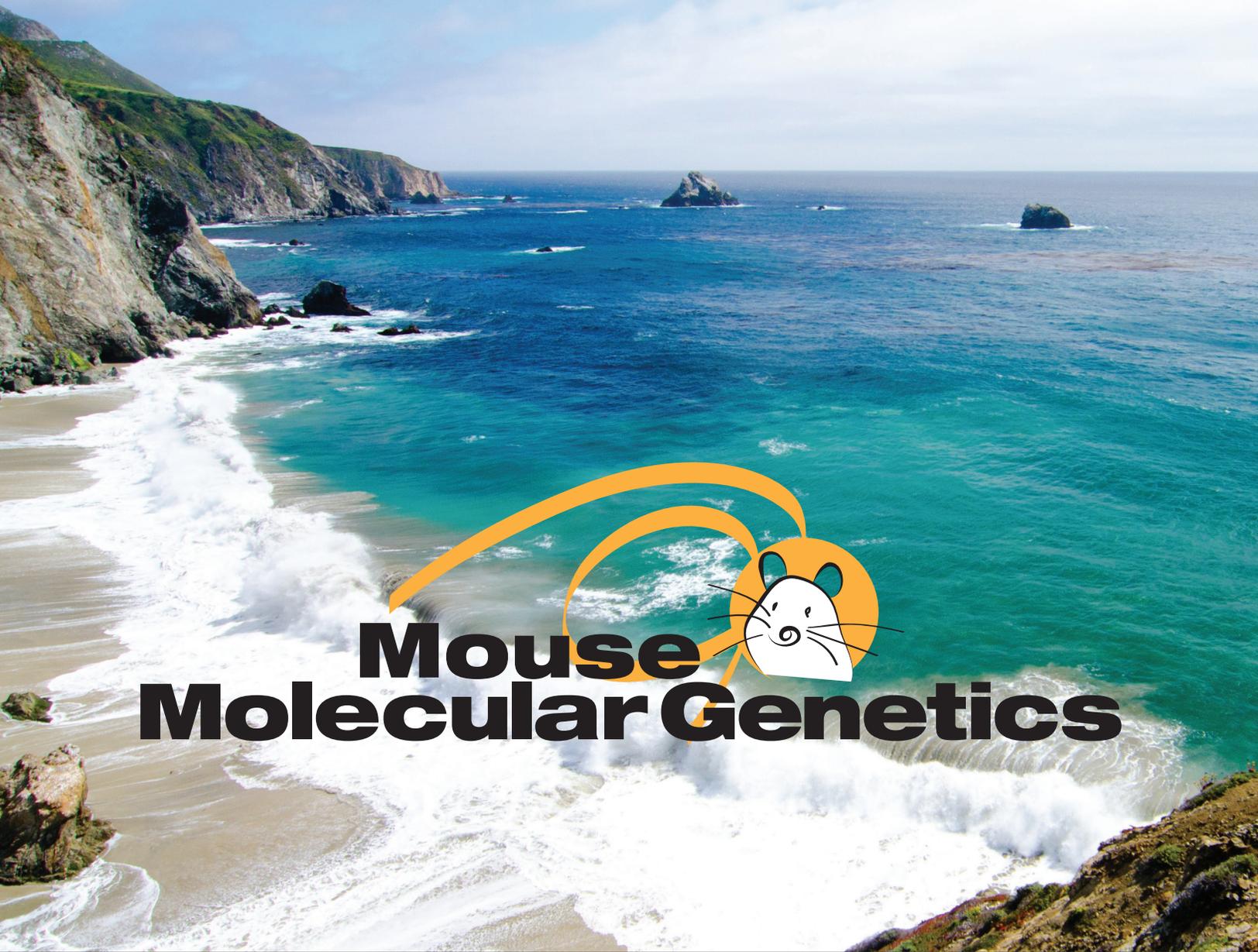
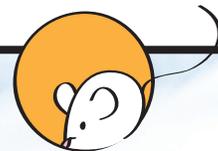




The Genetics Society of America Conferences



Mouse Molecular Genetics

27th Annual Mouse Molecular Genetics Conference
September 29-October 3, 2014 • Pacific Grove, CA

PROGRAM GUIDE

Thanks to the Following Companies for their Generous Support

Platinum Sponsors

REGENERON
science to medicine®

 **TACONIC**

Gold Sponsors



Additional Support Provided by:

Monterey Convention & Visitors Bureau



The Genetics Society of America Conferences



Program and Abstracts

Conference Organizers

Allan Bradley, Wellcome Trust Sanger Institute, UK

Kat Hadjantonakis, Sloan Kettering Institute, USA

Yumiko Saga, National Institute of Genetics, Japan

Philippe Soriano, Icahn School of Medicine at Mount Sinai, USA



9650 Rockville Pike, Bethesda, Maryland 20814-3998

Telephone: (301) 634-7300 • Fax: (301) 634-7079

E-mail: society@genetics-gsa.org • Web site: genetics-gsa.org

Invited Speakers

Keynote Lecture

Monica Justice - Baylor
College of Medicine

Rosa Beddington Lecture

Brigid Hogan - Duke
University Medical Center

Genetics and Genomics: **François Spitz** - EMBL Heidelberg and **David Threadgill** -
University of North Carolina

Cancer: **Scott Lowe** - Memorial Sloan-Kettering Cancer Center and **Martin McMahon** -
University of California, San Francisco

Epigenetics: **Marisa Bartolomei** - University of Pennsylvania and **Carlos Lopez-Otin** -
Universidad de Oviedo

Organogenesis: **Ralf Adams** - Max Planck Institute for Molecular Biomedicine and
Jonathan Epstein - University of Pennsylvania

Development: **Isao Matsuo** - Osaka Medical Center for Maternal and Child Health and
Xin Sun - University of Wisconsin

Disease Modeling: **Markus Grompe** - Oregon Health & Science University and **Terry
Magnuson** - University of North Carolina

Technology: **Mark Henkelman** - Hospital for Sick Children and **Maria Jasin** -
Memorial Sloan-Kettering Cancer Center

Stem Cells: **Olivier Pourquié** - Harvard University and **David Sassoon** - Hospital
Pitié Salpêtrière

Table of Contents

General Information	5
Schedule of Events	6-7
Exhibitors	8-9
Opening Session and Platform Sessions.....	10-23
Poster Sessions	24-27
Platform Presentation Abstracts	29-73
Poster Presentation Abstracts	74-92
Speaker and Author Index.....	93-98
Participant List	99-101



Genetics Society of America

The Genetics Society of America is pleased to be working with you to support the 27th Annual Mouse Molecular Genetics Conference.

Founded in 1931, the Genetics Society of America (GSA) is a professional scientific society with more than 5,000 members worldwide working to **deepen our understanding of the living world by advancing the field of genetics**, from the molecular to the population level. GSA represents the collective interests of the genetics and model organism communities in advocating support for research, educating students and the public about the importance of genetics, and providing a respected and authoritative voice on genetic issues increasingly in the public eye.

GSA promotes research and fosters communication through a number of GSA conferences including regular meetings that focus on cross-cutting areas of genetics and particular model organisms including *C. elegans*, *Chlamydomonas*, ciliates, *Drosophila*, fungi, mice, *Xenopus*, yeast, and zebrafish.

GSA publishes two peer-edited scholarly journals:

- *GENETICS*, which has published high quality original research across the breadth of the field since 1916, and
- *G3: Genes|Genomes|Genetics*, an open-access journal launched in 2011 to disseminate high quality foundational research in genetics and genomics.

GENETICS



The Society has a deep commitment to fostering the next generation of scholars in the field, including through providing career development activities and resources and offering travel grant programs including the GSA Undergraduate Travel Awards and DeLill Nasser Awards for Professional Development in Genetics.

2014 GSA Board of Directors

Vicki Chandler, President • **Jasper Rine**, Vice-President • **Michael Lynch**, Immediate Past President • **Anne M. Villeneuve**, Secretary • **Sue Jinks-Robertson**, Treasurer • **Angelika Amon** • **Lynn Cooley** • **Anna Di Rienzo** • **Sarah C.R. Elgin** • **Marnie E. Halpern** • **Lauren M. McIntyre** • **Mohamed A.F. Noor** • **Dmitri A. Petrov** • **John C. Schimenti** • **Deborah A. Siegele** • **Brenda J. Andrews**, Editor-in-Chief, *G3: Genes|Genomes|Genetics* • **Mark Johnston**, Editor-in-Chief, *GENETICS*

Trainee Advisory Representatives: **Andrew Adrian**, **Krista Dobi**, **Kathleen Dumas**

GSA Staff

Adam P. Fagen, Executive Director • **Mary Adams**, Accounting/Membership • **Raeka Aiyar**, Communications & Engagement Manager • **William Anderson**, Web Designer • **Suzy Brown**, Senior Meetings Director • **Sally Celia**, Executive Assistant • **Yimang Chen**, Director of Information Technology • **Tracey DePellegrin Connelly**, Executive Editor • **Cristy Gelling**, Journals Assistant Editor • **Ruth Isaacson**, Assistant Managing Editor • **Anne Marie Mahoney**, Senior Meetings Director • **Beth Ruedi**, Director of Education & Professional Development • **Wendy Scott**, Editorial Office Assistant • **Chuck Windle**, Director of Finance & Administration • **Hubert Zhang**, IT Project Lead • **Wujun Zhou**, Web Developer

www.genetics-gsa.org



GeneticsGSA



@GeneticsGSA

GENERAL INFORMATION

Registration Desk

The Conference registration desk will be open according to the following schedule:

Date	Time	Location
Monday, September 29	5:00 pm—8:00 pm	Merrill Hall
Tuesday, September 30	7:00 am-4:00 pm	Merrill Hall

Instructions for Speakers

All plenary/platform sessions will be held in Merrill Hall. Please upload your presentation the day before your session and check in with your session chair 30 minutes before your session begins in Merrill Hall.

Poster Sessions

The poster area will be open as follows:

Tuesday, September 30, 2014

8:00 am - 9:00 pm Open Poster Viewing
3:15 pm-5:00 pm Poster Presentations (odd poster numbers)

Wednesday, October 1, 2014

8:00 am-5:00 pm Open Poster Viewing
3:15 pm-5:00 pm Poster Presentations (even poster numbers)

Posters may be mounted on boards beginning at 7:00 am on September 30, 2014 and must stay in place through October 1, 2014 at 5 pm. **Posters must be removed by 7:00 pm on October 1, 2014.** Posters that are larger than 3’8” tall by 3’9” wide will be removed. Please note that the posters should be in a vertical layout. GSA cannot be responsible for items left in the poster area including but not limited to poster tubes, purses, backpacks, etc. Please keep personal items with you at all times. The meeting does not take responsibility for posters that are not removed on time.

Tickets

Please bring the event tickets for the Wine and Cheese Reception and Closing Dinner with you to the event. If you pre-ordered a box lunch for the final day, you will need that ticket to pick up your box lunch from Crocker Dining Hall.

Internet Access

Complimentary WiFi is available in Merrill Hall. The logon instructions are listed below:

1. Connect to the Asilomar Conference Network
2. Passphrase is the word “conference” - in all lower case.
3. Once connected, open your web browser.
4. You should see Asilomar Conference Grounds logon page.
5. Scroll to the bottom of the page and use the Username: conf8690 Password: conf8690

SCHEDULE OF EVENTS

<u>Monday, September 29</u>		
6:00 pm - 7:00 pm	Dinner	Crocker Dining Hall
7:10 pm - 7:15 pm	Opening Remarks Philippe Soriano, Mt. Sinai, New York	Merrill Hall
7:15 pm - 10:00 pm	Plenary/Platform Session 1 - Genetics and Genomics <i>Moderator: Francois Spitz, EMBL, Heidelberg, Germany</i>	Merrill Hall
<u>Tuesday, September 30</u>		
7:00 am - 9:00 pm	Open Poster Viewing	Kiln
7:30 am - 8:30 am	Breakfast	Crocker Dining Hall
8:30 am - 11:30 am	Plenary/Platform Session 2 - Cancer <i>Moderator: Martin McMahon, UCSF, San Francisco, CA</i>	Merrill Hall
8:30 am - 12:00 noon	Exhibits	Merrill Hall
12:00 noon - 1:00 pm	Lunch	Crocker Dining Hall
1:00 pm - 2:00 pm	Dedicated Exhibit Time	Merrill Hall
2:00 pm - 3:00 pm	Keynote Address <i>Moderator: Philippe Soriano, Mt. Sinai, New York</i>	Merrill Hall
2:00 pm	Using mouse models to identify disease suppressors: A genetic approach to therapeutic intervention Monica Justice, Genetics and Genome Biology, Hospital for Sick Children, Toronto, Ontario, Canada	
3:15 pm - 5:00 pm	Poster Session 1 - Odd Numbered Posters <i>Odd Poster Number Presentations</i>	Kiln
5:00 pm - 6:00 pm	Wine and Cheese Reception	Sand and Surf
6:00 pm - 7:00 pm	Dinner	Crocker Dining Hall
7:00 pm - 9:15 pm	Exhibits	Merrill Hall
7:15 pm - 9:15 pm	Plenary/Platform Session 3 - Epigenetics <i>Moderator: Marisa Bartolomei, U Penn, Philadelphia, PA</i>	Merrill Hall
<u>Wednesday, October 1</u>		
7:00 am - 5:00 pm	Open Poster Viewing	Kiln
7:30 am - 8:30 am	Breakfast	Crocker Dining Hall
8:30 am - 11:45 am	Plenary/Platform Session 4 - Organogenesis <i>Moderator: Ralf Adams, Max Planck Institute, Munster, Germany</i>	Merrill Hall
8:30 am - 12:00 noon	Exhibits	Merrill Hall
12:00 noon - 1:00 pm	Lunch	Crocker Dining Hall
1:00 pm - 2:00 pm	Dedicated Exhibit Time	Merrill Hall

SCHEDULE OF EVENTS

Wednesday, October 1 (continued)		
2:00 pm - 3:00 pm	Rosa Beddington Lecture <i>Moderator: Anna-Katerina Hadjantonakis, Sloan Kettering Institute, New York</i>	Merrill Hall
2:00 pm	<i>The life of breath: stem cells of the lung</i> Brigid Hogan, Department of Cell Biology, Duke University Medical Center, Durham, NC 27710	
3:15 pm - 5:00 pm	Poster Session 2 - Even Numbered Posters <i>Even Poster Number Presentations.</i> <i>All posters must be removed by 7:00 pm</i>	Kiln
6:00 pm - 7:00 pm	Dinner	Crocker Dining Hall
7:00 pm - 9:15 pm	Exhibits	Merrill Hall
7:15 pm - 10:30 pm	Plenary/Platform Session 5 - Development <i>Moderator: Xin Sun, University of Wisconsin, Madison</i>	Merrill Hall
<u>Thursday, October 2</u>		
7:30 am - 8:30 am	Breakfast	Crocker Dining Hall
8:30 am - 11:45 am	Plenary/Platform Session 6 - Disease <i>Moderator: Markus Grompe, OHSU, Portland, OR</i>	Merrill Hall
8:30 am - 12:00 noon	Exhibits	Merrill Hall
12:00 noon - 1:00 pm	Lunch	Crocker Dining Hall
2:00 pm - 4:30 pm	Plenary/Platform Session 7 - Technology <i>Moderator: Maria Jasin, Sloan Kettering Institute, New York</i>	Merrill Hall
6:00 pm - 7:15 pm	Dinner/Banquet	Crocker Dining Hall
<u>Friday, October 3</u>		
7:30 am - 8:30 am	Breakfast	Crocker Dining Hall
8:30 am - 11:00 am	Plenary/Platform Session 8 - Stem Cells <i>Moderator: Olivier Pourquié, IGBMC, Strasbourg, France</i>	Merrill Hall
11:00 am - 11:05 am	Closing Remarks <i>Moderator: Yumiko Saga, National Institute of Genetics, Mishima, Japan</i>	Merrill Hall
12:00 noon - 1:00 pm	Lunch <i>Those who pre-ordered box lunches can pick them up in Crocker Dining Hall at 11:15 am</i>	Crocker Dining Hall

EXHIBITORS

As exhibitors at the 27th Annual 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 new products, publications and services.

Gene Tools, LLC

1001 Summerton Way
Philomath, OR 97370
Phone: 541/929-7840
E-mail: custsupport@gene-tools.com
URL: www.gene-tools.com

GeneTools manufactures Morpholino oligos for blocking translation, modifying splicing or inhibiting miRNA activity. Morpholinos are effective, specific, stable and non-toxic. Backed by Ph.D. level customer support, GeneTools designs and synthesis Morpholinos and offers Cytosolic delivery options.

Integrated DNA Technologies (IDT)

1710 Commercial Park
Coralville, IA 52241
Phone: 800/328-2661
E-mail: custcare@idtdna.com
URL: www.idtdna.com

Integrated DNA Technologies (IDT) is the world leader in custom nucleic acid manufacturing for the life sciences research and clinical diagnostics markets, serving academic and clinical research, biotechnology, pharmaceutical development, and agricultural research. IDT product applications include qPCR, gene construction, genome editing, next generation sequencing, SNP detection, and functional genomics.

ISEEHEAR Inc.

5700 Yonge Street, Suite 200
Toronto, Ontario, M2M4K2
Phone: 416/226-3375
E-mail: kelly.rodriques@iseehear.com
URL: www.softmouse.net

Iseehear's flagship product, SoftMouseDB Mouse Colony Management Database, is a cloud based mouse breeding and colony maintenance software program. SoftMouse is ideally designed for researchers, animal technicians and laboratory clusters looking to increase efficiency and save cost in the production and management of mouse models and their associated experimental data.

StemExpress

778 Pacific Street
Placerville, CA 95667
Phone: 530/626-7000
E-mail: cdyer@stemexpress.com
URL: www.stemexpress.com

StemExpress has the largest variety of raw materials available in the industry. StemExpress is your one stop location for fresh, fixed and cryopreserved human primary cells, tissues, apheresis blood, and bone marrow. We guarantee every sample delivers the purity, viability & quality your investigators need.

EXHIBITORS

Taconic

One Hudson City Centre

Hudson, NY 12534

Phone: 518/697-3900

E-mail: william.collins@taconic.com

URL: www.taconic.com

Taconic is a global provider of genetically modified mouse and rat models and services. As a full-service industry leader, founded in 1952, Taconic helps clients acquire, test, develop, breed, cryopreserve, prepare, and distribute highly relevant research lines worldwide. Whether you require custom genetically engineered, humanized or research-ready models, Taconic's scientists will partner with you to rapidly and efficiently obtain the high quality models needed for your discovery or preclinical programs.

The Jackson Laboratory

600 Main Street

Bar Harbor, ME 04609

Phone: 207/288-6000

E-mail: lucy.albert@jax.org

URL: www.jax.org

The Jackson Laboratory (www.jax.org) is an independent, nonprofit biomedical research institution and National Cancer Institute-designated Cancer Center with more than 1,500 employees. Headquartered in Bar Harbor, Maine, it has a facility in Sacramento, Calif., and a new genomic medicine institute in Farmington, Conn. Its mission is to discover precise genomic solutions for disease and empower the global biomedical community in the shared quest to improve human health.

OPENING AND PLENARY/PLATFORM SESSIONS

See Pages 6-7 for presentation schedule. Program number and presenter are in **bold**. Full abstracts begin on page 29.

Monday, September 29 7:10 pm–7:15 pm
Merrill Hall

Opening Remarks - Philippe Soriano, Mt. Sinai, New York

Monday, September 29 7:15 pm–10:00 pm
Merrill Hall

Plenary/Platform Session 1 - Genetics and Genomics

Chair: Francois Spitz, EMBL, Heidelberg, Germany

Presentations:

1 - 7:15

Modeling population genetic heterogeneity to identify novel genes regulating alcohol metabolism. **David Threadgill**, Christine Rubinshteyn. Department of Molecular and Cellular Medicine, Texas A&M University, College Station, TX.

2 - 7:45

The Sanger Mouse Genetics Project: High Throughput Recessive Lethality Screen. **Antonella Galli**¹, Angela Green¹, Cecilia Icoresi Mazzeo¹, Catherine Jones¹, Charlotte Lillistone¹, Emma Siragher¹, Luke Souter¹, Elizabeth Tuck¹, Ed Ryder¹, Tim Mohun², Ramiro Ramirez-Solis¹, David Adams¹, Jacqueline White¹. 1) Mouse Genetics Project, Wellcome Trust Sanger Institute, Cambridge, UK; 2) Division of Developmental Biology, MRC National Institute For Medical Research, London, United Kingdom.

3 - 8:00

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.

4 - 8:15

Production and distribution of the EUCOMM/EUCOMMTOOLS knockout resources. **Andreas Hoerlein**¹, Antje Buerger¹, Wendy Bushell², Jun Fu³, Barry Rosen², Francis Stewart³, Wolfgang Wurst¹, William Skarnes². 1) Institute of Developmental Genetics, Helmholtz Zentrum München, Neuherberg, Germany; 2) Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, U.K; 3) Technische Universität Dresden, c/o Max Planck Institute of Molecular cell Biology and Genetics, 01307 Dresden, Germany.

5 - 9:00

CRISPR/Cas9(D10A) mediated engineering of human and mouse genomes. **C. Jung**, J. Sun, D. KostECKa, P. de Jong. Children's Hospital Oakland Research Institute, Oakland, CA.

OPENING AND PLENARY/PLATFORM SESSIONS

See Pages 6-7 for presentation schedule. Program number and presenter are in **bold**. Full abstracts begin on page 29.

6 - 9:15

RNF212 is a Meiosis-Specific SUMO E3-Ligase That Promotes Crossing-Over and Functions as a Meiotic Checkpoint Factor to Eliminate Defective Oocytes. **Huanyu Qiao**, Neil Hunter. Howard Hughes Medical Institute and Department of Microbiology and Molecular Genetics, UC Davis, Davis, CA.

7 - 9:30

Function and regulation of long-distance genomic interactions during mouse development. **François Spitz**. Developmental Biology Unit, EMBL, Heidelberg, Germany.

OPENING AND PLENARY/PLATFORM SESSIONS

See Pages 6-7 for presentation schedule. Program number and presenter are in **bold**. Full abstracts begin on page 29.

Tuesday, September 30 8:30 am–11:30 am
Merrill Hall

Plenary/Platform Session 2 - Cancer

Chair: Martin McMahon, UCSF, San Francisco, CA

Presentations:

8 - 8:30

Tumor suppressor & tumor maintenance genes. **Scott Lowe**. Memorial Sloan-Kettering Cancer Center, New York, NY.

9 - 9:00

Luminal cells are favored as the cell of origin for prostate cancer. **Zhu A. Wang**^{1,2}, Michael M. Shen¹. 1) Herbert Irving Cancer Center, Columbia University Medical Center, New York, NY; 2) MCD Biology, University of California Santa Cruz, Santa Cruz, CA.

10 - 9:15

Using a High Throughput Screen in Knockout Mice to Discover Genes Involved in the Host Response to Cancer. **Jennifer Schmahl**, Nicholas Gale. Therapeutic Targets, Regeneron Pharmaceuticals, Tarrytown, NY.

11 - 9:30

Phenotype reproducibility of human colon cancer in murine models using multiple species and microbiome rederivation. **J. Amos-Landgraf**^{1,2,4,5}, S. Busi¹, T. Parker¹, W. Dove², G. van den Brink³, M. McCoy¹, A. Ericsson^{1,4,5}, C. Franklin^{1,4,5}, E. Bryda^{1,4,5}. 1) Veterinary Pathobiology, University of Missouri, Columbia, MO; 2) McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI; 3) Amsterdam Medical Center, Amsterdam, Netherlands; 4) Rat Resource and Research Center, University of Missouri, Columbia, MO; 5) Mutant Mouse Regional Resource Center, University of Missouri, Columbia, MO.

12 - 10:15

High-throughput cancer gene validation using CRISPR/Cas9-based somatic genome editing in mice. **Roland Rad**^{1,2}, Rupert Öllinger^{1,2}, Julia Weber^{1,2}, Mathias Friedrich³, Ursula Ehmer¹, Maxim Barenboim^{1,2}, Katja Steiger³, Roman Maresch^{1,2}, Nina Gross^{1,2}, Sebastian Müller¹, Ulf Geumann^{1,2}, Irene Esposito³, Mathias Heikenwälder⁵, Ignacio Varela⁶, Dieter Saur^{1,2}, Allan Bradley³. 1) Department of Medicine; 2) Klinikum Rechts der Isar; Technische Universität München, München, Germany; 2) German Cancer Research Center (DKFZ), Heidelberg, & German Cancer Consortium (DKTK), Heidelberg, Germany; 3) Wellcome Trust Sanger Institute, Genome Campus, Hinxton/Cambridge, United Kingdom; 4) Department of Pathology; Klinikum Rechts der Isar; Technische Universität München, Trogerstrasse 18, 81675 München, Germany; 5) Institute of Virology; Klinikum Rechts der Isar; Technische Universität München, Schneckenburgerstraße 8, 81675 München, Germany; 6) Instituto de Biomedicina y Biotecnología de Cantabria, Santander, Spain.

OPENING AND PLENARY/PLATFORM SESSIONS

See Pages 6-7 for presentation schedule. Program number and presenter are in **bold**. Full abstracts begin on page 29.

13 - 10:30

PDGFR α signaling regulates a fibro/adipogenic switch to initiate fibrosis. **T. Iwayama**, L. Olson. Immunobiology & Cancer Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK.

14 - 10:45

Disabled-2 (Dab2) knockout shows an essential role in primitive endoderm development and multiple physiological functions through modulation of endocytosis and signaling. Wensi Tao, Robert Moore, Yue Meng, Elizabeth Smith, **Xiang-Xi (Mike) Xu**. Cell Biology, University of Miami, Miami, FL.

15 - 11:00

Modeling the initiation, progression and therapy of BRAF mutated cancers in mice. **Martin McMahon**¹, Joseph Juan², Rosalie Seers¹, Teruyuki Murachguchi¹, John Edward van Veen¹, Anny Shai¹, Shon Green¹, Wayne Phillips³. 1) Helen Diller Family Comprehensive Cancer Center, U.C. San Francisco, San Francisco, CA; 2) Knight Cancer Institute, Oregon Health & Science University, Portland, OR 97239; 3) Peter MacCallum Cancer Centre 1 St. Andrew's Place, East Melbourne Victoria 3002, Australia.

Tuesday, September 30 2:00 pm–3:00 pm
Merrill Hall

Keynote Address

Chair: Philippe Soriano, Mt. Sinai, New York

16 - 2:00

Using mouse models to identify disease suppressors: A genetic approach to therapeutic intervention. **Monica Justice**. Genetics and Genome Biology, Hospital for Sick Children, Toronto, Ontario, Canada.

OPENING AND PLENARY/PLATFORM SESSIONS

See Pages 6-7 for presentation schedule. Program number and presenter are in **bold**. Full abstracts begin on page 29.

Tuesday, September 30 7:15 pm–9:45 pm
Merrill Hall

Plenary/Platform Session 3 - Epigenetics

Chair: Marisa Bartolomei, U Penn, Philadelphia, PA

Presentations:

17 - 7:15

Mouse models and the hallmarks of aging. Carlos Lopez-Otin. Bioquímica y Biología Molecular, IUOPA, Universidad de Oviedo, Oviedo, Spain.

18 - 7:45

Derepression of early embryonic histone H3 lysine 27 trimethylation occurs via KDM6 demethylase independent mechanisms. Karl B. Shpargel, Joshua Starmer, Della Yee, Michael Pohlers, Terry Magnuson. Department of Genetics, Carolina Center for Genome Sciences, and Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC.

19 - 8:00

Long Noncoding RNAs in Mouse Lineage Allocation. Mary Donohoe^{1,2}, Hugo Borges Pinto^{1,2}. 1) Burke Medical Research Institute, White Plains, NY; 2) Neuroscience and Cell & Dev Biology. Weill Cornell Medical College, New York, NY.

20 - 8:45

A step toward understanding the *Hox* gene colinearity. Kyoichi Isono, Mami Kumon, Haruhiko Koseki. RIKEN IMS-RCAI, Yokohama, Japan.

21 - 9:00 - CANCELLED

Key Mediators of ATR DNA Damage Signaling are Spatially Confined to Unpaired Chromosomes in Spermatocytes. Andrew Fedoriw, Yochiro Shibata, Yuna Kim, Weipeng Mu, Terry Magnuson. Genetics, University of North Carolina Chapel Hill, Chapel Hill, NC.

22 - 9:15

Epigenetic regulation of imprinted gene loci. Marisa Bartolomei. Dept Cell & Develop Biol, Univ Pennsylvania Sch Med, Philadelphia, PA.

OPENING AND PLENARY/PLATFORM SESSIONS

See Pages 6-7 for presentation schedule. Program number and presenter are in **bold**. Full abstracts begin on page 29.

Wednesday, October 1 8:30 am–11:45 am
Merrill Hall

Plenary/Platform Session 4 - Organogenesis

Chair: Ralf Adams, Max Planck Institute, Munster, Germany

Presentations:

23 - 8:30

Cardiac Myocyte Lineage Commitment. Jonathan Epstein. Cell and Developmental Biology, Prelman School of Medicine, Philadelphia, PA.

24 - 9:00

Bicaudal C1 promotes pancreatic NEUROG3⁺ endocrine progenitor differentiation and ductal homeostasis. L. A. Lemaire¹, J. Goulley², Y. H. Kim^{1,2}, S. Carat³, P. Jacquemin⁴, J. Rougemont³, D. B. Constam², A. Grapin-Botton^{1,2}. 1) DanStem, University of Copenhagen, Copenhagen, Denmark; 2) ISREC, EPFL, Lausanne, Switzerland; 3) Bioinformatics and Biostatistics Core Facility, EPFL, Lausanne, Switzerland; 4) de Duve Institute, Université catholique de Louvain, Brussels, Belgium.

25 - 9:15

The linear ubiquitin-specific deubiquitinase Gumbby (Fam105b/Otulin) regulates angiogenesis and Wnt signaling. E. Rivkin^{1,2}, S. M. Almeida^{1,2}, D. F. Ceccarelli¹, Y.-C. Juang¹, T. A. MacLean^{1,2}, T. Srikumar³, H. Huang¹, R. Fukumura⁴, G. Xie¹, Y. Gondo⁴, B. Raught⁴, A.-C. Gingras^{1,2}, F. Sicheri^{1,2}, S. P. Cordes^{1,2}. 1) Lunenfeld-Tanenbaum Research Institute, Mt Sinai Hospital, Toronto, ON, Canada; 2) Department of Molecular Genetics, University of Molecular Genetics, University of Toronto, Toronto, ON, Canada; 3) Department of Medical Biophysics, University of Toronto and OCI, Princess Margaret Cancer Centre, Toronto, ON, Canada; 4) Mutagenesis and Genomics Team, RIKEN BioResource Center, Tsukuba, Ibaraki, Japan.

26 - 9:30

Autocrine role of fibronectin in neural crest development and smooth muscle differentiation. Sophie Astrof, Xia Wang, Dong Liang. Medicine, Thomas Jefferson University, Philadelphia, PA.

27 - 9:45

Foxn1 levels in thymus organogenesis and maintenance - too much is not enough. Nancy Manley, Jie Li, Brian Condie. Genetics, University of Georgia, Athens, GA.

28 - 10:30

The *Osr2* gene regulatory network in palate development. Yu Lan, Jing Zhou, Han Liu, Jingyue Xu, Eunah Chung, Joo-Seop Park, Shihai Jia, Bruce Aronow, Sunghee Oh, Rulang Jiang. Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

OPENING AND PLENARY/PLATFORM SESSIONS

See Pages 6-7 for presentation schedule. Program number and presenter are in **bold**. Full abstracts begin on page 29.

29 - 10:45

Pbx-dependent regulation of Snail1 controls mammalian face morphogenesis by promoting epithelial plasticity. **Licia Selleri**¹, Bingsi Li¹, Elisabetta Ferretti¹, Laura Quintana Rio¹, Jennifer Feenstra¹, Trevor Williams², Theresa Vincent¹, Anthony Brown¹. 1) Cell and Developmental Biology, Weill Medical College of Cornell University, New York, NY; 2) Craniofacial Biology and Cell and Developmental Biology, University of Colorado Denver, Denver, Aurora, CO.

30 - 11:00

Wnt signaling in craniofacial development and anomalies. Arjun Stokes^{1,2}, **Chengji Zhou**^{1,2}. 1) Department of Biochemistry and Molecular Medicine, UC Davis School of Medicine, Sacramento, CA; 2) Institute for Pediatric Regenerative Medicine, Shriners Hospitals for Children & UC Davis School of Medicine, Sacramento, CA.

31 - 11:15

Cellular and molecular principles of organ-specific blood vessel growth. **Ralf H. ADAMS**. Tissue Morphogenesis, Max Planck Institute for Molecular Biomedicine, Münster, Germany.

Wednesday, October 1 2:00 pm–3:00 pm
Merrill Hall

Rosa Beddington Lecture

Chair: Anna-Katerina Hadjantonakis, Sloan Kettering Institute, New York

Presentation:

32 – 2:00

The life of breath: stem cells of the lung. **Brigid I. M. Hogan**. Department of Cell Biology, Duke University Medical Center, Durham, NC 27710.

OPENING AND PLENARY/PLATFORM SESSIONS

See Pages 6-7 for presentation schedule. Program number and presenter are in **bold**. Full abstracts begin on page 29.

Wednesday, October 1 7:15 pm–10:30 pm
Merrill Hall

Plenary/Platform Session 5 - Development

Chair: Xin Sun, University of Wisconsin, Madison

Presentations:

33 - 7:15

Extracellular micro-environmental cues control specification of cell fate and behaviors in the early mouse embryo. **Isao Matsuo**¹, Ryuji Hiramatsu^{1,2}, Kayo Shimokawa¹, Chiharu Kimura-Yoshida¹. 1) Osaka Med Ctr for Maternal & Chi, Izumi, Osaka, Japan; 2) NIID, Musashi-Murayama, Tokyo, Japan.

34 - 7:45

Dynamic cell behaviors and commitment to pluripotency in the mouse blastocyst. **M. Kang**^{1,2}, A. Puliafito³, P. Xenopoulos¹, S. Di Talia⁴, A.-K. Hadjantonakis¹. 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; 3) Laboratory of Cell Migration, Candiolo Cancer Institute - FPO, IRCCS, Candiolo, Torino, Italy; 4) Department of Cell Biology, Duke University Medical Center, Durham, NC.

35 - 8:00

BMP signaling: Coordination of Ventral Folding Morphogenesis and L/R patterning. **Svetlana Gavrilov**, Elizabeth Lacy. Developmental Biology Program, Sloan-Kettering Institute, New York City, NY.

36 - 8:15

Loss of O-fucosylation on ADAMTS9 disrupts patterning in POFUT2 mutant embryos. **Bernadette Holdener**¹, Megumi Takeuchi¹, Deepika Vasudevan¹, Hideuki Takeuchi¹, Richard Grady^{1,2}, Suneel Apte², Robert Haltiwanger¹. 1) Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY 11794-5215; 2) Department of Biomedical Engineering, Cleveland Clinic, Cleveland, OH 44195.

37 - 8:30

Wnt/ β -catenin signalling directly regulates *Sox17* expression and is essential for organizer and endoderm formation in the mouse. **Silvia Engert**^{1,2}, Ingo Burtscher^{1,2}, W.Perry Liao¹, Stanimir Dulev³, Gunnar Schotta³, Heiko Lickert^{1,2}. 1) Institute of Stem Cell Research, Helmholtz Zentrum München, Germany; 2) Institute of Diabetes and Regeneration Research, Helmholtz Zentrum München, Germany; 3) Adolf-Budenandt Institute and Munich Center for Integrated Protein Science (CiPSM), Ludwig-Maximilian University, Germany.

OPENING AND PLENARY/PLATFORM SESSIONS

See Pages 6-7 for presentation schedule. Program number and presenter are in **bold**. Full abstracts begin on page 29.

38 - 9:15

Novel mode of defective neural tube closure in the non-obese diabetic (NOD) mouse strain.

J. Michael Salbaum, Nils J. Herion, Claudia Kruger, Jacalyn MacGowan, **Claudia Kappen**.

Pennington Biomedical Research Center, Baton Rouge, LA.

39 - 9:30

Arl13b functions in cilia to regulate canonical and non-canonical Shh signaling in Joubert Syndrome pathogenesis. Laura Mariani^{1,2}, Maarten Bijlsma³, **Tamara Caspary**¹. 1) Dept

Human Gen, Emory Sch Med, Atlanta, GA; 2) Graduate Program in Neuroscience, Emory Univ, Atlanta, GA; 3) Laboratory for Experimental Oncology and Radiobiology, Academic Medical Center, Amsterdam.

40 - 9:45

Eph/ephrin signaling mechanisms in the neurocristopathy craniofrontonasal syndrome.

Jeffrey O. Bush^{1,2}, Audrey K. O'Neill^{1,2}, Andrew Larson^{1,2}. 1) Department of Cell and Tissue Biology and Program in Craniofacial and Mesenchymal Biology, UCSF, San Francisco, US; 2)

Institute for Human Genetics, UCSF, San Francisco, UCSF.

41 - 10:00

Building of a Functional Lung: Lessons from Mouse Models of Lung-Related Birth Defects.

Xin Sun. Laboratories of Genetics, University of Wisconsin, Madison, WI.

OPENING AND PLENARY/PLATFORM SESSIONS

See Pages 6-7 for presentation schedule. Program number and presenter are in **bold**. Full abstracts begin on page 29.

Thursday, October 2 8:30 am–11:45 am
Merrill Hall

Plenary/Platform Session 6 - Disease

Chair: Markus Grompe, OHSU, Portland, OR

Presentations:

42 - 8:30

Concurrent ARID1A loss and PIK3CA activation initiates ovarian clear cell tumorigenesis. Ron Chandler, Jesse Raab, **Terry Magnuson**. Dept Genetics, CB 7264, Univ North Carolina, Chapel Hill, NC.

43 - 9:00

Dedifferentiated smooth muscle cells enhance atherosclerotic plaque initiation and progression in hypercholesterolemic mice. Lorin Olson, Chaoyong He. Immunobiology and Cancer, Oklahoma Medical Research Foundation, Oklahoma City, OK.

44 - 9:15

Studies of the functional consequences of aberrant pre-mRNA splicing in new mouse models. Katherine Yates, Dandan He, Zhengqiu Zhou, Randi Mackler, Chelsea Moherman, Yanqiang Wang, Keiko Akagi, Jingfeng Li, David Symer. Human Cancer Genetics Program, The Ohio State University Comprehensive Cancer Center, Columbus, OH.

45 - 9:30

Delayed ependymal development and juvenile hydrocephalus in *Jhy^{lacZ}* mice. Hilmarie Muniz-Talavera, Brian Grewe, **Jennifer Schmidt**. Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL.

46 - 9:45

Mouse Models of Human Diseases with Mutations in Nonmuscle Myosin II. Xuefei Ma, Yingfan Zhang, Connie Lerma, Mary Anne Conti, Robert Adelstein. LMC, NHLBI/NIH, Bethesda, MD.

47 - 10:30

Mechanism and prevention of the folate-resistant neural tube defects. Chengji Zhou^{1,2}, Tianyu Zhao², Arjun Stokes^{1,2}. 1) Department of Biochemistry and Molecular Medicine, UC Davis School of Medicine, Sacramento, CA; 2) Institute for Pediatric Regenerative Medicine, Shriners Hospitals for Children & UC Davis School of Medicine, Sacramento, CA.

48 - 10:45

A mouse model for gene-environment interaction in holoprosencephaly. Mingi Hong, Robert Krauss. Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY.

OPENING AND PLENARY/PLATFORM SESSIONS

See Pages 6-7 for presentation schedule. Program number and presenter are in **bold**. Full abstracts begin on page 29.

49 - 11:00

A recessive ENU screen identifies *Memo1* as a novel gene driving palatogenesis and cranial-base development. Eric Van Otterloo, David Clouthier, Lee Niswander, Trevor Williams.
University of Colorado - Denver, Anschutz Medical Campus, Aurora, CO.

50 - 11:15

Mice chimeric for human tissues for disease modeling and preclinical research. Markus Grompe. Oregon Stem Cell Ctr, Oregon Hlth & Sci Univ, Portland, OR.

OPENING AND PLENARY/PLATFORM SESSIONS

See Pages 6-7 for presentation schedule. Program number and presenter are in **bold**. Full abstracts begin on page 29.

Thursday, October 2 2:00 pm–4:30 pm
Merrill Hall

Plenary/Platform Session 7 - Technology

Chair: Maria Jasin, Sloan Kettering Institute, New York

Presentations:

51 - 2:00

Phenotyping Embryonic Lethals with Three-dimensional Imaging and Computer Analysis. **R. Mark Henkelman**, Michael Wong. Mouse Imaging Centre, Hospital for Sick Children, Toronto, Ontario, Canada.

52 - 2:30

Generation of newborn mice from embryonic stem cells using epiblast complementation chimeras. Yeonsoo Yoon, Joy Riley, Judith Gallant, Stephen N. Jones, **Jaime A. Rivera-Perez**. Dept of Cell and Developmental Biology, Univ of Massachusetts Medical School, Worcester

53 - 2:45

Isolation of rare recombinants without using selectable markers for one-step seamless BAC mutagenesis. George T. Lyozin¹, Paul C. Bressloff², Amit Kumar³, Yasuhiro Kosaka¹, Bradley L. Demarest⁴, H. Joseph Yost^{1,4}, Michael R. Kuehn³, **Luca Brunelli**¹. 1) Dept of Pediatrics, Univ of Utah School of Medicine, Salt Lake City; 2) Dept of Mathematics, Univ of Utah, Salt Lake City; 3) Laboratory of Protein Dynamics and Signaling, National Cancer Institute, Frederick, MD; 4) Dept of Neurobiology and Anatomy, Univ of Utah School of Medicine, Salt Lake City.

54 - 3:30

A ‘conditional-ON’ mouse model of Fibrosyplasia Ossificans Progressiva (FOP). **C. J. Schoenherr**¹, L. Huang¹, S. J. Hatsell¹, L. Xie¹, K. Nannuru¹, K. Feeley¹, T. Persaud¹, P. Yang¹, V. Idone¹, A. Lee², P. Yu³, A. J. Murphy¹, A. N. Economides¹. 1) Regeneron Pharmaceuticals, Tarrytown, NY; 2) National Center for Advancing Translational Sciences, NIH, Rockville, MD; 3) Brigham and Women’s Hospital, Boston, MA.

55 - 3:45

HNF4A and FOXA2 exhibit differentiation-dependent enhancer switching in response to Hippo signalling. **P. Hoodless**¹, O. Alder¹, R. Cullum¹, S. Lee¹, A. Kan¹, W. Wei¹, Y. Yi¹, V. Garside¹, M. Bilenky², M. Griffith², S. Morrissy², G. Robertson², N. Thiessen², Y. Zhao², Q. Chen³, D. Pan³, S. Jones², M. Marra². 1) Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada; 2) Michael Smith Genome Sciences Centre, BC Cancer Agency; 3) Johns Hopkins University School of Medicine.

56 - 4:00

Meiotic recombination mechanisms and hotspot evolutionary dynamics revealed by mouse tetrad analysis. **Maria Jasin**. Developmental Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY.

OPENING AND PLENARY/PLATFORM SESSIONS

See Pages 6-7 for presentation schedule. Program number and presenter are in **bold**. Full abstracts begin on page 29.

Friday, October 3 8:30 am–11:00 am
Merrill Hall

Plenary/Platform Session 8 - Stem Cells

Chair: Olivier Pourquié, IGBMC, Strasbourg, France

Presentations:

57 - 8:30

Dissecting the role of PW1/Peg3: a parentally imprinted gene that is expressed in all adult stem cells. **David Sassoon**, Rosamaria Correra, Anne-Lyse Denizot, Luigi Formicola, David Ollitrault, Jean-Remy Courbard, Karo Tanaka, Kateryna Kyrylkova, Sergiy Kyryachenko, Giovanna Marazzi. Stem Cells Regenerative Medicine, Univ. Paris/INSERM/ICAN, France.

58 - 9:00

Transit-Amplifying Cells Orchestrate Stem Cell Activity and Tissue Regeneration. **Ya-Chieh Hsu**¹, Lishi Li², Elaine Fuchs². 1) Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA; 2) Rockefeller University, New York, NY.

59 - 9:15

Robo4-mediated vascular integrity regulates hematopoietic stem cell trafficking. **Camilla Forsberg**, Stephanie Smith-Berdan, Andrew Nguyen, Matthew Hong. Univ of Cal, Santa Cruz

60 - 10:00

The dual role of SOX2 in pituitary development. **S. Goldsmith**, R. Lovell-Badge, K. Rizzoti. Stem Cell Biology and Developmental Genetics, MRC NIMR, London, United Kingdom.

61 - 10:15

Delineating the mesendoderm lineage hierarchy in culture. **Ingo Bartscher**^{1,2}, Dapeng Yang^{1,2}, Michael Schwarzfischer³, Carsten Marr³, Fabian Theis³, Martin Irmeler⁴, Johannes Beckers⁴, Heiko Lickert^{1,2}. 1) Inst of Diabetes and Regeneration Research, Helmholtz Centre Munich, Germany; 2) Inst of Stem Cell Research, Helmholtz Centre Munich, Germany; 3) Inst of Computational Biology, Helmholtz Centre Munich, Germany; 4) Inst of Experimental Genetics, Helmholtz Centre Munich, Germany.

62 - 10:30

A novel pathway involving the Chromatin-remodeling protein Rere acts downstream of retinoic acid signaling to control embryonic symmetry. **Oliver Pourquié**^{1,2}, Goncalo Vilhais-Neto¹. 1) Institut de Génétique et de Biologie Cellulaire et Moléculaire (IGBMC), Inserm U964, CNRS (UMR 7104), Université de Strasbourg, ILLKIRCH, FRANCE; 2) Dept of Genetics, Harvard Medical School, Dept of Pathology, Brigham and Woman's Hospital, BOSTON.

OPENING AND PLENARY/PLATFORM SESSIONS

See Pages 6-7 for presentation schedule. Program number and presenter are in **bold**. Full abstracts begin on page 29.

Friday, October 3 11:00 am–11:05 am
Merrill Hall

Closing Remarks

Chair: Yumiko Saga, National Institute of Genetics, Mishima, Japan

POSTER SESSIONS

See Page 5 for presentation schedule. Poster board number and presenter are in **bold**. Full abstracts begin on page 73.

POSTER LEGEND

Disease Modeling.....	63-67
Genetics and Genomics.....	68-74
Organogenesis Cancer	75-81
Patterning.....	82-83
Stem Cells.....	84-86
Technology	87-90

POSTER SESSIONS

See Page 5 for presentation schedule. Poster board number and presenter are in **bold**. Full abstracts begin on page 74.

Disease Modeling

63

Disruption of the mouse *Jhy* gene causes juvenile hydrocephalus. **Brian Grewe**¹, Oliver K. Appelbe², Bryan Bollman¹, Ali Attarwala¹, Lindy A. Tribes¹, Hilmarie Muniz-Talavera¹, Jennifer V. Schmidt¹. 1) Biological Sciences, University of Illinois-Chicago, Chicago, IL; 2) Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL.

64

An initiative for mechanistic study on non-cancer health effects by ionizing radiation: a comprehensive and systematic phenotyping of irradiated mice. **Yoshinobu Harada**¹, Hitomi Sudo², Toshiyuki Saito³, Makoto Akashi¹. 1) Fukushima Project Headquarters, NIRS, Chiba, Japan; 2) Molecular Imaging Center, NIRS, Chiba, Japan; 3) Research Center for Charged Particle Therapy, NIRS, Chiba, Japan.

65

Quantitative trait loci for early-stage atherosclerosis at different aortic locations. **Y. Kayashima**, K. Matsuki, H. Tomita, N. Maeda. Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC.

66

The Mouse Hydrocephalus Gene *JHY* Plays a Role in Ependymal Cell Differentiation. **Hilmarie Muniz-Talavera**¹, Jennifer V. Schmidt². 1) University of Illinois-Chicago, Chicago, IL; 2) University of Illinois-Chicago, Chicago, IL.

67

Runx1/Cbfb transcription factor complex is essential in maintaining articular cartilage. **Clara Y. Park**, Xiangguo Che, Na-Rae Park, Seung Hee Han, Gyoung-Hwa Kim, Je-Yong Choi. Kyungpook National University School of Medicine, Daegu, South Korea.

Genetics and Genomics

68

A cre tool resource for conditional mutagenesis in the mouse, www.creportal.org. **Randy Babiuk**, Martin Ringwald, Steve Murray, Janan T. Eppig. The Jackson Laboratory, Bar Harbor, ME.

69

Vitamin D receptor in mature osteoclasts suppresses osteoclastogenesis and enhances bone resorption in male mice. **Gyeong-hwa Kim**¹, Xiangguo Che¹, Yong-Joo Clara Park¹, Na-Rae Park¹, Seung-Hee Han¹, Shigeaki Kato², Je-Yong Choi¹. 1) Department of Biochemistry and Cell Biology, School of Medicine, Kyungpook National University, Daegu, South Korea; 2) Soma Central Hospital (S.K.), Fukushima, Japan.

70

Noncoding RNA transcription targets AID to divergently transcribed loci in B cells. **Evangelos Pefanis**^{1,3}, Jiguang Wang^{1,2}, Gerson Rothschild¹, Junghyun Lim¹, Jaime Chao¹, Raul Rabadan², Aris Economides³, Uttiya Basu¹. 1) Columbia University, Department of Microbiology & Immunology, New York, NY; 2) Columbia University, Department of Biomedical Informatics, New York, NY; 3) Regeneron Pharmaceuticals, Tarrytown, NY.

71

Using RNA-Sequencing in Linkage Analysis of Complex Traits: Evolution of response to LPS. **Alexander N. Poltorak**^{1,2}, Vladimir Ilyuha², Maria Churova², Irina Kurbatova², Uliana Bagina², Volkova Tatyana². 1) Tufts University, Boston, MA; 2) Petrozavodsk State University, Karelia, Russia.

72

A genome-edited allele of *Nxf1* precisely identifies a modifier of retrovirus mutations. **Kevin Ross**¹, Dorothy Concepcion², Bruce Hamilton^{1,2}. 1) Biomedical Sciences Graduate Program, University of California, San Diego, La Jolla, CA; 2) Department of Cellular & Molecular Medicine and Institute for Genomic Medicine, University of California, San Diego, La Jolla, CA.

POSTER SESSIONS

See Page 5 for presentation schedule. Poster board number and presenter are in **bold**. Full abstracts begin on page 74.

73

Insights into brain isoforms of neurotrophin receptor P75NTR. Mohamed Sabry¹, Mona Fares², Ronnie Folkesson³, Mariam Al-Ramadan⁴, Adel Alalwan¹, Jarrah Alabkal¹, Ahmed Baqer¹, Moustapha Hassan^{2,5}. 1) Department of Medical Biochemistry, College of Medicine & Medical Sciences, Arabian Gulf University, P.O.Box 26671 Manama, Bahrain; 2) Experimental Cancer Medicine (ECM), laboratory Medicine, Karolinska Institutet, Stockholm, Sweden; 3) Department of Neurobiology, Care Sciences and Society (NVS), Karolinska Institutet, Stockholm, Sweden; 4) Biotechnology Program, College of Postgraduate Studies, Arabian Gulf University, Manama, P O Box 26671, Bahrain; 5) Clinical Research Centre, Novum, Karolinska University Hospital-Huddinge, Sweden.

74

The emerging Chr1 substitution mice established from Chinese wild donors. Junhua Xiao¹, Kai Li¹, Yuxun Zhou¹, Yinming Liang², Tianzhu Chao¹, Fuyi Xu¹. 1) Donghua University, Shanghai, China; 2) Xinxiang Medical University, Henan, China.

Organogenesis

75

Characterization of platelet-derived growth factor-A expression in mouse tissues using a lacZ knock-in approach. Johanna Andrae¹, Leonor Gouveia¹, Liqun He¹, Christer Betsholtz^{1,2}. 1) Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden; 2) Department of Medical Biochemistry and Biophysics, Division of Vascular Biology, Karolinska Institute, Stockholm, Sweden.

76

Deficiency in Maged2 results in impaired myogenic differentiation. E. Srour¹, M. Delimoy¹, R. Rezsosazy², J. Marshal³, L. Kunkel³, O. De Backer¹. 1) URPHYM, University of Namur, Namur, Belgium; 2) Institut des Sciences de la Vie, University Catholic of Louvain, Louvain-la-Neuve, Belgium; 3) Boston Children's Hospital, Boston, MA.

77

Novel Role of Fgf Signaling in Eye Development. T. Abe, Y. Yamashita, Y. Mukumoto, A. Denda, H. Kiyonari, Y. Furuta. RIKEN Center for Developmental Biology, Kobe, Japan.

78

Rho GTPases and gastrulation. Navrita Mathiah, Isabelle Migeotte. IRIBHM, ULB, Brussels, Belgium.

79

Cell autonomous and nonautonomous factors shape cell fate decisions in the third pharyngeal pouch. John O'Neil¹, Nancy Manley². 1) University of Georgia Athens, GA; 2) University of Georgia.

80

PDGFRA signaling negatively regulates white and beige adipogenesis *in vivo*. Chengyi Sun^{1,2}, Lorin Olson^{1,2}. 1) IMCA, Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

81

Using conditional knockout mice to elucidate the function of Cdk5rap3 in liver development. Rui Yang, Yu-yan Jia, Lin Liu, Huang Yue. Department of Medical Genetics, Institute of Basic Medical Sciences, Peking Union Medical College, Beijing, China.

Patterning

82

A regulatory network controls *Nephrocyan* expression and midgut patterning. P. Hoodless¹, J. Hou¹, W. Wei¹, R. Saund², P. Xiang¹, T. Cunningham³, D. Lu¹, J. Savory⁴, N. Krentz¹, R. Montpetit¹, R. Cullum¹, D. Lohnes⁴, K. Humphries¹, G. Duester³, Y. Yamanaka⁵, Y. Saijoh². 1) Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada; 2) Dept of Neurobiology and Anatomy, University of Utah, Utah; 3) Development, Aging and Regeneration Program, Sanford-Burnham Medical Research Institute, La Jolla, California; 4) Cellular and Molecular Medicine, University of Ottawa, Ottawa, Canada; 5) Goodman Cancer Research Centre, Dept of Human Genetics, McGill University, Montreal, QC, Canada.

83

FGF signaling pathways in development. J. Richard Brewer, Pierre Mazot, Philippe Soriano. Developmental and Regenerative Biology, Icahn School of Medicine at Mt. Sinai, New York, NY 10029.

POSTER SESSIONS

See Page 5 for presentation schedule. Poster board number and presenter are in **bold**. Full abstracts begin on page 74.

Stem Cells

84

Generation and characterisation of germ line competent ES cell lines from C57Bl/6J. Purnima S. Sailasree¹, Partha Sarathi D.², Satish Kumar³. 1) Centre Cellular and Molecular Biology, Uppal main road, Hyderabad, Telangana, 500007India; 2) Centre Cellular and Molecular Biology, Uppal main road, Hyderabad, Telangana, 500007India; 3) National Institute for Animal Biotechnology, Miyapur, Hyderabad, Telangana 500049, India.

85

Spatiotemporal analysis of cell fate decisions during preimplantation development. Nestor Saiz Arenales, Nadine Schrode, Anna-Katerina Hadjantonakis. Developmental Biology, Sloan Kettering Institute, New York, NY.

86

***In vitro* screening of homozygous mutant mouse ES cells identified a novel gene essential for hematopoietic development. M. Tokunaga, C. Kokubu, J. Takeda.** Department of Social and Environmental Medicine, Osaka University, Suita, Osaka, Japan.

Technology

87

A novel vector configuration dramatically improves recovery of gene targeted ES cell clones. Jason Lawrence, Robert Williams, Clare Bender, Nicole Christ, Steve Sheardown, Kate Brown, Joanne Doran. Takeda Cambridge Ltd., 418 Cambridge Science Park, Cambridge, CB4 0PA, United Kingdom.

88

Generation of mutant mouse lines using endonuclease technologies at the ICS (the French Mouse Clinic). GUILLAUME PAVLOVIC^{1,2,3,4}, PHILIPPE ANDRE^{1,2,3,4}, SYLVIE JACQUOT^{1,2,3,4}, MARIE-CHRISTINE BIRLING^{1,2,3,4}, YANN HERAULT^{1,2,3,4}. 1) Institut Clinique de la Souris - ICS-MCI, PHENOMIN, Illkirch, France; 2) Centre National de la Recherche Scientifique, UMR7104, Illkirch, France; 3) Institut National de la Santé et de la Recherche Médicale, U964, Illkirch, France; 4) Université de Strasbourg, Illkirch, France.

89

Nuclease-Mediated Gene Knockout in an Allele Refractory to Gene Targeting in ES Cells. Filipiak Wanda¹, Zeidler Michael¹, Hughes Elizabeth¹, Gavrilina Galina¹, Mary Schmidt¹, Jifeng Zhang², Leslie Satin³, Thomas Saunders^{1,2}. 1) University of Michigan Medical School, Biomedical Research Core Facilities, Transgenic Core, Ann Arbor, MI; 2) University of Michigan Medical School, Department of Internal Medicine, Ann Arbor, MI; 3) University of Michigan Medical School, Department of Pharmacology, Ann Arbor, MI.

90

Rapid generation of mouse and rat mutants by CRISPR/Cas in one-cell embryos. Jochen Welcker¹, Sonja Ortmann¹, Simone Janzen¹, Jost Seibler¹, Jon Chesnut², Kai Schönig³. 1) TaconicArtemis GmbH, Neurather Ring 1, 51063 Cologne, Germany; 2) Thermo Fisher Scientific, Carlsbad, USA; 3) Central Institute of Mental Health, Heidelberg University, Department of Molecular Biology, J 5, 68159 Mannheim, Germany.

Platform Session 1 - Genetics and Genomics

1

Modeling population genetic heterogeneity to identify novel genes regulating alcohol metabolism.

David Threadgill, Christine Rubinshteyn. Department of Molecular and Cellular Medicine, Texas A&M University, College Station, TX.

Extensive evidence from human and animal studies supports the hypothesis that ethanol metabolism is a complex trait with both hereditary and environmental influences. Heritable factors underlying ethanol metabolism are of considerable interest as they most likely attribute to inter-individual variations in its toxicity, sensitivity, and preference. The majority of genetic mapping studies in rodents for ethanol metabolism have been limited to the BXD (C57BL/6J x DBA/2J) recombinant inbred lines or F2 crosses in rats. However, results from these studies have not lead to the identification of candidate genes for the detected QTLs or physiological characteristics that may be associated with differential ethanol metabolism. We have analyzed Collaborative Cross (CC) mice, a large panel of multi-parental recombinant inbred mouse lines, to characterize the phenotypic diversity of blood ethanol clearance (BEC) rates. Male CC mice were administered ethanol and the change in blood ethanol concentrations over time were used to calculate BEC rates. A total of 192 CC mice were examined with clearance rates ranging between 0 and 677 mg/kg/hr (113 ± 99 ; mean \pm SD) compared to 10 to 358 mg/kg/hr in founder strains. The spectrum of diversity in BEC rates among CC mice is unprecedented in comparison to other mouse genetic resources. Surprising, polymorphisms in the opioid receptor delta 1 (Oprd1) gene were most strongly associated with BEC. Using both knockout and pharmacologic inhibition, Oprd1 was validated as a regulator of BEC, indicating a novel mechanism regulating alcohol metabolism control by the liver. The CC presents a unique model to study genetic associations that contribute to diseases and phenotypes with complex etiologies.

2

The Sanger Mouse Genetics Project: High Throughput Recessive Lethality Screen. **Antonella Galli**¹, Angela Green¹, Cecilia Icoresi Mazzeo¹, Catherine Jones¹, Charlotte Lillistone¹, Emma Siragher¹, Luke Souter¹, Elizabeth Tuck¹, Ed Ryder¹, Tim Mohun², Ramiro Ramirez-Solis¹, David Adams¹, Jacqueline White¹. 1) Mouse Genetics Project, Wellcome Trust Sanger Institute, Cambridge, UK; 2) Division of Developmental Biology, MRC National Institute For Medical Research, London, United Kingdom.

The Sanger Institute Mouse Genetics Project (MGP) is a major contributor in the worldwide effort to develop mouse models to understand human genetic diseases. The MGP generates, cryopreserves and performs primary phenotypic characterization of over 160 lines of mice every year. A standardised battery of primary phenotyping tests is performed on all lines without any prior assumptions about gene function. To date, 35% of the 984 lines studied by the MGP are classified as lethal or sub-viable at postnatal day 14 due to non-Mendelian homozygous viability rates. To explore potential defects during embryogenesis, the viability and morphology of over 300 of these lines have been assessed during organogenesis at embryonic day 14.5 (E14.5). Any dysmorphology including growth retardation, oedema, craniofacial, skeletal and neural tube defects are recorded and annotated. This recessive lethality screen has been extended thanks to a strategic award from the Wellcome Trust involving the Deciphering Mechanisms of Developmental Disease (DMDD) consortium, an innovative and ambitious programme of research involving members of the UK developmental biology community. This programme includes additional phenotyping tests (encompassing embryonic days E9.5, E14.5 and E18.5) and will use a combination of comprehensive whole embryo 3D imaging, placental histopathology, transcriptomics and nervous system functionality assessment in order to identify abnormalities in embryo structure and development. All data is freely available, enabling individual researchers to identify lines relevant to their

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

research and provide valuable insight into novel gene functions and new mouse models of human developmental disorders.

3

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 resource of mouse developmental expression information. It covers all developmental stages and all organ systems and comprises data from wild-type and mutant mice. It collects and integrates data from RNA in situ hybridization, immunohistochemistry, in-situ reporter (knock-in), RT-PCR, northern blot, and western blot experiments. GXD has grown tremendously in recent years in terms of data content and search utilities. Through curation of the literature, electronic data submissions, and collaborations with large-scale data generators, GXD has acquired nearly 1.5 million expression results from over 67,200 expression assays for more than 13,800 genes, including expression data from nearly 2,000 mouse mutants. In addition, the database contains over 260,000 expression images. GXD annotates these images with extensive metadata such as the genes analyzed, probes used, strain and genotype of the specimens, and developmental stages and anatomical structures in which expression was analyzed. 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, thereby enabling users to search for expression data and images in many different ways, using a variety of biologically and biomedically relevant parameters. Recently, we have made many improvements to the search interface, such as implementing new type of data summaries, including one that shows all the images matching the search criteria; adding data filters to iteratively refine search summaries; and building an enhanced anatomy ontology and browser tool. Currently, we are developing interactive tissue-by-developmental stage and tissue-by-gene matrix views of expression data. These matrices will provide users with intuitive high-level summaries of expression data from where they can drill down to more detail. These new utilities, to be publicly released in October 2014, will also be presented at the meeting. Visit GXD at www.informatics.jax.org/expression.shtml. GXD is supported by NIH grant HD062499.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

4

Production and distribution of the EUCOMM/EUCOMMTOOLS knockout resources. Andreas Hoerlein¹, Antje Buerger¹, Wendy Bushell², Jun Fu³, Barry Rosen², Francis Stewart³, Wolfgang Wurst¹, William Skarnes². 1) Institute of Developmental Genetics, Helmholtz Zentrum München, Neuherberg, Germany; 2) Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, U.K; 3) Technische Universität Dresden, c/o Max Planck Institute of Molecular cell Biology and Genetics, 01307 Dresden, Germany.

EUCOMM and EUCOMMTOOLS are the European part of the International Knockout Mouse consortium (IKMC), a world-wide effort to generate knockout mouse resources for every protein coding mouse gene. To date, EUCOMM/EUCOMMTOOLS has generated a collection of more than 10,000 gene targeting vectors and conditional mutations in C57Bl/6N mouse embryonic stem (ES) cells available from the EuMMCR repository (www.eummcr.org). The EUCOMM/EUCOMMTOOLS resources are the foundation of international efforts to generate, archive and phenotype mouse mutants in a standardized and cost-effective manner. Recently, EUCOMMTOOLS and EuMMCR have initiated recovery efforts to rescue failed projects using CRISPR/Cas9 technology. The strategy involves the use linear-linear ET recombineering to shorten the 5' and 3' homology arms for high-efficiency CRISPR/Cas9-mediated gene targeting in ES cells. This method will enable the recovery of many hundreds of failed IKMC projects to complete the production goals of EUCOMM/EUCOMMTOOLS and will be offered as a service by the EuMMCR Repository.

This work has been performed in collaboration with all EUCOMM and EUCOMMTOOLS partners: http://www.mousephenotype.org/martsearch_ikmc_project/about/eucommtool.

5

CRISPR/Cas9(D10A) mediated engineering of human and mouse genomes. C. Jung, J. Sun, D. Kostecka, P. de Jong. Children's Hospital Oakland Research Institute, Oakland, CA.

The efficiency and specificity of CRISPR/Cas based technologies for engineering the genomes of various species is revolutionizing a wide array of scientific and clinical endeavors. In spite of the excitement engendered in these technologies however, much remains to be understood for their precise, targeted application. In this study, we seek to elucidate the underlying mechanisms governing the CRISPR/Cas mediated approach to stimulating homology directed repair (HDR), by knocking-in targeting constructs with varying lengths and designs of sequences homologous to regions flanking the double strand break. Preliminary data show more than 50 fold increase in the number of mESC colonies relative to negative control following selection, when transfected with targeting constructs carrying either ~5kb or ~800bp homology arm lengths, for which double strand DNA breaks were stimulated by either Cas9 nuclease or a pair of nickases (Cas9D10A). Analysis of individual clones via junction PCR and taqman loss of allele assay revealed more than 90% correct targeting. Interestingly, transfection of targeting constructs carrying homology arm sequences further away from the sgRNA cut site(s) led to a decreased number of colonies for constructs carrying ~800bp arms, while ~5kb arms remain unaffected. In view of these findings, we are evaluating the targeting efficiency of knock-in constructs with varying lengths of homology arm sequences in human ESCs, as well as in the mouse zygote. Furthermore, we are assessing the efficiency of targeting based on non-homologous end joining (NHEJ) approaches involving ligation (e.g., ObLiGaRe, single strand oligonucleotides). An in-depth analysis delineating the mechanism by which CRISPR/Cas systems mediate editing of various genomes allows for advances in technical development, as well as potential applications in basic sciences research and clinical therapeutics.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

6

RNF212 is a Meiosis-Specific SUMO E3-Ligase That Promotes Crossing-Over and Functions as a Meiotic Checkpoint Factor to Eliminate Defective Oocytes. **Huanyu Qiao**, Neil Hunter. Howard Hughes Medical Institute and Department of Microbiology and Molecular Genetics, UC Davis, Davis, CA.

The synapsis, recombination and segregation of homologous chromosomes (homologs) are essential features of meiosis that ensure formation of gametes with balanced euploid genomes. Defects in synapsis and recombination cause gametocytes to be eliminated via poorly characterized checkpoint processes. In females, pre- and post-partum attrition and atresia dictates the size of the resting oocyte pool and thus reproductive lifespan. Complete post-partum depletion of the oocyte pool is observed in a variety of mouse mutants that are defective for homologous recombination and/or chromosome synapsis. For example, *Spo11* mutants fail to initiate recombination, show severe defects in homolog synapsis and eliminate their oocyte pools within three months post partum¹. In *Msh4* mutants, recombination is initiated, but its progression is blocked and homolog synapsis is defective. The oocyte pools of *Msh4* mutants are depleted within four days of birth². Current evidence implies that two distinct branches of the meiotic checkpoint pathway respond to defects in recombinational repair or homolog synapsis, respectively³.

We have identified RNF212 as a novel component of the meiotic checkpoint machinery. Previously, we showed that RNF212 promotes crossing-over by selectively stabilizing pro-crossover factors at a subset of ongoing recombination sites. Unexpectedly, we now find that *Rnf212* mutation confers a striking restoration of the oocyte pools to both *Spo11* and *Msh4* mutant ovaries. Moreover, initial observations in males indicate that RNF212 also functions in spermatocyte quality control. Intriguingly, *Rnf212* encodes an RING-family E3-ligase that catalyzes protein modification by the ubiquitin-like molecule, SUMO. These data imply that RNF212-mediated SUMOylation is a novel component of the meiotic checkpoint-signaling pathway that leads to gametocyte apoptosis. Together, our results uncover a new aspect of meiotic checkpoint signaling and point to RNF212 as a central coordinator of chromosome synapsis, recombination and meiotic progression.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

7

Function and regulation of long-distance genomic interactions during mouse development. François Spitz. Developmental Biology Unit, EMBL, Heidelberg, Germany.

Expression of developmental genes is often controlled by sets of *cis*-acting elements that are distributed along large genomic intervals, far from the target gene promoter region. Integration of the inputs provided by these multiple, dispersed elements is an essential step to produce coherent, robust and specific regulatory programs. To understand the function of distant regulatory elements as well as the genomic and epigenomic features which organize their activities and ensure that enhancers act on the appropriate target genes, we generated mice carrying series of chromosomal rearrangements along of loci of interests. This functional, systematic dissection of large genomic intervals revealed their underlying regulatory architecture. Our studies underscore the tight link between regulatory organisation and spatial conformation of the genome, and show in particular that the formation of specific topological domains is essential to implement long-distance enhancer-gene interactions. In addition, the different mouse models generated shed light on the molecular consequences of genomic variations in non-coding intervals that have been associated with human pathologies and disease susceptibility, providing clues regarding their aetiolog.

Plenary/Platform Session 2 - Cancer

8

Tumor suppressor & tumor maintenance genes. **Scott Lowe.** Memorial Sloan-Kettering Cancer Center, New York, NY.

Cancer arises through an evolutionary process whereby normal cells acquire mutations that erode growth controls, leading to the inappropriate expansion of aberrantly proliferating cells. Such mutations can involve activation of oncogenes or inactivation of tumor-suppressor genes, each contributing one or more new capabilities to the developing cancer cell. However, cancer is not an inevitable consequence of oncogenic mutations; instead, cells acquiring such mutations can be eliminated or kept in check by innate tumor-suppressor programs that can be activated in these damaged cells. Our laboratory studies tumor-suppressor networks controlling apoptosis and senescence and how their disruption influences malignant behavior. To facilitate our research, we have recently developed new mouse cancer models based on the genetic manipulation of stem and progenitor cells *ex vivo* followed by transplantation of the altered cells into the appropriate organ of syngeneic recipient mice. This approach allows us to rapidly study the impact of many genes and gene combinations on tumorigenesis in a “mosaic” setting where tumor-initiating cells are embedded in normal tissues. Furthermore, we have developed powerful methods for using RNA interference to suppress gene function *in vivo* in either a stable and reversible manner. Current efforts in the laboratory strive to integrate mosaic mouse models, RNA interference, and cancer genomics to identify new components of tumor suppressor gene networks and characterize their impact on tumorigenesis and treatment response. In addition, we are optimizing and implementing new RNAi-based methods to identify tumor maintenance genes whose inhibition causes cancer regressions. Efforts to study the role of tumor suppressor gene loss in cancer maintenance, and new approaches to model complex mouse models, will be discussed.

9

Luminal cells are favored as the cell of origin for prostate cancer. **Zhu A. Wang**^{1,2}, Michael M. Shen¹. 1) Herbert Irving Cancer Center, Columbia University Medical Center, New York, NY; 2) MCD Biology, University of California Santa Cruz, Santa Cruz, CA.

The identification of cell of origin for cancer is significant since it has been implicated that distinct cell types within a tissue can give rise to different cancer subtypes that are distinguished by their histopathological phenotypes and patient outcomes. Many studies have investigated the cell of origin for cancer in mouse models by introducing a specific oncogenic insult within a defined cell type, and then determining whether cancer arises. Using this approach, our lab and others have shown that in the prostate both basal and luminal epithelial cells can initiate prostate cancer. However, it is still debated which cell type is the actual cell of origin under various tumor-initiating conditions *in vivo*.

Here, we use a lineage-tracing strategy to assess the cell of origin in a diverse range of mouse prostate cancer models, including *Nkx3.1*^{+/-}; *Pten*^{+/-}, *Pten*^{+/-}, *Hi-Myc*, and *TRAMP* mice, as well as in a hormonal carcinogenesis model where genetically-wildtype mice are administered a combination of testosterone and estradiol-17β. The novelty of our approach is that it dissociates the time of lineage-marking from the onset of tumorigenesis, thereby allowing multiple models to be analyzed and compared using the same overall strategy. Our results show that luminal cells, but not basal cells, are consistently the observed cell of origin for each model *in situ*; however, explanted mutant basal cells from these mice can generate tumors in grafts. Consequently, we propose that luminal cells are favored as cells of origin for prostate cancer in many physiological conditions, whereas basal cells only give rise to tumors after environmental conditions permit their luminal differentiation.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

10

Using a High Throughput Screen in Knockout Mice to Discover Genes Involved in the Host Response to Cancer. Jennifer Schmahl, Nicholas Gale. Therapeutic Targets, Regeneron Pharmaceuticals, Tarrytown, NY.

Recently an international consortium of mouse genetic research institutions (the IMPC) was formed to undertake a massive phenotyping screen in knockout mice, with the goal of gaining insight into the function of most mammalian genes. Most assays in this screen are focused on naïve adult mice, determining differences caused by these mutations in unchallenged states. We have undertaken a similar screen in mice with loss of function alleles with a focus on secreted and transmembrane genes, but we have included a challenge assay designed to identify genes that play a role in the host response to cancer. In this assay, tumors are engrafted in mice with loss of function alleles and changes in gene expression and tumor growth are assayed. Since this assay was put in place, we have screened nearly 400 knockout lines, identifying genes that may play a role in the development and/or maintenance of blood vessels within tumors, the immune response to tumor growth or in remodeling of tumor extracellular matrix (ECM). To our knowledge, this is the first report of a high throughput screen in tumors using targeted loss of function alleles.

Out of the nearly 400 genes screened, lacZ reporter staining identified roughly a third expressed within the tumor stroma. Expression patterns fell loosely into two categories: 19% were expressed in tumor vasculature and 15% were expressed in immune populations and other tumor infiltrating cells. Interestingly, about a quarter of the genes expressed within tumor vessels are restricted to the tumor, suggesting potential roles of these genes in tumor angiogenesis and highlighting them as specific markers of tumor vasculature. A small percentage of lines (~10%) show increases in tumor growth. Curiously, most of these lines appear to have metabolic function and known roles in glucose homeostasis, suggesting an extensive role of metabolic state in influencing tumor growth.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

11

Phenotype reproducibility of human colon cancer in murine models using multiple species and microbiome rederivation. **J Amos-Landgraf**^{1,2,4,5}, S Busi¹, T Parker¹, W Dove², G van den Brink³, M McCoy¹, A Ericsson^{1,4,5}, C Franklin^{1,4,5}, E Bryda^{1,4,5}. 1) Veterinary Pathobiology, University of Missouri, Columbia, MO; 2) McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI; 3) Amsterdam Medical Center, Amsterdam, Netherlands; 4) Rat Resource and Research Center, University of Missouri, Columbia, MO; 5) Mutant Mouse Regional Resource Center, University of Missouri, Columbia, MO.

Our studies address the recent recognition that mouse models often do not recapitulate human disease phenotypes, and that the same genetic model phenotype occasionally cannot be reproduced by other independent laboratories. We use mouse and rat models of human colon cancer with germline mutations in the tumor suppressor *Apc*. Unlike human colon cancer, mouse models develop tumors primarily in the small intestine. In contradistinction, the rat *Apc* mutant -Polyposis in the Rat Colon (*Pirc*)- model develops tumors primarily in the colon and also has a sex bias, with males developing twice as many tumors as females with a decreased time to morbidity, paralleling the human disease. We have determined in the rat, and in AOM treated mice, that this is largely due to male hormone promotion, as castration reduces the tumor multiplicities to female numbers and replacement of male hormones in castrated males restores tumor number levels. A decade of *Apc*^{Min} mouse data shows a similar sex bias in the mouse, however large numbers are required to show a statistical difference (n>50 per sex). We also investigated the role of the microbiome as a potential environmental modifier of our phenotype using 16S bacterial genome sequencing. Significant differences were observed in the microbiome of *Pirc* rats between congenic derivatives as well as between wild type and mutant animals within strains. Embryo rederivation of F344/NTac-*Apc*^{Pirc} using three different dam stocks from two different vendors as recipients showed sex-specific and enterotype-mediated differences in tumor numbers in the rederived pups. We have determined that sex and the microbiome play a significant role in disease phenotype in the rat and are investigating the role of the microbiome in mouse models of intestinal disease.

12

High-throughput cancer gene validation using CRISPR/Cas9-based somatic genome editing in mice. **Roland Rad**^{1,2}, Rupert Öllinger^{1,2}, Julia Weber^{1,2}, Mathias Friedrich³, Ursula Ehmer¹, Maxim Barenboim^{1,2}, Katja Steiger³, Roman Maresch^{1,2}, Nina Gross^{1,2}, Sebastian Müller¹, Ulf Geumann^{1,2}, Irene Esposito³, Mathias Heikenwälder⁵, Ignacio Varela⁶, Dieter Saur^{1,2}, Allan Bradley³. 1) Department of Medicine; 2) Klinikum Rechts der Isar; Technische Universität München, München, Germany; 2) German Cancer Research Center (DKFZ), Heidelberg, & German Cancer Consortium (DKTK), Heidelberg, Germany; 3) Wellcome Trust Sanger Institute, Genome Campus, Hinxton/Cambridge, United Kingdom; 4) Department of Pathology; Klinikum Rechts der Isar; Technische Universität München, Trogerstrasse 18, 81675 München, Germany; 5) Institute of Virology; Klinikum Rechts der Isar; Technische Universität München, Schneckenburgerstraße 8, 81675 München, Germany; 6) Instituto de Biomedicina y Biotecnología de Cantabria, Santander, Spain .

Novel approaches to cancer genome analysis, such as next generation sequencing or transposon-based genetic screening in mice are currently creating large catalogues of putative cancer genes for principally all malignancies. The challenge for the next decades of cancer research will be to validate these long lists of genes and dissect their biological function. Mouse models of cancer recapitulate the extensive biological complexity of the human disease. However, the speed and efficiency of such in vivo studies is currently limited by the long time-frames needed to genetically engineer, intercross and breed murine cancer models. Here, we demonstrate cancer induction in mice using CRISPR/Cas9-based somatic genome-editing. In an attempt to model intrahepatic cholangiocarcinoma (ICC) we delivered Cas9 nuclease and guide RNAs (gRNAs) against different tumour suppressor genes (TSGs) to the genome of hepatocytes by hydrodynamic tail vein injection and Sleeping Beauty transposition. Different gRNAs were designed against exonic sequences of 10 TSGs, of which several are typically altered in ICC. We observed multifocal ICC development, caused by CRISPR/Cas9-induced insertions/deletions of various

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

sizes (1 to >100 bases) in different combinations of TSGs. Many fundamental aspects of the disease could be studied using this approach, including differentiation tumour heterogeneity or tumour evolution. We provide evidence for selection of specific gRNAs in predisposing genetic contexts and also model the sequential accumulation of genetic alterations. Combinatorial use of specific gRNA pairs finally allowed us to perform targeted somatic deletions of large chromosomal regions. Somatic CRISPR/Cas9-based genome editing will substantially facilitate and accelerate cancer gene validation and gene function analysis in mice.

13

PDGFR α signaling regulates a fibro/adipogenic switch to initiate fibrosis. T. Iwayama, L. Olson. Immunobiology & Cancer Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK.

Fibrosis is an insidious disease process involving the destruction of normal tissue by deposition of extracellular matrix (ECM). All organs can be damaged by fibrosis, and recently adipose tissue fibrosis in mice and obese humans has been associated with insulin resistance and ectopic lipid accumulation. The molecular events that cause normal cells to become fibrogenic are unknown and it remains unclear what cell types are capable of undergoing this transition *in vivo*. We have shown previously that mice with a Cre/lox-inducible gain-of-function D842V point mutation in platelet-derived growth factor receptor α (PDGFR α ^{D842V}) develop spontaneous fibrosis in many organs, including adipose tissue. Lineage tracing studies from other laboratories have identified pericytes or pericyte-like cells as a cellular source of injury-induced fibrosis in the CNS, kidney, skeletal muscle, and skin. We hypothesized that increased PDGFR α signaling in pericytes would be sufficient to cause fibrosis in adipose tissue and other organs. To target pericytes with PDGFR α ^{D842V}, we tested a variety of Cre drivers including Ng2-Cre, PDGFR β -Cre, and Nestin-Cre, all of which led to systemic fibrosis with PDGFR α activation. However, Nestin-Cre was the most pericyte-specific Cre in adipose tissue and thus it became the focus of our study. Lineage-tracing in fibrotic skin, intestine, and adipose tissue of Nestin-Cre;PDGFR α ^{D842V} mice showed that most of the fibrogenic cells were derived from Nestin lineage pericytes. Initially, pericytes with PDGFR α activation did not show increased ECM gene expression but proliferation was increased compared to cells with normal PDGFR α . Nestin⁺ pericytes also expressed adipocyte progenitor markers, and readily differentiated into adipocytes *in vitro* and *in vivo* following transplantation. PDGFR α activation inhibited adipogenic differentiation and promoted the transition to a fibrogenic phenotype. We conclude that nestin⁺ pericytes in adipose tissue are a subset of adipocyte progenitors capable of causing fibrosis with PDGFR α activation. This transition occurs in a cell autonomous manner by increasing proliferation, losing potential for adipogenic differentiation, and switching to a fibrogenic cell phenotype.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

14

Disabled-2 (Dab2) knockout shows an essential role in primitive endoderm development and multiple physiological functions through modulation of endocytosis and signaling. Wensi Tao, Robert Moore, Yue Meng, Elizabeth Smith, **Xiang-Xi (Mike) Xu**. Cell Biology, University of Miami, Miami, FL.

Endocytic adaptor Disabled-2 (Dab2) is widely expressed in various tissues in adults and is first expressed in primitive endoderm in development. Constitutive knockout in mice is early embryonic lethal due to the failure in the formation of the extraembryonic endoderm layer. We constructed a conditional mutant *dab2* and a GFP knockin models to further investigate Dab2 in development and physiological function. In implanted Dab2 null embryos at E4.5, although Gata4- and Gata6-positive primitive endoderm cells were present, the cells were not organized into a monolayer epithelium and some located deep inside the Oct3/4-positive inner cell mass. By E5.5, the disorganization of the extraembryonic tissues derived from the primitive endoderm was apparent in Dab2 deficient embryos, and the mutant embryos showed a spectrum in the degree of endoderm disorganization ranging from mild to severe.

We constructed a floxed Dab2 conditional mutant line and generated Dab2 mosaic deletion using SOX2-Cre to restrict gene deletion within embryonic proper. By 3 week of age, Dab2 gene deletion was found in the majority (99%) of the cells of the conditional mutant mice. The mutant mice appear normal, fertile, and have a normal lifespan. Further investigation uncovered several defects in Dab2 conditional knockout mice including LDL and cholesterol metabolism, mammary involution, fat storage, and predisposition to tumorigenesis.

Mechanistic investigation determined that Dab2 presents these biological functions by modulation of cell polarity, endocytosis, and Ras/MAPK signaling. Thus, studies of Dab2 mutant mice demonstrated the wide influence of endocytosis on mammalian development and physiology.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

15

Modeling the initiation, progression and therapy of BRAF mutated cancers in mice. Martin

McMahon¹, Joseph Juan², Rosalie Seers¹, Teruyuki Murachguchi¹, John Edward van Veen¹, Anny Shai¹, Shon Green¹, Wayne Phillips³. 1) Helen Diller Family Comprehensive Cancer Center, U.C. San Francisco, San Francisco, CA; 2) Knight Cancer Institute, Oregon Health & Science University, Portland, OR 97239; 3) Peter MacCallum Cancer Centre 1 St. Andrew's Place, East Melbourne Victoria 3002, Australia.

Mutational activation of BRAF is a common event in a wide range of human malignancies. The most common mutation is a T1799->A transversion, which leads to expression of the BRAF^{V600E} oncoprotein. To model BRAF^{V600E}-induced tumorigenesis in vivo, we generated both *Braf*^{CA} and *BRAF*^{FA} mice carrying a conditional alleles of BRAf that express normal BRAF prior to either Cre (CA)- or Flp (FA)-mediated mediated recombination after which BRAF^{V600E} is expressed. More recently, we have generated *BRAF*^{CAT} mice in which expression of BRAF^{V600E} from a bicistronic mRNA is directly linked to tdTomato expression thereby indelibly marking cells with oncogenic BRAF^{V600E} expression. Using these mice we have generated models to explore the initiation, progression and therapy of BRAF mutated melanoma, lung, pancreatic, thyroid and hematopoietic malignancies. (Refs 1-5). In this presentation, the importance of WNT and PI3'-kinase signaling in models of BRAF^{V600E}-induced lung tumorigenesis or melanoma respectively will be discussed.

1. Dankort et al., (2007) A new mouse model to explore the initiation, progression and therapy of BRAF^{V600E}-induced lung tumors *Genes Dev.* 21: 379-384.
2. Dankort et al., (2009) BRAF^{V600E} cooperates with PTEN silencing to induce metastatic melanoma. *Nature Genetics*, 41: 544-523.
3. Charles et al., (2011) Mutationally activated BRAF^{V600E} elicits papillary thyroid cancer in the mouse. *Cancer Research*, 71: 3863-714.
4. Collisson et al., (2012) A Central Role for RAF→MEK→ERK Signaling in the Genesis of Pancreatic Ductal Adenocarcinoma. *Cancer Discovery*, 2: 685-93.5.
5. Kamata et al., (2013) Hematopoietic expression of oncogenic BRAF promotes aberrant growth of monocyte-lineage cells resistant to PLX4720 *Mol. Can. Res.* 11: 1530-41

Keynote Address

16

Using mouse models to identify disease suppressors: A genetic approach to therapeutic intervention. Monica Justice. Genetics and Genome Biology, Hospital for Sick Children, Toronto, Ontario, Canada.

Mutations in methyl CpG binding protein 2 (*MECP2*) cause Rett Syndrome (RTT), a severe neurological disease with autistic features and developmental regression. *Mecp2* null mouse models recapitulate many of the RTT symptoms and their study has provided insight into the physiological basis for disease. Reactivating silent *Mecp2* in symptomatic adult null mice reverses symptoms, suggesting that therapeutic intervention may be possible in RTT patients. Unfortunately, as a widespread epigenetic factor, MECP2 is extremely dosage sensitive, making direct manipulation a poor treatment option. MECP2 mutation impacts many biological pathways, but it is unclear which are relevant to symptom onset and progression. We used an unbiased forward genetic approach to identify dominant suppressors of phenotypes in *Mecp2* null male mice, dispensing with *a priori* beliefs about MECP2 function. Five suppressor mutations, which ameliorate symptoms of *Mecp2* loss, were identified using linkage mapping and whole exome sequencing. One is a loss-of-function mutation in squalene epoxidase (*Sqle*), a rate-limiting enzyme in committed cholesterol biosynthesis. We discovered that the underlying basis for the rescue is perturbed brain and systemic lipid homeostasis in *Mecp2* mutant mice. Although lipid metabolism has not been implicated in the pathogenesis of Rett Syndrome, it is abnormal in many other neurological disorders. Further, our data show that MECP2 is required to link a repressor complex that regulates lipid homeostasis to DNA. Accordingly, we treated *Mecp2* mutant mice with cholesterol-lowering statin drugs, which alleviate motor symptoms and confer increased longevity in both male and female mice. Cholesterol metabolism therefore represents a potential new therapeutic target for the treatment of RTT. Our overarching goal is to identify a large number of suppressors through additional screening such that a list of potential therapeutic targets can be developed for RTT. A similar genetic approach could be exploited to identify targetable pathways involved in other “untreatable” diseases, opening a new field for translational discovery.

Plenary/Platform Session 3 - Epigenetics

17

Mouse models and the hallmarks of aging. Carlos Lopez-Otin. Bioquímica y Biología Molecular, IUOPA, Universidad de Oviedo, Oviedo, Spain.

We have recently defined nine molecular and cellular hallmarks that represent common denominators of aging in different organisms. These hallmarks are: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. On the other hand, parallel studies of our laboratory on accelerated aging syndromes, including Hutchinson-Gilford Progeria Syndrome (HGPS) and Nestor-Guillermo Progeria Syndrome (NGPS), have provided relevant information about these hallmarks of aging. HGPS is caused by a point mutation in the *LMNA* gene that yields a truncated form of prelamin A called progerin, which is also produced during normal aging. Over the last years, the generation of mouse models of HGPS and other progeroid laminopathies has shed light on the molecular alterations functionally involved in these diseases. Thus, *knock-out mice* deficient in *Zmpste24* metalloproteinase implicated in prelamin A maturation, *mosaic mice* containing *Zmpste24*-deficient and *Zmpste24*-proficient cells, and *knock-in mice* carrying the human HGPS mutation which causes progerin accumulation, have allowed us to demonstrate that progeroid laminopathies result from the combined action of both cell-autonomous and systemic factors. Accordingly, we have shown that nuclear envelope defects causative of these complex diseases lead to alterations in stem cell functionality, epigenetic abnormalities, perturbations in p53-dependent cell senescence pathways, metabolic changes and chronic activation of inflammatory responses. We have also demonstrated that the genetic or pharmacological blockade of these altered pathways prevents the development of many age-associated features of these progeroid mice and extends their longevity. On this basis, we have developed therapeutic strategies for progeroid laminopathies which are now in clinical trials for the treatment of HGPS patients. These findings illustrate the importance of mouse models for designing therapeutic strategies to treat rare and dramatic progeroid syndromes as well as for improving our knowledge of the universal and complex process of human aging.

18

Derepression of early embryonic histone H3 lysine 27 trimethylation occurs via KDM6 demethylase independent mechanisms. Karl B. Shpargel, Joshua Starmer, Della Yee, Michael Pohlers, Terry Magnuson. Department of Genetics, Carolina Center for Genome Sciences, and Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC.

In embryonic stem cell populations, developmental genes are maintained in a quiescent repressive chromatin state through histone H3 lysine 27 trimethylation (H3K27me3). Upon differentiation, H3K27me3 is removed to activate lineage specific patterns of developmental gene expression, a process thought to be dependent on H3K27 demethylases. To test this hypothesis, we have removed activity of both H3K27 demethylases of the mouse *Kdm6* gene family, *Utx* (*Kdm6a*, X-linked gene) and *Jmjd3* (*Kdm6b*, autosomal gene). Male embryos null for *Utx* and *Jmjd3* H3K27 demethylation survive to term. At mid-gestation, mutant embryos demonstrate proper patterning and activation of *Hox* genes. These male embryos retain the Y-chromosome UTX homolog, UTY, which cannot demethylate H3K27me3 due to mutations in catalytic site of the Jumonji-C domain. Embryonic stem (ES) cells lacking all members of the KDM6 family exhibit a typical decrease in global H3K27me3 levels with differentiation. ChIP-seq histone profiling of KDM6 null cells demonstrated loss of H3K27me3 and gain of H3K4me3 to *Hox* promoters and other transcription factors with retinoic acid differentiation, and induced expression similar to control cells. Finally, *Utx* and *Jmjd3* mutant mouse embryonic fibroblasts (MEFs) demonstrate dramatic loss of H3K27me3 from promoters of several *Hox* genes and transcription factors. Our results

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

indicate that early embryonic H3K27me3 repression can be alleviated in the absence of active demethylation by the *Kdm6* gene family. We propose a model for passive loss of H3K27me3 in early development via displacement of the PRC2 H3K27 methyl-transferase complex and replication dependent histone turnover.

19

Long Noncoding RNAs in Mouse Lineage Allocation. **Mary Donohoe**^{1,2}, Hugo Borges Pinto^{1,2}. 1) Burke Medical Research Institute, White Plains, NY; 2) Neuroscience and Cell & Dev Biology. Weill Cornell Medical College, New York, NY.

A central question in stem cell biology is how cells choose between alternative fates. Induction of pluripotency is well established, but we have only a rudimentary understanding of how it is specified. A critical gap in our knowledge is understanding how the expression of key factors necessary for mediating cell fate decisions are regulated. Although the importance of transcription factors has long been appreciated, there is a growing interest in the role of regulatory noncoding RNAs. Indeed, the transcriptional networks that regulate embryonic stem cells and lineage specification have focused on protein-coding transcripts that comprise a mere 1% of the mammalian genome. But surprisingly, recent transcriptome studies show that approximately 70-90% of the genome is transcribed during development to produce noncoding RNAs. Of these noncoding RNAs, several thousand are long noncoding RNAs (lncRNAs) defined as >200 nucleotides in length and are expressed in a developmentally regulated manner. Here we describe a novel lncRNA important for lineage allocation. We propose that this regulatory lncRNA provides a blueprint for how pluripotency is regulated in mammals, and is lost through cellular differentiation.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

20

A step toward understanding the *Hox* gene colinearity. Kyoichi Isono, Mami Kumon, Haruhiko Koseki. RIKEN IMS-RCAI, Yokohama, Japan.

Cells with pluripotency are similar to a clock consistently revolving, but the clock starts to move in differentiation process. It is of great interest that differentiated cells have a potential to go back to the '0' time point. Transcriptional networks dominate the temporal axis of cells. It is thought that the Polycomb-group (PcG) proteins play central roles in controlling the time of cells, or cell fate determination by regulating large numbers of genes for cell cycle and development; little is, however, known about how to regulate gene expressions. There is a long-standing question in developmental biology, that is: how homeotic (*Hox*) genes are tempo-spatially regulated in axial body patterning. This phenomenon is called the *Hox* colinearity. Here we show the mechanisms of how PcG proteins could regulate *Hox* gene clusters during mouse embryogenesis. Last year, we reported that PcG proteins formed distinct foci (termed PcG bodies) across *Hox* gene clusters of ~200-kb in embryonic fibroblasts. This body formation that was mediated by the head-to-tail polymerization property of a PcG protein Phc2 could lead to *Hox* gene silencing concomitant with chromatin condensation (Isono et al., 2013). We then addressed PcG bodies at the *Hoxb* gene cluster and compaction level of the cluster in 9.5-dpc embryo sections along the antero-posterior axis. The *Hoxb* cluster condensed more intensively in anterior parts. Overlaps between PcG bodies and each *Hoxb* gene locus correlated to their repressive state. Importantly, the condensation and repression of the *Hoxb* cluster region were dependent on forming PcG bodies. These results suggest that PcG body formation is required for regulation of at least *Hoxb* genes along the axis. PcG body would open new perspectives for the *Hox* colinearity.

21 - CANCELLED

Key Mediators of ATR DNA Damage Signaling are Spatially Confined to Unpaired Chromosomes in Spermatocytes. Andrew Fedoriw, Yochiro Shibata, Yuna Kim, Weipeng Mu, Terry Magnuson. Genetics, University of North Carolina Chapel Hill, Chapel Hill, NC.

Meiotic silencing of unpaired chromatin (MSUC) occurs during the first meiotic prophase, as chromosomes that fail to pair are sequestered into a transcriptionally-repressive nuclear domain. This phenomenon is exemplified by the heterologous sex chromosomes of male mammals, where numerous components have been experimentally defined, at the core of which is the ATR DNA damage response kinase. However, the mechanisms underlying the activation of these pathways to recruit ATR, and initiate MSUC remain unknown. Here, we show that essential components of the ATR signaling in somatic cells are spatially confined to unpaired chromosomes in spermatocytes, including the ATR-dependent phosphorylation of the single-stranded DNA (ssDNA) binding complex, Replication Protein A (RPA). In addition to serving as a substrate for ATR kinase activity, RPA/ssDNA is a potent activator of ATR in somatic cells. Collectively, our data suggest RPA/ssDNA underlies the recruitment of ATR to unpaired chromosomes to initiate this chromosome-wide regulatory event.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

22

Epigenetic regulation of imprinted gene loci. Marisa Bartolomei. Dept Cell & Develop Biol, Univ Pennsylvania Sch Med, Philadelphia, PA.

Imprinted genes are expressed from a single parental allele and are typically found in clusters throughout the mammalian genome. We have studied one imprinted cluster in detail, which harbors the paternally expressed *Igf2* and maternally expressed *H19* genes. The opposite imprinting of these adjacent genes is mediated through shared 3' enhancers and a 2 kb imprinting control region (ICR). The ICR is hypermethylated on the paternal allele, where it mediates repression of *H19*. On the maternal allele, the ICR serves as a CTCF-dependent insulator, which allows exclusive access of *H19* to the 3' shared enhancers. Gain of maternal methylation and loss of paternal hypermethylation of the ICR are associated with the human growth disorders Beckwith-Wiedemann Syndrome and Silver-Russell Syndrome, respectively. Using gene targeting approaches, we have generated mouse lines that model the epigenetic mutations in these human syndromes. We have also studied the cis-acting sequences and trans-acting factors that mediate imprinting at this locus as well as other imprinted loci in mice. We have observed tissue-specific effects in loss of imprinted gene regulation. Moreover, we have also described various environmental insults that result in loss of imprinting in a mouse model system. These results will be described.

Plenary/Platform Session 4 - Organogenesis

23

Cardiac Myocyte Lineage Commitment. Jonathan Epstein. Cell and Developmental Biology, Prelman School of Medicine, Philadelphia, PA.

During heart development, multipotent cardiac progenitor cells (CPCs) are thought to contribute to endothelial, smooth muscle and cardiomyocyte lineages. However, the temporal-spatial details of commitment of a multipotent CPC to the myocyte lineage, and the regulatory signals *in vivo* that mediate commitment, remain poorly defined. Definitive early markers of the committed cardiomyocyte lineage in the second heart field have not been described. We have found that cardiomyocyte commitment occurs as multipotent CPCs enter the outflow and inflow tracts of the heart within a zone of strong BMP4 expression. Commitment is associated with a gradual down-regulation of Wnt signaling within a subset of Islet1+ CPCs. Commitment is defined by expression of the atypical homeodomain protein, Hopx, which acts to link BMP signaling to inhibition of Wnt by physically interacting with Smads and by directly repressing Wnt genes. In the absence of Hopx, commitment is impaired and both Islet1 and Wnt signaling are expanded in the second heart field. Thus, we describe a novel mechanistic link between Bmp activation and Wnt inhibition that functions to promote the cardiomyogenic lineage in the heart.

24

Bicaudal C1 promotes pancreatic NEUROG3⁺ endocrine progenitor differentiation and ductal homeostasis. L. A. Lemaire¹, J. Goulley², Y. H. Kim^{1,2}, S. Carat³, P. Jacquemin⁴, J. Rougemont³, D. B. Constam², A. Grapin-Botton^{1,2}. 1) DanStem, University of Copenhagen, Copenhagen, Denmark; 2) ISREC, EPFL, Lausanne, Switzerland; 3) Bioinformatics and Biostatistics Core Facility, EPFL, Lausanne, Switzerland; 4) de Duve Institute, Université catholique de Louvain, Brussels, Belgium.

The pancreas is a tree-like organ. Tips are composed of acini secreting digestive enzymes while the trunk is formed by the ducts collecting the enzymes and releasing them into the duodenum. Endocrine cells, clustered into the islets of Langerhans, are scattered throughout this tree. During pancreatic organogenesis, endocrine cells differentiate by two successive waves from NEUROG3⁺ endocrine progenitors which arise in the ducts. However little is known about how endocrine progenitors are formed. Bicaudal C1, a RNA-binding protein mutated in kidney dysplasia, is involved in this process. It is expressed in the progenitors lining the ducts. Its deletion leads to endocrine progenitor decrease in the second wave of production resulting in a 50% reduction of endocrine cells. Moreover, HNF6, a transcriptional activator of *Neurog3*, promotes *Bicc1* expression while it is down-regulated when *Bicc1* is absent. Like in human, *BICC1* mutations also lead to cyst formation in kidneys and in the pancreas. Transcriptome comparison before cystogenesis, reveals that PKD2 functions downstream of BICC1 in preventing cyst formation in the pancreas, as it has been described in kidneys. Moreover, the gene signature revealed immune cell infiltration and mesenchymal reaction developing early in the pancreas of *Bicc1* KO. Both are known to promote cyst formation in the kidneys. These results place BICC1 together with HNF6 in the pathway controlling both NEUROG3⁺ endocrine cell production and ductal cyst prevention. They also suggest a new candidate gene for syndromes associating kidney dysplasia with pancreatic disorders including diabete.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

25

The linear ubiquitin-specific deubiquitinase Gumby (Fam105b/Otulin) regulates angiogenesis and Wnt signaling. E. Rivkin^{1,2}, S.M. Almeida^{1,2}, D.F. Ceccarelli¹, Y-C Juang¹, T.A. MacLean^{1,2}, T.

Srikumar³, H. Huang¹, R. Fukumura⁴, G. Xie¹, Y. Gondo⁴, B. Raught⁴, A-C Gingras^{1,2}, F. Sicheri^{1,2}, **S.P. Cordes**^{1,2}. 1) Lunenfeld-Tanenbaum Research Institute, Mt Sinai Hospital, Toronto, ON, Canada; 2)

Department of Molecular Genetics, University of Molecular Genetics, University of Toronto, Toronto, ON, Canada; 3) Department of Medical Biophysics, University of Toronto and OCI, Princess Margaret Cancer Centre, Toronto, ON, Canada; 4) Mutagenesis and Genomics Team, RIKEN BioResource Center, Tsukuba, Ibaraki, Japan.

A complex interplay of signaling events, including the Wnt pathway, regulates sprouting of blood vessels from pre-existing vasculature during angiogenesis. Here we show that two distinct mutations in the (uro)chordate-specific *Gumby/Fam105b/Otulin* gene cause an embryonic angiogenic phenotype in *gumby* mice.

We show that Gumby encodes an Ovarian Tumor Domain (OTU) class of deubiquitinase (DUB). During ubiquitination, a covalent bond is generated most commonly between the most C-terminal amino acid residue and a lysine in the modified protein. Ubiquitin itself contains seven lysine residues that can undergo ubiquitination to form polyubiquitin oligomers (“chains”). An atypical ubiquitin chain involving the linear linkage of the carboxy-terminus of one ubiquitin to methionine 1 of another ubiquitin emerged evolutionarily in (uro)chordates. Gumby specifically cleaves linear ubiquitin linkages.

In line with a key role in the linear (de)ubiquitination axis, Gumby interacts with HOIP/Rnf31, the key component of the linear ubiquitin assembly complex (LUBAC), decreases linear ubiquitination and activation of NFκB dependent transcription.

Finally, we show that linear (de)ubiquitination modulates Wnt signaling. Gumby interacts with Disheveled 2 (Dvl2), and is expressed in canonical Wnt-responsive endothelial cells. Canonical Wnt signaling is reduced in *gumby* mutant endothelial cells.

Our work provides the first evidence for the importance of linear (de)ubiquitination in angiogenesis, craniofacial and neural development and in modulating Wnt signaling.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

26

Autocrine role of fibronectin in neural crest development and smooth muscle differentiation.

Sophie Astrof, Xia Wang, Dong Liang. Medicine, Thomas Jefferson University, Philadelphia, PA.

Chemical and mechanical properties of extracellular matrices (ECM) modulate diverse aspects of cellular fates such as adhesion, proliferation, survival, differentiation and migration. Components of the ECM are commonly used as three-dimensional scaffolds in tissue engineering. However, how distinct constituents of the ECM influence organ morphogenesis are not well understood. Fibronectin (FN1) is a secreted ECM glycoprotein, highly conserved in vertebrates and essential for vertebrate development. Our prior studies indicated that *FN1* mRNA and protein are expressed in strikingly non-uniform patterns during mouse embryogenesis, suggesting that cell-specific sources of FN1 regulate distinct morphogenetic processes. In order to test this hypothesis, we ablated *FN1* gene expression in the neural crest (NC), a population of multi-potent progenitors expressing high levels of *FN1* mRNA. Our experiments demonstrate that *FN1* synthesized by the NC regulates morphogenesis of the aortic arch arteries, an intricate vascular tree that routes de-oxygenated blood to the lungs and oxygenated blood to the body. We found that NC-synthesized FN1 functions in an autocrine manner by signaling through integrin $\alpha 5\beta 1$ expressed by the NC to facilitate Notch signaling and consequently, differentiation of the NC-derived cells into vascular smooth muscle cells (VSMCs), which in turn stabilize pharyngeal arch arteries and facilitate their remodeling. Our work demonstrates the indispensable role of cell-specific *FN1* in embryogenesis; particularly, in the development of essential, vertebrate-specific features, the multi-potent NC and high pressure-bearing vasculature.

27

Foxn1 levels in thymus organogenesis and maintenance - too much is not enough. Nancy Manley, Jie Li, Brian Condie. Genetics, University of Georgia, Athens, GA.

The transcription factor Foxn1 is essential for fetal thymus organogenesis, including thymic epithelial cell (TEC) differentiation and proliferation, and in its absence the thymic rudiment is poorly differentiated and extremely hypoplastic. In the postnatal thymus, Foxn1 levels vary widely between different TEC subsets, and its expression is required for maintenance of the postnatal microenvironment, as premature down-regulation of Foxn1 causes a rapid involution-like phenotype. Transgenic overexpression of Foxn1 in TECs has also been shown to cause thymic hyperplasia and delayed postnatal involution. In the current study, we investigated the effects of Foxn1 over expression using a K5.Foxn1 transgene. To our surprise, although this transgene drives Foxn1 over-expression in TECs, it does not cause thymic hyperplasia, nor does it delay or reduce the reduction in thymus size associated with aging-related involution. However, it does maintain TEC differentiation longer than in control mice. Similarly, this transgene cannot rescue thymus size in mice which undergo premature involution due to reduced Foxn1 levels, but TEC differentiation is significantly improved including expression of beta5t, AIRE, and UEA-1, and improved corticomedullary organization. Expression of this transgene in Foxn1 null "nude" mice also improves thymus size and differentiation, although the thymus is smaller and there is a bias towards medullary differentiation. These results demonstrate that the functions of Foxn1 in promoting TEC proliferation and differentiation are separable, and suggest that differential modulation of Foxn1 levels in different TEC subsets at different stages is critical for normal organogenesis and maintenance of the thymus.

28

The *Osr2* gene regulatory network in palate development. Yu Lan, Jing Zhou, Han Liu, Jingyue Xu, Eunah Chung, Joo-Seop Park, Shihai Jia, Bruce Aronow, Sunghee Oh, **Rulang Jiang**. Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

We previously reported that mice lacking the *Osr2* zinc finger transcription factor exhibit complete penetrance of cleft palate. Recently, we demonstrated that *Osr2* expression in the developing palatal mesenchyme depends on the function of the Pax9 transcription factor and that transgenic expression of

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

Osr2 from the endogenous *Pax9* locus partly rescued palate development in the *Pax9*^{-/-} mutant mice. In this study, we compared the whole transcriptome expression profiles of the developing secondary palate in the *Osr2*^{+/-} and *Osr2*^{-/-} mutant mouse embryos by using RNA-Seq and found that *Osr2* function is required for the proper expression of many transcription factors and signaling molecules during palate development. To identify direct target genes regulated by *Osr2* during palate development, we generated mice expressing the MYC-tagged *Osr2* protein from the endogenous *Osr2* locus and carried out chromatin immunoprecipitation assays followed by deep sequencing of the immunoprecipitated chromatin DNA (ChIP-Seq). We identified over 3000 high-quality *Osr2*-binding genomic regions in the developing palatal tissues. By integrating the RNA-Seq and ChIP-Seq data and verifying the differential gene expression profiles by qRT-PCR and in situ hybridization assays, we have identified over 100 candidate *Osr2* target genes, of which many have been implicated in craniofacial and/or palate development. These results provide significant new insights into the molecular mechanisms controlling palate development. This work is supported by National Institutes of Health (NIH)/National Institute of Dental and Craniofacial Research (NIDCR) grants DE013681 and DE018401 to RJ.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

29

Pbx-dependent regulation of Snail1 controls mammalian face morphogenesis by promoting epithelial plasticity. Licia Selleri¹, Bingsi Li¹, Elisabetta Ferretti¹, Laura Quintana Rio¹, Jennifer Feenstra¹, Trevor Williams², Theresa Vincent¹, Anthony Brown¹. 1) Cell and Developmental Biology, Weill Medical College of Cornell University, New York, NY; 2) Craniofacial Biology and Cell and Developmental Biology, University of Colorado Denver, Denver, Aurora, CO.

The human face is the gateway to verbal and nonverbal communication, and an immediate criterion for social acceptance. Cleft Lip and/or Palate (CL/P) is the most common human craniofacial malformation (1 in 500-700 births). Using the mouse as a model, we established that dysregulation of a previously unknown, essential Pbx-dependent Wnt-p63-Irf6 regulatory module leads to suppression of apoptosis at the site where the seams of the three opposing facial prominences fuse (λ junction), resulting in CL/P. We present embryologic, cellular, and genetic evidence that during mouse midface morphogenesis, Epithelial-Mesenchymal Transition (EMT) occurs at the λ . In addition to apoptosis, EMT promotes fusion of the facial prominences by eliminating unnecessary epithelial cells at this site. We found that Pbx transcription factors are key regulators of EMT at the λ . Notably, in *Pbx* compound mutant embryos the epithelium persists at the prominence seams, which prevents their fusion and results in CL/P. During the morphogenesis of the midface, Pbx-positive epithelial cells at the λ express multiple EMT inducers, such as Snail1 and Smad3/4, which leads to morphological and molecular changes typical of EMT. However, in the absence of Pbx1/2, the seam epithelial cells lose Snail1 and Smad3/4, and fail to acquire mesenchymal features and markers. By Chromatin immunoprecipitation (ChIP) on E11.5 midfaces and transient transfections in embryonic cells, we demonstrated that Pbx1 controls EMT by directly activating *Snail1* transcription. In addition, we are establishing a comprehensive regulatory network of Pbx-directed effectors of EMT in midface morphogenesis by determining which Pbx1-bound enhancers identified by ChIP-Seq regulate Pbx target genes by transcriptome assays on wild type and *Pbx*-mutant cephalic epithelium. We propose a new role for Pbx as a prime regulator of epithelial plasticity in face morphogenesis, as well as a potential player in early phases of head tumor invasion.

30

Wnt signaling in craniofacial development and anomalies. Arjun Stokes^{1,2}, Chengji Zhou^{1,2}. 1) Department of Biochemistry and Molecular Medicine, UC Davis School of Medicine, Sacramento, CA; 2) Institute for Pediatric Regenerative Medicine, Shriners Hospitals for Children & UC Davis School of Medicine, Sacramento, CA.

Using conditional gene-targeting approaches, we have demonstrated that Wnt/beta-catenin signaling is critical in transcriptional modulation of Fgf signaling in the anterior neural ridge and facial ectoderm for both facial and forebrain development. We have also demonstrated that Lrp6-mediated Wnt signaling is required for lip/palate formation and fusion, which may partially act through transcriptional activation of the homeobox genes *Msx1* and *Msx2* in facial mesenchymal cells. Ubiquitous inactivation of Lrp6 resulted in cleft lip/palate (CLP), a common birth defect in humans. We then addressed the cell lineage-specific roles of Lrp6 in the cause of CLP. Conditional ablation of Lrp6 solely in the neural crest cells or in the facial ectodermal cells did not cause CLP, indicating that synergistic Lrp6 signaling in both lineage cells is required for lip/palate formation and fusion. Meanwhile, we demonstrate a functional redundant role of Lrp6 and Lrp5 in transducing beta-catenin signaling during early facial development. Moreover, we demonstrate distinctly different craniofacial disorders in the Wls conditional mutants. Wls is a molecule required for the secretion of Wnt proteins. Together, our results demonstrate that Wnt signaling is required for multiple craniofacial developmental processes, and that disruptions of Wnt signaling genes or activities may cause a spectrum of craniofacial anomalies.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

31

Cellular and molecular principles of organ-specific blood vessel growth. Ralf H ADAMS. Tissue Morphogenesis, Max Planck Institute for Molecular Biomedicine, Münster , Germany.

Angiogenesis is the main process mediating the expansion of the blood vessel network during development, tissue regeneration or in pathological conditions such as cancer. The formation of new endothelial sprouts, a key step in the angiogenic growth program, involves the selection of endothelial tip cells, which are highly motile, extend numerous filopodia, and lead new sprouts. Angiogenic sprouting is induced by tissue-derived, pro-angiogenic signals such as vascular endothelial growth factor, and is strongly modulated by intrinsic signaling interactions between endothelial cells, which involve the Notch pathway. Our work is providing insight into the regulation of angiogenesis such as the roles of different Notch ligands or the modulation of growth factor receptor activity by endocytosis.

Mouse genetics is a powerful tool for the characterization of processes controlling blood vessel morphogenesis. As the global or constitutive endothelial cell-specific inactivation of genes controlling angiogenesis frequently lead to embryonic lethality at midgestation, we have focused on the generation of tamoxifen-inducible CreERT2 transgenic mice. To ensure robust and faithful expression of the resulting transgenic mice, we preferentially use large genomic DNA fragments in BAC or PAC vectors together with recombineering techniques. Successful examples include the *Cdh5(PAC)-CreERT2* line that can be used for functional studies in the developing and adult endothelium. *Bmx(BAC)-CreERT2* transgenics enable gene inactivation experiments in arterial endothelial cells without affecting veins or capillaries. To investigate the role of tip cells in endothelial sprouting, we have generated *Esm1(BAC)-CreERT2* transgenics. The *Pdgfrb(BAC)-CreERT2* line enables genetic experiments in mural support cells that stabilize the vessel wall.

We will provide examples of ongoing studies involving these transgenic lines, which provide insight into the regulation of blood vessel growth in the central nervous system and in bone.

Rosa Beddington Lecture

32

The life of breath: stem cells of the lung. **Brigid I M Hogan.** Department of Cell Biology, Duke University Medical Center, Durham, NC 27710.

Cell turnover in the lung is normally very slow, relative to other organ systems such as the intestine and skin. However, if lung epithelial cells are damaged by toxic agents or viral infection, or placed under stress by partial pneumonectomy, the lung reveals an impressive capacity for regrowth and repair. By combining a variety of injury/repair models in the mouse with in vivo genetic cell lineage tracing experiments, new imaging methods, and 3D organoid culture, we have obtained evidence that different regions of lung contain different populations of epithelial stem and progenitor cells. For example, in the larger airways lined by pseudostratified mucociliary epithelium the major stem cell population is the basal cell. By contrast, in the distal gas exchange alveolar region there are no basal cells and type 2 alveolar epithelial cells are largely responsible for long term maintenance and repair. This conclusion is complicated, however, by recent studies that show considerable plasticity in the phenotype of epithelial progenitor cells in response to different injuries. A major goal of our lab is to identify the cellular and molecular components of the niches in which different lung stem cells reside and to identify the signaling pathways by which the components interact with each other. Research is also directed towards understanding how these niches are formed during development and how they change in response to injury, infection, inflammation and aging.

Plenary/Platform Session 5 - Development

33

Extracellular micro-environmental cues control specification of cell fate and behaviors in the early mouse embryo. **Isao Matsuo**¹, Ryuji Hiramatsu^{1,2}, Kayo Shimokawa¹, Chiharu Kimura-Yoshida¹. 1) Osaka Med Ctr for Maternal & Chi, Izumi, Osaka, Japan; 2) NIID, Musashi-Murayama, Tokyo, Japan.

Increasing evidence has demonstrated that a microenvironment including extracellular matrix (ECM) plays crucial roles in regulating cell proliferation, differentiation and migration in the tissue development process. In addition to providing structural support, the ECM modulates signaling activity by regulating the reception, transport and diffusion of growth factors and by changing the mechanical properties of substrates. It remains unclear, however, which environmental cues control cell fate and behaviors in the early mouse embryo.

Heparan sulfate (HS) proteoglycans, one of the major ECM components, consists of HS chains and core-protein moieties, which are localized at the cell surface and in the basement membrane (BM) in the early mouse embryo. We have found that the spatio-temporal expression of cell surface HS chains is prominent near areas in which FGF signaling is active. Consistent with this notion, cell surface HS chains direct the local retention of FGF with their receptors and subsequent signaling activation. In addition, chimeric expression studies with HS-deficient and wild-type embryos have revealed that HS chains can activate FGF signaling in a non-cell-autonomous manner within a short-range. The results of further treatment with chemical inhibitors against cellular processes suggest that shedding with serine proteinases may involve the FGF signaling spreading. On the other hand, ECM molecules deposited in the BM control cell behaviors by demarcating tissue layers between the epiblast and visceral endoderm (VE) as physical and biochemical barriers. Notably, local breaches of the BM trigger establishment of the anterior-posterior axis, i.e., the emergence of distal VE through transmigration of epiblast cells. Moreover, this migration is directly governed by mechanical forces from uterine tissues exerted on the embryo rather than biochemical factors. Thus, we propose that biochemical and mechanical properties of ECM molecules control crucial developmental processes in the early mouse embryo.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

34

Dynamic cell behaviors and commitment to pluripotency in the mouse blastocyst. **M. Kang**^{1,2}, A. Puliafito³, P. Xenopoulos¹, S. Di Talia⁴, A.-K. Hadjantonakis¹. 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; 3) Laboratory of Cell Migration, Candiolo Cancer Institute - FPO, IRCCS, Candiolo, Torino, Italy; 4) Department of Cell Biology, Duke University Medical Center, Durham, NC.

The pluripotent epiblast (EPI) of the blastocyst is the founder tissue of most somatic cells. EPI and primitive endoderm (PrE) progenitors arise from the inner cell mass (ICM) of the blastocyst. Although the role of the gene regulatory network (GRN) composed of *Nanog-Gata6-Fgf4* during this cell lineage commitment process has been extensively studied, the dynamic cellular behaviors accompanying lineage specification within ICM cells have not been investigated quantitatively at single-cell resolution. To study the behaviors operating during ICM lineage specification, we applied a single-cell resolution 3D time-lapse (i.e. 4D) live imaging approach, coupled to an automated quantitative image analysis platform we recently developed.

Using single-cell resolution quantitative imaging of a *Nanog* transcriptional reporter, we observed an irreversible commitment to EPI/PrE lineages *in vivo*. A period of apoptosis occurred during the period of ICM fate choice, followed by a burst of EPI-specific cell proliferation. PrE-to-EPI transitions were very rarely observed. Their unidirectionality suggesting they were regulated, not stochastic. In sum, our data suggest that the unidirectionality and time-scale of embryo development may not permit fluctuations in cell fate. These dynamic cell behaviors were affected when lineage decisions were changed through the modulation of FGF signaling.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

35

BMP signaling: Coordination of Ventral Folding Morphogenesis and L/R patterning. Svetlana Gavrilov, Elizabeth Lacy. Developmental Biology Program, Sloan-Kettering Institute, New York City, NY.

Key steps in L/R patterning and heart morphogenesis coincide temporally with the onset of ventral folding morphogenesis (VFM), a fundamental process mediating gut endoderm internalization, linear heart tube formation, ventral body wall closure and encasement of the fetus in extraembryonic membranes. Aberrant VFM results in birth defects such as gastrochisis and ectopia cordis in humans and misplacement of head and heart in mouse. Defects in L/R patterning lead to heterotaxia, a mispositioning of one or more organs relative to the L/R axis, often accompanied by complex heart defects. The mechanisms achieving temporal coordination of VFM and L/R axis formation remain unknown. Using lineage-specific mouse mutants, we identified the Bone Morphogenetic Protein (BMP) pathway as a central regulator of anterior VFM. *Bmp2* Visceral Endoderm-specific knockout (VE-KO) results in a disorganized anterior phenotype characterized by the lack of foregut invagination and placement of the heart anterior/dorsal to the head. These findings led to the model that BMP2 expressed by anterior VE (AVE) signals to epiblast derivatives during gastrulation to orchestrate the initial stages of VFM: foregut invagination and placement of the heart caudal/ventral to the head. *Bmp2* expression in epiblast derivatives initiates at the neural plate stages, just before onset of VFM. *Bmp2* epiblast-specific knockout (EPI-KO) results in a posterior specific delay phenotype; such mutants display well developed head structures, foregut invagination and correctly positioned heart, but a severely truncated posterior region and defects in L/R patterning, manifested as randomized and aberrantly looped hearts. Experiments in progress will assess whether the generation and/or relay of L/R patterning signals require BMP2-specific functions, as predicted by the EPI-KO. The spatial/temporal pattern of *Bmp2* expression suggests independent roles for BMP2 in VFM and L/R patterning. Alternatively, correct L/R patterning may require sequential BMP2 signals; first from VE, then from epiblast. The latter scenario predicts AVE-BMP2 as a key signal directing the temporal coordination of VFM and L/R axis formation. Implications of these models will be discussed.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

36

Loss of *O*-fucosylation on ADAMTS9 disrupts patterning in POFUT2 mutant embryos. **Bernadette Holdener**¹, Megumi Takeuchi¹, Deepika Vasudevan¹, Hideuki Takeuchi¹, Richard Grady¹, Suneel Apte², Robert Haltiwanger¹. 1) Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY 11794-5215; 2) Department of Biomedical Engineering, Cleveland Clinic, Cleveland, OH 44195.

In mouse, mutations in *Protein O-fucosyltransferase 2 (Pofut2)* promote epithelial to mesenchymal transition and lead to a loss of epiblast pluripotency. Widespread expression of PECAM in E 8.5 *Pofut2* mutants, provides evidence that the abundant mesoderm is biased towards differentiation into the vascular endothelial cell lineage. These gastrulation defects are preceded by changes in Reichert's membrane that lead to embryo constriction and expanded expression of mesoderm inducing factors including: *Bmp4*, *Nodal*, and *Wnt3*. Combined, these data suggest that loss of *O*-fucosylation in the extra-embryonic tissues could be responsible for the patterning defects in *Pofut2* mutants. POFUT2 recognizes and transfers *O*-linked fucose to the S/T in the consensus sequence, CXX(S/T)XXG, located within thrombospondin type 1 motifs (TSRs). Using *in vitro* folding assay, we demonstrated that POFUT2 recognizes correctly folded TSRs and that *O*-fucosylation accelerates folding of TSR containing proteins through stabilization of the properly folded TSR. We predicted that the *Pofut2* mutant phenotype likely resulted from mis-folding of one or more of the 49 predicted POFUT2 targets. Among these candidates, ADAMTS9 (A Disintegrin and Metalloproteinase with ThromboSpondin type-1 motifs-9) contains 15 TSRs, suggesting that it would be highly dependent upon POFUT2 for efficient folding. Using mass spectral analysis, we demonstrated that ADAMTS9 is *O*-fucosylated, and was required POFUT2 in cell culture for efficient secretion. Consistent with our prediction that disruption of *O*-fucosylation of target proteins in extra-embryonic tissues contributes to the *Pofut2* mutant phenotype, we determined that *Adamts9* was strongly expressed in the trophoblast giant cells. Moreover, analysis of the *Adamts9* knockout phenotype identified remarkable morphological similarity to the POFUT2 mutants, suggesting that loss of ADAMTS9 function in the trophoblast giant cells is responsible for alteration in the properties of Reichert's membrane in POFUT2 mutant embryos and could contribute to early patterning defects in *Pofut2* mutants.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

37

Wnt/ β -catenin signalling directly regulates *Sox17* expression and is essential for organizer and endoderm formation in the mouse. **Silvia Engert**^{1,2}, Ingo Bartscher^{1,2}, W.Perry Liao¹, Stanimir Dulev³, Gunnar Schotta³, Heiko Lickert^{1,2}. 1) Institute of Stem Cell Research, Helmholtz Zentrum München, Germany; 2) Institute of Diabetes and Regeneration Research, Helmholtz Zentrum München, Germany; 3) Adolf-Budenandt Institute and Munich Center for Integrated Protein Science (CiPSM), Ludwig-Maximilian University, Germany.

Several signalling cascades have been implicated in the formation and patterning of the three principal germ layers, however, their precise temporal-spatial mode of action in progenitor populations still remains undefined. Here we have used conditional gene deletion of β -catenin in *Sox17*-positive embryonic and extra-embryonic endoderm as well as vascular endothelial progenitors to address the function of canonical Wnt signalling in cell-lineage formation and patterning. Conditional mutants fail to form anterior brain structures and exhibit posterior body axis truncations, whereas initial blood vessel formation appears to be normal. Tetraploid rescue experiments reveal that lack of β -catenin in the anterior visceral endoderm (AVE) results in defects in head organizer formation. Moreover, *Sox17*-lineage tracing in the definitive endoderm (DE) shows a cell-autonomous requirement for β -catenin in mid- and hindgut formation. Surprisingly, wild-type posterior visceral endoderm (PVE) in mid- and hindgut deficient tetraploid chimera rescues the posterior body axis truncation, indicating that the PVE is important for tail organizer formation. Upon loss of β -catenin in the AVE, PVE and DE lineages, but not in the vascular endothelial lineage, *Sox17* expression is not maintained, suggesting direct regulation by canonical Wnt signalling. Strikingly, we found accumulation of Tcf4/ β -catenin transactivation complexes on cis-regulatory elements in the *Sox17* up- and downstream regulatory regions specifically upon endoderm induction in an embryonic stem (ES) cell differentiation system. Taken together, these results indicate that the Wnt/ β -catenin signalling pathway directly regulates *Sox17* expression in the AVE, PVE and DE for induction of the head and tail organizer and formation of the definitive endoderm lineage.

38

Novel mode of defective neural tube closure in the non-obese diabetic (NOD) mouse strain. J Michael Salbaum, Nils J Herion, Claudia Kruger, Jacalyn MacGowan, **Claudia Kappen**. Pennington Biomedical Research Center, Baton Rouge, LA.

Mouse mutants have identified ~400 genes where mutations cause or contribute to neural tube defects (NTDs). In contrast, few genetic risk factors are known in humans, highlighting the critical role of environmental factors, such as folic acid deficiency or maternal diabetes, as causes for NTDs in humans. Moreover, 70% of the known NTD genes in the mouse are associated with anterior defects, and only 5% with spina bifida, while in humans, anencephaly is exceedingly rare, and spina bifida the most common NTD. This discrepancy underscores the need for mouse models that more closely resemble human disease.

We present evidence from two separate mouse models of diabetic pregnancy that identifies impaired migration of nascent mesodermal cells in the primitive streak as the morphogenetic basis for pathogenesis of neural tube and other structural birth defects in these pregnancies. Specifically, we demonstrate abnormalities in gastrulating embryos that form protrusions from the neural plate. By virtue of next-generation sequencing, comparison to laser captured tissue and gene expression studies, we identified these structures as consisting predominantly of mesodermal cells, encompassing precursors of axial, paraxial, heart and limb mesoderm. Thus, mesodermal cell types are specified normally in diabetes-exposed embryos, but subsequent migration out of the primitive streak is impaired. Explant cultures of protrusion and posterior primitive streak tissues confirmed the aberrant migratory properties of these cells. Perturbed gastrulation prior to neural tube closure can explain the majority of posterior NTDs and provide a unifying etiology for the broad spectrum of congenital malformations in diabetic pregnancies.

20 mouse alleles, in homozygous configuration, also cause protrusions from the primitive streak; at least half of these are associated with NTDs, as well as heart defects and caudal growth/tail defects. The

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

parallels between phenotypes in these mutants and our diabetes model make evident that mesoderm migration and neural tube closure are controlled by metabolic factors, and thus establish the NOD mouse strain as an excellent model for human environmentally induced NTDs.

39

Arl13b functions in cilia to regulate canonical and non-canonical Shh signaling in Joubert

Syndrome pathogenesis. Laura Mariani^{1,2}, Maarten Bijlsma³, **Tamara Caspary**¹. 1) Dept Human Gen, Emory Sch Med, Atlanta, GA; 2) Graduate Program in Neuroscience, Emory Univ, Atlanta, GA; 3) Laboratory for Experimental Oncology and Radiobiology, Academic Medical Center, Amsterdam.

Canonical Sonic Hedgehog (Shh) signaling occurs at the primary cilium. Mutations in cilia genes cause abnormal Shh signaling and a class of human diseases called ciliopathies. The cilia gene *ARL13B* has been linked to the ciliopathy Joubert Syndrome (JS). In mouse, loss of *Arl13b* is embryonic lethal and causes defects in cilia structure, misregulated localization of Smoothed, and abnormal neural tube patterning. JS patients have truncating or point mutations in *Arl13b* (or other cilia genes) but still show neurodevelopmental symptoms and signs of misregulated canonical Shh signaling (e.g., polydactyly).

Non-canonical Shh signaling refers to downstream effects of Shh that require Shh receptors and effectors but do not require cilia or transcription. These effects include cytoskeletal rearrangements important for axon pathfinding and cell migration. In addition to canonical Shh defects, JS patients have abnormal axon guidance and heterotopias consistent with dysfunctional neuron migration, but the relationship between these phenotypes and the Shh pathway has not been examined. To test whether mutations in *Arl13b* also affect non-canonical Shh signaling, we deleted *Arl13b* in cell types that are known to exhibit non-canonical Shh signaling. We saw impaired Shh-dependent migration in *Arl13b* null fibroblasts *in vitro* and brain phenotypes consistent with JS *in vivo*. We tested the effect of *Arl13b* mutations on canonical and non-canonical Shh signaling and saw that JS-causing alleles fail to rescue the *Arl13b* null phenotype. Surprisingly, a mutation that prevents cilia localization of *Arl13b* also fails to rescue Shh-dependent migration in fibroblasts. Therefore, while the cilium itself is dispensable for non-canonical Shh signaling, *Arl13b* must itself be in the cilium to regulate non-canonical Shh signaling. These data indicate that non-canonical Shh defects may contribute to human ciliopathies. Furthermore, they raise the possibility that phenotypes thought to be cilia-independent may in fact require ciliary protein function, consistent with ciliopathies being caused by cilia-associated genes.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

40

Eph/ephrin signaling mechanisms in the neurocristopathy craniofrontonasal syndrome. Jeffrey O Bush^{1,2}, Audrey K O'Neill^{1,2}, Andrew Larson^{1,2}. 1) Department of Cell and Tissue Biology and Program in Craniofacial and Mesenchymal Biology, UCSF, San Francisco, US; 2) Institute for Human Genetics, UCSF, San Francisco, UCSF.

The Eph receptor tyrosine kinase family and ephrin signaling partners are known for their roles in cell sorting, but the *in vivo* cellular and molecular mechanisms by which cell sorting occurs remain unknown. Ephrin-B1 provides a unique opportunity to study cell sorting mechanism and its consequences in a genetic system, since it is located on the X-chromosome, and *efnb1*^{+/-} heterozygous females are therefore mosaic for ephrin-B1 expression, leading to Eph/ephrin-mediated cell sorting. Heterozygous mutation of *EFNBI* in humans results in craniofrontonasal syndrome (CFNS), a congenital disease characterized by dramatic craniofacial, neurological and skeletal abnormalities associated with aberrant cell sorting. By analyzing a series of targeted inducible and signaling mutations in mice, we demonstrate that ephrin-B1 drives cell segregation in the neuroepithelium prior to neural crest emigration. Forward signaling by ephrin-B1 is necessary and sufficient for cell sorting and requires signaling through Rho-family small GTPases. Finally, we have generated human iPS cell lines as a developmentally relevant human model for understanding this aspect of CFNS etiology. Together, our data contribute to an *in vivo* understanding of the mechanistic basis of Eph/ephrin-mediated cell sorting and its role in the pathogenesis of this unusual disease.

41

Building of a Functional Lung: Lessons from Mouse Models of Lung-Related Birth Defects. Xin Sun. Laboratories of Genetics, University of Wisconsin, Madison, WI.

The building of a functional lung is dependent on precisely choreographed events within the organ, and its harmony with surrounding tissues. I will present recent findings that contribute to our understanding in both aspects. These data were generated from our studies of mouse genetic models of congenital diaphragmatic hernia (CDH), a prominent human birth defect. Newborns inflicted by CDH present herniation of their abdominal organs into the chest, through aberrant openings in the diaphragm. CDH leads all birth defects with the highest death rate (~50%). An overwhelming cause of lethality is insufficient lung function. Survivors suffer increased susceptibility to pulmonary hypertension and asthma. CDH is widely believed to originate from diaphragm malformations. Our data from genetic mouse models of CDH reveal evidence for diaphragm independent cause of CDH. Furthermore, these data add to our knowledge of the genetic requirements for building a respiratory organ that can function effectively, and in coordinately with other vital organs.

Plenary/Platform Session 6 - Disease

42

Concurrent ARID1A loss and PIK3CA activation initiates ovarian clear cell tumorigenesis. Ron Chandler, Jesse Raab, **Terry Magnuson**. Dept Genetics, CB 7264, Univ North Carolina, Chapel Hill, NC.

Ovarian clear cell carcinoma (OCCC) is a highly aggressive form of epithelial ovarian cancer with limited therapeutic options. ARID1A has emerged as a candidate tumor suppressor in OCCC, but its functional contributions to OCCC etiology remain unclear. We present the first genetically engineered mouse model of OCCC. We find that ARID1A inactivation is not sufficient for tumor formation, but requires concurrent activation of the phosphoinositide 3-kinase catalytic subunit, PIK3CA. Remarkably, the mice develop highly penetrant, chemo-resistant tumors with OCCC histopathology, culminating in hemorrhagic ascites and a median survival period of just 7.5 weeks. Treatment with a pan-PI3K inhibitor, NVP-BKM120 (*Buparlisib*), significantly improved survival, providing rationale for the use of PI3K/AKT/mTOR pathway inhibitors in OCCC treatment. Cross-species comparisons of OCCC gene expression signatures and cytokine profiling support a role for elevated Interleukin-6 (IL6) signaling in OCCC pathogenesis. We demonstrate that ARID1A and PIK3CA mutations activate IL6 transcription in a cooperative manner. Collectively, our findings not only establish a key causal relationship between SWI/SNF chromatin remodeling and PI3K signaling pathway mutations in OCCC tumor initiation, but also implicate SWI/SNF chromatin remodeling in the suppression of pro-tumorigenic cytokine signaling.

43

Dedifferentiated smooth muscle cells enhance atherosclerotic plaque initiation and progression in hypercholesterolemic mice. Lorin Olson, Chaoyong He. Immunobiology and Cancer, Oklahoma Medical Research Foundation, Oklahoma City, OK.

Clinical risk factors for atherosclerosis are systemic but plaques initiate at sites where the arterial anatomy creates a pro-atherogenic substrate. It is not known whether smooth muscle cells (SMCs) play a pathogenic role in plaque initiation. We hypothesized that dedifferentiated SMCs (ddSMCs) enhance the atherogenic milieu. To test this, we generated mice with ddSMCs in the aortic tunica media before plaque initiation. We used SMC-specific Cre driver (*Sm22 α -Cre*) to activate a Cre/lox-inducible platelet-derived growth factor receptor- β (PDGFR β) signaling cascade. We then crossed these mice (ddSMC mice) with the apolipoprotein E-null (*ApoE*^{-/-}) or low-density lipoprotein receptor-null (*Ldlr*^{-/-}) mice. Remarkably, *ApoE*^{-/-}ddSMC and *Ldlr*^{-/-}ddSMC mutants fed a Western diet for 6 weeks initiated plaques in the thoracic aorta, which is normally resistant to atherosclerosis. These plaques developed into fibrous cap atheromas with neovascularization and intraplaque hemorrhage. Feeding a normal chow diet gave similar results with delayed kinetics, and also resulted in coronary atherosclerosis by 10 months of age. We also used a tamoxifen-inducible SMC-specific Cre driver (SMMHC-CreER^{T2}) to activate PDGFR β signaling in adult *ApoE*^{-/-} mice, which also enhanced atherogenesis. Gene expression analysis revealed that ddSMCs overexpressed chemokines known to regulate monocyte and T cell trafficking. Aorta-flow analysis demonstrated increased homing of CD45⁺ leukocytes to the aortic adventitia of ddSMC mice, which could be reduced by pharmacological inhibition of PDGFR β . These results demonstrate that ddSMCs create a niche that enhances plaque initiation and progression. PDGFR β signaling regulates chemokine expression in ddSMCs and increases leukocyte numbers in the adventitia. Inflammatory signaling may represent a new mechanism by which PDGFR β and ddSMCs enhance site-specific plaque initiation.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

44

Studies of the functional consequences of aberrant pre-mRNA splicing in new mouse models.

Katherine Yates, Dandan He, Zhengqiu Zhou, Randi Mackler, Chelsea Moherman, Yanqiang Wang, Keiko Akagi, Jingfeng Li, David Symer. Human Cancer Genetics Program, The Ohio State University Comprehensive Cancer Center, Columbus, OH.

We recently reported that a human disease results from germline mutations in a non-protein coding gene (He et al., Science, 2011). Biallelic mutations in a small nuclear RNA gene, *RNU4ATAC*, result in microcephalic osteodysplastic primordial dwarfism type 1 (MOPD I). *RNU4ATAC* encodes a key component of the minor spliceosome, which when mutated causes aberrant pre-mRNA splicing. To model MOPD I, we engineered the most frequent mutations in the mouse ortholog (i.e. 51G>A and 55G>A). When heterozygous mice were intercrossed, the observed phenotypes in the compound heterozygous offspring mimicked several features of MOPD I, including dwarfism and bone abnormalities. A majority of biallelic mutant mice also developed maturity-onset diabetes mellitus of the young (DM; MODY). Histopathological analysis revealed a gradual dropout of their pancreatic beta cells without any signs of inflammation, suggesting that MODY was not due to an autoimmune attack. To evaluate DM onset and pathogenesis further, we conducted glucose tolerance tests (GTT) and other phenotypic assays, which showed that affected mice produced copious amounts of dilute urine, had high blood glucose levels and characteristically developed MODY at weaning age. Our goal is to identify the downstream genes whose mis-splicing leads to DM. Our *in vitro* biochemical assays demonstrated that orthologous mutations in human and mouse *U4atac* affect minor intron splicing differentially. To investigate such differences further, we engineered novel plasmids containing human and mouse *RNU4ATAC* transcription units including their promoters, resulting in an allelic series of *RNU4ATAC* mutations. We plan to use these constructs to “humanize” our current mouse models, using phi-31 integrase-mediated recombination *in vivo*, to phenocopy human MOPD I. We anticipate that our studies on the impacts of aberrant pre-mRNA splicing *in vitro* and *in vivo* will shed completely new light on the downstream mis-spliced target genes involved in formation of MOPD I and in DM.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

45

Delayed ependymal development and juvenile hydrocephalus in *Jhy^{lacZ}* mice. Hilmarie Muniz-Talavera, Brian Grewe, **Jennifer Schmidt**. Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL.

Hydrocephalus results from the overproduction, reduced absorption, or restricted flow of CSF through the ventricles of the brain. Increased CSF volume causes increasing intracranial pressure that can be fatal. Congenital hydrocephalus is common in human children, and current therapies only control the progression of the disease. Better treatment options for hydrocephalus await a more thorough understanding of its mechanisms. Many cases of human hydrocephalus can be attributed to known causes such as perinatal hemorrhage, but roughly half the incidence of the disease occurs in the absence of any predisposing event, and is believed to be genetic in origin. Animal models can provide insight into the genetic network underlying the regulation of CSF balance. The *Jhy^{lacZ}* mouse line carries an insertional mutation that disrupts the unstudied gene *Jhy*. Homozygous *Jhy^{lacZ/lacZ}* mice show progressive ventricular dilation by postnatal day 1.5, and most animals die by 4-8 weeks of age. The *Jhy* gene encodes a predicted 87 kD protein that is conserved across vertebrate species, but lacks any functional domains that might suggest its biological role. Electron microscopy determined that the ependymal cilia lining the ventricles of *Jhy^{lacZ/lacZ}* mice are reduced in number, and have abnormal patterns of microtubule organization. These abnormal cilia are likely to be immotile, resulting in loss of ciliary-mediated CSF flow. Ependymal cells mature during the first week after birth, when the radial glia lining the ventricles differentiate into a multiciliated ependyma. This maturation process is accompanied by a morphological change from elongated, multilayered cells to a flattened monolayer. Histological analysis showed that wild type medial wall ependyma assumes a mature morphology by postnatal day 5, while *Jhy^{lacZ/lacZ}* cells remain immature at this stage. Immunofluorescence analysis with differentiation-specific markers found that *Jhy^{lacZ/lacZ}* ependymal cells coexpress markers for both immature radial glia and for mature ependyma. These data suggest that loss of *Jhy* expression causes a delay in ependymal differentiation, with potential changes in ciliation and/or ependymal cell signaling leading to hydrocephalus.

46

Mouse Models of Human Diseases with Mutations in Nonmuscle Myosin II. **Xuefei Ma**, Yingfan Zhang, Connie Lerma, Mary Anne Conti, Robert Adelstein. LMC, NHLBI/NIH, Bethesda, MD.

Nonmuscle myosin II (a hexamer of two heavy chains and two pairs of light chains) plays a variety of roles in vivo during mouse development. Three isoforms of nonmuscle myosin II (NM IIA, IIB and IIC) which under certain circumstances can share roles but can also play unique roles, have been identified in mammalian cells. Mutations in the heavy chain of NM IIA and IIC (NMHC II, gene products of MYH9 and MYH14 respectively) in patients results in various abnormalities. Patients with a point mutation in MYH9 develop MYH9 related diseases (MYH9-RD), featuring macrothrombocytopenia (abnormally large and decreased numbers of platelets), aggregates of NM IIA in neutrophils (Dohle bodies), cataracts, deafness and a glomerulosclerosis which can be fatal. Mutations in MYH14 result in a sensorineural hearing impairment. To better study the pathology of MYH9-RD we have generated three mouse lines with mutations in either the myosin head (R702C) or rod (D1424N and E1841K) regions. All three heterozygous mouse lines display the phenotypes of MYH9-RD found in humans. Homozygous R702C NM IIA mice die at E10.5 with defects in placental vasculature, while homozygous D1424N and E1841K survive to adulthood showing the defects of MYH9-RD, including glomerulosclerosis. Furthermore the homozygous E1841K males are sterile. We have also generated a mouse line carrying a mutation in MYH10 (NMHC IIB) R709C, which is homologous to a mutation found in MYH9. Heterozygous R709C NM IIB mice develop an umbilical hernia and a diaphragmatic hernia. Homozygous R709C NM IIB mice die by E14.5 and show defects resembling the human syndrome Pentalogy of Cantrell. The abnormalities include a major defect in ventral body wall closure with ectopia cordis (heart located outside the thoracic chamber), split lower sternum and large umbilical hernia, diaphragmatic hernia, cardiac structural abnormalities such as a ventricular septal defect and a double outlet of the right ventricle. Defects in NM II dependent mesenchymal cell apoptosis, abnormal diaphragmatic skeletal muscle cell migration and a

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

faulty outflow tract myocardialization contribute to the phenotypes seen in R709C NM IIB mice. We are currently sequencing DNA from humans diagnosed with Pentalogy of Cantrell in an effort to explain these defects.

47

Mechanism and prevention of the folate-resistant neural tube defects. **Chengji Zhou**^{1,2}, Tianyu Zhao², Arjun Stokes^{1,2}. 1) Department of Biochemistry and Molecular Medicine, UC Davis School of Medicine, Sacramento, CA; 2) Institute for Pediatric Regenerative Medicine, Shriners Hospitals for Children & UC Davis School of Medicine, Sacramento, CA.

Neural tube defects (NTDs) are common structural birth defects, including spinal NTDs (spina bifida), cephalic NTDs (exencephaly and anencephaly), and craniorachischisis (an entirely open brain and spinal cord). Newborns with spina bifida are often viable but have severe defects related to caudal spinal cord functions. Fetuses with cephalic NTDs and craniorachischisis usually die around birth. It is estimated that folic acid supplementation and food fortification can prevent only 15% to 25% of NTDs worldwide. In turn, about 75% to 85% of NTDs are “folate-resistant”, or still unpreventable yet. In the animal models, maternal supplementation of folic acid may reduce the occurrence rate of cephalic NTDs in the Crooked tail mice, which bear a gain-of-function mutation in *Lrp6*. In contrast, folic acid supplementation cannot prevent spinal NTDs and may even cause early death of *Lrp6*-deficient mouse embryos. *Lrp6* is a crucial receptor for Wnts and maybe involved in both canonical Wnt/beta-catenin signaling and non-canonical Wnt/PCP (planar cell polarity) signaling pathways. PCP signaling plays a primary role in cell polarity and oriented tissue movements, such as convergent extension, a potential driving force for initiating neural tube closure and anterior-posterior body axis elongation. PCP mutant mice exhibit craniorachischisis, the severest, but rare NTD. However, the role of *Lrp6*-mediated canonical Wnt/beta-catenin signaling in neural tube closure and NTDs remains poorly understood. We demonstrate that conditional ablation of β -catenin in the dorsal neural tubes lead to spinal NTDs. In contrast, conditional activation of beta-catenin can rescue the spinal NTDs in the *Lrp6* mutants. Moreover, the folate-resistant spina bifida in the *Lrp6*-deficient mutants can be prevented by maternal supplementation of small-molecule Wnt signaling activators. Our results suggest that the canonical Wnt/beta-catenin signaling pathway is a significant target for the cause and prevention of the folate-resistant neural tube defects.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

48

A mouse model for gene-environment interaction in holoprosencephaly. Mingi Hong, Robert Krauss. Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY.

Holoprosencephaly (HPE), a common congenital disorder in which the midline of the forebrain and/or midface is deficient, is associated with heterozygous mutations in SHH and Nodal signaling pathway components. However, HPE phenotypes are highly variable and some mutation carriers are unaffected. A heterozygous mutation plus modifier model has been proposed to explain this scenario. Epidemiological studies suggest that maternal diabetes and fetal alcohol exposure are associated with HPE, so gene-environment interactions may be one explanation for modifier effects. We have modeled HPE in the mouse. CDON is a SHH coreceptor, and *CDON* mutations are found in individuals with HPE. *Cdon* mutant mice on a 129S6 background develop mild HPE with low penetrance but are sensitized to potential HPE modifiers. HPE phenotypes of *Cdon* mutants can be enhanced by a second mutation, such as in the additional SHH coreceptors, *Boc* or *Gas1*, or a synergistic environmental factor. We previously reported that, in 129S6 mice, loss of *Cdon* or fetal alcohol exposure alone do not cause HPE, but together produce defects in early midline patterning, inhibition of *Shh* signaling in the developing forebrain and a broad spectrum of HPE phenotypes. This model shows high specificity and fidelity to major aspects of human HPE. The sensitive time window for alcohol-induced HPE is over before E8.0, prior to major SHH function. Furthermore, expression of the *Nodal* signaling pathway target genes, *FoxA2*, *Gsc* and *Lefty2*, was decreased specifically in alcohol-treated *Cdon* mutant embryos at E7.25. The *Nodal* pathway is critical for induction of the precordal plate, a key structure for patterning the rostroventral midline that produces SHH. We hypothesize that *Cdon* mutation and alcohol exposure during gastrulation transiently disrupt *Nodal* signaling, leading to a situation where subsequent suboptimal SHH signaling due to loss of CDON results in variable HPE phenotypes in a stochastic manner. Consistent with CDON being a potential Nodal pathway regulator, we find that CDON directly interacts with components of the *Nodal* receptor complex in vitro. We propose that *Cdon* is involved in both the *Nodal* and *Shh* signaling pathways to promote midline patterning.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

49

A recessive ENU screen identifies *Memo1* as a novel gene driving palatogenesis and cranial-base development. **Eric Van Otterloo**, David Clouthier, Lee Niswander, Trevor Williams. University of Colorado - Denver, Anschutz Medical Campus, Aurora, CO.

Nearly 75% of all birth defects affect the head and orofacial complex, with ~1:750 live births having a facial cleft. To identify novel genes driving orofacial development we employed a recessive ENU screen and recovered a mutant line presenting with a shortened cranial base as well as a fully penetrant cleft secondary palate. Mapping identified the causative gene as *Mediator of ErbB2 Driven Cell Motility 1 (Memo1)*. Previous studies on *Memo1* have mainly focused on its role in breast cancer due to its ability to bind multiple receptor tyrosine kinases and it has not previously been associated with craniofacial development. We confirmed the role of *Memo1* in the embryonic phenotype using an available knockout (KO) allele coupled with non-complementation analysis. Detailed analysis of the various *Memo1* alleles we have at our disposal indicates that the phenotype is highly strain dependent. On a 129S1/SvImJ background it results in earlier lethality (as reported previously) than on a C57BL/6J background, when orofacial clefting predominates. Further analysis of the facial phenotype revealed a general failure in palatal shelf elevation. Examination of bone and cartilage development identified a general disruption of bones of the cranial-base and anterior craniofacial skeleton. As these phenotypes are consistent with a neural crest (NC) defect, we used *Wnt1-Cre* to delete *Memo1* in this tissue and confirmed a cell-autonomous role for *Memo1* in these cells. Early specification and migration of NC is not grossly affected, placing *Memo1*'s role developmentally downstream of these events. Current analyses are aimed at determining if *Memo1* mutation is affecting the function of a tyrosine kinase linked with facial development as well as associated cytoskeletal and cell migration processes, or whether the phenotype reflects the recently discovered role of *Memo1* in regulating redox potential during migration. In summary, our findings have identified a novel gene, *Memo1*, important in palatogenesis and cranial base morphogenesis likely exerting its effect through the NC. This work suggests *Memo1* may be an important candidate gene underlying cleft palate and cranial-base anomalies in humans.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

50

Mice chimeric for human tissues for disease modeling and preclinical research. Markus Grompe.
Oregon Stem Cell Ctr, Oregon Hlth & Sci Univ, Portland, OR.

Preclinical research in animals often fails to predict the outcomes observed in human patients. Chimeric animals bearing individual human tissues have been developed to provide improved models of human-specific cellular processes. Mice with hematolymphoid reconstitution can be used to study human immune responses, infections of blood cells and other processes of hematopoiesis. Animals with humanized livers are useful for modeling hepatotropic infections as well as drug metabolism and hepatotoxicity. However, many pathophysiologic processes involve both the liver and the hematolymphoid system. Examples include hepatitis C/HIV co-infection, immune mediated liver diseases, liver injuries with inflammation such as steatohepatitis and alcoholic liver disease.

We have developed a robust protocol enabling the concurrent double-humanization of mice with mature hepatocytes and human blood. Immune-deficient, fumarylacetoacetate hydrolase (Fah) deficient animals on the NOD-strain background (FRGN) were simultaneously co-transplanted with adult human hepatocytes and stem cells after busulfan pre-conditioning. Four months after transplantation liver repopulation exceeded 80% and hematopoietic chimerism also was high (40-80% in bone marrow). Importantly, human macrophages (Kupffer cells) were present in the chimeric livers.

Double-chimeric FRGN mice will serve as new preclinical model for any pathophysiology that involves interactions between hepatocytes and hematolymphoid cells.

Plenary/Platform Session 7 - Technology

51

Phenotyping Embryonic Lethals with Three-dimensional Imaging and Computer Analysis. R.

Mark Henkelman, Michael Wong. Mouse Imaging Centre, Hospital for Sick Children, Toronto, Ontario, Canada.

In the process of generating knockout lines for the International Mouse Phenotyping Consortium (IMPC) program, it is anticipated that about 7,000 of the lines will be embryonic lethal or subviable. Nonetheless, it is important to determine the lethal phenotype to understand the role of genes that are essential for development and because heterozygotes of these genes are frequently associated with human diseases and developmental disorders.

Three-dimensional imaging is an efficient and cost effective way of identifying critical lesions in these embryonic lethals. However, different types of imaging are required at different stages of development: Optical Projection Tomography (OPT) at E9.5; Magnetic Resonance Imaging (MRI) or preferably Micro Computed Tomography (μ CT) from E14 to E18; and, Diffusion-weighted MRI around p7. Each of these methodologies and the kind of performance achievable will be presented.

All of these 3D imaging methods result in large data sets—much larger than can be realistically viewed by human observers. Thus, it is important to develop computer-based analysis methods for screening these data sets to identify statistically significant abnormalities in the mutants compared with reference controls. Computer methods will be presented for identifying missing organs, for recognizing volume changes in various organs, and simple volume changes using voxel-based morphometry. The need for, and the implementation of, a time varying three-dimensional atlas of controls (a 4D atlas) will be presented. Such a comparative atlas allows for the identification of growth retardation delays in both whole embryos and in specific organs.

Samples of this kind of imaging and analysis will be presented from some of the first embryonic lethal mutants from the IMPC.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

52

Generation of newborn mice from embryonic stem cells using epiblast complementation chimeras.

Yeonsoo Yoon, Joy Riley, Judith Gallant, Stephen N. Jones, **Jaime A. Rivera-Perez**. Department of Cell and Developmental Biology, University of Massachusetts Medical School, Worcester, MA.

Recent advances in the derivation and maintenance of stem cell lines have generated a need to assess their developmental potency. Here we take advantage of epiblast-ablated mouse embryos to generate newborn pups from embryonic stem (ES) cells. In these epiblast complementation chimeras, the epiblast, the embryonic component of early post-implantation embryos is substituted with ES cells, leading to ES cell-derived newborn mice. Our novel chimera approach provides a stringent assay for testing the potency of stem cells and offers a simpler, efficient alternative to tetraploid complementation chimeras.

53

Isolation of rare recombinants without using selectable markers for one-step seamless BAC

mutagenesis. George T. Lyozin¹, Paul C. Bressloff², Amit Kumar³, Yasuhiro Kosaka¹, Bradley L. Demarest⁴, H. Joseph Yost^{1,4}, Michael R. Kuehn³, **Luca Brunelli**¹. 1) Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, UT; 2) Department of Mathematics, University of Utah, Salt Lake City, UT; 3) Laboratory of Protein Dynamics and Signaling, National Cancer Institute, Frederick, MD; 4) Department of Neurobiology and Anatomy, University of Utah School of Medicine, Salt Lake City, UT.

Current methods to isolate rare (1:10,000–1:100,000) bacterial artificial chromosome (BAC) recombinants require selectable markers. Seamless mutagenesis requires two steps: isolation of mutants using selectable markers followed by marker removal through counterselection. Here we illustrate founder principle–driven enrichment (FPE), a method to isolate rare BAC mutants without using selectable markers (*Nat Methods*, in press). FPE applies the founder principle from population genetics to rapidly isolate rare recombinants, allowing one-step seamless BAC mutagenesis. As proof of principle, we isolated 1:100,000 seamless fluorescent protein–modified *Nodal* BACs and confirmed BAC functionality by generating fluorescent reporter mice. We also isolated small indel P1 phage–derived artificial chromosome (PAC) and BAC recombinants. Statistical analysis revealed that 1:100,000 recombinants can be isolated with <40 PCRs, and we developed a web-based calculator to optimize FPE. By eliminating the need for selectable markers and counterselection, this work highlights a straightforward and low-cost approach to BAC mutagenesis, providing a tool for seamless recombineering pipelines in functional genomics.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

54

A ‘conditional-ON’ mouse model of Fibrosyplasia Ossificans Progressiva (FOP). C.J. Schoenherr¹, L. Huang¹, S.J. Hatsell¹, L. Xie¹, K. Nannuru¹, K. Feeley¹, T. Persaud¹, P. Yang¹, V. Idone¹, A. Lee², P. Yu³, A.J. Murphy¹, A.N. Economides¹. 1) Regeneron Pharmaceuticals, Tarrytown, NY; 2) National Center for Advancing Translational Sciences, NIH, Rockville, MD; 3) Brigham and Women’s Hospital, Boston, MA.

FOP (MIM 135100) is an autosomal dominant disorder characterized by early onset, episodic and progressive ossification of skeletal muscle and connective tissue. FOP is driven by mutations in the cytodomain of ACVR1, the most common mutation being Arg206His (R206H). To enable mechanistic studies and the development of therapeutic approaches for FOP, we engineered a Cre-regulated ‘conditional-ON’ allele of ACVR1[R206H] in the mouse – *Acvr1*[R206H]*COIN* – using a FIEEx-like design. This was necessary because an unregulated *Acvr1*[R206H] knock-in results in perinatal lethality. *Acvr1*^{[R206H]*COIN*} was generated by introducing the R206H mutation into exon 5 (e5) of *mmuAcvr1*, and placing this engineered mutated exon (e5[R206H]) and flanking intronic sequence in the antisense strand. To retain *Acvr1* function in the modified allele, the corresponding wild type region of hsaACVR1 e5 (‘WTe5’) was inserted into the sense strand of *mmuAcvr1*, upstream of e5[R206H]. To enable Cre-dependent replacement of WTe5 with e5[R206H], the WTe5 sequence was placed within a *lox2372-loxP* FIEEx array; a second FIEEx array was inserted after the inverted e5[R206H] in a mirror image configuration to the first array. Cre ‘activates’ *Acvr1*[R206H] by bringing e5[R206H] to the sense strand and deletes WTe5. Body-wide activation of the *Acvr1*[R206H] in *Acvr1*^{[R206H]*COIN*/+}; *Gt(ROSA)26Sor*^{CreERT2/+} adult mice using tamoxifen resulted in progressive heterotopic ossification (HO) resembling FOP. HO was spontaneous, did not require experimentally induced inflammation, and was evident on the axial and appendicular skeleton as early as 2 weeks after induction. Untreated mice were normal. The FOP phenotype was obtained in adult mice, hence uncoupling of the FOP in these mice from development. HO was prevented by treatment with the ACVR1 inhibitor LDN-212854, demonstrating that this model of FOP can be used for testing candidate therapeutic regimens. Additional novel therapeutic regimens will be presented in detail.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

55

HNF4A and FOXA2 exhibit differentiation-dependent enhancer switching in response to Hippo signalling. **P. Hoodless**¹, O. Alder¹, R. Cullum¹, S. Lee¹, A. Kan¹, W. Wei¹, Y. Yi¹, V. Garside¹, M. Bilenky², M. Griffith², S. Morrissy², G. Robertson², N. Thiessen², Y. Zhao², Q. Chen³, D. Pan³, S. Jones², M. Marra². 1) Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada; 2) Michael Smith Genome Sciences Centre, BC Cancer Agency; 3) Johns Hopkins University School of Medicine.

Cell fate acquisition is heavily influenced by direct interactions between master regulators and tissue-specific enhancers. However, it remains unclear how lineage-specifying transcription factors, often expressed in both progenitor and mature cell populations, influence cell differentiation. The hepatic master regulators, Hepatocyte Nuclear Factor (HNF) 4A and the pioneer factor, FoxA2 are highly expressed in both hepatoblasts and hepatocytes. The recent generation of induced hepatocytes (iHeps) from both mouse and human fibroblasts via ectopic expression of HNF4A, in conjunction with Forkhead box A family members highlights the critical role these master regulators play in hepatocyte differentiation. Using *in vivo* mouse liver development as a model, we compared HNF4a and FOXA2 DNA interactions in hepatoblasts isolated from E14.5 embryos and hepatocytes from adult livers. Using ChIP-Seq, we identified thousands of co-binding events occurring exclusively at each developmental stage. Analysis of surrounding H3K4me1 distributions suggested these sites function as differentiation-dependent enhancers. RNA-Seq analysis, confirmed that enhancer-switching during differentiation associates with temporal gene expression patterns. To determine whether chromatin accessibility may influence enhancer switching, we profiled repressive histone modifications and found no strong evidence of chromatin condensation, consistent with FOXA2's role as a pioneer factor. However, motif analysis showed that enhancers exclusively occupied in progenitors were enriched for TEAD motifs. Furthermore ectopic expression of TEAD co-activator YAP1 in adult liver induced enhancer switching. Our data suggests HNF4A and FOXA2 occupancy of temporal enhancers, and consequently hepatocyte differentiation, is influenced by Hippo signaling. In summary, transcription factor-enhancer interactions are not only tissue-specific but differentiation-dependent. Funding: Canadian Institutes of Health Research.

56

Meiotic recombination mechanisms and hotspot evolutionary dynamics revealed by mouse tetrad analysis. **Maria Jasin.** Developmental Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY.

The ability to examine all chromatids from a single meiosis in yeast tetrads has been indispensable for defining mechanisms of homologous recombination initiated by DNA double-strand breaks (DSBs). Using a broadly applicable strategy for the analysis of chromatids from a single meiosis at recombination hotspots in mouse oocytes and spermatocytes, we have demonstrated the unidirectional transfer of information — gene conversion — in both crossovers (COs) and noncrossovers (NCOs). Whereas gene conversion in COs is associated with reciprocal exchange, the unbroken chromatid is not altered in NCO gene conversions, providing strong evidence that NCOs arise from a distinct mechanism. A substantial fraction of NCO and CO gene conversions spares the binding site of the hotspot-specifying protein PRDM9, suggesting multi-template interactions during recombination, with the result that hotspot erosion is slowed. Thus, mouse tetrad analysis demonstrates how unique aspects of mammalian recombination mechanisms shape hotspot evolutionary dynamics.

Plenary/Platform Session 8 - Stem Cells

57

Dissecting the role of PW1/Peg3: a parentally imprinted gene that is expressed in all adult stem cells. David Sassoon, Rosamaria Corra, Anne-Lyse Denizot, Luigi Formicola, David Ollitrault, Jean-Remy Courbard, Karo Tanaka, Kateryna Kyrylkova, Sergiy Kyryachenko, Giovanna Marazzi. Stem Cells Regenerative Medicine, Univ. Paris/INSERM/ICAN, France.

PW1/Peg3 is a parentally expressed gene expressed in all adult progenitor/stem cells investigated to date including skin, skeletal muscle, bone, testis, bone marrow and the CNS. Previous studies by us and others have shown that PW1/Peg3 participates in the p53 as well as the inflammatory cell stress pathways. We generated a bac-mediated reporter gene which allows for a rapid and efficient identification of stem cells in a large array of tissues, and have extended these studies to adult tissues for which the identity of the stem cell is a matter of debate such as the heart. Ongoing studies show that PW1/Peg3 is expressed in these tissues and identifies cells with progenitor capacity. Moreover, we find that PW1/Peg3 expression is coincident with stem cell competence. Both knock down and knock out experiments suggest that PW1 plays a role in stabilizing stem cell quiescence as well as in regulating the anatomical organization of the stem cell niche.

In addition to studies focused on a potential role in stem cells, it was previously reported that PW1/Peg3 plays a key role in regulating maternal behaviors and the capacity to nurse pups (let down). As such, this gene has served as a key example to support the general notion that parentally imprinted genes play a role in driving mammalian parental behaviors. Our initial studies using a new conditional mutant allele we have generated reveals that all aspects of maternal behaviors are intact and robust in both paternal and homozygous mutant mice, thereby challenging the current view.

It is of interest that PW1/Peg3 is one of 10 parentally imprinted genes that has been shown to be preferentially expressed in adult mammalian stem cells. We propose that these genes participate as a network in controlling the expansion and possible cell fate capacities of stem cells in mammalian tissues.

58

Transit-Amplifying Cells Orchestrate Stem Cell Activity and Tissue Regeneration. Ya-Chieh Hsu¹, Lishi Li², Elaine Fuchs². 1) Dept of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA; 2) Rockefeller University, New York, NY.

In response to stimuli from their niche, stem cells (SCs) regenerate tissues. How the regenerative process is regulated is poorly understood. Here we show that transit-amplifying cells (TACs) produced by activated SCs play a hitherto unrecognized role in fueling this process. Using hair cycle as paradigm, we show that quiescent SCs proliferate only after TAC establishment, a phenomenon rooted in Sonic Hedgehog (SHH) production by emerging TACs. Intriguingly, TACs can proliferate without autocrine SHH signaling, but their pool wanes if they can't produce it. We trace this paradox to two SHH's effects: promoting SC proliferation directly and regulating dermal factors, which then stoke TAC expansion. Ingrained within the unique sensitivity of quiescent SCs to long-range SHH signaling is their high expression of GAS1. Our findings unveil TACs as indispensable SC niche components, and illuminate how factor sensitivity can be more important than the absolute distance between SCs and niche components.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

59

Robo4-mediated vascular integrity regulates hematopoietic stem cell trafficking. Camilla Forsberg, Stephanie Smith-Berdan, Andrew Nguyen, Matthew Hong. University of California Santa Cruz, Santa Cruz, CA.

Despite the use of hematopoietic stem cells (HSCs) in clinical therapy for over half a century, the mechanisms regulating HSC trafficking, engraftment and life-long persistence after transplantation are unclear. Recent findings have demonstrated that long-term HSC function within bone marrow (BM) niches depends on cells associated with the BM vasculature. Here, we show that the vascular endothelium also regulates HSC trafficking into and out of these BM niches. We previously reported that Robo4, expressed by HSCs, promotes HSC localization to BM niches at steady-state and upon transplantation. We showed that wt HSCs outcompete Robo4^{-/-} HSCs in competitive transplantation assays into wt mice. Here, we tested the hypothesis that wt HSCs would also outcompete Robo4^{-/-} HSCs upon transplantation into Robo4^{-/-} recipients and therefore engraft with high efficiency in Robo4-deficient hosts. Surprisingly, we found the opposite: engraftment of wt HSCs was significantly poorer in Robo4^{-/-} compared to wt recipient mice. Using a combination of in vivo and in vitro assays, we found that Robo4, expressed by endothelial cells, prevents vascular permeability yet is necessary for efficient HSC translocation across endothelial layers. Thus, instead of acting as barriers to cellular entry, vascular endothelial cells actively promote HSC extravasation across vessel walls into the BM space. In contrast, our experiments also revealed that the vasculature inhibits the reverse process of HSC mobilization from the BM to the blood stream. We found that induced vascular permeability by pharmacological targeting of endothelial cells led to a rapid increase in HSCs in the blood stream. Thus, the vascular endothelium reinforces HSC localization to BM niches both by promoting HSC extravasation from blood-to-BM and by forming vascular barriers that prevent BM-to-blood escape. Our results uncouple the mechanisms regulating the directionality of HSC migration across vessel walls and show that the vasculature can be targeted to regulate HSC trafficking to improve hematopoietic transplantation therapies.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

60

The dual role of SOX2 in pituitary development. S. Goldsmith, R. Lovell-Badge, K. Rizzoti. Stem Cell Biology and Developmental Genetics, MRC NIMR, London, United Kingdom.

SOX2 is expressed in the pituitary anlagen, Rathke's Pouch (RP), from its induction at 9.0dpc. RP progenitors initially expand, later giving rise to all endocrine cell types. As cells differentiate, SOX2 expression is down-regulated, but a population of SOX2⁺ cells persists until adulthood. These represent adult stem cells, displaying regenerative potential upon physiological demand. In humans and mice, heterozygous loss-of-function mutations in *SOX2/Sox2* are associated with hypopituitarism. It was therefore decided to investigate the role of SOX2 during murine pituitary development.

Homozygous null mutations of *Sox2* lead to embryo lethality following implantation; consequently conditional strategies were used to delete the gene specifically in RP. *Nkx3.1^{Cre}* and *FoxG1^{Cre}*, display different spatio-temporal patterns of activity, with the severity of the pituitary phenotype, notably a reduction in progenitor cell proliferation, correlated with the efficiency and timing of Cre-mediated deletion of *Sox2*. The expression of the transcription factor SIX6, known for its role in promoting cell proliferation, is downregulated, while expression of the cell cycle inhibitor p27kip1 is up-regulated, in the absence of *Sox2*. Furthermore, the proliferation defect in *Sox2* mutants can be rescued by homozygous loss of *p27kip1*, demonstrating a genetic interaction. Suggesting SOX2 promotes progenitor cell proliferation in the early RP and may do so, at least in part, by regulating *Six6* and *p27kip1* expression.

Sox2 RP mutants display a disproportionate reduction in melanotroph cell numbers in the intermediate lobe (IL). The *Sox2*-deleted cells display a downregulation of the melanotroph lineage specifier, PAX7. Consequently, the few differentiated cells present in mutant IL switch from a melanotroph identity to a corticotroph fate, despite these cells never being present in the normal IL. This phenotype was not rescued in *p27kip1;Sox2* compound mutants. This suggests that SOX2 plays two independent roles during pituitary development, initially promoting progenitor proliferation and later specifying IL melanotroph cell fate.

61

Delineating the mesendoderm lineage hierarchy in culture. Ingo Bartscher^{1,2}, Dapeng Yang^{1,2}, Michael Schwarzfischer³, Carsten Marr³, Fabian Theis³, Martin Irmeler⁴, Johannes Beckers⁴, Heiko Lickert^{1,2}. 1) Institute of Diabetes and Regeneration Research, Helmholtz Centre Munich, 85764 Neuherberg, Bavaria, Germany; 2) Institute of Stem Cell Research, Helmholtz Centre Munich, 85764 Neuherberg, Bavaria, Germany; 3) Institute of Computational Biology, Helmholtz Centre Munich, 85764 Neuherberg, Bavaria, Germany; 4) Institute of Experimental Genetics, Helmholtz Centre Munich, 85764 Neuherberg, Bavaria, Germany.

Our former results indicated that anterior *Foxa2*⁺ and posterior Brachyury (T)⁺ mesendoderm progenitors are already specified and separated in the epiblast before gastrulation (Bartscher and Lickert, 2009). However, the progenitor relationship and mesendoderm lineage hierarchy could technically not be solved due to our previous static endpoint analysis on fixed tissue samples. In this study, we have generated a dual fluorescent knock-in *T^{GFP/+}; Foxa2^{tagRFP/+}* reporter ESC line for single-cell time-resolved lineage tree analysis of ESC differentiation. This revealed that three different mesendoderm lineages are directly formed from pluripotent ESCs under endoderm and mesoderm promoting differentiation conditions in culture. Moreover, time-resolved isolation and molecular profiling of these distinct lineages further uncovered that mesoderm (Mes), axial mesendoderm (AME) and definitive endoderm (DE) are formed from three distinct T⁺, T⁺/*Foxa2*⁺, and *Foxa2*⁺ progenitor populations, respectively. In contrast to the common believe, DE is not formed via a T⁺ progenitor state and does not require T function for development. Thus, this analysis demonstrates an unprecedented progenitor relationship and lineage hierarchy during mesendoderm differentiation and is crucially important for the differentiation of the correct progenitor population into functional cell types in culture.

PLATFORM PRESENTATION ABSTRACTS

Presenters in bold

62

A novel pathway involving the Chromatin-remodeling protein Rere acts downstream of retinoic acid signaling to control embryonic symmetry. **Oliver Pourquié**^{1,2}, Goncalo Vilhais-Neto¹. 1) Institut de Génétique et de Biologie Cellulaire et Moléculaire (IGBMC), Inserm U964, CNRS (UMR 7104), Université de Strasbourg, ILLKIRCH, FRANCE; 2) Department of Genetics, Harvard Medical School, Department of Pathology, Brigham and Woman's Hospital, BOSTON,.

One of the most notable features of the vertebrate body plan organization is its bilateral symmetry, evident at the level of vertebrae and skeletal muscles. We showed that the chromatin remodelling protein Rere (Atrophin2) can act as a co-factor for retinoic acid (RA) signalling involved in the control of somite bilateral symmetry. Mutation of the Rere protein in the openmind mouse mutant leads to the formation of asymmetrical somites, as seen in embryos deprived of RA. Rere binds NR2F2 (COUP-TF2), p300 and the retinoic acid receptor (RAR) which is recruited to the retinoic acid regulatory element (RARE) of RA targets, such as the RAR-beta promoter. Knockdown of NR2F2 and/or Rere decreases RA signalling, suggesting that this complex is required to promote transcriptional activation of RA targets. NR2F2 is asymmetrically expressed in the right presomitic mesoderm (PSM) in a domain which overlaps with the PSM domain showing asymmetrical RA signalling detected in the RARE-LacZ reporter mouse. To gain further understanding on Rere function in RA signalling, we generated a Rere-HA tagged mouse line that conditionally expresses the tagged protein in mesodermal tissues. Together with mass spectrometry we identified proteins forming a complex with Rere. We are currently investigating the role of several of these proteins in RA signalling activation and as well in the control of somite bilateral symmetry.

Disease Modeling

63

Disruption of the mouse *Jhy* gene causes juvenile hydrocephalus. Brian Grewe¹, Oliver K. Appelbe², Bryan Bollman¹, Ali Attarwala¹, Lindy A. Tribes¹, Hilmarie Muniz-Talavera¹, Jennifer V. Schmidt¹. 1) Biological Sciences, University of Illinois-Chicago, Chicago, IL; 2) Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL.

Hydrocephalus results from a disruption of cerebrospinal fluid (CSF) dynamics within the central nervous system. Left untreated, hydrocephalus typically leads to increasing intracranial pressure with associated neuronal cell loss and eventual death. The integration of a *lacZ* reporter transgene into the mouse *Jhy* gene generated a novel mouse model for juvenile hydrocephalus, the *Jhy*^{lacZ} mouse line.

Homozygous *Jhy*^{lacZ/lacZ} mice exhibit ventricular dilation as early as postnatal day 1.5, and the majority of animals have died or must be sacrificed by 4-8 weeks of age. Electron microscopy determined that the ependymal cilia lining the lateral ventricles of *Jhy*^{lacZ/lacZ} mice are reduced in number with abnormal microtubule organization. *Jhy*^{lacZ/lacZ} ependymal cells develop predominantly 9+0 or 8+2 microtubule arrangements rather than the 9+2 configuration typical of motile cilia. These data suggest that loss of ciliary-mediated ependymal flow may cause CSF accumulation and hydrocephalus observed in *Jhy*^{lacZ/lacZ} mice. The *Jhy* gene is expressed in the brain, testes, spleen, lung, liver, thymus, and heart, and it is predicted to encode an 87 kDa protein lacking any identifiable functional domains. Western blot and mass spectrometry analysis of testes total protein lysate identify JHY as a 100 kDa protein, likely due to unidentified posttranslational processing. Immunofluorescence has shown that the JHY protein localizes to ependymal cells in the mouse brain, suggesting a direct role in the development of ependymal cilia.

Ongoing experiments are directed at examining the function of the JHY protein and understanding the mechanism of hydrocephalus in *Jhy*^{lacZ/lacZ} mice.

64

An initiative for mechanistic study on non-cancer health effects by ionizing radiation: a comprehensive and systematic phenotyping of irradiated mice. Yoshinobu Harada¹, Hitomi Sudo², Toshiyuki Saito³, Makoto Akashi¹. 1) Fukushima Project Headquarters, NIRS, Chiba, Japan; 2) Molecular Imaging Center, NIRS, Chiba, Japan; 3) Research Center for Charged Particle Therapy, NIRS, Chiba, Japan.

Substantial epidemiological studies on non-cancer health effects by ionizing radiation have been conducted and further mechanistic investigation is required on the issue at present. Radiation should affect various cells and might cause non-cancer health effects as accumulation of various influences and interactions. Laboratory mice must be an efficient tool to clarify the molecular mechanism of the non-cancer health effects by providing comprehensive and precise clinic system for irradiated bodies, that is unavailable in human. C57BL/6NTac mice acutely-irradiated with 0.5 or 5.0 Gy of X-ray were subjected to extensive clinical analysis in 'Japan Mouse Clinic', a comprehensive and systematic abnormality screening system conducted by the RIKEN BioResource Center (http://ja.brc.riken.jp/lab/jmc/mouse_clinic/en/index.html). The phenotyping pipeline used in this study was based on 'the International Mouse Phenotyping Resource of Standardised Screens' which was prescribed by the International Mouse Phenotyping Consortium (<http://www.mousephenotype.org/>). Seventeen procedures that included more than five hundreds inspection items (described in detail at <http://www.mousephenotype.org/impress>) were performed on the mice. Compared with control mice, the irradiated mice showed significant changes in various inspection items. Some of those were accelerated in a dose-dependent manner. Body weight and locomotive activities were significantly lower than of the control. Interestingly, the mean of the heart weight of irradiated mice was significantly smaller than of control mice. The male mice irradiated with 5.0 Gy have shown prolonged QT intervals in the

POSTER ABSTRACTS

Presenters in **bold**.

electrocardiogram test. In the presentation, we focus on some changed items and discuss possible molecular mechanisms of these phenotypes. And we also discuss about the efficacy and expandability of our experimental approach.

65

Quantitative trait loci for early-stage atherosclerosis at different aortic locations. Y Kayashima, K Matsuki, H Tomita, N Maeda. Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Atherosclerotic plaque development at different aortic locations of mice is differentially influenced by their genetic backgrounds. The plaque development at the aortic root of the apolipoprotein E null mice on 129S6 background (129-apoE) is slower than in the apoE-null mice on a C57BL/6J background (B6-apoE), which is in turn slower than in those on a DBA/2J background (DBA-apoE). However, both the 129-apoE and DBA-apoE mice develop equally extensive plaques at their aortic arch much earlier than the B6-apoE mice. QTL analysis of an F2 population from a cross between B6-apoE and 129-apoE revealed one locus on chromosome (Chr) 9 at 61 Mb (*Ath29*) significantly affects the plaque size at the aortic root, while two loci on Chr 1 at 105 Mb (*Aath1*) and 163 Mb (*Aath2*), and one locus on Chr 15 at 96 Mb (*Aath3*) affect the plaque size at the aortic arch. In contrast, a cross between 129-apoE and DBA-apoE identified two QTLs for the root lesion size on Chr 1 at 158 Mb (*Ath44*) and on Chr 2 at 162 Mb (*Ath45*), and two QTLs for the arch lesion size on Chr 2 at 137 Mb (*Aath4*) and on Chr 10 at 57 Mb (*Aath5*). In each F2 populations the loci for aortic root lesions and loci for aortic arch lesions were distinct, suggesting that different genetic factors likely determine the location-specific susceptibility to atherosclerosis. Utilizing genomic information of C57BL/6J, 129S5 and DBA/2J, and gene expression levels in parental strains, we screened for potential candidate genes for each locus. Haplotype distributions suggest *Fmo3*, *Sele* and *Selp* for *Ath44*, and *Lbp* and *Pkig* for *Ath45* are among those that require further investigation. In the aortic arch, altered *Mertk* and *CD93* within *Aath4* interval, which play roles in phagocytosis of apoptotic cells and thereby modulate inflammation, may be contributing to athero-susceptibility of DBA-apoE. Similarly, *Stab2*, a candidate in the *Aath5* interval, encodes a scavenger receptor for multiple ligands including apoptotic cells, modified lipoproteins, and hyaluronans. The DBA-allele of *Stab2* is associated with 10 times higher plasma hyaluronans than the 129-allele, as well as with a 60 fold increased *Stab2* expression in macrophages and athero-protection at the aortic arch.

POSTER ABSTRACTS

Presenters in **bold**.

66

The Mouse Hydrocephalus Gene *JHY* Plays a Role in Ependymal Cell Differentiation. Hilmarie Muniz-Talavera¹, Jennifer V. Schmidt². 1) University of Illinois-Chicago, Chicago, IL; 2) University of Illinois-Chicago, Chicago, IL.

Juvenile hydrocephalus results from abnormalities in the production, flow or absorption of cerebrospinal fluid (CSF) within the ventricles of the brain. Increased CSF volume causes ventricular dilation that can be fatal if untreated, and congenital hydrocephalus is a significant human health issue. The *Jhy*^{lacZ} mouse line carries an insertional mutation in the previously uncharacterized gene *JHY*, and homozygous *Jhy*^{lacZ/lacZ} mice develop rapidly progressive juvenile hydrocephalus. Ultrastructural analysis of postnatal *Jhy*^{lacZ/lacZ} mice found abnormal organization of the motile cilia lining the lateral ventricles of the brain, structures believed to be required for proper CSF flow. During the first postnatal week, radial glia lining the brain ventricles differentiate into ependymal cells, undergoing a morphological change from pseudostratified cuboidal cells to a monolayer of flat/scale-like cells. This differentiation event proceeds in a caudal to cranial, ventral to dorsal, and medial to lateral progression. Characterization of the ventricular ependyma of wild type (*Jhy*^{+/+}) mice using the ependymal marker S100 β shows that medial wall ependymal cells acquire a mature appearance by postnatal day 5 (P5). In *Jhy*^{lacZ/lacZ} mice, medial ventricular wall ependymal cells retain an immature appearance at P5, with an unorganized, multi-layered morphology. Lateral wall ependymal cells normally mature later than medial wall cells, and remain immature at this stage in both *Jhy*^{+/+} and *Jhy*^{lacZ/lacZ} mice. Histological analysis of *Jhy*^{lacZ/lacZ} ependyma at P5, P10 and P14 demonstrated that medial wall ependymal cells do progressively acquire mature ependymal characteristics, suggesting a delay, rather than a block, in the differentiation process. Mature ependymal cells are connected laterally by adherens junctions (AJ), structural and signaling complexes known to be involved in ependymal cell differentiation. Ependymal cells in *Jhy*^{lacZ/lacZ} mice fail to properly localize the key AJ protein N-cadherin, with resulting defects in AJ formation. These data suggest that loss of *JHY* alters the ependymal cell developmental program, with delayed ependymal cell maturation and the potential loss of signaling through AJ complexes.

67

Runx1/Cbfb transcription factor complex is essential in maintaining articular cartilage. Clara Y Park, Xiangguo Che, Na-Rae Park, Seung Hee Han, Gyoung-Hwa Kim, Je-Yong Choi. Kyungpook National University School of Medicine, Daegu, South Korea.

The function of core binding factor β (Cbfb), a partner protein of the Runx family transcription factor, has not been determined in articular cartilage. To explore the *in vivo* function of Cbfb in articular cartilage, we generated articular cartilage-specifically deleted *Cbfb* mice (*Cbfb*^{lac/lac}) by crossing Gdf5 promoter-driven Cre mice with Cbfb floxed mice. When we induced osteoarthritis (OA) through destabilization of the medial meniscus (DMM) surgery in the mice at 12 weeks of age, OA phenotypes were exacerbated in *Cbfb*^{lac/lac} mice compared to wild type mice. Histologically, the decrease in Cbfb, Runx1, and type II collagen and the increase of MMP13 in *Cbfb*^{lac/lac} mice were greater compared to wild type mice. The formation of a Runx1/Cbfb complex was detected in various types of chondrocytes *in vitro*, including primary cultured chondrocytes. Rescue of Cbfb in Cbfb-deficient chondrocytes increased Runx1 protein level and target gene expression. Additionally, *Cbfb*^{lac/lac} mice did not differ from wild type mice in joint and skeletal tissue formation. However, these mice naturally acquired phenotypes similar to mild OA from 8 months in which Runx1 and type II collagen diminished and MMP13 increased.

Collectively, these results indicate that Cbfb is required for Runx1 stability as a partner protein in articular cartilage and that the formation of Cbfb-Runx1 complex plays an essential role in articular cartilage homeostasis.

Genetics and Genomics

68

A cre tool resource for conditional mutagenesis in the mouse, www.creportal.org. Randy Babiuk, Martin Ringwald, Steve Murray, Janan T. Eppig. The Jackson Laboratory, Bar Harbor, ME.

Conditional mutagenesis is a powerful technique for interrogating gene function in a cell- or tissue-specific manner, potentially with a defined temporal component. To exploit fully the available conditional-ready mutations like those generated by the IKMC (www.knockoutmouse.org), information about the spatial (and temporal) specificity of recombinase alleles is essential to select the driver/allele combination best suited for experimental purposes. The cre portal (www.creportal.org) is a free public resource focusing on recombinase tools (primarily cre) generated by research labs, and large cre tool sets created by major initiatives such as the NIH Blueprint Cre Driver Network, the Allen Institute for Brain Science, the Pleiades Promoter Project, and EUCOMMTOOLS. Through integration with the Mouse Genome Informatics resource (MGI, www.informatics.jax.org), the cre portal provides a central access point to existing recombinase alleles and curated data for them. Important new enhancements improve searching, provide summary overviews, and allow users to submit data directly or to add observations about recombinase alleles used in their research. The new anatomy search allows querying for cre activity in specific anatomical substructures (e.g. smooth muscle) using the Mouse Anatomical Dictionary with the system(s) containing matching annotations highlighted in the summary. Querying by a specific driver is an alternate search option. Results display a summary of recombinase alleles with activity (observed or absent) in a specified structure or all alleles with a specified driver, and include information about the driver/promoter, inducibility, associated references, and public repository availability. Allele symbols link to a detail page containing a recombinase data summary in matrix format with each cell providing activity data for specific anatomical system by age combinations. Anatomical systems can be expanded to view substructures with annotations. The cre portal contains detailed information for >2100 recombinase alleles utilizing >830 unique drivers, as of June 2014.

Supported by NIH grant OD011190.

POSTER ABSTRACTS

Presenters in **bold**.

69

Vitamin D receptor in mature osteoclasts suppresses osteoclastogenesis and enhances bone resorption in male mice. Gyeong-hwa Kim¹, Xiangguo Che¹, Yong-Joo Clara Park¹, Na-Rae Park¹, Seung-Hee Han¹, Shigeaki Kato², Je-Yong Choi¹. 1) Department of Biochemistry and Cell Biology, School of Medicine, Kyungpook National University, Daegu, South Korea; 2) Soma Central Hospital (S.K.), Fukushima, Japan.

Vitamin D was discovered in an effort to prevent rickets and osteomalacia, a disease characterized by impaired bone mineralization. The active vitamin D metabolite, 1,25-dihydroxy vitamin D, induces the receptor activator of nuclear factor- κ B ligand (RANKL) in osteoblasts which stimulates osteoclast differentiation and thus bone resorption. Although the role of the vitamin D receptor (VDR) has been elucidated in osteoblasts, controversy still exists on its existence and role in osteoclasts. We examined the effect of Vdr deletion in mature osteoclasts by crossing cathepsin K-cre mice with *Vdr*^{flox/flox} mice (*Vdr*^{OC/DC}). The deletion of Vdr in mature osteoclasts was confirmed *in vivo* by immunohistochemistry and through RNA and protein assays from bone marrow monocyte (BMM) culture of wild type (WT) and *Vdr*^{OC/DC} mice. At 8 weeks of age, male *Vdr*^{OC/DC} mice had greater bone mass and osteoclast number than WT mice. *In vitro* BMM cultured osteoclasts of *Vdr*^{OC/DC} mice exhibited increased number of the multi-nuclear osteoclasts but lower mRNA expression of osteoclast maturation and resorption markers such as *PU.1*, *Runx1*, *Nfatc1*, *DC-stamp*, *Rank* and *Traf6*, and lower protein expression of Runx1 and Nfatc1 compared to WT mice. These results support that the VDR exists in osteoclasts and indicate that VDR in mature osteoclasts suppresses osteoclastogenesis and enhances bone resorption.

70

Noncoding RNA transcription targets AID to divergently transcribed loci in B cells. Evangelos Pefanis^{1,3}, Jiguang Wang^{1,2}, Gerson Rothschild¹, Junghyun Lim¹, Jaime Chao¹, Raul Rabadan², Aris Economides³, Uttiya Basu¹. 1) Columbia University, Department of Microbiology & Immunology, New York, NY; 2) Columbia University, Department of Biomedical Informatics, New York, NY; 3) Regeneron Pharmaceuticals, Tarrytown, NY.

The vast majority of the mammalian genome has the potential to express noncoding RNA (ncRNA). The 11-subunit RNA exosome complex is the main source of cellular 3'-5' exoribonucleolytic activity and potentially regulates the mammalian noncoding transcriptome. Here we generated a mouse model in which the essential subunit *Exosc3* of the RNA exosome complex can be conditionally deleted. *Exosc3*-deficient B cells lack the ability to undergo normal levels of class switch recombination and somatic hypermutation, two mutagenic DNA processes used to generate antibody diversity via the B cell mutator protein activation-induced cytidine deaminase (AID). The transcriptome of *Exosc3*-deficient B cells has revealed the presence of many novel RNA exosome substrate ncRNAs. RNA exosome substrate RNAs include xTSS-RNAs, transcription start site (TSS)-associated antisense transcripts that can exceed 500 base pairs in length and are transcribed divergently from cognate coding gene transcripts. xTSS-RNAs are most strongly expressed at genes that accumulate AID-mediated somatic mutations and/or are frequent translocation partners of DNA double-strand breaks generated at *Igh* in B cells. Strikingly, translocations near TSSs or within gene bodies occur over regions of RNA exosome substrate ncRNA expression. These RNA exosome-regulated, antisense-transcribed regions of the B cell genome recruit AID and accumulate single-strand DNA structures containing RNA-DNA hybrids. We propose that RNA exosome regulation of ncRNA recruits AID to single-strand DNA-forming sites of antisense and divergent transcription in the B cell genome, thereby creating a link between ncRNA transcription and overall maintenance of B-cell genomic integrity.

POSTER ABSTRACTS

Presenters in **bold**.

71

Using RNA-Sequencing in Linkage Analysis of Complex Traits: Evolution of response to LPS.

Alexander N Poltorak^{1,2}, Vladimir Ilyuha², Maria Churova², Irina Kurbatova², Uliana Bagina², Volkova Tatyana². 1) Tufts University, Boston, MA; 2) Petrozavodsk State University, Karelia, Russia.

The majority of mutations underlying monogenic disease traits alter protein structure. However, the first years of genome-wide association studies (GWAS) for complex disease have shown that only approximately 5% of the currently validated disease associations would have been explained by coding variants. Thus, the dissection of the genetic architecture of human disease is now focused on variants residing outside of coding regions; that is variants that potentially affect regulatory elements. Next Generation Sequencing (NGS) approach makes it possible to simultaneously monitor expression of thousands of genes using them as read-outs in eQTL analysis. To establish a model for genetic analysis of complex traits, we compared classical laboratory and so-called wild-derived mouse strains in their responses to bacterial lipopolysaccharide (LPS). In both strains, thousands of genes were up-regulated by LPS. We have mapped and quantified mouse transcriptomes by deeply sequencing them and recording how frequently each gene is represented in the sequence sample. Using RNA-seq of F1 hybrid mice, we directly detected cis-regulatory variation by allele counting in RNA-seq data. To confirm cis-regulated differential allelic expression, we used RNA-sequencing of the second-generation intercross F2 mice. The RNA-seq data provided support for cis-regulation as well as helped establishing a digital framework of genes that confer differences in LPS-responses in genetically diverse strains of mice.

72

A genome-edited allele of *Nxf1* precisely identifies a modifier of retrovirus mutations. **Kevin Ross**¹,

Dorothy Concepcion², Bruce Hamilton^{1,2}. 1) Biomedical Sciences Graduate Program, University of California, San Diego, La Jolla, CA; 2) Department of Cellular & Molecular Medicine and Institute for Genomic Medicine, University of California, San Diego, La Jolla, CA.

We previously showed that a wild-derived haplotype of the major mRNA nuclear export factor, *Nxf1*, acts as a modifier gene for mutations caused by integration of a specific retrovirus element into host gene introns. Integration of IAP I \square 1 elements in the sense orientation introduces an alternative splice acceptor site, interrupting host gene expression. The *Nxf1* variant from *Mus musculus castaneus* acts as a genetic modifier through increased abundance of exon-exon spliced mRNA at the expense of exon-virus spliced RNA. We show here that *Nxf1* suppressor alleles act on a wide range of IAP subclasses throughout the mouse reference genome. We show by CRISPR/Cas9-mediated genome editing that all of the modifier activity can be explained by a single amino acid substitution, E610G. This allele recapitulates all of the neurological, survival, and gene expression effects of the wild *castaneus* allele on the classical *vibrator* mutation and recapitulates gene expression effects at other B6 loci that include intronic IAP insertions, including *Adamts13*.

POSTER ABSTRACTS

Presenters in **bold**.

73

Insights into brain isoforms of neurotrophin receptor P75NTR. Mohamed Sabry¹, Mona Fares², Ronnie Folkesson³, Mariam Al-Ramadan⁴, Adel Alalwan¹, Jarrah Alabkal¹, Ahmed Baqer¹, Moustapha Hassan^{2,5}. 1) Department of Medical Biochemistry, College of Medicine & Medical Sciences, Arabian Gulf University, P.O.Box 26671 Manama, Bahrain; 2) Experimental Cancer Medicine (ECM), laboratory Medicine, Karolinska Institutet, Stockholm, Sweden; 3) Department of Neurobiology, Care Sciences and Society (NVS), Karolinska Institutet, Stockholm, Sweden; 4) Biotechnology Program, College of Postgraduate Studies, Arabian Gulf University, Manama, P O Box 26671, Bahrain; 5) Clinical Research Centre, Novum, Karolinska University Hospital-Huddinge, Sweden.

The low-affinity neurotrophin receptor P75NTR is a member of tumor necrosis factor superfamily. To explore the presence of different isoforms of P75NTR, we conducted western blotting (WB) on brain protein extracts from mouse, rat and human using polyclonal antibodies specific to the intracellular region of P75NTR. In addition to the 75kD full-length P75NTR (FL-p75NTR), which we identified in the brains of the three species investigated, we also identified a new, previously unreported 36kD isoform of P75NTR in human brain but not in the murine brains. Contrary to previous reports [von-Schack *et al. Nat Neurosci.* **4**, 977 – 78 (2001) & Naumann T *et al. J Neurosci.* **22**, 2409–18 (2002)], we could not find any WB evidence to support the existence of a 62kD short variant s-p75NTR in any of the mouse, rat, or human brains investigated. Indeed no other bands, apart from the 75kD FL-p75NTR band, were detected in brain protein extracts from mouse and rat. RT-PCR was conducted on cDNA from the murine and human brains using oligos flanking or including exon III, which was said to be alternatively spliced in the claimed 62kD short variant s-p75NTR. Also we used primers with exons 2/4 boundary sequences (i.e. discontinuous, exon III-skipping primers) as ‘specific’ oligos to the presumed s-p75NTR variant. Again, this approach did not identify any exon III-excluding isoform in any of the murine or human brains investigated. Our results also excluded the presence of the ENSEMBL-predicted human variant, ENSP00000421731. The new 36kD isoform of P75NTR we identified in human brain likely represents an un-posttranslationally modified FL-p75NTR.

POSTER ABSTRACTS

Presenters in **bold**.

74

The emerging Chr1 substitution mice established from Chinese wild donors. Junhua Xiao¹, Kai Li¹, Yuxun Zhou¹, Yinming Liang², Tianzhu Chao¹, Fuyi Xu¹. 1) Donghua University, Shanghai, China; 2) Xinxiang Medical University, Henan, China.

Fine mapping and identification of the causative genes for complex traits using mouse models remain extraordinarily challenging, and a mouse population with high sequence diversities and abundant historic recombination events is highly desirable for complex genetics.

Previous studies including ours indicated that there are two subspecies of house mouse in the China, 'M. m. musculus' in the north and 'M. m. castaneus' in the south. Since 2008, we collected Chinese wild mice from 23 different regions and started the construction of the chromosome 1 substitution mouse population. The Chr 1 of the recipient C57BL/6J mice was substituted by homologous counterparts from 23 wild mice, an outbred line Kunmin mouse in China, and five different inbred strains: C3H/He, FVB/N, AKR, NOD/LtJ, and NZW/LacJ.

After 6 years work, 15 wild mice derived Chr1 substitution strains have been established and the complete panel will be obtained by the end of this year. For each of these 15 strains, the donor mice were backcrossed to C57BL/6J genome for at least six times, and the progeny carrying the same whole Chr1 from the donor were intercrossed. This strategy keeps the residual genome from donor mice less than 2% in Chr1 substitution mice. In the next stage, the 23 wild derived Chr 1 will be sorted by flow cytometry and then sequenced by next generation sequencing technology. Blood biochemical phenotyping will be performed, and all the phenotypic and genomic data will be archived in a database which could be expanded by collaborative efforts on genetic dissection of complex traits.

Organogenesis

75

Characterization of platelet-derived growth factor-A expression in mouse tissues using a lacZ knock-in approach. Johanna Andrae¹, Leonor Gouveia¹, Liqun He¹, Christer Betsholtz^{1,2}. 1)

Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden; 2)

Department of Medical Biochemistry and Biophysics, Division of Vascular Biology, Karolinska Institute, Stockholm, Sweden.

Expression of the platelet-derived growth factor A-chain gene (*Pdgfa*) occurs widely in the developing mouse, where it is mainly localized to various epithelial and neuronal structures. Until now, *in situ* mRNA hybridization (ISH) has been the only reliable method to identify *Pdgfa* expression in tissue sections or whole mount preparations. Validated protocols for *in situ* detection of PDGF-A protein by immunohistochemistry is lacking. In particular, this has hampered understanding of *Pdgfa* expression pattern in adult tissues, where ISH is technically challenging.

Here, we report a gene targeted mouse *Pdgfa* allele, *Pdgfa*^{ex4COIN}, which is a combined conditional knockout and reporter allele. Cre-mediated inversion of the COIN cassette inactivates *Pdgfa* coding while simultaneously activating a beta-galactosidase (*lacZ*) reporter under endogenous *Pdgfa* transcription control. The generated *Pdgfa*^{ex4COIN-INV-lacZ} allele can next be used to identify cells with a *Pdgfa* null allele, and to map endogenous *Pdgfa* expression.

We evaluated the *Pdgfa*^{ex4COIN-INV-lacZ} allele as a reporter for endogenous *Pdgfa* expression patterns in mouse embryos and adults. We conclude that the expression pattern of *Pdgfa*^{ex4COIN-INV-lacZ} recapitulates known expression patterns of *Pdgfa*. We report on novel embryonic and adult *Pdgfa* expression patterns.

76

Deficiency in Maged2 results in impaired myogenic differentiation. E. Srour¹, M. Delimoy¹, R.Rezsöházy², J. Marshal³, L. Kunkel³, **O. De Backer**¹. 1) URPHYM, University of Namur, Namur,

Belgium; 2) Institut des Sciences de la Vie, University Catholic of Louvain, Louvain-la-Neuve, Belgium;

3) Boston Children's Hospital, Boston, MA.

In normal adult skeletal muscle, cell turnover is very slow. However, it accelerates upon physiological stimuli or in pathological conditions, such as injury and primary myopathies like Duchenne Muscular Dystrophy (DMD). In such cases, massive cell lysis occurs, which induces the activation of muscle stem cells, called satellite cells. These activated stem cells proliferate, differentiate and finally fuse with existing myofibers. We show here that Maged2, a member of the large family of Mage proteins, is expressed in developing embryonic and post-natal mouse skeletal muscle but is silent in normal adult muscle. Maged2 is induced in muscles from patients with Duchenne muscular dystrophy and Mdx mice (a mouse model of DMD), as well as in muscles regenerating after an acute experimental injury.

Expression of Maged2 is thus associated with myogenesis. We showed that Maged2 is strongly induced early after induction of differentiation of murine fibroblasts C2C12. siRNA-mediated depletion of Maged2 in C2C12 results in increased cell death and inhibits differentiation. To investigate the role of Maged2 *in vivo*, we produced mice with a floxed allele of Maged2. Mice with a constitutive deficiency of Maged2 die within the first 24h after birth. The reasons for this lethality remain to be clarified but it could result from hemorrhages resulting from parietal defects of large vessels. Adult mice with skeletal muscles deficient for Maged2 were obtained by crossing Maged2^{lox/lox} mice with Myf5-Cre transgenic mice. We started to investigate the ability of these mice to regenerate their muscle after experimental lesions induced by cardiotoxin (CTX) injection. We observed a strong reduction of skeletal myosin heavy chain (MHC) expression (a marker of terminally differentiated muscle cells) 7 days after CTX injection in Maged2-deficient muscle. This suggests that Maged2 is essential for efficient muscle regeneration.

POSTER ABSTRACTS

Presenters in **bold**.

77

Novel Role of Fgf Signaling in Eye Development. T. Abe, Y. Yamashita, Y. Mukumoto, A. Denda, H. Kiyonari, **Y. Furuta**. RIKEN Center for Developmental Biology, Kobe, Japan.

The Fgf signaling machinery comprises a battery of ligands and receptors that are promiscuous in their binding partners. Due to apparent functional redundancy, the collective function of endogenous Fgf family ligands in embryonic development has not been fully characterized genetically. To examine the role of Fgf signaling in the developing eye, we are generating mice lacking multiple Fgf gene family members, *Fgf3*, *Fgf9*, and *Fgf15*, expressed in the developing retina. Homozygous mutants for either of these genes, exhibit no drastic developmental defects during early eye development. However, *Fgf9;Fgf15* double homozygous mutants show misrouting of retinal ganglion cell axons (RGCs) soon after the initiation of RGC differentiation. Subsequently, RGC axons fail to target the central retina, resulting in the absence of the optic nerve. These results suggest a novel role of Fgf signaling during retinal development mediated by redundant activities of Fgf9 and Fgf15, restricting the expansion of RGC axons into the retinal proper. This mechanism may help RGC axons follow the path within the RGC layer and to properly target the central retina where multiple local guidance cues operate to guide the axons to exit the eye. Generation of *Fgf3;Fgf9;Fgf15* triple mutants has been hampered by the fact that *Fgf3* and *Fgf15* gene loci are closely linked within a 60 kb interval on chromosome 7. We have introduced multiple mutations into these three Fgf genes using CRISPR/Cas9 system in ES cells. Triple mutant ES cells have been used to generate chimeric embryos. Preliminary phenotypic characterization of *Fgf3;Fgf9;Fgf15* compound mutants using F0 chimeras with high ES contribution will be reported.

78

Rho GTPases and gastrulation. Navrita Mathiah, **Isabelle Migeotte**. IRIBHM, ULB, Brussels, Belgium.

Epithelial-mesenchymal transition (EMT) is a fundamental process during embryo morphogenesis, but also a crucial step for fibrosis, as well as cancer invasion and metastasis. We use the mouse embryo gastrulation as a model to study the cellular and molecular mechanisms of EMT followed by cell migration. In particular, we focus on the role of Rho GTPases in gastrulation. Rho GTPases are molecular switches and master regulators of the cytoskeleton.

Using genetics, whole embryo *ex vivo* culture, and confocal live imaging, we define the epiblast rearrangements that allow the formation of the primitive streak, as well as the cell shape changes during EMT and mesoderm migration. Initial observations suggest that posterior epiblast cells form “rosettes” structures at a higher frequency than the lateral and anterior epiblast cells. Interestingly, the rosettes forming at the posterior side tend to resolve in “daisies” structures, as cells undergo apical constriction and cell rounding to be able to pass through the PS. One possible hypothesis is formation of these new cells via asymmetric division of one of the rosette-forming cells. After exiting the streak, mesoderm cells extend long projections, which span several cell diameters, in multiple directions, before translating their cell body, in what seems a trial and error process. Long filopodia are observed between newly formed mesoderm cells, in particular between daughter cells, indicating long distance communication. Extraembryonic mesoderm cells exhibit very different cell shapes and migration behaviour when compared to embryo mesoderm cells.

We will show preliminary data on the role of GTPases during primitive streak formation and EMT through the study of two conditional mutants of genes known to play a fundamental role in cell migration (*Rac1* and *RhoA*), and of one of their activators, the GEF (Guanine Exchange Factor) *Ect2*, whom fly homologue Pebble is necessary for gastrulation.

POSTER ABSTRACTS

Presenters in **bold**.

79

Cell autonomous and nonautonomous factors shape cell fate decisions in the third pharyngeal pouch. **John O'Neil**¹, Nancy Manley². 1) University of Georgia Athens, GA; 2) University of Georgia.

The third pharyngeal pouch is an endoderm-derived epithelium that forms the thymus and parathyroids in the mouse. Little is currently known about the mechanism that specifies cells to thymus or parathyroid fate. Genetic manipulation of the Shh pathway suggests a necessary role for Shh signaling in parathyroid cell fate specification, as the Shh null mutation causes absence of parathyroid and expanded thymus fate. However, constitutive Shh signaling in the endoderm restricts thymus fate, but is not sufficient to expand the parathyroid domain. We also postulate that Bmp and Fgf signaling may function to inhibit parathyroid fate in the ventral pouch, while Shh signaling inhibits thymus fate in the dorsal pouch. To test this hypothesis, we have developed a serum-free *ex vivo* tissue culture system in which we are able to implant whole embryos with beads treated with recombinant protein or drugs designed to agonize or antagonize Bmp and Fgf signals. Our data shows that cell fate is plastic within these cells and can be influenced by agonists and antagonists of the Bmp and Fgf pathways as late as the 35 somite stage.

Treatment with either recombinant FGF8 or BMP4 protein shows a large inhibition of parathyroid fate, and a small expansion of thymus fate in E10.5 embryos while treatment with a synthetic BMP inhibitor causes pouch hypoplasia and an inhibition of thymus and parathyroid fate. This suggests a potentially redundant role for Bmp4 and Fgf8 in repressing parathyroid fate. Additionally, we have developed a novel approach combining fluorescence-activated cell sorting and ChIP-seq to identify regions of differentially modified chromatin between discrete populations of third pharyngeal pouch cells at early stages of pouch development. We aim to use these data to identify the cell signaling pathways and the genes involved in the specification process of the thymus and the parathyroids in the developing mouse.

POSTER ABSTRACTS

Presenters in **bold**.

80

PDGFR α signaling negatively regulates white and beige adipogenesis *in vivo*. Chengyi Sun^{1,2}, Lorin Olson^{1,2}. 1) IMCA, Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Understanding the molecular basis of adipogenesis is essential to identifying new biomarkers and therapeutic targets for treating obesity. Adipocyte precursors express PDGFR α (platelet-derived growth factor receptor α) but PDGFR α is downregulated during differentiation. This suggests a regulatory function for PDGFR α in the early adipocyte lineage, but germline deletion of PDGFR α is embryonic lethal and conditional mutation in the early adipocyte lineage is problematic. Therefore, we opted for a gain-of-function approach using mice with a V561D point mutation in PDGFR α , which causes increased tyrosine kinase activity. Analysis of developing white adipose tissue (WAT) at 4 days postnatal (P4) revealed that PDGFR α^{V561D} mutants had an enlarged adipocyte precursor compartment with more cell proliferation. However, at P18 when WAT development is complete, PDGFR α^{V561D} mutants had WAT that was reduced in mass with few mature white adipocytes, loss of mature adipocyte marker expression, and replacement with fibrotic extracellular matrix. Remarkably, UCP1⁺ beige adipocytes (thermogenic adipocytes important for energy homeostasis) were completely absent from subcutaneous WAT in PDGFR α^{V561D} mutants. However, interscapular brown adipose tissue (BAT) was completely normal. These results demonstrate the PDGFR α is a negative regulator of white and beige adipogenesis and suggest that PDGFR α signaling must be downregulated for adipocyte precursors to complete differentiation. To determine whether the effect of PDGFR α is tissue autonomous, we used Myf5-Cre to activate PDGFR α signaling in dorsal WAT and BAT but not ventral WAT. By comparison of WAT depots in the same mutants, we found that PDGFR α indeed inhibits WAT development in a tissue-autonomous fashion. PPAR γ is the master regulator of adipocyte terminal differentiation and is also required for maintenance of the terminally differentiated state. PPAR γ expression appeared normal in WAT at P4 but was downregulated at P18. This suggests that PDGFR α regulates adipogenesis by directly or indirectly affecting the expression of PPAR γ within WAT depots.

POSTER ABSTRACTS

Presenters in **bold**.

81

Using conditional knockout mice to elucidate the function of Cdk5rap3 in liver development. Rui Yang, Yu-yan Jia, Lin Liu, Huang Yue. Department of Medical Genetics, Institute of Basic Medical Sciences, Peking Union Medical College, Beijing, China.

Cdk5rap3 is a potential tumor suppressor, which has been reported to have a significant role in regulating cell cycle, cell proliferation, apoptosis, and cell adherence/invasion. Genetic studies on zebrafish have shown that Cdk5rap3-deficient embryos failed to initiate epiboly and gastrulation. Their results suggest that Cdk5rap3 is essential for early embryogenesis. However, the role of Cdk5rap3 in mammalian development remains unknown.

Here, by using mutant JM8 ES cells from EUCOMM, we generated a Cdk5rap3-deficient mouse, and found that Cdk5rap3 loss led to embryonic lethality at about E16.5-18.5. The defective embryos exhibited marked growth retardation, liver degeneration, and anemia. IHC examination of fetal liver sections revealed reduced hepatoblast proliferation, with most hepatoblasts remaining undifferentiated at E16.5. TUNEL analysis demonstrated massive apoptosis in the fetal livers, especially in the periphery. The isolated mutant hepatoblasts can not differentiate in vitro and that further confirmed the negative effect of Cdk5rap3 loss on hepatoblasts. Furthermore, FACS analysis of HSCs and HPCs was conducted to assess anemia, which showed decreased number and proportion of HSCs in knockout fetal livers, suggesting impaired hematopoiesis. To have a better understanding of Cdk5rap3 functions, we constructed a HSC-specific knockout mouse. We found that the mice have normal liver development and sufficient blood supply. Further, by using Alb-cre mice, we constructed hepatocyte-specific Cdk5rap3 knockout animal, which survived to adulthood possibly due to late-stage depletion and/or relatively low knockout efficiency.

We concluded that Cdk5rap3 knockout caused defects in hepatocytes and hematopoietic cells. Considering that hepatoblasts comprise a niche for fetal liver hematopoiesis, we surmised that the observed anemia was secondary to liver degeneration, rather than an intrinsic defect in hematopoietic cells. Next, we will elucidate the underlying mechanism leading to the observed dysfunction in Cdk5rap3-deficient mouse.

POSTER ABSTRACTS

Presenters in **bold**.

Patterning

82

A regulatory network controls *Nephrocan* expression and midgut patterning. P. Hoodless¹, J. Hou¹, W. Wei¹, R. Saund², P. Xiang¹, T. Cunningham³, D. Lu¹, J. Savory⁴, N. Krentz¹, R. Montpetit¹, R. Cullum¹, D. Lohnes⁴, K. Humphries¹, G. Duester³, Y. Yamanaka⁵, Y. Saijoh². 1) Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada; 2) Dept of Neurobiology and Anatomy, University of Utah, Utah; 3) Development, Aging and Regeneration Program, Sanford-Burnham Medical Research Institute, La Jolla, California; 4) Cellular and Molecular Medicine, University of Ottawa, Ottawa, Canada; 5) Goodman Cancer Research Centre, Dept of Human Genetics, McGill University, Montreal, QC, Canada.

While many regulatory networks involved in defining definitive endoderm have been identified, the mechanisms through which these networks interact to pattern the endoderm are less well understood. To explore the mechanisms involved in midgut patterning, we dissected the transcriptional regulatory elements of *Nephrocan* (*Nepn*), the earliest known midgut specific gene in mice. We observed that *Nepn* expression is dramatically reduced in *Sox17*^{-/-} and *Raldh2*^{-/-} embryos compared to wild-type embryos. We further show that *Nepn* is directly regulated by *Sox17* and the retinoic acid receptor via two enhancer elements located upstream of the gene. Moreover, *Nepn* expression is modulated by Activin signaling with high levels inhibiting and low levels enhancing RA-dependent expression. In *FoxH1*^{-/-} embryos in which Nodal signaling is reduced, the *Nepn* expression domain is expanded into the anterior gut region suggesting that Nodal signaling can modulate expression *in vivo*. Together, *Sox17* is required for *Nepn* expression in the definitive endoderm, while retinoic acid signaling restricts expression to the midgut region. A balance of Nodal/Activin signaling regulates the anterior boundary of the midgut expression domain.

83

FGF signaling pathways in development. J. Richard Brewer, Pierre Mazot, **Philippe Soriano**. Developmental and Regenerative Biology, Icahn School of Medicine at Mt. Sinai, New York, NY 10029.

Fgfrs are known to operate through the Frs2-Erk1/2 signaling pathway, but they can also activate multiple other pathways *in vitro*. To examine the signaling requirements for Fgfr1 and Fgfr2 *in vivo*, we have generated mice harboring allelic series of knock-in point mutations designed to disrupt a subset of signaling functions. For both receptors, loss of Fgfr signaling through Frs2 is less severe than loss of signaling through multiple pathways (Fgfr1-Frs2, -Crk/CrkL, -PLCγ, -Shb and -Grb14 signaling). This suggests that Frs2 regulates only a subset of Fgfr signaling and that Fgfrs utilize multiple signaling pathways additively *in vivo*. We are utilizing these alleles to study Fgfr signaling during early development and in the context of craniofacial morphogenesis.

Stem Cells

84

Generation and characterization of germ line competent ES cell lines from C57Bl/6J. Purnima S Sailasree¹, Partha Sarathi D², Satish Kumar³. 1) Centre Cellular and Molecular Biology, Uppal main road, Hyderabad, Telangana, 500007 India; 2) Centre Cellular and Molecular Biology, Uppal main road, Hyderabad, Telangana, 500007 India; 3) National Institute for Animal Biotechnology, Miyapur, Hyderabad, Telangana 500049, India.

Gene targeting in mouse ES cells had been established as an extremely powerful tool to understand gene function and for creation of models of human diseases. During the last two decades gene targeting and associated techniques have found utility in almost all fields of biology. At the Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India we have been working at the National Facility for Gene Knockout and Transgenic Mice with a mission to incorporate this technology in various research programs at the CCMB and other biology research institutions in India. Given the importance of derivation of ES cell lines from mouse strains other than SV129, we have isolated eight ES cell lines without the use of inhibitors /inducers and characterized them. Two of these have been tested for germ line transmission and it has been possible to derive the pups from the ES cell component. These resources would enhance our existing capabilities at the National Facility for Transgenic and Gene Knockout at CCMB, Hyderabad.

85

Spatiotemporal analysis of cell fate decisions during preimplantation development. Nestor Saiz Arenales, Nadine Schrode, Anna-Katerina Hadjantonakis. Developmental Biology, Sloan Kettering Institute, New York, NY.

Lineage specification in the mammalian preimplantation embryo occurs in two sequential steps that segregate the extraembryonic trophectoderm (TE) and primitive endoderm (PrE) from the pluripotent embryonic epiblast (EPI). Primitive endoderm specification is dependent on FGF signaling, which downregulates NANOG and promotes GATA6 expression. It has been proposed that ICM cells display high plasticity until late stages, which may be reflected by their heterogeneous gene expression pattern. However, it is still unclear when cells commit to either lineage and whether these fate decisions can be reverted. Unlike in previous studies, here we have used a single-cell, quantitative approach to analyze lineage commitment in the ICM with high spatiotemporal resolution, and to address when cell fates are stabilized. We show that commitment to either PrE or EPI coincides with the establishment of exclusive GATA6 or NANOG expression domains, respectively. We have observed that restriction of these gene expression profiles occurs at different times in individual ICM cells. These cells concomitantly stop responding to either stimulation or inhibition of the FGF-MAPK pathway, and therefore do not change fate. Moreover, altering the balance between NANOG and GATA6 using *Gata6*^{+/-} embryos affects the timing of lineage commitment, thus suggesting the intracellular ratio between these two factors is key in determining the outcome of stimulating the FGF-MAPK axis in individual cells. We therefore propose that lineage specification within the ICM of the mouse blastocyst is a sequence of discrete fate decision events, where incremental allocation of ICM cells to either the PrE or EPI guarantees the generation of both lineages, while allowing for the maintenance of an exquisitely balanced cell number.

POSTER ABSTRACTS

Presenters in **bold**.

86

***In vitro* screening of homozygous mutant mouse ES cells identified a novel gene essential for hematopoietic development.** **M. Tokunaga**, C. Kokubu, J. Takeda. Department of Social and Environmental Medicine, Osaka University, Suita, Osaka, Japan.

Purpose: A better understanding of molecular basis of hematopoietic lineage development contributes to efforts to achieve efficient production of functional blood cells from pluripotent stem cells. We have previously established a homozygous mutant mouse embryonic stem cell (ESC) library consisting of more than 200 mutant clones. Here, using an OP9 co-culture system, we screened the library for mutant ESC clones whose hematopoietic differentiation is affected. **Methods:** We co-cultured each mutant ESC clone with OP9, a stromal cell line supporting hematopoietic differentiation. On day 4, early mesodermal cells were FACS sorted as fetal liver kinase-1 (Flk-1) positive population and were distributed on newly prepared OP9 cells. Mesodermal cells were further differentiated into hematopoietic cells by adding SCF, IL-3 and EPO. On day 13 from the induction of ESC differentiation, non-adherent cells were collected and analyzed by flow cytometry. **Results and Conclusion:** During the screening, we found one ESC clone with severely disrupted hematopoiesis. This clone generated approximately 80-fold fewer hematopoietic cells, compared with its wild-type parental ESC clone. The biallelically-trapped gene in the mutant ESC clone was a novel, uncharacterized gene (designated as *Gene X*). Because the gene-trap cassettes are flanked by a pair of *FRT* sites, their Flp-mediated removal allowed generation of revertant ESC clones, whose potential of generating hematopoietic cells was restored to the wild-type ESC level. In addition, exogenous expression of *Gene X* cDNA also restored hematopoiesis from the mutant ESCs. These results suggest the requirement of *Gene X* for proper hematopoiesis *in vitro*. Next, to elucidate the physiological importance of *Gene X in vivo*, we generated knockout mice. Although heterozygous *Gene X*^{+/-} mice showed no detectable developmental abnormalities including hematopoiesis, null mutant mice were embryonic lethal by E6.5, demonstrating that this gene plays an essential role in early mouse development. Analyses using GFP-tagged *Gene X* product revealed its nuclear localization and located its NLS near the C-terminus, suggesting its potential roles in gene regulation. Further in-depth analyses are ongoing.

Technology

87

A novel vector configuration dramatically improves recovery of gene targeted ES cell clones. Jason Lawrence, Robert Williams, Clare Bender, Nicole Christ, Steve Sheardown. Takeda Cambridge Ltd., 418 Cambridge Science Park, Cambridge, CB4 0PA, United Kingdom.

Recent advances with targeted nucleases have greatly simplified genome modification in a wide variety of model organisms but there are still situations where gene targeting by classical homologous recombination is preferred, often where there is concern about off-target mutations. If targeting efficiency is poor cell handling and screening is labour intensive. Constraints associated with precise manipulations like conditional alleles or humanizations can make this difficult to avoid, thus any strategy that improves recovery of targeted clones is of value. Here we describe a novel targeting vector based on the pJAZZ linear plasmid system that dramatically improves targeted clone recovery by increasing the power of negative selection 20- to 30-fold.

We chose a conventional KO vector with a targeting efficiency of ~10% and cloned the entire targeting construct into pJAZZ. ES cells were transfected in parallel with the pJAZZ and conventional KO construct then selection applied with G418 alone (positive) or with G418 + Gancyclovir (positive/negative). We observed that negative selection with the pJAZZ based targeting vector was greatly increased compared to the conventional targeting vector (21% compared to 1.2%). This increased negative selection stringency was reflected by an improvement in targeted clone recovery (71% for the pJAZZ vector: 8% for the conventional vector). In a follow-up experiment the pJAZZ backbone transformed targeting efficiency of a poorly performing targeting construct from 0.6% to 28%.

We conclude that the pJAZZ targeting vector greatly reduces the number of clones that have to be handled, and enables easy recovery of targeted clones with constructs that normally display very low targeting efficiencies, making it a useful addition to the gene targeting toolkit. To date we have used the pJAZZ vector to produce >10 KO's in mouse ES cells.

POSTER ABSTRACTS

Presenters in **bold**.

88

Generation of mutant mouse lines using endonuclease technologies at the ICS (the French Mouse Clinic). GUILLAUME PAVLOVIC^{1,2,3,4}, PHILIPPE ANDRE^{1,2,3,4}, SYLVIE JACQUOT^{1,2,3,4}, MARIE-CHRISTINE BIRLING^{1,2,3,4}, YANN HERAULT^{1,2,3,4}. 1) Institut Clinique de la Souris - ICS-MCI, PHENOMIN, Illkirch, France; 2) Centre National de la Recherche Scientifique, UMR7104, Illkirch, France; 3) Institut National de la Santé et de la Recherche Médicale, U964, Illkirch, France; 4) Université de Strasbourg, Illkirch, France.

In the last decade, a large part of the research to understand the mammalian genome function was permitted by the generation of mutant mouse models by targeted modification via homologous recombination in ES cells. However, this ES route approach harbors a number of disadvantages, as it is time-consuming, laborious and restricted to few backgrounds (requires wild type ES cells).

Starting in 2009, 3 new genome editing technologies have been developed: zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats/CRISPR-associated (CRISPR/Cas). These endonucleases are now a very attractive alternative to generate mouse mutant models as they offer the possibility to target a large panel of mouse background (if embryos can be generated easily), show an increased efficiency in gene editing, are less expensive and promise a reduced generation time of mutant mice.

Here, we will present our results using ZFNs, TALENs and CRISPR/Cas. Direct injection of ZFNs, TALEN mRNA or gRNA with Cas9 into fertilized mouse oocytes were performed to generate knock-out animals or to introduce point mutation / knock-in sequence. We will discuss PROs and CONs of these approaches in regard of our results: efficiency for inactivation, efficiency for replacement, mosaicism of G0 animals, off-target cleavage, genotyping, timeline, cost, design & construction, potential applications... We will also compare endonuclease technologies to targeted modification via homologous recombination in ES cells for generating mouse mutant models.

89

Nuclease-Mediated Gene Knockout in an Allele Refractory to Gene Targeting in ES Cells. Filipiak Wanda¹, Zeidler Michael¹, Hughes Elizabeth¹, Gavrilina Galina¹, Mary Schmidt¹, Jifeng Zhang², Leslie Satin³, **Thomas Saunders**^{1,2}. 1) University of Michigan Medical School, Biomedical Research Core Facilities, Transgenic Core, Ann Arbor, MI; 2) University of Michigan Medical School, Department of Internal Medicine, Ann Arbor, MI; 3) University of Michigan Medical School, Department of Pharmacology, Ann Arbor, MI.

Muscle phosphofructokinase is an essential enzyme for glucose homeostasis. Patients with phosphofructokinase deficiency present with Tarui disease and exercise intolerance. To generate a mouse model for this disease, exon 3 in the mouse *Pfkm* gene was selected for gene targeting in mouse embryonic stem (ES) cells. Mutation of exon 3 is expected to disrupt both isoforms of *Pfkm* by nonsense-mediated decay of mRNA encoding a premature termination codon. A gene-targeting vector designed to replace exon 3 with a sequence flanked by loxP sites was introduced into mouse ES cells. Genetic screening of 480 drug-resistant ES cell clones found none with homologous recombination with the targeting vector. Subsequent efforts to generate a mutant mouse model turned to the direct manipulation of the mouse genome in fertilized eggs by the microinjection of nucleases targeted to exon 3. Four transcription activator-like effector nucleases (TALENs), one zinc finger nuclease (ZFN), and one CRISPR-associated Cas9 nuclease were designed to target *Pfkm* exon 3 and prepared for microinjection. Nucleases were obtained from commercial vendors and also prepared in-house from publicly available resources. Microinjection of plasmid vectors expressing TALENs targeted to exon 3 did not produce mutant mice although this method has been reported to be effective for other genes. The microinjection of mRNA coding for TALENs also failed to generate mouse mutants, independent of the origin of the TALENs reagents. The use of ZFN mRNA for microinjection produced multiple mouse mutants. Co-injection of Cas9 mRNA and guide RNA produced numerous mice that are undergoing genetic analysis. Factors affecting the efficiency and success of nuclease-mediated gene targeting include the activity of nucleases on target sequences and toxicity of microinjection preparations.

POSTER ABSTRACTS

Presenters in **bold**.

90

Rapid generation of mouse and rat mutants by CRISPR/Cas in one-cell embryos.. Jochen Welcker¹, Sonja Ortmann¹, Simone Janzen¹, Jost Seibler¹, Jon Chesnut², Kai Schönig³. 1) TaconicArtemis GmbH, Neurather Ring 1, 51063 Cologne, Germany; 2) Thermo Fisher Scientific, Carlsbad, USA; 3) Central Institute of Mental Health, Heidelberg University, Department of Molecular Biology, J 5, 68159 Mannheim, Germany.

Simple handling and high efficiencies make type II bacterial CRISPR/Cas systems the most powerful genome engineering tool currently available. With this technology, in principle, all genome modifications previously established through gene targeting in mouse embryonic stem cells can now be performed in zygotes of mice and rats. Our data demonstrate high efficiency of ODN-mediated introduction of frame-shift and point mutations in C57BL/6 mice and Sprague Dawley rats. All sgRNAs tested, so far, showed indel rates above 30%. Currently, we are extending the range of applications to allow larger deletions and replacement of mouse genes with their human orthologs.

SPEAKER AND AUTHOR INDEX

This index includes all authors' names for all programmed abstracts. The number following the author's name refers to the abstract program number. Numbers 81 and above are poster presentations. The presenting author of an abstract is noted with an asterisk

A

Abe, T.....	77
Adams, David.....	2
ADAMS, Ralf H.....	31*
Adelstein, Robert.....	46
Akagi, Keiko.....	44
Akashi, Makoto.....	64
Alabkal, Jarrah.....	73
Alalwan, Adel.....	73
Alder, O.....	55
Almeida, S. M.....	25
Al-Ramadan, Mariam.....	73
Amos-Landgraf, J.....	11*
Andrae, Johanna.....	75*
ANDRE, PHILIPPE.....	88
Appelbe, Oliver K.....	63
Apte, Suneel.....	36
Aronow, Bruce.....	28
Astrof, Sophie.....	26*
Attarwala, Ali.....	63

B

Babiuk, Randy.....	68*
Bagina, Uliana.....	71
Baqer, Ahmed.....	73
Barenboim, Maxim.....	12
Bartolomei, Marisa.....	22*
Basu, Uttiya.....	70
Beckers, Johannes.....	61
Bender, Clare.....	87
Betsholtz, Christer.....	75
Bijlsma, Maarten.....	39
Bilenky, M.....	55
BIRLING, MARIE-CHRISTINE.....	88
Bollman, Bryan.....	63
Borges Pinto, Hugo.....	19
Bradley, Allan.....	12
Bressloff, Paul C.....	53
Brewer, J. Richard.....	83
Brown, Anthony.....	29
Brown, Kate.....	87
Brunelli, Luca.....	53*
Bryda, E.....	11
Buerger, Antje.....	4

Burtscher, Ingo.....	37, 61*
Bush, Jeffrey O.....	40*
Bushell, Wendy.....	4
Busi, S.....	11

C

Carat, S.....	24
Caspary, Tamara.....	39*
Ceccarelli, D. F.....	25
Chandler, Ron.....	42
Chao, Jaime.....	70
Chao, Tianzhu.....	74
Che, Xiangguo.....	67, 69
Chen, Q.....	55
Chesnut, Jon.....	90
Choi, Je-Yong.....	67, 69
Christ, Nicole.....	87
Chung, Eunah.....	28
Churova, Maria.....	71
Clouthier, David.....	49
Concepcion, Dorothy.....	72
Condie, Brian.....	27
Constam, D. B.....	24
Conti, Mary Anne.....	46
Cordes, S. P.....	25*
Correra, Rosamaria.....	57
Courbard, Jean-Remy.....	57
Cullum, R.....	55, 82
Cunningham, T.....	82

D

De Backer, O.....	76*
de Jong, P.....	5
Delimoy, M.....	76
Demarest, Bradley L.....	53
Denda, A.....	77
Denizot, Anne-Lyse.....	57
Di Talia, S.....	34
Donohoe, Mary.....	19*
Doran, Joanne.....	87
Dove, W.....	11
Duester, G.....	82
Dulev, Stanimir.....	37

SPEAKER AND AUTHOR INDEX

This index includes names and speakers for all programmed abstracts. The number following the author's name refers to the abstract program number. Number 81 and above are poster presentations. The presenting author of an abstract is noted with an asterisk

E

Economides, A. N.....	54
Economides, Aris.....	70
Ehmer, Ursula.....	12
Elizabeth, Hughes.....	89
Engert, Silvia.....	37*
Eppig, Janan.....	3
Eppig, Janan T.....	68
Epstein, Jonathan.....	23*
Ericsson, A.....	11
Esposito, Irene.....	12

F

Fares, Mona.....	73
Fedoriw, Andrew.....	21*
Feeley, K.....	54
Feenstra, Jennifer.....	29
Ferretti, Elisabetta.....	29
Finger, Jacqueline.....	3
Folkesson, Ronnie.....	73
Formicola, Luigi.....	57
Forsberg, Camilla.....	59*
Franklin, C.....	11
Friedrich, Mathias.....	12
Fu, Jun.....	4
Fuchs, Elaine.....	58
Fukumura, R.....	25
Furuta, Y.....	77*

G

Gale, Nicholas.....	10
Galina, Gavrilina.....	89
Gallant, Judith.....	52
Galli, Antonella.....	2*
Garside, V.....	55
Gavrilov, Svetlana.....	35*
Geumann, Ulf.....	12
Gingras, A.-C.....	25
Goldsmith, S.....	60*
Gondo, Y.....	25
Goulley, J.....	24
Gouveia, Leonor.....	75
Grady, Richard.....	36
Grapin-Botton, A.....	24
Green, Angela.....	2

Green, Shon.....	15
Grewe, Brian.....	45, 63*
Griffith, M.....	55
Grompe, Markus.....	50*
Gross, Nina.....	12

H

Hadjantonakis, A.-K.....	34
Hadjantonakis, Anna-Katerina.....	85
Haltiwanger, Robert.....	36
Hamilton, Bruce.....	72
Han, Seung-Hee.....	69
Han, Seung Hee.....	67
Harada, Yoshinobu.....	64*
Hassan, Moustapha.....	73
Hatsell, S. J.....	54
Hayamizu, Terry.....	3
He, Chaoyong.....	43
He, Dandan.....	44
He, Liquan.....	75
Heikenwalder, Mathias.....	12
Henkelman, R. Mark.....	51*
HERAULT, YANN.....	88
Herion, Nils J.....	38
Hiramatsu, Ryuji.....	33
Hoerlein, Andreas.....	4*
Hogan, Brigid I. M.....	32*
Holdener, Bernadette.....	36*
Hong, Matthew.....	59
Hong, Mingi.....	48*
Hoodless, P.....	55*, 82*
Hou, J.....	82
Hsu, Ya-Chieh.....	58*
Huang, H.....	25
Huang, L.....	54
Humphries, K.....	82
Hunter, Neil.....	6

I

Icoresi Mazzeo, Cecilia.....	2
Idone, V.....	54
Ilyuha, Vladimir.....	71
Irmmler, Martin.....	61
Isono, Kyoichi.....	20*
Iwayama, T.....	13*

SPEAKER AND AUTHOR INDEX

This index includes names and speakers for all programmed abstracts. The number following the author's name refers to the abstract program number. Number 81 and above are poster presentations. The presenting author of an abstract is noted with an asterisk

J

Jacquemin, P.....	24
JACQUOT, SYLVIE.....	88
Janzen, Simone.....	90
Jasin, Maria.....	56*
Jia, Shihai.....	28
Jia, Yu-yan.....	81
Jiang, Rulang.....	28*
Jones, Catherine.....	2
Jones, S.....	55
Jones, Stephen N.....	52
Juan, Joseph.....	15
Juang, Y.-C.....	25
Jung, C.....	5*
Justice, Monica.....	16*

K

Kadin, James.....	3
Kan, A.....	55
Kang, M.....	34*
Kappen, Claudia.....	38*
Kato, Shigeaki.....	69
Kayashima, Y.....	65*
Kim, Gyeong-hwa.....	69*
Kim, Gyoung-Hwa.....	67
Kim, Y. H.....	24
Kim, Yuna.....	21
Kimura-Yoshida, Chiharu.....	33
Kiyonari, H.....	77
Kokubu, C.....	86
Kosaka, Yasuhiro.....	53
Koseki, Haruhiko.....	20
Kostecka, D.....	5
Krauss, Robert.....	48
Krentz, N.....	82
Kruger, Claudia.....	38
Kuehn, Michael R.....	53
Kumar, Amit.....	53
Kumar, Satish.....	84
Kumon, Mami.....	20
Kunkel, L.....	76
Kurbatova, Irina.....	71
Kyryachenko, Sergiy.....	57
Kyrylkova, Kateryna.....	57

L

Lacy, Elizabeth.....	35
Lan, Yu.....	28
Larson, Andrew.....	40
Lawrence, Jason.....	87*
Lee, A.....	54
Lee, S.....	55
Lemaire, L. A.....	24*
Lerma, Connie.....	46
Li, Bingsi.....	29
Li, Jie.....	27
Li, Jingfeng.....	44
Li, Kai.....	74
Li, Lishi.....	58
Liang, Dong.....	26
Liang, Yinming.....	74
Liao, W.Perry.....	37
Lickert, Heiko.....	37, 61
Lillistone, Charlotte.....	2
Lim, Junghyun.....	70
Liu, Han.....	28
Liu, Lin.....	81
Lohnes, D.....	82
Lopez-Otin, Carlos.....	17*
Lovell-Badge, R.....	60
Lowe, Scott.....	8*
Lu, D.....	82
Lyozin, George T.....	53

M

Ma, Xuefei.....	46*
MacGowan, Jacalyn.....	38
Mackler, Randi.....	44
MacLean, T. A.....	25
Maeda, N.....	65
Magnuson, Terry.....	18, 21, 42*
Manley, Nancy.....	27*, 79
Marazzi, Giovanna.....	57
Maresch, Roman.....	12
Mariani, Laura.....	39
Marr, Carsten.....	61
Marra, M.....	55
Marshal, J.....	76
Mathiah, Navrita.....	78
Matsuki, K.....	65
Matsuo, Isao.....	33*

SPEAKER AND AUTHOR INDEX

This index includes names and speakers for all programmed abstracts. The number following the author's name refers to the abstract program number. Number 81 and above are poster presentations. The presenting author of an abstract is noted with an asterisk

Mazot, Pierre	83
McCoy, M.....	11
McCright, Ingeborg	3
McMahon, Martin.....	15*
Meng, Yue	14
Michael, Zeidler.....	89
Migeotte, Isabelle	78*
Moherman, Chelsea	44
Mohun, Tim.....	2
Montpetit, R.....	82
Moore, Robert.....	14
Morrissy, S.	55
Mu, Weipeng	21
Mukumoto, Y.	77
Müller, Sebastian	12
Muniz-Talavera, Hilmarie	45, 63, 66*
Murachguchi, Teruyuki	15
Murphy, A. J.....	54
Murray, Steve	68

N

Nannuru, K.	54
Nguyen, Andrew.....	59
Niswander, Lee.....	49

O

Oh, Sunghee	28
Öllinger, Rupert.....	12
Ollitrault, David.....	57
Olson, L.	13
Olson, Lorin.....	43*, 80
ONeil, John.....	79*
O'Neill, Audrey K.....	40
Ortmann, Sonja.....	90

P

Pan, D.	55
Park, Clara Y.	67*
Park, Joo-Seop.....	28
Park, Na-Rae.....	67, 69
Park, Yong-Joo Clara	69
Parker, T.	11
PAVLOVIC, GUILLAUME	88*
Pefanis, Evangelos.....	70*
Persaud, T.	54

Phillips, Wayne.....	15
Pohlers, Michael	18
Poltorak, Alexander N.	71*
Pourquié, Oliver	62*
Puliafito, A.	34

Q

Qiao, Huanyu.....	6*
Quintana Rio, Laura	29

R

Raab, Jesse.....	42
Rabadan, Raul.....	70
Rad, Roland	12*
Ramirez-Solis, Ramiro	2
Raught, B.....	25
Rezsohazy, R.	76
Richardson, Joel	3
Riley, Joy.....	52
Ringwald, Martin.....	3*, 68
Rivera-Perez, Jaime A.	52*
Rivkin, E.....	25
Rizzoti, K.....	60
Robertson, G.....	55
Rosen, Barry	4
Ross, Kevin.....	72*
Rothschild, Gerson	70
Rougemont, J.....	24
Rubinshteyn, Christine	1
Ryder, Ed.....	2

S

Sabry, Mohamed.....	73*
Saijoh, Y.	82
Sailasree, Purnima S.	84*
Saito, Toshiyuki.....	64
Saiz Arenales, Nestor	85*
Salbaum, J. Michael.....	38
Sarathi D., Partha.....	84
Sassoon, David	57*
Satin, Leslie	89
Saund, R.	82
Saunders, Thomas.....	89*
Saur, Dieter.....	12
Savory, J.	82

SPEAKER AND AUTHOR INDEX

This index includes names and speakers for all programmed abstracts. The number following the author's name refers to the abstract program number. Number 81 and above are poster presentations. The presenting author of an abstract is noted with an asterisk

Schmahl, Jennifer	10*
Schmidt, Jennifer	45*
Schmidt, Jennifer V.	63, 66
Schmidt, Mary	89
Schoenherr, C. J.	54*
Schönig, Kai	90
Schotta, Gunnar	37
Schrode, Nadine.....	85
Schwarzfischer, Michael.....	61
Seers, Rosalie	15
Seibler, Jost.....	90
Selleri, Licia	29*
Shai, Anny	15
Sheardown, Steve	87
Shen, Michael M.....	9
Shibata, Yochiro	21
Shimokawa, Kayo.....	33
Shpargel, Karl B.	18*
Sicheri, F.....	25
Siragher, Emma	2
Skarnes, William	4
Smith, Constance	3
Smith, Elizabeth	14
Smith-Berdan, Stephanie	59
Soriano, Philippe	83*
Souter, Luke	2
Spitz, François	7*
Srikumar, T.....	25
Srouf, E.....	76
Starmer, Joshua.....	18
Steiger, Katja	12
Stewart, Francis	4
Stokes, Arjun	30, 47
Sudo, Hitomi.....	64
Sun, Chengyi	80*
Sun, J.	5
Sun, Xin.....	41*
Symer, David	44

T

Takeda, J.....	86
Takeuchi, Hideuki	36
Takeuchi, Megumi.....	36
Tanaka, Karo	57
Tao, Wensi.....	14
Tatyana, Volkova.....	71
Theis, Fabian	61

Thiessen, N.	55
Threadgill, David.....	1*
Tokunaga, M.....	86*
Tomita, H.....	65
Triebes, Lindy A.....	63
Tuck, Elizabeth.....	2

V

van den Brink, G.....	11
Van Otterloo, Eric.....	49*
van Veen, John Edward	15
Varela, Ignacio	12
Vasudevan, Deepika	36
Vilhais-Neto, Goncalo	62
Vincent, Theresa	29

W

Wanda, Filipiak	89
Wang, Jiguang	70
Wang, Xia.....	26
Wang, Yanqiang	44
Wang, Zhu A.	9*
Weber, Julia.....	12
Wei, W.....	55, 82
Welcker, Jochen.....	90*
White, Jacqueline.....	2
Williams, Robert.....	87
Williams, Trevor.....	29, 49
Wong, Michael	51
Wurst, Wolfgang	4

X

Xenopoulos, P.....	34
Xiang, P.	82
Xiao, Junhua	74*
Xie, G.	25
Xie, L.	54
Xu, Fuyi.....	74
Xu, Jingxia.....	3
Xu, Jingyue.....	28
Xu, Xiang-Xi (Mike)	14*

SPEAKER AND AUTHOR INDEX

This index includes names and speakers for all programmed abstracts. The number following the author's name refers to the abstract program number. Number 81 and above are poster presentations. The presenting author of an abstract is noted with an asterisk

Y

Yamanaka, Y.	82
Yamashita, Y.	77
Yang, Dapeng	61
Yang, P.	54
Yang, Rui.....	81*
Yates, Katherine	44*
Yee, Della	18
Yi, Y.	55
Yoon, Yeonsoo	52
Yost, H. Joseph.....	53

Yu, P.	54
Yue, Huang.....	81

Z

Zhang, Jifeng	89
Zhang, Yingfan.....	46
Zhao, Tianyu.....	47
Zhao, Y.....	55
Zhou, Chengji	30*, 47*
Zhou, Jing	28
Zhou, Yuxun.....	74
Zhou, Zhengqiu	44

PARTICIPANT LIST

As of August 10, 2014

Ralf H Adams

Max Planck Inst for Mol Biomed
i.acres@mpi-muenster.mpg.de

Robert S. Adelstein, MD

NHLBI/NIH
AdelsteR@mail.nih.gov

Dana M Alessi

Regeneron Pharmaceuticals
dana.alessi@regeneron.com

Poltorak Alexander

Tufts Univeristy
alexander.poltorak@tufts.edu

James M Amos-Landgraf, Ph.D.

University of Missouri
amoslandgrafj@missouri.edu

Keith R Anderson, PhD

Genentech
anderk18@gene.com

Johanna Andrae, PhD

Uppsala University
Johanna.Andrae@igp.uu.se

Sophie Astrof, ph.d.

Thomas Jefferson University
sophie.astrof@gmail.com

Randy Babiuk, PhD

The Jackson Laboratory
rbabiuk@jax.org

Ulyana S Bagina

GUEST Petrozavodsk State Univ
lepsyas@mail.ru

Marisa S Bartolomei, PhD

UPenn Perelman Sch Med
bartolom@mail.med.upenn.edu

Allan Bradley, PhD

Wellcome Trust Sanger Inst
abradley@sanger.ac.uk

Madeleine J Braun, Ph.D.

The Jackson Laboratory
madeleine.braun@jax.org

Luca Brunelli, MD, PhD

University of Utah
luca.brunelli@hsc.utah.edu

Antje Buerger, PhD

Helmholtz Zentrum Muenchen
antje.buerger@helmholtz-
muenchen.de

Ingo M Burtscher, PdD

Helmholtz Center Munich
ingo.burtscher@helmholtz-
muenchen.de

Jeffrey O Bush, Ph.D.

UCSF
jeffrey.bush@ucsf.edu

Tamara J Caspary, PhD

Emory University Sch of Medicine
tcaspar@emory.edu

Kai Cheng, Ph.D.

The Jackson Laboratory
kai.cheng@jax.org

Je-Yong Choi, PhD

School of Medicine, Kyungpook
National University
jechoi@knu.ac.kr

Melina Claussnitzer, PhD

MIT
melina@broadinstitute.org

Sabine P Cordes, Ph.D.

Mt Sinai Hospital, Rm 876
cordes@lunenfeld.ca

Sean P Curran, PhD

University of Southern California
spcurran@usc.edu

Olivier RY De Backer

University of Namur
olivier.debacker@unamur.be

Pieter J de Jong, Ph.D

Children's Hospital Oakland
Research Institute
pdejong@chori.org

Mary Donohoe

Burke Medical/Weill Cornell Med
med2008@med.cornell.edu

**Fatemeh Dr. Darakhshan-
Rassam, Ph.D**

Qatar National Research Fund
fdarakhshan@yahoo.co.uk

Silvia Engert

Helmholtz Center Munich
silvia.engert@helmholtz-
muenchen.de

Jonathan Epstein, MD

University of Pennsylvania
epsteinj@mail.med.upenn.edu

Camilla Forsberg

UC Santa Cruz
cforsber@soe.ucsc.edu

Yas Furuta

RIKEN CDB
frty@cdb.riken.jp

Antonella Galli

Wellcome Trust Sanger Institute
ag10@sanger.ac.uk

Svetlana Gavrilov, MD, PhD

Memorial Sloan Kettering
gavrilos@mskcc.org

Sam Goldsmith, PhD

MRC NIMR
sgoldsm@nimr.mrc.ac.uk

Brian Grewe, B.S. M.S.

University of Illinois-Chicago
bgrewel@uic.edu

Markus Grompe, MD

Oregon Hlth & Sci Univ
gropem@ohsu.edu

Kat Hadjantonakis, PhD

Memorial Sloan-Kettering Cancer
Ctr
had@mskcc.org

Yoshinobu Harada, Ph.D.

National Institute of Radiological
Sciences
y_harada@nirs.go.jp

R. Mark Henkelman, PhD

Hospital for Sick Children
mhenkel@mouseimaging.ca

Andreas J Hoerlein, Ph.D.

Helmholtz Zentrum München
andreas.hoerlein@helmholtz-
muenchen.de

Brigid L M Hogan, PhD

Duke University
brigid.hogan@dm.duke.edu

Bernadette c Holdener, PhD

Stony Brook University
bernadette.holdener@stonybrook.edu

Mingi Hong

Mount Sinai Sch Med
mingi.hong@gmail.com

PARTICIPANT LIST

As of August 10, 2014

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

Jered Housewoth
StemExpress
bmalenstein@stemexpress.com

Ya-Chieh Hsu
Harvard University
yachieh_hsu@harvard.edu

Kyoichi Isono
RIKEN IMS-RCAI
isono@rcai.riken.jp

Tomoaki Iwayama, DDS, PhD
Oklahoma Medical Research
Founda
toallwavebands@gmail.com

Maria Jasin, PhD
Memorial Sloan-Kettering Cancer
Center
m-jasin@ski.mskcc.org

Rulang Jiang, PhD
Cincinnati Children's Hospital
Medical Center
Rulang.Jiang@CCHMC.org

Monica J Justice, PhD
Baylor Col Med
monica.justice@sickkids.ca

Minjung Kang
Sloan-Kettering Institute
kangm@mskcc.org

Claudia Kappen, PhD
Pennington Biomed. Res. Center
claudia.kappen@pbrc.edu

Yukako Kayashima
University of North Carolina at
Chapel Hill
yukaya@email.unc.edu

Gyeong-hwa Kim
School of Medicine, Kyungpook
National University
aoet111@gmail.com

Markku Kurkinen, PhD
Wayne State University School of
Medicine
markku@genetics.wayne.edu

Patricia Labosky, PhD
National Institutes of Health
laboskypa@od.nih.gov

Elizabeth Lacy, PhD
Memorial Sloan Kettering
e-lacy@mskcc.org

Lynda LAMRI
Developmental Genetics Group
lamri.lynda@gmail.com

Jason MK Lawrence, MBiochem
Takeda Cambridge Ltd.
jlawrence@takedacam.com

Stacy Lawrence
The Open University
stacylewis18@hotmail.com

Laurence A. Lemaire
University of Copenhagen
laurence.lemaire@sund.ku.dk

Carlos Lopez-Otin, Professor
Universidad de Oviedo
clo@uniovi.es

Scott Lowe
Memorial Sloan-Kettering Cancer
Center
lowes@mskcc.org

Xuefei Ma, PhD
NHLBI/NIH
max@nhlbi.nih.gov

Terry R Magnuson, PhD
UNC Chapel Hill
trm4@med.unc.edu

Barbara Malerstein
StemExpress
bmalenstein@stemexpress.com

Nancy R Manley, PhD
University of Georgia
nmanley@uga.edu

Willie H Mark, PhD
Sloan-Kettering Institute
markw@mskcc.org

Isao Matsuo
Osaka Med Ctr for Maternal & Chi
imatsuo@mch.pref.osaka.jp

Martin McMahon, Ph.D.
U.C. San Francisco
mcmahon@cc.ucsf.edu

Isabelle M.E. Migeotte, MD PhD
ULB
isamigeotte@gmail.com

Oleg Mirochnitchenko, PhD
NIH
bohincec@mail.nih.gov

Alejandro O Mujica, PhD
Regeneron Pharmaceuticals, Inc.
alejo.mujica@regeneron.com

Hilmarie Muniz-Talavera
University of Illinois-Chicago
muniztal@uic.edu

Robert J Newman
Genentech
newmanr4@gene.com

Gladys Chioma Nzeh, PhD
University of Ilorin
c.g.nzeh@gmail.com

Rupert Oellinger, Dr.
Klinikum rechts der Isar
rupert.oellinger@lrz.tu-
muenchen.de

Lorin E Olson, PhD
Oklahoma Medical Research
Foundation
Lorin-Olson@omrf.org

John D O'Neil, B.S.
University of Georgia
oneilj88@uga.edu

Virginia E Papaioannou, Ph.D.
Columbia University
vep1@columbia.edu

**GUILLAUME PAVLOVIC,
Engineer**
CERBM - Mouse Clinical Institute
pavlovic@igbmc.fr

Evangelos Pefanis
Columbia University
ep419@columbia.edu

Elizabeth Pitts
Thermo Fisher Scientific
elizabeth.pitts@thermofisher.com

Oliver Pourquie
Harvard Medical School/Brigham
pourquie@igbmc.fr

Huanyu Qiao, Ph.D.
UC Davis
hyqiao@gmail.com

Roland Rad
Technical University Munich
Roland.Rad@lrz.tum.de

PARTICIPANT LIST

As of August 10, 2014

Martin Ringwald
The Jackson Laboratory
Martin.Ringwald@jax.org

Jaime A Rivera-Perez
University of Massachusetts
Medical School
jaime.rivera@umassmed.edu

Kelly Rodriques
Iseehear Inc.
kelly.rodriques@iseehear.com

Kevin Donald Ross
UC San Diego
kdross@ucsd.edu

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

Nestor Saiz Arenales, PhD
Sloan Kettering Institute
saizaren@mskcc.org

David A Sassoon
U Pierre & Marie Curie - Sorbonn
david.a.sassoon@gmail.com

Thom Saunders, Ph.D.
University of Michigan
tsaunder@umich.edu

jennifer schmahl, PhD
Regeneron Pharmaceuticals
jennifer.schmahl@regeneron.com

Jennifer Schmidt, PhD
University of Illinois Chicago
jvs@uic.edu

Chris J Schoenherr, PhD
Regeneron Pharmaceuticals
chris.schoenherr@regeneron.com

Licia Selleri, MD, PhD
Weill Medical College of Cornell
University
lis2008@med.cornell.edu

Shyam K. Sharan, PhD
Frederick National Lab, NCI
sharans@mail.nih.gov

William Shawlot, Ph.D.
University of Texas at Austin
wshawlot@austin.utexas.edu

Karl B. Shpargel, Ph.D.
University of North Carolina
shpargel@email.unc.edu

Kathy Snow
The Jackson Laboratory
kathy.snow@jax.org

Philippe Soriano
Icahn School of Medicine at Mt.
Sinai
philippe.soriano@mssm.edu

François Spitz
EMBL
spitz@embl.de

Chengyi Sun, BS
Oklahoma Medical Research
Foundation
suna@omrf.org

Xin Sun, Ph.D.
University of Wisconsin-Madison
xsun@wisc.edu

David W Threadgill, PhD
Texas A&M University
dwt@tamu.edu

Masahiro Tokunaga, M.D. Ph.D.
Osaka University
tokunaga@mr-envi.med.osaka-
u.ac.jp

John Tonkinson, Ph.D.
The Jackson Laboratory
john.tonkinson@jax.org

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

Eric Van Otterloo
University of Colorado - Denver
eric.vanotterloo@ucdenver.edu

Zhu Wang, Ph.D.
University of California Santa Cruz
zwang36@ucsc.edu

Soren Warming, Ph.D.
Genentech, inc.
sorenw@gene.com

Julia M Weber
German Cancer Research Center
j.weber@dkfz.de

Jochen E Welcker, PhD
TaconicArtemis
jochen.welcker@taconicartemis.com

Xiang-Xi Mike Xu
University of Miami
xxu2@med.miami.edu

Rui Yang, master
Peking Union Medical College
ruiyang@ibms.pumc.edu.cn

Katherine Yates
The Ohio State University
yates.183@osu.edu

Sezen Yertutanol
Department of Biobehavioral Health
siy5089@psu.edu

Chengji Zhou, PhD
University of California Davis
cjzhou@ucdavis.edu

SOMETIMES
NEW TECHNOLOGIES
CAN CHANGE
EVERYTHING

INTRODUCING

CRISPR

MOUSE MODEL
GENE EDITING
FROM TACONIC

To download a white paper on the use of
CRISPR technology to generate genetically
engineered mouse models visit taconic.com/crispr



TACONIC

COMPREHENSIVE MOUSE RESOURCES ARE ONLY A MOUSE CLICK AWAY

UC Davis Mouse Biology Program is an innovator of mouse models and molecular technologies that advance human health through reproducible research, higher education, and next-generation discovery.

www.MouseBiology.org is your one stop source for:

- Custom Breeding
- Genome Editing (Cas9)
- Cryopreservation and Cryorecovery
- Diagnostic and Anatomic Pathology
- Embryonic Stem Cells
- Genotyping
- Microinjection
- Embryo Manipulation
- Phenotyping
- Reanimation
- Rederivation
- Transgenic and Knockout Mice
- Vivarium Services
- And more...

The MBP at UC Davis and its NIH funded, Mutant Mouse Resource and Research Center (MMRRC), Mouse Metabolic Phenotyping Center (MMPC), and Knockout Mouse Production and Phenotyping (KOMP2) Project is the research, resource and repository epicenter for the highest quality precision mouse models and services for the biomedical research community.



To learn more visit www.mousebiology.org today.



*Your next Mouse Model is
only a Mouse click away!*

GSA® Two Journals, One Mission

Genetics Society of America

Scientific peer-review and publishing is a critical component of your research process that deserves to be handled with care, responsiveness, and speed. Trust your articles to GSA's peer-edited sister journals. Your peers – practicing scientists – manage the review and make all decisions on manuscripts. We publish cross-journal article blocks including Genomic Selection, QTL mapping in multi-parental population, Mouse Collaborative Cross, Genetics of Sex Determination, and others.

One month from submission until first decision means timely discovery of your research. And *G3* and *GENETICS* articles feature interactive, one-click links to FlyBase, plus Altmetric statistics, and, in mid-2014, ORCID IDs to link all your research output. Want to see your artwork on the cover? Submit an entry! Not sure if your work is appropriate for *GENETICS* or *G3*? Submit a pre-submission inquiry. Need to fast-track a paper? Let us know.

G3
Genes | Genomes | Genetics



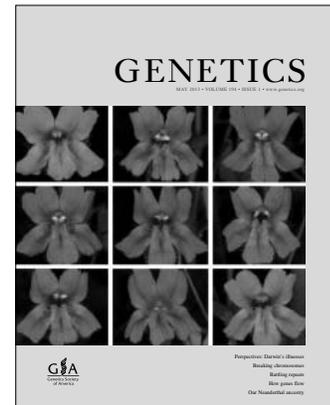
Why submit to *G3*? *G3* provides a forum for the publication of high-quality research that generates useful genetic and genomic findings. Topics include genome maps, single gene studies, genome-wide association and QTL studies, mutant screen reports, sequence of novel species, plus datasets, research in human and medical genetics, and more. *G3* believes that rapid dissemination of its published articles is the necessary foundation for analysis that leads to mechanistic insights.

- Fully open-access journal
- Rapid turnaround on submitted manuscripts
- Early online publication within 2 weeks of acceptance; final publication within 4-8 weeks
- Well-described studies with useful results
- Mutant Screen Report format for quick submissions

Because your research is important to you, it's important to us. Don't let your work get lost in a sea of papers! Submit to *G3*, increase your visibility, and support the genetics community.

www.g3journal.org

GENETICS



Why submit to *GENETICS*? To communicate significant, novel findings in genetics and genomics to a wide range of scientific investigators. *GENETICS* also publishes Methods, Technology and Resources; significant software advances; Toolbox reviews; Educational Primers; Commentaries, Perspectives, and more.

- Fast decisions - average 34 days from submission to first decision
- Thoughtful, thorough reviews improve the impact of your research
- Clear decisions by peer-editors
- Space to tell the whole story - no limits on page length, tables, or figures
- Early online within 2 weeks of acceptance
- Open-access option
- Highlights and press releases promote exceptional stories and discoveries

www.genetics.org

Submit your next manuscript to the GSA Journals

DMM Disease Models & Mechanisms

dmm.biologists.org

Editor-in-Chief: Ross Cagan

Senior Editors: **Monica Justice** and **Geoffrey Smith**

Basic research with translational impact

DMM publishes research and reviews that focus on the use of model organisms to understand, diagnose and treat human disease. The journal is committed to promoting rigorous preclinical assessment of models and mechanisms to drive the development of novel and effective therapies.

Key Author Benefits

- Impact factor: 5
- Open Access
- PMC deposition
- Accepted manuscripts online within 1 week
- Indexed in Medline, ISI and Scopus

Read the latest in translational research at:
dmm.biologists.org



An **OPEN ACCESS** Journal

