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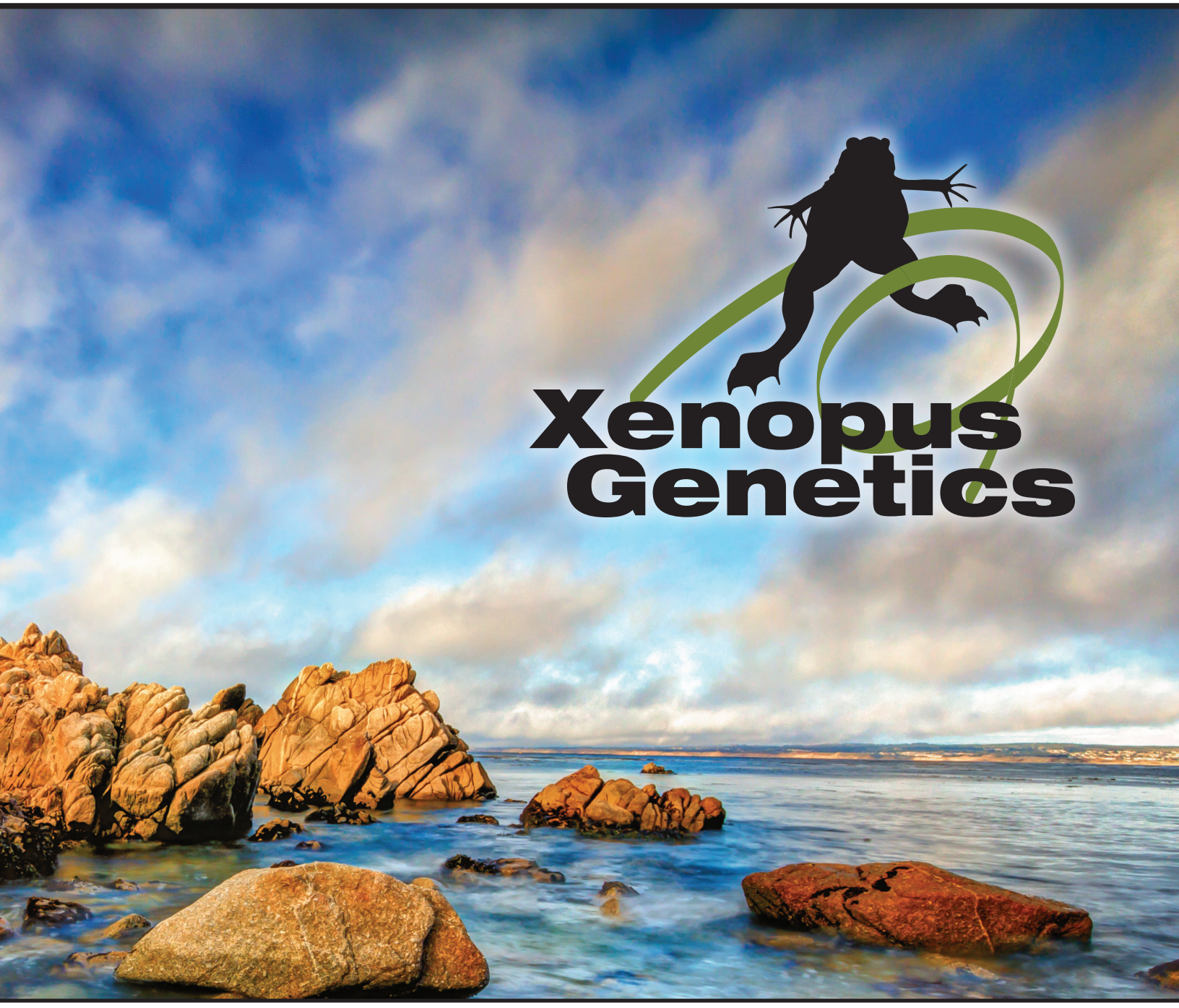
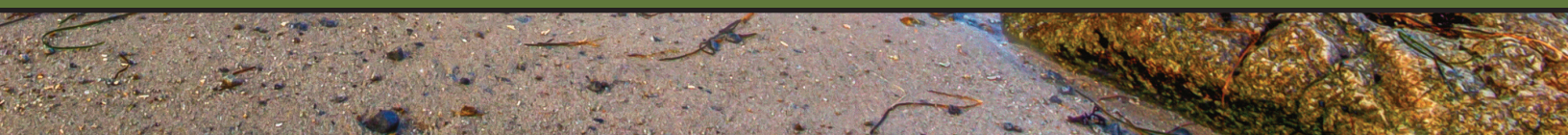


15th International Xenopus Conference

August 24-28, 2014 • Pacific Grove, CA

PROGRAM GUIDE



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Program and Abstracts

Meeting Organizers

Carole LaBonne, Northwestern University
John Wallingford, University of Texas at Austin

Organizing Committee:
Julie Baker, Stanford Univ
Carmen Domingo, San Francisco State Univ
Chris Field, Harvard Medical School
Anna Philpott, Univ of Cambridge

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Invited Speakers

Keynote Lecture **Rebecca Heald,** University of California, Berkeley
Special Lectures **John Gurdon,** The Gurdon Institute
Marc Kirschner, Harvard Medical School

Enrique **Amaya**, Univ of Manchester
Ruchi **Bajpai**, Univ of Southern California
Bill **Bement**, Univ of Wisconsin
Mike **Blower**, Harvard Medical School
Cliff **Brangwynne**, Princeton Univ
Josh **Brickman**, The Danish Stem Cell Center DanStem
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Hollis **Cline**, The Scripps Research Institute
Frank **Conlon**, Univ of North Carolina
Lance **Davidson**, Univ of Pittsburgh
Eddy **DeRobertis**, UCLA
Amanda **Dickinson**, Virginia Commonwealth Univ
Carmen **Domingo**, San Francisco State Univ
Karel **Dorey**, Univ of Manchester
Jim **Ferrell**, Stanford University
Jenny **Gallop**, Univ of Cambridge
Jay **Gatlin**, Univ of Wyoming
Jean **Gautier**, Columbia Univ
Xi **He**, Harvard Univ
Ralf **Hofmann**, Karlsruhe Institute of Technology
Jubin **Kashef**, Karlsruhe Institute of Technology
Mustafa **Khokha**, Yale Univ
Mary Lou **King**, Univ of Miami
Laurent **Kodjabachian**, Developmental Biology Institute of Marseille (IBDM)
Branko **Latinkic**, Cardiff Univ
Dan **Levy**, Univ of Wyoming
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Dan **Rokhsar**, Univ of California, Berkeley
Hazel **Sive,** Massachusetts Institute of Technology
Elena **Silva Casey**, Georgetown Univ
Francesca **Spagnoli**, Max-Delbrück-Center for Molecular Medicine (MDC)
Elly **Tanaka**, Center for Regenerative Therapies
Gert **Veenstra**, Radboud Univ
Monica **Vetter**, Univ of Utah
Sara **Woolner**, Univ of Manchester
Phil **Zegerman**, Univ of Cambridge
Aaron **Zorn**, Cincinatti Children's

GENERAL INFORMATION

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

Date	Time	Location
Sunday, August 24	**1:00 pm—5:00 pm**	**Merrill Hall**
Monday, August 25	**8:00 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

Monday, August 25, 2014

8:00 am - 8:30 am	AUTHORS affix Odd Numbered Posters
8:30 am - 11:30 pm	Open Poster Viewing (odd poster numbers)
8:30 pm-11:30 pm	Poster Presentations (odd poster numbers)

Tuesday, August 26, 2014

8:00 am-10:40 am	Open Poster Viewing (odd poster numbers)
10:45 am-noon	**AUTHORS must remove Odd Numbered Posters**
noon-1:00 pm	AUTHORS affix Even Numbered Posters
1:00 pm-11:30 pm	Open Poster Viewing (even poster numbers)
8:30 pm-11:30 pm	Poster Presentations (even poster numbers)

Wednesday, August 27, 2014

8:00 am-3:00 pm	Open Poster Viewing (even poster numbers)
3:00 pm-4:00 pm	**AUTHORS must remove Even Numbered Posters**

All posters will be displayed in Fred Farr/Kiln. Two authors will share a 4 x 8 ft (1.2 x 2.4 m) poster board. Posters that are larger than 3'8" tall by 3'9" wide will be removed. Authors will present according to the schedule listed above. The meeting does not take responsibility for posters that are not removed on time.

Tickets
The event tickets for the Opening/Welcome Reception and Closing Dinner/Party can be exchanged for two drink tickets per 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

Sunday, August 24		
1:00 pm - 5:00 pm	**Registration**	**Merrill Hall**
2:30 pm - 2:45 pm	**Welcome and Opening Remarks**	**Merrill Hall**
2:45 pm - 3:00 pm	**Xenopus: The View from NIH - James Coulombe, NICHD**	**Merrill Hall**
3:00 pm - 6:00 pm	**Session 1** *Moderator:* Richard Harland, UC Berkeley	**Merrill Hall**
6:00 pm - 7:00 pm	**Dinner**	**Crocker Dining Hall**
7:30 pm - 8:30 pm	**Opening Reception**	**Meadows**
Monday, August 25		
7:30 am - 8:30 am	**Breakfast**	**Crocker Dining Hall**
8:30 am - 10:40 am	**Session 2** *Moderator:* Sally Moody, George Washington University	**Merrill Hall**
8:30 am - 11:30 pm	**Odd Numbered Posters Open for Viewing**	**Fred Farr/Kiln**
10:10 am - 10:40 am	**Break - Exhibits**	**Merrill Hall**
10:40 am - 11:50 am	**Session 3** *Moderator:* Jeremy Green, Kings College, London	**Merrill Hall**
12:00 noon - 1:00 pm	**Lunch**	**Crocker Dining Hall**
1:00 pm - 1:30 pm	**Exhibits**	**Merrill Hall**
1:30 pm - 2:30 pm	**Special Lectures** *Moderator:* Carole LaBonne, Northwestern University	**Merrill Hall**
3:00 pm - 4:30 pm	**Session 4** *Moderator:* Chenbei Chang, University of Alabama	**Merrill Hall**
4:30 pm - 5:00 pm	**Break - Exhibits**	**Merrill Hall**
5:00 pm - 5:50 pm	**Session 5 - Large Scale Resources for Xenopus Research** *Moderator:* Rob Grainger, University of Virginia	**Merrill Hall**
6:00 pm - 7:10 pm	**Dinner**	**Crocker Dining Hall**
7:30 pm - 8:30 pm	**Keynote Lecture** *Moderator:* John Wallingford, Howard Hughes Medical Institute & Dept of Molecular Biosciences	**Merrill Hall**
7:30 pm	**Mechanisms of mitosis and size control in Xenopus** Rebecca Heald, University of California, Berkeley, Berkeley, CA	
8:30 pm - 11:30 pm	**Poster Session 1 - Odd Numbered Posters** *Cash Bar (8:30-10:30 pm)*	**Fred Farr/Kiln**
Tuesday, August 26		
7:30 am - 8:30 am	**Breakfast**	**Crocker Dining Hall**
8:00 am - 10:40 am	**Odd Numbered Posters Open for Viewing** *All odd numbered posters **must be removed by noon**. Posters that are not removed will be recycled.*	**Fred Farr/Kiln**
8:30 am - 10:10 am	**Session 6** *Moderator:* Julie Baker, Stanford University	**Merrill Hall**
10:10 am - 10:40 am	**Break - Exhibits**	**Merrill Hall**
10:40 am - 11:50 am	**Session 7** *Moderator:* Jerry Thomsen, Stony Brook University	**Merrill Hall**
12:00 noon - 1:00 pm	**Lunch**	**Crocker Dining Hall**
1:00 pm - 1:30 pm	**Exhibits**	**Merrill Hall**
1:00 pm - 11:30 pm	**Even Numbered Posters Open for Viewing** *Even numbered posters can be placed on boards between noon and 1 pm*	**Fred Farr/Kiln**
1:30 pm - 3:00 pm	**Career Advice for Postdocs and Students**	**Merrill Hall**

SCHEDULE OF EVENTS

	Tuesday, August 26 (continued)	
3:00 pm - 5:00 pm	**Free Time to Visit Exhibits**	**Merrill Hall**
3:00 pm - 5:00 pm	**Free Time to Visit Posters**	**Fred Farr/Kiln**
5:00 pm - 6:00 pm	**Session 8** *Moderator:* Asako Shindo, Nagoya University	**Merrill Hall**
6:00 pm - 7:00 pm	**Dinner**	**Crocker Dining Hall**
7:10 pm - 8:30 pm	**Session 9** *Moderator:* Mike Sheets, University of Wisconsin	**Merrill Hall**
8:30 pm - 11:30 pm	**Poster Session 2 - Even Numbered Posters** *Cash Bar (8:30-10:30 pm)*	**Fred Farr/Kiln**
Wednesday, August 27		
7:00 am - 8:00 am	**Breakfast**	**Crocker Dining Hall**
8:00 am - 10:00 am	**Session 10** *Moderator:* Amy Sater, University of Houston	**Merrill Hall**
8:00 am - 3:00 pm	**Even Numbered Posters Open for Viewing** ***Even numbered posters must be removed by 4:00 pm****. Posters that are not removed will be recycled.*	**Fred Farr/Kiln**
10:00 am - 10:30 am	**Break - Exhibits**	**Merrill Hall**
10:30 am - 11:50 am	**Session 11** *Moderator:* Mike Gilchrist, National Institute of Medical Research, London	**Merrill Hall**
12:00 noon - 1:00 pm	**Open Lunch and Women in Science Roundtable Lunch**	**Crocker Dining Hall**
1:30 pm - 3:00 pm	**Career Advice for New PIs**	**Merrill Hall**
3:20 pm - 5:00 pm	**Session 12** *Moderator:* Anna Philpott, Cambridge University	**Merrill Hall**
6:00 pm - 7:00 pm	**Dinner** (must bring banquet ticket)	**Crocker Dining Hall**
7:30 pm - 10:30 pm	**Dancing/General Conviviality**	**Merrill Hall**
Thursday, August 28		
7:30 am - 8:30 am	**Breakfast**	**Crocker Dining Hall**
8:30 am - 10:40 am	**Session 13** *Moderator:* Dan Weeks, University of Iowa Medical School	**Merrill Hall**
10:10 am - 10:40 am	**Break**	**Merrill Hall**
10:40 am - 12:00 noon	**Session 14** *Moderator:* Carmen Domingo, San Francisco State University	**Merrill Hall**
12:00 noon - 1:00 pm	**Lunch** *If you pre-ordered a box lunch, bring your ticket to Crocker Dining Hall*	**Crocker Dining Hall**
1:00 pm - 5:00 pm	**PI Meeting**	**Merrill Hall**

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OPENING AND PLENARY/PLATFORM SESSIONS

See Pages 6-7 for presentation schedule. Program number and presenter are in **bold**. Full abstracts begin on page 38.

Sunday, August 24 2:30 pm–2:45 pm
Merrill Hall

Opening Remarks – Carole LaBonne, Northwestern University and **John Wallingford,** HHMI & University of Texas

1 - 2:45
Xenopus - The View from NIH. James Coulombe. NICHD.

Sunday, August 24 3:00 pm–6:00 pm
Merrill Hall

Session 1

Chair: Richard Harland, UC Berkeley

2 - 3:00
Polarized Wnt signaling regulates ectodermal cell fate in Xenopus. Ya-Lin Huang[2], **Christof Niehrs**[1,2]. 1) IMB Institute of Molecular Biology, 55128 Mainz, Germany; 2) Division of Molecular Embryology, DKFZ-ZMBH Alliance, Deutsches Krebsforschungszentrum, 69120 Heidelberg, Germany.

3 - 3:20
Trigger Waves in Mitosis and Apoptosis. James Ferrell, Jeremy Chang. Stanford University.

4 - 3:40
Dead-End1 is Required and Sufficient to Activate *nanos* Translation in the Xenopus Germline. Mary Lou King[1], Tristan Aguero[1], Jing Yang[2]. 1) University of Miami School of Medicine; 2) University of Illinois.

5 - 4:00
Genome assemblies and the origin of tetraploidy. Don Rokhsar. University of California, Berkeley.

6 - 4:20
Oct4 duplication and the delination of distinct conserved mechanisms of pluripotency. Joshua Brickman[1], Alessandra Livigni[2], Hanna Peradziry[1], Woranop Sukparangsi[1]. 1) DanStem - The Novo Nordisk Foundation Section for Basic Stem Cell Research, University of Copenhagen; 2) MRC Centre for Regenerative Medicine University of Edinburgh.

7 - 4:40
Role and Mechanism of Branching Microtubule Nucleation. Sabine Petry. Molecular Biology, Princeton University, Princeton, NJ.

8 - 5:00
Combining *Xenopus* Embryology with Human Genetics – The Case of Anks6, a Novel Nephronothisis Gene. Sylvia Hoff[1], Jan Halbritter[2], Ronald Roepman[4], Sophie Saunier[5], Gerd Walz[1], Friedhelm Hildebrandt[2], Carsten Bergmann[3], **Soeren Lienkamp**[1]. 1) Department of Nephrology, University Hospital Freiburg, Freiburg, Germany; 2) Department of Medicine, Boston Children's Hospital, Harvard Medical School, Boston; 3) Center for Human Genetics, Bioscientia, Ingelheim, Germany; 4) Department of Human Genetics, Nijmegen Centre for Molecular Life Sciences and Institute for Genetic and Metabolic Disease, Radboud University Medical Centre, 6525 GA, Nijmegen, The Netherlands; 5) Inserm U983, Hôpital Necker-Enfants Malades and Paris Descartes- Sorbonne Paris Cité University, Imagine Institute, Paris, France.

9 - 5:20
Spinal circuit remodeling during developmental transitions in motor behavior. Lora B. Sweeney[1], Jay B. Bikoff[2], Susan B. Morton[2], Thomas M. Jessell[2], Christopher Kintner[1]. 1) Molecular Neurobioloy Laboratory, Salk Institute for Biological Studies, La Jolla, CA; 2) Howard Hughes Medical Institute, Kavli Institute for Brain Science, Mortimer B. Zuckerman Mind Brain Behavior Institute, Departments of Neuroscience and Biochemistry and Molecular Biophysics, Columbia University, New York, NY.

10 - 5:30
Understanding regeneration from the inside out: chromatin and transcription landscapes in the regenerating tadpole tail. Andrea E. Wills, Jessica Chang, Rakhi Gupta, Julie Baker. Genetics, Stanford School of Medicine, Stanford, CA.

150 - 5:40
Functional anatomy of a transcription factor: learning how GATA4 induces cardiogenesis. Branko Latinkic[1], Wensheng Deng[1], Joseph Gallagher[1], Simon Fellgett[1], Abir Yamak[2], Mona Nemer[2]. 1) Cardiff University, Cardiff, Wales, United Kingdom; 2) Laboratory of Cardiac Development and Differentiation, Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, Ontario, Canada.

Monday, August 25 8:30 am–10:40 am
Merrill Hall

Session 2

Chair: Sally Moody, George Washington University

11 - 8:30
Non-canonical Wnt signaling mediates morphogenesis of the extreme anterior domain during mouth formation. Hazel Sive[1], Laura Jacox[1,2], Radek Sindelka[1,3], Alyssa Rothman[1]. 1) Whitehead Institute and MIT, Cambridge , MA; 2) Harvard School of Dental Medicine, Boston MA; 3) Institute of Biotechnology Videnska, Prague, Czech Republic.

12 - 8:50
The calcium-dependent ribonuclease XendoU promotes ER network formation through local RNA degradation. Mike Blower[1,2], Dianne Schwarz[1,2]. 1) Molecular Biology, Massachusetts General Hosptial, Boston, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA.

13 - 9:10
Radial Intercalation is regulated by the Par complex and the microtubule-stabilizing protein CLAMP/Spef1. Michael Werner, Jen Mitchell, **Brian Mitchell**. Cell and Molecular Biology, Northwestern University, Chicago, IL.

14 - 9:30
Age-Defying Proline, Because It's Worth It. Nathalie Escande-Beillard[1], Mohammad Shboul[1], Amira Masri[2], **Bruno Reversade**[1]. 1) Institute of Medical Biology, www.reversade.com, A*STAR, Singapore; 2) Department of Pediatrics, University of Jordan, Amman, Jordan.

15 - 9:50
Neural Crest Migration and Neuronal Differentiation is Mediated by *tfap2e* in Xenopus. Sofia Medina Ruiz[1], Anastasia Lobanova[1], Anne-Helene Monsoro-Burq[2], Michael Eisen[1], Richard Harland[1]. 1) Department of Molecular and Cell Biology, University of California, Berkeley, California, United States of America; 2) CNRS-Institut Curie. Orsay, France.

16 - 10:00
Parent-of-origin effects in Xenopus laevis gene expression. Leonid Peshkin[1], Virginia Savova[2], Anwesha Nag[2], Esther Pearl[3], Marko Horb[3], Marc Kirschner[1], Alexander Gimelbrant[2]. 1) Systems Biology, Harvard Medical School, Boston, MA; 2) Dana Farber Cancer Institute, Boston, MA; 3) Marine Biological Laboratory, Woods Hole, MA.

Monday, August 25 10:40 am–11:50 am
Merrill Hall

Session 3

Chair: Jeremy Green, Kings College, London

17 - 10:40
Regulation of cell division orientation during epithelial morphogenesis. Sarah Woolner[1], Georgina Goddard[1], Alexander Nestor-Bergmann[1], Oliver Jensen[2]. 1) Faculty of Life Sciences, The University of Manchester, Manchester, United Kingdom; 2) School of Mathematics, The University of Manchester, Manchester, United Kingdom.

18 - 11:00
Novel Regulations in Early Neural and Neural Crest Development. Anne Monsoro-Burg, C. Pegoraro, A. Figueiredo, F. Maczkowiak, J-L Plouhinec, D. Roche. Insitut Curie, Université Paris Sud, CNRS, INSERM.

19 - 11:20
Not Separate but Equal: Cohesion Establishment during DNA Replication. Saili Moghe[1], Jianhua Song[1], **Susannah Rankin**[1,2]. 1) Cell Cycle and Cancer Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

20 - 11:40
Spinal cord regeneration in *Xenopus laevis*. Juan Larrain[1,3], Dasfne Lee-Liu[1,3], Rosana Muñoz[1,3], Emilio Méndez[1,3], Gabriela Edwards[1,3], Mauricio Moreno[1,3], Victor Tapia[1,3], Nikole Zuñiga[1,3], Leonardo Almonacid[2,3], Francisco Melo[2,3]. 1) Center for Aging and Regeneration and Millenium Nucleus in Regenerative Biology; 2) Molecular Bioinformatics Laboratory and Millennium Institute on Immunology and Immunotherapy; 3) Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

Monday, August 25 1:30 pm–2:30 pm
Merrill Hall

Special Lectures

Chair: Carole LaBonne, Northwestern University

21 - 1:30
Past, present, and possible future of Xenopus research. John B. Gurdon. Zoology [Gurdon Institute], University of Cambridge, Cambridge, United Kingdom.

22 - 2:00
How do we get to an explanation in biology. Marc Kirschner. Systems Biology, Harvard Medical School, Boston, MA.

Monday, August 25 3:00 pm–4:30 pm
Merrill Hall

Session 4

Chair: Chenbei Chang, University of Alabama

23 - 3:00
microRNA input into an ultradian oscillator provides an autonomous but tunable timer for differentiation. Nancy Papalopulu. University of Manchester.

24 - 3:20
Epigenetic fine-tuning of cell fate choices: impact on developmental disorders. Ruchi Bajpai. Center for Craniofacial Molecular Biology, Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA, 90033.

25 - 3:40
Embryogenesis kinetics revealed by high-resolution absolute quantitation of transcripts. Nick Owens[1], Ira Blitz[2], Maura Lane[3], John Overton[4], Mustafa Khokha[3], Ken Cho[2], Michael Gilchrist[1]. 1) Division of Systems Biology, MRC National Institute for Medical Research, London, UK; 2) Department of Developmental and Cell Biology, University of California, Irvine, CA, USA; 3) Program in Vertebrate Developmental Biology, Department of Pediatrics and Genetics, Yale University School of Medicine, New Haven, CT, USA; 4) Yale Center for Genome Analysis,Yale University School of Medicine, New Haven, CT, USA.

26 - 3:50
Unliganded thyroid hormone receptor alpha regulates gene repression and developmental timing as revealed by gene knockout in *Xenopus tropicalis*. Jinyoung Choi[1], Ken-ichi Suzuki[2], Tetsushi Sakuma[2], Leena Shewade[1], Takashi Yamamoto[2], **Daniel R. Buchholz**[1]. 1) Biological Sciences, University of Cincinnati, Cincinnati, OH; 2) Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, Hiroshima, Japan.

27 - 4:00
More than one frog in the pond: evolutionary modulations of gut morphogenesis in tadpoles with different diets. Nanette Nascone-Yoder. Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

28 - 4:10
Protein Networks In Cardiac Morphogenesis. Frank Conlon[1,2,3], Stephen Sojka[1,2], Nirav M. Amin[1,3], Devin Gibbs[1,3], Kathleen S. Christine[1,3], Marta S. Charpentier[1]. 1) University of North Carolina McAllister Heart Institute, UNC-Chapel Hill, Chapel Hill, NC 27599-3280, USA; 2) Department of Biology, UNC-Chapel Hill, Chapel Hill, NC 27599-3280, USA; 3) Department of Genetics, UNC-Chapel Hill, Chapel Hill, NC 27599-3280, USA.

Monday, August 25 5:00 pm–5:50 pm
Merrill Hall

Session 5 - Large Scale Resources for Xenopus Research

Chair: Rob Grainger, University of Virginia

29 - 5:00
Xenbase. Peter Vize. Univ. Calgary.

30 - 5:10
The National Xenopus Resource. Esther Pearl. Marine Biological Laboratory, Woods Hole, MA.

31 - 5:20
The Institute for Amphibian Biology. Atsushi Suzuki. Hiroshima University.

32 - 5:30
The European Xenopus Resource Center. Matt Guille. University of Portsmouth, UK.

33 - 5:40
The Xenopus ORFeome. Todd Stukenberg. University of Virginia.

Monday, August 25 7:30 pm–8:30 pm
Merrill Hall

Keynote Lecture

Chair: John Wallingford, Howard Hughes Medical Institute & Dept of Molecular Biosciences

34 - 7:30
Mechanisms of mitosis and size control in Xenopus. Rebecca Heald. University of California, Berkeley, Berkeley, CA.

Tuesday, August 26 8:30 am–10:10 am
Merrill Hall

Session 6

Chair: Julie Baker, Stanford University

35 - 8:30
Understanding Wnt/beta-catenin signaling in development, stem cell, and cancer. Xi He[1,2]. 1) F. M. Kirby Center, Boston Children's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA.

36 - 8:50
Anillin Regulates Cell-Cell Junction Integrity by Organizing Junctional Accumulation of RhoA-GTP and Actomyosin. Ciara Reyes[1], Torey Arnold[2], Tomohito Higashi[2], Rachel Stephenson[2], **Ann Miller**[1,2]. 1) The Cellular and Molecular Biology Program (CMB), University of Michigan; 2) Molecular, Cellular, & Developmental Biology (MCDB), Univ of Michigan.

37 - 9:10
RNA transport in the oocyte cytoplasm: How to get there from here. K. Mowry, S. Jeschonek, C. Neill, C. Pratt, E. Powrie. Department of Molecular Biology, Cell Biology & Biochemistry, Brown University, Providence, RI.

38 - 9:30
Pancreatic development: cell fate choices and plasticity. Francesca Spagnoli. Molecular and Cellular Basis of Embryonic Development, MDC, Berlin, Germany.

39 - 9:50
Nuclear functions of the "other catenins" in stem cell/ developmental decisions. Moon Sup Lee[1], Yasuhide Furuta[2], Jae-il Park[1], **Pierre McCrea**[1]. 1) UT MD Anderson Cancer Center, Houston TX; 2) Riken Center for Developmental Biology, Kobe Japan.

40 - 10:00
Quantitative proteomics of Xenopus laevis embryos: expression kinetics of 4000 proteins during early development. Norman J. Dovichi, Liangliang Sun, Paul Huber, Michelle Bertke, Matthew Champion, Guijie Zhu. Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN.

Tuesday, August 26 10:40 am–11:50 am
Merrill Hall

Session 7

Chair: Jerry Thomsen, Stony Brook University

41 - 10:40
System-level analysis of pancreatic cell type differentiation in Xenopus. Matthew Salanga[1], Leonid Peshkin[2], **Marko Horb**[1]. 1) Bell Center for Regenerative Biology and Tissue Engineering and National Xenopus Resource, Marine Biological Laboratory; 2) Department of Systems Biology, Harvard University

42 - 11:00
Using Frog Faces to Better Understand Orofacial Development. Amanda J. G. Dickinson. VCU, Richmond, VA.

43 - 11:20
Reconstituting the mechanisms of actin remodeling. Jennifer Gallop. Gurdon Institute, University of Cambridge, Cambridge, United Kingdom.

44 - 11:40
Genomics Study of the Spemann-Mangold Organizer: Occupancy of Tissue-Specific cis-Regulatory Modules by Otx2 and TLE/Groucho for Embryonic Head Specification. Yuuri Yasuoka[1,2], Yutaka Suzuki[2], Shuji Takahashi[2,3], Haruka Someya[4], Norihiro Sudou[2,5], Yoshikazu Haramoto[2,6], Ken Cho[7], Makoto Asashima[2,6], Sumio Sugano[2], Masanori Taira[2]. 1) OIST, Japan; 2) Univ. of Tokyo, Japan; 3) Hiroshima Univ., Japan; 4) TMU, Japan; 5) TWMU, Japan; 6) AIST, Japan; 7) UC Irvine, USA.

Tuesday, August 26 5:00 pm–6:00 pm
Merrill Hall

Session 8

Chair: Asako Shindo, Nagoya University

45 - 5:00
***Xenopus* Smad4 at the intersection of FGF, Wnt and TGF-β.** Hadrien Demagny, Tatsuya Araki, **Edward De Robertis**. Biological Chemistry, HHMI/UCLA, Los Angeles, CA.

46 - 5:20
TACC3 is a microtubule plus-end tracking protein that promotes axon elongation and regulates microtubule plus-end dynamics in multiple embryonic cell types. Belinda Nwagbara, Anna Faris, Elizabeth Bearce, Burcu Erdogan, Patrick Ebbert, Matthew Evans, Charlie Baker, Tiffany Enzenbacher, **Laura Anne Lowery**. Dept of Biology, Boston College, Chestnut Hill, MA.

47 - 5:40
Regulation of replication intitation and cell cycle events at the mid-blastula transition (MBT). Philip Zegerman[1], Clara Collart[1,2], James C. Smith[2]. 1) Gurdon Institute, Cambridge, United Kingdom; 2) MRC National Institute for Medical Research, London, United Kingdom.

Tuesday, August 26 7:10 pm–8:30 pm
Merrill Hall

Session 9

Chair: Mike Sheets, University of Wisconsin

48 - 7:10
Proteomic analysis of the DNA double-strand break response. Jean Gautier[1], Tomas Aparicio[1], Yinyin Li[2], Gaganpreet Sidhu[1], Krithika Rajagopalan[1], Brian Chait[2], Max Gottesman[1]. 1) Institute for Cancer Genetics, Columbia University Medical Center, New York, NY; 2) Rockefeller University.

49 - 7:30
Coco is a dual-activity modulator of TGFβ signaling. Alessia Deglincerti, Aryeh Warmflash, Tomomi Haremaki, Qixiang Zhang, Ali H. Brivanlou. Laboratory of Stem Cell Biology and Molecular Embryology, The Rockefeller University, 1230 York Ave, New York, NY.

50 - 7:40
Identification of novel genes involved in epicardial development. P. Tandon[1,2,4], C. Wilczewski[1,4], F. Conlon[2,3,4]. 1) Molecular Biology, UNC, Chapel Hill, NC; 2) Department of Genetics, UNC, Chapel Hill, NC; 3) Department of Biology, UNC, Chapel Hill, NC; 4) McAllister Heart Institute, UNC, Chapel Hill, NC.

51 - 7:50
Mechanisms and functions of nuclear size regulation in early *Xenopus* development. Daniel Levy, Lisa Edens, Predrag Jevtic. Molecular Biology, University of Wyoming, Laramie, WY.

52 - 8:10
Role of cytoskeletal regulators in cranial neural crest migration. Shuyi Nie[1], Marianne Bronner[2]. 1) Georgia Institute of Technology, Atlanta, GA; 2) California Institute of Technology, Pasadena, CA.

Wednesday, August 27 8:00 am–10:00 am
Merrill Hall

Session 10

Chair: Amy Sater, University of Houston

53 - 8:00
A Gene Regulatory Network controlling lung development. Scott Rankin, Lu Han, Kyle McCraken, Marcin Mancini, Pamela Wlizla, James Wells, **Aaron Zorn**. Developmental Biology, Cincinnati Children's, Cincinnati, OH.

54 - 8:20
Cell behaviors associated with somite formation in *Xenopus laevis*. Carmen Domingo, Armbien Sabillo, Vanja Krneta, Daniel Saw, Ceazar Nave, Hernando Vergara, Julio Ramirez. Biology, San Francisco State University, San Francisco, CA.

55 - 8:40
The Role of Heparan Sulfate Nanostructures in Morphogen Gradient Formation and Signaling Reception. Masanori Taira[2], Yusuke Mii[1], Takayoshi Yamamoto[2], Shinji Takada[1]. 1) NIBB; 2) Univ. of Tokyo.

56 - 9:00
X-ray phase contrast microtomography: 4D livecell imaging of structural development. Ralf Hofmann. Karlsruhe Institute of Technology.

57 - 9:20
Regulation of neurogenesis with Sox transcription factors and F-box-mediated protein degradation. Elena Silva Casey, Niteace Whittington, Banu Saritas-Yildirim, Doreen Cunningham, Jing Jin. Dept of Biology, Georgetown University, Washington, DC.

58 - 9:40
Cadherin-11 localizes to focal adhesions and promotes cell-substrate adhesion. Jubin Kashef[1], Rahul P. Langhe[1], Tetyana Gudzenko[2], Sarah F. Becker[1], Carina Gonnermann[2], Claudia Winter[1], Michael Bachmann[3], Clemens M. Franz[1,4]. 1) Zoological Institute, Karlsruhe Institute of Technology (KIT), Cell and Developmental Biology, Kaiserstr. 12, 76131 Karlsruhe, Germany; 2) Center for Functional Nanostructures, Karlsruhe Institute of Technology (KIT), Wolfgang-Gaede-Strasse 1a, 76131 Karlsruhe, Germany; 3) Zoological Institute, Karlsruhe Institute of Technology (KIT), Cell and Neurobiology Biology, Haid-und-Neu-Strasse 9, 76131 Karlsruhe, Germany; 4) Institute for Photon Science and Synchrotron Radiation, Karlsruhe Institute of Technology (KIT), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany.

Wednesday, August 27 10:30 am–11:50 am
Merrill Hall

Session 11

Chair: Mike Gilchrist, National Institute of Medical Research, London

59 - 10:30
Regulation of IP3-dependant calcium signalling during early embryogenesis. Karel Dorey, Tomasz Gwozdz. The Healing Foundation Centre, The University of Manchester, United Kingdom.

60 - 10:50
Cellularization of a Cell-Free System to Study Mechanisms of Organelle Scaling. Jay Gatlin[1], James Hazel[1], Kaspars Krutkramelis[2], John Oakey[2], Dan Levy[1]. 1) Molecular Biology, University of Wyoming, Laramie, WY; 2) Chemical & Petroleum Engineering, University of Wyoming, Laramie, WY.

61 - 11:10
Polycomb regulation of retinal progenitor proliferation and differentiation. Monica L. Vetter, Issam Aldiri, Kathryn Moore, David Hutcheson, Zhang Jianmin. Dept of Neurobiology & Anatomy, University of Utah, Salt Lake City, UT.

62 - 11:30
A systems-level dissection of multiciliated cell specification. Ian Quigley, Lina Ma, Chris Kintner. Molecular Neurobiology Lab, Salk Institute, La Jolla, CA.

63 - 11:40
A novel genetically tractable tumor model for Familial Adenomatous Polyposis using TALEN-mediated gene disruption in *Xenopus tropicalis*. Kris Vleminckx[1,2], Thomas Naert[1], Griet Van Imschoot[1], Ellen Sanders[1,3], Frans Van Roy[1,3], Hong Thi Tran[1], Tom Van Nieuwenhuysen[1]. 1) Dept. Biomed. Mol. Biol., Ghent University, Ghent, Belgium; 2) Centre for Medical Genetics, Ghent University; 3) Inflammation Research Centre VIB, Ghent, Belgium.

Wednesday, August 27 3:20 pm–5:00 pm
Merrill Hall

Session 12

Chair: Anna Philpott, Cambridge University

64 - 3:20
A role for reactive oxygen species in regeneration and early embryogenesis. Enrique Amaya, Nick R. Love, Yaoyao Chen, Yue Han, Shoko Ishibashi, Saori Hokari, Rob Lea. The Healing Foundation Centre, The University of Manchester.

65 - 3:40
Nuclear Mechanics: An F-actin scaffold and gravitational stabilization of RNA/Protein Droplets in Large Cells. Clifford Brangwynne, Marina Feric. Chemical and Biological Engineering, Princeton University, Princeton, NJ.

66 - 4:00
Sox5 modulates TGF-β signaling during early embryogenesis to correctly establish ectodermally derived tissue lineages. Kara Nordin[1], Carole LaBonne[1,2]. 1) Molecular Biosciences, Northwestern University, Evanston, IL; 2) Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL.

67 - 4:10
Reversible control of GSK-3 in the neural crest. Sandra Gonzalez Malagon, Anna Lopez Munoz, Triona Bolger, **Karen Liu**. Craniofacial Development and Stem Cell Biology, King's College London, London, United Kingdom.

68 - 4:30
Post-transcriptional regulation of left-right axis formation. Maike Getwan, Axel Schweickert, Martin Blum. University of Hohenheim, Stuttgart, Germany.

69 - 4:40
Molecular studies of spinal cord regeneration in the axolotl using over-expression and CRISPR-mediated knock out approaches. Elly Tanaka, Aida Rodrigo Albors, Jifeng Fei, Akira Tazaki. DFG Center for Regenerative Therapies Dresden, Technische Universität Dresden, Dresden, Saxony, Germany.

Thursday, August 28 8:30 am–10:40 am
Merrill Hall

Session 13

Chair: Dan Weeks, University of Iowa Medical School

70 - 8:30
Genome-wide view of Nodal/Foxh1 regulation of the early mesendoderm program. Ken Cho[1], Rebekah Charney Le[1], Ira Blitz[1], Yi Li[2], Jacob Biesinger[2], Xiaohui Xie[2], William Chiu[1]. 1) Dev and Cell Biology, Univ of California, Irvine; 2) Computer Science, Univ of California.

71 - 8:50
Cyclin-dependent Kinase Constrained Cortical Chaos. William (Bill) Bement[1], George von Dassow[2], Andrew Goryachev[3], Ann Miller[4], Matt Larson[1], Angela Kita[1], Alison Moe[1], Adriana Golding[1]. 1) University of Wisconsin-Madison; 2) Oregon Inst for Marine Biology-Charleston, OR; 3) University of Edinburgh, Edinburgh, Scotland; 4) University of Michigan-Ann Arbor.

72 - 9:10
The *cdc20b*/miR-449 locus controls multiciliogenesis in *Xenopus* embryonic epidermis. Laurent Kodjabachian[1], Anna Adamiok[1], Diego Revinski[1], Benoit Chevalier[2], Laure-Emmanuelle Zaragosi[2], Andrea Pasini[1], Brice Marcet[2], Pascal Barbry[2]. 1) IBDM, CNRS, Aix-Marseille Univ, Marseille, France; 2) IPMC, CNRS, Nice Sophia-Antipolis Univ, Sophia-Antipolis, France.

73 - 9:30
Analysis of newly-synthesized proteins during brain development and experience-dependent plasticity in *Xenopus laevis*. Hollis T. Cline[1,3,4], Han-Hsuan Liu[1,3,5], Wanhua Shen[2], Lucio Schiapparelli[1,3], Danial McClatchy[1,4], John R. Yates III[1,4]. 1) The Scripps Research Institute, La Jolla, CA; 2) Institute of Developmental and Regenerative Biology, College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou, Zhejiang, China; 3) The Dorris Neuroscience Center, Department of Molecular and Cellular Neuroscience; 4) Department of Chemical Physiology; 5) Kellogg School of Science and Technology.

74 - 9:50
Protein tyrosine kinase 7 (PTK7) modulates Wnt signaling activity via LRP6 in the developing neural plate. Hava Lichtig, Naama Bin-Nun, **Dale Frank**. Department of Biochemistry, Faculty of Medicine - Technion, Haifa, Israel.

75 - 10:00
Role of lbh during Xenopus Craniofacial Development. Helene Cousin[1], Gretchen McLinden[1], Rebekah Pack[2], Kara Powder[2], Craig Albertson[2]. 1) Vet. & Animal Sciences department, UMass- Amherst, Amherst, MA; 2) Biology department, UMass- Amherst.

Thursday, August 28 10:40 am–12:00 noon
Merrill Hall

Session 14

Chair: Carmen Domingo, San Francisco State University

76 - 10:40
A novel control mechanism to enable adaptive morphogenesis. Lance Davidson. Departments of Bioengineering, Developmental Biology, and Computational and Systems Biology, University of Pittsburgh, Pittsburgh, PA.

77 - 11:00
Shaping Kidney Tubules through Planar Cell Polarity Signaling and Cilia. Rachel Miller[1,2,3,4], Malgorzata Kloc[5], Tanya Baldwin[2], Le Huang[2,3,6], Andrew Gladden[2,3,6], Vicki Huff[2,3,6], Pierre McCrea[2,3,4]. 1) Pediatrics- Research Center, The University of Texas Medical School at Houston, Houston, TX; 2) Graduate School of Biomedical Sciences; 3) Graduate Program in Genes & Development; 4) Department of Biochemistry & Molecular Biology, MD Anderson Cancer Center; 5) Immuno-Biology Laboratory, Methodist Hospital Research Institute; 6) Department of Genetics, MD Anderson Cancer Center.

78 - 11:10
The Nuclear Proteome of a Vertebrate Oocyte. Martin Wühr[1,2], T. Güttler[1], L. Peshkin[2], GC McAlister[1], AC Groen[2], R. Rad[1], TJ Mitchison[2], SP Gygi[1], MW Kirschner[2]. 1) Department of Cell Biology; 2) Department of Systems Biology, Harvard Medical School, Boston, MA.

79 - 11:20
***Xenopus* epigenome and enhancer dynamics.** Saartje Honteleze, Sarita Paranjpe, George Georgiou, Simon van Heeringen, **Gert Jan Veenstra**. Radboud University, Nijmegen, Netherlands.

80 - 11:40
Gene Discovery in Birth Defect Patients and Mechanism Disovery in *Xenopus*. Mustafa Khokha. Pediatrics & Genetics, Yale University, New Haven, CT.

POSTER SESSIONS

See Page 5 for presentation schedule. Poster board number and presenter are in **bold**. Full abstracts begin on page 38.

POSTER LEGEND

See Page 5 for presentation schedule. Poster board number and presenter are in **bold**. Full abstracts begin on page 38.

Behavior

81 ***CANCELLED***
Learning, memory, and behavior in tadpoles; positioning *Xenopus* as a model for cognitive studies. D. Blackiston, N. Rahman, M. Levin. Tufts University, Medford, MA.

Cell Biology

82
Positive and Negative Modulators of WNT/β-Catenin Pathway Identified by Functional Screening of Natural Compounds. Jose G. Abreu, Barbara Fonseca, Danilo Predes, Nathalia Amado. Program of Cell and Developmental Biology, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil.

83
Detection of amphibian chytridiomycosis before and after formalin fixation. Sean C. Adams[1], Richard Luong[1], Raul Figueroa[2], Stephen Felt[1], Vance Vredenburg[2], Sherril Green[1]. 1) Comparative Medicine, Stanford University, Stanford, CA; 2) Department of Biology, San Francisco State University, San Francisco, CA, United States.

84
Making the connection: Ciliary adhesion complexes are responsible for anchoring basal bodies to the actin cytoskeleton. Ioanna Antoniades, Panayiota Stylianou, Paris Skourides. Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus.

85
Dynamic Dvl1, Pk2 and Vangl1 localizations pattern a vertebrate multiciliated epithelium. Mitchell Butler[1], John Wallingford[2]. 1) The University of Texas at Austin, Austin, TX; 2) Howard Hughes Medical Institute and The University of Texas at Austin, Austin, TX.

86
EphrinB2 affects apical constriction in *Xenopus*embryos and is regulated by ADAM10 and flotillin-1. Ira Daar[1], Yon Ju Ji[1], Yoo-Seok Hwang[1], Kathleen Mood[1], Hee-Jun Cho[1], Hyun-Shik Lee[2], Emily Winterbottom[1], Helene Cousin[3]. 1) Laboratory of Cell & Developmental Signaling, National Cancer Institute, Frederick, MD; 2) ABRC, CMRI, School of Life Sciences, College of Natural Sciences, Kyungpook National University, Daegu 702-701, South Korea; 3) Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA.

87
Investigating Rho GTPase Pattern Formation During Wound Repair: The Role of RhoGAP1/8. Nicholas R. Davenport[1], William Bement[1,2,3]. 1) Graduate Program in Cellular and Molecular Biology, University of Wisconsin-Madison, Madison, WI; 2) Department of Zoology, University of Wisconsin-Madison, Madison, WI; 3) Laboratory of Cell and Molecular Biology, University of Wisconsin-Madison, Madison, WI.

88
RFX7 is required for the formation of primary cilia in the neural tube. R. Earwood, Z. Manojlovic, A. Kato, B. Stefanovic, Y. Kato. Department of Biomedical Science, Florida State University, Tallahassee, FL.

89
Cell to Cell Adhesion in *Xenopus* Mesendoderm Cells is Regulated via CXCR7 Signaling. A. Fukui, K. Furusawa, N. Sasaki. Grad. Sch. Life Sci., Hokkaido Univ., Sapporo, Hokkaido, Japan.

90
Investigating biological size control using *Xenopus* hybrids. Romain Gibeaux, Rebecca Heald. Department of Molecular & Cell Biology, University of California, Berkeley, CA.

91
Investigating physical and biochemical mechanisms underlying the adaptability of mitotic spindle length to cell size in *Xenopus*. M. Good[1], C. Brownlee[1], M. Vahey[2], J. Wilbur[1], D. Fletcher[2], R. Heald[1]. 1) Molecular and Cellular Biology Dept., UC Berkeley, Berkeley, CA; 2) Bioengineering Dept., UC Berkeley, Berkeley, CA.

92
Truncation of RASSF7 induces centrosome amplification; a role in cancer? Tulay Gulsen[1], Paul Whitley[2], Andrew Chalmers[3]. 1) University of Bath, bath, avon, United Kingdom; 2) #1; 3) #2.

93

Remodeling of cell-cell junctions during cytokinesis. T. Higashi, T. Arnold, R. Stephenson, A. Miller. Mol Cell Dev Biol, University of Michigan, Ann Arbor, MI.

94

Analysis of the role of ROS during tail regeneration in Xenopus. S. Ishibashi[1], A. Yamamoto[1,2], R. Lea[1], E. Amaya[1]. 1) The Healing Foundation Centre, The University of Manchester, Manchester, United Kingdom; 2) College of Biological Sciences, University of Tsukuba, Japan.

95

Cadherin-11 localizes to focal adhesions and promotes cell-substrate adhesion. Rahul Langhe[1], Tetyana Gudzenko[2], Sarah Becker[1], Carina Gonnermann[2], Michael Bachmann[3], Clemens Franz[2],**Jubin Kashef**[1,4]. 1) Zoological Institute, Karlsruhe Institute of Technology (KIT), Cell and Developmental Biology, Kaiserstr. 12, 76131 Karlsruhe, Germany; 2) Center for Functional Nanostructures, Karlsruhe Institute of Technology (KIT), Wolfgang-Gaede-Strasse 1a, 76131 Karlsruhe, Germany; 3) Zoological Institute, Karlsruhe Institute of Technology (KIT), Cell and Neurobiology Biology, Haid-und-Neu-Strasse 9, 76131 Karlsruhe, Germany; 4) Institute for Photon Science and Synchrotron Radiation, Karlsruhe Institute of Technology (KIT), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany.

96

A new role of TGF-beta signaling in ciliogenesis. R. Earwood[1], J. Tözser[2], J. Brown[1], A. Kato[1], M. Blum[2], **Y. Kato**[1]. 1) Biomedical Sciences, Florida State University, Tallahassee, FL USA; 2) Institute of Zoology, University of Hohenheim, Stuttgart, Germany.

97

Developing methods to study live chromosome dynamics in *Xenopus* using the CRISPR/Cas system. Andrew Lane, Esther Kieserman, Rebecca Heald. Molecular And Cellular Biology, University of California, Berkeley, Berkeley, CA.

98

Cholesterol Selectively Activates Canonical Wnt Signaling over Non-Canonical Wnt Signaling during *Xenopus* development. Hyeyoon Lee[1], Hyunjoon Kim[1], Sheng Ren[2], Yao Xin[2], Yong Chen[2], Wen Tian[2], Yao Cui[2], Jong-Cheol Choi[3], Junsang Doh[3], Wonhwa Cho[2], Jin-Kwan Han[1]. 1) Life Sciences, POSTECH, Pohang, South Korea; 2) Dept of Chemistry, University of Illinois at Chicago, Chicago, IL, US; 3) Mechanical Engineering, POSTECH, Pohang, South Korea.

99

Polyglutamylation of Nucleosome Assembly Protein 1 modulates Histone H1 dynamics and chromosome condensation in *Xenopus* egg extracts. Kelly Miller, Rebecca Heald. UC Berkeley, Berkeley, CA.

100

ERK7, a novel regulator of ciliogenesis, is required for basal body migration. Koichi Miyatake, Morioh Kusakabe, Chika Takahashi, Eisuke Nishida. Department of Cell and Developmental Biology, Graduate School of Biostudies, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan.

101

FAK is required for the transduction of extracellular forces that orient the mitotic spindle and control tissue morphogenesis. Nicoletta Petridou, Paris Skourides. University of Cyprus, Nicosia, Cyprus.

102

Analysis of alternative splicing in the Xenopus SMRT gene. Colin Sharpe. EXRC, University of Portsmouth, Portsmouth, Hampshire, United Kingdom.

103

Septins control contractile force during epithelial wound closure. Asako Shindo[1], John Wallingford[1,2]. 1) Molecular Biosciences, University of Texas at Austin, Austin, TX; 2) Howard Hughes Medical Institute.

104

Understanding the interaction of Wnt ligands with their cell surface receptors. Kelsey F. Speer[1], Jeannine Mendrola[3], Peter Klein[1,2], Mark Lemmon[1,3]. 1) Graduate Group in Cell and Molecular Biology, University of Pennsylvania, Philadelphia, PA; 2) Department of Medicine, Raymond and Ruth Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 3) Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA.

See Page 5 for presentation schedule. Poster board number and presenter are in **bold**. Full abstracts begin on page 38.

105
The role of core spliceosomal components in spindle assembly. Magdalena Strzelecka, Andrew Grenfell, Rebecca Heald. Molecular & Cell Biology Department, UC Berkeley, Berkeley, CA.

106
A New Member of the Tubulin Superfamily Orients Cilia in Multiciliated Epithelial Cells. Erin Turk[1], Airon Wills[2], Stuart Howes[4], Eva Nogales[3,5,6], John Wallingford[1,2], Tim Stearns[1,7]. 1) Department of Biology, Stanford University; 2) Section of Molecular Cell and Developmental Biology, University of Texas at Austin; 3) Howard Hughes Medical Institute; 4) Biophysics Graduate Program, UC Berkeley; 5) Life Science Division, Lawrence Berkeley National Laboratory; 6) Dept of Molecular and Cell Biology, UC Berkeley, Berkeley, CA; 7) Dept of Genetics, Stanford School of Medicine.

107
***miR-34/449* miRNAs are required for motile ciliogenesis in vertebrate mucociliary epithelia by direct repression of *cp110* and regulation of basal body function. P. Walentek**[1,6], R. Song[2,6], N. Sponer[2,6], A. Klimke[3], JS Lee[2], G. Dixon[2], RM Harland[1], Y. Wan[4], P. Lishko[2], M. Lize[5], M. Kessel[3], L. He[2]. 1) Molecular & Cell Biology Department, Genetics Genomics and Development Division, University of California, Berkeley, Berkeley, CA; 2) Molecular & Cell Biology Department, Cellular and Developmental Biology Division, University of California, Berkeley, Berkeley, CA; 3) Department of Molecular Cell Biology, Max Planck Institute for Biophysical Chemistry, Goettingen 37077, Germany; 4) The Third Military Medical University, Chongqing 400038, China; 5) Department of Molecular Oncology, University of Goettingen, Goettingen 37073, Germany; 6) These authors contributed equally to this work.

Developmental Biology

108
A novel role for GSK3 and Polo-like kinase in promoting cranial neural crest cell migration and regulating ADAM13 function. Genevieve Abbruzzese[1], Hélène Cousin[1,2], Dominique Alfandari[1,2]. 1) Molecular and Cell Biology, University of Massachusetts, Amherst, MA; 2) Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA.

109
A conserved function for T-type calcium channels in anterior neural tube closure. Sarah Abdul-Wajid, Heidi D. Morales Diaz, William C. Smith. UCSB, Santa Barbara, CA.

110
Different requirements for GATA factors in cardiogenesis are mediated by non-canonical Wnt signaling. Boni A. Afouda, Stefan Hoppler. University of Aberdeen School of Medical Sciences Institute of Medical Sciences Foresterhill Health Campus Aberdeen, AB25 2ZD Scotland (UK).

111
High-resolution Analysis of Oocyte RNAs Reveals Gene Pathways Specific for the Vegetal Pole. Tristan Aguero[1], Dawn Owens[1], Derek Van Booven[2], Karen Newman[1], Mary Lou King[1]. 1) Depart. of Cell Biology, Univ of Miami, School of Medicine, 2) Hussman Institute for Human Genomics, University of Miami, School of Medicine.

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Identification and Expression Analysis of Two *Xenopus laevis* Homologs of the Novel F-Box Containing Protein, FBXO30. Osamah Badwan[1], Theodor Zbinden[1,2], Noelia Flores[1], Tamia Lozada[1], Dariana Núñez[1], Jesús Ayala[1], Josué Hernández[2], Grisselle Valentín[2], José García[2], Edwin Traverso[1]. 1) Biology, Univ of Puerto Rico at Humacao; 2) Biology, Univ of Puerto Rico-Río Piedras.

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***Xenopus* Egr4 is required to specify rhombomere 5 in the hindbrain. Chang-Joon Bae**, Jean-Pierre Saint-Jeannet. Basic Science and Craniofacial Biology, New York University College of Dentistry, New York, NY.

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Prdm12 specifies V1 interneurons downstream of Pax6 and through cross-repressive interactions with *Dbx1* and *Nkx6.1* in the developing vertebrate spinal cord. E. Bellefroid[1], A. Thelie[1], S. Desiderio[1], J. Hanotel[1], S. Kricha[1], G. Cerva[2], K. Lewis[2], C. Hui[3], M. Götz[4], M. Sander[5], Alexandra Pierani[6]. 1) University of Brussels, Gosselies, Belgium; 2) Syracuse Univ; 3) The Hospital for Sick Children, Toronto, Ontario, Canada; 4) Helmholtz Center Munich, Munich, Germany; 5) Depts of Pediatrics and Cellular and Molecular Medicine, Pediatric Diabetes Research Center, Univ of California, La Jolla; 6) Institut Jacques Monod, Université Paris Diderot, Paris, France.

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The Wnt co-receptor PTK7 is dynamically localized in neural crest cells and required for their migration. Annette Borchers[1], Hanna Berger[1], Ewa Maj[1], Martina Podleschny[1], Peter Wehner[2], Hanna Peradziryi[2]. 1) Department of Biology, Molecular Embryology, Philipps University Marburg, Karl-von-Frisch-Str. 8, 35032 Marburg, Germany; 2) Department of Developmental Biochemistry, Center for Molecular Physiology of the Brain (CMPB), GZMB, University of Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany.

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Characterising targets of FGF signalling in *Xenopus* neural development. Hannah Brunsdon, Harry V. Isaacs. Department of Biology, University of York, York, North Yorkshire, United Kingdom.

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A comparative study on the Krüppel-like factors during *Xenopus* embryogenesis. Yan Gao, Qing Cao, Lei Lu, Xuena Zhang, Zan Zhang, **Ying Cao**. Model Animal Research Center, Nanjing University, Nanjing, Jiangsu, China.

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Snail2/Slug cooperates with Polycomb Repressive Complex 2 (PRC2) to regulate neural crest development. Chih-Liang Tien[1], Amanda Jones[2], Hengbin Wang[2], **Chenbei Chang**[1]. 1) Cell, Developmental and Integrative Biology, Univ. Alabama at Birmingham, Birmingham, AL; 2) Department of Biochemistry and Molecular Genetics, Univ. Alabama at Birmingham, Birmingham, AL 35294.

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Control of neural crest migration and mouth formation by Kinin-Kallikrein signaling from the extreme anterior domain. Laura Jacox[1], Radek Sindelka[2], **Justin Chen**[1], Alyssa Rothman[1], Amanda Dickinson[3], Hazel Sive[1]. 1) Whitehead Institute, 9 Cambridge Center, Cambridge, 02142 MA; 2) Institute of Biotechnology, Videnska 1083, Prague, 14220, Czech Republic; 3) Virginia Commonwealth University.

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The transcriptional regulation of the BMP signaling pathway in the early *Xenopus tropicalis* embryos using genome-wide approaches. Jin Cho, Ira Blitz, Ken Cho. University of California Irvine, Irvine, ca.

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Calpain2: a novel regulator of Apical Constriction. Neophytos Christodoulou, Paris Skourides. University of Cyprus, Nicosia, Cyprus.

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Increasing the repertoire of asymmetrically distributed maternal mRNAs in *Xenopus tropicalis* through RNA-seq analysis of single blastomeres from the 8-cell embryo. Elena De Domenico, Nick D. L. Owens, Michael J. Gilchrist. MRC-National Institute for Medical Research The Ridgeway Mill Hill London, United Kingdom.

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TAF15 is required for proper dorso-ventral patterning in *Xenopus* through the repression of *ventx2*. Caitlin DeJong, Darwin Sorento Dichmann, Richard Harland. U.C. Berkeley, Berkeley, CA.

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Exposure to the Common Preservative Methylisothiazolinone (MIT) Inhibits Development and Blocks Both Regeneration and Wound Healing in *Xenopus laevis*. Nicole Delos Santos, Jessica Drenth, Ai-Sun Tseng. School of Life Sciences, University of Nevada, Las Vegas.

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The alternative spicing regulator Tra2b is required for somitogenesis and regulates a novel inhibitory *wnt11b* isoform. Darwin Dichmann, Peter Walentek, Richard Harland. Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA.

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The Heterotaxy Candidate Gene, Deadly Nightshade, is a novel regulator of Wnt signaling. Anna R. Duncan, John Griffin, Andrew Robson, Mustafa Khokha. Pediatrics, Yale School of Medicine, New Haven, CT.

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Computational Visualization of Cell Motility driving Neural Convergent Extension and Growth Cone Motility underlying Optic Axon Pathfinding in *Xenopus Laevis*.. Tamira Elul[1], Amar Bains[2], Avik Patel[1]. 1) Department of Basic Sciences, Touro University California, Vallejo, CA; 2) Department of Molecular and Cellular Biology, UC Berkeley, Berkeley, CA.

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Investigating the roles of the direct Wnt/β-catenin signaling targets *sall1* and *sall4* in neural tube development. Cameron R. T. Exner, Richard M. Harland. Dept. of Molecular & Cell Biology, University of California, Berkeley, Berkeley, CA.

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Role of thyroid hormone in neural stem and progenitor cells during Xenopus metamorphosis. Fernando Faunes[1,2], Renzo Bruno[1,2], Ivo Carrasco-Wong[1,2], Juan Larrain[1,2]. 1) P. Universidad Catolica de Chile, Santiago, Santiago, Chile; 2) Center for Aging and Regeneration, Millennium Nucleus in Regenerative Biology.

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A *Xenopus tropicalis* mutation in *rax*, a key gene in the eye formation regulatory network, reveals its role in spatially restricting alternative forebrain fates. Margaret Fish[1], Takuya Nakayama[1], Marilyn Fisher[1], Nicolas Hirsch[1], Amanda Cox[1], Rollin Reeder[1], Samantha Carruthers[2], Amanda Hall[2], Derek Stemple[2], Robert Grainger[1]. 1) Dept of Biology, UVA, Charlottesville, VA, USA; 2) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK.

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Function of Thyroid Hormone (TH)-Regulated HAL in Frog Intestinal Stem Cell Development. Liezhen Fu, Nga Luu, Yun-Bo Shi. LGRD/PCRM/NICHD, National Institutes of Health, Bethesda, MD.

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FGF/MAPK signaling is essential for Neural Crest formation in *Xenopus*.. Lauren Geary[1], Carole LaBonne[1,2]. 1) Molecular Biosciences, Northwestern University, Evanston, IL; 2) Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL.

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The Xenopus Orfeome. Ian Grant[1], Dawit Balcha[2], Tong Hao[2], Yun Shen[2], David Hill[2], Aaron Zorn[3], Todd Stukenberg[4]. 1) MRC Natl Institute for Medical Research, London, UK; 2) Center for Cancer Systems Biology (CCSB), Dana-Farber Cancer Institute, Boston, MA; 3) Cincinnati Children's Research Foundation, Cincinnati, OH; 4) Univ of Virginia School of Medicine, Charlottesville, VA.

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The distribution of Dishevelled in convergently extending mesoderm. Eleni Panousopoulou[1], Richard Tyson[1], Till Brettschneider[2], **Jeremy Green**[1]. 1) Dept of Craniofacial Development, King's College London, London, United Kingdom; 2) Warwick Systems Biology Centre, University of Warwick, UK.

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XUch37 regulates the formation of XTcf1/b-catenin complex for mediating Xenopus mesoderm patterning. Wonhee Han, Seungjoon Lee, Jin-Kwan Han. LIFE SCIENCES, POSTECH, POHANG, Gyeongbuk, South Korea.

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The Role of Reactive Oxygen Species (ROS) in Early Vertebrate Development. Y. Han[1], S. Ishibashi[1], Y. Chen[1,2], E. Amaya[1]. 1) The Healing Foundation Centre, Faculty of Life Sciences, University of Manchester, Manchester, M13 9PT, UK; 2) Wellcome Trust-Medical Research Council Stem Cell Institute, University of Cambridge.

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Comparative analysis of insulin-like growth factor binding proteins. Y. Haramoto[1], T. Oshima[1], S. Takahashi[2], M. Asashima[1], Y. Ito[1]. 1) AIST, Tsukuba, Ibaraki, Japan; 2) Hiroshima University, Higashi-Hiroshima, Hiroshima, Japan.

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Propagation based X-ray phase contrast in vivo microtomography: Gastrulation and neurulation in Xenopus laevis. Ralf Hofmann. Institute for Photon Science, KIT, Karlsruhe, Germany.

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Regulation of axis formation and microtubule dynamics during cortical rotation by vegetally-localized components in the Xenopus egg. Douglas Houston, Abby Matthews, Denise Oh, David Olson. The University of Iowa

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Expression cloning of camelid nanobodies against *Xenopus* gastrula antigens. Keiji Itoh, Sergei Sokol. Developmental and Regenerative Bilogy, Icahn School of Medicine at Mount Sinai, New York, NY.

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Active Repression by RARγ Signaling is Required for Vertebrate Axial Elongation. Amanda S. Janesick[1], Tuyen TL Nguyen[1], Ken-ichi Aisaki[2], Katsuhide Igarashi[2], Satoshi Kitajima[2], Roshantha AS Chandraratna[3], Jun Kanno[2], Bruce Blumberg[1]. 1) Developmental and Cell Biology, University of California, Irvine, Irvine, CA 92697-2300, USA; 2) Division of Cellular and Molecular Toxicology, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan; 3) IO Therapeutics, Santa Ana, CA 92705-5851, USA.

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Initiation of the Xenopus mid-blastula transition by histone titration. David Jukam[1], Amanda Amodeo[1], Aaron Straight[2], Jan Skotheim[1]. 1) Dept. of Biology, Stanford University, Stanford, CA; 2) Dept. of Biochemistry, Stanford Medical School, Stanford, CA.

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Identification of renal cell fate inducing transcription factors. Michael Kaminski, Hannes Engel, Florian Grahammer, Tobias Huber, Gerd Walz, Sebastian Arnold, Soeren Lienkamp. Department of Medicine, Renal Division, University of Freiburg Medical Center, Freiburg, Germany.

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Amphibian (*Euphlyctis cyanophlyctis*) *in vitro* ovarian follicle culture: A potential assay to assess impact of aquatic contaminants on female reproduction?. Pancharatna Katti, Basavaraj B. Goundadkar, Manjunath G. Ghodgeri. Dept of Studies in Zoology, Karnatak University, Dharwad, Karnataka, India.

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Identification of Cell Motility Genes *coronin1a* and *destrin* Specific to Migrating Primitive aVBI-Myeloid Lineage in *X. laevis* and Identification of the aVBI as an Essential Inducer of Foregut Endoderm Progenitors. Zachary Agricola, Amrita Jagpal, Andrew Allbee, Allison Prewitt, Scott Rankin, Emily Shifley, Aaron Zorn, **Alan Kenny**. Perinatal Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

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Determining the direct targets of Wnt/β-catenin signaling during neural plate patterning. Rachel Kjolby, John Young, Richard Harland. MCB, University of California, Berkeley, CA, CA.

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Ciliary proteins with unexpected roles in gene regulation. Jianli Shi, Ying Zhao, Domenico Galati, Janet Meehl, Robin Dowell, Tyson Vonderfecht, Mark Winey, **Michael Klymkowsky**. Molecular, Cellular & Developmental Biology, University of Colorado Boulder, Boulder, CO. 80309-0347.

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Tissue reconstruction of myocardium during *Xenopus laevis* metamorphosis. Manami Kobayashi, Misa Sugiura, Tsutomu Kinoshita. Rikkyo University, Toshima, Tokyo, Japan.

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beta-adrenergic signaling promotes posteriorization in *Xenopus* early development. Hiroki Kuroda[1], Yuka Sato[1], Shoko Mori[2]. 1) Faculty of Environment and Information Studies, Keio University, Fujisawa, Kanagawa, Japan; 2) Department of Education (Sciences), Shizuoka University 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan.

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Peroxiredoxin1 regulates pronephros development via modulating proximal tubule formation. Hyeon-Gyeong Lee, Inji Park, **Hyun-Shik Lee**. School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, Kyungpook National University, Daegu, South Korea.

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Localization of Oct25-expressing cells during development and regeneration of hindlimb phalanges in *Xenopus laevis*. Jaehoon Lee, Yui Shoji, Tsutomu Kinoshita. Rikkyo University, Toshimaku, Tokyo, Japan.

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Imparting regenerative capacity to limbs by progenitor cell transplantation. Gufa Lin, Ying Chen, Jonathan Slack. Stem Cell Institute, University of Minnesota, Minneapolis, MN.

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MiR-124 overexpression represses bipolar cell fate specification by targeting *Otx2* in *Xenopus* retina. Kaili Liu, Ying Liu, Mengru Sun, Xiumei Wang, Rongqiao He. Institute of Biophysics, CAS, Beijing, China.

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Regulation of *Eya1* expression in the preplacodal region of *Xenopus laevis*. Santosh Kumar Maharana, Gerhard Schlosser. Zoology, School of Natural Sciences & Regenerative Medicine Institute, National University of Ireland, Galway, Ireland,.

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Controlled levels of canonical Wnt signaling are required for neural crest migration. Ewa Maj[1], Lutz Künneke[2], Timo Aspelmeier[2], Annette Zippelius[2], Annette Borchers[1]. 1) Department of Molecular Embryology, University of Marburg, Marburg, Hessen; 2) Institute for Theoretical Physics, University of Göttingen, Göttingen, Niedersachsen.

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Pre-placodal ectoderm and neural crest patterning: requirement for histone modification by PRDM12 and Kdm4a in *Xenopus* embryos. Shinya Matsukawa[1], Kyoko Miwata[2], Makoto Asashima[2], Tatsuo Michiue[1]. 1) The University of Tokyo, Meguro-ku, Tokyo, Japan; 2) Research Center for Stem Cell Engineering National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba City, Ibaraki, Japan.

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Aquaporin-3b is required for tissue boundary formation and fibronectin assembly during gastrulation. Christa Merzdorf, Daniel Van Antwerp, Sean Lujan. Cell Biology and Neuroscience, Montana State University, Bozeman, MT.

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The role of *xRhoGEF3* in convergent extension movement of *Xenopus* embryo. Ikuko Seki[1], Saori Akiduki[2], Shuko Tokuda[1], Toshiyasu Goto[3], Shinya Matsukawa[1], Makoto Asashima[2], **Tatsuo Michiue**[1]. 1) Grad. School Arts Sci., the University of Tokyo, Tokyo, Japan; 2) Stem Cell Research Center, AIST, Tsukuba, Japan; 3) Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan.

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The development of TALEN methods to enhance the mutation efficiency and to perform genome editing preferentially in germ cells using *Xenopus*. Keisuke Nakajima, Yoshio Yaoita. Institute for Amphibian Biology, Higashihiroshima, Japan.

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The role of microRNA-206 in *Xenopus laevis* somite morphogenesis. Ceazar E. Nave, Julio R. Ramirez, Hernando Martinez-Vergara, Parag Saraf, Daniel Saw, Carmen Domingo. Biology, San Francisco State University, San Francisco, CA.

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Elongation movement of *Xenopus* posterior neural tissue is directed by anteroposterior tissue polarity. Hiromasa Ninomiya[1,2]. 1) AIST Tsukuba Central, Tsukuba, Ibaraki, Japan; 2) Graduate School of Arts and Sciences, the University of Tokyo, Meguro, Tokyo, Japan.

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Investigating the function of Wnt/β-catenin signaling in the rhombomeric boundaries. Rivka Noelanders[1], Hong Thi Tran[1], Sylvie Janssens[2], Kris Vleminckx[1]. 1) Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium; 2) Department of Developmental and Regenerative Biology, Mount Sinai School of Medicine, New York, United States.

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A thioredoxin fold protein Sh3bgr is necessary for embryonic muscle development. Dong Gil Jang, **Tae Joo Park**. Department of Biological Science, Ulsan National Institute of Science and Technology(UNIST), Ulsan, Korea.

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Multiple Lineage-Specific Roles of Syndecan 2 in Early *Xenopus* Development. Annita Peterson, H. Joseph Yost. Molecular Medicine, University of Utah, Salt Lake City, UT.

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Role of Myosin Regulatory Light Chain during Convergence and Extension. Katherine Pfister[1], Ray Keller[1,2], Skoglund Paul[2]. 1) Cell Biology, University of Virginia, Charlottesville, VA; 2) Biology, University of Virginia, Charlottesville, VA.

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Xenbase: the Xenopus model organism database. Virgilio Ponferrada[1], Joshua Fortriede[1], Christina James-Zorn[1], Kevin Burns[1], Kamran Karimi[2], J. Brad Karpinka[2], Jacqueline Lee[2], Yu Liu[2], Peter Vize[2], Aaron Zorn[1]. 1) Cincinnati Children's Hospital, Cincinnati, OH; 2) Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada.

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An epigenetic root of cilia formation - Suv4-20h enzymes control multiciliogenesis in skin and pronephros. Ralph Rupp[1], Ohnmar Hsam[1], Dario Nicetto[1,2]. 1) Molecular Biology, Adolf-Butenandt-Institut, LMU, München, Germany; 2) Department of Cell and Developmental Biology, Smilow Center for Translational Research, Philadelphia.

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Self-regulatory morphogenetic field in embryogenesis and organogenesis. Yoshiki Sasai, Hidehiko Inomata. RIKEN CDB, Kobe, Hyogo, Japan.

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SKL-tagging: a method for generating dominant-negative inhibitors of dimeric transcriptional factors such as Siamois and Vent. Y. Sato[1], S. Matsukawa[2], T. Furukawa[3], H. Kuroda[1]. 1) Keio University, Fujisawa, Kanagawa, Japan; 2) Tokyo University, Graduate School of Arts and Sciences, Bunkyo-ku, Tokyo, Japan; 3) Shizuoka University, GSTT, Shizuoka-city, Shizuoka, Japan.

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Stabilization of Spop and SpopL by Dzip1 is Essential for Gli Turnover and the Proper Output of Hedgehog Signaling. Tyler Schwend[1], Zhigang Jin[1], Kai Jiang[2], Brian Mitchell[3], Jianhang Jia[2], Jing Yang[1]. 1) Department of Comparative Biosciences, University of Illinois, Urbana, IL; 2) Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY; 3) Department of Cell and Molecular Biology, Northwestern University, Feinberg School of Medicine, Chicago, IL.

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B56α and B56γ regulatory subunits of protein phosphatase 2A play roles in both canonical and noncanonical Wnt pathways. Joni Seeling[1,2], Sungmin Baek[1], Lauren Sommer[2]. 1) Department of Biology, City University of New York, Queens College, Flushing, NY 11367; 2) Biological Sciences, Sam Houston State University

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Identification of microRNAs and mRNA targets in the specification of neural vs. epidermal fates.Identification of microRNAs and mRNA targets in the specification of neural vs. epidermal fates. Vrutant Shah[1], Ashley Benham[2], Benjamin Soibam[2], Jamina Oomen-Hajagos[3], Ruth A. Ritter[1], Gerald H. Thomsen[3], Amy K. Sater[1]. 1) Dept. of Biology and Biochemistry, University of Houston, Houston TX USA; 2) Stem Cell Engineering Dept., Texas Heart Institute at St. Luke's Episcopal Hospital, Houston TX USA; 3) Dept. of Biochemistry and Cell Biology, Stony Brook University, Stony Brook NY USA.

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Control of embryonic polarity and cell fates by the translational repressor Bicaudal-C. Sookhee Park[2], Megan Dowdle[2], Susanne Blaser[2], Douglas Houston[1], **Michael Sheets**[2]. 1) Department of Biology, University of Iowa; 2) Biomolecular Chemistry, Univ. of Wisconsin-Madison, Madison, WI.

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Epigenetic changes in gene regulation by thyroid hormone receptor during adult intestinal stem cell development. Yun-Bo Shi. NICHD, NIH, Bethesda, MD.

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Exogenously-added spermine and poly-L-lysine equally inhibit neural differentiation, especially eye formation, in marginal zone explants isolated from *Xenopus* late blastulae. Koichiro Shiokawa[1,2,3], Jun-Ichi Takai[1], Takeshi Kondo[1], Kazuei Igarashi[4]. 1) Department of Biosciences, Teikyo University, Utsunomiya 320-8551, Japan; 2) Graduate School of Judo Therapy, Teikyo University, Utsunomiya 320-8551, Japan; 3) Department of Judo Therapy, Fukuoka School of Health Sciences, Sawara-ku, Fukuoka 814-0005, Japan; 4) Graduate School of Pharmaceutical Sciences, Chiba University, Chiba 260-8675,Japan.

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Regulation of neurogenesis with Sox transcription and F-box mediated protein degradation. Elena Silva Casey, Niteace Whittington, Doreen Cunningham, Banu Saritas-Yildirim, Jing Jin. Biology, Georgetown University, Washington, DC.

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***Vestigial-like 4* is required for neural crest formation and neurogenesis in early *Xenopus* embryo. Emilie Simon**, Corinne Faucheux, Sandrine Fedou, Pierre Thiebaud, Nadine Theze. Bordeaux University, Bordeaux, France.

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Defining the mechanisms of transcriptional regulation by the T-box transcription factor Eomesodermin during early cardiac development. Christopher Slagle, Frank Conlon. Depts. of Biology and Genetics, McAllister Heart Institute, University of North Carolina - Chapel Hill, Chapel Hill, NC.

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A secreted integrin-beta like molecule, Itgbl-1 modulates the Integrin-ECM interactions for the cartilage formation. Eun kyung Song, Tae Joo Park. Department of Biological Science, Ulsan National Institute of Science and Technology(UNIST), Ulsan, Korea.

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A dominant-negative provides new insights into Focal Adhesion Kinase (FAK) regulation and function in early embryonic morphogenesis. Panayiota Stylianou, Nicoletta Petridou, Paris Skourides. Biological Sciences, University of Cyprus, Nicosia, Cyprus.

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SCP3 regulates cell competence to Nodal/Activin and BMP signals via dephosphorylating linker sites of Smad2 and Smad1 in *Xenopus* embryos. Guanni Sun[1], Zheying Min[1], Zhirui Hu[2], Zhenpo Guan[1], Hanxia Su[1], Xiaopeng Ma[1], Michael Zhang[2,3], Qinghua Tao[1], Wei Wu[1]. 1) School of Life Sciences, Tsinghua Univ, Beijing, China; 2) Bioinformatics Division, Center for Synthetic and Systems Biology, Tsinghua Natl Laboratory for Information Science and Technology, China; 3) Dept of Molecular and Cell Biology, Center for Systems Biology, The Univ of Texas at Dallas.

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Establishment of vertebrate body plan via coordinated regulation of dorsal-ventral and anterior-posterior patterning during early *Xenopus* embryogenesis. Kimiko Takebayashi-Suzuki, Hidenori Konishi, Hitoshi Yoshida, Maya Okada, **Atsushi Suzuki**. Institute for Amphibian Biology, Hiroshima University

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Genome editing reveals a novel function of keratin in fin formation in *X. laevis*. Ken-ichi T. Suzuki[1], Keiko Kashiwagi[1], Tetsushi Sakuma[1], Akihiko Kashiwagi[1], Makoto Mochii[2], Takashi Yamamoto[1]. 1) Graduate School of Science, Hiroshima University, Higashihiroshima,Hiroshima, Japan; 2) Graduate School of Life Science, University of Hyogo, Aioi, Hyogo, Japan.

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Dynamic intracellular localization of Xdazl protein during the *Xenopus* germline development. Haru Tada, Hidefumi Orii. Graduate School of Life Science, University of Hyogo, Japan.

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***Xenopus mab21-l3* is required for cell fate specification of multiciliate cells and ionocytes. Chika Takahashi**[1], Morioh Kusakabe[1,2], Toshiyasu Suzuki[1], Koichi Miyatake[1], Eisuke Nishida[1,2]. 1) Department of Cell and Developmental Biology, Graduate School of Biostudies, Kyoto University, Sakyo-ku, Kyoto, 606-8502, Japan; 2) JST, CREST, Tokyo, Japan.

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Ascl1 regulates mesendoderm through antagonizing VegT and activating Delta/Notch in the early Xenopus embryos. Qinghua Tao[1,2], Li Gao[1,2], Xuechen Zhu[1,2], Hao Lin[1,2]. 1) Tsinghua University School of Life Sciences, Beijinh, China; 2) 1 Qinghuayuan, Haidian District.

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Gtpbp2: a unique GTPase functioning in BMP and Wnt signaling in Xenopus embryos. William Gillis[1,2], Arif Kirmizitas[1,2], Yasuno Iwasaki[1], Dong-Hyuk Ki[1], Jonathan Wyrick[1], **Gerald Thomsen**[1]. 1) Biochemistry & Cell Biology, Center for Developmental Genetics, Stony Brook University, Stony Brook, NY, USA; 2) equal contributors.

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Ric-8A/Gα13 signaling pathway is required to proper cranial neural crest migration in Xenopus. Gabriela Toro-Tapia[1], Marion Rodriguez[1], Sylvain Marcellini[2], Roberto Mayor[3], Marcela Torrejon[1]. 1) Biochemistry and Molecular Biology, University of Concepcion, Concepcion, Chile; 2) Cell and Developmental Biology, University College London, UK; 3) Cell Biology, University of Concepcion, Concepcion, Chile.

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Ric-8A is required for neural crest cells induction. Marion Rodriguez, Gabriela Toro-Tapia, Maria Hinrichs, **Marcela Torrejon**. Biochemistry and Molecular Biology, University of Concepcion,

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Sodium Currents Initiate Tail and Limb Regeneration. Ai-Sun Tseng[1], Michael Levin[2]. 1) School of Life Sciences, University of Nevada, Las Vegas; 2) Center for Regenerative and Developmental Biology, Tufts University, MA.

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Systematic analysis of *RFX2* targets in *Xenopus* multiciliated cells. Fan Tu, Mei-I Chung, Taejoon Kwon, Edward Marcotte, John Wallingford. MBS, University of Texas at Austin,

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Repression of Both BMP and Activin Signaling is Required for Retina Formation. Andrea S. Viczian[1], Michael Trembley[2], Kimberly Wong[1]. 1) Ophthalmology, Upstate Medical University, Syracuse, NY; 2) Cellular and Molecular Pharmacology and Physiology, Univ of Rochester,

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Xenbase 2014. Peter Vize[1], Aaron Zorn[2], Kamran Karimi[1], Kevin Burns[2], Joshua Fortriede[2], Christina James-Zorn[2], Brad Karpinka[1], Jacqueline Lee[1], Yu Liu[1], Virgilio Ponferrada[2], Erik Segerdell[2]. 1) Biological Sciences, University of Calgary, Alberta, Calgary, Alberta, Canada; 2) Cincinnati Children's Research Foundation, College of Medicine.

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MicroRNAs in Neural Crest development. Nicole Ward, Ayisha Ahmed, Dario Dotlic, Grant Wheeler. School of Biological sciences, University of East Anglia, Norwich Research Park, NR4 7TJ.

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Unliganded thyroid hormone receptor α controls developmental timing in *Xenopus tropicalis*. Luan Wen, Yun-Bo Shi. Laboratory of Gene Regulation and Development, National Institutes of Health (NIH/NICHD), Bethesda, MD.

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Regulation of Dead End1 (Dnd1) protein turnover and primordial germ cell development by autophagy. Jing Yang[1], Zhigang Jing[1], Wenyan Mei[1], Mary Lou King[2]. 1) University of Illinois at Urbana-Champaign; 2) University of Miami.

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***fezf2* promotes neuronal differentiation through localised activation of Wnt/β-catenin signalling during forebrain development. Siwei Zhang**[1], Jingjing Li[1], Kris Vleminckx[2], Enrique Amaya[1]. 1) The Healing Foundation Centre, Faculty of Life Sciences, University of Manchester, M13 9PT, UK; 2) Department for Biomedical Molecular Biology, Ghent University, Ghent, Belgium.

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Syndecan4 in foregut organ development. Zheng Zhang, Scott Rankin, Aaron Zorn. Perinatal Institute, Division of Developmental Biology, Cincinnati Children's Hospital Medical Center and the College of Medicine, University of Cincinnati, Cincinnati OH

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Heat shock 70kDa protein 5 (Hspa5) is essential for pronephros formation by mediating retinoic acid signaling. Weili Shi[1], Gang Xu[2], Chengdong Wang[1], Yi Deng[3], **Hui Zhao**[1]. 1) School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, P. R. China; 2) School of Chinese Medicine, Hong Kong Baptist University, 7 Baptist University Road, Kowloon Tong, Hong Kong SAR, P. R. China; 3) South University of Science and Technology of China, Shenzhen, 518055, P. R. China.

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Evolution of Maturin suggests a role for ubiquitination during primary neurogenesis. Michael E. Zuber[1], Reyna I. Martinez-De Luna[1], Robert M. Freeman, Jr.[2]. 1) Center for Vision Research, SUNY Eye Institute, Department of Ophthalmology, SUNY Upstate Medical University, Syracuse, NY; 2) Department of Systems Biology, Harvard Medical School, Boston, MA.

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Neurogenin3-directed differentiation of endoderm to insulin-expressing cells. **Matthew Salanga**[1], Leonid Peshkin[2], Marko Horb[1,3]. 1) Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA; 2) Department of Systems Biology, Harvard Medical School, Boston, MA; 3) National Xenopus Resource, Marine Biological Laboratory, Woods Hole, MA

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Deep Proteomics of the *Xenopus Laevis* Egg using an mRNA-derived Reference Database. Marc Presler[1], Martin Wühr[1,3], Robert M. Freeman Jr.[1], Marko E. Horb[2], Leonid Peshkin[1], Steven Gygi[3], Marc W. Kirschner[1]. 1) Department of Systems Biology, Harvard Medical School, Boston, MA; 2) Marine Biological Laboratory, Woods Hole, MA; 3) Department of Cell Biology, Harvard Medical School, Boston, MA.

Session 1

2
Polarized Wnt signaling regulates ectodermal cell fate in Xenopus. Ya-Lin Huang[2], **Christof Niehrs[1,2]**. 1) IMB Inst of Molecular Biology, 55128 Mainz, Germany; 2) Div of Molecular Embryology, DKFZ-ZMBH Alliance, Deutsches Krebsforschungszentrum, 69120 Heidelberg, Germany.
How cells convert polarity cues into cell fate specification is incompletely understood. We find that Wnt/b-catenin and Wnt/PCP signaling cooperate in this process in early Xenopus embryos. The Wnt co-receptor Lrp6 is asymmetrically localized to the basolateral membrane in ectodermal blastomeres. Lrp6 asymmetry is controlled by Wnt/PCP signaling, indicating that this pathway not only regulates planar- but also apicobasal cell polarity. Following asymmetric cell division Lrp6 preferentially sorts to the deep ectodermal cell layer and becomes depleted in the epithelial cell layer. This is accompanied by elevated Wnt/b-catenin signaling in deep cells, which in turn promotes their differentiation into ciliated cells. We conclude that coordinated Wnt/PCP and Wnt/b-catenin signaling convert apicobasal polarity information to specify ectodermal cell fate.

3
Trigger Waves in Mitosis and Apoptosis. James Ferrell, Jeremy Chang. Stanford University.
Xenopus laevis eggs are so large that even a freely diffusing protein would take hours to make it from the center of the egg to the cortex. Despite this, mitosis takes place quickly and in a spatially coordinated fashion in fertilized Xenopus eggs. Because there is bistability in the activation of Cdk1 at the onset of mitosis, the egg has the possibility of supporting trigger waves of Cdk1 activation the spread over large distances faster than diffusion alone would allow. We carried out experiments to look for these trigger waves, using cell free Xenopus egg extracts in thin Teflon tubes and a fluorescence microscopy assay for mitosis. We found that Cdk1 activation does, as hypothesized, spread linearly through these extracts at a constant speed of ~1 μm/sec, allowing Cdk1 activity to spread from the center to the cortex of an egg in about 10 min. We suspect that trigger waves may be found in other signaling systems where events need to be coordinated over long distances, and we will present some new studies on how apoptosis spreads through Xenopus egg extracts.

4
Dead-End1 is Required and Sufficient to Activate *nanos* Translation in the Xenopus Germline. Mary Lou King[1], Tristan Aguero[1], Jing Yang[2]. 1) Univ of Miami School of Medicine, Miami, FL 33136; 2) Univ of Illinois.
The translational repressor Nanos plays an essential role in preserving the germline by preventing determinants of somatic fates from being expressed there (Lai et al., 2012). Xenopus *nanos* RNA translation is tightly regulated. Ectopic expression in the oocyte leads to abnormal development; failure to express Nanos after fertilization results in the loss of the germline. Understanding the mechanism(s) that regulates the correct activation of *nanos* translation is critically important to both the preservation of the germline and somatic cell fates. We have previously shown that *nanos* RNA contains a large secondary structure within its ORF that is required and sufficient to structurally block ribosome entry and repress translation (Luo et al., 2011). We identified the RNA binding protein Dead-end in an oocyte assay for components that relieved repression of injected *nanos* RNA. Further, Dnd was both required and sufficient to activate *nanos* translation in a plant based in vitro translation system arguing against any germline specific co-factor. In embryos resulting from oocytes depleted of Dnd, Nanos protein was not detected in the germ plasm but nanos RNA levels were unaffected indicating Dnd functions at the level of translation. Re-introducing wild-type Dnd1 rescued Nanos expression while a mutation in the Dnd RNA recognition motif(s) (RRM) did not. Dnd can bind directly to *nanos* RNA; binding requires the RRM. We performed a deletion analysis of both *nanos* RNA and Dnd protein to determine what domains were required for *nanos* activation. Surprisingly, the *nanos* 3'UTR was not essential for Dnd activation, but a region including the secondary structure, is required. Xenopus Dnd, like zebrafish, has ATPase activity. Partial deletions of the putative ATPase site greatly reduced *nanos* translation. Taken together, our results show that Dnd is both required and sufficient to activate germline *nanos* translation. Further, that Dnd may function as a novel helicase capable of altering RNA secondary structure. Our findings indicate a new function for Dnd in translational activation besides its known roles in RNA stability (Kedde et al., 2007) and RNA localization (Mei et al., 2013). NIH GM102397 to MLK

5 – **Genome assemblies and the origin of tetraploidy. Don Rokhsar**. University of California, Berkeley. No Abstract

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Oct4 duplication and the delination of distinct conserved mechanisms of pluripotency. Joshua Brickman[1], Alessandra Livigni[2], Hanna Peradziry[1], Woranop Sukparangsi[1]. 1) DanStem - The Novo Nordisk Foundation Section for Basic Stem Cell Research, University of Copenhagen, Copenhagen, Copenhagen N, Denmark; 2) MRC Centre for Regenerative Medicine University of Edinburgh.
The class V POU domain transcription factor Oct4 (Pou5f1) is a pivotal regulator of embryonic stem cell (ESC) self-renewal

and reprogramming of somatic cells to induced pluripotent stem (iPS) cells. Oct4 is also an important evolutionarily conserved regulator of progenitor cell differentiation during embryonic development. We examined the function of Oct4 homologs in Xenopus embryos and compare this to the role of Oct4 in maintaining and inducing pluripotency. Based on a combination of expression profiling of Oct4/POUV- depleted Xenopus embryos and in silico analysis of existing mammalian Oct4 target data sets, we defined a set of evolutionary-conserved Oct4/POUV targets. Most of these targets were regulators of cell adhesion. This was consistent with Oct4/POUV phenotypes observed in the adherens junctions in Xenopus ectoderm, mouse embryonic, and epiblast stem cells. As there are three Oct4 homologs in Xenopus we also explored the diversificiation of the function. We found that the adhesion activity correlates with the homologue expressed at highest levels in epiblast/ectoderm and maybe key to supporting primed pluripotency. However, the capacity to induce naive pluripotency in mammallian cells was linked to expression in the primordial germ cells. Taken together, our findings suggest that gene duplication in Xenopus enables the untangling of specific moleculear pathways regulating stem cell potency in mammals.

7

Role and Mechanism of Branching Microtubule Nucleation. Sabine Petry. Molecular Biology, Princeton University, Princeton, NJ.

The microtubule (MT) cytoskeleton is essential for giving cells their shape, positioning organelles, serving as tracks that move materials, generating force for movement, and making mitotic and meiotic spindles to segregate chromosomes during cell division. Each function relies on a specific MT architecture, e.g. long and stable MTs in an axon or short and dynamic MTs arranged in a spindle. The MT cytoskeleton originates from several MT nucleation sites, a process which, despite its central biological importance, is poorly understood. I am using the mitotic spindle in Xenopus egg extracts as a MT model structure to research how MT nucleation is locally activated to build the MT cytoskeleton. The presentation will cover a new mechanism, by which microtubules are nucleated within the mitotic spindle. This mechanism plays a vital role in the self-organization of MTs into a bipolar structure, which is essential to ensure that the segregation of genetic material into daughter cells is as reliable as possible.

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Combining *Xenopus* Embryology with Human Genetics – The Case of Anks6, a Novel Nephronothisis Gene. Sylvia Hoff[1], Jan Halbritter[2], Ronald Roepman[4], Sophie Saunier[5], Gerd Walz[1], Friedhelm Hildebrandt[2], Carsten Bergmann[3], **Soeren Lienkamp[1]**. 1) Department of Nephrology, University Hospital Freiburg, Freiburg, Germany; 2) Department of Medicine, Boston Children's Hospital, Harvard Medical School, Boston, USA; 3) Center for Human Genetics, Bioscientia, Ingelheim, Germany; 4) Department of Human Genetics, Nijmegen Centre for Molecular Life Sciences and Institute for Genetic and Metabolic Disease, Radboud University Medical Centre, 6525 GA, Nijmegen, The Netherlands; 5) Inserm U983, Hôpital Necker-Enfants Malades and Paris Descartes- Sorbonne Paris Cité University, Imagine Institute, Paris, France.

Cystic kidney disease leads to severe impairment in renal function. Affected patients frequently rely on dialysis or renal transplant. One rare genetic childhood form of cystic kidney disease is nephronophthisis (NPHP). There is no causal treatment, and despite the identification of 17 disease causing genes (NPHPs), the molecular pathogenesis is unclear.We recently analyzed the function of some known NPHP proteins in *Xenopus*. NPHPs are essential for renal tubule morphogenesis, affecting distinct tubular segments and knockdown results in impaired excretory ability of the embryonic kidney.We recently identified a novel candidate NPHP (Anks6) in a biochemical interaction screen. Anks6 knockdown replicated the phenotype of known NPHPs extremely well and prompted the successful search for mutations in a patient cohort with nephronophthisis. Thus, disease modelling in Xenopus can serve as an important link between basic science and clinical genetics.Because the 17 known genes only account for only 40% of disease causing mutations in nephronophthisis patients, we will continue to use the combination of phenotypic analysis in *Xenopus*, biochemistry, and human genetics in the search for the more elusive NPHP genes.

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Spinal circuit remodeling during developmental transitions in motor behavior. Lora B. Sweeney[1], Jay B. Bikoff[2], Susan B. Morton[2], Thomas M. Jessell[2], Christopher Kintner[1]. 1) Molecular Neurobioloy Laboratory, Salk Institute for Biological Studies, La Jolla, CA; 2) Howard Hughes Medical Institute, Kavli Institute for Brain Science, Mortimer B. Zuckerman Mind Brain Behavior Institute, Departments of Neuroscience and Biochemistry and Molecular Biophysics, Columbia University, New York, NY.

The frog *Xenopus laevis* undergoes a major transition in motor behavior from swimming to walking during metamorphosis. A spike in thyroid hormone triggers a burst in neurogenesis, differentiation, and ultimately, a massive remodeling of the motor circuit. The specific changes to the motor circuit during metamorphosis—for example, how proliferation and differentiation are regulated at these late stages, how motor and interneuron cell populations change, or whether the larval swim circuit is rewired or lost—are largely unknown. Using molecular markers, we have found that motor neuron (MN) and interneuron (IN) subtypes are added, specified and diversified in the metamorphosing spinal cord. We describe three distinct motor circuit stages of developing tadpoles: (1) the larval swim circuit that controls escape swimming (stage 35-42), (2) an expanded and diversified thoracic circuit at the time of free-feeding (stage 42-50), and (3) a limb circuit during metamorphosis (stage 50-54). The results from these studies will inform us about how circuits in the vertebrate spinal cord evolved to mediate limb locomotion, identify neuronal classes uniquely associated with limb locomotion, and provide the tools to probe the function of individual classes of spinal neurons during the unique locomotor behavioral transitions of the metamorphing frog.

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Understanding regeneration from the inside out: chromatin and transcription landscapes in the regenerating tadpole tail. Andrea E. Wills, Jessica Chang, Rakhi Gupta, Julie Baker. Genetics, Stanford School of Medicine, Stanford, CA.

The *Xenopus tropicalis* tail represents an excellent model for molecular and genomic investigations of regeneration. Following amputation, tail regeneration progresses rapidly through stereotyped stages of inflammation, wound healing, blastema formation, proliferation, and new cell fate commitment. By 60 hours after amputation, the elongating tail bud contains new muscle, spinal cord, nerves, vasculature, and polarity along all three axes. The large clutch size and tractable genome of *X. tropicalis* make genomics approaches to regeneration in this organism uniquely feasible. We are interested in defining the changes in chromatin landscape and transcriptome that occur during regeneration, and especially whether the molecular mechanisms that underlie differentiation and patterning during regeneration are the same as those that occur during embryogenesis. To characterize the events of regeneration, we have first used RNA-Seq to generate a transcriptome of the regenerating tail at several time points. We have also used FACS to isolate neural cells so that we can define the transcriptome of a single regenerating lineage. These datasets form a framework for understanding gene expression during regeneration, but ultimately, we are most interested in understanding how chromatin from the differentiated tail is remodeled to allow new gene expression during regeneration. Does a stem cell-like signature arise? Do different cell cell types undergo the same changes in chromatin landscape during differentiation, or is each lineage unique? To approach these questions, we have employed ATAC-Seq, which identifies regions of open chromatin. This technique is tractable with small amounts of cells, allowing us to profile open chromatin with fine resolution over time, and in specific cell types. We are using ATAC-Seq in concert with ChIP-Seq of histone marks to identify the chromatin remodeling events that occur during regeneration, and to clarify whether regenerating cells re-establish a chromatin signature like that of the early embryo, or progress through a unique path as they differentiate.

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Functional anatomy of a transcription factor: learning how GATA4 induces cardiogenesis. Branko Latinkic[1], Wensheng Deng[1], Joseph Gallagher[1], Simon Fellgett[1], Abir Yamak[2], Mona Nemer[2]. 1) Cardiff University, Cardiff, Wales, United Kingdom; 2) Laboratory of Cardiac Development and Differentiation, Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, Ontario, Canada.

GATA4-6 transcription factors regulate numerous aspects of development and homeostasis in multiple tissues of mesodermal and endodermal origin. In the heart, the best studied of these factors, GATA4, has multiple distinct roles, but the mechanisms and cofactors required for its diverse functions are not fully understood. We have previously shown that cardiogenic activity of GATA4 requires a discrete N-terminal region which is needed for transcriptional synergy and physical interaction with BAF60c, a component of SWI/SNF chromatin remodelling complex. This region of GATA4 also mediates its synergy with Cyclin D2. More recently we have shown that the carboxy terminal region comprised of amino acids 362-400 is essential for mediating cardiogenesis. The same domain interacts with CDK4 and is required for transcriptional synergy between GATA4 and CDK4. The existence of separable N- and C-terminal cardiogenic domains suggests that the activity of GATA4 is encoded in a complex manner. Indeed, we have identified additional determinants of cardiogenesis in the central Zn finger domain of GATA4. Several amino acids in this region of GATA4 are both necessary for cardiogenesis, as single amino acid mutations abolish cardiogenic activity of GATA4 without affecting its biochemical properties (DNA binding, nuclear localisation, stability). These residues are also sufficient for cardiogenesis, as they can endow non-cardiogenic GATA1 with GATA4-like activity. Potential mechanisms of action of cardiogenic determinants used by GATA4 will be discussed.

Session 2

11

Non-canonical Wnt signaling mediates morphogenesis of the extreme anterior domain during mouth formation. Hazel Sive[1], Laura Jacox[1,2], Radek Sindelka[1,3], Alyssa Rothman[1]. 1) Whitehead Institute and MIT, Cambridge , MA; 2) Harvard School of Dental Medicine, Boston MA; 3) Institute of Biotechnology Videnska, Prague, Czech Republic.

The Extreme Anterior Domain (EAD) is a craniofacial signaling center we described in *Xenopus* that comprises ectoderm and endoderm (Dickinson and Sive, 2009). EAD ectoderm arises medially from the anterior neural ridge prior to cranial neural crest (CNC) migration. The region contributes to the mouth opening and lining, the nostrils and the anterior pituitary. By tailbud stages EAD ectoderm has formed a wide mass of epithelial cells, however as the CNC migrates to lie adjacent to this region, the EAD becomes longer and narrower, indicating convergent extension. Subsequently, the narrow band of EAD opens along the apical midline to form the initial mouth opening. We hypothesized that morphogenesis of the EAD depends on non-canonical Wnt signaling. In support of this, loss of function in genes encoding the ligand Wnt11, the receptor Fzl7, and the signaling connector Dvl result in failure of EAD extension and aberrant mouth development. Face transplant assays demonstrate a local requirement in the EAD for *fzl7, dvl* and the effector Jnk during EAD extension. We therefore hypothesize that Wnt11 is secreted by the incoming CNC and acts on EAD cells to direct their reorganization. Our study is the first in any system to describe and probe the mechanisms underlying EAD morphogenesis during craniofacial development. Supported by a grant from the NIDCR 1RO1 DE021109.

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The calcium-dependent ribonuclease XendoU promotes ER network formation through local RNA degradation. Mike Blower[1,2], Dianne Schwarz[1,2]. 1) Molecular Biology, Massachusetts General Hosptial, Boston, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA.

One of the most dramatic intracellular reorganizations in higher eukaryotes is triggered by an increase in cytosolic calcium at fertilization leading to large-scale changes in gene expression and organelle structure. Calcium signaling directly triggers the transition from metaphase to anaphase at fertilization through activation of CAMKII, but it is unknown if intracellular calcium directly regulates additional processes at fertilization. Using *Xenopus laevis* egg extract, we found that increases in cytosolic calcium lead to the activation of an endogenous ribonuclease that we purified and identified as XendoU. Interestingly, a fraction of XendoU localizes to the Endoplasmic Reticulum (ER), which is the main intracellular store of calcium. We used immunodepletion and rescue experiments to show that XendoU is required for nuclear envelope assembly and ER network formation in a catalysis-dependent manner. Using a purified vesicle fusion assay in the absence of cytosol we show that XendoU functions on the surface of ER membranes to promote RNA cleavage and ejection from the surface of the ER. Interestingly, we find that removal of RNA from the surface of vesicles by RNase treatment leads to increased ER network formation, demonstrating that RNA removal from the ER promotes ER network formation and remodeling. In addition, we found that human EndoU localizes to the ER where it promotes the formation of ER tubules in a catalysis-dependent manner. Recent studies have identified several proteins that influence the structure and remodeling of the ER, but it was unknown if regulation of the RNA content of the ER influences ER structure. Our work directly demonstrates that removal of RNA from the ER promotes ER remodeling and the formation of tubular ER. These results demonstrate that XendoU is an important calcium-activated factor that promotes ER remodeling during the cell cycle and is likely to play a key role in shaping the ER in other cell types.

13

Radial Intercalation is regulated by the Par complex and the microtubule-stabilizing protein CLAMP/Spef1. Michael Werner, Jen Mitchell, **Brian Mitchell**. Cell and Molecular Biology, Northwestern University, Chicago, IL.

The directed movement of cells is critical for numerous developmental and disease processes. A developmentally reiterated form of migration is radial intercalation; the process by which cells move in a direction orthogonal to the plane of the tissue from an inner layer to an outer layer. We use the radial intercalation of cells into the skin of *Xenopus* embryos as a model to study directed cell migration within an epithelial tissue. We identify a novel function for both the microtubule binding protein CLAMP and members of the microtubule-regulating Par complex during intercalation. Specifically, we show that Par3 and aPKC promote the apical positioning of centrioles, whereas CLAMP stabilizes microtubules along the axis of migration. We propose a model in which the Par complex defines the orientation of apical migration during intercalation and in which sub-cellular localization of CLAMP promotes the establishment of an axis of microtubule stability required for the active migration of cells into the outer epithelium.

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Age-Defying Proline, Because It's Worth It. Nathalie ESCANDE-BEILLARD[1], Mohammad SHBOUL[1], Amira MASRI[2], **Bruno REVERSADE[1]**. 1) Institute of Medical Biology, www.reversade.com, A*STAR, Singapore; 2) Department of Pediatrics, University of Jordan, Amman, Jordan.

In mice, *TAP63* is required for adult stem cell renewal and prevents premature ageing . Here, we show that in humans and frogs, proline metabolism and its rate-limiting enzyme PYCR1 acts upstream of TAP63.The loss of PYCR1 in humans causes De Barsy syndrome, a congenital progeroid disease which can be modelled in *Xenopus*. In both systems, PYCR1 and its dedicated antagonist PRODH, control the proliferative potential of skin lineages. Using mutant PYCR1 patients' primary cells, we establish that TAP63 translocates to the mitochondria in conditions of stress, where it binds to PYCR1. This interaction prevents degradation of TAP63 protein to sustain the growth of skin progenitors.Our results reveal an unexpected and conserved role for proline metabolism whereby PRODH ⊣ PYCR1 → TAP63 → cellular proliferation to control skin development and prevent premature ageing in humans.

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Neural Crest Migration and Neuronal Differentiation is Mediated by *tfap2e* in Xenopus. Sofia Medina Ruiz[1], Anastasia Lobanova[1], Anne-Helene Monsoro-Burq[2], Michael Eisen[1], Richard Harland[1]. 1) Department of Molecular and Cell Biology, University of California, Berkeley, California, United States of America; 2) CNRS-Institut Curie. Orsay, France.

The neural crest is a transient and multipotent cell population in vertebrates. Many transcriptional regulators required for the specification, migration and differentiation of these cells have been identified. However, the specificity of these transcription factors in regulating down stream effectors is not well understood. In an RNA-seq screen we identified the *tfap2e* transcription factor as a gene specifically expressed in the neural crest and later determined, though gain and loss of function, that it is essential for mediating cranial neural crest migration and trunk neural crest specification.During early neurula stages *tfap2e,* together with other *tfap2* family members, collectively specify the neural crest. But during mid to late neurula stages, *tfap2e* becomes critical for both cranial and trunk neural crest migration and differentiation. In the cranial region, it is required for the formation of the neural crest streams and migration of individual neural crest cell towards the dorsal midline. In the trunk region, it promotes the specification of Rohon-Beard sensory neurons and pronephros. Chromatin immuno-precipitation led us to identify genomic regions in the vicinities of the genes, whose expression is downregulated in *tfap2e* knockdowns, which may respond to TFAP2

transcription factors in the neural crest. The current study demonstrates that *tfap2e* directly regulates expression of neural crest, sensory neuron specifier genes.

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Parent-of-origin effects in Xenopus laevis gene expression. Leonid Peshkin[1], Virginia Savova[2], Anwesha Nag[2], Esther Pearl[3], Marko Horb[3], Marc Kirschner[1], Alexander Gimelbrant[2]. 1) Systems Biology, Harvard Medical School, Boston, MA; 2) Dana Farber Cancer Institute, Boston, MA; 3) Marine Biological Laboratory, Woods Hole, MA.

Parent-of-origin effects are epigenetic phenomena that appear as phenotypic differences between heterozygotes depending on the allelic parent of origin. The primary mechanism that can lead to the parent-of-origin effects is genomic imprinting. Imprinted genes play an important part in a number of complex traits, notably in early development.Though the taxonomic distribution of gene imprinting remains uncertain, a widely-accepted view holds that among animals it is limited to mammals. There are significant technical challenges in assessment of imprinting in a wider variety of species. The most direct way to identify imprinting involves generation of reciprocal crosses between inbred lines with significant nucleotide-level differences. Transcriptome-wide surveys of allele-specific expression have only recently become feasible, and the analytical and statistical tools for analysis of such data are still in their infancy.Here we report the results of two rounds of deep RNA sequencing experiments on tadpoles from mixed crosses between inbred lines. Our identification of SNPs and quantitative analysis strongly suggest that there are genes in X.laevis that exhibit parent-of-origin bias in expression.Identification of parent-of-origin effect in an amphibian would represent a significant conceptual advance in understanding the evolutionary origins of that gene regulatory mechanism and its role in development. Moreover, Xenopus is the only vertebrate model system that allows for high-throughput in vivo analyses of gene function and biochemistry. Xenopus as a model for the study of imprinting would allow for the development of screens to identify new targets important for disease and for the design of new therapies involving imprinted genes.

Session 3

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Regulation of cell division orientation during epithelial morphogenesis. Sarah Woolner[1], Georgina Goddard[1], Alexander Nestor-Bergmann[1], Oliver Jensen[2]. 1) Faculty of Life Sciences, The University of Manchester, Manchester, United Kingdom; 2) School of Mathematics, The University of Manchester, Manchester, United Kingdom.

The orientation of cell division, determined by the mitotic spindle, must be carefully controlled in epithelia in order to generate shape, maintain tissue organisation and regulate cell fate. Defective spindle orientation leads to failures in organogenesis and morphogenesis and has been associated with cancer. Using *Xenopus* as our model system, we are investigating the intra- and extracellular forces that position the mitotic spindle in epithelia during embryonic development. We have found that antagonistic microtubule and actomyosin forces function to hold the spindle in place during symmetric divisions. We speculate that this mechanism provides more flexibility than a model based on tethering the spindle to a specific cortical landmark, allowing spindle orientation to be quickly adapted to changes in the tissue environment. We are now investigating how these intracellular mechanisms are linked to the external environment of the tissue. In particular, we are testing how tissue tension provides a cue for spindle orientation.

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Novel Regulations in Early Neural and Neural Crest Development. Anne Monsoro-Burg, C Pegoraro, A Figueiredo, F Maczkowiak, J-L Plouhinec, D Roche. Insitut Curie, Université Paris Sud, CNRS, INSERM.

Embryonic patterning involves the complex interplay of multiple signaling pathways triggering gene regulatory networks (GRNs), which, in turn, drive cell fate choices and progenitor cell differentiation. In vertebrates, neural and neural crest induction have been the focus of detailed analyses for decades, and are excellent models to establish such networks. In frog, access to early embryos has favored the analysis of neural and neural crest progenitors initial induction during gastrulation. Recently, we have implemented large-scale transcriptome analyses, in parallel to experimental validation in vivo, to elaborate the early neural/neural crest GRN. In addition, we found that besides the transcriptional network controlling neural and neural crest induction, a novel checkpoint allows uncommitted progenitors to follow their developmental progression. This checkpoint is driven by a non-conventional role of a glycolysis regulator, PFKFB, coupled to Akt signaling. Our findings and models will be discussed.

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Not Separate but Equal: Cohesion Establishment during DNA Replication. Saili Moghe[1], Jianhua Song[1], **Susannah Rankin[1,2]**. 1) Cell Cycle and Cancer Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Sister chromatids are held together along their length from the time they are made during DNA replication until anaphase by a protein complex called Cohesin. In vertebrates, the Cohesin complex associates with chromatin throughout interphase, and is thought to play a role in normal chromosome architecture. A small pool of Cohesin is converted during DNA replication to a form that is capable of tethering sister chromatids together. Although mechanistic details of this conversion are unclear, acetylation of the Smc3 subunit of Cohesin by members of the Eco1 family of acetyltransferases is essential. In vertebrates there are two Eco family members, Esco1 and Esco2. We are investigating the relationships between DNA replication, Esco-dependent acetylation, and cohesion establishment using frog egg extracts and cultured cells, with the goal of defining the unique and overlapping functions of Esco1 and Esco2. These proteins have unique expression profiles during cell cycle progression and are functionally non-redundant. We show that the enzymes are developmentally regulated and that in egg extracts Esco1 cannot rescue cohesion in the absence of Esco2. Although most models suggest that cohesin acetylation happens during DNA replication, we show that Cohesin acetylation occurs readily when DNA replication is blocked, and after DNA replication is complete. Finally we show that Pds5, a Cohesin-interacting protein, is essential for establishing normal levels of cohesion: it paradoxically promotes both pro-cohesion activities, such as cohesin acetylation and Sororin loading, and anti-cohesion activities, such as Wapl-dependent destabilization, during DNA replication. We are currently developing extract-based experimental strategies to explore these reactions at increased resolution.

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Spinal cord regeneration in *Xenopus laevis*. Juan Larrain[1,3], Dasfne Lee-Liu[1,3], Rosana Muñoz[1,3], Emilio Méndez[1,3], Gabriela Edwards[1,3], Mauricio Moreno[1,3], Victor Tapia[1,3], Nikole Zuñiga[1,3], Leonardo Almonacid[2,3], Francisco Melo[2,3]. 1) Center for Aging and Regeneration and Millenium Nucleus in Regenerative Biology; 2) Molecular Bioinformatics Laboratory and Millennium Institute on Immunology and Immunotherapy; 3) Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile.

Xenopus laevis at tadpole stages (stage 50-54, R-stages) regenerate in response to spinal cord injury (SCI) a capability that is lost at the metamorphic climax (stage 56-66, NR-stages), providing a unique model system to study spinal cord regeneration. We have studied the role of $Sox2^+$ ependymal cells. We have found that in R-stages $Sox2^+$ cells have a rapid and transient activation in response to injury followed by migration of $Sox2^+$ cells into the ablation gap and restoration of the ependymal canal. Importantly, no activation of $Sox2^+$ cells and no migration to the ablation gap occur in NR-stages. Reduction of Sox2 levels by morpholino electroporation diminishes regeneration suggesting that $Sox2^+$ cells are necessary for spinal cord regeneration. Furthermore, cells isolated from spinal cord at stage 50 and transplanted into non-regenerative animals restore axon growth. In addition, we have performed a transcriptomic profile using RNA-SEQ of the response to SCI in R- and NR-stages. We have found extensive changes in the transcriptome of regenerative tadpoles already at 1 day after injury, which was only observed in non-regenerative froglets at 6 days after damage. In addition, we found differential regulation of the following when comparing R- and NR-stages: 1) genes related to neurogenesis and the axonal growth cone; 2) gene ontology enrichment analysis revealed differences in genes from biological processes including cell cycle, response to stress, metabolism, development and immune response and inflammation and 3) we have also identified previously uncharacterized transcripts regulated differentially after SCI. We have validated differential expression of several genes involved in these processes using low-scale validation (RT-qPCR). Currently we are testing by gain and loss-of-function studies the role in spinal cord regeneration of a subset of genes

Special Lectures

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Past, present, and possible future of Xenopus research. John B Gurdon. Zoology [Gurdon Institute], University of Cambridge, Cambridge, United Kingdom.

The origin of *Xenopus* for research in developmental biology is a curious story starting before the Second World War with work by the polymath Lancelot Hogben. Important at this early stage was work by P. D. Nieuwkoop and this was accompanied by the normal table of *Xenopus laevis* published by Nieuwkoop and Faber. My own involvement in the field followed the direction of my supervisor M. Fischberg in Oxford. At about that time Donald Brown (Baltimore) started using *Xenopus* for molecular experiments. The rest of this talk will try to outline other important steps that led to widespread use of*Xenopus laevis* and *Xenopus tropicalis* in the field of developmental and cell biology. In spite of a massive movement towards zebrafish, there are still substantial advantages of using *Xenopus* for laboratory work and I will try to suggest some likely future directions.

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How do we get to an explanation in biology. Marc Kirschner. Systems Biology, Harvard Medical School, Boston, MA.
It has been 90 years since Hilde Mangold and Hans published their paper on embryonic induction in amphibian embryos and 44 years since Janni Nusslein-Vollhardt and Eric Wieschaus published their genetic study of segment number and polarity in flies. Both of these studies were conceptual breakthroughs in understanding how the complexity of the adult is generated from the single celled egg. Both unleashed a fury of detailed molecular studies of signaling molecules and genetic circuitry that has greatly enriched our understanding of development. Today most of us feel that a fuller molecular description but applied more broadly to differentiation, movement, intercellular signaling, and cell division, will provide an explanation of how the egg and the adult are linked through causal chemical reactions. Avoiding for a moment the philosophical question of whether fundamental explanations can exist for any phenomenon, we can ask as biologists whether these answers can offer satisfactory *descriptions* of how various cellular processes are linked together. Is there such a thing as a qualitative understanding, absent a quantitative one? Can genetic circuitry give us a real model without the including underlying biochemical processes? Can we claim to understand why certain processes are highly conserved and others appear not to be? Can we explain the robustness of developmental systems? Are new overarching principles still to be discovered or will we merely fill out our current understandings by accumulating countless details to fill in our qualitative intuitions? I have no simple response but I predict that answers to these questions will arise most naturally in the application of new methodologies to the study development. As conceptual as we believe science to be, historically it has been novel techniques and the lure of open questions, rather than big ideas that have illuminated our initial steps into the darkness. Surgical manipulations of amphibian egg led us to refute preformation and to discover the independence and dependence of different tissues. Focusing genetic tools toward the earliest unattractive stages of fly development, opened up a simpler conceptual understanding of patterning and polarity. Today we can make the argument that the amphibian egg has some of the ideal characteristics for applying modern proteomic and computational tools to begin to describe a more complete and quantitative picture of development as an integrative process. One can hope that these understandings will lead to new fundamental explanations or at least much more satisfactory descriptions of how and why the egg develops as it does.

Session 4

23
microRNA input into an ultradian oscillator provides an autonomous but tunable timer for differentiation. Nancy Papalopulu. University of Manchester.
Progenitor maintenance, timed differentiation and the potential to enter quiescence are three fundamental processes that underlie the development of the nervous system. Neural progenitor cells show short period (ultradian) oscillations in the expression of the transcriptional repressor Hes1. I will present evidence that a neural specific microRNA, miR-9, is a novel component of ultradian oscillations and it controls the stability of Hes1 mRNA and eventually leads cells to exit the oscillatory phase and initiate differentiation. I will also describe the development of a computational model, based on experimental data, which shows that the input of miR-9 into the Hes1 oscillator tunes the oscillatory dynamics, and it endows the system with bistability and the ability to measure time to differentiation.

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Epigenetic fine-tuning of cell fate choices: impact on developmental disorders. Ruchi Bajpai. Center for Craniofacial Molecular Biology, Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA.
The regulatory sophistication of the vertebrate genome is only just beginning to be unraveled. The quantitative expression of genes in distinctive spatial and temporal domains is controlled by genomic regulatory elements called enhancers that can be located proximal or distal to the promoter. Genomic enhancers for the nearly 25,000 human genes have been identified by characteristic epigenetic 'signatures of active enhancers' and total to over 400,000 regions and counting. Co-ordinated activation of thousands of enhancers must occur during cell fate transitions and involves extensive modifications of the chromatin. Not surprisingly, defects in the organization of the chromatin are responsible for some of the common birth defects, many of which are caused by genetic mutations in chromatin modifying proteins. Using Xenopus as a model system we have investigated the function of two chromatin-associated proteins (CHD7-a chromatin remodeler and PHF6-a chromatin binder) that preferentially function at enhancers and result in devastating developmental disorders when mutated. CHARGE syndrome affects the lives of 1:10,000 newborn children and is caused by spontaneous mutations that result in the loss of function of a single copy of the gene encoding CHD7, an ATP dependent chromatin remodeling Swi/Snf like helicase. While the rare disease Börjeson-Forssman-Lehmann syndromes (BFLS), occurs upon the loss of a dual plant homeodomain finger protein, PHF6 that can bind DNA and modified histones. Our studies uncover basic mechanisms of enhancer regulation in cell fate determination. Understanding how these proteins are involved in epigenetic processes has the potential to help patients with these syndromes, and to shed light on enhancer function during development and disease more generally.

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Embryogenesis kinetics revealed by high-resolution absolute quantitation of transcripts. Nick Owens[1], Ira Blitz[2], Maura Lane[3], John Overton[4], Mustafa Khokha[3], Ken Cho[2], Michael Gilchrist[1]. 1) Division of Systems Biology, MRC National Institute for Medical Research, London, UK; 2) Department of Developmental and Cell Biology, University of California, Irvine, CA, USA; 3) Program in Vertebrate Developmental Biology, Department of Pediatrics and Genetics, Yale University School of Medicine, New Haven, CT, USA; 4) Yale Center for Genome Analysis,Yale University School of Medicine, New Haven, CT, USA.

Embryogenesis is a highly dynamic process involving dramatic changes in cell organisation driven by gene regulation. However, a quantitative understanding of transcript kinetics over broad windows of development is lacking. Kinetic information is essential to build dynamic gene regulatory networks that control cellular differentiation and morphogenesis. Here we sample *Xenopus* embryos from 0 to 66 hours post fertilisation at a high temporal resolution sufficient to capture the underlying dynamics of gene expression. We profile both polyadenylated and total transcript levels, and by spiking with exogenous RNA standards, we calculate kinetics in terms of absolute transcripts per embryo.Our results provide an unprecedented comprehensive view of gene regulation during an extended period of development. We find gene expression trajectories varying smoothly throughout our timecourse, and dynamic stage-specific switching of isoforms. To illustrate the potential of this dataset, we select one remarkable finding to report here. We identify a novel gene locus, *escape velocity (esv)*, which exhibits massive zygotic transcription of a lncRNA that at its peak at the blastula stage has twice as many transcripts as the most abundant non-ribosomal RNAs. In addition, esv transcripts become detectable at the 8-16 cell stage, well before the classic mid-blastula transition. Our results redefine our ability to apply quantitative systems biology to the early vertebrate embryo.

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Unliganded thyroid hormone receptor alpha regulates gene repression and developmental timing as revealed by gene knockout in *Xenopus tropicalis*. Jinyoung Choi[1], Ken-ichi Suzuki[2], Tetsushi Sakuma[2], Leena Shewade[1], Takashi Yamamoto[2], **Daniel R. Buchholz[1]**. 1) Biological Sciences, University of Cincinnati, Cincinnati, OH; 2) Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, Hiroshima, Japan.

Thyroid hormone (TH) receptor (TR) expression occurs in low circulating TH levels during development in all vertebrates, yet few developmental roles for unliganded TRs have been established. Unliganded TRs are expected to repress TH-response genes, increase tissue responsivity to TH, and regulate the timing of developmental events. Here, we determined the role of unliganded TR alpha (TRα) in gene repression and development post-organogenesis in *Xenopus tropicalis*. We used TALEN gene disruption to generate founder animals with mutations in the *TRα* gene and bred them to produce homozygous mutants. We examined gene expression of TH-response genes and early larval development with and without exogenous TH. As hypothesized, we found increased expression of TH-response genes in the TRα mutants in the absence of TH and decreased expression of these same genes compared to wild-type animals after 24 hrs. of TH treatment (10 nM T3). Corresponding morphological effects of the TRa mutantion included precocious hind limb growth in the absence of TH and reduced responsivity to exogenous TH. These results provide clear and dramatic evidence for the role of unliganded TR in the regulation of gene expression and developmental timing.

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More than one frog in the pond: evolutionary modulations of gut morphogenesis in tadpoles with different diets. Nanette Nascone-Yoder. Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

Variation in the morphology of the digestive tract reflects the diverse diets of different species. To discern the developmental mechanisms that generate such variation during evolution, we assessed gut morphogenesis by immunohistochemical staining in an array of phylogenetically diverse, non-model frog species with distinct diets. Herbivorous tadpoles, like *Xenopus*, develop a long intestine as the yolky endoderm cells in the embryonic gut become polarized, and then radially rearrange to convert the endoderm mass into a long, hollow tube while forming the intestinal epithelium. In contrast to this ancestral condition, omnivorous tadpoles of terrestrial frogs with larger eggs tend to have delayed or decreased intestine lengthening, despite storing a greater mass of endoderm in the embryonic gut. Endoderm morphogenesis is delayed in these species (*Engystomops*, *Theloderma*), consistent with mathematical models which predict that the efficiency of tube lengthening by radial cell rearrangement should decrease as the initial diameter of the primitive gut tube increases. Indeed, direct-developing embryos, which arise from the largest eggs, often form a very short, adult-like digestive tract. In such species (*Ceratobatrachus*), the massive yolk-laden endoderm cells take much longer to polarize, and cell rearrangement serves to expand epithelial surface area rather than gut length. Interestingly, although their eggs are not particularly large, species that generate cannibalistic tadpoles which feed on siblings or nutritive eggs also form a short, adult-like gut. These tadpoles develop in isolated or ephemeral microhabitats where selection favors rapid development. In this context (*Ceratophrys, Lepidobatrachus, Anotheca*), the innermost endoderm cells never get the chance to rearrange and are instead eliminated by apoptosis, resulting in a shorter, but immediately functional, intestine. Thus, the novel tadpole gut morphologies that accompanied the ecological diversification of frogs may have arisen, in part, due to constraints on endoderm morphogenesis in embryos with varying developmental rates and/or larger yolk reserves. Similar modulations of tubulogenesis may be a common source of novelty in other vertebrates.

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Protein Networks In Cardiac Morphogenesis. Frank Conlon[1,2,3], Stephen Sojka[1,2], Nirav M Amin[1,3], Devin Gibbs[1,3], Kathleen

S Christine[1,3], Marta S Charpentier[1]. 1) University of North Carolina McAllister Heart Institute, UNC-Chapel Hill, Chapel Hill, NC 27599-3280, USA; 2) Department of Biology, UNC-Chapel Hill, Chapel Hill, NC 27599-3280, USA; 3) Department of Genetics, UNC-Chapel Hill, Chapel Hill, NC 27599-3280, USA.

The identification and characterization of the cellular and molecular pathways involved in the differentiation and morphogenesis of specific cell types of the developing heart are critical to understanding the process of cardiac development and the pathology associated with human congenital heart disease. Here we show that the cardiac transcription factor CASTOR (CASZ1) directly interacts with Congenital Heart Disease 5 protein (CHD5), also known as tryptophan rich basic protein (WRB), a gene located on chromosome 21 in the proposed region responsible for congenital heart disease in Down Syndrome patients. We demonstrate that loss of CHD5 in Xenopus leads to compromised myocardial integrity, improper deposition of basement membrane, and a resultant failure of hearts to undergo cell movements associated with cardiac formation. We further report that CHD5 is essential for CASZ1 function and the CHD5-CASZ1 interaction is necessary for cardiac morphogenesis. Recently we have gone on to develop a general binary transgenic-based approach in Xenopus using the Avi-tag/BirA system for the identification and purification of potentially any endogenous protein complex from Xenopus. Using this system we have identified additional proteins in complex with CASZ1 inb vivo that potentially mediate CASZ1 transcriptional activity. Collectively, these results establish a role and mechanism for CASZ1 in the early stages of vertebrate cardiac development.

Session 5 – No Abstracts

Keynote Lecture

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Mechanisms of mitosis and size control in Xenopus. Rebecca Heald. University of California, Berkeley, Berkeley, CA.

The goal of research in my laboratory is to elucidate the molecular mechanisms of cell division and morphogenesis. We use cytoplasmic extracts prepared from eggs of the frog *Xenopus laevis* to reconstitute and study mitotic chromosome condensation and spindle assembly and function in vitro, applying imaging, biochemical, and biophysical approaches. To study mechanisms of spindle and organelle size control, we take advantage of a smaller, related frog, *Xenopus tropicalis*, to investigate interspecies scaling, and extracts prepared from fertilized eggs at different stages of embryogenesis to study developmental scaling. Our research provides novel insight into how cell/organelle scaling contributes to intracellular morphogenesis and cell division, processes essential for viability and development, and defective in human diseases including cancer.

Session 6

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Understanding Wnt/beta-catenin signaling in development, stem cell, and cancer. Xi He[1,2]. 1) F. M. Kirby Center, Boston Children's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA.

We focus on understanding the molecular mechanism of Wnt signaling in vertebrate development, stem cell regulation, and human cancer and diseases. We are particularly interested in canonical Wnt/beta-catenin signaling and noncanonical Wnt signaling pathways. We employ molecular, structural, cellular, and developmental approaches in Xenopus, mice, and human cell culture. I will discuss our effort in the study of the Wnt/LRP6 receptor complex and the mechanism by which Wnt/LRP6 stabilizes the transcriptional co-activator beta-catenin through coordination of phosphorylation-dephosphorylation events. I will also discuss the function and regulation of Tiki, a membrane-tethered and Organizer-specific Wnt inactivating enzyme. If time permits I will highlight our continuing effort in identifying in Xenopus and mammalian systems novel Wnt signaling components that have roles in vertebrate embryogenesis and stem cell functions.

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Anillin Regulates Cell-Cell Junction Integrity by Organizing Junctional Accumulation of RhoA-GTP and Actomyosin. Ciara Reyes[1], Torey Arnold[2], Tomohito Higashi[2], Rachel Stephenson[2], **Ann Miller[1,2]**. 1) The Cellular and Molecular Biology Program (CMB), University of Michigan, Ann Arbor, MI; 2) Molecular, Cellular, & Developmental Biology (MCDB), University of Michigan, Ann Arbor, MI.

Anillin is a scaffolding protein that organizes and stabilizes the actomyosin contractile rings during cytokinesis. However, almost nothing is known about Anillin's function during cytokinesis in vertebrate organisms *in vivo*, and potential roles outside cytokinesis are completely uncharacterized. Using *Xenopus laevis* embryos as a model system to examine Anillin's role in the

intact vertebrate epithelium, we find that a population of Anillin surprisingly localizes to epithelial cell-cell junctions throughout the cell cycle, whereas it was previously thought to be nuclear during interphase. Furthermore, we show that Anillin plays a critical role in regulating cell-cell junction integrity. Both tight junctions and adherens junctions are disrupted when Anillin is knocked down, leading to altered cell shape and increased intercellular spaces. Moreover, our unpublished results show that Anillin regulates the dynamics of the tight junction protein ZO-1. Anillin interacts with RhoA, F-actin, and Myosin II, all of which regulate cell-cell junction structure and function. When Anillin is knocked down, active RhoA (RhoA-GTP), F-actin, and Myosin II are misregulated at junctions. Despite an overall reduction in junctional F-actin and phospho-Myosin II accumulation when Anillin is depleted, we observe increased dynamic ''flares'' of RhoA-GTP at cell-cell junctions. Our unpublished results suggest that these RhoA-GTP flares occur after local breaks in tight junctions and could be involved in reestablishment of the junction. Together, these results reveal a novel role for Anillin in regulating epithelial cell-cell junctions and suggest that Anillin may be required for orchestrating proper tension distribution at cell-cell junctions. To that end, we are developing approaches to directly investigate Anillin's role in regulating epithelial tension.

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RNA transport in the oocyte cytoplasm: How to get there from here. K. Mowry, S. Jeschonek, C. Neill, C. Pratt, E. Powrie. Department of Molecular Biology, Cell Biology & Biochemistry, Brown University, Providence, RI.

RNA localization is a widely conserved mechanism for generating polarized gene expression. We have studied the localization of mRNA in the *Xenopus* oocyte as a model to gain mechanistic insight into how RNA molecules can be targeted to specific regions of the cell cytoplasm to generate spatially restricted gene expression. A major focus of our studies is Vg1 mRNA, which is transported to the vegetal hemisphere cytoplasm and encodes a secreted grown factor whose restricted expression is required for proper embryonic patterning. Localized Vg1 expression is ensured through at least two mechanisms: efficient transport of the mRNA to the vegetal cytoplasm and tight translational repression during transport. Our recent studies have focused on tracing the molecular pathway for cytoplasmic RNA localization, from motor-driven RNP transport in the cytoplasm to events at the oocyte cortex that trigger spatially-restricted translation.

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Pancreatic development: cell fate choices and plasticity. Francesca Spagnoli. Molecular and Cellular Basis of Embryonic Development, MDC, Berlin, Germany.

Understanding how distinct cell types arise from common multipotent progenitor cells is a major quest in stem cell biology. The endoderm germ layer gives rise to a number of vital organs, including the lungs, liver, pancreas and intestine. This remarkable diversity derives from a homogenous population of multipotent cells. The aim of our research is to elucidate the fundamental principles that establish and maintain pancreatic cell identity. Cell identity is determined by specific gene expression patterns that are imparted by temporal and spatial integration of extrinsic signals and intrinsic determinants. One of the challenges in my laboratory is to understand how distinct cell types, such as liver and pancreas, arise from common endoderm progenitors in the embryo and acquire specialized shape to form functional organs. This fascinating question in developmental biology has direct clinical relevance. In the long run, our investigations will have direct implications in developing novel strategies to generate pancreas progenitors and ß-cells either from programming of stem cells or from re-programming of adult hepatic cells, closing the gap between studies of basic processes in model systems and clinical research.

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Nuclear functions of the "other catenins" in stem cell/ developmental decisions. Moon Sup Lee[1], Yasuhide Furuta[2], Jae-il Park[1], **Pierre McCrea[1]**. 1) UT MD Anderson Cancer Center, Houston TX; 2) Riken Center for Developmental Biology, Kobe Japan.

beta-Catenin's actions in canonical-Wnt signaling and in cadherin-catenin complexes is relatively well characterized, yet there is a need to understand the larger family of "other catenins" in vertebrate development. We have used Xenopus and further models to focus upon the essential contributions of the p120-subfamily or related plakophilin-subfamily catenins, finding that select p120- and plakophilin-catenin isoforms are stabilized by canonical-Wnt signals, and bind to varied transcription factors essential to embryogenesis. We have thus been asking if the canonical-Wnt pathway in vertebrates should be defined to involve a network of catenins rather than beta-catenin alone. Here, we present unpublished findings that p120-catenin binds and regulates the powerful transcriptional repressor complex REST/CoREST, needed for making differentiation decisions in stem cells, as well as for repressing neural genes in non-neural tissues. In human pathology, REST has key roles in tumor and neurodegenerative disease. Using both Xenopus embryos and mouse embryonic stem cells, in combination with knockdown, overexpression and rescue approaches, we reveal that p120-catenin de-represses REST/CoREST to favor neural differentiation. Our findings point to p120's displacement of REST from gene control regions and REST's proteasomal destruction. As for p120's upstream regulation in stem cells, we intriguingly see that p120's signaling pool is modulated by the endogenous level of E-cadherin. That is, E-cadherin sequesters p120 at cell-cell contacts, lowering p120's signaling pool and thus ability to displace and degrade REST, so that REST gene-targets remain repressed. Since E-cadherin levels are known to plummet immediately before embryonic stem cells depart their pluripotent state, we are testing the premise that p120 thereby enters a signaling pool more readily, to enable its nuclear function in relieving REST-mediated repression. In summary, we report upon a newly resolved E-cadherin-p120-catenin-REST/CoREST axis in stem cell differentiation. We will also share findings upon novel nuclear partners of yet additional catenins (e.g. delta-catenin and plakophilin-3 catenin), and their functional contributions to Xenopus development.

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Quantitative proteomics of Xenopus laevis embryos: expression kinetics of 4000 proteins during early development. Norman J Dovichi, Liangliang Sun, Paul Huber, Michelle Bertke, Matthew Champion, Guijie Zhu. Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN.

While there is a rich literature on transcription dynamics during the development of many organisms, protein data is limited. We used iTRAQ isotopic labeling and mass spectrometry to generate the largest developmental proteomic dataset for any animal. Expression dynamics of 4,000 proteins of Xenopus laevis were generated from fertilized egg to neurula embryo. Expression clusters into groups. The cluster profiles accurately reflect the major events that mark changes in gene expression patterns during early Xenopus development. We observed decline in the expression of ten DNA replication factors after the midblastula transition, including a marked decline of the licensing factor XCdc6. Ectopic expression of XCdc6 leads to apoptosis; temporal changes in this protein are critical for proper development. Measurement of expression in single embryos provided no evidence for significant protein heterogeneity between embryos at the same stage of development.

Session 7

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System-level analysis of pancreatic cell type differentiation in Xenopus. Matthew Salanga[1], Leonid Peshkin[2], **Marko Horb**[1]. 1) Bell Center for Regenerative Biology and Tissue Engineering and National Xenopus Resource, Marine Biological Laboratory; 2) Department of Systems Biology, Harvard University**.**

Cell fate specification is a dynamic, complex and coordinated process that involves continuous interactions with neighboring cells resulting in rapid changes in gene expression, both transcriptional and translational. To accurately model these dynamic molecular interactions requires a detailed high-resolution map of transcriptomic and proteomic changes. Once a detailed map is constructed for a specific cell lineage then that information can be used to promote ectopic development of that cell. As one of the main goals of basic biomedical research is to identify a method to replace cell types that are lacking or defective in specific diseases, such as diabetes, elucidating the regulatory networks for specific cell lineages is critical. Most symptoms that characterize diabetes are due to a decline in function of pancreatic beta cells, identifying a method to replace beta cells is a key area of research. To model the dynamic regulatory interactions of beta cell development requires a more detailed high-resolution map of the transcriptomic and proteomic changes from endocrine progenitor to insulin expression. All pancreatic endocrine cells develop from a common progenitor that expresses the bHLH transcription factor Neurogenin 3 (Ngn3). Exactly how Ngn3 promotes the development of one endocrine cell fate over another is not known. To be useful for diabetes treatment it is important to define how Ngn3 promotes the development and differentiation of beta cells over other endocrine cell fates, and this requires a more detailed knowledge of the dynamic beta cell lineage. We recently developed a protocol to maximize production of beta cells via controlled overexpression of Ngn3 in Xenopus laevis endoderm. To define the Ngn3 beta cell lineage in high resolution, we used RNA-Seq at hourly intervals following Ngn3 activation to measure the transcriptomic changes using J strain Xenopus laevis. Our results highlighting interesting and unappreciated aspects regarding the dynamic nature of beta cell development. Several new genes were identified and knocked out in Xenopus and found to be critical for beta cell development. In conclusion these results demonstrate that the usefulness of the X. laevis J strain for RNA-Seq analysis and functional analysis of gene regulatory networks**.**

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Using Frog Faces to Better Understand Orofacial Development. Amanda J.G. Dickinson. VCU, Richmond, VA.

In humans, the orofacial region serves as our gateway to the environment, permitting ingestion, taste, communication and facial recognition. Therefore, birth defects affecting the mouth and face such as orofacial clefts can be devastating. *Xenopus* has become a powerful tool to better understand the molecular mechanisms underlying orofacial development. For example, my lab has uncovered novel roles for retinoic acid signaling and folate metabolism in midface and primary palate development. Decreased retinoic acid signals result in a reduction in cell proliferation in the dorsal facial prominences and a median cleft in the primary palate. In parallel studies we determined that inhibition of folate metabolism can also result in median facial clefts as well as midface hypoplasia due to decreased cell proliferation. Interestingly, excess folic acid can reduce the severity of retinoic acid receptor inhibitor induced clefts. Preliminary studies further suggest that both retinoic and folic acid epigenetically regulate gene expression during orofacial development and therefore we are currently investigating changes in DNA and histone methylation. From this work we have also become more generally interested in epigenetic regulators of orofacial development. Accordingly, we have untaken the first analysis of the developmental role of an important histone code reader, Retinoic acid induced-1 (RAI1). When this gene is mutated in humans it results in Smith-Magenis Syndrome (SMS), a neurobehavioral disorder accompanied by signature craniofacial abnormalities. Knockdown of Rai1 in frogs results in midface hypoplasia and malformed mouth shape analogous to defects in humans with SMS. These craniofacial defects were accompanied by aberrant neural crest development and reduction in the size of facial cartilage. Finally, we are interested in better describing the similarities and differences in the orofacial defects we see in frogs and therefore we have developed a method to quantify orofacial size and shape. This method combines traditional measurements with geometric morphometrics to describe anatomical

changes in the orofacial region. By combining our quantitative analyses with molecular and epigenetic studies we will be better equipped to understand the complex morphogenetic processes involved in orofacial development.

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Reconstituting the mechanisms of actin remodeling. Jennifer Gallop. Gurdon Institute, University of Cambridge, Cambridge, United Kingdom.
Actin dynamics generates force during different cell biological processes including vesicle trafficking, cytokinesis and the formation of cell protrusions. The physiological roles are therefore many and varied e.g. in synapse formation, cancer metastasis, during pathogen infection as well as normal morphogenesis. The proteins involved are often the same between different functions, so how is a specific actin structure made at the required time and place in the cell? The nature of the membrane-cytosol interface appears to be important as it is the site of incorporation of new actin monomers and is the physical site of signaling events that are capable of controlling actin polymerization. Reconstituting the membrane-cytosol interface using supported lipid bilayers or liposomes and frog egg extracts means that it is possible to control the composition and topology of the membrane to trigger actin polymerization in different ways. I will talk about what we are learning from two reconstitution systems, flat $PI(4,5)P_2$ containing membranes that nucleate filopodia-like structures and curved $PI(3)P/PI(4,5)P_2$ membranes that appear to mimic a phase of endocytosis.

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Genomics Study of the Spemann-Mangold Organizer: Occupancy of Tissue-Specific cis-Regulatory Modules by Otx2 and TLE/Groucho for Embryonic Head Specification. Yuuri Yasuoka[1,2], Yutaka Suzuki[2], Shuji Takahashi[2,3], Haruka Someya[4], Norihiro Sudou[2,5], Yoshikazu Haramoto[2,6], Ken Cho[7], Makoto Asashima[2,6], Sumio Sugano[2], Masanori Taira[2]. 1) OIST, Japan; 2) Univ. of Tokyo, Japan; 3) Hiroshima Univ., Japan; 4) TMU, Japan; 5) TWMU, Japan; 6) AIST, Japan; 7) UC Irvine, USA.
The Spemann-Mangold organizer initiates gastrulation movement and establishes basic body plans. The organizer consists of two different regions - head and trunk organizers, which effect anteroposterior patterning of the neuroectoderm. Genes for homeodomain proteins, Otx2, Lim1 (=Lhx1), and Goosecoid (Gsc), are expressed in the head organizer to specify head structures. The transcriptional regulatory networks underlying the *Xenopus* organizer have been studied extensively, but the genomic overview is still unclear.Head specification by the head-selector gene, *orthodenticle* (*otx*) is highly conserved among bilaterian lineages. However, the molecular mechanisms by which Otx and other transcription factors (TFs) interact with the genome to direct head formation, are largely unknown. Here we employ ChIP-seq and RNA-seq approaches in *Xenopus tropicalis* gastrulae, and find that occupancy of the corepressor, TLE/Groucho, is a better indicator of tissue-specific cis-regulatory modules (CRMs) than the coactivator p300, during early embryonic stages. Based on TLE binding and comprehensive CRM profiling, we define two distinct types of Otx2- and TLE-occupied CRMs. Using these devices, Otx2 and other head organizer TFs [e.g., Lim1 (activator) or Gsc (repressor)] are able to upregulate or downregulate a large battery of target genes in the head organizer. An underlying principle is that Otx marks target genes for head specification to be regulated positively or negatively by partner TFs through specific types of CRMs. The simplicity of this mode of head specification may explain the evolutionary conservation of the head-selector Otx.

Session 8

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***Xenopus* Smad4 at the intersection of FGF, Wnt and TGF-β.** Hadrien Demagny, Tatsuya Araki, **Edward De Robertis**. Biological Chemistry, HHMI/UCLA, Los Angeles, CA.
Smad4, also known as Deleted in Pancreatic Carcinoma 4 (DPC4), is a major tumor suppressor essential for TGF-β signaling currently thought to function constitutively. Here we report that Smad4 activity is tightly regulated by the Wnt and FGF pathways through novel GSK3 and MAPK phosphorylations sites. FGF activates MAPK, which primes three sequential GSK3 phosphorylations that generate a Wnt-regulated phosphodegron bound by the Ubiquitin E3 ligase β-TrCP. In the presence of FGF, Wnt potentiates TGF-β signaling by preventing Smad4 GSK3 phosphorylations that inhibit a transcriptional activator domain located in the linker region. When MAPK is not activated, the Wnt and TGF-β signaling pathways remain insulated from each other. In *Xenopus* embryos, these Smad4 phosphorylations regulate germ layer specification and Spemann organizer formation. We propose that the competence modifier effect of Wnt by Activin/TGF-β (discovered 20 years ago by Doug Melton and Randy Moon) may be explained in part at the level of Smad4 phosphorylations. We conclude that three major signaling pathways critical in development and cancer are integrated at the level of Smad4.

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TACC3 is a microtubule plus-end tracking protein that promotes axon elongation and regulates microtubule plus-end dynamics in multiple embryonic cell types. Belinda Nwagbara, Anna Faris, Elizabeth Bearce, Burcu Erdogan, Patrick Ebbert, Matthew Evans, Charlie Baker, Tiffany Enzenbacher, **Laura Anne Lowery**. Dept of Biology, Boston College, Chestnut Hill,

MA.

A fundamental problem in cell biology is how global cytoskeletal dynamics are coordinated to drive directed cell migration. An interesting group of microtubule regulators that play key roles during directed migration of various cell types are the highly-conserved family of microtubule plus-end-tracking proteins (also known as +TIPs). These proteins bind to and regulate the "plus-ends" of microtubules, which are the dynamic ends that extend towards the periphery of every cell type. Despite their importance, it is still unclear how various +TIPs interact with each other and with plus-ends to control microtubule behavior, and also how microtubule behavior relates to changes in cell motility. We study +TIP function by analyzing high-resolution live-imaging data of cultured embryonic *Xenopus laevis* neuronal growth cones and neural crest cells. The centrosome-associated protein TACC3, a member of the transforming acidic coiled coil (TACC) domain family, has been previously implicated in regulating several aspects of microtubule dynamics. However, TACC3 has not been described as a +TIP in vertebrates, despite several points of evidence that it may affect microtubule dynamics. Here, we show that TACC3 promotes axon outgrowth and regulates microtubule dynamics by increasing microtubule plus-end velocities *in vivo*. We also demonstrate that TACC3 specifically acts as a +TIP in multiple embryonic cell types, and that this requires the conserved C-terminal TACC domain. Using high-resolution live-imaging data of tagged +TIPs, we reveal that TACC3 localizes distally at the plus-ends of microtubules, where it lies in front of the microtubule polymerization marker, EB1, and appears to directly co-localize with the microtubule polymerase, XMAP215. Together, our results implicate TACC3 as a +TIP that functions with XMAP215 to regulate microtubule plus-end dynamics.

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Regulation of replication intitation and cell cycle events at the mid-blastula transition (MBT). Philip Zegerman[1], Clara Collart[1,2], James C Smith[2]. 1) Gurdon Institute, Cambridge, United Kingdom; 2) MRC National Institute for Medical Research, London, United Kingdom.

Developmental transitions in many metazoans are accompanied by remodeling of the cell cycle, including changes to the density and timing of replication initiation. It has previously been proposed that the titration of cell cycle regulators by the increasing nuclear:cytoplasmic (N/C) ratio is critical for the events of the mid-blastula transition (MBT) during *Xenopus laevis* embryonic development. We have directly tested this model and show that four essential DNA replication factors, Cut5, RecQ4, Treslin and Drf1 are limiting for replication initiation *in vitro* and are titrated from *Xenopus* egg extracts at increasing N/C ratios. We show that these factors are limiting for replication initiation *in vivo* in MBT stage embryos and regulate multiple events of the MBT including the slowing of the cell cycle, the onset of zygotic transcription and the developmental activation of the checkpoint kinase Chk1. Together this work provides a molecular mechanism for how the N/C ratio controls events at the MBT. The implications of stringent developmental regulation of DNA replication initiation for normal embryogenesis will be discussed.

Session 9

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Proteomic analysis of the DNA double-strand break response. Jean Gautier[1], Tomas Aparicio[1], Yinyin Li[2], Gaganpreet Sidhu[1], Krithika Rajagopalan[1], Brian Chait[2], Max Gottesman[1]. 1) Institute for Cancer Genetics, Columbia University Medical Center, New York, NY; 2) Rockefeller University.

DNA double-strand breaks (DSBs) are harmful lesions that threaten genomic integrity. If unrepaired or misrepaired they may lead to chromosome loss or rearrangements including, but not limited to, oncogenic translocations. To counteract this threat, cells have developed the DNA damage response (DDR) to sense, process and repair DSBs. DSBs are repaired primarily by non-homologous end-joining (NHEJ) and homology-dependent repair (HDR). Competition between these pathways is a critical determinant of downstream signaling and repair. We employ two complementary strategies to get insight into the mechanisms dictating DSB repair pathway choice utilizing cell-free extracts derived from Xenopus laevis eggs.First, we analyzed the regulation of DNA end-processing (DNA resection), which funnels DSB repair towards HDR. Specifically, we focused on CtIP phosphorylation. We demonstrate that cell cycle- and damage-dependent phosphorylations of CtIP are critical for resection. We also establish that CtIP recruitment to damaged chromatin is dictated by post-translational modifications.Second, we have initiated a comprehensive analysis of the protein landscape at DSBs using iTRAQ (isobaric tags for relative and absolute quantitation) combined with LC-MS/MS. This approach is highly sensitive, reproducible and suited for comparative proteomics studies. We document proteins that are enriched or depleted from DSB-containing chromatin. Notably, our analysis provides biochemical evidence for competition between DSB repair pathways. We will also document the involvement of novel signaling pathways in the DNA damage response to DSBs.

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Coco is a dual-activity modulator of TGFβ signaling. Alessia Deglincerti, Aryeh Warmflash, Tomomi Haremaki, Qixiang Zhang, Ali H. Brivanlou. Laboratory of Stem Cell Biology and Molecular Embryology, The Rockefeller University, 1230 York Ave, New York, NY.

In earlier work, we have determined that the dynamics of TGFβ signaling correlate with Smad4 nuclear localization. To dissect the molecular mechanisms involved in ligand-dependent Smad4 nuclear translocation, we performed an unbiased, genome-wide RNAi screen. We uncovered a total of 256 genes that significantly affect Smad4 translocation. Strikingly, one of these genes had been previously identified in the lab in a gain-of-function *Xenopus* screen for inducers of secondary heads: Coco. We have previously shown that Coco is an inhibitor of BMP4 and Activin/Nodal signaling. Surprisingly, we now find that Coco synergizes with TGFβ1 in mammalian cells and *Xenopus* explants. I will present data supporting these findings and discuss the significance of the discovery that Coco functions as both an inhibitor and an enhancer of a subset of TGFβ ligands.

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Identification of novel genes involved in epicardial development. P. Tandon[1,2,4], C. Wilczewski[1,4], F. Conlon[2,3,4]. 1) Molecular Biology, UNC, Chapel Hill, NC; 2) Department of Genetics, UNC, Chapel Hill, NC; 3) Department of Biology, UNC, Chapel Hill, NC; 4) McAllister Heart Institute, UNC, Chapel Hill, NC.

The inability of the adult heart to regenerate post-infarction results in multiple complications after injury. Recent work to promote endogenous cardiac cell populations to reinitiate embryological mechanisms of healing has highlighted the use of epicardial cells to assist in cardiac repair. The epicardium forms as a sheet of cells surrounding the heart during development and is conserved throughout vertebrates. This dynamic population of cells is a source of essential cardiac progenitors and mitogenic factors critical for the continued development of the heart. The specific lineage differentiation potential of epicardial derived-cells is established during its development as the proepicardial (PE) precursor structure. We have recently identified a critical function for the transcription factor Tcf21 in establishing correct specification and maturation of PE cells in *Xenopus*. Cardiac-specific RNAseq data has provided potential factors and pathways through which Tcf21 regulates PE cell development, including the transcription factor Lim homeobox 9 (Lhx9). Depletion of Lhx9 resulted in aberrant epicardial formation and mislocalization of PE cells, demonstrating the use of *Xenopus* as a high-throughput model to study the function of novel epicardial genes. In addition, to elucidate the transcriptional mechanisms through which Tcf21 regulates epicardial development we have identified and verified through a directed proteomics-based approach, an interaction between Tcf21 and C-terminal binding protein 2 (Ctbp2), an established transcriptional modulator, with the aim to further understand how this interaction is pertinent to epicardial specification and identify potential downstream target genes. Using the highly established developmental and regenerative model *Xenopus*, together with new genomic editing technology such as TALENs (from which we currently have germline transmission of Tcf21 mutations) we can identify critical genes involved in the formation and function of this important cardiac progenitor population.

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Mechanisms and functions of nuclear size regulation in early *Xenopus* development. Daniel Levy, Lisa Edens, Predrag Jevtic. Molecular Biology, University of Wyoming, Laramie, WY.

Mechanisms regulating nuclear size are largely unknown and may be relevant to disease, most notably cancer. Early *Xenopus laevis* embryogenesis is a robust system for investigating nuclear size reductions that occur during development. We developed a nuclear re-sizing assay wherein nuclei assembled in *Xenopus* egg extract become smaller in the presence of cytoplasmic extract isolated from post-gastrula*Xenopus* embryos. Nuclear shrinkage depends on conventional protein kinase C (cPKC), demonstrated using specific inhibitors and neutralizing antibodies. Nuclear size reductions during development correlate with increased nuclear cPKC localization and activity and decreased nuclear association of lamins. Furthermore, manipulating cPKC activity *in vivo* by microinjection and with small molecule inhibitors alters nuclear size in the embryo. We propose a model in which nuclear expansion is balanced by cPKC-dependent nuclear size reductions to regulate steady-state nuclear size. We have also been studying the impact of nuclear size on cell function in the context of midblastula transition (MBT) timing. A critical ratio of DNA:cytoplasm determines MBT timing, possibly regulated by titration of limiting cytoplasmic components by DNA. Several such factors have been identified, and notably they all act within the nucleus. We reasoned that the nuclear concentrations of these factors might regulate MBT timing, consistent with the observation that total nuclear volume in the embryo reaches a maximum at the MBT. To test this idea, we manipulated nuclear volume in embryos by mRNA microinjection of a variety of different factors, including nuclear import proteins, lamins, and reticulons. We find that increasing nuclear size leads to early onset of MBT-specific transcription, detected by in situ hybridization and qRT-PCR, as well as early onset of asynchronous, longer cell cycles. Conversely, reducing nuclear size delays the MBT, as measured by these same parameters. Thus altering nuclear size leads to changes in MBT timing. We propose that the MBT is regulated not by the absolute amounts of key DNA-binding factors but by their nuclear concentrations, as determined by developmentally regulated changes in nuclear volume.

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Role of cytoskeletal regulators in cranial neural crest migration. Shuyi Nie[1], Marianne Bronner[2]. 1) Georgia Institute of Technology, Atlanta, GA; 2) California Institute of Technology, Pasadena, CA.

During cell movements, actin cytoskeleton is the primary force-generating machinery that pushes the cell-front forward and pulls the cell-rear in. My goal is to elucidate the roles of actin cytoskeletal regulators during cell migration in vivo, using the highly migratory neural crest cells in Xenopus embryos as a model. Based on gene expression patterns, I studied the activities of a few actin regulators, Caldesmon, Myosin X, and Cdc42 effector protein 1 (CEP1). Loss of function studies show that they are all required for proper migration of cranial neural crest cells, such that depletion of either gene results in impaired neural crest migration and subsequent defects in cranial cartilage formation. Analysis of cranial neural crest cells reveals that these regulators

are important in different aspect of cell behaviors. For example, CEP1 is required for the formation of membrane protrusions, and the organization of actin filaments. In addition, misexpression of CEP1 disrupts the distribution of Cdc42, suggesting that CEP1 interacts with Cdc42 in neural crest cells to control cell morphology and motility. In summary, selective expression of cytoskeletal regulators in neural crest cells may facilitate efficient migration of this cell population.

Session 10

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A Gene Regulatory Network controlling lung development. Scott Rankin, Lu Han, Kyle McCraken, Marcin Mancini, Pamela Wlizla, James Wells, **Aaron Zorn**. Developmental Biology, Cincinnati Children's, Cincinnati, OH.

Embryonic development of the respiratory system is regulated by a series of mesenchymal-epithelial interactions that are only partially understood. Mesenchymal FGF, Retinoic acid (RA), BMP and Wnt2/2b signaling are all involved in specification of $Nkx2.1^{+}$ respiratory progenitors from the ventral foregut endoderm, but how these factors are regulated, their epistatic relationships and downstream targets are largely unknown. We have used the experimental advantages of Xenopus, which enables rapid functional genomics to interrogate the complex signaling pathways regulating embryonic lung specification. We have defined a preliminary gene regulatory network consisting of a series of reciprocal paracrine signaling events between the splanchnic mesenchyme and the foregut endoderm that coordinates both local lung inducing signals and the competence of the epithelium to respond to those signals. We are confirming key components of this model with mouse genetics, which suggest that genetic pathways controlling lung development are highly conserved from amphibians to mammals. Finally we are using this knowledge to direct the differentiation of lung progenitors from human iPS cells. These results inform our understanding of congenital defects and should impact strategies to direct the differentiation of respiratory lineages from stem cells.

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Cell behaviors associated with somite formation in *Xenopus laevis*. Carmen Domingo, Armbien Sabillo, Vanja Krneta, Daniel Saw, Ceazar Nave, Hernando Vergara, Julio Ramirez. Biology, San Francisco State University, San Francisco, CA.

Somite formation is a critical step during early development as it gives rise to skeletal muscle, vertebrae and dermis as well as establishes the segmented body plan of the animal. To understand the cell behaviors underlying this process we used a fate map approach to follow the trajectory of pre-somitic cells beginning in the gastrula and ending with the formation of myotome fibers in the *Xenopus laevis*tadpole. We show that pre-somitic cells initially adjacent to the prospective notochord form myotome fibers within the central domain of somites positioned along the entire anteroposterior axis. These cells undergo convergent and extension cell movements much like pre-notochord cells. In contrast, cells positioned in the lower lip region of the gastrula migrate to the posterior presomitic mesoderm (PSM). These cells will eventually form myotome fibers positioned ventrally and dorsally within trunk and tail somites. Using a morpholino knockdown approach we show that the chemokine, stromal derived factor-1α (sdf-1α) and its receptor, cxcr4, are important for the movement of cells from the lower lip region of the gastrula to the PSM. Furthermore, we show that the sdf-1α signaling pathway is also important for somite rotation and myotome alignment. Lastly, using a cell-transplantation approach, we show that signals within the PSM at late neurula stages are essential for proper myotome formation in the tadpole.

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The Role of Heparan Sulfate Nanostructures in Morphogen Gradient Formation and Signaling Reception. Masanori Taira[2], Yusuke Mii[1], Takayoshi Yamamoto[2], Shinji Takada[1]. 1) NIBB; 2) Univ. of Tokyo.

Morphogens are essential for various developmental processes. Our data suggest that Wnt ligands on the cell membrane exhibit dot-like distributions, which co-localize with dot-like structures of heparan sulfate (HS) with N-sulfo modification, called N-sulfo heparan sulfate nanostructures (HSNSs), whereas N-acetyl HSNSs colocalize a secreted Frizzled-related protein (sFRP), an Wnt inhibitor/expander, thereby generating wider Wnt distribution through its association with a Wnt ligand. We will present recent progresses in investigation of the molecular nature of HSNSs.

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X-ray phase contrast microtomography: 4D livecell imaging of structural development. Ralf Hofmann. Karlsruhe Institute of Technology.

We explain a novel imaging modality for in vivo time-lapse tomography of development which is based on coherent and monochromatic, hard synchrotron radiation and which continuously images the electron density throughout the embryo at micron-range spatial resolution. The focus is on gastrulation in Xenopus l., but we'll also demonstrate applicability to neurulation and early-stage zebrafish development. We discuss various modes of image analysis (global and differential optical flow, cell trajectories, volumetry of cavities, motion based segmentation, etc.) to address specific aspects of cell and tissue dynamics such as propulsion (convergent extension), fluid exchange (pumping), and transient structure (confrontation of head and ventral mesendoderm). Also, a brief view on our present understanding of X-ray dose expression is provided. Finally, we give an outlook

on present research concerning maturation, emigration, migration of and invasion of target tissue by cranial neural crest cells in Xenopus l. which requires the attainment of a substantially higher spatial resolution.

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Regulation of neurogenesis with Sox transcription factors and F-box-mediated protein degradation. Elena Silva Casey, Niteace Whittington, Banu Saritas-Yildirim, Doreen Cunningham, Jing Jin. Dept of Biology, Georgetown University, Washington, DC.

Neurons are generated from proliferating progenitor cells that initially span the entire neural plate and later reside in the ventricular zone of the CNS. When the differentiation program is initiated, progenitors exit the cell cycle, migrate out of the proliferation zone and differentiate into neurons. The regulatory protein profile of the neural cells change to drive their progression through these steps. These changes are controlled in part at the transcriptional level but as neural cells progress to their final fate, regulatory proteins must also be inactivated and/or removed. To investigate both the transcriptional regulation and protein degradation required for neurogenesis, we have investigated the role of SoxB and C transcription factor family members and the F-box E3 ubiquitin ligases. With gain and loss of function studies, we discovered that the SoxB2 protein Sox21 and the SoxC protein Sox11 have dose and temporal dependent roles in neurogenesis. Sox protein target specificity is dependent on partner proteins and using both a candidate approach and mass spectrometry, we are identifying Sox11 and Sox21 partners that play a role in their context dependent functions. To investigate the role of protein degradation in altering the neural cell protein profile, we used an in silico screen for REST/NRSF (neuron-restrictive silencing factor) targets and identified 4 neuron specific F-box proteins. Fbox proteins are components of the SCF family of ubiquitin ligases and target proteins for ubiquitin-mediated protein degradation. Our data demonstrate that to regulate neurogenesis and maintain a balance of proliferating neural progenitors and neurons, a coordination of selective protein degradation mediated by F-box proteins and transcriptional regulation by Sox proteins is required.

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Cadherin-11 localizes to focal adhesions and promotes cell-substrate adhesion. Jubin Kashef[1*], Rahul P Langhe[1], Tetyana Gudzenko[2], Sarah F Becker[1], Carina Gonnermann[2], Claudia Winter[1], Michael Bachmann[3], Clemens M Franz[1,4]. 1) Zoological Institute, Karlsruhe Institute of Technology (KIT), Cell and Developmental Biology, Kaiserstr. 12, 76131 Karlsruhe, Germany; 2) Center for Functional Nanostructures, Karlsruhe Institute of Technology (KIT), Wolfgang-Gaede-Strasse 1a, 76131 Karlsruhe, Germany; 3) Zoological Institute, Karlsruhe Institute of Technology (KIT), Cell and Neurobiology Biology, Haid-und-Neu-Strasse 9, 76131 Karlsruhe, Germany; 4) Institute for Photon Science and Synchrotron Radiation, Karlsruhe Institute of Technology (KIT), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany.

Cadherins represent a family of Ca2+-dependent glykoproteins mediating cell-cell adhesion processes. Despite their high structural similarity the function of different types of cadherins is considerably diverse. For instance, epithelial cadherins such as E-Cadherin mediate cell-cell adhesion, while mesenchymal cadherins like Cadherin-11 promote cell migration. However, the mechanisms how mesenchymal cadherins promote cell migration are still poorly understood. Here, we unravel an unexpected function for Cadherin-11 in mediating cell-substrate adhesion during Xenopus cranial neural crest (CNC) migration. By using Total Internal Reflection Fluorescence (TIRF) microscopy we can show that in addition to its localisation at the cell-cell contacts Cadherin-11 is also found in focal adhesions at the cell-substrate surface. Interestingly, within these focal adhesions Cadherin-11 is co-localized with focal adhesion markers like Paxillin and b1-integrin, indicating a novel function in cell-substrate adhesion. Indeed, single cell force spectroscopy with Cadherin-11 morphant CNC cells revealed reduced cell-substrate adhesion. In reconstitution experiments we identified the transmembrane and the cytoplasmic domain of Cadherin-11 to be necessary for this novel cadherin function. We suggest, that this Cadherin-mediated cell-substrate adhesion depends on the interaction of Cadherin-11 and Syndecan-4, a heparan sulfate proteoglycan, which binds to fibronectin and mediates cell-substrate adhesion.

Session 11

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Regulation of IP3-dependant calcium signalling during early embryogenesis. Karel Dorey, Tomasz Gwozdz. The Healing Foundation Centre, The University of Manchester, United Kingdom.

During embryogenesis, a small number of signalling molecules control a vast array of biological responses. How this is achieved is a major question in developmental biology and different mechanisms have been proposed. These include crosstalk of different pathways, multiple positive and negative feedback loops or the ability of a signal to stimulate different intracellular signalling cascades. For example, Receptor Tyrosine Kinase (RTK) activates three intracellular pathways: the Ras/Erk pathway (proliferation and differentiation), the PI3K/Akt pathway (survival) and the PLCg/Ca^{2+} pathway (cell movements).Whilst the regulation of the Erk and Akt pathways are well understood, much less is known about the mechanisms regulating Ca^{2+} signalling, despite its importance in controlling cell movements (during gastrulation) or cell morphology (in neurons). Sprouty family members (Sprys) are negative regulators of RTK important for the regulation of cell migration during gastrulation, morphogenesis of multicellular organs (such as the lungs or kidney) and axonal branching in neurons. We and others

have shown that Sprys are negative regulator of Ca^{2+} signalling downstream different RTKs (FGFR, TrkB or VEGFR) by a yet unknown mechanism.Using naïve animal cap cells, fully differentiated Xenopus neurons and cells in culture, we have started to elucidate the mechanisms by which Spry regulate Ca^{2+}signalling. Our results show that RTKs can elicit A Ca^{2+} response via two distinct mechanisms: a PLCg-dependant manner or by activating voltage-gated channels. However, Sprys specifically inhibit PLCg-dependant Ca^{2+} release. Surprisingly, Sprys can also inhibit Ca^{2+} release downstream of G-Protein Coupled Receptors (GPCRs) raising the intriguing possibility that Spry could coordinate the ability of different signalling pathways to induce Ca^{2+} release from intracellular stores during embryogenesis. Using live imaging, structure/function analyses and functional assays we are now establishing the biological relevance of these data.

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Cellularization of a Cell-Free System to Study Mechanisms of Organelle Scaling. Jay Gatlin[1], James Hazel[1], Kaspars Krutkramelis[2], John Oakey[2], Dan Levy[1]. 1) Molecular Biology, University of Wyoming, Laramie, WY; 2) Chemical & Petroleum Engineering, University of Wyoming, Laramie, WY.

Cell-free extract derived from *Xenopus laevis* eggs is a well-established model system for studying a variety of cell biological processes. The open nature of this system confers several advantages to the experimentalist and makes it especially tractable to many biochemical and biophysical approaches. Here we describe recent results that demonstrate the utility of "re-cellularizing" these extracts. Using newly developed microfluidics-based platform we were able to encapsulate extract into discrete volumes of defined size and shape. This advance enabled us to address fundamental questions regarding the relationship between cytoplasmic volume and the sizes of organelles assembled within it. We found that changes in cytoplasmic volume, not shape, are sufficient to account for the scaling of the mitotic spindle as it is observed during early *X. laevis* development. Additionally, we found that steady-state nuclear size also scales with extract volume. Both results are consistent with a component limitation model, whereby organelle size is determined passively by limited availability of some integral structural component.

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Polycomb regulation of retinal progenitor proliferation and differentiation. Monica L Vetter, Issam Aldiri, Kathryn Moore, David Hutcheson, Zhang Jianmin. Dept of Neurobiology & Anatomy, University of Utah, Salt Lake City, UT.

The histone methyltransferase complex PRC2 controls key steps in developmental transitions and cell fate choices by catalyzing the trimethylation of histone H3 at lysine 27 (H3K27me3), which is a repressive histone modification. We investigated the role of PRC2 during Xenopus retinal development. We found that the PRC2 core components are enriched in retinal progenitors and are downregulated in differentiated cells. Knockdown of the PRC2 core component Ezh2 leads to reduced retinal progenitor proliferation, in part due to upregulation of the Cdk inhibitor p15(Ink4b). PRC2 knockdown did not alter eye patterning, retinal progenitor gene expression or expression of the neural competence factor Sox2. However, it did cause suppression of proneural bHLH gene expression, indicating that PRC2 is crucial for the initiation of neural differentiation in the retina. Consistent with this, knocking down or blocking PRC2 function constrained the generation of most retinal neural cell types and promotes a Müller glial cell fate decision. We also show that Wnt/β-catenin signaling acting through the receptor Frizzled 5, but independent of Sox2, regulates expression of key PRC2 subunits in the developing retina. This is consistent with a role for this pathway in coordinating proliferation and the transition to neurogenesis in the Xenopus retina.JARID2 is an accessory component of PRC2, and has been shown to play a role in the recruitment of PRC2 to target genes that are silenced during embryonic stem (ES) cell differentiation. We are now investigating the expression and function of JARID2 during Xenopus eye development to determine how it modulates PRC2 function in vivo, or whether it has non PRC2-dependent activities.

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A systems-level dissection of multiciliated cell specification. Ian Quigley, Lina Ma, Chris Kintner. Molecular Neurobiology Lab, Salk Institute, La Jolla, CA.

The multiciliated cell, a cell type found in the mammalian airway, brain, female reproductive tract and elsewhere, is critical for producing fluid flow in these tissues. Multiciliated cell development requires two distinct processes: the massive duplication of centrioles (from 2 to ~200) and the extension of long, motile cilia from these centrioles. However, we lack a complete understanding of how these two transcriptional programs are controlled and intersect during development. Here, we establish the transcriptome of the developing multiciliated cell by disrupting the number of these cells in Xenopus skin with a variety of perturbations and performing RNAseq on each. We then obtain all active promoters in Xenopus ectoderm with H3K4me3 ChIPseq and, with an unbiased search of the contents of promoters flanking multiciliated cell genes, demonstrate dramatic enrichments of motifs corresponding to the rfx, e2f, and forkhead transcription factor families. To determine the genomic targets and genes regulated by some of these factors, we perform ChIPseq on the cell cycle regulator e2f4 and the forkhead transcription factor foxj1, and perform RNAseq on caps with those genes perturbed. We show that in multiciliated cells, e2f4 changes its binding preference away from regulatory elements flanking canonical cell cycle genes and towards those near genes in centrioles, promoting the transcription of centriole components. Incorporating published rfx2 ChIPseq data, we show that foxj1 rarely binds to promoters in the absence of rfx2, but that rfx2 binding alone does a poor job of promoting transcription of multiciliated cell genes. Finally, we show that e2f4 binds to the promoters of genes found in motile cilia and that foxj1 binds to the promoters of genes in centrioles, hinting at an underappreciated overlap between the two transcriptional programs.

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A novel genetically tractable tumor model for Familial Adenomatous Polyposis using TALEN-mediated gene disruption in *Xenopus tropicalis*. **Kris Vleminckx[1,2]**, Thomas Naert[1], Griet Van Imschoot[1], Ellen Sanders[1,3], Frans Van Roy[1,3], Hong Thi Tran[1], Tom Van Nieuwenhuysen[1]. 1) Dept. Biomed. Mol. Biol., Ghent University, Ghent, Belgium; 2) Centre for Medical Genetics, Ghent University, Ghent, Belgium; 3) Inflammation Research Centre VIB, Ghent, Belgium.

Familial Adenomatous Polyposis (FAP) is an autosomal dominant disorder characterized by the presence of hundreds to thousands of benign, adenomatous polyps in the colon, which may progress into adenocarcinomas. FAP is caused by a germline mutation in the APC gene. Similar mutations are found in sporadic colorectal cancer. In FAP patients, adenoma formation is frequently accompanied by extra-colonic manifestations such as desmoid tumors. The APC protein is a negative regulator of the Wnt/β-catenin pathway by mediating destruction of the effector protein β-catenin in the absence of a Wnt stimulus. Mutations in the APC tumor suppressor gene typically result in the expression of a truncated protein and subsequent accumulation of the β-catenin protein. This results in constitutive activation of the Wnt/β-catenin pathway associated with the hyper-induction of several Wnt-responsive target genes that ultimately lead to tumor formation.The emerging TALEN and CRISPR/Cas9 techiques offer unmatched experimental opportunities for genome editing, allowing true reverse genetics in organisms other than the mouse. Using TALENs we developed two novel APC/β-catenin tumor models in the genetically tractable model organism *Xenopus tropicalis*. Several tumors and abnormalities that are found in FAP patients, are also observed at early stage (tadpole) and in high frequency in the *Xenopus* tumor model, including intestinal neoplasia, desmoid tumors and medulloblastomas. We are using this novel cancer model to get a better understanding in colorectal cancer and especially in the etiology of desmoid fibromatosis found in FAP patients. This work should identify novel pathways and strategies to treat desmoid fibromatosis and possibly other cancers associated with mutational activation of the Wnt/β-catenin pathway. Moreover, our work will form the basis for furthering the use of *Xenopus* as a principal organism for modeling human cancer.

Session 12

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A role for reactive oxygen species in regeneration and early embryogenesis. **Enrique Amaya**, Nick R Love, Yaoyao Chen, Yue Han, Shoko Ishibashi, Saori Hokari, Rob Lea. The Healing Foundation Centre, The University of Manchester.

A major goal in regenerative medicine is to understand and ultimately facilitate our body's ability to repair itself following injury. As a first step toward this goal, we are investigating the molecular and cellular mechanisms responsible for scarless wound healing and tissue regeneration in frog embryos and tadpoles. A few years ago, we set out to identify the changes in the transcriptome, during tadpole tail regeneration in Xenopus tropicalis. That study uncovered a number of coordinately modulated genes, implicated in the production of reactive oxygen species (ROS). Consistent with this finding, we found that regenerating tadpole tails induce a sustained production of reactive oxygen species (ROS) during the entire regenerative response. We then used genetic and pharmacological approaches (including the addition of antioxidants) to show that lowering ROS levels significantly impaired tail regeneration. These finding were unexpected as antioxidants are often thought to be beneficial to health. More recently we have found remarkable parallels in the induction, maintenance and role of reactive oxygen species during tissue regeneration and early embryonic development. Thus, we postulate that the production of reactive oxygen species following injury promotes a return to an embryonic-like state of cellular oxidation, which facilitates growth factor signaling and tissue growth. These findings further suggest that manipulating ROS levels may provide a means to induce a sustained regenerative response in animals that normally have poor regenerative capacity, such as humans.

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Nuclear Mechanics: An F-actin scaffold and gravitational stabilization of RNA/Protein Droplets in Large Cells. **Clifford Brangwynne**, Marina Feric. Chemical and Biological Engineering, Princeton University, Princeton, NJ.

Ribonucleoprotein (RNP) granules are membrane-less organelles assembled from the dynamic interactions of RNA and protein. We have shown that many of these structures behave as liquid phase droplets, which assemble from soluble RNA and protein by an intracellular phase transition. The nucleolus is an RNP droplet that functions in ribosome biogenesis, and thus plays a central role in cell growth and size control. This connection to cell size manifests in the *X.laevis* oocyte, a very large (~1mm) single cell that assembles hundreds of extra-chromosomal nucleoli, which are distributed throughout the nuclear volume. We show that nuclear F-actin forms a network that spatially organizes the nucleus by kinetically stabilizing an emulsion of these nucleoli; in actin-disrupted oocytes, embedded nucleoli and other RNP droplets undergo gravitational sedimentation and fusion at the bottom of the nucleus. Using several complementary micro-rheology approaches, together with quantitative imaging, we probe the length-scale dependent architecture and mechanics of these stabilized biological emulsions. We show how these measurements provide important insights into the geometry and mechanics of intracellular organization, and suggest that biophysical constraints play a role in cell growth and size control.

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Sox5 modulates TGF-β signaling during early embryogenesis to correctly establish ectodermally derived tissue lineages. Kara Nordin[1], Carole LaBonne[1,2]. 1) Molecular Biosciences, Northwestern University, Evanston, IL; 2) Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL.

Formation and patterning of the germ layers, including the ectoderm, is controlled by a host of signaling events that function to progressively restrict cell potential. TGF-β signaling plays a central role in the formation of all three germ layers, with the BMP branch of this pathway being of particular importance in the ectoderm. High levels of BMP signaling are believed to instruct ectodermal fates, while modulation of BMP signaling levels by secreted antagonists leads to formation of the CNS and the neural plate border. Here we show that BMP signaling is also required in *Xenopus* for the pluripotency of blastula animal pole cells prior to the onset of cell fate restriction.The nuclear effectors of TGF-β signaling are Smad proteins, DNA binding proteins that require partners to direct their target specificity. While partners for the Nodal R-Smads, Smad2/3, that function during germ layer formation have been well characterized, much less is known about partners for Smad1/5, the BMP R-Smads, during very early developmental events.Here we demonstrate that the SoxD family protein, Sox5, functions as a DNA binding partner for BMP R-Smads regulating plasticity and pattern in the early ectoderm. We show that Sox5 is expressed throughout pluripotent animal pole cells and subsequently maintained at the neural plate border and in the neural crest. Sox5 deficient embryos display patterning defects characteristic of perturbations of BMP signaling including a loss of epidermal and border fates and an expansion of the neural plate. We demonstrate that Sox5 is essential for BMP signaling, and that it binds Smad1/4 to direct BMP target gene specificity. Finally, our findings provide support for a model in which some of the same extrinsic and intrinsic factors that control the pluripotency of early blastula cells are maintained at the neural plate border where they contribute to the unique properties of neural crest cells.

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Reversible control of GSK-3 in the neural crest. Sandra Gonzalez Malagon, Anna Lopez Munoz, Triona Bolger, **Karen Liu**. Craniofacial Development and Stem Cell Biology, King's College London, London, United Kingdom.

Glycogen synthase kinase 3 (GSK-3) is a highly conserved serine/threonine protein kinase which controls key developmental programmes. GSK-3 proteins have established roles during neural crest induction and epithelial-to-mesenchymal transitions. However, subsequent roles for GSK-3 during neural crest development are difficult to study. Pharmacological inhibition of GSK-3 in Xenopus and mouse neural plate explants is sufficient to inhibit neural crest cell migration without perturbing neural crest induction. Furthermore, spatial and temporal regulation of GSK-3 in mouse and frog embryos uncovers a β-catenin independent role for GSK-3 in migratory neural crest cells, likely through control of cytoskeletal dynamics.

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Post-transcriptional regulation of left-right axis formation. Maike Getwan, Axel Schweickert, Martin Blum. University of Hohenheim, Stuttgart, Germany.

In vertebrates most inner organs are placed asymmetrically along the left-right axis. The basis for this is a highly conserved symmetry breakage cascade during neurulation. Central to this process is an epithelium of mesodermal fate that develops at the posterior pole of the notochord. In *Xenopus laevis* it is represented by the gastrocoel roof plate (GRP), in mammals by the posterior notochord and in teleost fish by the Kupffer's vesicle. Cells in all cases bear polarized monocilia. They rotate in a clockwise manner and produce a fluid flow directed to the left side. The flow-sensing lateral cells of the GRP express two genes, the growth factor *Xnr1* (*Nodal*) and its inhibitor *Coco* (*Cerl2* in mice, *charon* in fish). During symmetry breakage *Coco* mRNA is partially down-regulated through flow on the left side. As a result Xnr1 repression is released, which in due course leads to left-asymmetric induction of the Nodal gene cascade in the lateral plate mesoderm.It is presently unclear by which flow-dependent mechanism Coco is degraded. However the expression pattern of *Coco* led to the suggestion that it should be a post-transcriptional event. This hypothesis was verified by microinjections of *Coco* 3'UTR antisense RNA (whole sequence and fragments of it), which should protect endogenous *Coco* mRNA. Indeed especially one fragment prevented the left-sided down-regulation of *Coco*. Sequence analysis showed the presence of a microRNA-15 binding site. To prove the general involvement of microRNAs in the regulation of *Coco* we used a morpholino oligonucleotide (MO) against the miRNA processing enzyme Dicer. We further tested the importance of the miRNA-15 binding site with a protector MO. Both interfered with the down-regulation of *Coco* on the left side.In conclusion we demonstrate that symmetry breakage is regulated post-transcriptionally via down-regulation of the Nodal antagonist Coco by microRNAs.

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Molecular studies of spinal cord regeneration in the axolotl using over-expression and CRISPR-mediated knock out approaches. Elly Tanaka, Aida Rodrigo Albors, Jifeng Fei, Akira Tazaki. DFG Center for Regenerative Therapies Dresden, Technische Universität Dresden, Dresden, Saxony, Germany.

The axolotl has very similar embryology and developmental aspects as Xenopus and represents a robust model for studying regeneration of complex body structures. We have invested into the molecular genetic development of the axolotl system to study limb and spinal cord regeneration. Here I describe our work on spinal cord regeneration. We have combined the use of germline transgenic animals and tissue grafting to identify the source cells of regeneration. Tail amputation results in a cell cycle acceleration of the slowly dividing neural stem cell source pool. We show that BMP signalling is involved in this cell cycle acceleration using electroporation to overactivate and inhibit the BMP pathway. We have further shown that induction of the

planar cell polarity pathway in this source cells is required for inducing oriented cell divisions, and the propogation of a self-renewing state in these stem cells. Finally, using CRISPR-mediated deletions, we show that deletion of Sox2, a transcription factor assciated with neural stem cells yields a spinal cord specific regeneration phenotype. We find that Sox2 is required for the regeneration-specific cell cycle acceleration during the rapid amplification of neural stem cells during regeneration.

Session 13

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Genome-wide view of Nodal/Foxh1 regulation of the early mesendoderm program. Ken Cho[1], Rebekah Charney Le[1], Ira Blitz[1], Yi Li[2], Jacob Biesinger[2], Xiaohui Xie[2], William Chiu[1]. 1) Developmental and Cell Biology, University of California, Irvine, CA; 2) Computer Science, University of California, Irvine, CA.

Our goal is to elucidate the mechanisms controlling endoderm formation by combining experimental and computational approaches. Experimentally, we obtain genome-wide datasets that consist of both "inputs" detailing how endodermal genes are controlled, and "outputs", which are the changes in gene activity in response to perturbation of the inputs. Nodal/Activin signaling is critical for the proper induction of mesoderm and endoderm, and the Foxh1 transcription factor (TF) and receptor-activated Smad2/4 complex are key mediators of Nodal signaling. Therefore, we examined their relative contributions and dynamic activities in mediating Nodal signaling. By combining RNA-seq on Foxh1 and Nodal signaling loss-of-function embryos, with ChIP-seq of Foxh1 and Smad2/3, we provide the following comprehensive genome-wide view of Nodal signaling action. First, our analysis significantly increases the total number of "direct" Nodal target genes regulated by Foxh1 and Smad2/3 and reinforces the notion that Foxh1-Smad2/3-mediated Nodal signaling directly coordinates the expression of a cohort of genes involved in the control of gene transcription, signaling pathway modulation, and tissue morphogenesis during gastrulation. Second, the role of Foxh1 is much more complex than anticipated - not only is it involved in mediating Nodal signaling via Smad2/3, but also has novel dual (activation and repression) functions that are independent of Nodal signaling. Third, while hundreds of Foxh1 binding sites are commonly present throughout early blastula to gastrula stages, many Foxh1 bound regions are also stage-specific, suggesting that Foxh1 has both common and unique regulatory roles at different developmental stages. By combining all these data, we aim at examining the dynamic interplay between Foxh1 TF and the chromatin landscape. Thus, our goal is to uncover changes, both spatially and temporally, in the regulation of chromatin accessibility that manifest the progress of endodermal cell fate specification.

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Cyclin-dependent Kinase Constrained Cortical Chaos. William (Bill) Bement[1], George von Dassow[2], Andrew Goryachev[3], Ann Miller[4], Matt Larson[1], Angela Kita[1], Alison Moe[1], Adriana Golding[1]. 1) University of Wisconsin-Madison, Madison, WI; 2) Oregon Institute for Marine Biology-Charleston, OR; 3) University of Edinburgh, Edinburgh, Scotland; 4) University of Michigan-Ann Arbor, MI.

Cytokinesis in animal cells results from activation of the small GTPase Rho in a discrete zone at the plasma membrane that directs the formation of the cytokinetic apparatus, a structure based on actin filaments (F-actin) and myosin-2. Cytokinesis is normally restricted to a discrete portion of the cell division cycle that follows anaphase onset and in which the cortex is responsive to signals arising from the spindle. Here we show that shortly after anaphase onset frog and echinoderm cells exhibit waves of Rho activity and F-actin polymerization that traverse the cortex. Waving is suppressed by high cyclin dependent kinase 1 (Cdk1) and promoted by the RhoGEF, Ect2. Remarkably, the F-actin waves antagonize the Rho activity waves. Empirical and modeling results show that waving represents a form of excitability which can develop into spiral chaos, manifest as repeating patterns of Rho waves. We propose that the cell cortex is uniquely responsive to signals from the spindle after anaphase due to development of excitability.

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The *cdc20b*/miR-449 locus controls multiciliogenesis in *Xenopus* embryonic epidermis. Laurent Kodjabachian[1], Anna Adamiok[1], Diego Revinski[1], Benoit Chevalier[2], Laure-Emmanuelle Zaragosi[2], Andrea Pasini[1], Brice Marcet[2], Pascal Barbry[2]. 1) IBDM, CNRS, Aix-Marseille University, Marseille, France; 2) IPMC, CNRS, Nice Sophia-Antipolis University, Sophia-Antipolis, France.

The *Xenopus* embryonic epidermis has proven an excellent model to study the biogenesis of mucociliary epithelia, such as the one lining human upper airways. In particular, it has become evident that multiciliated cells (MCCs) in both tissues form in response to similar molecular cues. A striking example has emerged with the discovery of a conserved locus containing three genes co-expressed in MCCs, called *multicilin*, *ccno* and *cdc20b*. The first two genes have been shown to be required for multiciliogenesis of frog epidermal MCCs. We now report that *cdc20b* also encodes a protein important for MCC biology. The CDC20B protein localizes in the deuterosome, a structure required for centriole multiplication in MCCs, as well as in basal bodies. *cdc20b* knockdown reduces the number of basal bodies, impairs their apical docking and consequently causes defective ciliogenesis. Interestingly, the second intron of *cdc20b* hosts the miR-449 triplet of microRNAs that was also shown to be required for multiciliogenesis in frog and human MCCs. Our data suggest that miR-449 coordinately down-regulate multiple

transcripts and thus control discrete steps of MCC differentiation including centriole multiplication and apical actin web assembly. The conserved *cdc20b*/miR-449 locus thus harbors multiple complementary functions that collectively allow the complex process of multiciliogenesis to unfold.

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Analysis of newly-synthesized proteins during brain development and experience-dependent plasticity in *Xenopus laevis*. Hollis T Cline[1,3,4], Han-Hsuan Liu[1,3,5], Wanhua Shen[2], Lucio Schiapparelli[1,3], Danial McClatchy[1,4], John R Yates III[1,4]. 1) The Scripps Research Institute, La Jolla, CA; 2) Institute of Developmental and Regenerative Biology, College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou, Zhejiang, China; 3) The Dorris Neuroscience Center, Department of Molecular and Cellular Neuroscience; 4) Department of Chemical Physiology; 5) Kellogg School of Science and Technology.

Brain development and experience-dependent synaptic plasticity, the cellular basis of learning and memory, are dynamic at both transcriptial and translational levels. We are interested in identifying proteins that are newly-synthesized during specific time windows of brain development and how protein synthesis is regulated in response to changes in visual experience. Unbiased investigation of global protein synthesis is challenging due to the lack of available techniques. We adapted the fluorescent or biotin-tagging of non-canonical amino acid (FUNCAT and BONCAT), which are nonbiased methods to label and identify newly-synthesized proteins, to examine newly-synthesized proteins in *Xenopus laevis* tadpole brain. Using FUNCAT, we found that newly-synthesized proteins are ubiquitous in the tadpole brains but the fluorescence intensity was higher in SOX2+ progenitor cells than neurons. Pentylenetetrazol (PTZ), a GABA receptor antagonist, which elevates brain activity and induces seizure, increased protein synthesis in the brain. For MS/MS proteomics, we developed a method for direct detection of biotin-tagged peptides which increases the sensitivity of detection of newly-synthesized proteins. This improvement allowed us to identify directly proteins that are newly-synthesized in response to visual experience-dependent plasticity compared to control animals.

Supported by MH091676, MH099799, NS076006 and W81XWH-12-1-0207 to HTC and P41 GM103533 to JRYIII).

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Protein tyrosine kinase 7 (PTK7) modulates Wnt signaling activity via LRP6 in the developing neural plate. Hava Lichtig, Naama Bin-Nun, **Dale Frank**. Department of Biochemistry, Faculty of Medicine - Technion, Haifa, Israel.

Protein tyrosine kinase 7 (PTK7) is a transmembrane protein expressed in the developing *Xenopus* neural plate. PTK7 regulates vertebrate planar-cell-polarity (PCP), controlling mesodermal and neural convergent-extension (CE) cell movements, neural crest migration and neural tube closure in vertebrate embryos. Besides CE phenotypes, we now show that PTK7 protein knock-down also inhibits canonical Wnt activity. Canonical Wnt signaling caudalizes the neural plate via direct transcriptional activation of the *meis3* TALE-class homeobox gene, which subsequently induces neural CE. PTK7 controls *meis3*gene expression to specify posterior tissue and downstream PCP activity. Furthermore, PTK7 morphants phenocopy embryos depleted for Wnt3a, LRP6 and Meis3 proteins. PTK7 protein depletion inhibits embryonic canonical Wnt signaling by strongly reducing LRP6 protein levels. LRP6 protein positively modulates canonical Wnt, but negatively modulates Wnt/PCP activities. We suggest that the maintenance of high LRP6 protein levels by PTK7 can trigger PCP inhibition. PTK7 and LRP6 proteins physically interact, suggesting that PTK7 stabilization of LRP6 protein reciprocally regulates both canonical and non-canonical Wnt activities in the embryo. Recent studies in cancer cells have shown that PTK7 protein undergoes proteolysis to smaller isoforms that are localized to the cytoplasm and nucleus. Very little is known about the physiological function of these proteins. We are investigating the role of these different PTK7 protein isoforms protein to regulate Wnt signaling during neural plate specification and folding in early *Xenopus* development.

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Role of lbh during Xenopus Craniofacial Development. Helene Cousin[1], Gretchen McLinden[1], Rebekah Pack[2], Kara Powder[2], Craig Albertson[2]. 1) Vet. & Animal Sciences department, UMass- Amherst, Amherst, MA; 2) Biology department, UMass- Amherst, Amherst, MA.

The *lbh* gene encodes a transcription factor expressed in the **l**imb **b**ud and **h**eart of the developing mouse embryo. It is also expressed in the first branchial arch, embryonic precursor of facial structures. While *lbh* function during limb and heart development has been investigated, nothing is known about *lbh* role during craniofacial development. We have shown that *lbh* knock down in zebrafish lead to severe craniofacial defects without affecting chondrogenesis. To understand the origins of these defects, we are investigating the role of *lbh* using a multiple species approach. Here we describe the three main findings we uncovered in the amphibian *Xenopus laevis*.First, *lbh* is expressed in the cranial neural crest cell before and during their migration and is down regulated after their migration. Second, *lbh* knock down using translational morpholino inhibits CNC migration but not induction. Third, the misexpression of Xenopus *lbh* induces a very modest inhibition of CNC migration while the misexpression of a long-jawed (*Metriaclima zebra*) and short-jawed (*Labeotropheus fuelleborni*) lake Malawi cichlid *lbh* produces a significant change in the number and the quality of the migration of the cranial neural crest. These observations could help us understand the mechanisms behind the craniofacial developmental plasticity and the microevolution of lake Malawi cichlids.

Session 14

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A novel control mechanism to enable adaptive morphogenesis. Lance Davidson. Departments of Bioengineering, Developmental Biology, and Computational and Systems Biology, University of Pittsburgh, Pittsburgh, PA.

Programs that regulate early development must be able to adapt to variation in maternal contributions as well as environmental conditions. To adapt morphogenetic movements to variations in mechanical conditions the embryo must be able to sense and modulate the physical processes that drive those movements. Through recent studies on mechanisms that regulate contractility in the embryonic epithelium we have uncovered a feedback loop whereby external strain in the gastrulating embryo drives the apical release of extracellular ATP which is sensed by G-protein coupled receptors of the P2Y family. Cells receiving these signals respond autonomously by contracting but also transmit a "contractile signal" to regions 5 to 8 cells distant. Hyperactivated P2YR leads chronically contracting epithelial to stiffen; P2YR-MO injected embryos no longer respond to ATP and soften. Both hyperactivated and P2YR morphants encounter severe defects in epiboly and blastopore closure. We hypothesize that extracellular ATP and apically localized receptors play a key role in maintaining basal levels of mechanical tension in the epithelium and enable the embryo to respond rapidly to changing conditions during development.

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Shaping Kidney Tubules through Planar Cell Polarity Signaling and Cilia. Rachel Miller[1,2,3,4], Malgorzata Kloc[5], Tanya Baldwin[2], Le Huang[2,3,6], Andrew Gladden[2,3,6], Vicki Huff[2,3,6], Pierre McCrea[2,3,4]. 1) Pediatrics- Research Center, The University of Texas Medical School at Houston; 2) Grad School of Biomedical Sciences; 3) Grad Program in Genes & Development; 4) Dt of Biochemistry & Molecular Biology, MD Anderson Cancer Center; 5) Immuno-Biology Laboratory, Methodist Hospital Research Inst; 6) Dept of Genetics, MD Anderson Cancer Center.

Wnt ligands are expressed in the developing kidney in amphibians and mammals. Canonical and noncanonical Wnt pathways are linked to kidney diseases such as Wilms tumor, polycystic kidney disease and nephronophthisis. Prior work from our group and others indicates that canonical/β-catenin signaling is essential to nephric tubulogenesis. Noncanonical pathways contribute to processes such as cell polarization and cytoskeletal control, but the specifics of their involvement in nephrogenesis remain in question. Here, using *X. laevis*, we tested the roles of downstream noncanonical Wnt components in kidney tubule morphogenesis. One of the planar cell polarity (PCP) branches of the noncanonical Wnt pathways, namely the Daam1/WGEF/Rho trajectory, is assessed in the current study. Daam1, a formin protein, and WGEF, a Rho GEF, are expressed in the developing nephron during tubulogenesis. Knockdown of Daam1 or WGEF using antisense morpholinos, or inhibition of the pathway using dominant negative Daam1 or Rho, results in reduced nephric tubulogenesis. Our preliminary data further indicate that Daam1 and WGEF are important for forming cilia in embryonic skin and kidney, as well as in polarized MDCK cells. We are beginning investigate the mechanisms by which Daam1 is involved in ciliogenesis, which may lead to its functional role in nephrogenesis. Our preliminary data suggest that Daam1 interacts with TUBA, a Cdc42 GEF, which is component of the Exocyst vesicle trafficking complex involved in ciliogenesis. We are also investigating a mouse model of pediatric Wilms tumors to determine if cilia and PCP are altered, and our preliminary data indicate that ciliogenesis is increased. Together, our data suggest that Daam1/WGEF/Rho PCP signaling is necessary for tubulogenesis within the developing kidney, possibly in part due to effects upon ciliogenesis.

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The Nuclear Proteome of a Vertebrate Oocyte. Martin Wühr[1,2], T Güttler[1], L Peshkin[2], GC McAlister[1], AC Groen[2], R Rad[1], TJ Mitchison[2], SP Gygi[1], MW Kirschner[2]. 1) Department of Cell Biology; 2) Dept of Systems Biology, Harvard Medical School,

The defining eukaryotic organelle, the nucleus, is bounded by the nuclear envelope, which separates nuclear from cytoplasmic activities and allows for complex regulation unavailable to prokaryotes. Despite the nucleus' central role in multi-cellular biology, how proteins partition between nucleus and cytoplasm on a proteome-wide scale is still poorly understood. This lack of understanding is mostly due to the difficulty of isolating nuclei without loss or gain of material, and the challenge to reliably and comprehensively measure proteins' subcellular localization.A remarkable exception for the nuclear isolation problem is the giant Xenopus laevis oocyte which allows manual dissection of nuclear and cytoplasmic contents. With two different methods of accurate multiplexed proteomics (TMT^C and MultiNotch-MS3), this model allowed us to quantify the nucleocytoplasmic partitioning for more than 9000 proteins. Surprisingly, we find a distinctly trimodal distribution. Most proteins are localized exclusively in nucleus or cytoplasm, with a third smaller subset of proteins nearly equidistributed. By measuring the proteins' physiological stokes radii in undiluted cell lysate we found that nearly all proteins with physiological size of up to ~100kDa are equidistributed; by contrast nearly all partitioned proteins are physiologically larger, suggesting that protein aggregation plays an important and so far underappreciated role in proteins' retention within a membrane bound organelle.To test if our method can capture dynamic changes, we inactivated Exportin-1 (Exp1) mediated nuclear export with the inhibitor Leptomycin B. Indeed, we found many established Exp1 cargos to reliably re-localize to the nucleus but also identified ~300 novel putative Exp1 substrates.Thus, we provide the first resource for the quantitative nucleocytoplasmic partitioning of a vertebrate proteome,

measure its dynamics on a proteome-wide scale and these studies shed new light on the mechanisms of subcellular protein localization

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***Xenopus* epigenome and enhancer dynamics.** Saartje Honteleze, Sarita Paranjpe, George Georgiou, Simon van Heeringen, **Gert Jan Veenstra**. Radboud University, Nijmegen, Netherlands.

The state of chromatin reflects genome usage and is essential for pluripotency, competence and cell lineage commitment. Little is known about how chromatin state emerges and is regulated during development. We have generated epigenome reference maps of eight histone modifications, the enhancer co-activator protein p300 and RNA polymerase II at five stages of development in*X.tropicalis*. The epigenome starts in a relatively naive, unmodified state. H3K4me3 and H3K9ac appear hours before the onset of embryonic transcription. During the pluripotent phase of development, enhancer and heterochromatic modifications are relatively underrepresented. Upon cell lineage commitment p300 is more strongly recruited to clusters of enhancers and also heterochromatic modifications become more prominent. In contrast to promoter marking, enhancer activation requires new transcription, linking the maternal-to-zygotic transition to enhancer function. At many loci H3K27 methylation is independent of new transcription, suggestive of a time-dependent autonomous response to inducing signals, independent of actual cell lineage commitment. To shed more light on the selective pressures on the regulatory landscape following whole genome duplication, we have generated maps of histone modifications, RNA polymerase II and p300 in *X.laevis* early gastrulae. Among the duplicate (homeolog) gene pairs we find differential expression, differential enrichment of H3K4me1 and H3K4me3 histone modifications and differential gain/loss of p300-bound enhancers. The data uncover the multi-level selection pressures in the epigenome of the two competing sub genomes of *Xenopus laevis* and show the dynamics of the regulatory landscape on the vastly different time scales of development and evolution.

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Gene Discovery in Birth Defect Patients and Mechanism Disovery in *Xenopus*. Mustafa Khokha. Pediatrics & Genetics, Yale University, New Haven, CT.

Birth defects (congenital malformations) affect 180,000 infants yearly in the US. It is now the major cause of infant mortality in the US and Europe, surpassing prematurity, and the most common cause of non-preventable mortality in childhood. Yet our understanding of the genetic causes of birth defects remains poor. Fortunately, recent advances in human genomics have transformed our ability to analyze these patients with birth defects. These studies frequently identify genes that are either completely novel or novel to developmental biology. In addition, the high degree of locus heterogeneity complicates assigning disease causality. Here, we show that *Xenopus* is an extraordinary system for modeling genetic variants identified in patients with congenital malformations. *Xenopus* is highly cost effective and high-throughput for *in vivo* screening of congenital malformation genes. It reproduces human phenotypes, and enables the discovery of novel biological mechanisms. We present a few examples illustrating the power of this system. We conclude that gene discovery in human disease subjects followed by screening and mechanism analysis in Xenopus is a powerful method for understanding these terrible diseases of childhood.

Behavior

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Learning, memory, and behavior in tadpoles; positioning *Xenopus* as a model for cognitive studies. D. Blackiston, N. Rahman, M. Levin. Tufts University, Medford, MA.

One of the major goals in developmental biology is to link alterations in early neural development with higher level cognitive function. Breakthroughs in this understanding will greatly increase our ability to diagnose the problems underlying human disorders, and potentially lead to their treatment through pharmaceutical, chemical, or molecular methods. *Xenopus laevis* is an ideal model system for such studies, as numerous molecular tools exist to perturb early neural patterning throughout embryogenesis. However, almost nothing is known about the cognitive abilities of these animals. To overcome this barrier, we have created the first associative learning assay with *Xenopus* tadpoles. Using a custom machine vision system we developed an automated program in which a computer, without input from the investigator, can train tadpoles to avoid specific wavelengths of light. Performance of animals improves with repeated training and memory lasts at least 24 hours. Further, we have used this device to examine visual learning in *Xenopus* tadpoles which have had ectopic eyes induced at various positions along the body axis. Results reveal that animals can learn visual tasks using ectopic eyes, even when they are far removed from the head of the animal. Taken together, we present this data as proof of principle for *Xenopus laevis* as a tractable model in behavioral and cognitive studies.

Cell Biology

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POSITIVE AND NEGATIVE MODULATORS OF WNT/β-CATENIN PATHWAY IDENTIFIED BY FUNCTIONAL SCREENING OF NATURAL COMPOUNDS. Jose G Abreu, Barbara Fonseca, Danilo Predes, Nathalia Amado. Program of Cell and Developmental Biology, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil.

Wnt/β-catenin pathway plays essential role in embryogenesis and adult tissue homeostasis. Its deregulation is often related to organ disorder/malfunction, degeneration and cancer. Manipulation of Wnt/β-catenin signaling in Xenopus embryos either by mRNA injection or lithium chloride (LiCl) treatment promotes axial phenotypes. Based on these aspects we performed a functional screening of small molecules of natural origin by combining Wnt/β-catenin specific reporter assay with classical Xenopus embryology experiments.We screened 20 compounds combining two experimental approaches: an *in vitro* assay based on Wnt/β-catenin responsive luciferase reporter and an *in vivo* approach based on phenotypic effects in *Xenopus laevis* embryos at specific stages, where Wnt/β-catenin is essential for dorsal determination. Among twenty substances tested, four showed potential inhibitory activity on the pathway through Wnt3a-responsive luciferase assays. In addition, three substances appear to increase the activity of the pathway, therefore being potential activator compounds. Inhibitory substances of Wnt/beta-catenin partialy rescued LiCl and inhibited Wnt8-induced ectopic axis as well as transcription of Wnt-specific target gene *Xnr3*. Activator substances inhibited β-catenin phosphorylation and enhanced activity of Wnt/β-catenin specific reporter.Our results present some promising natural compounds that differentially modulate Wnt/β-catenin signaling and revealed a potential screening strategy combining *Xenopus laevis* embryology and *in vitro* reporter assay.Support: CAPES, FAPERJ, CNPq.

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Detection of amphibian chytridiomycosis before and after formalin fixation. Sean C Adams[1], Richard Luong[1], Raul Figueroa[2], Stephen Felt[1], Vance Vredenburg[2], Sherril Green[1]. 1) Comparative Medicine, Stanford University, Stanford, CA; 2) Department of Biology, San Francisco State University, San Francisco, CA, United States.

Batrachochytrium dendrobatis (*Bd*) is a fungal pathogen of amphibians and is the causative agent of chytridiomycosis, which is responsible for worldwide amphibian population declines and extinctions. *Bd* has been identified in amphibian samples collected from the wild, the pet trade, as well as the laboratory setting. Characterization of this pathogen has included the use of archived, formalin-fixed amphibian samples to determine its origin and spread. Analysis of these samples historically has relied on histology. However, recently, quantitative PCR (QPCR) has replaced histology as the gold standard for *Bd* detection. To determine the effect of formalin fixation on *Bd* detection by QPCR, *Bd* load was quantified by QPCR before and after formalin fixation of five species of frog (n=19 total) and odds ratios were calculated to determine the probability of detection post fixation for a given pre fixation load. Analysis of 114 swabs by QPCR identified that samples with less than 100 zoospores per swab as detected by QPCR prior to formalin fixation were 160 times less likely to be detectable by QPCR after fixation. To examine the effect of swab location of *Bd* load and frequency of detection, a one-way analysis of variance (ANOVA) was performed which identified that there was no statistical difference in the frequency of *Bd* detection or in *Bd* load between swabs collected from the dorsum, ventrum, inner thigh, and toe web. Histologic evaluation identified characteristic lesions of chytridiomycosis (presence of zoosporangium and zoospores within stratum corneum, acanthosis, hyperkeratosis, and epithelial sloughing) and was less sensitive than QPCR alone, however when combined with post-fixation QPCR increased overall post-fixation detection. Based on these findings, archival samples should be analyzed by a combination of QPCR and histology in order to maximize sensitivity and when possible swabs should be collected and stored prior to formalin fixation of specimens. Furthermore, when collecting swabs, anatomical location has minimal impact on the outcome of QPCR.

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Making the connection: Ciliary adhesion complexes are responsible for anchoring basal bodies to the actin cytoskeleton. Ioanna Antoniades, Panayiota Stylianou, Paris Skourides. Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus.

Cilia are structures associated with diverse processes during development and tissue homeostasis. Motile cilia are found on the surface of specialized epithelial tissues such as the human respiratory track and the brain ependyma, where they generate directed fluid flow, while ciliary dysfunction is linked to a variety of diseases and can lead among others to infertility and respiratory problems. Several pieces of evidence support a critical role of the actin cytoskeleton in ciliogenesis and ciliary function. Electron microscopy analysis identified interactions between basal bodies and the actin cytoskeleton at the apical surface of multiciliated cells, while different networks of actin have been shown to be crucial for different aspects of basal body organization, such as migration, docking, spacing and polarization. Although the role of actin in these processes has been studied extensively, how cilia and basal bodies link to the actin cytoskeleton in not known. Here, using the multiciliated epithelium of the *Xenopus* epidermis, live imaging and immunofluorescence experiments we show that four well characterized focal adhesion proteins, including FAK, are found associated with the basal bodies at the apical region of multiciliated cells where they form complexes. In addition, we show that they are also found at the end of the ciliary rootlets. At both sites these proteins are shown to co-localize with actin and FRET experiments support an interaction between FAK and actin in these regions. Embryo dissections reveal a similar

localization in monociliated cells of the gastrocoel roof plate and in cells carrying primary cilia. Knock-down experiments reveal an important role of FAK in ciliogenesis since its down-regulation in multiciliated cells leads to defects, similar to those observed when the actin cytoskeleton is disrupted, including defects in basal body migration, docking and spacing. Collectively, our data suggest that focal adhesion proteins associate with the basal bodies and their accessory structures in ciliated cells and form complexes, which we named ciliary adhesions, that functionally link cilia to the actin cytoskeleton.

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Dynamic Dvl1, Pk2 and Vangl1 localizations pattern a vertebrate multiciliated epithelium. Mitchell Butler[1], John Wallingford[2]. 1) The University of Texas at Austin, Austin, TX; 2) Howard Hughes Medical Institute and The University of Texas at Austin, Austin, TX.

The Planar Cell Polarity (PCP) signaling pathway controls polarized cellular behaviors and the coordination of cellular asymmetry throughout development. A common feature of PCP-patterned epithelia is the asymmetric localization of complementary core PCP complexes within the residing cells, but there are clearly tissue-specific differences in these molecular patterns and how they relate to planar polarity. Studies in the fly wing have highlighted the importance of the dynamics of these localizations in the establishment of PCP, yet similar processes have not been well described for more complex vertebrate tissues. For the present study, we have established a robust and tractable model system for measuring core Planar Cell Polarity dynamics in the *Xenopus* multiciliated epidermis. We have identified *Xenopus*Dvl1 and Pk2 as cytoplasmic core PCP proteins that exhibit asymmetrical localizations in this epithelium, and we show that intact PCP signaling is necessary for the progressive generation of these asymmetries. We subsequently demonstrate that the expression levels of Pk2 influence the anisotropic clustering of stable transmembrane Vangl1 accumulations. These findings serve as a foundation for the discovery of novel characteristics of vertebrate PCP and for the advancement of quantitative, high-resolution analysis of core PCP patterning.

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EphrinB2 affects apical constriction in *Xenopus*embryos and is regulated by ADAM10 and flotillin-1. Ira Daar[1], Yon Ju Ji[1], Yoo-Seok Hwang[1], Kathleen Mood[1], Hee-Jun Cho[1], Hyun-Shik Lee[2], Emily Winterbottom[1], Helene Cousin[3]. 1) Laboratory of Cell & Developmental Signaling, National Cancer Institute, Frederick, MD; 2) ABRC, CMRI, School of Life Sciences, College of Natural Sciences, Kyungpook National University, Daegu 702-701, South Korea; 3) Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA.

The Eph/ephrin signaling pathways have a critical function in cell adhesion and repulsion, and thus play key roles in various morphogenetic events during development. Here, we demonstrate that a decrease in ephrinB2 protein causes neural tube closure defects during *Xenopus laevis* embryogenesis. Moreover, immunocytochemistry and confocal microscopy reveal that this defect resides in the process of apical constriction during neural tube formation. In addition, we demonstrate that morpholino (MO)-mediated loss of flotillin-1, a lipid raft scaffold protein that interacts with ephrinB2, phenocopies the ephrinB2 MO-mediated neural tube defect. Using metalloprotease inhibitors and individual MOs, we also show that loss of flotillin-1 leads to the cleavage of the ephrinB2 ectodomain specifically through the ADAM10 metalloprotease, before being subsequently degraded. Thus, we link ephrinB2 to an important developmental process (neural tube closure), and show that it's protein levels are sustained by a lipid raft that regulates cleavage by ADAM10. These findings indicate that flotillin-1 regulates ephrinB2 protein levels through ADAM10, and that ephrinB2 plays a role in appropriate neural tube morphogenesis in the*Xenopus* embryo.

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Investigating Rho GTPase Pattern Formation During Wound Repair: The Role of RhoGAP1/8. Nicholas R. Davenport[1], William Bement[1,2,3]. 1) Graduate Program in Cellular and Molecular Biology, University of Wisconsin-Madison, Madison, WI; 2) Department of Zoology, University of Wisconsin-Madison, Madison, WI; 3) Laboratory of Cell and Molecular Biology, University of Wisconsin-Madison, Madison, WI.

The ability to respond to physical damage is an essential and evolutionarily conserved feature of single cells and tissues. The Rho family of GTPases has been shown to coordinate the cytoskeletal rearrangements needed to reestablish the integrity of the plasma membrane and underlying cortex in response to cell damage. The precision with which Rho and Cdc42 are activated and maintained in distinct activity zones throughout the healing process suggests the involvement of multiple Rho GTPase regulators at the wound. To date, a candidate screen for guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) involved in regulating Rho GTPase activity at the wound has identified a single wound regulator. By continuing the candidate screen, we have identified two additional GAPs, RhoGAP1 and RhoGAP8, as potential wound regulators. eGFP-tagged RhoGAP1 localizes between the zones of active Rho and Cdc42 in wounded *Xenopus laevis* oocytes. These results have been confirmed by the discovery of endogenous RhoGAP1 at wounds via immunofluorescence microscopy. Targeting of RhoGAP1 to wounds is mediated by its C-terminal region which contains both a GAP domain and a proline-rich region. Reduction of active Rho due to overexpression of untagged RhoGAP1 and alteration of GTPase activity zone position by dominant negative RhoGAP1 supports a hypothesis that RhoGAP1 acts as an enzymatic barrier to promote proper GTPase zone segregation throughout wound repair. Further, we have discovered that RhoGAP1 and RhoGAP8 colocalize at single-cell wounds which, in addition to previously reported evidence of their interaction, suggests they may form a complex to regulate Rho GTPase activity. We have also used RNAi to effectively knockdown proteins in *Xenopus* embryos 24 hours post-fertilization. Knockdown of RhoGAP1 in embryos appears to increase the amount of cytoplasmic F-actin in cells, suggesting a vital role for RhoGAP1 in regulating the actomyosin cytoskeleton.

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RFX7 is required for the formation of primary cilia in the neural tube. R. Earwood, Z. Manojlovic, A. Kato, B. Stefanovic, Y. Kato. Department of Biomedical Science, Florida State University, Tallahassee, FL.

Regulatory Factor X (RFX) transcription factors are developmentally crucial and are likely involved in the pathogenesis of serious human diseases like ciliopathies. In vertebrates, seven RFX genes have been identified and several RFX transcription factors have been shown to be involved in ciliogenesis. Up until now the developmental role of RFX7 has not been shown. We found RFX7 expression in the neural tube, eye, otic vesicles, and somites during *Xenopus* development. Knockdown of RFX7 by Morpholino Oligos in *Xenopus* embryos caused a failure of neural tube closure and an absence of neural tube cilia. We also found that RFX7 controls ciliogenesis by regulating RFX4 expression, which has previously been reported to be required for ciliogenesis in the neural tube. Furthermore, ectopic expression of Foxj1, a master regulator of motile ciliogenesis, suppressed the expression of RFX4 but not RFX7. We concluded that RFX7 plays a key role in the process of neural tube closure at the top of the molecular cascade which controls ciliogenesis in the neural tube.

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Cell to Cell Adhesion in *Xenopus* Mesendoderm Cells is Regulated via CXCR7 Signaling. A. Fukui, K. Furusawa, N. Sasaki. Grad. Sch. Life Sci., Hokkaido Univ., Sapporo, Hokkaido, Japan.

A chemokine stromal cell-derived factor-1 (SDF-1), also known as CXCL12, functions as chemoattractant through its G-protein coupled receptor CXCR4. We have found that the expression of *Xenopus*CXCR4 was detected in the mesendoderm, and was complemented by *Xenopus* SDF-1a expression in the inner surface of the blastocoel roof (BCR) at early gastrula. Furthermore, explants of mesendodermal cells migrated toward the BCR in the presence of xSDF-1a (Fukui *et al.*, BBRC, 354:472-477, 2007). By using this migration assay, we have investigated the xCXCR7 functions for collective cell migration. CXCR7 had been identified as a second receptor for SDF-1, and the function of that has been focused since its novel roles have been reported as controlling cell migration to inhibit the signaling by sequestrating the SDF-1 and to signal *via* non-G-protein signaling pathway. xCXCR7 mRNAs were injected into presumptive mesoendoderm region, and descendant mesoendoderm cells at early gastrula were cut and set adjacent to the xSDF-1 expressing BCR explant on the fibronectin-coated cover glass. The overexpression of xCXCR7 in the mesendoderm population resulted in cell scattering and the cells migrated discretely toward the BCR. Mesoendodermal explant expressing deletion mutants of xCXCR7 did not dissociate, suggesting that signaling via xCXCR7 is required for cell scattering. Moreover, a inhibitor for ERK signaling also abrogated dissociation of cells of the xCXCR7-injedted explant. These finding suggest that the ERK signaling is necessary for controlling cell-cell adhesion by SDF-CXCR7 signaling. These results demonstrate that the xCXCR7/SDF-1-dependent novel function that CXCR7 signaling modulates intercellular adhesion, but allows directional cell migration by SDF-1/CXCR4 signaling.

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Investigating biological size control using *Xenopus* hybrids. Romain Gibeaux, Rebecca Heald. Department of Molecular & Cell Biology, University of California, Berkeley, CA.

Determining how size is controlled is a fundamental question in biology that is poorly understood at the organism, cellular and subcellular levels. The two frog species *Xenopus laevis* and *Xenopus tropicalis*differ in size at all three of these levels. *X. laevis* is allo-tetraploid (36 chromosomes) and larger (~10 cm), whereas *X. tropicalis* is diploid (20 chromosomes) and smaller (~4 cm). Scaling at the organismal and genome levels is accompanied by differences in the size of the egg as nuclei and mitotic spindles formed in egg extracts. Despite these size differences, the close phylogenetic relationship between these two species allows the production of hybrid embryos by cross-fertilization. Using *Xenopus* hybrids, we aim to elucidate genetic and cell biological mechanisms that govern biological size. First, taking advantage of the viable hybrid produced when *X. laevis* eggs are fertilized by *X. tropicalis* sperm (l_ext_s), we are investigating how an embryo that is initially identical in size and developmental rate to that of *X. laevis*generates a hybrid frog closer in size to *X. tropicalis*. Using live embryo imaging to monitor early cleavage phases, and immunofluorescence to determine nucleocytoplasmic and genome-spindle size ratios, and by comparing l_ext_s hybrids to diploid and haploid *X. laevis*, we are evaluating the effects of the smaller hybrid genome on spindle, nucleus, cell and embryo size before and after zygotic genome activation. Second, we are investigating the reverse hybrid generated by the fertilization of *X. tropicalis* eggs with *X. laevis* sperm (t_exl_s), which is not viable and dies at the late blastula stage. We observed gross mitotic defects prior to embryo death, including lagging chromosomes and the formation of micronuclei. We are testing the hypothesis that t_exl_s hybrid death is caused by scaling defects that lead to chromosome segregation errors, resulting in extensive DNA damage and large-scale apoptosis. Thus, *Xenopus* hybrid embryos provide a novel system to investigate biological scaling and its consequences for cell division and development, and in the longer term to identify underlying molecular mechanisms that have important implications for human developmental diseases and cancer.

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Investigating physical and biochemical mechanisms underlying the adaptability of mitotic spindle length to cell size in *Xenopus*. M. Good[1], C. Brownlee[1], M. Vahey[2], J. Wilbur[1], D. Fletcher[2], R. Heald[1]. 1) Molecular and Cellular Biology Dept., UC Berkeley, Berkeley, CA; 2) Bioengineering Dept., UC Berkeley, Berkeley, CA.

Rapid and reductive cell divisions during embryogenesis require that intracellular structures adapt to a wide range of cell sizes. The mitotic spindle presents a central example of this flexibility, scaling with the dimensions of the cell to mediate accurate chromosome segregation. To determine whether spindle size regulation is achieved through a developmental program or is

intrinsically specified by parameters coupled to cell size and shape, we developed a system to encapsulate cytoplasm from *Xenopus* eggs and embryos inside cell-like compartments of defined sizes. We find that spindle size shrinks with decreasing compartment size, similar to what occurs during early embryogenesis. This scaling trend depends on compartment volume rather than shape, and occurs as spindle components such as tubulin become limited by the amount of cytoplasm. Superimposed on volume-dependent scaling are changes in cytoplasmic composition that set spindle upper and lower size limits. We identified the kinesin-13 kif2a as one spindle scaling factor whose microtubule-destabilizing activity is inhibited in the cytoplasm of large cells during early divisions by the transport receptor importin alpha, and subsequently activated to decrease spindle size in small cells at later stages, when importin alpha shifts from the cytoplasm to the plasma membrane, possibly through direct binding to lipids or proteins at internal membranes. Current experiments utilize encapsulation techniques to investigate how limiting components modulate spindle size, and whether membrane localization of importin alpha serves as a sensor for the surface area to volume ratio of individual cells. We propose that these mechanisms may be generalizable, explaining the scaling behavior of other subcellular structures, such as the nucleus, to cell size.

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Truncation of RASSF7 induces centrosome amplification; a role in cancer? Tulay Gulsen[1], Paul Whitley[2], Andrew Chalmers[3]. 1) University of Bath, bath, avon, United Kingdom; 2) #1; 3) #2.

RASSF7 is a Ras-association domain containing protein which localises to the centrosome, is required for completing mitosis and whose expression is upregulated in a range of cancers. Investigating the function of RASSF7 will help us to gain a deeper understanding of mitosis and whether increased levels of RASSF7 protein can promote cancer formation. To achieve this aim, I am focusing on understanding the role of the four domains of RASSF7 protein in mediating its subcellular localization. GFP fusion proteins were expressed in early *Xenopus laevis* embryos and showed that the coiled-coil domain is responsible for the centrosomal localization of RASSF7. Interestingly, removing the C-terminal domain caused accumulation of GFP-RASSF7 at the centrosomes. In addition, this lead to enlargement of the centrosomes, an increase in centrosome number and increased cell death later in development. This may suggest that increased centrosomal levels of RASSF7 could promote tumourigenesis, as centrosome amplifications/defects are common in tumours.

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Remodeling of cell-cell junctions during cytokinesis. T. Higashi, T. Arnold, R. Stephenson, A. Miller. Mol Cell Dev Biol, University of Michigan, Ann Arbor, MI.

Cell-cell junctions are required for maintenance of mechanical integrity and barrier function in epithelial tissues. During cytokinesis, the dividing cell and its neighbors undergo major changes in shape and tension as the contractile ring pinches the cell in two. Junctional integrity and barrier function are thought to be preserved even during cell division. However, our understanding of how junctions are maintained and remodeled during cytokinesis in epithelial cells is lacking. In this study, we investigated the behaviors of three types of cell-cell junctions, tight junctions (TJs), adherens junctions (AJs), and tricellular tight junctions (tTJs), during cytokinesis in the *Xenopus* gastrula by expressing fluorescently-tagged junction proteins. Using a small fluorescent tracer molecule, we demonstrated that the barrier function of TJs remained intact throughout cell division. We found that both TJs and AJs invaginated together with the cleavage furrow; this is in contrast to reports in the *Drosophila* epithelium indicating that AJs are locally disengaged at the division site. Furthermore, we found that the invagination of AJs precedes that of TJs. We also observed that two nascent tTJs are formed between daughter cells after cytokinesis, and LSR/angulin-1 is recruited to the newly formed tTJs first followed by tricellulin. Finally, we investigated changes in cellular tension that occur in a dividing cell and its neighbors during cytokinesis. Use of a fluorescent probe that reports tension changes at AJs revealed that the AJs around the cleavage furrow are under high tension, suggesting that AJs are mechanically linked to the contractile ring. Our data provide the first in-depth characterization of dynamic cell-cell junction remodeling during vertebrate cytokinesis.

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Analysis of the role of ROS during tail regeneration in Xenopus. S. Ishibashi[1], A. Yamamoto[1,2], R. Lea[1], E. Amaya[1]. 1) The Healing Foundation Centre, The University of Manchester, Manchester, United Kingdom; 2) College of Biological Sciences, University of Tsukuba, Japan.

Reactive oxygen species (ROS) is highly reactive and causes damages to cells, but also functions as a second messenger in the intracellular signaling pathway. We have previously shown that tadpole tail amputation induces a sustained production of ROS, which is essential for tail regeneration. We now wish to determine the mechanisms that act upstream and downstream of ROS production during tail regeneration. Primary candidates of what acts upstream include the NADPH oxidase (NOX) family genes. Of those Nox5 and Duoxes are regulated by calcium signalling. We have recently found that calcium acts upstream of ROS production. To investigate the roles of *nox5* and *duox1* in ROS signalling during tail regeneration, we have been generating *nox5* and *duox1* mutant lines using TALENs and CRISPRs. In order to generate germline mutants of *nox5*, we have generated bicistronic construct containing a fluorescence marker and *nox5* TALENs followed by a signal sequence in the 3'UTR to direct localization to the germ plasm. We have also constructed bicistronic *tyrosinase* (*tyr*) TALENs with the 3'UTR. Then *nox5* and *tyr* TALEN RNAs were coinjected into the vegetal pole of 1-cell stage embryos. Tadpoles expressing fluorescence proteins in the primordial germ cells generated from bicistronic *nox5* and *tyr* TALEN RNAs were raised. To determine the mutation rate in the germ line, a mature pigmented male was mated with an albino female carrying a mutation in both *tyr* alleles. Obtained tadpoles were sorted by pigmentation and 98% of tadpoles showed albinism, suggesting that 98% of

germ cells in the TALEN injected male carries mutations in at least *tyr* locus. This result demonstrated that injecting with germ line localized TALENs are the most efficient way to generate F0 founder line carrying a mutation. We are currently trying to determine whether these albino offspring also possess a mutation in the *nox5* locus.

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Cadherin-11 localizes to focal adhesions and promotes cell-substrate adhesion. Rahul Langhe[1], Tetyana Gudzenko[2], Sarah Becker[1], Carina Gonnermann[2], Michael Bachmann[3], Clemens Franz[2],**Jubin Kashef[1,4]**. 1) Zoological Institute, Karlsruhe Institute of Technology (KIT), Cell and Developmental Biology, Kaiserstr. 12, 76131 Karlsruhe, Germany; 2) Center for Functional Nanostructures, Karlsruhe Institute of Technology (KIT), Wolfgang-Gaede-Strasse 1a, 76131 Karlsruhe, Germany; 3) Zoological Institute, Karlsruhe Institute of Technology (KIT), Cell and Neurobiology Biology, Haid-und-Neu-Strasse 9, 76131 Karlsruhe, Germany; 4) Institute for Photon Science and Synchrotron Radiation, Karlsruhe Institute of Technology (KIT), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany.

Cadherins represent a family of Ca^{2+}-dependent glykoproteins mediating cell-cell adhesion processes. Despite their high structural similarity the function of different types of cadherins is considerably diverse. For instance, epithelial cadherins such as E-Cadherin mediate cell-cell adhesion, while mesenchymal cadherins like Cadherin-11 promote cell migration. However, the mechanisms how mesenchymal cadherins promote cell migration are still poorly understood.Here, we unravel an unexpected function for Cadherin-11 in mediating cell-substrate adhesion during *Xenopus* cranial neural crest (CNC) migration. By using Total Internal Reflection Fluorescence (TIRF) microscopy we can show that in addition to its localisation at the cell-cell contacts Cadherin-11 is also found in focal adhesions at the cell-substrate surface. Interestingly, within these focal adhesions Cadherin-11 is co-localized with focal adhesion markers like Paxillin and b1-integrin, indicating a novel function in cell-substrate adhesion. Indeed, single cell force spectroscopy with Cadherin-11 morphant CNC cells revealed reduced cell-substrate adhesion. In reconstitution experiments we identified the transmembrane and the cytoplasmic domain of Cadherin-11 to be necessary for this novel cadherin function. We suggest, that this Cadherin-mediated cell-substrate adhesion depends on the interaction of Cadherin-11 and Syndecan-4, a heparan sulfate proteoglycan, which binds to fibronectin and mediates cell-substrate adhesion.

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A new role of TGF-beta signaling in ciliogenesis. R. Earwood[1], J. Tözser[2], J. Brown[1], A. Kato[1], M. Blum[2], **Y. Kato[1]**. 1) Biomedical Sciences, Florida State University, Tallahassee, FL USA; 2) Institute of Zoology, University of Hohenheim, Stuttgart, Germany.

A cilium is a small cellular organelle which broadly exists throughout the human body as either a motile or an immotile form with microtubule doublet structures. While motile cilia generate force by beating for directional movement of fluid, immotile cilia, referred to as primary cilia, are involved in sensory processes and cellular signal transduction. Congenital disruption of ciliary structure and/or function in humans causes pleiotropic developmental disorders with a variable degree of spectrum, called ciliopathies. While numerous studies have started revealing the protein machinery underlying ciliary structure and function, very little is known about how cilia formation and/or assembly are regulated. Transcriptional regulation of ciliary genes is believed to be an important mechanism that induces the assembly of specific cilia types in a temporal and spatial manner during development. We have recently discovered that TGF-beta signaling regulates the elongation of cilia on the cells of gastrocoel roof plate (GRP) which is a *Xenopus*analog of the mammalian node. Motile cilia at the GRP generate a leftward flow of extracellular embryonic fluid, called Nodal flow, to control establishment of left-right (LR) asymmetry within the disposition and the placement of internal organs. TGF-beta superfamily members activate Smad2 in the GRP cells while cilia are formed at the GRP. Blocking of TGF-beta signaling resulted in short cilia at the GRP and reduced the expression level of genes required for ciliogenesis. These data show that TGF-beta signaling is activated in the ciliated cells of the GRP and this activation is crucial for ciliogenesis. Since the role of TGF-beta signaling in ciliogenesis has not been reported before, our findings indicate an undiscovered regulatory mechanism of cilia formation by this signal.

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Developing methods to study live chromosome dynamics in *Xenopus* using the CRISPR/Cas system. Andrew Lane, Esther Kieserman, Rebecca Heald. Molecular And Cellular Biology, University of California, Berkeley, Berkeley, CA.

As rapid divisions without growth generate progressively smaller cells within an embryo, mitotic chromosomes must also decrease in size to permit their proper segregation, but this scaling phenomenon is poorly understood. To recapitulate chromosome scaling during development, we combined nuclei isolated from different stage *Xenopus laevis* embryos with metaphase-arrested egg extracts. We found that mitotic chromosomes derived from nuclei of cleaving embryos through the blastula stage were similar in size to replicated sperm chromosomes, but decreased in area approximately 50% by the neurula stage, reproducing the trend in size changes observed in fixed embryos. Allowing G2 nuclei to swell in interphase prior to mitotic condensation did not increase mitotic chromosome size, but progression through a full cell cycle in egg extract did, suggesting that epigenetic mechanisms determining chromosome size can be altered during DNA replication. Measurements of chromosome size are limited by our inability to positively identify specific chromosomes in living samples, and are thus whole-genome averages of chromosome dimensions, necessitating large sample sizes to detect changes. To overcome this obstacle and enable higher-throughput analysis of potential chromosome scaling factors, we have developed methods to label specific chromosomal regions using an RNA-guided fluorescently-labeled protein, dCas9-Neon. We have found that this method can be used to label repetitive chromosome loci live throughout the cell cycle in egg extracts, and are extending this technique to allow labeling of

whole chromosomes. Live chromosome paints will facilitate investigation into chromosome condensation during mitosis as well as changes in chromosome size and organization during development.

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Cholesterol Selectively Activates Canonical Wnt Signaling over Non-Canonical Wnt Signaling during *Xenopus* development. Hyeyoon Lee[1], Hyunjoon Kim[1], Sheng Ren[2], Yao Xin[2], Yong Chen[2], Wen Tian[2], Yao Cui[2], Jong-Cheol Choi[3], Junsang Doh[3], Wonhwa Cho[2], Jin-Kwan Han[1]. 1) Life Sciences, POSTECH, Pohang, South Korea; 2) Dept of Chemistry, University of Illinois at Chicago, Chicago, IL, US; 3) Mechanical Engineering, POSTECH, Pohang, South Korea.

Wnt signaling controls a variety of developmental and homeostatic processes through the β-catenin-dependent canonical pathway and other non-canonical pathways, including the planar cell polarity (PCP) pathway. Dishevelled (Dvl) is a scaffold protein that serves as the branch point of canonical Wnt and PCP signaling but it remains unclear how Dvl differentially directs the two pathways. Here, we show that Dvl is a novel cholesterol binding protein and this Dvl-cholesterol binding on the plasma membrane selectively drives the progression of canonical Wnt signaling over PCP signaling. In *Xenopus* embryos, cholesterol binding of Dvl is required for canonical Wnt signaling activities but not for PCP signaling activities. Furthermore, confocal and single molecule imaging using culture cells shows that cholesterol is essential for sustained association of Dvl with the Frizzled receptor and other components of canonical Wnt signaling in the plasma membrane and endocytic vesicles in response to a canonical Wnt ligand, but not for the formation of the PCP signaling complex. It also shows local enrichment of cholesterol near the Frizzled receptor upon its binding to a canonical Wnt ligand. Collectively, our results suggest a new role of cholesterol binding of Dvl in Wnt signaling during *Xenopus* embryogenesis and a potential link between cellular cholesterol levels and canonical Wnt signaling activities.

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Polyglutamylation of Nucleosome Assembly Protein 1 modulates Histone H1 dynamics and chromosome condensation in *Xenopus* egg extracts. Kelly Miller, Rebecca Heald. UC Berkeley, Berkeley, CA.

Linker histone H1 is required for mitotic chromosome architecture in *Xenopus* egg extracts, and unlike core histones exhibits rapid turnover on chromatin. The role of mechanisms regulating the recruitment, deposition, and dynamics of linker histones in mitosis are largely unknown. We found that the cytoplasmic histone chaperone Nucleosome Assembly Protein 1 (Nap1) associates with the embryonic isoform of linker histone H1 (H1M) in egg extracts. Immunodepletion of Nap1 decreased H1M binding to mitotic chromosomes by nearly 50%, reduced H1 dynamics as measured by FRAP, and caused chromosome decondensation similar to the effects of H1M depletion. Defects in H1M dynamics and chromosome condensation were rescued by adding back wild-type Nap1, but not mutants lacking sites subject to posttranslational modification by polyglutamylation. We propose that charge-shifting post-translational modification of Nap1 is required for cycles of deposition and turnover of H1M required for normal chromatin assembly and higher order chromosome architecture.

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ERK7, a novel regulator of ciliogenesis, is required for basal body migration. Koichi Miyatake, Morioh Kusakabe, Chika Takahashi, Eisuke Nishida. Department of Cell and Developmental Biology, Graduate School of Biostudies, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan.

The family of mitogen-activated protein kinases (MAPKs) plays an essential role in various processes, such as cell proliferation, stress response, and cell differentiation. In vertebrates, there are 14 members of the MAPK family. Among these, ERK7 (also known as MAPK15) is the most recently identified MAPK family member. Although ERK7 is evolutionarily conserved, its *in vivo* function remains largely unknown. Here, we show that ERK7 is a key regulator of ciliogenesis. ERK7 is strongly expressed in ciliated tissues of *Xenopus* embryos, and the expression of ERK7 is under the control of FoxJ1, a master regulator of the motile ciliogenic program. Knockdown of ERK7 in *Xenopus* epidermis inhibits cilia-generated fluid flow. We then find that the number and the length of cilia in multi-ciliated cells (MCCs) are dramatically diminished by ERK7 knockdown, and that apical migration of basal bodies, a key step in ciliogenesis, is inhibited in ERK7 knocked-down embryos. Moreover, ERK7 knockdown results in loss of apical actin meshwork, which is required for proper migration of basal bodies. Remarkably, we find that the actin regulator CapZIP, which is shown to regulate ciliogenesis in a phosphorylation-dependent manner, is an ERK7 substrate, and that Dishevelled, which is also shown to regulate ciliogenesis, facilitates ERK7 phosphorylation of CapZIP through binding to both ERK7 and CapZIP. Collectively, these results suggest that ERK7 regulates ciliogenesis through phosphorylating the actin regulator CapZIP in cooperation with Dishevelled.

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FAK is required for the transduction of extracellular forces that orient the mitotic spindle and control tissue morphogenesis. Nicoletta Petridou, Paris Skourides. University of Cyprus, Nicosia, Cyprus.

Spindle positioning is critical for proper morphogenesis and growth of organs and tissues during development, as well as for the maintenance of tissue morphology. Defective spindle orientation is also linked to the appearance of severe diseases, including neurological disorders, polycystic kidneys and tumorigenesis. Significant progress has been made in understanding the mechanisms linking the cell cortex to the spindle, resulting in proper positioning of the latter. However, recent work has also revealed the critical role that extracellular cues and forces play in spindle positioning. Specifically, when cells in culture enter mitosis, they round up and remain attached to the substrate through actin-containing retraction fibers. Although it has been shown

that mechanical forces exerted by retraction fibers can guide spindle orientation in adherent cells, how external forces are transmitted to the cell cortex and subsequently bias cortical cues to orient the spindle is still poorly understood. In this study we show that the focal adhesion protein FAK is necessary for correct spindle orientation in adherent cells and the vertebrate embryo. By performing rescue experiments with several FAK mutants both in vitro and in vivo we go on to show that FAK's role in spindle positioning is dependent on its ability to localize at focal adhesions and its interaction with paxillin but surprisingly, is kinase activity independent. In addition, by either applying external forces on the Xenopus epithelium or by laser ablation experiments to generate unidirectional application of force we show that FAK is required for external force induced spindle reorientation suggesting that FAK's involvement in spindle positioning stems from a role in the transduction of external forces to the cell cortex. Finally, by using an inducible dominant negative, we show that FAK's role in spindle positioning is indispensable for proper epithelial morphogenesis during Xenopus epiboly and pronephros development.

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Analysis of alternative splicing in the Xenopus SMRT gene. Colin Sharpe. EXRC, University of Portsmouth, Portsmouth, Hampshire, United Kingdom.

Acting on transcripts from a single gene, alternative splicing is a common cellular process that generates protein isoforms that often have very different functions. With a few exceptions, however, the process by which an exon acquires the capacity for alternative splicing has not been widely investigated. As a system to analyse the factors that influence alternative splicing, we have characterized transcripts from the nuclear receptor corepressor, NCoR2, also known as SMRT. Using an antisense morpholino oligonucleotide to prevent the inclusion of SMRT exon 37b generates Xenopus embryos with a distinct neural phenotype, demonstrating the functional significance of this alternatively spliced exon. We have used orthologues of SMRT and its paralogue NCoR1 from a range of organisms to identify how the alternative splicing of SMRT exon 37b has arisen. Although Xenopus NCoR1 has a similar exon arrangement to SMRT it does not undergo alternative splicing of exon 37. Introduction of a single point mutation at the equivalent point in a Xenopus NCoR1 minigene, however, recreates a functional internal 37b splice donor. This may suggest a capacity for alternative splicing of this exon in the ancestral NCoR, that was lost in NCoR1 following the gene duplication event that gave rise to the two paralogues. The injection of 'splicing minigenes' into Xenopus laevis embryos provides an efficient and simple vehicle to examine the factors that influence alternative splicing. Unlike Xenopus, zebrafish use only the internal splice donor in SMRT exon 37b whilst sea lampreys, in contrast, use only the terminal splice donor. By injecting embryos with minigenes containing these exons we can assess the contribution of cis- and trans-acting factors and propose a sequential process for the acquisition of alternative splicing of SMRT exon 37b across the deuterostome phylogeny.

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Septins control contractile force during epithelial wound closure. Asako Shindo[1], John Wallingford[1,2]. 1) Molecular Biosciences, University of Texas at Austin, Austin, TX; 2) Howard Hughes Medical Institute.

Embryonic or fetal epithelial wounds are rapidly closed by contractile forces generated by the actomyosin purse-string at the wound margin. Despite the clear functional involvement of the actomyosin purse-string during wound closure, it is unclear how multicellular actomyosin activations are coordinately regulated at the wound margin. To address this question, we have focused on the cytoskeletal element Septin7 (Sept7), a known regulator of intracellular actomyosin distribution and cortical rigidity. We found that Sept7 is required for the rapid reduction of epithelial wound area in *Xenopus* tadpoles. The cell edges at the wound margin shrink more slowly under Sept7 knockdown conditions, suggesting the attenuation of actomyosin functions. Interestingly, the cells along the wound in the Sept7 knockdown tadpoles fail to stretch parallel to the wound margin, as is normally observed immediately after wounding. Additionally, at the late phase of wound closure Sept7 knockdown cells fail to elongate perpendicular to the wound margin, and retain a rounded shape compared with controls.Our results suggest a model in which septins control the cortical rigidity of individual cells, and that this cortical rigidity is required for mediating cell shape changes in response to extrinsic forces such as wounding. Further, our findings raise the possibility that these individual cell shape changes trigger localized actomyosin activation, suggesting a mechanism for the coordinated generation of actomyosin purse strings during wound healing.

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Understanding the interaction of Wnt ligands with their cell surface receptors. Kelsey F Speer[1], Jeannine Mendrola[3], Peter Klein[1,2], Mark Lemmon[1,3]. 1) Graduate Group in Cell and Molecular Biology, University of Pennsylvania, Philadelphia, PA; 2) Department of Medicine, Raymond and Ruth Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 3) Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA.

Wnts are a family of morphogens that play many conserved roles in patterning the early embryo. Wnts act as ligands for the Frizzled (Fzd) family of seven-pass transmembrane receptor by specifically engaging their extracellular cysteine-rich domain (CRD). However, other Wnts such as Wnt5a have the unique ability to engage the extracellular regions of the Ror, Ryk, and PTK7/CCK4 families of receptor tyrosine kinases (RTKs). We are interested in understanding how Wnt ligands can control the activation of such divergent classes of cell surface receptor. To address this question, we developed complementary *in vivo* and *in vitro* approaches. We use development of *Xenopus laevis* embryos as a model *in vivo* system to define functional interactions between receptors and ligands. This work, in turn, informs our analysis of protein structure and interaction using classic *in vitro* biochemical techniques. We are currently working on understanding receptor/ligand interactions as well as

identifying RTK-dependent signaling complexes. Our ultimate goal is to use these analyses to determine how different Wnt receptors cooperate with one another to drive different modes of Wnt signaling.

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The role of core spliceosomal components in spindle assembly. Magdalena Strzelecka, Andrew Grenfell, Rebecca Heald. Molecular & Cell Biology Department, UC Berkeley, Berkeley, CA.

The mitotic spindle is a complex macromolecular structure required for accurate distribution of the genetic material during cell division. Until recently, most research has focused on protein components of the spindle. Interestingly, our lab has shown that an RNA component is also essential for spindle integrity. Furthermore, a number of genome-wide screens have implicated RNA processing factors in the regulation of mitotic events, and the spindle assembly factor TPX2 has been found to co-purify with active spliceosomes, large RNA-protein complexes that catalyze RNA splicing reactions. In order to investigate whether spliceosome components play a direct role in mitosis, we use transcriptionally silent and metaphase-arrested Xenopus egg extracts to reconstitute spindle assembly and study mitotic RNAs. Strikingly, molecular and biochemical perturbations of spliceosome assembly and function led to defects in spindle integrity. Moreover, next generation sequencing of RNAs from Xenopus tropicalis egg extract and of RNAs co-immunoprecipitated with spliceosomal small nuclear ribonucleoproteins (snRNPs) revealed the presence of intron-containing pre-mRNAs at metaphase, suggesting that their processing might be required for mitotic progression. However, translation inhibition does not recapitulate the spindle integrity phenotype caused by spliceosome perturbation, indicating that spliceosome components play a role in mitosis in the context of unspliced or non-coding RNAs. Consistently, we find that intron-containing non-coding RNAs are also associated with spliceosomal snRNPs in metaphase-arrested extracts and our proteomic analysis revealed that mitotically relevant factors co-immunoprecipitate with core spliceosomal components. Thus, our data support the direct involvement of core spliceosomal components in mitosis and current experiments aim to address whether this occurs through a splicing-dependent or -independent mechanism.

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A New Member of the Tubulin Superfamily Orients Cilia in Multiciliated Epithelial Cells. Erin Turk[1], Airon Wills[2], Stuart Howes[4], Eva Nogales[3,5,6], John Wallingford[1,2], Tim Stearns[1,7]. 1) Department of Biology, Stanford University, Stanford CA; 2) Section of Molecular Cell and Developmental Biology, University of Texas at Austin, College Station, TX; 3) Howard Hughes Medical Institute; 4) Biophysics Graduate Program, UC Berkeley, Berkeley, CA; 5) Life Science Division, Lawrence Berkeley National Laboratory, Berkeley, CA; 6) Department of Molecular and Cell Biology, UC Berkeley, Berkeley, CA; 7) Department of Genetics, Stanford School of Medicine, Stanford, CA.

Tubulins are a conserved superfamily of proteins that are important in the structure and function of the microtubule cytoskeleton. Members of the alpha-tubulin, beta-tubulin, and gamma-tubulin families are found in all eukaryotes, and are the structural elements of the microtubule polymer, and the main nucleator of that polymer, respectively. Members of the delta-tubulin and epsilon-tubulin families are present in many, but not all, eukaryotes and are associated with centrioles. We have characterized in vertebrates members of another tubulin family, which most closely resembles eta-tubulin, previously described in Paramecium. Based on this similarity we will refer to this as the eta-tubulin subfamily. Eta-tubulin is present in many single-celled eukaryotes, chordates, and marsupial mammals, but is absent in Drosophila, C. elegans, zebrafish, and placental mammals. Analysis of genome sequences indicates that eta-tubulin is the last remaining tubulin family member to be characterized in vertebrates. Eta-tubulin is most similar to delta-tubulin, although it is clearly a distinct tubulin family. Eta-tubulin is also the least well-conserved of the tubulin families, both in terms of the number of eukaryotic lineages that have lost the gene and in the divergence of the protein within branches that have retained it. We have characterized Xenopus laevis eta-tubulin in a variety of cell lines and developmental stages. In the multiciliated skin cells of the frog embryo, eta-tubulin is a basal body component whose depletion produces ciliary phenotypes, including disorganization of basal body distribution and polarity. In tissue culture cells, however, eta-tubulin is associated with the TRiC-CCT complex, a large cytoplasmic chaperone that is responsible for folding actin, tubulin, and a large number of other proteins. Finally, we show that eta-tubulin's localization pattern is conserved in placental mammals by exogenous expression in mouse multiciliated and tissue culture cells.

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***miR-34/449* miRNAs are required for motile ciliogenesis in vertebrate mucociliary epithelia by direct repression of *cp110* and regulation of basal body function. P Walentek[1,6]**, R Song[2,6], N Sponer[2,6], A Klimke[3], JS Lee[2], G Dixon[2], RM Harland[1], Y Wan[4], P Lishko[2], M Lize[5], M Kessel[3], L He[2]. 1) Molecular & Cell Biology Department, Genetics Genomics and Development Division, University of California, Berkeley, Berkeley, CA; 2) Molecular & Cell Biology Department, Cellular and Developmental Biology Division, University of California, Berkeley, Berkeley, CA; 3) Department of Molecular Cell Biology, Max Planck Institute for Biophysical Chemistry, Goettingen 37077, Germany; 4) The Third Military Medical University, Chongqing 400038, China; 5) Department of Molecular Oncology, University of Goettingen, Goettingen 37073, Germany; 6) These authors contributed equally to this work.

Multiciliated cells (MCCs) harbor dozens of motile cilia that beat in coordinated fashion to transport mucus in mucociliary epithelia, e.g. in the vertebrate airway. The *Xenopus* embryonic epidermis is also a mucociliary epithelium, and has emerged as a valuable model to study transcriptional and signaling control of MCC induction, differentiation and function. In addition to transcriptional networks consisting of factors like Notch, Multicilin and Foxj1, post-transcriptional mechanisms also control ciliogenesis in vertebrate MCCs. The *miR-34/449* family consists of six homologous miRNAs located at three genomic loci.

Redundancy of *miR-34/449* and their dominant expression in multiciliated epithelia suggest a functional significance in ciliogenesis. In both *Xenopus* and mice, *miR-34/449*-deficient MCCs exhibited a significant decrease in cilia length and number, due to defects in basal body maturation and docking to the apical membrane. *miR-34/449* function in ciliogenesis, at least in part, by direct post-transcriptional repression of Cp110, a centriolar protein that suppresses cilia assembly. Knockdown of *cp110* in *miR-34/449*-deficient MCCs restored ciliogenesis by rescuing basal body maturation, Centrin incorporation and docking to the apical membrane. Our findings provide key insights into the evolutionarily conserved functions of *miR-34/449* and elucidate the mechanism through which *miR-34/449* enable motile ciliogenesis.

Developmental Biology

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A novel role for GSK3 and Polo-like kinase in promoting cranial neural crest cell migration and regulating ADAM13 function. Genevieve Abbruzzese[1], Hélène Cousin[1,2], Dominique Alfandari[1,2]. 1) Molecular and Cell Biology, University of Massachusetts, Amherst, MA; 2) Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA.

The cranial neural crest (CNC) is a highly migratory population of cells that forms the craniofacial structures in all vertebrates. Our lab has previously shown that the cell surface metalloprotease ADAM13 is promotes CNC cell migration by cleaving the cell adhesion molecule Cadherin-11 to release its adhesive extracellular domain. In addition, we have also discovered a novel and essential role for the cytoplasmic domain of ADAM13 in gene regulation. Previously, the cytoplasmic domains of ADAMs have only been shown to control localization or modify the enzymatic activity of the protease. Our work has shown, however, that the ADAM13 cytoplasmic domain (C13) is cleaved off from the membrane-bound protease and translocates into the nucleus where it regulates gene expression. One of the genes regulated by ADAM13, the protease Calpain8, is essential for CNC migration. While the nuclear function of ADAM13 is conserved from nematodes to marsupial, it is unclear if this is a general function of ADAMs and how this process may be regulated.We show that ADAM13 function to promote CNC migration is regulated by two successive phosphorylations involving GSK3 and Polo-like kinase (Plk). We further show that inhibition of either kinase blocks CNC migration and that the respective phosphomimetic forms of ADAM13 can rescue these inhibitions. However, these phosphorylations are not required for ADAM13 proteolysis of its substrates, γ-secretase cleavage or nuclear translocation of its cytoplasmic domain. Significantly, migration of the CNC can be restored in the absence of Plk phosphorylation by expression of Calpain8, pointing to an impaired nuclear activity of ADAM13. We have identified several interesting binding partners for ADAM13 that may be important for regulating Calpain8 expression and will determine whether phosphorylation mediates these binding interactions.

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A conserved function for T-type calcium channels in anterior neural tube closure. Sarah Abdul-Wajid, Heidi D. Morales Diaz, William C. Smith. UCSB, Santa Barbara, CA.

Through a forward genetic screen in the marine invertebrate chordate *Ciona*, we identified the candidate T-type calcium channel gene, *CAV3*, as a regulator of anterior CNS closure. A spontaneous mutation in the *CAV3* gene resulted in neural tube closure defects reminiscent of human embryonic exencephaly. A role for *CAV3* in neural tube closure had not been described previously, so we examined orthologs in *Xenopus* to investigate possible conservation of function. Expression patterns for *CAV3* orthologs in *Xenopus* during the early stages of anterior neural plate closure correlate well with those observed for *CAV3* in *Ciona*. Injection of a splice-blocking morpholino against *CAV3.2*, the earliest expressed T-type calcium channel in *Xenopus*, results in anterior neural tube closure delay and a later defect that manifests in a dramatic protrusion and expulsion of neural tissue from the anterior head. Our study characterizes this phenotype in *Xenopus*, and correlates it with the amount of disrupted splicing achieved in each morpholino-injected embryo. Finally, we explore potential mechanisms by which *CAV3.2* might play a role in sealing the anterior neural tube.

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Different requirements for GATA factors in cardiogenesis are mediated by non-canonical Wnt signaling. Boni A Afouda, Stefan Hoppler. University of Aberdeen School of Medical Sciences Institute of Medical Sciences Foresterhill Health Campus Aberdeen, AB25 2ZD Scotland (UK).

GATA factors and Wnt signals are key regulators of vertebrate cardiogenesis, but specific roles for individual GATA factors and how they interact with Wnt signaling remain unknown. We use loss of function and overexpression approaches to elucidate how these molecules regulate early cardiogenesis in Xenopus. In order to minimize indirect effects due to abnormal early embryogenesis, we use pluripotent embryonic tissues as cardiogenic assays. We confirm central roles for GATA4, 5, and 6 in cardiogenesis, but also discover individual and different requirements. We show that GATA4 or 6 regulate both cardiogenic potential and subsequent cardiomyocyte differentiation but that GATA5 is involved in regulating cardiomyocyte differentiation. We also show that Wnt11b signaling can rescue reduced cardiac differentiation resulting from loss of function of GATA4 and 6 but not GATA5. We conclude that Wnt11b mediates the differential requirements for GATA factors during vertebrate cardiogenesis.

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High-resolution Analysis of Oocyte RNAs Reveals Gene Pathways Specific for the Vegetal Pole. Tristan Aguero[1], Dawn Owens[1], Derek Van Booven[2], Karen Newman[1], Mary Lou King[1]. 1) Depart. of Cell Biology, University of Miami, School of Medicine, Miami, FL 33136; 2) Hussman Institute for Human Genomics, University of Miami, School of Medicine, Miami, FL 33136.

During Xenopus oogenesis, specific maternal RNAs are localized to the vegetal pole that will determine germ layer identity, dorsal/ventral patterning, and the germ cell lineage in the embryo. To date, identification of localized maternal mRNAs has relied on microarray analysis, an approach limited to known mRNAs. Here we present our results using RNA-seq to identify, in an unbiased way, RNAs highly enriched at the vegetal pole. In situ hybridization and qPCR were used to validate localized RNAs. Complete details of the RNA-seq procedures and parameters will be provided at the meeting. Vegetal and animal pole tips were cut from stage VI oocytes. Samples from three different frogs were pooled for one set; a total of 3 sets were made from a total of nine frogs. Data were aligned to the Xl. (v6.0) and Xt. (v7.1) annotated genomes, in addition to de novo sequencing. Over three thousand transcripts were found differentially expressed between the animal and vegetal poles. Reads aligning to a ribosomal specific reference or mitochondria sequences represented <5% and 1.28% of the total respectively. The top 355 RNAs enriched >900 to 5-fold over the animal pole sample were selected for GeneGo analysis. Protein-modifying enzymes, receptors, ligands, RNA binding proteins and 4 key transcription factors defining hubs were selected for functional analyses. The maternal transcription factor Creb1 was found in diverse gene pathways involving localized mRNA, suggesting its function as an embryonic determinant is based on its regulating expression of localized RNAs. Interestingly, known genes in neurogenic pathways were well represented. miRNA analysis identified only 8 and all uniformly distributed. 16 localized mRNAs were identified containing at least one recognition sequence conserved between Xt. and humans for these miRNAs. Early embryonic patterning appears not regulated by localized maternal miRNAs, but rather localized mRNAs. Interestingly, 5 miRNAs are found densely clustered on the human X chromosome and are dysregulated in cancers. (NIH HD072340 to MLK)

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Identification and Expression Analysis of Two *Xenopus laevis* Homologs of the Novel F-Box Containing Protein, FBXO30. Osamah Badwan[1], Theodor Zbinden[1,2], Noelia Flores[1], Tamia Lozada[1], Dariana Núñez[1], Jesús Ayala[1], Josué Hernández[2], Grisselle Valentín[2], José García[2], Edwin Traverso[1]. 1) Biology, University of Puerto Rico at Humacao, Humacao, PR; 2) Biology, University of Puerto Rico-Río Piedras, San Juan, PR.

Tumorhead (TH) is a maternal factor that regulates cell proliferation during early embryogenesis in *Xenopus laevis.* To understand how TH functions at the molecular level, we have been studying its relationship with the novel F-Box containing protein FBXO30, found in a two-hybrid screen for TH binding proteins. Using RT-PCR, we identified two FBXO30 homolog genes in *X. laevis*, named FBXO30-A and FBXO30-B. The FBXO30-A and FBXO30-B proteins are 91% identical to each other, and share 64% and 63% identity with their *Homo sapiens* homolog, respectively. Sequence analysis and alignment of several vertebrate FBXO30 proteins show that they contain very conserved Traf-like zinc finger domains at their N-terminus, and F-Box domains at their C-terminus, while the internal part of the proteins diverge extensively. We found through RT-PCR that FBXO30-A and FBXO30-B are maternal factors as their messages are present in the unfertilized egg. Their mRNAs persist during the cleavage stages but decrease dramatically once gastrulation starts. The FBXO30-A and FBXO30-B messages are present at low levels during organogenesis, showing a slight peak of expression during the mid-tailbud stages. *In situ* hybridization studies show that the maternal FBXO30-A mRNA is localized to the animal pole, and that the later expression of FBXO30-A occurs in the developing somites. Using antibodies raised against a synthetic FBXO30-A peptide, we have preliminarily localized the FBXO30-A protein to the nuclei of ectodermal cells at the gastrula (st. 12) stage. Our studies show the presence of two homologs of FBXO30 in *X. laevis*, which could be key regulators of early development, working with TH to regulate cell proliferation.

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***Xenopus* Egr4 is required to specify rhombomere 5 in the hindbrain. Chang-Joon Bae**, Jean-Pierre Saint-Jeannet. Basic Science and Craniofacial Biology, New York University College of Dentistry, New York, NY.

The transcription factor Egr4 (early growth response 4) was isolated in a screen for genes synergistically activated by Pax3 and Zic1 in *Xenopus* animal explants. *egr4* is specifically and transiently expressed in rhombomeres 5 and 6 (r5/r6), and in the neural crest cells that populate the third branchial arch. In both regions, *egr4* expression depends on Pax3 and Zic1 function. Egr4 knockdown in whole embryos using two morpholino antisense oligonucleotides, blocking either *egr4* translation or splicing, caused a similar loss of expression of the posterior hindbrain gene *mafb1*, normally confined to r5/r6. The r5 expression domain of another hindbrain-specific transcription factor, *krox20 (egr2)*, was also reduced in these embryos, while its expression in r3 was unaffected. This phenotype could be fully rescued by injection of either frog or mouse Egr4 mRNA. In contrast, Egr4 gain-of-function resulted in a dramatic expansion of *mafb1* and *krox20*, associated with a loss of *hnf1b*, a well-characterized direct activator of*mafb1* in amniotes. We found that Hnf1b can activate *egr4* expression in the embryo, and demonstrated that Hnf1b regulates *mafb1* expression indirectly through Egr4. We propose that Egr4 is a novel player in the regulatory circuit controlling r5 identity in the *Xenopus* hindbrain: Hnf1b -> Egr4 -> Mafb1 -> Krox20 --| Hnf1b.

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Prdm12 specifies V1 interneurons downstream of Pax6 and through cross-repressive interactions with *Dbx1* and *Nkx6.1* in the developing vertebrate spinal cord. E. Bellefroid[1], A. Thelie[1], S. Desiderio[1], J. Hanotel[1], S. Kricha[1], G. Cerva[2], K. Lewis[2], C. Hui[3], M. Götz[4], M. Sander[5], Alexandra Pierani[6]. 1) University of Brussels, Gosselies, Belgium; 2) Syracuse University, Syracuse, NY; 3) The Hospital for Sick Children, Toronto, Ontario, Canada; 4) Helmholtz Center Munich, Munich, Germany; 5) Departments of Pediatrics and Cellular and Molecular Medicine, Pediatric Diabetes Research Center, University of California, La Jolla, CA; 6) Institut Jacques Monod, Université Paris Diderot, Paris, France.

Prdm zinc finger transcription factors act as epigenetic regulators of gene expression in many developmental processes and their deregulation has been associated with human diseases. Here we show that *Prdm12* is expressed in specific regions of the fore- and midbrain, cranial placodes, and in p1 spinal cord progenitors in the *Xenopus* embryo and that this restricted expression profile is conserved in the zebrafish, chick and mouse embryos, Prdm12 being in addition strongly expressed in those species in dorsal root ganglia. We found that *Prdm12* is dependent on Pax6 and that it is restricted to p1 progenitors due to the repressive action of the homeobox transcription factors Dbx1 and Nkx6.1. Knockdown experiments in the frog revealed that Prdm12 is required for *eng1* expressing V1 interneurons. Conversely in *Prdm12* overexpressing embryos, V1 interneurons are expanded while *Dbx1* and *Nkx6.1,* expressed rescpectively in the adjacent p0 and p2 domains and promoting respectively V0 and V2 interneurons, are reduced. This ability of Prdm12 to induce *eng1* is blocked by coinjection of Dbx1 or Nkx6.1. Overexpression of *Prdm12* in the chick neural tube leads as well to a dramatic increase of eng1 and of other markers representative of the different V1 interneuron subpopulations. Finally, we show that an Engrailed-Prdm12, but not VP16-Prdm12, has a V1 inducing activity similar to that of native Prdm12, and that Prdm12 activity requires both its PR and zinc finger domains. Together, our results indicate that Prdm12 acts in ventral spinal cord progenitors a general determinant of V1 cell fate by functioning as a transcriptional repressor and through cross-regulatory interactions with Dbx1 and Nkx6.1.

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The Wnt co-receptor PTK7 is dynamically localized in neural crest cells and required for their migration. Annette Borchers[1], Hanna Berger[1], Ewa Maj[1], Martina Podleschny[1], Peter Wehner[2], Hanna Peradziryi[2]. 1) Department of Biology, Molecular Embryology, Philipps University Marburg, Karl-von-Frisch-Str. 8, 35032 Marburg, Germany; 2) Department of Developmental Biochemistry, Center for Molecular Physiology of the Brain (CMPB), GZMB, University of Göttingen, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany.

PTK7 (protein tyrosine kinase 7) is an evolutionarily conserved transmembrane receptor with a broad range of functions including the determination of planar cell polarity and the regulation of cell migration. In *Xenopus laevis* PTK7 is required for neural crest migration. The PTK7 protein is dynamically localized during different phases of neural crest migration. It transiently accumulates at cell-cell contact sites, but is rapidly removed from the membrane if cell-cell contacts are broken. The molecular mechanism controlling this dynamic localization is unclear, but our data support a model whereby Wnt ligands may play a role. First, we find evidence that PTK7 interacts with members of the Wnt family. PTK7 co-precipitates canonical Wnt3a and Wnt8, but not non-canonical Wnt5a or Wnt11. Second, we observe that the PTK7 protein localization is affected by the presence of Wnt ligands. Membrane localized PTK7 translocates from the plasma membrane to the cytoplasm in the presence of canonical Wnt, while it is not affected by non-canonical Wnt ligands. Furthermore, we find that PTK7 inhibits canonical Wnt signaling in functional assays. As it was previously shown that PTK7 regulates non-canonical Wnt signaling, these data indicate a broader role for PTK7 in the control of Wnt signaling pathways, which will be discussed here in the context of neural crest migration.

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Characterising targets of FGF signalling in *Xenopus* neural development. Hannah Brunsdon, Harry V. Isaacs. Department of Biology, University of York, York, North Yorkshire, United Kingdom.

There is evidence of an important role of FGF signalling in the specification and patterning of posterior neural tissues. Transcriptional targets of FGF signalling during germ layer specification have been identified recently by Branney et al., (2009). However, less is known about FGF targets active during neural development. The aim of this project is to investigate proximal downstream targets of FGF signalling in the context of neural development by using drug-inducible forms of *Xenopus* FGF receptors (iFGFRs). RNA-seq was used to investigate global changes to the *Xenopus laevis* transcriptome as a result of iFGFR1 or iFGFR4 activation during a period of early neural specification. Lists of transcripts with an FPKM >30 and fold change of >2 were compiled. 188 genes were found to be affected by iFGFR1 and 274 genes affected by iFGFR4. Functional characterisation of putative FGF targets using morpholinos and TALEN technology is being undertaken to better understand the different aspects of iFGFR1 and iFGFR4 signalling and their roles in neural development.

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A comparative study on the Krüppel-like factors during *Xenopus* embryogenesis. Yan Gao, Qing Cao, Lei Lu, Xuena Zhang, Zan Zhang, **Ying Cao**. Model Animal Research Center, Nanjing University, Nanjing, Jiangsu, China.

The Krüppel-like family transcription factors, which include 17 members in mammal (Klf1-Klf17), play important roles in cell differentiation and proliferation and are aberrantly regulated in many diseases including cancers. However, their functions during *Xenopus* early embryogenesis have been reported in very few studies. We analyzed the expression patterns of *Klf2*, *Klf5*, *Klf6*, *Klf7*, *Klf8*, *Klf11*, *Klf15*and *Klf17* during *Xenopus* embryogenesis, and examined their functions in germ layer formation using loss and gain of function analyses. Results demonstrate that these Klf genes are all maternally transcribed, but in

later stages they show distinct expression patterns, especially in different organs. They exhibits different effects on germ layer formation and expression of genes that are involved in dorsoventral patterning. The study presents a comprehensive view for the expression and functions of the Klf family during *Xenopus* embryogenesis.

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Snail2/Slug cooperates with Polycomb Repressive Complex 2 (PRC2) to regulate neural crest development. Chih-Liang Tien[1], Amanda Jones[2], Hengbin Wang[2], **Chenbei Chang[1]**. 1) Cell, Developmental and Integrative Biology, Univ. Alabama at Birmingham, Birmingham, AL; 2) Department of Biochemistry and Molecular Genetics, Univ. Alabama at Birmingham, Birmingham, AL 35294.

Neural crest cells arise from the border of the neural plate and epidermal ectoderm, migrate extensively, and differentiate into diverse cell types during vertebrate embryogenesis. Though much has been learnt about growth factor signals and gene regulatory networks that regulate neural crest development, limited information is available on how epigenetic mechanisms control this process. In this study, we show that Polycomb Repressive Complex 2 (PRC2) cooperates with the transcription factor Snail2/Slug to modulate neural crest development in Xenopus. PRC2 core components, Eed, Ezh2 and Suz12, are expressed in the neural crest cells and required for neural crest marker expression. Knockdown of Ezh2, the catalytic subunit of PRC2 for histone H3K27 methylation, results in defects in neural crest specification, migration and craniofacial cartilage formation. EZH2 interacts directly with Snail2, and Snail2 fails to expand the neural crest domains in the absence of Ezh2. Chromatin immunoprecipitation (ChIP) analysis shows that Snail2 regulates EZH2 occupancy and histone H3K27 trimethylation levels at the promoter region of the Snail2 target E-cadherin. Our results indicate that Snail2 cooperates with EZH2 and PRC2 to control expression of the genes important for neural crest specification and migration during neural crest development.

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Control of neural crest migration and mouth formation by Kinin-Kallikrein signaling from the extreme anterior domain. Laura Jacox[1], Radek Sindelka[2], **Justin Chen[1]**, Alyssa Rothman[1], Amanda Dickinson[3], Hazel Sive[1]. 1) Whitehead Institute, 9 Cambridge Center, Cambridge, 02142 MA; 2) Institute of Biotechnology, Videnska 1083, Prague, 14220, Czech Republic; 3) Virginia Commonwealth University.

The extreme anterior domain (EAD) is a putative embryonic signaling center that includes the presumptive mouth. We show that the Kinin-Kallikrein pathway is active in the EAD and necessary for craniofacial development in *Xenopus* and zebrafish. The mouth failed to form and neural crest development and migration was abnormal after loss of function in the pathway genes *kng*, encoding Bradykinin peptide, *carboxpeptidase-N* (*cpn)* that cleaves Bradykinin and *neuronal nitric oxide synthase*. Consistent with a role for nitric oxide in face formation, endogenous NO levels declined after LOF in pathway genes but these and a normal face could be restored by medial implantation of xBdk-beads into LOF embryos. Facial transplants demonstrated that Cpn function within the EAD is essential to guide first arch cranial NC into the face and to promote mouth opening. The study identifies a novel localized signaling mechanism essential for craniofacial development.

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The transcriptional regulation of the BMP signaling pathway in the early *Xenopus tropicalis* embryos using genome-wide approaches. **Jin Cho**, Ira Blitz, Ken Cho. University of California Irvine, Irvine, ca.

The bone morphogenetic protein (BMP) signaling pathway is required to specify ventral fate during germ layer (ectoderm, mesoderm and endoderm) specification. However, the mechanism of transcriptional regulation through BMP signaling to specify ventral endoderm is still unclear. To better understand the molecular events behind this specification, we first performed Smad1 chromatin immunoprecipitation followed by sequencing (ChIP-seq) using early gastrula embryos to identify the possible cis-regulatory regions regulating BMP target genes. Furthermore, to better understand the influence of chromatin organization on gene expression, we have adapted DNase-seq to map the DNase I hypersensitive sites within the Xenopus tropicalis genome. Thus, by combinining ChIP-seq and DNase-seq, we hope to identify open chromatin regions that contain transcriptionally active cis-regulatory elements. Our preliminary results show that Smad1 uses a conserved BMP-responsive element (BRE) as an active cis-regulatory motif to regulate ventral endoderm target genes such as *ventx2.1*, *id3* and *bambi*. Smad1 also binds other cis regulatory elements for transcriptional regulation. These data suggest Smad1 uses different elements and multiple regulatory modes for target gene transcription.

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Calpain2: a novel regulator of Apical Constriction. **Neophytos Christodoulou**, Paris Skourides. University of Cyprus, Nicosia, Cyprus.

Apical Constriction (AC) is a morphogenetic movement driving cell shape changes during embryogenesis. Calpain2 is a calcium dependent intracellular cysteine protease implicated in several biological processes. Downregulation of Calpain2 in the neural tissues of Xenopus embryos results in anterior Neural Tube Defects (NTDs). Such defects have been previously correlated with defective AC. To asses the role of Calpain2 in AC, we examined the effects of Calpain2 downregulation on the AC induced cell shape changes occurring in neuroepithelia. Downregulation of Calpain2 blocked AC of neuroepithelial cells.This phenotype was rescued by the expression of a Calpain2 rescue construct, confirming that the AC phenotype is due to Calpain2 downregulation. AC drives the formation of several organs such as the eye, the cement gland, the gut and all the placodes. Whole mount in-situ hybridization revealed elevated Calpain2 exprerssion in these tissues. To explore the possibility that Calpain2 is a

general regulator of AC, we carried out loss of function experiments using morpholino and a Calpain specific inhibitor. From these experiments we concluded that Calpain2 is a general regulator of AC as it regulates this morphogenetic movement during the formation of the gut, the cement gland and the olfactory placode. To position Calpain2 into the signaling pathway of AC we examined the necessity of Calpain2 function during Shroom3 and Lulu induced AC, showing that Calpain2 regulates AC downstream of both Shroom3 and Lulu. AC is driven by pulsed constractions of a medioapical actomyosin complex. In order to explore Calpain2 involvement in the formation of the actomyosin complex we stained Calpain2 morphant embryos with phalloidin and for phosphorylated myosin. Calpain2 downregulation resulted in the reduction of apical enrichment of both actin and phosphorylated myosin. From our experiments we conclude that Calpain2 downregulation results in anterior NTDs due to impaired AC. In addition, we show that Calpain2 is a general regulator of AC acting downstream of Shroom3 and Lulu. Finally, our results suggest that Calpain2 is involved in AC, through regulation of the formation of the actomyosin complex.

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Increasing the repertoire of asymmetrically distributed maternal mRNAs in *Xenopus tropicalis* through RNA-seq analysis of single blastomeres from the 8-cell embryo. Elena De Domenico, Nick D. L. Owens, Michael J. Gilchrist. MRC-National Institute for Medical Research The Ridgeway Mill Hill London, United Kingdom.

An important question in developmental biology is how specification of the major body axes is controlled in the early embryo. In *Xenopus*, maternal mRNAs are distributed with radial symmetry around the animal-vegetal axis connecting the darkly pigmented animal pole and the lightly pigmented vegetal pole. Cortical rotation after fertilization reorganizes the cytoplasmic content of the egg, and translocates maternal dorsalizing factors to the opposite side with respect to the sperm entry point. This study builds on earlier work in *Xenopus laevis* involving microarray analysis of axially dissected pools of blastomeres, or qPCR on selected genes from single blastomeres.In this work, we investigate the asymmetrical distribution of maternal mRNAs by micro-dissection of 8-cell *Xenopus tropicalis* embryos, and RNA-seq analysis of the individual blastomeres using low imput RNA protocols. This has the advantages that each of the three primary axes can be investigated from one set of blastomeres, and that correlation amongst the four blastomeres at either end of each axis can be used to increase confidence in the results. Data were compiled by analysis of blastomeres from three embryos. Overall we detect the expression of ~12,200 genes in the blastomeresWe find several hundred genes showing asymmetric distribution over the different primary axes of the embryo, although a large majority of these were between the animal and vegetal poles of the developing embryo, with most accumulation in the vegetal blastomeres. Comparison with published data identifies many genes not previously known to be asymmetrically distributed, including over half of the 113 of the genes found with at least three-fold higher expression in the vegetal blastomeres. Many previously reported asymmetrically distributed genes were found with a lower fold-change.

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TAF15 is required for proper dorso-ventral patterning in *Xenopus* through the repression of *ventx2*. Caitlin DeJong, Darwin Sorento Dichmann, Richard Harland. U.C. Berkeley, Berkeley, CA.

Proper embryonic development requires a series of precisely concerted events and players. Previous work in the lab found RNA-binding proteins to be key regulators of embryogenesis (Dichmann et al. 2008). One such gene, Fused in Liposarcoma (Fus), was shown to be essential; playing a role in alternative pre-mRNA splicing and is required for gastrulation and cell adhesion (Dichmann and Harland 2012). Fus belongs to the FET family of proteins that includes Ewings sarcoma (EWS) and TATA box binding protein-associated factor 15 (TAF15). Given the requirement for Fus in *Xenopus* embryogenesis, we asked if TAF15 fulfils a similar developmental role. TAF15 is a non-canonical TAF and has been implicated in many aspects of transcriptional regulation. TAF15 is known to associate with TFIID, a subset of the spliceosome, microRNA machinery, and acts as an RNA-binding protein. Additionally, translocated fusion forms of TAF15, like Fus and Ews, have been implicated in tumorigenesis, sarcomas, and leukemias. Although TAF15 has been implicated in many processes, very little is known about direct genetic interactions of TAF15 *in vivo*. Our studies find that *taf15* is expressed in the future dorsal region of the embryo and is expressed in the neural crest, dorsal mesoderm, as well as in the epithelial and sensorial layers of the epidermal ectoderm during neurulation. Upon knockdown of taf15, morphant embryos exhibit a shortened anterior-posterior axis, fail to respond to touch, and die before tadpole stages. Using genetic knockdown and transcriptome analysis approaches, we are unraveling an interesting genetic pathway where TAF15 negatively regulates the ventrally expressed *ventx2* gene. *ventx2* expression is complimentary to *taf15* and upon knockdown of*taf15*, *ventx2* expression increases dorsally. Continuing work aims to understand the mechanism by which Taf15 represses *ventx2* from dorsal regions of the embryo and the effects of this genetic relationship on dorso-ventral patterning.

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Exposure to the Common Preservative Methylisothiazolinone (MIT) Inhibits Development and Blocks Both Regeneration and Wound Healing in *Xenopus laevis*. Nicole Delos Santos, Jessica Drenth, Ai-Sun Tseng. School of Life Sciences, University of Nevada, Las Vegas.

The powerful biocide, methylisothiazolinone (MIT), is a preservative commonly found in household items such as shampoos, dish soap, and baby wipes. Recent studies have linked MIT to the surprising upsurge in human contact dermatitis cases. Moreover, MIT has also been identified as a neurotoxin, inhibiting neural outgrowth at lower concentrations while inducing neural cell death at higher ones. Even though MIT is found in many baby care items, it is not known how this chemical affects animal development. To investigate the effects of MIT exposure on embryogenesis, we used *Xenopus laevis* as a model to

determine its environmental toxicity. Our results show that treatment with MIT at the same concentration contained in most household products (15ppm or 100µM) induced developmental delays with abnormal morphogenesis and swimming behavior in *Xenopus* embryos and larvae. Importantly, tadpoles treated with 100µM MIT immediately following tail amputation completely failed to regenerate. This regenerative failure was found to be due to a lack of wound healing at the injury site. We performed a wound healing assay that compared the effects of MIT with the known regeneration-inhibiting drug MS222 (a sodium channel blocker). These data revealed that while both control and MS222-treated tadpoles could cover their wounds within 24 hours post-amputation, MIT-treated animals maintained open wounds that did not reduce in size. Our data suggest that exposure to MIT at currently accepted concentrations is detrimental to developing organisms, highlighting a need to further understand the mechanisms of MIT activity and evaluate the consequences of MIT exposure to the public.

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The alternative spicing regulator Tra2b is required for somitogenesis and regulates a novel inhibitory *wnt11b* isoform. Darwin Dichmann, Peter Walentek, Richard Harland. Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA.

Alternative splicing is pervasive in vertebrates and expands proteomic complexity, yet little is known about most isoforms or how they are regulated. *transformer-2b (tra2b)*, encodes a serine-arginine-rich SR-like protein that regulates splicing, but whose endogenous function is poorly understood. Here we combine the power of frog embryology with RNA-seq to identify novel splice changes and determine their biological function. Morpholino-oligonucleotide mediated knockdown of Tra2b in *Xenopus* results in embryos with multiple defects, including shortened A-P axes and defective somitogenesis. Using a sensitive RNA-seq analysis pipeline for detecting novel isoforms, we identify 142 significant splice changes in 133 genes, of which 89% are novel. The most common splice changes in *tra2b* morphants are whole intron retention (43%) followed by exon skipping (34%). One of the novel isoforms is a *wnt11b* isoform that retains the last intron, resulting in a truncated Wnt11b ligand (Wnt11b-short). Using animal cap experiments, we show that Wnt11b-short inhibits Wnt signaling in cardiac gene induction by Wnt+Activin and functions similar as a dominant-negative to inhibit pronephric tubule formation in whole embryos. To determine the contribution of Wnt11b-short to the *tra2b* morphant phenotype, we use a splice-blocking morpholino to induce retention of intron4 in *wnt11b*. Retention of intron4 in *wnt11b*recapitulates the failure to form somites but not other defects in *tra2b* morphants, indicating that changed splicing of *wnt11b* specifically cause somitogenic defects. This is the first example of alternative splicing of a Wnt ligand and adds additional intricacy to an already complex signaling pathway and highlights intron retention a regulatory mechanism.

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The Heterotaxy Candidate Gene, Deadly Nightshade, is a novel regulator of Wnt signaling. Anna R Duncan, John Griffin, Andrew Robson, Mustafa Khokha. Pediatrics, Yale School of Medicine, New Haven, CT.

Congenital heart disease (CHD) affects 1 in 130 newborns and is a major cause of morbidity in the pediatric population. One cause of CHD is heterotaxy (Htx), a disorder of abnormal left-right (LR) development. Unfortunately, many of the genetic causes of Htx remain undefined, but a recent screen of Htx patients (Fakhro et al 2010) suggests that a mutation in a novel gene that we have recently named Deadly Nightshade (Ddns) may lead to Htx. We examined the role of Ddns using morpholino (MO) knockdown and mRNA overexpression in Xenopus tropicalis. At a low dose of MO, we can recapitulate our patient's Htx phenotype in Xenopus. Interestingly, at a higher dose of MO, a remarkable gastrulation defect occurs, in which the blastopore is unable to close following multiple attempts. The gastrulation defect appears to be due to changes in the Wnt signaling pathway. Western blot and TOPflash analysis of Ddns knockdown show a significant decrease in beta-catenin expression and confocal imaging of these embryos showed decreased nuclear localization of beta-catenin during gastrulation. Ddns overexpression induces twinning in the embryos and increased TCF/LEF activation by TOPflash assay. Together our results confirm Ddns as a heterotaxy gene and demonstrate it to be a novel activator of the Wnt signaling pathway.

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Computational Visualization of Cell Motility driving Neural Convergent Extension and Growth Cone Motility underlying Optic Axon Pathfinding in *Xenopus Laevis*.. Tamira Elul[1], Amar Bains[2], Avik Patel[1]. 1) Department of Basic Sciences, Touro University California, Vallejo, CA; 2) Department of Molecular and Cellular Biology, UC Berkeley, Berkeley, CA.

We have made visualizations of cell motility underlying convergent extension of the neural ectoderm, and growth cone motility driving optic axon pathfinding in *Xenopus* embryos. To do so, we used the programming language, Processing, developed at MIT for visual artists to incorporate programming into their art, and easily amenable to programming dynamic shapes. We first visualized cell motility to mimic that observed in timelapse videos of of neural convergent extension in explants from Xenopus embryos (Elul et al., 19995). Specific geometric and mechanical parameters such as aspect ratio and viscosity were used to simulate cell and growth cone motility. These parameters define a computational mechanism of dynamic cell behaviors that drive neural and neuronal development in embryos. Future work will focus on inputting parameters to visualize interactions between the cells and axons, and comparing the computational properties needed to simulate cell motility driving neural and neuronal development in other cell types and species.

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Investigating the roles of the direct Wnt/β-catenin signaling targets *sall1* and *sall4* in neural tube development. Cameron R. T. Exner, Richard M. Harland. Dept. of Molecular & Cell Biology, University of California, Berkeley, Berkeley, CA.

The vertebrate neural plate is patterned along its anteroposterior axis in part by Wnt/β-catenin signaling activity. Though disruption of this pathway is known to lead to characteristic defects in patterning of the neural plate, few downstream factors have been identified and described in terms of a role in neural plate development. Our lab has identified direct targets of Wnt signaling involved in neural plate posteriorization, including genes encoding the transcription factors Sall1 and Sall4. Knockdown of either gene results in partial loss of posterior neural fates, consistent with a role downstream of Wnt signaling, and also causes defects in convergent extension and neural tube closure. Similar to previous reports about *sall* gene function in other contexts, *pou* family gene expression is increased in *sall1* or *sall4*morphant tissue; consequent failure of these tissues to differentiate properly may be an underlying cause of both the patterning and morphogenetic defects. Recent work has focused on investigating the roles of*sall1* and *sall4* at the nexus of neural differentiation, patterning, and morphogenesis. In particular, confocal microscopy has been employed to further characterize the open neural tube phenotype of morphants. Ongoing work will better characterize *pou*-associated differentiation defects and the consequences for patterning and morphogenesis.

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Role of thyroid hormone in neural stem and progenitor cells during Xenopus metamorphosis. Fernando Faunes[1,2], Renzo Bruno[1,2], Ivo Carrasco-Wong[1,2], Juan Larrain[1,2]. 1) P. Universidad Catolica de Chile, Santiago, Santiago, Chile; 2) Center for Aging and Regeneration, Millennium Nucleus in Regenerative Biology.

Xenopus laevis metamorphosis is the transformation of the tadpole into a juvenile frog, controlled by the thyroid hormone (T3). T3 levels exhibit a peak (~8nM) at stage 58. During metamorphosis, tissues respond to T3 at different times *in vivo.* The central nervous system (CNS) is able to respond to T3 at pre-metamorphic stages (stages 46-55). Interestingly, a peak of T3 is also observed in postnatal stages in mammals, when CNS maturation is crucial for normal development. The early cellular mechanisms of the response to T3 in CNS neural stem and progenitor cells (NSPCs) are not completely known. Our general aim is to understand the cellular and molecular basis of metamorphosis by studying the role of T3 on proliferation and differentiation of NSPCs. We have characterized the expression pattern of neural stem cell and differentiation markers in brain and spinal cord during metamorphosis by RT-qPCR. Responsiveness to T3 was studied with the T3-target gene *thrb*. The levels of neuroblast markers *nestin* and *doublecortin* (*dcx*) and proliferation marker *ccnb3* increase with a peak in stage 60, very similar to the pattern of *thrb*, suggesting that T3 induces the exit of quiescence and proliferation. The levels of progenitor cell markers *neurogenin2a* and *neuroD1* decrease and differentiation markers *myelin binding protein* (*mbp*), *plp1a* and *sox10* increase during metamorphosis. Exogenous T3 2nM at stage 50 increase the RNA levels of *nestin, ccnb3* and protein levels of *dcx*. Consistent with these results, the levels of lin28B, a protein with roles in stem cell maintenance and developmental transitions in other animal models, decrease from stage 50 as observed by qRT-PCR, western blot and immunofluorescence. One of the roles of Lin28B is to inhibit the biogenesis of the microRNA *let-7* and, consistently, we observe an increase in the levels of *let-7* in SNC during metamorphosis. These results indicate that T3 induces the exit of quiescence and proliferation of NSPCs during Xenopus metamorphosis. FUNDINGS: FONDECYT 11130564 (FF) and 1141162 (JL), MILENIO RC120003 (JL)

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A *Xenopus tropicalis* mutation in *rax*, a key gene in the eye formation regulatory network, reveals its role in spatially restricting alternative forebrain fates. Margaret Fish[1], Takuya Nakayama[1], Marilyn Fisher[1], Nicolas Hirsch[1], Amanda Cox[1], Rollin Reeder[1], Samantha Carruthers[2], Amanda Hall[2], Derek Stemple[2], Robert Grainger[1]. 1) Dept of Biology, UVA, Charlottesville, VA, USA; 2) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK.

The retinal anterior homeobox (*rax*) gene encodes a transcription factor that is a key part of the regulatory network specifying anterior neural plate and retina, and is necessary for vertebrate eye development. We describe here a *Xenopus tropicalis rax* mutant, the first mutation analyzed in detail from a reverse genetic screen in *Xenopus*. As in other vertebrates, this mutation results in eyeless animals, and is lethal peri-metamorphosis. The consistency of a stable mutant background allowed us to discern several new properties of retinal and neural patterning. We find that tissue normally fated to form retina in these mutants instead forms tissue with characteristics of diencephalon and telencephalon. This implies that a key role of *rax* is to repress alternative forebrain identities, in addition to positively defining the eye field. Our data also examine the timing of patterning of the anterior neural plate, and highlight that brain and retina regions are not determined by the mid-gastrula stage but are by the neural plate stage. An RNA-Seq analysis and *in situ* hybridization assays for early gene expression in the mutant revealed that activation of several key eye field transcription factors (e.g. *pax6*, *lhx2* and *six6*) occurs independently of *rax*. These analyses also identified novel genes that are differentially expressed in mutant presumptive retinal tissue, including key forebrain specification factors *hesx1* and *fezf2*. Expression of these genes is not properly repressed in the *rax* mutant, but remains active in the presumptive eye region, in contrast to wild-type expression patterns. *hesx1* and *fezf2* knock-down experiments restore some normal forebrain properties in the *rax* mutant, indicating that the expanded expression of these genes in the *rax* mutant contributes to the set of defects leading to the transformation of presumptive retinal tissue into non-retinal forebrain identities.

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Function of Thyroid Hormone (TH)-Regulated HAL in Frog Intestinal Stem Cell Development. Liezhen Fu, Nga Luu, Yun-Bo Shi. LGRD/PCRM/NICHD, National Institutes of Health, Bethesda, MD.

Adult organ-specific stem cells are critical for organ homeostasis as well as tissue repair and regeneration. However, it has been difficult to study the formation of adult stem cells during vertebrate development. The development of adult intestine during thyroid hormone (TH)-dependent frog metamorphosis, which involves apoptotic degeneration of the larval epithelium and de novo formation of adult stem cells, offers a unique opportunity to study adult stem cell development. We have shown previously that the adult intestinal stem cells are formed through dedifferentiation of some larval epithelial cells with the connective tissue plays an essential role, likely via the contribution to the stem cell niche. To investigate the underlying molecular basis, we have carried out microarray analysis on tissue- and stage-specific RNAs and discovered a range of dynamic gene expression patterns associated with adult epithelial progenitor/stem cell development. To investigate the function of the regulated genes, we focused on HAL2, an enzyme involved in histidine metabolism that was highly upregulated at the climax of metamorphosis in the epithelium. We demonstrated that HAL2 are induced by TH specifically in the proliferating adult progenitor/stem cells in the metamorphosing epithelium. We further showed that HAL2 is directly regulated through TR/RXR binding to a putative thyroid hormone response element (TRE) in the proximity of its promoter. We generated transgenic animals to overexpress HAL2 using an inducible transgenic construct, and we showed that overexpression of HAL2 promoted intestinal epithelial proliferation, mimicking TH-induced adult epithelial proliferation at the climax of intestinal metamorphosis. This and the discovery that another gene encoding an enzyme in the same histidine metabolic pathway is also highly upregulated in the epithelium argue for a role of this pathway in stem cell development and/or proliferation. These data further support the use of frog intestinal metamorphosis as a model to identify and characterize genes involved in adult organ-specific stem cell development in vertebrates.

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FGF/MAPK signaling is essential for Neural Crest formation in *Xenopus*.. Lauren Geary[1], Carole LaBonne[1,2]. 1) Molecular Biosciences, Northwestern University, Evanston, IL; 2) Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL.

Neural crest cells are a population of stem cell-like progenitors unique to vertebrates that exhibit multi-germ layer developmental potential and are of central importance to vertebrate development and evolution. Neural crest cells originate at the neural plate border, and their development displays dependence on multiple signaling pathways, including BMPs and Wnts. FGF signals have also been implicated in early neural crest development, yet the precise role of these signals has remained poorly understood.A major pathway activated in response to FGF signaling is the Ras-MAPK cascade, and it is this pathway that has been proposed to play a central role in mediating the FGF response in neural crest cells. Much remains to be learned, however, about when this signaling pathway is active, what ligands activate it in early *Xenopus* embryos, and how this pathway contributes to the formation, behavior, and differentiation of neural crest cells. Here, we show that FGF ligands are broadly expressed in *Xenopus* blastula and subsequently become excluded from the neural crest cells. FGF signaling has a required role in these cells, as its inhibition leads to aberrant neural crest cell formation. Misregulation of FGF signaling alters the expression of early neural crest regulatory factors at stages prior to neurula stages. We present evidence for an early role of FGF/MAPK signaling that affects neural crest formation prior to inductive events at the neural plate border.

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The Xenopus Orfeome. Ian Grant[1], Dawit Balcha[2], Tong Hao[2], Yun Shen[2], David Hill[2], Aaron Zorn[3], Todd Stukenberg[4]. 1) MRC National Institute for Medical Research, London, UK; 2) Center for Cancer Systems Biology (CCSB), Dana-Farber Cancer Institute, Boston, MA; 3) Cincinnati Children's Research Foundation, Cincinnati, OH; 4) University of Virginia School of Medicine, Charlottesville, VA.

The *Xenopus* Orfeome project aims to provide a complete set of validated, full-length cDNA transcripts for every open reading frame (ORF) encoded in the *Xenopus* genome. This single set of reagents will be an invaluable resource for the *Xenopus* community. An important benefit of the *Xenopus* Orfeome will be the ready availability of clones in the Gateway entry vector system for all *Xenopus* genes. In addition, there are currently many important proteins that cannot be studied efficiently in *Xenopus* as no full-length clones exist, the *Xenopus* Orfeome project will resolve this. In this first stage of the project, we have transferred existing *Xenopus* laevis (Xl) clones from the *Xenopus* Gene Collection (XGC) into the Gateway system. Around 9,000 Xl ORFs, ranging from 180 to 7,244 nucleotides in length, covering approximately 30% of Xl genes have been cloned. Each clone was sequenced at the 5′ and 3′ ends; from these data we have confirmed a ~90% transfer success rate. In addition to this, we have analysed the reference ORFs themselves with comparison to the *Xenopus* full-length EST database and encoded proteins from other species. From this computational analysis, we have classified the ORFs as demonstrably full-length or not. Around 85% are predicted to be full-length, but for approximately 10% we observed truncation at the 5′ end; for example, the matching EST cluster indicates that the true start of translation should be at a start codon upstream of that defined in the XGC clone. For two-thirds of the truncated ORFs, computational analysis of the XGC sequence including the 5′ and 3′ UTRs predicts a different, longer ORF which matches the EST or prediction from non-*Xenopus* protein data. The first set of clones will be made available to the community shortly. This will contain around 90% of the genes in the XGC clone set, along with analysis confirming their full-length status, which will be an important factor in strengthening the quality of this Orfeome resource.

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The distribution of Dishevelled in convergently extending mesoderm. Eleni Panousopoulou[1], Richard Tyson[1], Till Brettschneider[2], **Jeremy Green[1]**. 1) Dept of Craniofacial Development, King's College London, London, United Kingdom; 2) Warwick Systems Biology Centre, University of Warwick, UK.

Convergent extension (CE) is a conserved morphogenetic movement that drives axial lengthening of the primary body axis and depends on the planar cell polarity (PCP) pathway. In Drosophila epithelia, a polarised subcellular accumulation of PCP core components, such as Dishevelled (Dvl) protein, is associated with PCP function. Dvl has long been thought to accumulate in the mediolateral protrusions in Xenopus chordamesoderm cells undergoing CE. Here we present a quantitative analysis of Dvl intracellular localisation in Xenopus chordamesoderm cells. We find that, surprisingly, accumulations previously observed at mediolateral protrusions of chorda-mesodermal cells are not protrusion-specific but reflect yolk-free cytoplasm and are quantitatively matched by the distribution of the cytoplasm-filling lineage marker dextran. However, separating cell cortex-associated from bulk Dvl signal reveals a statistical enrichment of Dvl in notochord–somite boundary-(NSB)-directed protrusions, which is dependent upon NSB proximity. Dvl puncta were also observed, but only upon elevated overexpression. These puncta showed no statistically significant spatial bias, in contrast to the strongly posteriorlyenriched GFP-Dvl puncta previously reported in zebrafish. We propose that Dvl distribution is more subtle and dynamic than previously appreciated and that in vertebrate mesoderm it reflects processes other than protrusion as such.

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XUch37 regulates the formation of XTcf1/b-catenin complex for mediating Xenopus mesoderm patterning. **Wonhee Han**, Seungjoon Lee, Jin-Kwan Han. LIFE SCIENCES, POSTECH, POHANG, Gyeongbuk, South Korea.

The Wnt/b-catenin pathway has crucial roles in diverse biological processes, including vertebrate development as well as cancer. Ubiquitination and De-ubiquitination events are involved in multiple steps during the Wnt signal transduction but it is still unclear that how these events can be linked to the vertebrate developmental processes. Here, we report that Xenopus Uch37 (XUch37), a deubiquitinase interacts with Xenopus Tcf1 (XTcf1) to regulate the formation of XTcf1/b-catenin complex and is required for the Xenopus mesoderm patterning process. In biochemical analysis, XUch37 positively regulates the Wnt/b-catenin pathway at the transcriptional level and specifically interacts with XTcf1. Chromatin-IP assay shows that depletion of XUch37 inhibits interactions between b-catenin and XWnt target gene promoters such as Xnr3 and Siamois. Furthermore, during Xenopus embryogenesis, depletion of XUch37 causes shorten body axis as well as reduction of the ventrolateral mesoderm marker genes, MyoD and Xpo. Taken together, our results suggest that XUch37 is a new positive regulator of the Wnt/b-catenin pathway through the formation of XTcf1/b-catenin complex and is essential for the mesoderm patterning during Xenopus embryogenesis.

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The Role of Reactive Oxygen Species (ROS) in Early Vertebrate Development. **Y. Han[1]**, S. Ishibashi[1], Y. Chen[1,2], E. Amaya[1]. 1) The Healing Foundation Centre, Faculty of Life Sciences, University of Manchester, Manchester, M13 9PT, UK; 2) Wellcome Trust-Medical Research Council Stem Cell Institute, University of Cambridge, Cambridge CB2 1QR, UK.

Embryonic development involves a variety of complex cellular processes, including cell growth, proliferation, differentiation, migration and cell death. The precise regulation of these events is essential for morphogenesis and development. Reactive Oxygen Species (ROS) have been reported to modulate several signaling pathways and other cellular processes. Previous studies have shown a respiratory oxidative burst following fertilization in the sea urchin. However, whether a burst of ROS also happens in other organisms during early embryogenesis, and whether ROS levels play an important role during embryogenesis remains to be elucidated. *Xenopus laevis* provides an ideal vertebrate model to address these questions. We previously established a Hyper YFP line in *X. laevis*. This line permits the visualisation of ROS levels in living oocytes, fertilized eggs and early embryos. Using this line, we have been able to show that fertilization triggers a dramatic increase in the production of ROS, and that ROS levels remain high throughout early embryogenesis. Through gain and loss of function analysis, we confirm that the IP3 receptor derived calcium initiates the burst of ROS post fertilisation in *Xenopus*. Addition of antioxidants reduces ROS levels in early embryos, and such treatments results in defects in mesoderm formation and gastrulation. In addition we have shown that inhibiting ROS levels in early embryos reduces the activation of several signalling pathways, including canonical Wnt signalling, PI3K/AKT signalling, and TGF-β signalling. These findings suggest that sustained high level of ROS is crucial for early embryonic development in *Xenopus*.

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Comparative analysis of insulin-like growth factor binding proteins. **Y. Haramoto[1]**, T. Oshima[1], S. Takahashi[2], M. Asashima[1], Y. Ito[1]. 1) AIST, Tsukuba, Ibaraki, Japan; 2) Hiroshima University, Higashi-Hiroshima, Hiroshima, Japan.

Insulin-like growth factor binding protein (IGFBP) family consists of six members, that are designated IGFBP1-6. IGFBPs exert various activities involved in many vital biological functions. IGFBPs interact with IGFs (IGF1 and IGF2), and act as carriers thereby protecting IGFs. Thus, they function as modulators of IGF activity. Furthermore, IGFBPs have been reported to have IGF-independent activities. IGFBPs interact with proteins other than IGFs, including cell surface proteins, extra-cellular matrix proteins, and potentially intracellular molecules. In *Xenopus*, only four IGFBPs (IGFBP1, IGFBP2, IGFBP4, and IGFBP5) have been identified so far, and less well characterized. Whether these *Xenopus* IGFBPs correlate to any mammalian IGFBPs is unclear. We report that *X. tropicalis* lacks *IGFBP3*and *IGFBP6* genes in the genome by synteny analyses. We also examined the spatio-temporal expression patterns of *IGFBPs* in the early *Xenopus* development. The expression analyses indicate

that they are expressed differentially during early development. Each *IGFBP* shows a characteristic spatial expression pattern. Especially, *IGFBPs* except for *IGFBP5* are expressed overlapping in the pronephros.*Xenopus* pronephros is composed of four domains (proximal tubule, intermediate tubule, distal tubule, and connecting tubule). Our results showed that at least two *IGFBPs* are co-expressed in all pronephric domains, suggesting that redundant function of *IGFBPs* will be required in early pronephric kidney development.

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Propagation based X-ray phase contrast in vivo microtomography: Gastrulation and neurulation in Xenopus laevis. Ralf Hofmann. Institute for Photon Science, KIT, Karlsruhe, Germany.

We explain principles of a new 4D in vivo imaging modality based on propagation based X-ray phase contrast (XPCμT). Owing to X rays' deeply penetrating nature, their small wavelength, and the particular contrast mechanism 3D images can be obtained that continuously map the electron density throughout (optically opaque) living embryos at submicron resolution and a low heat load. A discussion of imaging and image analysis results in time-lapse-series applications of XPCμT to gastrulation and neurulation of *Xenous laevis* is given. We also sketch our present understanding of X ray dose and how advanced phase retrieval and tomographic reconstruction algorithms plus optimimized experimental conditions at modern synchrotrons will help to reduce the dose problem, presently limiting time lapse series to about two hours duration, for future imaging applications.

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Regulation of axis formation and microtubule dynamics during cortical rotation by vegetally-localized components in the Xenopus egg. Douglas Houston, Abby Matthews, Denise Oh, David Olson. The University of Iowa, Department of Biology, 257 BB, Iowa City IA, 52242.

In Xenopus, asymmetry established by rotation of the egg cortex about the deep cytoplasm during the first cell cycle induces dorsal Wnt/beta-catenin signaling and dorsal axis formation. This cortical rotation critically requires microtubule assembly and polarization, with the plus ends becoming oriented toward the future dorsal side. Cortical rotation is thought to cause the plus end-directed transport of dorsal determinants, including *wnt11b* mRNA and/or protein activators of Wnt/beta-catenin signaling. Despite the importance of cortical rotation, the mechanisms of microtubule assembly and alignment during this process, as well as the mechanisms of Wnt activation, are unknown. Our lab has identified several proteins encoded by localized mRNAs that are required for cortical rotation, however a general lack of understanding of the mechanisms of cortical rotation initiation and potentiation have complicated further analysis. Using maternal loss-of-function approaches and live imaging of microtubules, we have further investigated the roles of these components in cortical rotation. We show that several of these, including *trim36* and *dead end homolog 1 (dnd1)*, which are both required for microtubule assembly in the egg, as well as *wnt11b*, comprise a distinct class of localized mRNAs with an intermediate pattern of distribution in the vegetal cortex. Also, we examine the dynamics of microtubule assembly and plus end orientation during the early stages of cortical rotation. We identified several novel features of microtubule organization and found that Trim36 likely plays a role in limiting plus end polymerization and overall microtubule dynamics during cortical rotation. Additionally, we present a reinvestigation of the role of Wnt11b and other Wnt activators in axis formation.

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Expression cloning of camelid nanobodies against *Xenopus* gastrula antigens. Keiji Itoh, Sergei Sokol. Developmental and Regenerative Bilogy, Icahn School of Medicine at Mount Sinai, New York, NY.

Cell and developmental biology research relies heavily on the use of conventional antibodies, but their production and maintenance involves significant effort. Here we use an expression cloning approach aimed to identify variable regions of llama single domain antibodies reacting with specific embryonic antigens. A cDNA library was prepared from lymphocytes of a llama immunized with *Xenopus* gastrula lysates. Sib-selection of small cDNA pools expressed in E. coli led to the isolation of three different antibodies with specific staining patterns in gastrula embryos. One antibody predominantly stained the cytoplasm and cell cortex, whereas two others stained yolk platelets. All three antibodies detected specific protein bands by immunoblot analysis. Reverse proteomic approach identified one target antigen as EP45/Seryp, a serine protease inhibitor. Given the unique stability of these antibody fragments (also known as nanobodies) and the ease of their expression in diverse systems, we propose that nanobody cDNA libraries represent a promising future resource for molecular markers for developmental biology.

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Active Repression by RARγ Signaling is Required for Vertebrate Axial Elongation. Amanda S Janesick[1], Tuyen TL Nguyen[1], Ken-ichi Aisaki[2], Katsuhide Igarashi[2], Satoshi Kitajima[2], Roshantha AS Chandraratna[3], Jun Kanno[2], Bruce Blumberg[1]. 1) Developmental and Cell Biology, University of California, Irvine, Irvine, CA 92697-2300, USA; 2) Division of Cellular and Molecular Toxicology, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan; 3) IO Therapeutics, Santa Ana, CA 92705-5851, USA.

Retinoic acid receptor gamma 2 (RARγ2) is the major RAR isoform that is expressed throughout the caudal axial progenitor domain in vertebrates. During a microarray screen to identify RAR targets, we identified a subset of genes that pattern caudal structures or promote axial elongation and that were up-regulated by increased RAR-mediated repression. Prior studies have suggested that RAR is present in the caudal domain, but is quiescent until its activation in late-stage embryos terminates axial elongation. In contrast, we show here that RARγ2 is engaged in *all* stages of axial elongation, not solely as a terminator of axial

growth. In the absence of RA, RARγ2 represses transcriptional activity *in vivo* and maintains the pool of caudal progenitor pool cells and presomitic mesoderm. In the presence of RA, RARγ2 serves as an activator, facilitating somite differentiation. Treatment with an RARγ-selective inverse-agonist (NRX205099) or over-expression of dominant-negative RARγ increases expression of posterior Hox genes and expression of marker genes for presomitic mesoderm and the chordoneural hinge. Conversely, when RAR-mediated repression is reduced by over-expressing a dominant-negative corepressor (c-SMRT), a constitutively-active RAR (VP16-RARγ2), or by treatment with an RARγ-selective agonist (NRX204647), expression of caudal genes is diminished and extension of the body axis is prematurely terminated. Hence, gene repression mediated by the unliganded RARγ2-corepressor complex is a novel mechanism to regulate and facilitate the correct expression levels and spatial restriction of key genes that maintain the caudal progenitor pool during axial elongation in *Xenopus* embryos.

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Initiation of the Xenopus mid-blastula transition by histone titration. David Jukam[1], Amanda Amodeo[1], Aaron Straight[2], Jan Skotheim[1]. 1) Dept. of Biology, Stanford University, Stanford, CA; 2) Dept. of Biochemistry, Stanford Medical School, Stanford, CA.

Embryonic development requires exquisite timing and coordination of developmental transitions. In Xenopus laevis embryos, zygotic genome activation occurs concurrently with other dramatic cellular changes—in particular, cell cycle lengthening and the onset of cellular motility—during the mid-blastula transition (MBT). MBT timing depends on changes in the nuclear-to-cytoplasmic ratio and a proposed titration mechanism: as DNA content increases exponentially with each round of cleavage divisions in an embryo of constant size, maternally deposited inhibitory factor(s) are titrated away, allowing zygotic gene activation and other MBT events. We generated a Xenopus egg extract system that recapitulates DNA-to-cytoplasm ratio-dependent transcription, and biochemically purified the inhibitory factor(s) as histones H3 and H4. These histones can quantitatively alter the DNA-to-cytoplasm ratio threshold required to induce transcription *in vitro*. In embryos, reduction of H3 protein levels with morpholinos shifts the MBT earlier by one cell cycle, as measured by total zygotic transcription and cell cycle lengthening. We conclude that histone titration regulates initiation of the MBT. Our working model is that histone titration before the MBT results in nucleosome depletion or nucleosome re-organization at loci of zygotic gene activation. We are testing this model by analyzing transcriptional and nucleosome occupancy dynamics in embryos before and during the MBT. Preliminary data using these approaches will be described.

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Identification of renal cell fate inducing transcription factors. Michael Kaminski, Hannes Engel, Florian Grahammer, Tobias Huber, Gerd Walz, Sebastian Arnold, Soeren Lienkamp. Department of Medicine, Renal Division, University of Freiburg Medical Center, Freiburg, Germany.

Forced overexpression of tissue-specific transcription factors has the potential to convert one differentiated cell type into another, referred to as direct reprogramming. However, no direct reprogramming towards a terminally differentiated kidney cell type has been achieved yet.To find cell type defining transcription factors we identified 50 genes showing high and specific expression within the kidney based on publically available quantitative expression data. An in-situ screen in *Xenopus* showed that more than 70% of these transcription factors had a strong staining in the embryonic kidney. Genes with expression in the renal precursor tissue at stage 22 and known disease relation were tested for their reprogramming capability using the *Xenopus* animal cap assay. Three factors induced tissue detected by pronephros specific antibodies 3G8 and 4A6. In addition, tubule specific genes were upregulated as assessed by qRT-PCR and in-situ hybridization. Overexpressing the candidate transcription factors tested in Xenopus resulted in promoter induction of kidney specific genes in mouse embryonic fibroblasts (MEFs) derived from transgenic reporter mice.Thus, we conclude that the directed specification towards renal tissues may be feasible, and three identified transcription factors are prime candidates to initiate renal differentiation programs. Further tests aim to reveal to which degree these cells resemble their native counterparts and how transcription factors cooperate to induce renal cell fate in vertebrates.

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Amphibian (*Euphlyctis cyanophlyctis*) *in vitro* ovarian follicle culture: A potential assay to assess impact of aquatic contaminants on female reproduction?. Pancharatna Katti, Basavaraj B Goundadkar, Manjunath G Ghodgeri. Dept of Studies in Zoology, Karnatak University, Dharwad, Karnataka, India.

The present study is a novel attempt to screen the impact of aquatic chemical contaminants such as, organophosphorous pesticide (acephate), weedicide/herbicide (atrazine) and pyrethroid insecticide (cypermethrin) on the development, growth (previtellogenic and vitellogenic) and degeneration of follicles in the ovarian fragments of frog (*Euphlyctis cyanophlyctis)* cultured *in vitro*. Pieces of ovary (consisting all types of growing oocytes) removed surgically from adult females were cultured *in vitro* in graded (0.01, 0.1, 1.0, 2.5 and 5.0 µg/ml of culture medium) concentrations of test chemicals or estradiol – 17 β (positive controls) or culture medium alone (controls) in quadruplicate sets at 23 ± 1°C temperature for 20 days. On 21st day the ovarian fragments were fixed in Bouin's fluid, and used for differential follicle counting (n = 3 sets) and histological analysis (n = 1 set). *In vitro* exposure of ovarian fragments to above test chemicals elicited generalized effects such as, (i) dose dependent decline in the number of previtellogenic follicles (ii) increase in follicular atresia (iii) incorporation of chemical granules in early vitellogenic follicles (iv) decrease in the mean diameter of largest follicles compared to corresponding positive controls and initial controls. Interestingly, large vitellogenic follicles were rescued, but histology revealed that the compact

arrangement of yolk granules was disturbed in these follicles. The results suggest that follicles are greatly sensitive to chemicals during their transition from previtellogenic to vitellogenic growth phase resulting in either follicular atresia or sub-quality of egg production. We conclude that i*n vitro* ovarian culture system may be considered as a potential tool to assess/evaluate the effects of aquatic chemical contaminants (present in micro-quantities) on ovarian function.

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Identification of Cell Motility Genes *coronin1a* and *destrin* Specific to Migrating Primitive aVBI-Myeloid Lineage in *X. laevis* and Identification of the aVBI as an Essential Inducer of Foregut Endoderm Progenitors. Zachary Agricola, Amrita Jagpal, Andrew Allbee, Allison Prewitt, Scott Rankin, Emily Shifley, Aaron Zorn, **Alan Kenny**. Perinatal Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Background: Vertebrate blood development occurs in 2 conserved spatially and temporally distinct waves: primitive and definitive hematopoiesis. In Xenopus neurula and tail bud stages, *cebpα* and *spiba*-expressing primitive myeloid cells emerge in the anterior ventral blood islands (aVBI) in direct apposition to the *hhex*-expressing foregut progenitor territory, the equivalent of the mammalian yolk sac. Over time these cells later migrate throughout the embryo. We aim to answer important remaining questions addressing: (1) identifying genes specifically expressed in the migrating primitive myeloid lineage and (2) determine whether primitive myeloid cells are essential to induce the foregut progenitor.*Methods and Results*: A series of microarray experiments in neurula-stage embryos identified 8 genes including actin-binding *coronin1a* and *destrin* genes expressed in the aVBI. Developmental *in situ* hybridization analysis and double *in situ* analysis revealed these genes progress from an aVBI-centered to a more broadly dispersed distribution from stages 15 to 34. Morpholino-generated *spiba* loss-of-function and *cebpα* mRNA gain-of-function studies revealed these genes are downstream of these myeloid master regulatory transcription factors. *In situ* analysis revealed loss of the *hhex*-expressing foregut progenitor in the context of *spiba* morpholino loss-of-function. Einsteck rescue of *hhex* expression and aVBI myeloid expression was achieved in these loss-of-function embryos using *cebpα*-injected animal caps.*Conclusions*: Several genes specific to the aVBI-myeloid population have been identified and are specific to the migrating population of differenting cells. The aVBI plays an essential role in inducing the foregut progenitor. These results suggest an important role for primitive hematopoiesis in foregut organogenesis.

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Determining the direct targets of Wnt/β-catenin signaling during neural plate patterning. Rachel Kjolby, John Young, Richard Harland. MCB, University of California, Berkeley, CA, CA.

Patterning of the presumptive central nervous system, or brain and spinal cord, occurs after its initial induction at gastrulation. The dorsal ectoderm, or future neural plate, is first "activated" to become anterior neural tissue and then subsequently "transformed" into tissue with posterior gene expression by Wnt, FGF and Retinoic Acid. While it is hypothesized that Wnt acts as a morphogen whereby the most posterior tissue receives the greatest concentration of Wnt, the direct targets of Wnt signaling that mediate this pattering still remain unknown. To address this, I tagged the downstream transcription factor of Wnt signaling, β-catenin, with a FLAG epitope for use in ChIP-seq. By identifying the closest gene to a β-catenin binding event, I have made a list of Wnt/β-catenin candidate target genes to be further validated by *in-situ* and RNA-seq.

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Ciliary proteins with unexpected roles in gene regulation. Jianli Shi, Ying Zhao, Domenico Galati, Janet Meehl, Robin Dowell, Tyson Vonderfecht, Mark Winey, **Michael Klymkowsky**. Molecular, Cellular & Developmental Biology, University of Colorado Boulder, Boulder, CO. 80309-0347.

A combination of translation blocking morpholinos and GFP-tagged rescuing constructs for EFHC1 (implicated in Juvenile Myoclonic Epilepsy), Chibby (Cby), and the centrins CETN2, CETN3, and CETN4 were used in both intact embryos and embryonic explants to define the functions of these cilia-associated proteins. Each morpholino led to a distinct and rescuable set of phenotypic defects. Three major (and a number of minor) new findings emerged from these studies. First, and most surprisingly, Cetn2, but not Cetn3, is required for normal FGF and FGFR gene expression, apparently through interactions with chromatin. Not surprisingly then Cetn2 morphants led to defects in mesoderm (heart and notochord) formation. Second, knocking down levels of the axonemal protein EFHC1 or the basal body associated protein Cby disrupted the patterning of the central nervous system, inhibited neural crest formation and migration, and led to various other defects as well as defects in ciliogenesis. EFHC1 regulates β-catenin mediated Wnt signaling, but through a mechanism distinct from that utilized by the Cby protein. Reduction of either EFHC1 or Cby led to a Wnt-mediated feed forward loop that up-regulates Wnt8 RNA levels. Finally, EFHC1 over-expression induced the ectopic expression of the bHLH transcription factor Twist1, while Cby expression was negatively regulated by the zinc-finger transcription factor Snail2. We will present the results of our on-going light and ultrastructural, chromatin-IP, RNAseq, and mutational studies on the molecular and inductive mechanisms behind these phenotypes. NIH support through a collaborative supplement to NIH grant 3R01GM074746 - 06W1.

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Tissue reconstruction of myocardium during *Xenopus laevis* metamorphosis. Manami Kobayashi, Misa Sugiura, Tsutomu Kinoshita. Rikkyo University, Toshima, Tokyo, Japan.

In anuran development, basic structure of heart is formed at the embryonic stage. Thereafter, the small larval hearts with rapid beating grow up to large adult hearts with slow beating, during metamorphosis. Contractile protein in cardiac muscles changes

from larval type αMHC (myosin heavy chain) to adult type vMHC. These changes suggest that a tissue reconstruction from larval heart to adult one occurs during metamorphosis. However, there is no report about the reconstructing change of the heart. Here, we examined the cytological change of *Xenopus laevis* heart during metamorphosis. In order to understand the myocardial change from larval heart to adult one, we analyzed apoptotic cell death by TUNEL method. In the ventricular myocardium, apoptotic cells were detected from st.60, remarkably increased in number at st.62, and decreased at st.64. Prior to the beginning of apoptotic cell death, a great number of dividing cells were recognized from st.58 heart, and the high frequency of cell division was maintained by the end of metamorphosis. At the same time, Islet-1-expressing heart precursor cells increased in number within the ventricle. Immunocytological analysis of dissociated cardiomyocytes showed that vMHC-expressing cells increased in number during metamorphosis, which was contrast to the decrease in number of αMHC-expressing cells. However, significant number of myocardiac cells indicated the expression of αMHC and vMHC within the same cell. Co-staining of the dissociated cardiomyocytes with BrdU and Islet-1, αMHC or vMHC antibodies showed that dividing cells became both Islet-1 and vMHC-expressing cells. There results suggest that the tissue reconstruction of myocardium may occur by two ways during *Xenopus* metamorphosis. One is cellular replacement of larval cardiomyocytes with newly formed adult ones. The other is change of gene expression in the same cardiomyocytes.

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beta-adrenergic signaling promotes posteriorization in *Xenopus* early development. Hiroki Kuroda[1], Yuka Sato[1], Shoko Mori[2]. 1) Faculty of Environment and Information Studies, Keio University, Fujisawa, Kanagawa, Japan; 2) Department of Education (Sciences), Shizuoka University 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan.

Adrenaline (also known as Epinephrine) is a hormone, which works as major regulator of various biological events such as heart rate, blood vessel and air passage diameters, and metabolic shifts. Although its specific receptors are expressing at the early developmental stages of vertebrate, the role of adrenaline for early embryogenesis has been poorly understood.Here we show that loss-of-functional effects of adrenergic receptor b-2 (Adrb2), which was known as the major receptor for adrenaline and highly expressed in embryonic stages, led posterior defects at tadpole stage of *Xenopus* embryos, while embryos injected with *Adrb2* mRNA or treated with adrenaline hormone adversely lost anterior structures. This posteriorization effect by adrenaline hormone was dose-dependently increased but effectively rescued by microinjection of antisense morpholino oligomer for Adrb2 (Adrb2-MO). Combination of adrenaline treatments and microinjection of *Adrb2* mRNA maximized efficiency in its posteriorizing activity. Interestingly, both gain- and loss-of-functional treatment for b-adrenergic signaling could not influence anterior neural fate induced by overexpression of *Chordin* mRNA in presumptive ectodermal region, meaning that it worked via mesoderm.Taken together with these results, we conclude that adrenaline is novel regulator of anteroposterior axis formation in vertebrate.

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Peroxiredoxin1 regulates pronephros development via modulating proximal tubule formation. Hyeon-Gyeong Lee, Inji Park, **Hyun-Shik Lee**. School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, Kyungpook National University, Daegu, South Korea.

Peroxiredonxins (Prdxs) are small peroxidases that consist of six isoforms in mammalians. During catalytic cycle, intracellular H_2O_2 removal is controlled by 2-Cys Prdx proteins. All Prdxs share conserved N-terminus having H_2O_2-sensitive cystein residue (Cys52 in Prdx1), which is oxidized to Cys-SOH by H_2O_2. This oxidized Prdx interacts with the conserved thiol group (-SH) at C-terminus (Cys178 residue in Prdx1) of other homodimer subunit through intermolecular disulfide bonds. Despite the importance of oxidative stress during development, numerous age-related diseases including cancers have been associated with the modulation of ROS generation. In the current study, we demonstrated that antioxidant enzyme Prdx1 that reduces H_2O_2 and alkyl hydroperoxides, plays a crucial role in pronephros development. We observed that silencing of Prdx1 greatly reduces proximal tube formation in pronephros. In line with this, catalytic mutants of Prdx1 (deficient antioxidant function) such as C52S, C173S, and C52S/C173S, failed to prevent loss of Prdx1-induced disruption of proximal tubule formation. On the other hand, wild type Prdx1 rescued disruption of proximal tubule formation. Additionally, we found that loss of Prdx1 function causes significant induction of cellular ROS level consequently reduces primary cilia formation in human retina epithelial cells. By using *Xenopus* version of knock-in experiments, we found the presence of Prdx1 in the retinoic acid signaling pathway, known to be important for *Xenopus* pronephros development. The aforementioned results suggest that antioxidant function or dimerization of Prdx1 may be essential component in proximal tubule formation in pronephros.

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Localization of Oct25-expressing cells during development and regeneration of hindlimb phalanges in *Xenopus laevis*. Jaehoon Lee, Yui Shoji, Tsutomu Kinoshita. Rikkyo University, Toshimaku, Tokyo, Japan.

In *Xenopus*, and amputated limb can be regenerated completely at early limb bud staged. This capacity is gradually lost during metamorphosis which results in the formation of spike-like structure composed of cartilage. In the tip of the limb, however, amputated phalange can be regenerated completely even after metamorphosis, suggesting that the phalange may have high regenerative capacity after metamorphosis. However, molecular mechanism of the regenerative capacity in the hindlimb bone remains unknown. Here, we examined the spatio-temporal expression of Oct25 in the hindlimb phalanges of*Xenopus laevis*. Oct25 belongs to one of the POU family subclass V transcription factors, which are known to be essential for multipotency of the cell. In the semi-quantitative analysis by RT-PCR, gene expression of Oct25 was detected weakly at st.61, and increased during

metamorphosis until st.66. After metamorphosis, the Oct25 expression was maintained for several weeks, but disappeared until 2months after metamorphosis. In order to analyze localization of the Oct25-expressing cells in the hindlimb phalange, monoclonal antibody raised against to the N-terminus of Oct25 was produced. Immunohistological examination showed that Oct25-positive cells and BrdU- incorporated cells were co-localized in the periosteum and intracapsular. In regeneration of the amputated phalange, transient expression of Oct25 was detected 7days after the amputation. Immunostaining demonstrated that Oct25-expressing cells accumulated in the blastema.These results suggest that Oct25-expressing multipotent cells might play a role in the formation and maintenance of hindlimb phalanges.

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Imparting regenerative capacity to limbs by progenitor cell transplantation. Gufa Lin, Ying Chen, Jonathan Slack. Stem Cell Institute, University of Minnesota, Minneapolis, MN.

The frog *Xenopus* can normally regenerate its limbs at early developmental stages but loses the ability during metamorphosis. This behavior provides a potential gain-of-function model for measures that can enhance limb regeneration. Here we show that frog limbs can be caused to form multi-digit regenerates after receiving transplants of larval limb progenitor cells. It is necessary to activate Wnt/beta-catenin signaling in the cells, and to add Sonic hedgehog, FGF10 and thymosin beta 4 in the transplants. These factors promote survival and growth of the grafted cells and also provide pattern information. Importantly, the eventual regenerates are not composed solely of donor tissue; the host cells also make a substantial contribution despite their lack of regeneration-competence. Cells from adult frog legs or from regenerating tadpole tails do not promote limb regeneration, demonstrating the necessity for progenitor cells. These findings have obvious implications for the development of a technology to promote limb regeneration in mammals.

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MiR-124 overexpression represses bipolar cell fate specification by targeting *Otx2* in *Xenopus* retina. Kaili Liu, Ying Liu, Mengru Sun, Xiumei Wang, Rongqiao He. Institute of Biophysics, CAS, Beijing, China.

MicroRNAs (MiRNAs) are short endogenous non-coding RNAs that have diverse functions in regulating animal development and diseases by degrading target gene transcript or repressing the protein translation via specifically binding to 3'UTR of target mRNA. MiR-124 is a neural specific miRNA that plays important roles in brain and eye development. We previously reported that miR-124 regulates early neurogenesis at the optic vesicle stage and is sufficient to induce the malformation of optic nerve and optic cup in *Xenopus*. However, whether miR-124 overexpression influences retinal cell fate specification and the underlying molecular mechanisms are largely unknown. In this study, we find that miR-124 overexpression by lipofecting its expression vector can increase the proportion of horizontal cell and decrease the proportion of bipolar cell, while not significantly affect ratios of other cell types in the retina. Gain of miR-124 significantly decreases *Otx2* mRNA level and Otx2 protein-expressing bipolar cell number and proportion. Furthermore, miR-124 interacts with a conserved binding site in the 3'UTR of *Otx2* and suppresses its expression by luciferase reporter assay. In addition, *Otx2* mRNA can rescue the miR-124-induced effect on retinal cell fate specification. These results reveal that miR-124 overexpression negatively regulates expression of *Otx2* as its target and specifically represses the bipolar cell production.

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Regulation of *Eya1* expression in the preplacodal region of *Xenopus laevis*. Santosh Kumar Maharana, Gerhard Schlosser. Zoology, School of Natural Sciences & Regenerative Medicine Institute, National University of Ireland, Galway, Ireland,.

Cranial placodes contribute to many sensory organs and ganglia of the vertebrate head. Recent studies indicate that all placodes originate from a common precursor region immediately peripheral to the anterior neural plate. The transcription factor Six1 and its cofactor Eya1 are initially expressed in this preplacodal region and both continue to be expressed in placodes at later stages suggesting that they play a central role in placode development. Our recent studies demonstrated that the pre-placodal region can only be induced in competent non-neural ectoderm but we know little about direct upstream regulators. To gain insights into the latter, we have begun to analyze *Xenopus Eya1* cis-regulatory regions.Using comparative genomic analyses we identified several conserved noncoding regions in the vicinity of the*Eya1* coding region. We have cloned some of these candidate regulatory sequences from *Xenopus laevis* and currently test *GFP*-fusion constructs to check for their expression in transgenic frog embryos. We will then further characterize cis-regulatory sequences by testing various deletion constructs in transgenic frog embryos. Putative transcription factor binding sites will be verified by EMSA and/or luciferase reporter assays. In addition, we are currently analyzing the role of several transcription factors as upstream regulators of *Eya1* in gain and loss of function experiments (by microinjection of mRNA and morpholino antisense oligonucelotides, respectively, followed by in situ hybridization for *Eya1*). Our findings promise new insights into how the pre-placodal region is established in the early embryonic ectoderm.

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Controlled levels of canonical Wnt signaling are required for neural crest migration. Ewa Maj[1], Lutz Künneke[2], Timo Aspelmeier[2], Annette Zippelius[2], Annette Borchers[1]. 1) Department of Molecular Embryology, University of Marburg, Marburg, Hessen; 2) Institute for Theoretical Physics, University of Göttingen, Göttingen, Niedersachsen.

Wnt signaling plays a dominant role for the development of the neural crest (NC), a highly migratory cell population that generates a vast array of cell types. Canonical Wnt signaling is required for NC induction as well as differentiation of NC cells,

however, its role in NC migration remains largely unknown. To define the effect of canonical Wnt-signaling on *Xenopus* NC migration, canonical Wnt signaling was activated or inhibited at different time points after NC induction using inducible glucocorticoid fusion constructs of Lef/Tcf transcription factors or chemical modulators. Subsequently, NC migration was analyzed by whole mount *in situ* hybridization or life-cell imaging of explanted neural crest cells followed by biophysical data analysis. Our results show that ectopic activation of canonical Wnt signaling leads to an inhibition of NC migration in whole embryos. Interestingly inhibition of canonical Wnt signaling also causes NC migration defects indicated by fused NC branches or delayed NC migration. Furthermore, life-cell imaging of explanted NC cells confirmed that modulation of canonical Wnt signaling affects migration speed and ability to perform single cell migration. In summary our data support the hypothesis that canonical Wnt signaling needs to be tightly controlled to allow for migration of NC cells.

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Pre-placodal ectoderm and neural crest patterning: requirement for histone modification by PRDM12 and Kdm4a in *Xenopus* embryos. Shinya Matsukawa[1], Kyoko Miwata[2], Makoto Asashima[2], Tatsuo Michiue[1]. 1) The University of Tokyo, Meguro-ku, Tokyo, Japan; 2) Research Center for Stem Cell Engineering National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba City, Ibaraki, Japan.

In vertebrate, pre-placodal ectoderm and neural crest patterning is known to determine by BMP and Wnt morphogen gradients and regulation of transcriptional factors, whereas transcriptional regulation by histone modification remains unclear. Here, we identified two histone modification enzymes and reported the requirement of histone modification for this patterning. *PRDM12*, which is a member of the PRDM family related to histone methylation, is expressed at lateral pre-placodal ectoderm in early neurula stage. *PRDM12* overexpression represses the expression of neural crest specifier genes by promoting the trimethylation of histone H3K9 known as an effective mark for chromatin remodeling. PRDM12 binds to the *Foxd3* promoter and methylates H3K9 on this region. Conversely, histone demethylase*Kdm4a/Jmjd2a* is expressed at neural crest region and inhibits presumptive trigeminal placode formation. Kdm4a can also bind to the *Foxd3* promoter by recognizing the trimethylation of histone H3K4. Interestingly, *Kdm4a* overexpression can offset the effects of PRDM12. Our study indicates the importance of the regulation of histone modification by PRDM12 and Kdm4a for pre-placodal ectoderm and neural crest patterning.

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Aquaporin-3b is required for tissue boundary formation and fibronectin assembly during gastrulation. Christa Merzdorf, Daniel Van Antwerp, Sean Lujan. Cell Biology and Neuroscience, Montana State University, Bozeman, MT.

Aquaporins are a highly conserved family of proteins that form pores in cell membranes to facilitate rapid cellular water movement along osmotic gradients. Very little is understood concerning the roles of aquaporins during embryonic development. We have found that the *Xenopus laevis aqp3b* gene is expressed in a highly specific manner during early embryogenesis, both during gastrulation and in neurula embryos, where it is expressed exclusively in the neural folds and participates in neural tube closure. Here we focus on our newest data that concern the role of *aqp3b* in gastrulating embryos, where *aqp3b*expression is strikingly restricted to the deep cells (sensorial layer) of the blastocoel roof and the dorsal margin. Inhibiting Aqp3b translation with morpholino oligos (MO) determined that the Aqp3b protein is necessary for proper blastopore closure. Confocal microscopy revealed a lack of tissue organization and abnormal cell shape in *aqp3b* MO-injected gastrula embryos, where the boundary between involuted and non-involuted cells was particularly disorganized. The border between involuted and non-involuted gastrula cells is defined by an ECM, of which fibril fibronectin (FN) is a major constituent. This fibril FN was absent adjacent to cells injected with *aqp3b* MO in a cell-autonomous manner and coincided with those areas that showed tissue disorganization. FN is required for cell movements during gastrulation, including epiboly, radial convergence and convergent extension. Regulation of FN fibril synthesis has been linked to noncanonical Wnt signaling, which can enhance cadherin-based cell-cell adhesion that, in turn, initiates polymerization of fibril FN. Preliminary analysis of *aqp3b* MO treated Keller explants has identified defects in convergent extension, which appear to correlate with noncanonical Wnt/Ca2+ signaling and we are exploring the role of this signaling. Thus, our results suggest a role for aqps in the ability of cells to correctly interact with and assemble the extracellular matrix. Our continued studies are focused on further defining this role at the molecular and cellular level, including signaling pathway interactions, analysis of cell shape and volume and imaging during cell migration.

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The role of *xRhoGEF3* in convergent extension movement of *Xenopus* embryo. Ikuko Seki[1], Saori Akiduki[2], Shuko Tokuda[1], Toshiyasu Goto[3], Shinya Matsukawa[1], Makoto Asashima[2], **Tatsuo Michiue[1]**. 1) Grad. School Arts Sci., the University of Tokyo, Tokyo, Japan; 2) Stem Cell Research Center, AIST, Tsukuba, Japan; 3) Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan.

Gastrulation movement is one of the most dynamic morphogenesis in early development. Rho signaling plays important roles for cell migration during gastrulation and is upregulated by Wnt-PCP pathway via dishevelled and Daam. Previous report showed that WGEF is involved with activation of RhoA. Here we show the role of another GEF, *xRhoGEF3* (also described as *xARHGEF3.2*) in gastrulation movement. *XRhoGEF3* expression was highly restricted in dorsal lip region during gastrulation. When *xRhoGEF3* mRNA was injected into dorsal blastomere, curved and short axis was caused. Injection with morpholino antisenseoligo against *xRhoGEF3* (xRhoGEF3MO) caused similar defect with overexpression and inhibited convergent extension. Immunoprecipitation analysis revealed that xRhoGEF3 directly interacted with both disheveled and Daam1 protein. The analysis with GST-RBD construct revealed that xRhoGEF3 actually increased GTP-bound RhoA. Moreover, xRhoGEF3

protein were co-localized with F-Actin. Together with these results, xRhoGEF3 play an important role in convergent extension movement via Wnt-PCP pathway.

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The development of TALEN methods to enhance the mutation efficiency and to perform genome editing preferentially in germ cells using *Xenopus*. Keisuke Nakajima, Yoshio Yaoita. Institute for Amphibian Biology, Higashihiroshima, Japan.

A gene of interest can be modified in *Xenopus* by microinjecting mRNAs of transcription activator-like effector nucleases (TALENs) into fertilized eggs. Here, we will introduce two methods. One is the combination of TALEN mRNA-microinjection into oocytes and the host-transfer technique to maximizing the efficiency of gene modification, and the other is the method using TALEN mRNA that is stable specifically in the primordial germ cell (PGC). It is important to know whether the mutagenesis is observed before or after mid-blastula transition (MBT), because the zygotic transcription begins after MBT. We examined the time course of TALEN-induced mutation using *Xenopus laevis*. The mutagenesis started from stage 6, and most of mutations were created before the MBT. The gene mutation rate was saturated after MBT. We reasoned that a level of TALEN protein is not high enough for 100% mutation before MBT, as the TALEN translation starts after the microinjection. The host-transfer technique was adopted after the oocyte injection of TALEN mRNAs to increase the protein level of TALENs during early development, resulting in 100% mutation. If a target gene is essential for development, growth and fertility, TALENs with a high mutagenesis activity should bring about developmental disorder or sterility, which makes F1 phenotype analysis very difficult. To solve this problem, we inserted the 3'-UTR of*Xenopus tropicalis DEADSouth* gene downstream of the termination codon of TALEN coding sequence, because this 3'-UTR is known to stabilize mRNA in PGC. When embryos were microinjected with TALEN mRNA fused to the 3'-UTR of *DEADSouth*, their sperms and oocytes showed a high rate (84-100%) of the target gene modification in contrast to a lower rate of nucleotide alternation in somatic cells.

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The role of microRNA-206 in *Xenopus laevis* somite morphogenesis. Ceazar E Nave, Julio R. Ramirez, Hernando Martinez-Vergara, Parag Saraf, Daniel Saw, Carmen Domingo. Biology, San Francisco State University, San Francisco, CA.

MiRNAs have been shown to play a major role in the post-transcriptional regulation of cells during embryogenesis and diseases. Recent work has shown that miR-206 is exclusively expressed in somites—early progenitors for skeletal muscle of vertebrates (Sempere et al., 2004; Baskerville and Bartel, 2005). We aim to characterize the role of miR-206 during early muscle development. To study the importance of miR-206 we use a morpholino approach to knock down miR-206 during early *X. laevis* embryogenesis. We find that the downregulation of miR-206 disrupts embryonic development characterized by a shortened anteroposterior axis. Furthermore, using a membrane-tagged GFP to examine cell shapes, we show that knocking down miR-206 leads to disorganized somite boundaries, and misaligned myotome fibers. Additionally, we observe a separation between the somites and the notochord. Our findings suggest that the knockdown of miR-206 during early embryonic development affects the formation of cell attachments between the newly forming somites and the notochord, as well as between adjacent somites. We propose that the inability to form stable intersomitic boundaries leads to the inability of muscle fibers to elongate and align parallel to the notochord. Thus, miR-206 appears to play a significant role in regulating the expression levels of genes necessary for embryonic muscle morphogenesis.

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Elongation movement of *Xenopus* posterior neural tissue is directed by anteroposterior tissue polarity. Hiromasa Ninomiya[1,2]. 1) AIST Tsukuba Central, Tsukuba, Ibaraki, Japan; 2) Graduate School of Arts and Sciences, the University of Tokyo, Meguro, Tokyo, Japan.

Basic tissue morphology is formed by extensive and global cellular rearrangements in vertebrate embryos. For example, dorsal tissues in vertebrate embryos tissue-autonomously elongate by convergent extension movement. Convergent extension has been extensively studied as a model system to understand principles of morphogenetic movements. I have previously shown that convergent extension in*Xenopus* chordamesoderm is directed by anteroposterior tissue polarity, an intrinsic gradient of cellular specification within tissue. To explore mechanisms of morphogenesis by the tissue polarity further, I have analyzed relations between posterior neural elongation movement and the tissue polarity in *Xenopus*. Explanted neural tissue was elongated only in heterophilic combinations of posterior and anterior tissues along the anteroposterior axis, indicating requirement of the anteroposterior tissue polarity for directing neural elongation. Difference in received signals, such as FGF and Wnt, could also confer the tissue polarity which leads to neural elongation movement. These results suggest that convergent extension in dorsal tissues is oriented by the tissue polarity which is formed by tissue specific combinations of inducing signals.

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Investigating the function of Wnt/β-catenin signaling in the rhombomeric boundaries. Rivka Noelanders[1], Hong Thi Tran[1], Sylvie Janssens[2], Kris Vleminckx[1]. 1) Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium; 2) Department of Developmental and Regenerative Biology, Mount Sinai School of Medicine, New York, United States.

The vertebrate brain comprises a vast array of neuronal and glial cell types. Yet all these are derived from a homogeneous neuroepithelium. During development a range of different growth and differentiation factor gradients pattern this homogeneous epithelium, each one adding a new layer of complexity. An important feature of brain development is the formation of neuromeres, subcompartments of the developing brain that can be distinguished both through differential gene expression as

through physical separation. At the boundaries of these neuromeres specific signaling centers are established that regulate the further growth and patterning of the adjacent tissues.My research focuses on the hindbrain, which during the course of development becomes subdivided in rhombomeres. We found that Wnt/β-catenin signaling is highly active in the rhombomere boundaries. My goal is to reveal the functional role of Wnt in the generation and maintenance of these boundaries and in the patterning of the hindbrain as a whole. I am also exploring a possible connection between Wnt and Notch signaling in these rhombomere boundaries.

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A thioredoxin fold protein Sh3bgr is necessary for embryonic muscle development. Dong Gil Jang, **Tae Joo Park**. Department of Biological Science, Ulsan National Institute of Science and Technology(UNIST), Ulsan, Korea.

Sh3bgr (SH3 domain binding glutamate-rich) gene encodes a small protein containing thioredoxin like fold, sh3 binding domain and glutamate-rich domain. It has been suggested that Sh3bgr may be a causative gene for the cardiac phenotypes in Down syndrome but the over-expression of Sh3bgr did not caused any disease phenotypes. We have discovered that Sh3bgr is strongly expressed in embryonic muscle tissues including the heart and somite. Morpholino mediated-knockdown of sh3bgr caused severe malformation of somite and heart muscles. Also the segmentation patterns of somite muscles were severely disrupted in the morphants. Further analysis of the Sh3bgr morphants revealed that Sh3bgr is critical for the sarcomere formation. Sh3bgr specifically localized to the Z-line in mature sarcomeres of developing somite muscles. Moreover, we show that Sh3bgr is an upstream regulator for Enah protein in striated muscle formation.

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Multiple Lineage-Specific Roles of Syndecan 2 in Early *Xenopus* Development. Annita Peterson, H. Joseph Yost. Molecular Medicine, University of Utah, Salt Lake City, UT.

Syndecan 2 (Sdc2) and other heparan sulfate proteoglycans (HSPGs) have been implicated in a number of cell processes such as cell adhesion, proliferation, migration and signaling. Sdc2 contains multiple conserved cytoplasmic domains, a single transmembrane domain, and an extracellular domain with multiple covalent attachment sites for long linear chains of heparan sulfate glycosaminoglycans. Here we report three functions for Sdc2 during early Xenopus development.**First**, the cytoplasmic domains of Sdc2 have discrete roles during gastrulation to regulate subsequent left-right (LR) patterning. Two conserved serines in the cytoplasmic domain are phosphorylated in right side ectoderm cells and not phosphorylated in left side cells. LR patterning can be altered by expressing constitutively phosphorylated Sdc2 in left lineages or constitutively nonphosphorylated Sdc2 in right side lineages. Conversely, expressing mutant Sdc2 lacking a conserved cytoplasmic PDZ-binding domain on either side alters LR patterning. **Second**, Sdc2 is involved in the development of the gastrocoel roof plate (GRP). When Sdc2 morpholinos are injected into GRP lineages, GRP development is abnormal and cilia are shorter. Cells within the GRP normally have a regular cobblestone pattern with interspersed rosettes. Knockdown of Sdc2 results in loss of rosettes and irregular GRP boundaries. This structural perturbation of the GRP disrupts LR patterning: Xnr1, which is normally expressed in left lateral plate mesoderm, becomes bilateral in 40% of sdc2 morphants, and the orientation of heart looping is randomized. **Third**, Sdc2 is important for radial intercalation of ectodermal cells in skin development. In bilayered epithelium development, ciliated and nonciliated precursor cells intercalate into the outer layer from the inner layer. When Sdc2 is knocked down in the inner cell layer, ciliated cells have the same number and pattern, but fail to intercalate completely. In contrast, non-ciliated precursor cells increase in number and intercalate as groups. The mechanisms and pathways by which Sdc2 plays multiple lineage-specific roles are currently being explored.

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Role of Myosin Regulatory Light Chain during Convergence and Extension. Katherine Pfister[1], Ray Keller[1,2], Skoglund Paul[2]. 1) Cell Biology, University of Virginia, Charlottesville, VA; 2) Biology, University of Virginia, Charlottesville, VA.

During Xenopus development, blastopore closure is driven by active intercalation of the presumptive mesoderm. The initially round cells become elongate in the mediolateral direction. Through multiple rounds of extending protrusions and contracting on neighboring cells a longer and narrower array is created. Intercalation is an active process, requiring myosin motors on a specialized cortical actin network. Myosin contractility is activated by phosphorylation of Myosin Regulatory Light Chain (MRLC) on the Serine-19 residue. Many kinase pathways have been implicated in this phosphorylation event, including a calcium-dependent Myosin Light Chain Kinase and a Rho-dependent kinase, ROCK. We have preliminary evidence indicating a timing difference in the predominant kinase used to phosphorylate MRLC during Xenopus gastrulation. These different stages of myosin activation produce independent effects on the cortical actin network, but function in concert to drive intercalation. Point mutations of the Serine 19 residue result in major changes in the structure and movement of the cortical actin network of presumptive mesodermal cells. Alanine substitution creates a phosphonull mutant resulting in a highly crosslinked, but quiescent network. These changes are responsible for defect in CE causing blastopore closure delays and shorter axes. We suggest a critical role for highly regulated activation of MRLC, and the interaction of a set of specialized cortical actin networks during Convergence and Extension.

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Xenbase: the Xenopus model organism database. Virgilio Ponferrada[1], Joshua Fortriede[1], Christina James-Zorn[1], Kevin Burns[1], Kamran Karimi[2], J. Brad Karpinka[2], Jacqueline Lee[2], Yu Liu[2], Peter Vize[2], Aaron Zorn[1]. 1) Cincinnati Children's Hospital, Cincinnati, OH; 2) Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada.

Xenbase (www.xenbase.org) is a comprehensive resource to the global community studying early vertebrate development, molecular cell biology, and functional genomics in *Xenopus*. Xenbase hosts**15,485 gene pages** supporting *X. laevis* (allotetraploid) and *X. tropicalis* (diploid) gene models. Gene pages link to mouse, zebrafish and human orthologs as well as to **human disease information**.Gene expression annotations are shown from ~60,500 literature and expression screen images. Gene expression curation uses the Xenopus Anatomy Ontology (XAO), an anatomical ontology modeling the dynamic processes of frog development. Our powerful **gene expression search** allows users to mine the *Xenopus* literature (~45,200 published articles) for anatomy-specific detection.Xenbase allows users to conduct **BLAST searches** and browse frog **gene models**. Users can find research **protocols**, clones, **morpholino and antibody information** and links to **CRISPr/TALEN genome editing resources**. Xenbase coordinates with *Xenopus* **stock centers** with searchable databases for frog lines, clones and transgenes.Xenbase houses **community submitted datasets** (e.g. large expression screens, high-throughput sequence data) made freely available to the research community. As an online resource, we highlight community news, the latest research developments, conferences, and workshops.

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An epigenetic root of cilia formation - Suv4-20h enzymes control multiciliogenesis in skin and pronephros. Ralph Rupp[1], Ohnmar Hsam[1], Dario Nicetto[1,2]. 1) Molecular Biology, Adolf-Butenandt-Institut, LMU, München, Germany; 2) Department of Cell and Developmental Biology, Smilow Center for Translational Research, Philadelphia.

Histone H4 trimethylation at Lysine 20 is catalyzed by Suv4-20h1/h2 enzymes. This modification is repressive and mostly decorates heterochromatic regions of the genome, but it can also be found on genes such as the pluripotency-associated Oct-25. We have recently shown that removal of H4K20me3 by translation-blocking morpholinos against Suv4-20h enzymes leads to prolonged expression of Oct25 in sensorial ectoderm, which in turn arrests neuronal differentiation at the neural precursor state. Here we report on a second specific phenotype in Suv4-20h morphants that connects the H4K20me3 mark to ciliogenesis. While gastrocoel roof plate cilia form normal in morphants, the multiciliated cells (MCC) of the skin are strongly impaired. Notably, while MCC precursors are specified at even slightly higher numbers than normal, the number of cilia per cell is strongly reduced, indicating a defect in ciliogenesis rather than cell fate specification. Epistatic analysis details the defect to be located upstream of MCI and Foxj1. In addition, ciliary markers are absent from the nephrostomes of the embryonic kidney in the Suv4-20h morphants. Taken together, our results indicate a specific requirement for H4K20me3 in multiciliogenesis, aside from its role during exit from pluripotency.

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Self-regulatory morphogenetic field in embryogenesis and organogenesis. Yoshiki Sasai, Hidehiko Inomata. RIKEN CDB, Kobe, Hyogo, Japan.

I would like to also discuss the formation of the Xenopus DV-axis pattern via a self-regulatory BMP signaling field. I introduce our recent finding of self-regulatory mechanism of degradation of the organizer Chordin and discuss its role in scaling of DV pattern development according to the embryonic size.The DV patterning is so robust that an amphibian embryo with its ventral half surgically removed can develop into a smaller but proportionally patterned larva. Here we show that this robust patterning depends on facilitated Chordin degradation and requires the expression of the Chordin-proteinase inhibitor Sizzled on the opposite side. Sizzled, which is stable and diffuses widely along the DV axis, stabilizes Chordin and expands its distribution in the ventral direction. This expanded Chordin distribution, in turn, limits BMP-dependent Sizzled production, forming an axis-wide feedback loop for shaping Chordin's activity. Using bisection assays, we demonstrate that Chordin degradation is dynamically controlled by embryo size-coupled Sizzled accumulation. We propose a scaling model that enables the DV pattern to adjust proportionally to embryonic axis size. In addition, we would like to discuss the dynamic interactions with Chordin and other BMP regulators in the self-regulatory field. Finally, we would like to extend the view of self-regulation study to a wider point of tissue self-organization in multi-cellular systems.

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SKL-tagging: a method for generating dominant-negative inhibitors of dimeric transcriptional factors such as Siamois and Vent. Y. Sato[1], S. Matsukawa[2], T. Furukawa[3], H. Kuroda[1]. 1) Keio University, Fujisawa, Kanagawa, Japan; 2) Tokyo University, Graduate School of Arts and Sciences, Bunkyo-ku, Tokyo, Japan; 3) Shizuoka University, GSTT, Shizuoka-city, Shizuoka, Japan.

Refer to KDEL-tag trap assay, which is very effective way to block secretion of dimeric secreted proteins (PMID 22811269), in this study we tried to generate new inhibition method for dimeric transcriptional factor. Many peroxysomal proteins contain a carboxy-terminal signal sequence called the peroxisomal targeting signal motif, counting of the Ser-Lys-Leu (SKL) motif. Using this molecular mechanism, here we propose a new dominant-negative assay, designated the SKL-tag trap assay, to negatively regulate nuclear localization of dimeric transcriptional factors as typified by homeobox proteins. First, we tested this method on Siamois, which is a well-studied dorsalizing factor in Xenopus. Siamois protein tagged with SKL at carboxy terminus blocked activity of Siamois, resulting in slight inhibition of dorsalization on Xenopus embryogenesis. Second, we examined the

usefulness of the SKL-tag trap assay on Vent, well-known ventralizing factor in Xenopus, showing that functions of the Vent1 and Vent2 were both blocked by the SKL-tag trap assay. Taken together, these results suggest that the SKL-tag trap assay can be adapted to inhibit a variety of dimeric proteins located in cytoplasmic regions.

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Stabilization of Spop and SpopL by Dzip1 is Essential for Gli Turnover and the Proper Output of Hedgehog Signaling. Tyler Schwend[1], Zhigang Jin[1], Kai Jiang[2], Brian Mitchell[3], Jianhang Jia[2], Jing Yang[1]. 1) Department of Comparative Biosciences, University of Illinois, Urbana, IL; 2) Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY; 3) Department of Cell and Molecular Biology, Northwestern University, Feinberg School of Medicine, Chicago, IL.

The Hedgehog (Hh) pathway is essential for embryonic development and adult tissue homeostasis. The Gli family of transcription factors acts at the downstream end of the pathway to mediate Hh signaling. In addition to Hh ligands, Gli proteins are regulated by many Hh-independent mechanisms, which influence the output of Hh signaling through controlling the stability or activity of Gli. It has been shown that the cullin3 E3 ubiquitin ligase adaptor protein Speckle-type POZ protein (Spop) directly interacts with Gli proteins and can promote their degradation. Interestingly, genetic ablation of Spop in mouse embryos does not lead to any phenotypes resembling misregulation of Hh signaling. This suggests the existence of other Gli regulatory mechanisms capable of compensating for the loss of Spop during development.We recently found that Daz interacting protein 1 (Dzip1) regulates Gli turnover and prevents degradation of Spop. Depletion of Dzip1 in *Xenopus* embryos led to phenotypes that resemble activation of Hh signaling. Similar to studies in mouse embryos, depletion of Spop in *Xenopus* embryos did not lead to any phenotypes reminiscent of Hh signaling disruption. Interestingly, we found that Dzip1, in addition to preventing degradation of Spop, also prevents the degradation of Spop-like (SpopL), a protein that shares high homology with Spop and is capable of promoting Gli turnover. Importantly, overexpression of Spop or SpopL was able to restore proper Gli protein turnover and rescue phenotypes in Dzip1-depleted embryos. Collectively, our findings suggest that Dzip1 stabilizes Spop and SpopL proteins, which in turn function redundantly to control Gli turnover. These findings shed insight into a novel Gli regulatory mechanism, involving Dzip1, Spop and SpopL to control Hh pathway output.

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B56α and B56γ regulatory subunits of protein phosphatase 2A play roles in both canonical and noncanonical Wnt pathways. Joni Seeling[1,2], Sungmin Baek[1], Lauren Sommer[2]. 1) Department of Biology, City University of New York, Queens College, Flushing, NY 11367; 2) Biological Sciences, Sam Houston State University, Huntsville, TX 77341.

The Wnt pathway is a key regulator of development and tumorigenesis. Activation of canonical Wnt signaling results in β-catenin-dependent transcription, while noncanonical Wnt signaling regulates convergent extension movements. Wnt signaling, like many signaling pathways, is regulated by phosphorylation at multiple levels. Protein phosphatase 2A (PP2A), which consists of a catalytic C, a structural A, and a regulatory B subunit, plays diverse roles in Wnt signaling through its B subunits. We determined the orthologous relationships of the evolutionarily conserved B56 B subunit gene family through phylogenetic analyses. Ectopic B56α and B56γ reduce β-catenin abundance, and B56α reduces β-catenin-dependent transcription, suggesting that B56α and B56γ inhibit canonical Wnt signaling. In contrast, B56ε is required for canonical Wnt signaling. B56α, B56δ/γ, and B56ε are expressed in early *Xenopus* development, but their precise roles are not known. Although the role of Wnt signaling in development is well characterized, the mechanisms by which the pathway is regulated by dephosphorylation is not. In order to more fully understand the regulation of Wnt signaling by dephosphorylation, we characterized the role of PP2A's B56 subunits in *Xenopus* development. We localized PP2A A, C, and B56 subunit expression using *in situ* hybridization. Their localization patterns suggest roles for B56α in eye and cement gland development, and for B56γ in forebrain, eye, and branchial arch development. We used loss-of-function analyses to characterize the roles of B56α and B56δ/γ during *Xenopus* development using morpholino antisense oligonucleotides (MOs). We found that B56α and B56δ/γ MOs dorsalize *Xenopus* embryos, and cause convergent extension defects in explants and whole embryos. B56α and B56δ/γ inhibit canonical Wnt signaling, as well as influence convergent extension Wnt signaling. B56α and B56δ/γ have both unique and overlapping spatiotemporal expression patterns, suggesting that they are not redundant. Future studies to identify relevant substrates will further define their roles in Wnt signaling.

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Identification of microRNAs and mRNA targets in the specification of neural vs. epidermal fates.Identification of microRNAs and mRNA targets in the specification of neural vs. epidermal fates. Vrutant Shah[1], Ashley Benham[2], Benjamin Soibam[2], Jamina Oomen-Hajagos[3], Ruth A. Ritter[1], Gerald H. Thomsen[3], Amy K. Sater[1]. 1) Dept. of Biology and Biochemistry, University of Houston, Houston TX USA; 2) Stem Cell Engineering Dept., Texas Heart Institute at St. Luke's Episcopal Hospital, Houston TX USA; 3) Dept. of Biochemistry and Cell Biology, Stony Brook University, Stony Brook NY USA.

During early embryonic development, the ectoderm becomes specified to adopt either a neural or epidermal fate. The fundamental gene regulatory networks (GRNs) that underlie these initial states of specification have been delineated; however, the roles of microRNAs (miRs) in establishing and maintaining these networks have not been investigated *in vivo*. We have initiated two studies to examine possible functions of ectodermal miRs. First, we carried out high-throughput sequencing of small RNA from presumptive neural and epidermal ectoderm to identify ectodermal miRs. Libraries were prepared from RNA

isolated from midgastrula animal caps of *X. laevis* expressing either noggin or an activated BMP4 receptor. Alignments to the human miR set revealed 425 miRs expressed only in presumptive neural ectoderm, 20 miRs expressed only in presumptive epidermal ectoderm, and 301 miRs that are expressed in both tissues. Alignments to the *X. tropicalis* miR set yield 5 additional miRs each for neural and epidermal ectoderm, and an additional 123 shared miRs. The miRs uniquely expressed in either neural or epidermal ectoderm may play specific roles in maintaining the GRNs that regulate the specification and differentiation of these tissues, while shared miRs may function to terminate responsiveness to mesoderm inducing signals. Our second study has used co-immunoprecipitation of Argonaute-associated RNAs to identify mRNA targets of miR-dependent regulation in both tissues. Preliminary results based on RNA sequencing indicate that many RNAs are shared between neural and epidermal ectoderm; these shared targets include GDF3, which has been implicated in mesoderm induction. Elucidation of miR-target mRNA relationships may reveal new functions for miRs in neural or epidermal development.

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Control of embryonic polarity and cell fates by the translational repressor Bicaudal-C. Sookhee Park[2], Megan Dowdle[2], Susanne Blaser[2], Douglas Houston[1], **Michael Sheets[2]**. 1) Department of Biology, University of Iowa; 2) Biomolecular Chemistry, Univ. of Wisconsin-Madison, Madison, WI.

The origins of embryonic polarity begin during oogenesis with the localization of specific mRNAs to the vegetal cortex. The proteins encoded by the localized mRNAs establish animal/vegetal differences in embryonic cells and guide cell fate decisions. However, the functional importance of proteins encoded by most localized mRNAs for embryonic development remains obscure. We are focused on the Bicaudal-C (Bic-C) translational repressor encoded by a vegatally localized mRNA (Zhang et al 2013, 2014). Antisense oligonucleotide ablation coupled with the host-transfer technique was used to create embryos that lack maternal Bic-C mRNA and protein. The resulting Bic-C deficient embryos develop with expanded anterior/dorsal structures, suggesting that Bic-C represses the translation of mRNAs required for normal anterior/dorsal development. Bic-C is an mRNA-specific translational repressor and the key to understanding its affect on embryonic development is identifying the specific mRNAs it represses. To identify such mRNAs, Bic-C was immunoprecipitated from *Xenopus* embryos and the bound mRNAs analyzed by RNA-SEQ. Several of these Bic-C target mRNAs encode proteins, such Cripto-1, that function as components or regulators of developmentally important processes such as the Nodal/TGFβ and Wnt pathways. In-depth studies focusing on the Cripto-1 mRNA demonstrate that Bic-C represses translation directly through a regulatory element, called the TCE (translational control element), within Cripto-1 mRNA's 3′ UTR. Biochemical analysis of the TCE defined a 32-nucleotide region that functions as a minimal Bic-C binding site. This site forms a stem-loop secondary structure, and mutational analyses supported the importance of this structure for Bic-C-RNA interactions and repression *in vivo*. Our results indicate that Bic-C creates embryonic polarities and guides cell-fate decisions during the maternal stages of vertebrate embryogenesis by directly regulating the translation of mRNAs that encode key cell-fate determinants.

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Epigenetic changes in gene regulation by thyroid hormone receptor during adult intestinal stem cell development. Yun-Bo Shi. NICHD, NIH, Bethesda, MD.

Adult organ-specific stem cells are essential for organ homeostasis and tissue repair and regeneration. Intestinal remodeling during frog metamorphosis offers a unique opportunity to study the formation of such stem cells during vertebrate development. During the transition from an herbivorous tadpole to a carnivorous frog, the intestine is completely remodeled with the larval epithelial cells undergo apoptotic degeneration and are replaced by adult epithelial cells developed de novo. The entire metamorphic process is under the control of thyroid hormone. We show that adult epithelial stem cells are induced by thyroid hormone through dedifferentiation of the larval epithelial cells. Thyroid hormone exerts its effects through thyroid hormone receptors (TRs). We demonstrate that TRs recruit, in a hormone-dependent manner, cofactors including epigenetic modification enzymes such as histone acetyltransferase p300 and histone methyltransferase PRMT1 to the target genes for chromatin remodeling/histone modifications in the developing stem cells. We demonstrate for the first time *in vivo* during vertebrate development that TR induces the removal of core histone as well as histone modifications at the promoter regions in vivo. Our findings suggest a role of such epigenetic modifications in the development and/or proliferation of adult intestinal stem cells.

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Exogenously-added spermine and poly-L-lysine equally inhibit neural differentiation, especially eye formation, in marginal zone explants isolated from *Xenopus* late blastulae. Koichiro Shiokawa[1,2,3], Jun-Ichi Takai[1], Takeshi Kondo[1], Kazuei Igarashi[4]. 1) Department of Biosciences, Teikyo University, Utsunomiya 320-8551, Japan; 2) Graduate School of Judo Therapy, Teikyo University, Utsunomiya 320-8551, Japan; 3) Department of Judo Therapy, Fukuoka School of Health Sciences, Sawara-ku, Fukuoka 814-0005, Japan; 4) Graduate School of Pharmaceutical Sciences, Chiba University, Chiba 260-8675,Japan.

When marginal zones were isolated from *Xenopus* late blastulae, and cultured as explants for 3 days in the 1 X Steinberg's solution which contained a relatively high concentration (202 μg/ml; 1.0 mM) of spermine, neural differentiation especially eye formation was inhibited in ca. 60% of the explants, in spite of the fact that pH of the medium was not altered appreciably during culture. Similar effects were observed when spermine was replaced by spermidine. To examine if this inhibitory effect is specific to polyamines, we incubated marginal zone explants in the medium which contained poly-L-lysine of either smaller molecular weights (MW: 500-2,000; poly-L-lysine A), or larger molecular weights (MW: 15,000-30,000; poly-L-lysine B). We found here that while 154-256 μg/ml (0.3-0.5 mM) of poly-L-lysine A suppressed eye formation in 60-90% of the explants, 2.56-

25.6 μg/ml (0.17-1.7 μM) of poly-L-lysine B suppressed eye formation in 50-100% of the explants. In most of the experiments with polyamines and poly-L-lysine, inhibition of eye formation was accompanied with inhibition of the elongation of the explants. These results indicate that poly-L-lysine, just like polyamines, has inhibitory effect on eye formation in the explanted marginal zones of *Xenopus* late blastulae, and the inhibitory effect becomes quite strong when molecular weight of poly-L-lysine becomes larger. These results are consistent with previous reports which showed that both polyamines and poly-L-lysine suppress neural differentiation in animal cap cells (Cheung *et al*., 2010).

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Regulation of neurogenesis with Sox transcription and F-box mediated protein degradation. Elena Silva Casey, Niteace Whittington, Doreen Cunningham, Banu Saritas-Yildirim, Jing Jin. Biology, Georgetown University, Washington, DC.

Neurons are generated from proliferating progenitor cells that initially span the entire neural plate and later reside in the ventricular zone of the CNS. When the differentiation program is initiated, progenitors exit the cell cycle, migrate out of the proliferation zone and differentiate into neurons. The regulatory protein profile of the neural cells change to drive their progression through these steps. These changes are controlled in part at the transcriptional level but as neural cells progress to their final fate, regulatory proteins must also be inactivated and/or removed. To investigate both the transcriptional regulation and protein degradation required for neurogenesis, we have investigated the role of SoxB and C transcription factor family members and the F-box E3 ubiquitin ligases. With gain and loss of function studies, we discovered that the SoxB2 protein Sox21 and the SoxC protein Sox11 have dose and temporal dependent roles in neurogenesis. Sox protein target specificity is dependent on partner proteins and using both a candidate approach and mass spectrometry, we are identifying Sox11 and Sox21 partners that play a role in their context dependent functions. To investigate the role of protein degradation in altering the neural cell protein profile, we used an in silico screen for REST/NRSF (neuron-restrictive silencing factor) targets and identified 4 neuron specific F-box proteins. Fbox proteins are components of the SCF family of ubiquitin ligases and target proteins for ubiquitin-mediated protein degradation. Our data demonstrate that to regulate neurogenesis and maintain a balance of proliferating neural progenitors and neurons, a coordination of selective protein degradation mediated by F-box proteins and transcriptional regulation by Sox proteins is required.

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***Vestigial-like 4* is required for neural crest formation and neurogenesis in early *Xenopus* embryo. Emilie SIMON**, Corinne FAUCHEUX, Sandrine FEDOU, Pierre THIEBAUD, Nadine THEZE. Bordeaux University UMR CNRS 5164, Bordeaux, France.

Vestigial-like 4 belongs to the vestigial family of transcription cofactor that is highly conserved from drosophila to mammals. In drosophila, vestigial forms a complex with the transcription factor scalloped that activates a number of target genes involved in wing development and muscle differentiation. In mammals four *vestigial-like* (*vgll1-4*) genes have been described and four orthologs of *scalloped*, namely*tead*. Similar to drosophila, vertebrate vestigial proteins form a complex with tead through a conserved domain named TONDU, but their functions remain largely unknown. We have previously described the four *vgll* in *Xenopus laevis* and two *tead* and their developmental expression (Naye *et al*. 2007 and Faucheux *et al*. 2010). In the present work, we provide data on the function of vgll4 during early development. *Vgll4* is expressed maternally and in different regions of the embryo such as brain, eye, ear and somites. In loss of function experiments, using antisens morpholino-nucleotides, *vgll4* morphants show a decrease in neural crest markers (*Pax3*, *Slug* and *Sox9*) and neuronal markers expression (*sox2* and *N-tubulin*). These results suggest that vgll4 is involved in neural crest formation and neurogenesis. Although *vgll4* is expressed in somites, it doesn't seem to have any effects on muscle differentiation with respect to *myod* expression. Similarly vgll4 seems not to have a function on early endodermal development. This study provides a novel and uncharacterized role for vgll4 during vertebrate development and more specifically in neuroectodermal differentiation.

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Defining the mechanisms of transcriptional regulation by the T-box transcription factor Eomesodermin during early cardiac development. Christopher Slagle, Frank Conlon. Depts. of Biology and Genetics, McAllister Heart Institute, University of North Carolina - Chapel Hill, Chapel Hill, NC.

Numerous processes during development depend on the precise control of gene expression regulated by a myriad of signaling pathways and downstream transcription factors. These proteins often function in multiple processes at different times during development, raising the question of how their functional specificities are achieved. The conserved T-box transcription factor Eomesodermin (Eomes), is required in a TGF-β-associated manner for the induction of vertebrate mesoderm and endoderm. Eomes was recently shown to directly activate transcription of *Mesp1*, the master regulator of the cardiogenic transcriptional program, independently of TGF-β signaling. Eomes' functional specificity therefore relies not only on the tissue-specific targets it regulates, but on the transcriptional co-regulators with which it interacts. I have employed an immunoprecipitation-based mass spectrometry (IP/MS) analysis to probe the identities of Eomes-containing proteins during cardiac specification. I performed a pilot IP/MS analysis of Eomes binding partners in HEK293 cells overexpressing EGFP-tagged Eomes. This analysis identified several chromatin-modifying complex subunits (e.g. SWI/SNF and NuRD components) as candidate Eomes-interacting proteins. To focus on cardiac-specific co-regulators, I will repeat the IP/MS analysis in mouse embryonic stem cells differentiating along the cardiac lineage. Protein interactions will be validated in *Xenopus* to demonstrate conserved mechanisms of Eomes-mediated transcriptional regulation during early development. Domains of Eomes responsible for these interactions will be identified

through co-IP assays using deletions and mutant versions of Eomes. Finally, the biologically relevant domains of Eomes will be validated *in vivo* and assessed for functionality in morpholino and TALEN mutagenesis-based models of Eomes loss of function in *X. laevis* and *X. tropicalis.* This analysis can be expanded to other Eomes-dependent tissues, including endoderm, forebrain neurons, and cytotoxic T lymphocytes, to determine the common and unique modes by which Eomes regulates its targets.

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A secreted integrin-beta like molecule, Itgbl-1 modulates the Integrin-ECM interactions for the cartilage formation. Eun kyung Song, Tae Joo Park. Department of Biological Science, Ulsan National Institute of Science and Technology(UNIST), Ulsan, Korea.

Craniofacial development is among the most complicated processes in vertebrate development. In an effort to identify novel genes involved in facial development, we analyzed the transcriptome profiles of pharyngeal arches by performing RNAseq experiments. Several dozens of genes were strongly expressed in the first pharyngeal arch. Among them, a secreted integrin beta like molecule, Itgbl-1 is specifically expressed in the cartilage tissues of the first pharyngeal arch. Morpholino-mediated knockdown of Itgbl-1 caused hypoplastic facial cartilages. Especially, the maxillary tissues were severely affected rather than mandibular, or hyoid cartilages. Furthermore the overexpression of Itgbl1 accelerated cartilage formation resulting in significantly enlarged craniofacial cartilages. Further analysis revealed that Itgbl1 plays central roles in the integrin dependent signaling cascade for the cartilage differentiation. Igbl1 seemed to reduce the integrin-ECM interactions and thereby inhibits stable focal adhesion formation in developing cartilage cells. In this study, we provide the first evidences for the molecular functions of Itgbl1 as a unique secreted modulator for the integrin-ECM interactions.

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A dominant-negative provides new insights into Focal Adhesion Kinase (FAK) regulation and function in early embryonic morphogenesis. Panayiota Stylianou, Nicoletta Petridou, Paris Skourides. Biological Sciences, University of Cyprus, Nicosia, Cyprus.

Focal Adhesion Kinase (FAK) is a 125kDa non-receptor tyrosine kinase involved in a wide variety of biological processes and crucial for embryonic development. In this study, we have generated a new FAK dominant negative (FF), composed of the C terminus (FRNK) and the FERM domain of the protein. FF, unlike FRNK and FERM, mimics the localization of active endogenous FAK in the embryo, primarily localizing at sites of cell-cell contact. This is demonstrating that both domains are necessary to target FAK to its complexes *in vivo*.We show through FRAP experiments that the FERM domain has a role in the recruitment of FAK on focal adhesions and controls the dynamics of the protein on these complexes. Expression of FF blocks focal adhesion turnover and, unlike FRNK, acts as a dominant negative *in vivo*. FF expression in *Xenopus* results in an overall phenotype remarkably similar to the FAK knockout in mice, including loss of mesodermal tissues. Expression of FF in the animal cap revealed a previously unidentified role of FAK in early morphogenesis and specifically epiboly. By using microdissecting approaches we show that a fibronectin-derived signal transduced by FAK governs polarity and cell intercalation.Finally, failure of epiboly results in severe gastrulation problems that can be rescued by either mechanical or pharmacological relief of tension within the animal cap, demonstrating that epiboly is permissive for gastrulation. Overall, this work introduces a powerful new tool for the study of FAK, uncovers new roles for FAK in morphogenesis and reveals new mechanisms through which the FERM domain regulates the localization and dynamics of FAK.

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SCP3 regulates cell competence to Nodal/Activin and BMP signals via dephosphorylating linker sites of Smad2 and Smad1 in *Xenopus* embryos. Guanni Sun[1], Zheying Min[1], Zhirui Hu[2], Zhenpo Guan[1], Hanxia Su[1], Xiaopeng Ma[1], Michael Zhang[2,3], Qinghua Tao[1], Wei Wu[1]. 1) School of Life Sciences, Tsinghua University, Beijing, China; 2) Bioinformatics Division, Center for Synthetic and Systems Biology, Tsinghua National Laboratory for Information Science and Technology, Tsinghua University, Beijing, China; 3) Department of Molecular and Cell Biology, Center for Systems Biology, The University of Texas at Dallas, Richardson, TX.

Nodal/Activin and BMP signals, playing key roles in the pattern formation and germ layer induction, are activated significantly soon after the MBT in *Xenopus*. The signals are regulated by not only the burst of ligands but also the competence — the ability of a cell to respond to the stimulus. Here we found that among the known Smad1 and Smad2 linker region phosphatases, only SCP3 (small C-terminal domain phosphatase 3) is expressed asymmetrically along the animal-vegetal axis in *Xenopus*, reminiscent the localization of initially activated Smad1 and Smad2. In *Xenopus* early embryos, SCP3 is able to enhance the response to both Nodal/Activin and BMP signals and is required for the timely induction of germ layers. Mechanistically, SCP3 dephosphorylates linker regions of Smad1 and Smad2, ensuring a prepared status of Smads for their activation by the coming ligands.

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Establishment of vertebrate body plan via coordinated regulation of dorsal-ventral and anterior-posterior patterning during early *Xenopus* embryogenesis. Kimiko Takebayashi-Suzuki, Hidenori Konishi, Hitoshi Yoshida, Maya Okada, **Atsushi Suzuki**. Institute for Amphibian Biology, Hiroshima University, Higashi-Hiroshima, Japan.

The formation of the dorsal–ventral (DV) and anterior–posterior (AP) axes, fundamental to the body plan of animals, is regulated by several regulatory pathways including the TGF-β, FGF, and Wnt families. In order to ensure the establishment of

the body plan, the processes of DV and AP axis formation must be linked and coordinately regulated during early development. However, the molecular mechanisms responsible for these interactions remain unclear. Here, we report the molecular mechanisms of neural induction and patterning regulated by Biz, a novel candidate protein involved in the coordinative regulation of the DV and AP body axes. Overexpression of Biz resulted in inhibition of BMP signaling and activation of Wnt signaling, thereby promoted neural induction and posterior neural development in isolated ectodermal explants. Knockdown of Biz resulted in repression of neural gene expression. Interestingly, we found that Biz functionally interacted with forkhead transcription factor FoxB1, another important factor in the coordinative regulation of DV and AP patterning. Our results suggest that Biz plays essential roles in the coordinative regulation of the DV and AP body axes by modulating BMP and Wnt signaling during early development.

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Genome editing reveals a novel function of keratin in fin formation in *X. laevis*. Ken-ichi T Suzuki[1], Keiko Kashiwagi[1], Tetsushi Sakuma[1], Akihiko Kashiwagi[1], Makoto Mochii[2], Takashi Yamamoto[1]. 1) Graduate School of Science, Hiroshima University, Higashihiroshima,Hiroshima, Japan; 2) Graduate School of Life Science, University of Hyogo, Aioi, Hyogo, Japan.

Keratin, a component of intermediate filament, provides epithelium with physical and mechanical strength. It is assumed to be involved in the dynamic formation of 3D structures in organogenesis; however, there is no report about function of keratins in development. To understand the function of keratin in organogenesis, we focused on a novel type I keratin that is exclusively expressed in median fin fold (MFF) at the tail bud stage of *X. laevis*, referred to as *fg keratin* (*fgk*). First we generated transgenic lines with EGFP and DsRed2 reporter genes driven by *fgk* 4.2-kb upstream sequence. EGFP reporter activities recapitulated the endogenous gene expression and were particularly observed in the prospective fin region and MFF, where dynamic organogenesis accompanying extensive cell shape change and movement occurs. Also we performed targeted mutagenesis of *fgk* using TALENs. Interestingly, TALEN mRNA-injected embryos showed drastic loss of fin but normal axial elongation. These results suggest a new role of keratin filament in organogenesis during early development.

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Dynamic intracellular localization of Xdazl protein during the *Xenopus* germline development. Haru Tada, Hidefumi Orii. Graduate School of Life Science, University of Hyogo, Japan.

In *Xenopus*, the germline cells are generated by inheritance of specialized cytoplasm called 'germ plasm', which contains mRNAs, proteins and abundant mitochondria. Previously, we have demonstrated that the germ plasm is sufficient for germ cell determination (Tada et al., 2012). *Xdazl* has been identified as an RNA component of the germ plasm (Houston et al., 1998) and encodes an RNA-binding protein homologous to DAZL (*deleted in azoospermia like*). It was shown that *Xdazl* was involved in the migration and differentiation of the primordial germ cells (PGCs) (Houston and King, 2000). The protein was detected in the germ cells during the gametogenesis as well as PGCs (Houston and King, 2000; Mita and Yamashita, 2000). However, despite of the importance of Xdazl in the PGC development and gametogenesis, the detailed intracellular localization remains unknown. In this study, we performed immunohistochemistry for Xdazl protein with paraffin-embedded sections of the embryos and gonadal tissues at various stages, and found that the intracellular localization of Xdazl protein changed developmentally. Xdazl protein did not show any specific distribution in the embryo until stage 8. It is initially detected in the germ plasm at stage 9. At stage 10, the protein was present in the perinuclear region where the germ plasm moved. Interestingly, Xdazl signal was detected in the nucleus as well as the perinuclear region around stage 12. Until stage 48, the protein was observed in both cytoplasm and nucleus of PGCs in developing gonad. At stage 55, the Xdazl protein was observed only in the cytoplasm of PGCs in both female and male gonads. In the developing ovary at stage 66, Xdazl protein did not show any specific distribution in oocytes, however, it was distributed throughout the cytoplasm of oogonia. In spermatogenesis, Xdazl protein was distributed in the cyotoplasm of spermatogonia and spermatocytes, although it was distributed in both nucleus and cytoplasm of the primary spermatogonium. Then, it was observed in the nuclei as dots in the spermatids. These observations suggest that Xdazl has multiple functions in the PGC development and gametogenesis of *Xenopus*.

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***Xenopus mab21-l3* is required for cell fate specification of multiciliate cells and ionocytes. Chika Takahashi[1]**, Morioh Kusakabe[1,2], Toshiyasu Suzuki[1], Koichi Miyatake[1], Eisuke Nishida[1,2]. 1) Department of Cell and Developmental Biology, Graduate School of Biostudies, Kyoto University, Sakyo-ku, Kyoto, 606-8502, Japan; 2) JST, CREST, Tokyo, Japan.

Flow-generating multiciliate cells (MCCs) and ion-transporting ionocytes are found in *Xenopus* embryonic epidermis. Cell fate specifications of MCCs and ionocytes are commonly suppressed by the Notch pathway in developing epithelia, but are governed by different master regulators: a coiled-coil protein multicilin and a forkhead transcription factor foxj1 for MCCs, and another forkhead transcription factor foxi1 for ionocytes, suggesting the existence of a common regulator linking the Notch pathway to both MCC and ionocyte specifications. Here we identify an evolutionarily conserved mab21 family gene,*mab21-l3*, as a key common regulator that connects this missing link. In *Xenopus* embryonic epidermis, *mab21-l3* expression is specifically found in MCCs and ionocytes, and is downregulated by the Notch pathway. Knockdown of *mab21-l3* in *Xenopus* downregulates *multicilin*, *foxj1* and *foxi1*, resulting in drastic loss of MCCs and ionocytes. Moreover, conditional gain-of-function of *mab21-l3* rescues Notch-induced loss of MCCs and ionocytes in *Xenopus*. The rescue experiment also suggests that the expression level of *mab21-l3* is one of the factors influencing Notch responsiveness and cell fate decision in

epithelia. These results indicate that a Notch-repressed gene *mab21-l3* is a key regulator for cell fate specification of MCCs and ionocytes.

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Ascl1 regulates mesendoderm through antagonizing VegT and activating Delta/Notch in the early Xenopus embryos. Qinghua Tao[1,2], Li Gao[1,2], Xuechen Zhu[1,2], Hao Lin[1,2]. 1) Tsinghua University School of Life Sciences, Beijinh, China; 2) 1 Qinghuayuan, Haidian District.

During the early Xenopus embryogenesis, endoderm is specified in the vegetal pole by the vegetally localized maternal transcription factor VegT, mesoderm is induced to form around the equator mainly by VegT-activated zygotic Nodal signaling, and the animal hemisphere is left for ectoderm formation. How the maternal VegT-initiated mesendoerm formation is regulated remains unknown. Here we provide evidence that Ascl1, in additional to its well known functions in neurogenesis and the differentiation of neuroendocrine cells, plays a novel role in regulating mesendoderm formation in the earlier phase of embryogenesis. Maternal Ascl1 mRNA is enriched in the animal hemisphere and overlapped with VegT in the sub-equatorial region. Overexpression of Ascl1 inhibits the expression of mesendoderm genes downstream of maternal VegT, but not of Xnr1, indicating that Ascl1 specifically function through VegT. Coinjection of Ascl1 with VegT reduces the level of acetyl-Histone marks and VegT binding on the promoter regions of its target genes. HDAC activity is required for Ascl1-mediated repression of VegT activity. Depletion of Ascl1 results in increased expression of mesendoderm genes as exemplified Bix4 and Mixer. In addition, Ascl1 is also required for pan-mesoderm gene Brachyury expression induced by Nodal signaling. We demonstrate that Ascl1 and Nodal signaling jointly activare zygotic Delta1 and then Notch/Delta signaling in the equatorial zone, which renders the equatorial cells competent for mesoderm induction by Nodal signaling. Together we propose that maternal Ascl1 is a crucial regulator of mesendoderm formation initiated by maternal VegT.

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Gtpbp2: a unique GTPase functioning in BMP and Wnt signaling in Xenopus embryos. William Gillis[1,2], Arif Kirmizitas[1,2], Yasuno Iwasaki[1], Dong-Hyuk Ki[1], Jonathan Wyrick[1], **Gerald Thomsen[1]**. 1) Biochemistry & Cell Biology, Center for Developmental Genetics, Stony Brook University, Stony Brook, NY, USA; 2) equal contributors.

Gtpbp2 is a little understood 'orphan' GTPase, distantly related to elongation factor 1 alpha (eEf1a), and not yet linked to any pathway or biological process. We have uncovered Gtpbp2 as component of BMP and Wnt signaling that is essential for normal *Xenopus* embryonic development. We identified Gtpbp2 as a Smad1 interacting protein in a yeast two-hybrid screen and have evaluated its expression and action in *Xenopus* embryos by in situ hybridization and gain and loss of function tests. Gtpbp2 overexpression induces BMP response genes in animal caps, and morpholino knockdown causes phenotypic and gene expression effects indicating an essential role in BMP signaling and ventral-posterior patterning. The Gtpbp2 C-terminus interacts with the MH1 domain of Smad1, and Gtpbp2 can localize to the cytoplasm or nucleus. Although required for BMP signaling, Gtpbp2 knockdown has no effect on nodal responses in animal caps, yet morphants have dorsal and anterior phenotypes. This led to further tests which uncovered a requirement for Gtpbp2 in canonical Wnt signaling. Epistatic analysis points to Gtpbp2 functioning at the level of the β-catenin destruction complex, supported by its ability to interact with Dishevelled, Axin and Gsk3β. Mechanistically, Gtpbp2 governs the levels of Axin protein in embryonic cells, mimicking the destabilizing effects that Tankyrase (a poly-ADP ribose polymerase) and Rnf146 (a ubiquitin ligase) exert on Axin. The ability of Gtpbp2 to affect both BMP and Wnt signaling suggests it may function as an integrator or coordinator of signaling in both pathways, perhaps via Gsk3β. Mechanistic studies are ongoing. In the wider scope, Gtpbp2 is a new target for developing small molecules and drugs to probe its normal functions and to potentially regulate pathological conditions stemming from aberrant Wnt or BMP signaling.

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Ric-8A/Gα13 signaling pathway is required to proper cranial neural crest migration in Xenopus. Gabriela Toro-Tapia[1], Marion Rodriguez[1], Sylvain Marcellini[2], Roberto Mayor[3], Marcela Torrejon[1]. 1) Biochemistry and Molecular Biology, University of Concepcion, Concepcion, Chile; 2) Cell and Developmental Biology, University College London, UK; 3) Cell Biology, University of Concepcion, Concepcion, Chile.

Cell migration is important during development and requires several signals to control cell movements. One of these signals is the heterotrimeric G protein pathway which has been studied mainly in gastrulation movement. Recently we have reported that Ric-8A, a GEF for Gα proteins, plays an important role in neural crest (NC) migration in Xenopus development. However the Gα protein activated by Ric-8 remains unknown. Ric-8A and Gα13 are involved during gastrulation movements of *Droshopila* and both proteins are required to trigger apical constriction and acto-myosin contractility during gastrulation events.Therefore we propose:"Ric-8A, through Gα13 subunit of the heterotrimeric G protein pathway, regulates NC migration in Xenopus". Our preliminary experiments suggest that Gα13 could be the Ric-8 target. *Xenopus tropicalis* and *Xenopus laevis* embryos were analyzed by *in-situ* hybridization to evaluate *in-vivo* cranial NC migration. On the other hand, cranial NC was dissected and cultured on fibronectin coated wells to analyze *in vitro* cell migration.Our data show that both Ric-8A and Gα13 are required for *in vivo* and *in vitro* cranial NC cells migration. By epistasis assays we have shown that Gα13 mRNA was able to rescue the Ric-8A morphant phenotype. Furthermore Gα13 loss-of-function inhibits cranial NC cells migration. Gα13 and Ric-8A co-localize in protrusion at the leading edge of cranial NC cells. In addition to these results co-immunoprecipitation assays demonstrated that Ric-8A and Gα13 interact. Interestingly Ric-8A down-regulation alters Gα13 localization, which changes to cytoplasmic area. Besides, in Ric-8A and Gα13 morphant cells we observed changes in the cortical F-actin phenotype,

number and size of lamellipodia, cellular shape and aPKC polarity marker subcellular localization. Therefore, all these results suggest that Ric-8A regulates Gα13 signaling and together control cell polarity and cytoskeletal reorganization required for proper cell migration.

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Ric-8A is required for neural crest cells induction. Marion Rodriguez, Gabriela Toro-Tapia, Maria Hinrichs, **Marcela Torrejon**. Biochemistry and Molecular Biology, University of Concepcion, Concepcion, Chile.

The neural crest (NC) is a transient embryonic cell population that emerges from the dorsal neural tube during early development in response to molecular signals from the mesoderm, neural and non-neural ectoderm. We have reported that Ric-8A, a guanine nucleotide exchange factor (GEF) over different Gα subunits from Xenopus, is involved in the cranial NC cells migration and is expressed in the marginal zone of the prospective mesoderm and in NC during induction stages. Here, we aim to study the function of Ric-8A during NC induction. For this, gain and loss-of-function assays were performed in embryos obtained from X. tropicalis. Ric-8A morpholino (Ric-8AMO) or mRNA for Ric-8A was injected in one blastomere of 2 cells stage embryos and then fixed at stage16. Whole-mount in situ hybridization assays against Snail2 were performed. Rescue was visualized by co-injecting Ric-8AMO with mRNA for Ric-8A. On the other hand, epistasis assays were tested by injecting Ric-8AMO together with Gαq subunit mRNA. We showed that the silencing of Ric-8A alters the expression pattern of Snail2 during NC induction and rescued by injecting the Ric-8A mARN or Gαq mRNA in morphant embryos. Finally, to study Ric-8A in the tissue that delivers the induction signal for NC induction, or in the tissue that receives it, tissue recombination assays were performed. We dissected DLMZ (Dorso-Lateral Marginal Zone) from early gastrula Ric-8A morphant embryos, and conjugated them together with Animal Caps (ACs) dissected from blastula embryos. The same strategy was used with dissected DLZM from wild-type embryos conjugated together with Ric-8A morphant ACs. The conjugates corresponding to DLMZ/AC tissue were incubated and fixed at stage19-20. In situ hibridization assays showed that all DLMZ/AC conjugates with Ric8AMO in DLMZ presented an inhibition phenotype of Snail2. However, all DLMZ/AC conjugates with Ric-8AMO in the AC were able to normally express Snail2. Together, these results suggest that Ric-8A has a specific effect over NC cells induction. and is required at the DLMZ (prospective mesoderm) and not in ACs (ectoderm) in order to induce NC cells in X. tropicalis conjugates

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Sodium Currents Initiate Tail and Limb Regeneration. Ai-Sun Tseng[1], Michael Levin[2]. 1) School of Life Sciences, University of Nevada, Las Vegas, Las Vegas, NV; 2) Center for Regenerative and Developmental Biology, Biology Department, Tufts University, Medford, MA.

Mammals are highly restricted in their ability to regenerate tissues, whereas amphibians such as frogs can restore lost larval structures including appendages. To advance organ repair strategies, a detailed understanding of the regenerative process is needed. Although it is known that some developmental pathways are reactivated during regeneration, the signals that initiate their deployment are poorly understood. We have identified one such initiating signal as a novel role for the voltage-gated sodium channel (Na_V), historically known for propagating action potentials in excitable cells. After amputation,*Xenopus* tadpoles regrow a new tail within a week—complete with spinal cord, nerves, vasculature and muscle. *Xenopus* Na_V1.2 is specifically expressed at the injury site by 18 hours after tail ablation, and its activity results in a strong increase of intracellular sodium levels. Na_V inhibition causes regenerative failure by blocking cell proliferation, nerve innervation, and reactivation of normal tail developmental pathways without affecting overall development of the larvae. Na_V is absent under non-regenerative conditions, suggesting that its function is a determinant of regenerative ability. Conversely, ectopic expression of human Na_V1.5 restored regeneration in non-regenerative tadpoles, indicating that it is the sodium transport function alone that is required. Consistent with a role for sodium in driving regenerative growth, chemical induction of a transient sodium current even as late as 18 hours after amputation restores tail regeneration during non-regenerative states. Furthermore, we show that a similar chemical induction of sodium flux also promotes limb regrowth in older (non-regenerative) tadpoles, which holds promise for future therapeutic strategies. Our study demonstrates that sodium transport is a critical mechanism for controlling the initiation of regeneration. Thus, short-term modulations of ion transport could represent an exciting new approach to tissue repair in mammals.

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Systematic analysis of *RFX2* targets in *Xenopus* multiciliated cells. Fan Tu, Mei-I Chung, Taejoon Kwon, Edward Marcotte, John Wallingford. MBS, University of Texas at Austin, Austin, TX.

Multiciliated cells (MCCs) are critically important for normal functioning in vertebrate tissues such as the trachea, brain, and reproductive tracts. The embryonic epidermis of *Xenopus* tadpoles is an excellent model for studying MCCs. Previously we showed that the conserved transcription factor *RFX2* was required for ciliogenesis and cilia function. By RNA-seq and Chip-seq, we identified 911 directly regulated downstream target genes of *RFX2*. As expected, this dataset contains many genes (~100) known to be involved in cilia-related processes, such as IFT components, axonemal dyneins, and centriolar proteins. However the functions of the vast majority of *RFX2* targets remain unknown. To address this issue, we systematically made fluorescence protein tagged gene clones and screened for targets with distinct subcellular localization in *Xenopus* MCCs. Our preliminary results have identified targets localized to the axoneme, basal bodies, cell cortex, tight junctions, and other specific cellular structures. This localization data will provide important clues to the function of these target proteins in MCCs, and will provide a foundation for future functional studies.

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Repression of Both BMP and Activin Signaling is Required for Retina Formation. Andrea S Viczian[1], Michael Trembley[2], Kimberly Wong[1]. 1) Ophthalmology, Upstate Medical University, Syracuse, NY; 2) Cellular and Molecular Pharmacology and Physiology, University of Rochester, Rochester, NY.

Purpose: Retina formation requires the correct spatiotemporal patterning of key regulatory factors. While it is known that repression of several signaling pathways lead to formation of retinal fates, addition of only Noggin, a known BMP antagonist, can convert pluripotent animal cap cells to function retinal cells. The aim of our study is to determine if Noggin affects signaling pathways other than BMP during this conversion.**Methods**: We treated pluripotent *Xenopus laevis* animal caps with chemical inhibitors and function-altering components of the BMP and Activin signaling pathways. Animal caps from the blastula were cultured until neural plate stage. Their effect on retina formation was determined using the Animal Cap Transplant (ACT) assay, in which the animal caps were transplanted into the eye field of host embryos. Signaling activity was determined by Western blot and semi-quantitative PCR (RT-PCR) to measure downstream protein and gene target expression.**Results**: Overexpressing Noggin in animal caps resulted in a concentration-dependent suppression of both Smad1 and Smad2 phosphorylation, which act downstream of BMP and Activin receptors, respectively. This caused a decrease in downstream transcriptional ability, reflected by the reduced expression of mesodermal marker, *xbra*, and endothelial marker, *xk81*. Overexpression of dominant negative BMP and Activin receptors revealed that retinal specification was increased when both pathways were inhibited simultaneously. Similar results were observed when the chemical inhibitors Dorsomorphin and SB431542 were used to inhibit Smad1 and Smad2 phosphorylation, respectively.**Conclusion**: Thus, the dual inhibition of BMP and Activin pathways promotes retinal specification in *Xenopus* tissue. Future studies will translate these findings to a mammalian culture assay, in order to efficiently produce retinal cells in culture.

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Xenbase 2014. Peter Vize[1], Aaron Zorn[2], Kamran Karimi[1], Kevin Burns[2], Joshua Fortriede[2], Christina James-Zorn[2], Brad Karpinka[1], Jacqueline Lee[1], Yu Liu[1], Virgilio Ponferrada[2], Erik Segerdell[2]. 1) Biological Sciences, University of Calgary, Alberta, Calgary, Alberta, Canada; 2) Cincinnati Children's Research Foundation, College of Medicine. Cincinnati, OH 45229, USA.

Xenbase has many new features, including laevis genome build 7.1, new user interface and news service, full database support for antibodies and morpholinos, CRISPr design support, thousands of new figures from PubMedCentral papers, upgraded genome browser and BLAST services, more links to human disease data, a massive data repository, thousands of manually curated gene expression patterns and much more. We will present a summary of new features and demonstrate some of their uses, and Xenbase staff will be on hand in poster sessions to provide hands on training throughout the conference.http://www.xenbase.org - landing page URLhttp://gbrowse.xenbase.org/fgb2/gbrowse/xl7_1/ - laevis genomehttp://www.xenbase.org/reagents/antibody.do - >700 antibodies

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MicroRNAs in Neural Crest development. Nicole Ward, Ayisha Ahmed, Dario Dotlic, Grant Wheeler. School of Biological sciences, University of East Anglia, Norwich Research Park, NR4 7TJ.

MicroRNAs (miRNAs) are involved in controlling various mechanisms during development by regulating gene expression at the post-transcriptional level. Many are highly conserved amongst diverse organisms and have highly specific spatio-temporal expression patterns during embryonic development. miRNAs are short, non-coding RNAs around 22 nucleotides long. They inhibit gene expression either by translational repression or by causing the degradation of the mRNAs they bind to.We have determined the expression patterns of 190 miRNAs in *Xenopus laevis* embryos using LNA oligonucleotides. These are available as a resource in XenMark and Xenbase. Using miRNA-seq we have also identified a number of novel miRNAs and show them to be developmentally expressed.Some of the miRNAs have expression patterns that overlap with developing neural crest cells (NCC). NCC are a transient, multipotent stem cell–like population of highly migratory embryonic cells found exclusively in vertebrates. Their derivatives contribute to a wide variety of tissues and organs in the developing embryo. These include melanophores, enteric ganglia, neuroendocrine cells, neurons, and craniofacial cartilage and bone. The induction and specification of NC cells occurs during gastrulation through to organogenesis due to a complex gene regulatory network. However, the role of miRNAs within this network is yet to be explored.To confirm that these miRNAs are located within developing NCC we have carried out double *in situ* hybridisations for the identified miRNAs with the NC marker, Sox10. We show clear overlap indicating the miRNAs are present within the developing NC. We now plan to investigate the targets of these miRNAs using luciferase assays and assess their function using both loss of function and gain of function experiments.

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Unliganded thyroid hormone receptor α controls developmental timing in *Xenopus tropicalis*. Luan Wen, Yun-Bo Shi. Laboratory of Gene Regulation and Development, National Institutes of Health (NIH/NICHD), Bethesda, MD.

Thyroid hormone (T3) affects adult metabolism and postembryonic development in vertebrates. T3 functions mainly via binding to its receptors (TRs) to regulate gene expression. There are two TR genes, TRα and TRβ, with TRα more ubiquitously expressed. During development, TRα expression appears earlier than T3 synthesis and secretion into the plasma. This and the ability of TRs to regulate gene expression both in the presence and absence of T3 have implicated a role of unliganded TR during vertebrate development. On the other hand, it has been difficult to directly study the role of unliganded TR and the associated

mechanisms during development in mammals due to the difficulty to manipulate the uterus-enclosed, late stage embryos. We are using amphibian development as a model to address this question as TR is both necessary and sufficient for T3-dependent metamorphosis. Here, we have designed TALENs (transcriptional activator like effector nucleases) to mutate the TRα gene in *Xenopus tropicalis.* We show that knockdown of TRα enhances tadpole growth in premetamorphic tadpoles, in part because of increased growth hormone gene expression. More importantly, the knockdown also accelerates animal development, with the knockdown animals initiating metamorphosis at a younger age and with a smaller body size. On the other hand, such tadpoles are resistant to exogenous T3 and have delayed natural metamorphosis. Thus, TRα has dual functions, first regulating both the tadpole growth rate and the timing of onset of metamorphosis by repressing target gene expression in the absence of T3 and then promoting metamorphosis when T3 is available.

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Regulation of Dead End1 (Dnd1) protein turnover and primordial germ cell development by autophagy. Jing Yang[1], Zhigang Jing[1], Wenyan Mei[1], Mary Lou King[2]. 1) University of Illinois at Urbana-Champaign; 2) University of Miami.

The primordial germ cells (PGCs) are the "stem cells of the species". Unlike somatic stem cells, which often reside in a protected microenvironment, PGCs are exposed to numerous signals from surrounding tissues that specify somatic cell fates. To prevent somatic differentiation, PGCs must establish a transient genome-wide transcriptionally repressive state during early stages. Currently, it is largely unclear how this transient transcriptional repression is established in PGCs. Here we report that Dnd1, a germ cell specific RNA-binding protein, is dynamically expressed during early development. Dnd1 protein expression is very low in oocytes, but increased dramatically after fertilization. It is persistently expressed at high levels when zygotic transcription is repressed in PGCs and is down-regulated right before the beginning of zygotic transcription in PGCs. This suggests a role for Dnd1 in establishing the transient transcriptionally repressive state in PGCs. To understand how this dynamic expression of pattern Dnd1 protein is regulated, we measured the half-life of Dnd1. We found that Dnd1 protein is intrinsically unstable. Rapid turnover of Dnd1 protein is mediated by an evolutionarily conserved motif located in the RNA-recognition domain of Dnd1. Strikingly, this motif acts as an autophagy-targeting signal to control Dnd1 turnover. Truncation of this motif or inhibition of autophagy all lead to accumulation of Dnd1 protein. Taking advantage of the host transfer technique, we further demonstrate that overexpression of Dnd1 in oocytes causes defective embryonic development. It appears that autophagy-dependent turnover is an important mechanism that ensures proper expression level of Dnd1, which is essential for early development. Inspired by the finding that Dnd1 is degraded by autophagy, we examined a number of maternal RNA-binding proteins and germ plasm components. Our results indicate that a subset of germ plasm components is selectively degraded by autophagy. Furthermore, we found that knockdown of Beclin1, a component of the autophagy pathway, induced abnormal germ cell development. To our knowledge, this is the first demonstration of a role of autophagy in controlling vertebrate PGC development.

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***fezf2* promotes neuronal differentiation through localised activation of Wnt/β-catenin signalling during forebrain development. Siwei Zhang[1]**, Jingjing Li[1], Kris Vleminckx[2], Enrique Amaya[1]. 1) The Healing Foundation Centre, Faculty of Life Sciences, University of Manchester, M13 9PT, UK; 2) Department for Biomedical Molecular Biology, Ghent University, Ghent, Belgium.

Neuronal subtype diversification, brain regionalisation, and circuit connectivity are critical events during forebrain development and form the structural and functional basis for higher brain activities. Here we report the requirement for the transcriptional repressor *fezf2* for proper differentiation of neural progenitor cells during the development of the *Xenopus* forebrain. Depletion of *fezf2* induces apoptosis in post-mitotic neural progenitors, with concomitant reduction in forebrain size and neuronal progenitor differentiation. Mechanistically, we found that *fezf2* modulates Wnt/β-catenin signalling, and this function is critical for its role in forebrain development. We show that the neuronal differentiation inducing activity of Fezf2 is Wnt-dependent, and we further demonstrate that Fezf2 promotes localised Wnt activation via repressing the expression of Wnt negative regulators *lhx2* and *lhx9*. Together, our findings suggested localised Wnt/β-catenin activation is responsible for Fezf2-induced neuronal differentiation, and this Wnt/β-catenin activity is possibly crucial for other Fezf2-induced functions.

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Syndecan4 in foregut organ development. Zheng Zhang, Scott Rankin, Aaron Zorn. Perinatal Institute, Division of Developmental Biology, Cincinnati Children's Hospital Medical Center and the College of Medicine, University of Cincinnati, Cincinnati OH 45229.

Syndecan-4 (sdc4), a transmembrane heparin sulphate proteoglycan (HSPG), is important for convergent extension during Xenopus gastrulation as a co-receptor of non-canonical Wnt pathway. It's also widely known as a regulator of fibronectin mediated extracellular matrix, which is critical for BMP signaling. In this study, we showed that Sdc4 coordinate non-canonical Wnt pathway and BMP pathway in Xenopus foregut during endoderm patterning stage. Foregut-specific Sdc4 depletion results in foregut organ agenesis. Sdc4-depleted embryos failed to maintain the foregut progenitor marker hhex and exhibited decreased proliferation as early as gastrula stage. Moreover, foregut cells were enlarged with a randomized orientation, mimicking Wnt receptor Frizzled-7 loss of function. We showed that Sdc4 mediates foregut organ development by regulating both fzd7 mediated Wnt signaling and fibronectin mediated BMP signaling.

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Heat shock 70kDa protein 5 (Hspa5) is essential for pronephros formation by mediating retinoic acid signaling. Weili Shi[1], Gang Xu[2], Chengdong Wang[1], Yi Deng[3], **Hui Zhao[1]**. 1) School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, P. R. China; 2) School of Chinese Medicine, Hong Kong Baptist University, 7 Baptist University Road, Kowloon Tong, Hong Kong SAR, P. R. China; 3) South University of Science and Technology of China, Shenzhen, 518055, P. R. China.

The heat shock 70kDa protein 5 (Hspa5) also known as binding immunoglobulin protein (Bip) or glucose regulated protein 78 (Grp78), belongs to the heat shock protein 70kDa family. As a multifunctional protein, it participates in protein folding, calcium homeostasis and serves as an essential regulator of the endoplasmic reticulum (ER) stress response. It has also been implicated in signal transduction by acting as a receptor or co-receptor residing at the plasma membrane. Its function during embryonic development, however, remains largely elusive. In this study, we used morpholino antisense oligonucleotides (MO) to knockdown Hspa5 activity in *Xenopus* embryos. In Hspa5 morphants, pronephros formation was strongly inhibited with the reduction of pronephric marker genes *lhx1, pax2* and *atp1b1*. Pronephros tissue is induced *in vitro* by treating animal caps with *all-trans* retinoic acid (atRA) and activin. Depletion of Hspa5 in animal caps, however, blocked the induction of pronephros as well as reduced the expression of RA-responsive genes, suggesting that knockdown of Hspa5 attenuated RA signaling. Knockdown of Hspa5 in animal caps resulted in decreased expression of *lhx1*, a transcription factor directly regulated by RA signaling and essential for pronephros specification. Co-injection of Hspa5MO with *lhx1* mRNA partially rescues the phenotype induced by Hspa5MO. These results suggest that the RA-lhx1 signaling cascade is involved in Hspa5MO induced pronephros malformation. This study shows that Hspa5, a key regulator of the unfolded protein response, plays an essential role in pronephros formation, which is mediated in part through RA signaling during early embryonic development.

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Evolution of Maturin suggests a role for ubiquitination during primary neurogenesis. Michael E. Zuber[1], Reyna I. Martinez-De Luna[1], Robert M. Freeman, Jr.[2]. 1) Center for Vision Research, SUNY Eye Institute, Department of Ophthalmology, SUNY Upstate Medical University, Syracuse, NY; 2) Department of Systems Biology, Harvard Medical School, Boston, MA.

Maturin is a novel protein required for the proneural pathway to regulate neural differentiation, however the molecular mechanism(s) by which it functions are unknown. Maturin has no identifiable functional or structural motif, yet has been highly conserved during vertebrate evolution. To infer possible molecular mechanism(s), we identified Maturin orthologs from more evolutionarily distant organisms to identify conserved regions and generate phylogenetic trees. *In situ* hybridization was used to determine and compare the expression patterns of Maturin in select species. More than 100 vertebrate Maturin orthologs were identified. Vertebrate predicted proteins range in size from 129 (pufferfish) to 141 (lamprey) residues. Although multiple small regions of similarity were observed, the most highly conserved region was the Maturin Domain, a 29-residue sequence that was invariant in most vertebrates. Invertebrate Maturins were also identified, including orthologs from cephalochordates, hemichordates, and marine polychaetes. All identified invertebrate Maturins contained an extended C-terminus with sequence homology to the E2 family of ubiquitin-conjugating enzymes (UBE2). Phylogenetic analysis indicates the C-terminus extensions are most similar to the UBE2 Q subfamily. *X. laevis UBE2 QL1* and *Q2* are co-expressed with *Maturin* during neural development and *Maturin•UBE2Q* transcripts were detected in the hemichordate *Saccoglossus kowalevskii* during embryonic development. Our observations suggest a model in which a single *Maturin•UBE2Q* gene evolved into independent *Maturin* and *UBE2Q* genes, as well as a possible molecular mechanism by which Maturin may have functioned in neural development for more than 500 million years.

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Neurogenin3-directed differentiation of endoderm to insulin-expressing cells. Matthew Salanga[1], Leonid Peshkin[2], Marko Horb[1,3]. 1) Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA; 2) Department of Systems Biology, Harvard Medical School, Boston, MA; 3) National Xenopus Resource, Marine Biological Laboratory, Woods Hole, MA.

Neurogenin3 (NGN3) is a bHLH transcription factor sufficient for development of the pancreatic beta cell. Efforts to utilize NGN3 activity for directed differentiation of multipotent cells to endocrine lineages have primarily favored formation of glucagon-producing alpha cells. However, careful developmental timing of NGN3 activity can shift the resultant cell types toward insulin-producing beta cells. Using a drug-inducible version of NGN3 in *Xenopus laevis*, we show that brief (<4hrs) and early (immediately after gastrulation, stage 12) activation of NGN3 induces ectopic and precocious formation of insulin-producing cells detectable approximately 14 hours earlier compared to control embryos. Therefore, we have established a narrow window of development (~13hrs) where we can interrogate in detail the gene regulatory network leading up to insulin expression. Using a combination of time-series and single time-point gain-of- and loss-of-function studies within the 13 hour window, we have identified many direct NGN3 targets potentially involved in beta cell differentiation, as well as two transcription factors which appear necessary for normal insulin expression. We continue to build on these data sets with additional time-series replicates, computation, and functional analyses. Our goal is to assemble a gene regulatory network useful for predicting causal relationships in the path from undifferentiated endoderm to mature beta cells. (HD073104; OD010997)

Genetics and Genomics

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A toolbox for CRISPR/Cas9-mediated genome engineering in *Xenopus tropicalis*. Margaret Fish, Ken Cho, **Ira Blitz**. Developmental and Cell Biology, University of California, Irvine, CA.

We and others have recently shown that the CRISPR/Cas9 system from *Streptococcus pyogenes* can mediate efficient targeted mutagenesis in *Xenopus tropicalis*. The endonuclease Cas9 is directed to target sites in the genome via a short RNA containing target-site complementarity at its 5' end. Target site sequences are seemingly only limited by the requirement for a downstream "PAM element" that consists of the trinucleotide NGG, although unique target sequences should be chosen when possible to reduce the possibility of off-target effects. Mutagenesis is accomplished via Cas9's creation of a double strand break within the target site, which is repaired by non-homologous end joining, an error prone process that typically results in small indels, creating mosaic tadpoles and frogs with wild-type and a variety of mutant alleles. These methods already offer many exciting opportunities for manipulating the frog genome, but the system can be improved. We are pursuing technologies towards decreasing the amount of mosaicism observed in G0 animals, expanding target site options, gene targeting by homologous recombination, and creating large-scale deletions. We will present our progress on advancing the CRISPR/Cas9 system in *Xenopus.*

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Updated *Xenopus laevis* Genome Annotation Based on Brain Deep Transcriptomes. John Cornelius[1], Raghu Metpally[2], Amanda Courtright[2], Jennifer Bestman[3], Lin-Chien Huang[3], Kendall Van Keuren-Jensen[2], Kenro Kusumi[1], Hollis Cline[3]. 1) School of Life Sciences, Arizona State Univ., Tempe, AZ; 2) Neurogenomics Div., Translational Genomics Research Inst., Phoenix, AZ; 3) The Scripps Research Inst., La Jolla, CA.

Xenopus laevis is a model system for the study of brain development, plasticity, physiology, and regeneration. Many features make *X. laevis* an attractive model for observing, manipulating, and testing neurodevelopmental paradigms, due to a prolonged and accessible period of cell proliferation and differentiation that extends through the larval period of CNS development. A significant obstacle to the advancement of the model has been the shortage of a well-annotated genome with transcriptomic information. While initial genomic efforts focused on the eudiploid *X. tropicalis*, sequencing of *X. laevis* has recently advanced to cover over 89% of the allotetraploid genome, with the initial annotation based primarily on predictive gene models. Our goal is to create a comprehensive profile of the transcriptomes in the developing *X. laevis* brain, an organ that in other species is known to have the highest number of expressed transcripts and isoforms. We are updating the annotation of the *X. laevis* genome (build 7.1) based on three sources. First, *de novo* assembly was carried out on 233 Gbp of RNA-Seq data using Trinity. Specifically, we isolated whole brain from stages 46 and 49 and FACS enriched for neural progenitor cells and differentiated neurons from tadpoles between stages 46 and 49. Second, we incorporated over 987,000 full-length cDNA and ESTs from public databases. Third, genome-guided assembly (Trinity & GSNAP) further improved the updated annotation, which incorporates over 500 Mbp of assembled sequence, out of a predicted 3.1 Gbp genome. Using the updated annotation, we are quantifying differential expression between neural cell types and brain regions in development. We anticipate that an updated annotation of the *X. laevis* genome will increase data on alternate splice variants, UTR sequences, and noncoding RNAs and will provide an essential resource for the *Xenopus* community and for compartive genome analysis.

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Making the most of the European Xenopus Resource Centre. Matt Guille[1], Anna Noble[1], Maya Piccinni[1], Alan Jafkins[1], Gretel Nicholson[1], Colin Sharpe[1], Viki Allan[2], European Xenopus Resource Centre. 1) EXRC, University of Portsmouth, Portsmouth, Hampshire, United Kingdom; 2) Faculty of Life Sciences,Michael Smith Building, Oxford Road, Manchester, M13 9PT.

The EXRC collects, makes, quality assures and distributes resources for the research community at cost. It holds almost 200 lines of transgenic frogs and more than 100 lines of mutants together with inbred J-line X. laevis, albino and wild type animals. Oocytes and egg extracts are also available from the centre as are fosmid and BAC libraries, antibodies and well characterised in situ probes and expression plasmids. The transgenic lines in the centtre are now fully characterised and current focus is on improving gene editing protocols in Xenopus, developing nanobodies against Xenopus proteins and providing accredited training on Xenopus husbandry and experimentation. Visit the poster to meet members of the EXRC team and talk about ways in which we may be able to help you deliver your research - we can make lines, provide resources to order and you can come and share our facilities and expertise.

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Mechanisms of context-specific Wnt/β-catenin target gene regulation in the ventral mesoderm of *Xenopus tropicalis*. Yukio Nakamura, **Stefan Hoppler**. Institute of Medical Sciences, University of Aberdeen, ABERDEEN, Scotland, United Kingdom.

Wnt/β-catenin signalling (Wnt signalling) is repeatedly deployed during embryonic development, and yet the developmental response to Wnt signalling is tissue- and stage-specific. Xenopus embryos provide a unique model system to study a dramatic

change of context-specific Wnt target gene expression: while maternal Wnt signalling establishes the dorsal axis by directly regulating dorsal specific genes such as*siamois* and *nodal3*, zygotically activated wnt8 signalling promotes ventral mesoderm development by regulating different downstream targets, which are not fully understood.In order to explore the mechanisms by which the same Wnt signalling pathway activates transcription of distinct target genes and elicits specific responses, we have performed RNA-seq analysis to identify targets of zygotic Wnt8 signalling regulation and combined it with β-catenin ChIP-seq to identify the direct target genes among them. We compared transcriptomes of early gastrula control embryos with embryos where *wnt8* was knocked down by a MO and with embryos where its activity was rescued again with a *wnt8*-expressing DNA construct, and thereby identified at least 23 Wnt8-regulated genes. ChIP-seq analysis revealed β-catenin-bound regions in the proximity to 22 of those identified gene loci, confirming them as direct Wnt targets. Cooperated regulation by Wnt and BMP signalling has previously been suggested for ventral mesoderm-specific Wnt target genes. We found that BMP signalling is indeed required for some but not all of the identified Wn8 target genes to respond to Wnt signalling. However, remarkably, BMP signalling did not regulate β-catenin-binding to those Wnt8 target gene loci we analysed. Instead, significant BMP-dependent H3K4me3 chromatin modification was observed in promoters of these Wnt8 targets. These results suggest that Wnt8 signalling requires BMP signalling not for integration of its signal to these target genes but for changes in chromatin states that allow target gene transcription.This project is funded by BBSRC.

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Standardized SAGE-seq analysis for empowered transcriptome profiling in *X. tropicalis*. Zhihua Jiang[1], Rui Li[1], Ming Zhang[1], Zhongzhen Liu[2], Hui Zhao[2], Yin Xia[2], Jennifer Michal[1], Xiang Zhou[1], Bo Ding[1], Daniel Rokhsar[3], Richard Harland[3]. 1) Dept Animal Sci, Washington State Univ, Pullman, WA; 2) School of Biomedical Sciences, Faulty of Medicine, The Chinese University of Hong Kong, Hong Kong; 3) Department of Molecular & Cell Biology, University of California Berkeley, Berkeley, CA 94720-3200, USA.

The mid-blastula transition (MBT) is the period when there is a prominent switch from synchronous to asynchronous divisions of cells and is one of the most critical events associated with early development of embryos. In the present study, we pooled 50 *X. tropicalis* embryos each from two pairs of parents at stages 6 (before MBT), 8 (during MBT) and 11 (gastrula) for SAGE (serial analysis of gene expression)-seq analysis with 22.9 – 36.4 million clean tags produced per sample. Using 2 mitochondrial genome sequences, 47,063 mRNA sequences, 170,807 in-house assembled RNA-seq sequences and 1,271,480 EST sequences of *X. tropicalis* as references, we collected a total 1,863,195 unique tags of 21 bp each in length, which are referred to as "dry tags." For the SAGE-seq data, we excluded tags that had <10 TPM (tags per million) in all of these six samples. As such, only 22,848 tags were classified as "wet tags" that were subsequently analyzed. The information on genes or transcripts was transferred from the dry tags to the wet tags. Initial annotation tentatively revealed that a total of 9,566 genes/transcripts were expressed in *X. tropicalis* embryos, with 9,342, 9,398 and 9,565 expressed at stages 6, 8 and 11, respectively. DE-seq analysis showed transcriptome profiles were not different between stages 6 and 8 (FDR>0.05). However, 254 genes/transcripts were significantly down-regulated in embryos at stage 11, while 480 genes were up-regulated as compared to stage 6 (FDR<0.01). Of the up-regulated genes/transcripts, 116 were exclusively expressed at stage 11. Advantages of the standardized SAGE-seq analysis include 1) easy identification of noisy tags, 2) potential identification of alternative polyadenylation sites and usage frequencies, 3) retrieval of polymorphic alleles, and 4) fewer lanes required for next generation sequencing platforms. The samples were also sequenced using RNA-seq and data will be further investigated using different approaches.

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Systematic classification of protein coding genes and orthologous gene (and name) assignment in *Xenopus laevis*. Taejoon Kwon, John Wallingford, Edward Marcotte. Department of Molecular Biosciences, University of Texas at Austin, Austin, TX.

One of the biggest challenges in the *Xenopus laevis* genome annotation is to discriminate the duplicated genes (homoeologs) arising from its allotetraploid genome, as well as to define their true orthologs in other species, including humans and Xenopus tropicalis. For example, a conventional reciprocal best hit approach to define orthologous genes between two species (e.g. InParanoid) is less effective if one species exhibits systematic gene duplications. In order to comprehensively annotate the *X. laevis* gene set, we used > 25 million putative transcript sequences derived by de novo assembly of RNA sequencing (RNA-Seq) data, in combination with already known full length cDNAs, expressed sequence tags (ESTs), and putative coding sequences derived from draft genome sequences and reference proteomes of other species. The challenge was to reduce the redundancy of the gene set and define orthologs in other species, while at the same time retaining all duplicated genes, which are important for understanding*Xenopus* genome evolution. Furthermore, many putative transcripts from RNA-Seq and EST collections represent non-coding RNAs, pervasive transcripts, or pre-processed mRNAs that cannot be translated directly to protein sequences, complicating the compilation of a reference proteome for *X. laevis*. We will present the methods we developed to address these problems as part of the *X. laevis*genome project, and also describe the current state of the *X. laevis* gene annotation and orthology definitions to the reference proteomes of mammals (human and mouse), bird (chicken), and fish (zebrafish).

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A genome-wide approach reveals the contribution of Nodal/Foxh1 signaling to vertebrate mesendoderm development. Rebekah Le, William Chiu, Ira Blitz, Ken Cho. Department of Developmental and Cell Biology, UCI, Irvine, CA, USA.

Emergence of the primary germ layers is among the earliest events of cell specification in animal development. Understanding the mechanisms of germ layer formation and subsequent patterning has implications for the treatment of human disease, particularly to the field of regenerative medicine. Nodal signaling, a member of the TGF-β superfamily, is evolutionarily ancient and specifies the formation of the mesoderm and endoderm germ layers. The maternal Foxh1 transcription factor and receptor-activated Smad2/4 complex are key mediators of Nodal signaling. As other known Smad2/4 co-factors are zygotically expressed, we sought to investigate Foxh1's potential as a mediator of early cell signaling. To gain a comprehensive understanding of the transcriptional contribution of Foxh1 to the Nodal signaling gene regulatory network, we employed chromatin immunoprecipitation coupled with high-throughput sequencing (ChIP-seq) to profile endogenous Foxh1 binding during *Xenopus tropicalis* mesendoderm development. First, we compared Foxh1 binding at three developmental stages - corresponding to the time-course of mesendoderm development. While hundreds of genes are bound at all three stages, many bound genes are stage-specific. These data suggest that Foxh1 functions dynamically and has both common and unique regulatory roles at each stage of mesendoderm development. Second, we found thousands of Foxh1-bound genes at mid-blastula, including components of major signaling pathways such as Nodal, BMP, FGF and Wnt. Third, we investigated whether Foxh1 can bind DNA in the absence of Nodal signaling. *Xenopus* embryos were treated with SB-431542, a small molecule inhibitor of Smad2 phosphorylation, and subjected to ChIP-qPCR. Foxh1 binding to regulatory regions of target genes was unaffected by the treatment, supporting the notion of Nodal-independent functions for Foxh1. Thus, our results uncovered dynamic function of Foxh1 binding throughout mesendoderm development, and point to Nodal-dependent and -independent functions of Foxh1. Our future goal aims to incorporate the epigenetic status of chromatin and changes in Foxh1 binding to understanding the regulatory programs utilized in germ layer formation.

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Comparative sex sequencing and assembly of *Pyxicephalus adspersus*. Jacob Malcom, Randy Kudra, **John Malone**. Institute of Systems Genomics, Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut.

How do genomes respond to changes in gene dose? Reducing or increasing DNA dose by altering copy number can lead to changes in gene expression levels that may percolate across gene expression networks to alter phenotypes. Interestingly, dosage compensation that offsets copy number change is often observed so that the effects of copy number changes are dampened or removed. Previous work has shown that dosage compensation, or the lack thereof, is a gene-specific response, largely mediated by interactions with the rest of the transcriptome. Frog sex chromosomes offer an ideal system for advancing our understanding of genome evolution and function because of the variety of sex determination systems in the group, the diversity of sex chromosome maturation states, and the ease of experimental manipulation during early development. Sequencing of the African Bullfrog (*Pyxicephalus adspersus)* provides the identification of sex-linked sequences for comparative and functional analysis of dosage compensation and other mechanisms of genome dose tolerance. Investigating how the frog genome responds to changes in gene dose will provide important insights into the evolution of sex chromosomes and dosage compensation systems.

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Elucidating Transcriptional Regulatory Networks in the Developing Kidney. Nasima Mayer[1,3], Leila Taher[2], Gabriela Loots[1,3]. 1) School of Natural Sciences, University of California, Merced, Merced, CA, USA; 2) Institute for Biostatistics and Informatics in Medicine and Ageing Research, University of Rostock, Rostock, Germany; 3) Biology and Biotechnology Division, Lawrence Livermore National Laboratory, Livermore, CA, USA.

Elucidating the architecture of transcriptional regulatory sequences and determining how *cis*-regulatory elements control tissue specific gene expression continues to be a significant challenge in functional genomics. To obtain new insights into the transcriptional regulatory networks that control early patterning of the vertebrate kidney we mapped ChIP-Seq hits in the vicinity of transcripts known to be highly expressed in the murine metanephric mesenchyme (E11.5), cap mesenchyme (E15.5) and renal vesicle (E12.5). Of the 205651 human/mouse evolutionary conserved elements (ECRs) present in the loci of kidney-enriched transcripts, 9959 ECRs harbored at least one ChIP-Seq hit for CTCF, Hnf4A, P300 and histone markers H3K27ac/H3K4me3. 57% of ECRs were found in intronic and intragenic regions while 31% mapped within 1Kb of transcriptional start sites, while the rest overlapped with exons and UTRs. To determine whether different ChIP-Seq signatures correlate with particular genomic features, we conducted pair-wise correlations between all the ChIP-Seq datasets, and found that 30% of promoter-positioned CTCF sites co-localized with Hnf4A and H3K4me3 ChIP-Seq hits, suggesting CTCF/Hnf4A/H3K4me3 cohorts may correlate with kidney-specific promoter elements. Furthermore we found that 5% of Hnf4A noncoding ChIP-Seq hits mapped near H3K27ac sites, suggesting that these ECRs may function as kidney-specific transcriptional regulatory elements. Currently, we are validating these ECRs to determine if they drive kidney-specific transcription in the developing Xenopus pronephros.Disclosures: *NoneThis study received funding from NIH DK075730 and LDRD 11-ERD-060. This work was conducted under the auspices of the USDOE by LLNL (DE-AC52-07NA27344).*

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Differentially Expressed Maternal Factors Along the Animal-Vegetal Axis in *Xenopus tropicalis*. Kitt Paraiso, Ira Blitz, Ken Cho. Developmental and Cell Biology, University of California, Irvine, Irvine, CA.

In Xenopus, differentially deposited maternal factors are important in specifying the three germ layers and initiating the complex gene regulatory networks leading to differentiation. Among which, VegT and Foxi2 are transcriptional factors deposited in the vegetal and animal halves, respectively, and are required for the activation of endodermal and ectodermal genes. The vegetally-expressed Vg1 is a signaling molecule that activates zygotic Nodal signaling important for mesodermal and endodermal fates. The animally-expressed Trim33 (ectodermin) has been shown to inhibit zygotic Nodal signaling in ectodermal precursors. Recently, whole genome sequencing of whole *Xenopus tropicalis* embryos showed a breadth of potential maternal regulators of germ layer differentiation. Here, we quantify the transcriptome of the animal and vegetal halves of an 8-cell stage *Xenopus tropicalis* embryo using RNA-seq. We analyze the sequences using the Trinity suite and identify differentially-expressed genes. The data confirms that the vegetal genes, VegT and Vg1, are differentially expressed. However, the animal genes, Foxi2 and Trim33 are not. The transcriptomes reveal more transcription factors are expressed specifically in either of the two halves suggesting involvement of a whole list of potential regulators in the differentiation process. These results will aid in the identification of maternal factors that activate the specific germ layer gene regulatory networks.

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Targeted mutagenesis of multiple and paralogous genes in *Xenopus laevis* using two pairs of TALENs. Yuto Sakane[1], Tetsushi Sakuma[1], Keiko Kashiwagi[2], Akihiko Kashiwagi[2], Takashi Yamamoto[1], Ken-ichi Suzuki[1]. 1) Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, Higashi-Hiroshima, Japan; 2) Laboratory of Amphibian Biology, Hiroshima University, Higashi-Hiroshima, Japan.

Xenopus laevis has long been used extensively as a model animal, because researchers can easily perform knock-down experiments for genes of interest by injecting antisense oligonucleotides. To precisely analyze gene function, however, gene knock-out techniques are required. Recently, genome editing using site-specific nucleases has been introduced in various organisms. In *X. laevis*, paralogous genes duplicated by polyploidization may exhibit functional redundancy, therefore, simultaneous disruption of both genes should be done using site-specific nucleases. To address this issue, we report here multiple and paralogous gene disruption by injecting two pairs of transcription activator-like effector nucleases (TALENs) mRNA. When TALENs mRNA targeting egfp and *tyrosinase* paralogs were co-injected into fertilized eggs obtained from egfp-reporter transgenic line, most tadpoles showed albino phenotypes reducing GFP fluorescence. The results of genomic PCR and DNA sequencing reveal that somatic mutations of each gene were already introduced at the morula stage. In addition, we also show that two different pairs of TALENs can simultaneously introduce somatic mutations to both paralogs, encoding histone chaperone with high efficiency. These results suggest that targeted mutagenesis using two pair TALENs enables us to analyze functions of genes in *X. laevis*, most of which are duplicated and may have functional redundancy.

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Frog wrangling in Woods Hole at the NXR. Cristy Salanga[1], Esther Pearl[1], Robert Grainger[1,2], Marko Horb[1]. 1) Marine Biological Laboratory, Woods Hole, MA; 2) University of Virginia, Charlottesville, VA.

The National *Xenopus* Resource (NXR) is a recently established stock center located at the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts. The goals of the NXR are to produce, acquire, maintain, and distribute mutant, inbred, and transgenic *X. laevis* and *X. tropicalis* lines and to provide research training as well as promote intellectual interchange through hosting mini-courses and workshops. Current workshops include Bioinformatics and Advanced Imaging, with future courses aimed to teach other diverse skills including TALEN and CRISPR/Cas genome editing and transgenesis techniques, among others. The NXR also offers a research hotel service, where visiting researchers can come to the MBL and perform short-term projects using NXR resources. Last, the NXR offers custom genome editing and transgenesis resource services; the NXR will clone, inject, and breed TALEN, CRISPR, or transgenic animals for individual researchers. The NXR is working to foster cooperation and collaboration within the *Xenopus* community and improve productivity by providing resources and expertise to all levels, from graduate students to experienced PIs. Some of the important facets and future plans of the NXR are presented in this poster.

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***Xenopus* Genome Resources at NCBI. David Webb**, K. Pruitt, T. Murphy. National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Department of Health and Human Services, Bethesda, MD. 20814.

The National Center for Biotechnology Information (NCBI) provides several extensively cross-linked resources, tools, and submission approaches that support *Xenopus* genome research at multiple levels. This resource network facilitates navigation, data-mining, and discovery and includes comparative displays of map data in a variety of coordinates (Sequence Viewer), a gene-oriented database (Gene), reference sequences (RefSeq), putative orthologs (Homologene), and more. NCBI also provides analysis and display tools to support interrogating the annotated genome data including alignment analysis (species-specific genome BLAST; precomputed protein alignments in Blink; splice-site-inferred alignments in Splign) and a robust client (Genome Workbench) that has integrated display and analysis functionality.In addition to facilitating data access through linked resources, NCBI provides several mechanisms to submit and re-distribute annotation information. The submission of transcript sequences to GenBank automatically makes the data available for inclusion in Gene, RefSeq, and genome annotation pipelines at

NCBI and elsewhere. Alternatively, re-assembly and/or annotation projects with an associated publication can be submitted to the Third Party Annotation (TPA) database, which accepts annotation of sequences already in GenBank. The *Xenopus* research community can also make contributions to an annotated bibliography (available in Gene records) by submitting GeneRIFs (Gene Reference into Function); GeneRIF submissions enable scientists to add brief functional comments, associated with a PubMed ID, to a GeneID. In addition, the NCBI LinkOut service allows external resources to be linked to any NCBI data object (a publication, sequence, gene, etc.).

Human Disease Modeling

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CRISPR/ Cas9 is a cheap and efficient strategy to screen genes implicated in congenital heart malformations. Dipankan Bhattacharya, Mustafa Khokha. Yale School of Medicine, New Haven, CT.

Congenital heart disease is a major cause of infant mortality. With the transformation of human genomics, numerous genes have been implicated in causing congenital heart malformations. However, assigning disease causality to these genes remains a challenge. Also, many of these genes have either no known function or their identified functions do not suggest mechanisms that might result in congenital heart disease. To screen these candidate genes for function, we are testing the CRISPR/Cas9 system in *Xenopus tropicalis*.Traditionally, knockdown in *Xenopus* is achieved using translation or splice-site blocking morpholino oligos. However, there are numerous congenital heart disease candidate genes and the cost of MOs becomes prohibitive. We demonstrate that the CRISPR/ Cas9 system can efficiently knockdown genes in the F0 generation to screen congenital heart disease genes at minimal cost. We use a PCR based sgRNA construction strategy and use fragment analysis to assay the effectiveness of the CRISPR/ Cas9 system in generating site-specific deletions. We conclude that CRISPR/Cas9 can be a powerful method for disease modeling of human congenital heart disease genes.

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A *Xenopus* model of alkaptonuria. Stefan M. Schmitt[1], Katrin König[2], Mazhar Gull[1], Michael Vogeser[2], **André W. Brändli[1]**. 1) Walter Brendel Centre of Experimental Medicine, Ludwig-Maximilians-University Munich, Munich, Germany; 2) Institute of Laboratory Medicine, University Hospital Munich, Munich, Germany.

Alkaptonuria (AKU) is a rare metabolic disease caused by mutations in the gene encoding homogentisate 1,2-dioxigenase (HGD), an enzyme participating in the catabolism of tyrosine. Patients with HGD deficiencies accumulate homogentisic acid (HGA) in the blood circulation and excrete large amounts in the urine, causing darkening of the urine upon exposure to air. Progressive accumulation of HGA polymers damages cartilages, heart valves, and kidneys. Presently, no approved treatment modality is available for AKU patients. The full scope of AKU cannot be modeled in cell or organ cultures. A genetic mouse model of AKU was recently established and has been instrumental in the study of the histopathology of AKU and validation of nitisinone as a promising drug candidate for the long-term treatment of AKU. Cost considerations and technical limitations prohibit however the use of the mouse AKU model for large-scale drug screening purposes. Here we demonstrate that two *hgd* genes are present in the*Xenopus laevis* genome, and *Xenopus* embryos express *hgd* transcripts in the developing liver and kidneys. Furthermore, we have evidence that the other enzymes of the tyrosine degradation pathway are expressed in a similar manner in *Xenopus* embryos. Using antisense morpholino knockdown methodology, we have developed a first *Xenopus* model of AKU for drug discovery and drug testing purposes. In addition, TALE nuclease (TALENs) and CRISPR/Cas9 technologies are being evaluated to disrupt *hgd* gene functions *in vivo*. Using mass spectroscopy, we were able to detect HGA excretion by *hgd*knockdown *Xenopus* embryos. Furthermore, HGA excretion is efficiently suppressed by nitisinone supplementation. In summary, the *Xenopus* AKU model adequately recapitulates the early pathophysiology of human AKU patients. Future applications of the *Xenopus* AKU model are in the preclinical testing of promising AKU drug candidates, such as improved nitisinone derivatives, and in high-throughput drug discovery screening to identify novel small organic molecules that suppress the AKU phenotype *in vivo*.

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Analysis of Fetal Alcohol Spectrum Disorder in *Xenopus* embryos identifies novel functions for retinoic acid. A. Fainsod, M. Gur, H. Kot Leibovich, G. Pillemer, Y. Shabtai. Dev. Biol. and Cancer Res., Faculty of Medicine, Hebrew Univ, Jerusalem, Israel.

Exposure of human embryos during pregnancy to alcohol (ethanol, EtOH) results in a high incidence of Fetal Alcohol Spectrum Disorder (FASD). Children with FASD exhibit facial dysmorphology, microcephaly, short stature, central nervous system, neurodevelopmental, behavioral and psychological abnormalities. We established a *Xenopus* embryo-based model to study the etiology of the EtOH effects. We determined that EtOH competes and reduces the biosynthesis of retinoic acid (RA) resulting in low, teratogenic levels. The strongest competition takes place at the onset of gastrulation and centers on the retinaldehyde dehydrogenase 2 (Raldh2) activity in Spemann's organizer. We determined that retinaldehyde and acetaldehyde compete using *in vivo* and biochemical assays. Many FASD phenotypes affect brain functionality and architecture, including microcephaly. RA treatment is widely accepted as a negative regulator of anterior brain regions. Reduced RA from EtOH

exposure and the resulting microcephaly, identify an RA requirement during early head development. We identified a novel RA activity in the leading-edge mesendoderm crucial for the normal induction of the rostral head regions. We can show that this activity is RALDH2 independent.Genetic variation is known to influence FASD severity. Analysis of the human *Raldh2* gene in the population revealed multiple single amino acid changes whose effect is unknown. We used *Xenopus* embryos to determine the genetic/enzymatic predisposition to FASD by analyzing the different RALDH2 isoforms in the presence of EtOH *in vivo*. We compared different human *Raldh2* alleles to identify those with altered enzymatic activity affecting the risk to develop FASD. The results of these studies will increase our knowledge on the effects of ethanol on embryonic development, will help establish the experimental conditions to screen for genetic predisposition to FASD and understand the developmental malformations.

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Loss of the dystrophin-associated protein DTNA causes paralysis in embryos. Matthew Guille, Sarah Thresh, Darek Gorecki, Colin Sharpe. EXRC, University of Portsmouth, Portsmouth, Hampshire, United Kingdom.

Mutations in proteins of the dystrophin associated complex cause disease in humans. The locus encoding one of these proteins, DTNA, has been sequenced in humans with muscle diseases but no mutations in the protein encoding region have been found. Rare individuals with mutations that reduce the level of DTNA expression however have reduced muscle function. These data suggest the hypothesis that mutations that affect DTNA function are lethal in humans. DTNA knockout mice show only mild symptoms, but it has been discovered that the murid DTNA locus has insertions and re-arrangements when compred with the human one and so fewer than half of the transcripts from this complex locus are made; mice are therefore not good models for studying the function of this gene.We therefore studied the DTNA locus in Xenopus, like the human locus it has two promoters and produces a large number of alternatively spliced transcripts. We have analysed the expression of the mRNA and protein in embryos and also knocked down the DTNA protein. LOF causes complete paralysis which can be rescued by replacing DTNA.

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Dissecting the biological function of Huntingtin during early *Xenopus* embryogenesis. TOMOMI HAREMAKI, Melissa Popowski, Ali Brivanlou. Laboratory of Stem Cell Biology and Molecular Embryology, The Rockefeller University, 1230 York Ave, New York, NY 10065.

Huntington's disease is an inherited neurodegenerative disorder caused by an abnormal expansion of the poly-glutamine repeats in the huntingtin (HTT) protein. Although the *HTT* gene is ubiquitously expressed, its normal biological function is still unclear. Moreover, the mechanism by which the mutant HTT is toxic is also not totally understood. To identify the normal function of Htt, we conducted Htt knockdown experiments by injecting a translation-blocking morpholino oligonucleotide (MO) into *Xenopus* eggs. The HttMO injected embryos showed a dose-dependent delay of blastopore closure, neural tube closure and axis elongation. The Htt morphants also showed defective ciliary flow and whole body paralysis. These phenotypes were partially rescued by co-injection of either wild-type or mutant-type human *HTT* RNA, indicating that mutant HTT protein still maintains its biological function. Because cells need to be polarized for convergent extension movements and ciliated epithelial cells need to be polarized to generate directed ciliary flow, these results suggest that Htt has important roles in cell polarization during early *Xenopus* development.

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Candidate co-factors for vertebrate Six family transcription factors are required for otic development. Karen Neilson[1], Kristy Kenyon[2], Josie Stout[2], Dominique Alfandari[3], **Sally Moody[1]**. 1) Anatomy and Regenerative Biology, George Washington University, Washington, DC; 2) Hobart and William Smith Colleges, Geneva, NY; 3) Veterinary and Animal Sciences, University of Massachusetts Amherst, Amherst MA.

Several members of the Six family of transcription factors play important roles in vertebrate craniofacial development. It is well documented that Six protein transcriptional activity can be modified by co-factor proteins. Two *Six* genes (*Six1*, *Six5*) and one co-factor gene (*Eya1*) are involved in the human craniofacial syndromes BOS (Branchio-Otic Syndrome) and BOR (Branchio-Otic-Renal Syndrome). Both syndromes are characterized by mild craniofacial defects and hearing loss.However, mutations in *Six* and *Eya* genes only account for about half of the BOS/BOR patients. To discover potential new causative genes, we identified over 30 *Xenopus* genes with high sequence identity to proteins previously shown to interact with the *Drosophila* Six homologue, *SO*, and we determined that many of these are expressed at some stage of craniofacial development in patterns that overlap with Six1 (Neilson et al., 2010, Dev Dyn 239: 3446). We now show that 4 of the novel candidate co-factors interact with vertebrate Six1 protein. When their endogenous expression is reduced in the embryo, there is a loss of otic placode genes and an expansion of neural plate genes. When their endogenous expression in increased in the embryo, *Six1* expression is reduced. These results suggest that these *Xenopus* proteins, which have homologues in humans, are previously uncharacterized Six-interacting partners with potential important roles in vertebrate craniofacial development and congenital syndromes.Funding: NIH R03 HD 055321; NIH R01 DE022065; NIH R01 DE016289.

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TALEN- and CRISPR/Cas9-mediated mutagenesis of *pax6* and *six3* genes for studying eye and brain development in *X. tropicalis*. Takuya Nakayama[1], Keisuke Nakajima[2], Marilyn Fisher[1], Akinleye Odeleye[1], Sumanth Manohar[1], Keith Zimmerman[1], Yoshio Yaoita[2], Robert Grainger[1]. 1) Department of Biology, University of Virginia, Charlottesville, VA, USA; 2) Institute for Amphibian Biology, Hiroshima University, Higashihiroshima, Hiroshima, Japan.

Both *pax6* and *six3* are transcription factors essential for forebrain and eye-field development. In humans, semi-dominant mutations in *PAX6* cause aniridia, a syndrome resulting in multiple eye, brain and pancreas deficits. Mutations in the homeodomain of *SIX3* are known to cause holoprosencephaly (HPE) with a number of serious brain deficits. While mouse mutants have been used as models of these human diseases, they miss essential information about early developmental roles of those genes simply because mouse embryos at early stages are difficult to study and manipulate. *Xenopus* mutants can bridge this gap so that embryonic gene regulatory interactions and patterning can be examined both to understand these early developmental events and to clarify the primary deficits leading to the human diseases noted above.Here we performed targeted mutagenesis of *pax6* by TALEN and *six3* by CRISPR/Cas9, creating loss-of-function mutants in both genes in *X. tropicalis*. Using dual TALENs we were able to successfully target exons 7 and 9 of *pax6* in order to inactivate all variants of *pax6*, resulting in small indel mutations in exon7 and/or 9, or a large 10-kb deletion or inversion between exon7 and 9. We have confirmed that some of those mutations show germline transmission. Compound heterozygous F1 animals showed a consistent phenotype, namely, the lens is lost, the retina is malformed, and several brain and pancreas markers clearly delineate specific brain and pancreas abnormalities. Similarly, when targeting two different coding regions in the *six3* gene, or when deleting its promoter or enhancer, we also observed expected eye and brain phenotypes consistent with HPE, suggesting that the phenotypes are due to on-target mutations and not off-target effects. Germline transmission of some of these*six3* mutations has been confirmed, and lines are being established for future study.

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Exploring the function of *pqbp1* to elucidate the developmental basis of Renpenning Syndrome. Jamina Oomen-Hajagos[1,2], Yasuno Iwasaki[1], Gerald Thomsen[1]. 1) Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook , NY; 2) Genetics Graduate Program, Stony Brook University, Stony Brook, NY.

The *pqbp1* (polyglutamine tract-binding protein 1) gene is located on the X chromosome in humans, and its protein product is involved regulating transcription and pre-mRNA splicing. Mutations in *pqbp1*have been identified as causing a variety of X-linked intellectual disabilities (ID), which comprise a group of disorders collectively referred to as Renpenning syndrome. The distinct physical anomalies that occur with these syndromes strongly suggest that early development is disrupted in these individuals. Even though the PQBP1 protein has been implicated in RNAPII-dependent transcription and pre-mRNA splicing, its role or gene targets in developing embryos have not been clearly defined.Here, we use *Xenopus* as a model system to investigate the developmental impacts of aberrant PQBP1 activity. We are exploring the role of *pqbp1* in both neural and mesodermal development. *Pqbp1* has a neural expression pattern that is consistent with the fact that in humans, *pqbp1* mutations cause mental retardation and microcephaly.*Pqbp1* knockdown by morpholino caused both mesodermal and neural defects in *Xenopus* embryos, with phenotypes including shortened AP body axes, small heads, and no tail structures. Further investigation showed that PQBP1 appears to be required for FGF signaling and regulates splicing of FGF receptor transcripts, with *pqbp1* knockdown reversing the splicing pattern observed in wild type embryos. This would be expected to affect the ligand specificity of these receptors and could thus be critical in influencing development. *Pqbp1* knockdown in the neuralized context of Chordin-injected animal caps also affected a subset of neural markers, and we postulate that it impacts the ability of neural precursors to properly differentiate. Our results yield insight into the potential mechanisms causing Renpenning syndrome in humans.

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Loss Of Katenin P80 in Mice, Fish, Humans and Frogs Causes Extreme Microcephaly. Oz POMP, Carine BONNARD, Mohammad SHBOUL, **Bruno REVERSADE**. Institute of Medical Biology, A*STAR, Singapore.

In multiple families presenting with recessive and extreme microcephaly, we have identified several loss-of-function mutations in the gene *KATNB1* which encodes Katanin P80. Together with its partner Katanin P60, these two enzymes are beleived to promote the re-organization of microtubules networks. Using patients-derived fibroblasts, iPSCs and Neural Stem cells we bring evidence that Katanin P80 controls the stability of Katanin P60 at the post-translational level and that their loss results in defective proliferation, spindle structure, excess centrioles and supernumerary cilia.Knockout of *KATNB1* in various model organisms confirmed that Katanin P80 has neural-specific requirements and that its absence compromises brain expansion during embryogensis as a result of fewer cortical progenitors.

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Spatially distinct homeolog expression of Nkx2-5 in *Xenopus laevis*. Daniel Weeks[1,2], Michael Hayes[2], Ryan Marling[1], Sue Travis[1]. 1) Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA; 2) Molecular and Cell Biology,Medical Scientist Training Program, Carver College of Medicine, University of Iowa, Iowa City, IA.

Xenopus laevis is an allotetrapolid, containing two homeologs (closely related yet distinct copies) of most genes. Nkx2-5 is an irreplaceable transcription factor that helps establish, modify and maintain the heart. Previous studies have shown that elevated levels of Nkx2-5 enlarges the heart while reduced levels decreases heart size and alters normal heart development. The relatively recent assembly of the*Xenopus laevis* genome sequence has allowed a better opportunity to examine expression of both Nkx2-5 homeologs. Our analysis indicates that, when examined on a whole organism basis, both copies are expressed. However, Nkx2-5b accounts for the majority of Nkx2-5 expression. This differential expression pattern is especially pronounced in non-cardiac tissues, and persists throughout all stages analysed thus far, including the adult. Differences in homeolog expression coupled with differences in the amino acid composition of each homeolog may provide insight into tissue specific regulation and function of Nkx2-5.

Neurobiology

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An *in vivo* screen to reveal genes controlling cell proliferation and differentiation in the developing visual system. Jennifer Bestman[1], Jane Lee-Osbourne[2], Lin-Chien Huang[3], Hollis Cline[3]. 1) Medical University of South Carolina, Charleston, SC; 2) University of Nebraska Medical Center, Omaha, NE; 3) The Scripps Research Institute, La Jolla, CA.

Cell proliferation in the CNS of *Xenopus laevis* continues throughout larval stages of development even as the tadpoles carry out a full range of behaviors. Our previous work using *in vivo* time-lapse imaging of sox2:eGFP-expressing neural progenitor cells (NPCs) in the optic tectum of stage 47-48 tadpoles revealed that brief visual deprivation increases cell proliferation, while short-term visual enhancement promotes the differentiation of stem cells into neurons. Therefore, we reared animals with visual stimulation or visual deprivation to bias the fate of the NPCs toward differentiation or division, respectively. Sox2:eGFP+ cells were collected from optic tecta, and using Affymetrix Xenopus 2.0 microarrays, we compared expression data from actively-dividing NPCs and differentiated neurons to identify candidate neurogenic regulatory genes. We tested whether the candidate neurogenic genes regulate neurogenesis in the optic tectum by co-electroporating antisense morpholinos to knockdown the expression of 34 different candidate genes along with the sox2:eGFP reporter that targets neural progenitor cells. The effects of the morpholinos on neurogenesis was quantified as changes in the number of sox2:eGFP-expressing NPCs and their neuronal progeny over 48 hours, as visualized by *in vivo* time-lapse confocal microscopy. Control animals show a ~20% increase in eGFP+ cells generated from sox2-expressing NPCs, and the proportion of labeled NPCs decreases as the labeled neuronal progeny increase. Of the 34 candidate genes targeted, 18 affected the number of eGFP+ cells detected over 48h: 7 morpholinos decreased and 11 increased the eGFP+ cell numbers compared to controls. Furthermore, 22 of the 34 morpholinos changed the proportion of neurons to NPCs detected after 2 days. Together these data identify candidate neurogenic regulatory genes and demonstrate that *Xenopus* is an effective experimental animal model to identify and characterize genes that regulate neural stem cell proliferation and differentiation *in vivo*.

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Generation of BAC transgenic larval *Xenopus* enabling live imaging of motoneurons. Marion Bougerol[1], Frédéric Auradé[2], Francois Lambert[3], Didier Le Ray[3], Denis Combes[3], Muriel Thoby-Brisson[3], Frédéric Relaix[2], Nicolas Pollet[4], Hervé Tostivint[1]. 1) UMR 7221 CNRS-MNHN, Paris, France; 2) UMRS 974 INSERM-UPMC FRE3617 CNRS, Paris, France; 3) UMR 5287 CNRS-Université de Bordeaux, Bordeaux, France; 4) Genopole CNRS-Université d'Evry-Val d'Essonne, Evry, France.

Due to its developmental morpho-physiological advantages, *Xenopus* represents an excellent tetrapod model to study normal and pathological motoneuron ontogeny. Consequently it is relevant to build*Xenopus* reporter lines in which motoneurons are specifically labeled. In mammals CNS, the urotensin II-related peptide (urp) gene is primarily expressed in motoneurons of the brainstem and the spinal cord. Concomitantly, we showed that this expression pattern was conserved in *Xenopus* and established in early development, from stage 24. At stage 50, about 45% of the motoneurons expressed the *urp* gene. The aim of the present study was to test the ability of the *urp* promoter to drive the expression of a fluorescent reporter in motoneurons by recombineering a green fluorescent protein (GFP) reporter gene into a BAC clone containing the entire *X. tropicalis urp* locus. After injection of this construction in one-cell stage oocytes, a transient GFP expression was observed in the spinal cord of up to 1/3 of the embryos from stage 41 and up to stage 63. The GFP expression pattern was globally consistent with that of the endogenous *urp* in the spinal cord but no fluorescence was observed in the brainstem yet. In spinal GFP^+cells, both somata and axons projecting to axial muscles were fluorescent. We used choline acetyltransferase (ChAT) immunohistochemistry, intramuscular dye injection that specifically labels motoneurons, and patch clamp techniques to further characterize GFP^+ cells in larvae. More than 98% of the GFP-expressing cells were $ChAT^+$. When we injected rhodamine dextran amine crystals in tail myotomes, we revealed the occurrence of numerous double-stained GFP^+ cells. In addition, intracellular electrophysiological recordings of GFP^+ neurons revealed various locomotion-related rhythmic discharge patterns during fictive swimming. Taken together our results provide evidences that *urp* is an appropriate promoter to express reporter genes in *Xenopus* motoneurons.

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Sprouty 3 negatively regulates BDNF-dependent axonal branching by modulation of calcium signaling pathway. Tomasz Gwozdz, Karel Dorey. Faculty of Life Sciences, The University of Manchester, Manchester, United Kingdom.

The formation, refinement, and maintenance of neural circuits require exquisite control of axonal growth, guidance and branching. Extracellular guiding cues and growth factors have been shown to affect branching. For example the Brain Derived Neurotrophin Factor (BDNF) induces axonal branching in the optic nerve and motoneurons, however the molecular mechanism regulating this process is not understood.We have identified Sprouty 3 (Spry3) as a new regulator of axonal branching downstream of BDNF. Loss-of-function experiments in Xenopus embryos revealed that Spry3 represses axonal branching in motor neurons. Biochemical analysis in cell culture revealed that Spry3 inhibits PLC-dependent calcium release from the intracellular stores by lowering inositol-1,4,5-triphosphate (IP3) levels. We therefore hypothesized that Spry3 can inhibit axonal branching by preventing calcium release from the intracellular stores. Using live imaging of Xenopus spinal cord neurons with genetically encoded calcium-sensor (GEM-GECO), we monitored changes in intracellular calcium concentration upon

modulation of Spry3 expression. So far our results have shown that BDNF treatment increases the frequency of calcium oscillations in spinal cord neurons. However, these oscillations require extracellular calcium, are action potential dependent and most probably are not induced by calcium release from the stores. Consequently, they are not affected by downregulation of Spry3. We therefore decided to assess the role of Spry3 in neurons by analysing its subcellular distribution using time-lapse imaging. Spry3 shuttles in vesicles along the axon shaft but seems to be excluded from the dynamic growth cone. Interestingly, we have observed that in some cases Spry3 appeared to accumulate at branching points resulting in the collapse of filopodia. Furthermore, downregulation of Spry3 expression results in an increase in the number and the stability of filopodia in neurons. Taken together, these data suggest that Spry3 may affect axonal branching by modulation of calcium levels locally at the branching points presumably controlling cytoskeleton dynamics.

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The olfactory system as a model to study axonal growth patterns and morphology *in vivo*. Thomas Hassenklöver[1,2], Ivan Manzini[1,2]. 1) Institute of Neurophysiology and Cellular Biophysics, University of Göttingen, Humboldtallee 23, 37073 Göttingen, Germany; 2) Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), 37073 Göttingen, Germany.

The olfactory system has the unusual capacity to generate new neurons throughout the lifetime of an organism. Olfactory stem cells in the basal portion of the olfactory epithelium continuously give rise to new sensory neurons that extend their axons into the olfactory bulb, where they face the challenge to integrate into existing circuitry. Because of this particular feature, the olfactory system represents a unique opportunity to monitor axonal wiring and guidance, and to investigate synapse formation. Here we describe a procedure for *in vivo* labeling of sensory neurons and subsequent visualization of axons in the olfactory system of larvae of the amphibian *Xenopus laevis*. To stain sensory neurons in the olfactory organ we adopt the electroporation technique. *In vivo* electroporation is an established technique for delivering fluorophore-coupled dextrans or other macromolecules into living cells. Stained sensory neurons and their axonal processes can then be monitored in the living animal either using confocal laser-scanning or multiphoton microscopy. By reducing the number of labeled cells to few or single cells per animal, single axons can be tracked into the olfactory bulb and their morphological changes can be monitored over weeks by conducting series of *in vivo* time lapse imaging experiments. While the described protocol exemplifies the labeling and monitoring of olfactory sensory neurons, it can also be adopted to other cell types within the olfactory and other systems.[Supported by DFG Schwerpunktprogramm 1392 (I.M.) and Cluster of Excellence and DFG Research Center Nanoscale Microscopy and Molecular Physiology of the Brain (I.M.).]

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Convergence of Nutrient and Injury Response Pathways in *Xenopus* CNS Repair. Caroline R. McKeown, Abigail C. Gambrill, Evan Fitchett, Hollis T. Cline. The Scripps Research Institute, La Jolla, CA.

We are studying recovery from brain injury in the visual system of *Xenopus laevis*. Focal ablation of part of optic tectum prevents a visual avoidance response, yet tadpoles recover the avoidance behavior one week after injury (McKeown, et al., 2013). Tectal injury causes a burst of cell proliferation proximal to the injury site, and these injury-responsive progenitors generate mature neurons. Recovery of the visual avoidance behavior is inhibited by cell proliferation-blocking drugs, and visual deprivation, which expands the tectal neural progenitor pool, facilitates behavioral recovery. These data indicate that the generation of new neurons and their integration into the visual circuit is critical for recovery from injury in the developing tadpole brain. Here we demonstrate that nutritional status plays a key role in neurogenesis and neuronal maturation in the developing visual system. In contrast to nutrient restriction (NR) which decreases proliferation, feeding significantly increases neurogenesis in the optic tectum via an mTOR-dependent mechanism. The NR-induced quiescent progenitors can be triggered to re-enter the cell cycle upon re-exposure to food. Moreover, increased proliferative responses to food and injury are additive, indicating that the developing CNS possesses greater proliferative capacity than triggered by either single neurogenic stimulus. We tested whether nutrition-induced neurogenesis could facilitate behavioral recovery from injury. Indeed, increased access to food after injury accelerated recovery. Furthermore, NR decreased the intrinsic excitability and impaired integration of newly generated neurons into the tectal circuit. Thus, nutrition plays a key role in neurogenic proliferation, differentiation, and circuit connectivity, and nutritional status is critical for CNS injury recovery. Additionally, the cellular responses to injury and nutrition appear to trigger distinct pathways that converge at the level of the cell division machinery. Investigations into the mechanisms underlying the activation of quiescent neural progenitor cells and the integration of newly generated neurons into the tectal circuit may facilitate treatment of brain injury.

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Endogenous gradients of resting potential instructively pattern *Xenopus* neural tissue via Notch signaling and regulation of proliferation. Vaibhav P Pai[1], Joan M Lemire[1], Jean-François Pare[1], Gufa Lin[2], Ying Chen[2], Michael Levin[1]. 1) Biology, Tufts University, Medford, MA; 2) Stem Cell Institute, University of Minnesota, Minneapolis, MN.

Biophysical forces play important roles in pattern formation of the embryonic brain and other organs. Previously we showed that endogenous bioelectric signals are crucial eye patterning signals, being necessary and sufficient for eye induction. Here, we identify a new role for endogenous bioelectricity as an instructive factor during brain patterning in *Xenopus laevis*. Early frog embryos exhibit a characteristic polarization of a key group of cells in the neural tube; disruption of this bioelectric gradient induces specific brain defects with changes in the expression of early brain markers, causing anatomical mispatterning of the

brain. This effect is mediated by voltage-gated calcium signaling and gap-junctional communication. Neural cell proliferation within the developing brain is regulated by both, the local V_{mem}states within the neural tube, and the V_{mem} states of distant regions. Misexpression of the constitutively-active form of Notch, a suppressor of neural induction, impairs the normal voltage polarization pattern and neural patterning; moreover, reinforcing proper polarization rescues brain defects induced by activated Notch signaling. Interestingly, ectopic polarization implemented by misexpression of one ion channel's mRNA induces ectopic neural tissue well outside the neural field. We also found that polarization states synergize with the actions of reprogramming factors that are known to promote an undifferentiated cell state to direct ectopic tissues toward neural fate *in vivo*. These data identify a new functional role for bioelectric signaling in brain patterning, characterize the interaction between V_{mem} and key biochemical pathways (Notch and Ca^{2+} signaling) during organogenesis of the vertebrate brain, and suggest voltage modulation as a tractable strategy for intervention in certain classes of neural birth defects.

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Characterisation of a transgenic Xenopus tropicalis line (Hb9-GFP) for the analysis of motor neuron regeneration. Diane Pelzer, Lucy McDermott, Karel Dorey. Faculty of Life Sciences, The University of Manchester, Manchester, United Kingdom.

In contrast to most vertebrates Xenopus tadpoles have the ability to regenerate their central nervous system. After tail amputation lost tissues including the spinal cord are repaired. The tail regains it ability to move suggesting that the neuronal circuits linking the muscles to the spinal cord have been re-established. In order to further understand spinal cord regeneration we want to analyse the behaviour of motor neurons during this process To this end, we aimed to generate a transgenic line expressing GFP specifically in motor neurons. We used the zebrafish promoter of the *HB9* gene, a homeobox transcription factor expressed specifically in differentiated motor neurons. Using both REMI (in Xenopus laevis) and I-SceI (in Xenopus tropicalis) confirmed the ability of this promoter to drive GFP expression in motor neurons. We are currently analysing F1 embryos, confirming germline transmission. To characterise this line further we use immunohistochemistry to analyse the spatial expression of GFP. Double staining using antibodies recognising GFP together with antibody labelling differentiated neurons, proliferating neuronal progenitors as well as antibodies against specific subtypes of spinal cord neurons will be used. This line will be a valuable tool to analyse the behaviour of motor neurons live during spinal cord regeneration

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Splicing of a specific intron is required for protein but not RNA expression of a neurofilament reporter gene in the developing *Xenopus* nervous system. Ben G Szaro, Chen Wang. Biological Sciences, State University of New York at Albany, Albany, NY.

Post-transcriptional control of axonal cytoskeletal-related genes is crucial for both developmental and successful regenerative axonal outgrowth. Many of the RNA binding proteins that direct the trafficking of such RNAs through the cell first associate with them within the nucleus, but it is unclear how and when, relative to transcription, these associations initially form. Although intron splicing in general is known to be important for promoting the nuclear export, stability, and translation of mRNAs, the splicing of specific introns has not been linked directly with the post-transcriptional control of individual genes. Using an assay of injecting reporter plasmids into *Xenopus* embryos to identify *cis*-regulatory elements of the middle neurofilament (*nefm*) RNA, we discovered that splicing of specifically the last intron of the *nefm*gene was required for robust reporter protein expression *in vivo*, but not that of the mRNA, regardless of promoter or cell type. Reporter plasmids bearing no introns or other introns, whether from the same or a different gene, produced poor protein expression, despite yielding essentially identical, fully spliced, mature mRNAs having the same 3'UTR. Thus, splicing of an intron in general was insufficient for robust protein expression, and the deficient protein expression seen with these other introns was attributable to neither defective transcription nor premature decay of the RNA. Further analysis revealed that the crucial elements for robust protein expression lay within the 3'-most region of the last intron of the *nefm* gene. This region, but not the comparable region of the other introns, contained a consensus sequence for binding hnRNP K, an RNA binding protein required for *nefm* mRNA translation through its association with the *nefm* 3'UTR in *Xenopus* neurons. Co-immunoprecipitation experiments indicated that splicing of the last *nefm* intron indeed promoted the association of hnRNP K with the *nefm* 3'UTR reporter RNA *in vivo*. These findings indicated that splicing of a specific intron may be used to initiate the association of the RNA with ribonucleoproteins that subsequently regulate trafficking of the mature mRNA through the cell. Supported by NSF IOS-1257449.

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Thyroid hormone acts locally to increases rates of neurogenesis and dendritic arborization in the tadpole visual system. Christopher Thompson, Hollis Cline. Dept of Molecular and Cellular Neuroscience, The Scripps Research Institute, La Jolla, CA., CA.

Thyroid hormone is a critical regulator of brain development. For instance, low levels of maternal thyroid hormone during gestation leads to cretinism in humans. The specific effects thyroid hormone as it acts directly on brain areas is not well understood, however. To address this issue we have developed a method for direct application of thyroid hormone to distinct parts of the *Xenopus laevis* tadpole visual system, which is particularly sensitive to changes in thyroid hormone signaling. Crystalline triiodothyronine (T_3) was diluted into melted coconut oil, which was then injected via micropipette and picospritzer into either the 3rd ventricle near the optic tectum or into the eye. These animals were treated with CldU, a thymdine analogue, in order to label newly-divided cells and then sacrificed 2 days later. We found that T_3placed in the brain increased the rate of proliferation nearly tenfold relative to animals that received T_3 in the eye. In contrast, T_3 in the eye increased the rate of

proliferation in the ciliary region by fivefold, whereas T_3 in the brain had no effect on retinal proliferation. In another experiment, we electroporated the tectum with morpholinos against type 3 iodothyronine deiodinase (DIO3), which converts T_3 to reverse T_3, an inactive form of thyroid hormone. We found that DIO3-knockdown increased the rate of proliferation in the tectum. Systemic treatment with methimizole, which blocks thyroid synthesis in the thyroid gland, reversed the effects of DIO3 morpholino. Last, we sought to examine the effects of local T_3 on arborization of tectal neurons. We injected T_3 coconut oil into the tecta of tadpoles that had been electorporated with GFP-overexpressing plasmids 7 days prior and imaged these neurons with a 2-photon microscope each day for 4 days. We found that T_3 treatment significantly increased dendrite arbor length and branch tip number by 66% and 73%, respectively. These experiments demonstrate that thyroid hormone can act directly on brain circuits and have a substantial impact on brain development. Funding sources: EY011261 and K99ES022992

Proteomics

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Unraveling a protein's interactome *in vivo*. Nathalie Escande-Beillard, Abigail Loh, Artina Metoska, Bruno Reversade. Institute of Medical Biology, A*STAR,Singapore.

Loss of *PYCR1* in humans causes De Barsy syndrome, an autosomal recessive progeroid disease which can be modelled in *Xenopus*. The molecular functions of PYCR1, a mitochondrial enzyme central for proline synthesis, are not fully elucidated. Our goal was to shed light on differential pathways utilized by PYCR1 by deciphering its interactome.We chose to use a new assay called BioID that allows to tag proteins, coming in close proximity to PYCR1, in its native cellular environment. This approach is based on the fusion of PYCR1 to the protein biotin ligase BirA. Upon expression of PYCR1-BirA in the presence of biotin, proximity-dependent proteins are promiscuously biotinylated. Potential direct, or indirect interacting, partners are isolated by affinity capture using streptavidin columns and subsequently identified by mass spectrometry.To date, the BioID has only been employed in cultured cells. Here we show that we have successfully established all the conditions to adapt BioID *in vivo*, using *Xenopus*embryos. This innovative approach has contributed to the identification of PYCR1 partner proteins such as components of the respiratory chains. We anticipate that this approach will become a powerful tool to the *Xenopus* community for mapping out interactomes during embryogenesis.

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Deep Proteomics of the *Xenopus Laevis* Egg using an mRNA-derived Reference Database. Marc Presler[1], Martin Wühr[1,3], Robert M. Freeman Jr[1], Marko E. Horb[2], Leonid Peshkin[1], Steven Gygi[3], Marc W. Kirschner[1]. 1) Department of Systems Biology, Harvard Medical School, Boston, MA; 2) Marine Biological Laboratory, Woods Hole, MA; 3) Department of Cell Biology, Harvard Medical School, Boston, MA.

Mass spectrometry-based proteomics offers an opportunity to globally identify and quantify proteins and their posttranslational modifications in complex biological samples. However, proteomics requires a complete and accurate reference set of proteins, and is therefore largely restricted to model organisms with sequenced genomes. For example, difficulties in the sequencing the quasi-tetraploid genome of *X. laevis* has rendered proteomics in the frog challenging, despite its advantages in the relative ease of gathering sufficient, synchronized material for proteomic study in the context of embryonic time series or egg extracts.To facilitate proteomics in *X. laevis* and other nonmodel organisms, we developed methods to convert heterogeneous mRNA data into a high quality protein reference set. We use protein similarity to other species to inform protein translation of mRNAs in the correct reading frame and dismiss irrelevent sequences. We are able to generate a reference set with fewer sequences and significantly higher overall quality than when using 6-frame translation, as has been previously attempted.We demonstrate the feasibility of deep genome-free proteomics and identify more than 11,000 proteins with 99% confidencefrom the unfertilized *Xenopus laevis* egg and estimate protein abundance with ~2-fold precision. Our reference database outperforms the provisional gene models based on genomic DNA sequencing and other methods. In the egg, we surprisingly detect many proteins for which we could not find any mRNA support from deep RNA-seq. Many of these proteins are found in the blood, suggesting that they are taken up during oocyte growth in the ovary. A strategy of direct maternal protein contribution to the embryos was previously only known for the yolk protein vitellogenin. Our platform to convert heterogeneous mRNA data into a protein reference set is available online.

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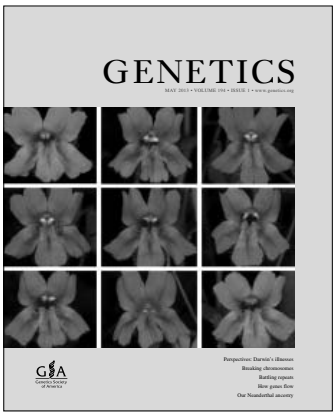

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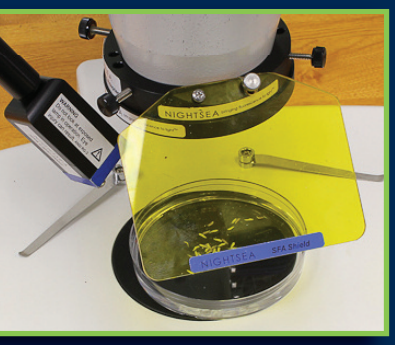
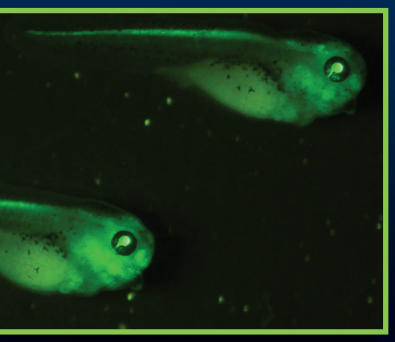
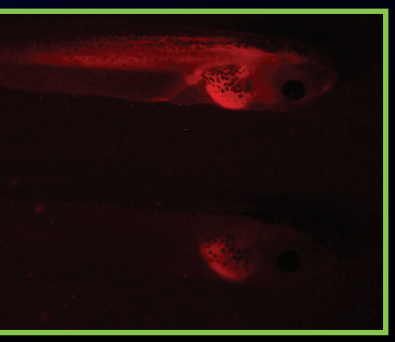


Stereo microscope configured for green fluorescence, viewing *Xenopus* through shield filter for sorting.



Stage 41 *X. tropicalis*, transgenic OTX-GFP eyes. Photograph © NIGHTSEA/ Charles Mazel



Fluorescent and non-fluorescent Stage 46 *X. laevis* with messenger RNA injected ubiquitous GFP and membrane RFP. Photograph © NIGHTSEA/ Charles Mazel

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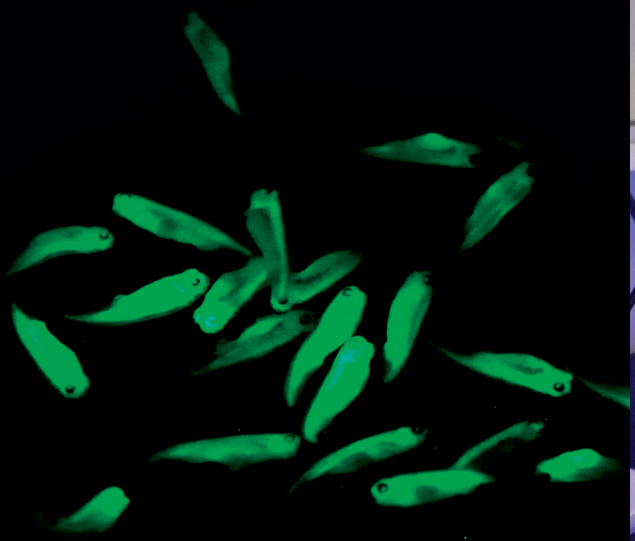

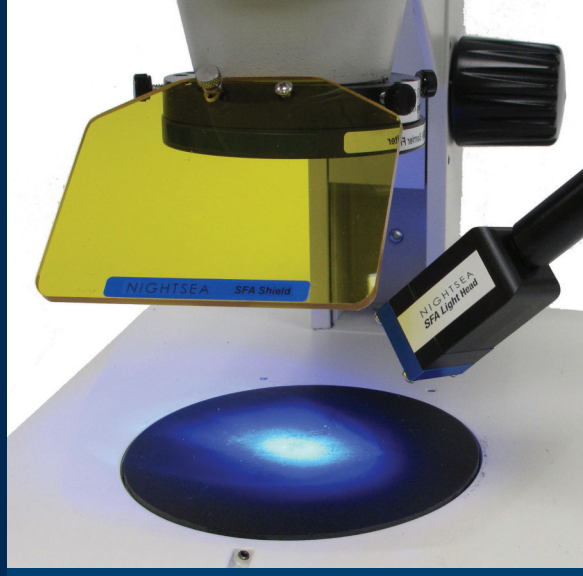

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