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.

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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.

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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.

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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

debate. Recently, it has been discovered that nodal cilia in the mouse node have a counterclockwise twirling movement causing leftward nodal flow. This flow has been proposed to be the initial break in symmetry required for proper L–R patterning. Motile cilia are also observed in Kupffer's vesicle, formed by dorsal forerunner cells (DFCs) in the zebrafish *Danio rerio*. Currently, it is assumed that Kupffer's vesicle is homologous to the mouse node, and ciliary function is conserved throughout vertebrate L–R development. However, there are several questions on whether structure and mechanisms are conserved between nodal cilia and cilia in Kupffer's vesicle. Our ultrastructural and immunohistochemical studies in zebrafish reveal that both dorsal and ventral DFCs lining the luminal surface of Kupffer's vesicle produce primary cilia. However, Kupffer's vesicle cilia seem very different from those previously studied. In mouse and medakafish, cilia are more numerous and posteriorly tilted (Tanaka et al., Nature, 2005). In our studies, cilia in Kupffer's vesicle were not obviously tilted in the same direction. It is also questionable whether cilia projecting from all luminal surfaces of Kupffer's vesicle have the same direction of rotation. If all cilia rotate similarly, how can be a leftward flow produced in the spherical space of Kupffer's vesicle? Recent progress in understanding the structure and function of cilia in Kupffer's vesicle will be reported.

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Squint protects early embryos from temperature-induced dysmorphology

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A key step in vertebrate development is the allocation of embryonic cells into germ layers. When signaling by the Nodal group of TGF-beta-like ligands is blocked, there is a failure in the allocation of mesoderm and endoderm. In zebrafish, this leads to holoprosencephaly (HPE) and the absence of vital organs. Partial Nodal impairment has milder effects, but HPE remains a hallmark. Two zebrafish nodal-related genes – *squint* (*sqt*) and *cyclops* (*cyc*) – are expressed during germ-layer allocation. Embryos null for *cyc* always display HPE, whereas *sqt*-null embryos have variable HPE, often developing normally. We sought out development contexts where Squint might be essential, by examining two independent *sqt* alleles. With the exception of certain high-penetrant crosses, we find maternal-zygotic *sqt* (*MZsqt*) and zygotic *sqt* mutants to have similar embryonic phenotypes, supporting previous evidence that maternal Squint is dispensible. We also find that HPE penetrance in *MZsqt* embryos depends on interacting genes that influence extant Nodal signaling levels. Extant Nodal activity is also reduced in heat- or cold-shifted *MZsqt* embryos and these temperature shifts dramatically increase the incidence of HPE. A novel *sqt*-associated phenotype, *spina bifida*, is also found in heat-shifted *MZsqt* embryos. Finally, we find that the Heat Shock Protein 90 (HSP90) normally protects *MZsqt* embryos from acquiring HPE. Thus, a key function of Squint is to guarantee the integrity of early-stage Nodal signaling over a range of temperatures.

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New insights into left–right patterning: The role of *pkd2* in the zebrafish

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An important step in patterning the vertebrate embryo involves the formation of the left–right body axis. To gain insight into the genetic events involved in establishing the left–right axis, we are analyzing mutations obtained from a large-scale Zebrafish screen.[1] The zebrafish mutant *curly up* (*cup*) affects the zebrafish ortholog of *polycystic kidney disease 2*, a gene that encodes the Ca^{2+} activated non-specific cation channel, Polycystin-2. We have characterized two alleles of *cup*, and even though one allele of *cup* appears to be a null, cup mutants do not display defects in kidney patterning nor do they develop kidney cysts. *cup* alleles do display left–right defects in organ positioning that resemble human heterotaxia, as well as abnormalities in asymmetric gene expression in the lateral plate mesoderm and dorsal diencephalon of the brain. However, *nodal* expression phenotypes between the mouse and zebrafish $pkd2^{-/-}$ do not coincide. In the majority of *cup* embryos, *nodal* is activated bilaterally in lateral plate mesoderm, as opposed to the complete absence of *nodal* reported in the LPM of the *pkd2* null mouse. The mouse data indicate that *pkd2* is responsible for an asymmetric calcium transient that is upstream of *nodal* activation. In zebrafish, it appears that *pkd2* is not responsible for the activation of *nodal* transcription but is required for a mechanism to bias *nodal* expression towards the left half of the embryo. Our data suggest that *pkd2* may not be playing a conserved role in left–right axis determination amongst all vertebrates.

[1] Haffter P. et al. *Development*, 1996.

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