

Abstracts

## Morphogenesis

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### A novel FLRT3 and Rnd1 pathway involved in TGF- $\beta$ signaling-mediated cellular morphogenesis

Ken W. Cho<sup>1</sup>, Souichi Ogata<sup>1</sup>, Junji Morokuma<sup>2</sup>, Tadayoshi Hayata<sup>1</sup>, Gabriel Kolle<sup>3</sup>, Christof Niehrs<sup>3</sup>, Ueno<sup>2</sup>

<sup>1</sup> University of California, Irvine, CA, USA

<sup>2</sup> National Institute for Basic Biology, Okazaki, Japan

<sup>3</sup> German Cancer Research Center, Heidelberg, Germany

The molecular mechanisms governing cell behaviors underlying morphogenesis remain a major focus of research in both developmental biology and cancer biology. TGF- $\beta$  signaling guides cellular morphogenesis in variety of biological contexts, including vertebrate gastrulation. We find that activin/nodal members of the TGF- $\beta$  superfamily induce expression of two genes regulating cell adhesion during gastrulation: FLRT3, a putative type I transmembrane protein containing extracellular leucine-rich repeats, and the small GTPase Rnd1. We show that these two proteins participate in a novel physical interaction to control cell adhesion during early embryogenesis. Both loss- and gain-of-function analyses of FLRT3 and Rnd1 show that these proteins modulate cadherin function by regulating the availability of C-cadherin protein on the cell surface without changing the overall levels of cadherin protein in the cell. We propose that activin/nodal members of the TGF- $\beta$  superfamily induce expression of FLRT3 and Rnd1, which in turn, affect the availability of cadherin on the cell surface to regulate the adhesion property of the cells. As numerous studies have linked aberrant expression of small GTPases, adhesion molecules such as cadherins and TGF- $\beta$  signaling to oncogenesis and metastasis, it is tempting to propose that this FLRT3-Rnd1 pathway controls cell behavior and tissue morphogenesis in both embryos and adults.

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### Yes-associated protein 65 (YAP65) is required for early *Xenopus* development

Stephen T. Gee, Frank L. Conlon, Sharon L. Milgram  
Univ. of North Carolina-Chapel Hill, USA

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We previously showed that YAP is required for proper mouse development (Morin-Kensicki et al. 2006). YAP null mice exhibit defects in yolk sac vasculogenesis, chorioallantoic fusion, and axis elongation and fail to develop past embryonic day 8.5. To examine the role of YAP in axis elongation, we set up a parallel study using *Xenopus laevis* as our model system. We cloned *Xenopus YAP* (xYAP) and designed morpholinos (MOs) around the xYAP 5' UTR. Embryos injected with 40 ng xYAP MOs exhibited defective blastopore closure, while embryos injected with lower xYAP MO concentrations (20, 10, and 5 ng) resulted in embryos with defective axis elongation. RT-PCR analysis of stage 11 xYAP MO and control MO injected embryos showed that induction of the mesoderm and the endoderm is not perturbed. In addition, in situ characterization for *chordin*, *eomesodermin*, and *Vent2* expression in YAP MO and control MO injected embryos illustrated that the axes were properly specified, yet the YAP MO injected embryos were developmentally delayed. Additionally, we cloned the *Xenopus* homolog of a related protein, transcriptional co-activator with a PDZ-binding motif (TAZ), and designed morpholinos (MOs) around its 5' UTR. MO-mediated knockdown of xTAZ resulted in a less severe phenotype evident at the later tadpole stage. Thus, TAZ is required later in development, while YAP is critically important for early *Xenopus* development.

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### Paraxial mesoderm signals are required for intermediate mesoderm formation in the mouse

Deborah L. Chapman

Dept. of Biological Sciences, University of Pittsburgh,  
Pittsburgh, PA, USA

During development of the vertebrate embryo, paraxial mesoderm (PAM) generates the bilateral somites, which flank the axial neural tube and the notochord. Somites are the precursor tissue to the vertebrae, ribs, skeletal muscle and dermis of the back and body wall. As such, precise development and patterning of these mesodermal structures are critical for proper formation of the organism. Although

our lab primarily focuses on how PAM is specified and patterned during development, we are also interested in how this tissue exerts its effects on the formation of neighboring tissues, specifically the intermediate mesoderm (IM) that will give rise to the kidney and gonads. We used mouse embryos that are deficient in posterior somite formation to study how this affects IM formation. *Tbx6* and *wnt3a* mutant embryos form approximately 9 anterior somites, and from the forelimb bud caudally ectopic neural tissue forms at the expense of PAM. *Pax2* an early marker of IM was expressed in each mutants anteriorly, but more caudal expression terminated prematurely. This demonstrates that PAM is required for the continued formation and elaboration of IM. Through genetic combinations, we reduced the number of anterior somites to 4–5 and show that IM fails to form in these embryos as judged by a lack of *Pax2* expression. Using a transgene that marks IM (*hoxb7-GFP*), we further show that mouse embryos containing both ectopic neural tissue and somites will form some IM structures, but these are often malformed. These results support work in the chick that also shows a dependence of IM on signals emanating from the PAM. Sponsored by NIH NICHD Grant: RO1-HD38786.

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##### **Vascular differentiation defects associated with activated Notch signaling in endothelia of the mouse embryo**

Jessica N. Copeland, Hiroo Katayama, Jay L. Vivian  
*Institute of Maternal-Fetal Biology; Univ. of Kansas Medical Center, USA*

Notch signaling is known to play a role in the formation of the vascular system in the mouse. Loss-of-function and gain-of-function mutations in certain receptors and ligands of Notch lead to defects in the vascular system and embryonic lethality, though a detailed description of these functions remains to be determined. To further understand the role of Notch signaling during endothelial differentiation, we are using a binary transgenic mouse model that expresses an activated Notch1 intracellular domain in the embryonic vasculature. Several defects are seen in these transgenic embryos, which do not survive after 10.5 days postcoitum. These abnormalities include poor growth and development most notably in the posterior, an enlarged pericardium, and abnormal head and yolk sac vasculature. The vasculature of the yolk sac fails to differentiate fully, with few matured vessels. The vascular defects resulting from activated Notch1 expression further demonstrate the importance of Notch signaling in vascular differentiation, and provide a valuable *in vivo* tool to identify genes that are downstream of Notch signaling in the vasculature.

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##### **Inhibition of oral–facial clefting gene Interferon Regulatory Factor 6 disrupts gastrulation, pectoral fin formation, and somite boundary formation in zebrafish**

Jaime Sabel<sup>3</sup>, Claudia d'Alencon<sup>1</sup>, Nicholas Rorick<sup>3</sup>, Phyllis Hemerson<sup>2</sup>, Brian Schutte<sup>2</sup>, Robert Cornell<sup>1</sup>

<sup>1</sup> *Department of Anatomy and Cell Biology, Univ. of Iowa, Iowa City, IA, USA*

<sup>2</sup> *Department of Pediatrics, Univ. of Iowa, Iowa City, IA, USA*

<sup>3</sup> *Interdisciplinary Graduate Program in Genetics, Univ. of Iowa, Iowa City, IA, USA*

Genetic variation in Interferon Regulatory Factor 6 (*IRF6*) contributes to oral–facial clefting in humans and null alleles cause Van der Woude syndrome (VWS) which includes clefting. Other members of the IRF family of transcription factors are involved in regulating the immune response but the function of *IRF6* is unknown. To determine the *in vivo* function of *IRF6*, we identified the zebrafish ortholog and found it to be expressed maternally and by 24 h in oral, nasal, otic and pharyngeal arch epithelia. To inhibit *Irf6* function, we tested 5 unique morpholinos targeting *irf6* but saw widely variable phenotypic effects. We then built a putative dominant negative variant of *Irf6* (*dnIrf6*) composed of only the *Irf6* DNA binding domain. Embryos injected with *dnIrf6* RNA die with loss of cell adhesion during gastrulation; this phenotype is efficiently rescued by co-injection of full length *irf6* RNA. Embryos injected with a low dose of *dnIrf6* RNA survive gastrulation and develop with reduced or absent pectoral fins, blistered skin and diffuse somite boundaries. Interestingly, *p63* is also a human clefting locus and zebrafish *p63* MO-injected embryos superficially resemble embryos injected with *dnIrf6*. Results from *Irf6/p63* epistasis experiments and efforts to rescue zebrafish phenotypes with wild-type and VWS alleles of human *IRF6* will be presented.

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##### **A reverse-genetic screen identifying RhoGEF genes that are essential for zebrafish epiboly**

Benjamin Feldman, Justin Costa  
*National Human Genome Research Institute, NIH, Bethesda MD, USA*

We are interested in molecules controlling the cellular changes underlying morphogenesis during vertebrate gastrulation. RhoGTPases have emerged as key regulators of cell shape changes and already have been implicated in one gastrulation movement: convergence. A survey of known metazoan phenotypes resulting from mutations within the RhoGTPase pathway suggested the RhoGEF regulators of RhoGTPase activity as promising targets for a reverse genetic screen to find additional roles for RhoGTPase signaling during gastrulation. An *in silico* search identified 71 predicted zebrafish RhoGEFs with complete DH domains—the conserved signature of RhoGEFs. RT-PCR, subcloning, and sequencing showed that at least 48 of the