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SCIENCE DIRECT

Developmental Biology 295 (2006) 440 – 451

DEVELOPMENTAL BIOLOGY

www.elsevier.com/locate/ydbio

Abstracts

# Early Embryo Patterning

## 323

### Time-dependent patterning of the germ layers by Nodal signals

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The formation and patterning of the three germ layers are a critical early step during vertebrate embryogenesis. In all vertebrates, secreted proteins of the Nodal-related subclass of the TGF-β superfamily induce the mesendoderm and pattern all three germ layers. It is not clear, however, when these signals are required to perform each of these roles. In zebrafish, two nodal-related genes, called *squint* (*sqt*) and *cyclops* (*cyc*), have overlapping roles in mesendoderm formation. Like all Activin-like signals, Sqt and Cyc activate a bipartite receptor complex containing the ALK4 ser/thr kinase. Activated ALK4 phosphorylates the cytoplasmic factors Smad2 and Smad3, which associate with Smad4 and translocate to the nucleus where they activate transcription. To determine when activation of this pathway is required to pattern the embryo, we treated zebrafish embryos at different stages of development with SB-431542, a small molecule inhibitor of the ALK4 receptor. Our results indicate that this drug specifically blocks endogenous Nodal signals without inhibiting the ability of cells to respond to BMPs, which activate a structurally similar receptor complex. By treating embryos with SB-431542 at different developmental stages, we show that cells adopt progressively more vegetal fates when they are exposed to endogenous Nodal signals for increasing periods. Thus, our data support previous experiments suggesting that Nodal-related signals pattern the animal–vegetal, but not the dorsoventral body axis in zebrafish. In addition, our data suggest that during normal development, the effective dose of Nodal signals is regulated by how long a cell is exposed to endogenous Nodal signals.

doi:10.1016/j.ydbio.2006.04.353

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### PTEN in zebrafish gastrulation

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PTEN is a well-characterized tumor suppressor protein with multiple functions in cell proliferation, cell polarization and cell death. PTEN is the main negative regulator of the phosphoinositol-3-kinase/protein kinase-AKT (PI3K/AKT) pathway, which plays a key role in zebrafish early development. To test whether or not Pten regulates cell movements during gastrulation, we first cloned two paralogous pten genes from zebrafish. Sequence analysis showed that they are identical to the Ptena and Ptenb isoforms reported by Croushore et al., 2005. RT-PCR analysis showed that ptena and ptenb are maternally derived and ubiquitously expressed during early stages, and that each gene is alternatively spliced. Overexpression results showed that Ptena produced a higher frequency of phenotypic effects than Ptenb. Since a similar trend was seen when the RNAs were expressed off the HSP promoter, subsequent studies focused on the longest variant, Ptena. Ptena, when expressed in U87MG human glioma cells, decreased the level of phosphorylated AKT. In embryos, ptena overexpression caused similar effects to those induced by the PI3K inhibitor wortmannin. Ptena-induced phenotypes included cardia bifida, heart edemas, defective otoliths, small eyes and/or the loss of anterior or posterior structures. Further analysis using in situ hybridization and probes to hgg, ntl and krox 20 showed altered expression patterns by 9 hpf and suggest that a delay in gastrulation and abnormal convergence is the likely cause of the perturbations. Together, these results implicate the PI3K/AKT pathway plays a key role during gastrulation, participating in the coordinated cell movements that influence subsequent development.

doi:10.1016/j.ydbio.2006.04.352

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### Uncovering genes essential for neuronal development in zebrafish using a GFP-based forward genetic screen

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Neuronal development in vertebrates is a complex multistep process beginning with irreversible commitment of naïve ectodermal cells to a neural fate followed by proliferative precursors exiting the cell-cycle and undergoing terminal differentiation. Expression of the neural-specific α1tubulin gene spans the entire developmental phase of zebrafish neurons such that a GFP reporter driven by its

doi:10.1016/j.ydbio.2006.04.354

promoter acts as a stable in vivo molecular tag, marking neural cells from birth to synaptogenesis. We have exploited this α1tubulin-GFP transgenic zebrafish system in a mutagenesis screen to identify disruptions in genetic loci essential for neurogenesis, which would manifest as visually appreciable perturbations in GFP fluorescence. 32 Recessive mutations have been identified and a subset screened through an RNA quantification based assay to eliminate housekeeping gene defects. Three representative loci have revealed missteps in discrete, sequential events of embryonic neurogenesis. Mutation in sookshma panneurally diminishes the neural precursor pool by affecting cell proliferation in the developing embryo. Disruption of drishti ameliorates the mitotic neural population by stalling cell-cycle exit of progenitors, delaying their progression to the post-mitotic neuronal stage. Finally, dhruva is required during neuronal differentiation for axonal branching and terminal innervation in spinal motoaxons and the retinotectal projection. Molecular identification of these loci and characterization of the remaining mutational repertoire is underway and will help delineate genetic inputs that go on to make a mature, differentiated neuron.

doi:10.1016/j.ydbio.2006.04.355

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## Her9-dependent regulation of neurogenesis by Zic family proteins

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During early vertebrate embryonic development, neurogenesis occurs in three longitudinal columns within the caudal neural plate and in the adjacent placodal ectoderm. The mechanisms by which cells become neurons have been well studied, however, the patterning mechanisms that delineate neurogenic and non-neurogenic domains in the neural ectoderm are poorly understood. Analyses of Zic zinc-finger transcription factors in *Xenopus* have suggested a role for this gene family in defining non-neurogenic domains within the neural plate. We tested this hypothesis in zebrafish embryos by a loss-of-function approach to assay the role of Zic proteins in neurogenesis using morpholino mediated protein knockdown. Zebrafish Zic2a, Zic2b and Zic3 were found to function together to promote neuronal differentiation in the neural plate while at the same time contributing to the suppression of neurogenesis in the placodal ectoderm. Surprisingly, the function of the Zic proteins in both contexts was dependent on their ability to repress her9, an inhibitor of neurogenesis in the neural plate. In morphant embryos, the expression of her9 was expanded in both the neural plate and placodal ectoderm. The concurrent knockdown of Her9 in the Zic2a/Zic2b/Zic3 morphant embryos rescued neuronal differentiation in both tissues, in spite of the their qualitatively opposite phenotypes: neuronal differentiation was restored in the neural plate and the over-production of neurons in the placodal ectoderm was suppressed. Thus, Zic proteins function by repressing her9 to either promote or suppress neuronal differentiation in a tissue-dependent manner.

doi:10.1016/j.ydbio.2006.04.356

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## Requirement of calcium modulation in organ laterality

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Vertebrates require asymmetrical arrangement of internal organs with respect to the left–right (L–R) axis. An important step in early embryonic development breaks bilateral symmetry. While the triggering mechanism of this event is yet to be uncovered, several downstream signaling cascade components have been identified and shown to be highly conserved across species. Using in vivo image analysis in zebrafish (*Brachydanio rerio*), we have identified several key stages of endogenous calcium release. Molecular and pharmacological manipulation of one of these pre-somite phases of calcium activity has identified an exciting link between calcium modulation and L–R axis determination. Treatment with a calcium inhibitor impacted L–R patterning, as it resulted in heart and gut laterality defects and in perturbations in asymmetric gene expression. Furthermore, we find that Kupffer's vesicle, a ciliated structure implicated in L–R axis determination, is dramatically reduced upon treatment with calcium inhibitor. The treatment also disrupts the organization of cilia in Kupffer's vesicle. Immunolocalization of no tail (ntl), a key player in L–R axis determination, demonstrates that the protein is still expressed after treatment. Interestingly, antibody staining against β-catenin reveals a dramatic increase in the nuclear localization of this transcription factor. Furthermore, we show that the naked cuticle (nkd1), an inducible antagonist of the Wnt/β-catenin pathway, is also upregulated after calcium inhibition. Collectively, our data uncovers an early "pre-somitic" role for calcium signaling and implicates Wnt signaling in L–R patterning.

doi:10.1016/j.ydbio.2006.04.357

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## KUPFFER'S vesicle in zebrafish

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Vertebrates appear bilaterally symmetric, but have internal asymmetries along the left–right (L–R) axis. This axis is revealed by the asymmetric placement of organs along the midline. However, how L–R asymmetry during early vertebrate embryogenesis is established is under considerable