serves in part to buffer melanogaster against environmental variation to ensure robustness of D/V patterning.

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Program/Abstract # 350
Arp2/3-mediated actin dynamics affect polarity maintenance in the *Caenorhabditis elegans* embryo

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The PAR proteins and the actin cytoskeleton are necessary to generate cell diversity throughout development. Cdc42 and its effectors, WASp/WAVE and the Arp2/3 complex, are well-conserved regulators of actin nucleation and branching, which is necessary for proper cell polarization and membrane trafficking. Members of the PAR polarity complex can regulate membrane traffic as well. In the *Caenorhabditis elegans* one-celled embryo, asymmetric localization of the PAR polarity cues distinguishes the anterior end from the posterior. The anterior PAR complex, CDC-42 and RHO-1, as well as the actomyosin network become enriched at the anterior cortex while PAR-2 and PAR-1 are localized to the posterior cortex. CDC-42 acts specifically to maintain PAR asymmetry, yet it remains unclear how exactly CDC-42 functions in this role. We have found that depletion of the *C. elegans* orthologs of Cdc42, Arp2/3 and WASp/WAVE leads to similar defects in cortical actin dynamics specifically during polarity maintenance phase. During this time, PAR asymmetry also fails to be stably maintained suggesting that the conserved Cdc42/WASp/WAVE pathway is functioning to stabilize polarity in the embryo. Disruptions in the cortical localization of DYN-1-GFP and cytoplasmic aggregates of endosomal markers have been observed. This raises the possibility that Arp2/3-mediated actin dynamics, regulated by Cdc42 and WASp/WAVE, play a role in the organization of endocytic trafficking in the early embryo that may also contribute to PAR domain stability during polarity maintenance phase.

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Program/Abstract # 351
Cell cycle arrest in node cells governs node cilia development to break the left-right symmetry

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Recent studies have show that a rotational movement of the node cilia generates a leftward fluid flow to break the left-right symmetry. Despite the importance of cilia at the node, very little is known about the mechanisms that regulate the node cilia development. In this presentation, we show, for the first time, the molecular mechanisms of how monocilia at the node is developed. We took an advantage of conditional gene knockout approach to assess the requirement of bone morphogenetic protein (BMP) signaling for breaking the left-right symmetry. The resulted embryos develop a left-isomerism evidenced by bilateral expressions of left-side specific markers. We found that BMP signaling through a type I receptor ACVRI is critical to develop node cilia and subsequent generation of a leftward fluid flow at node. Using mouse embryonic fibroblast cells from the mutant embryos, we found that BMP signaling positively controls the levels of cyclin-dependent kinase (cdk) inhibitors and therefore maintains cells into quiescence, which is critical to induce primary cilia. We also confirmed that the cdk inhibitor is exclusively produced at the node under the tight control of BMP signaling when embryos start to break symmetry. Taken together, our results suggest that BMP signaling governs the cell cycle arrest in the node cells to develop node cilia, and thus provide insight into the fundamental question about how node is defined as the "node" for breaking of left-right symmetry in vertebrates.

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Program/Abstract # 352
Pkd1l1 and Pkd2 physically interact and establish left-right asymmetry

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In mammals, left–right (L–R) asymmetry is generated by posteriorly tilted monocilia on the surface of the embryonic node. These cilia drive a unidirectional leftwards flow of extra-cellular fluid across the node and thereby activate asymmetric gene expression. How the mechanical force of fluid flow is translated into intracellular biochemical signals is unknown. The two-cilia hypothesis argues that, as well as motile cilia generating fluid flow, immotile sensory cilia respond to this flow through a Pkd2-mediated mechanism, eliciting a Ca2+ spike at the left periphery of the node. However, a putative mechanosensory Pkd2 partner has remained elusive. We have identified the polycystin 1-related locus Pkd1l1 as a crucial component of L–R patterning in mouse. Systematic comparison of Pkd1l1 and Pkd2 mutants reveals strong phenocopying; both mutants fail to activate asymmetric gene expression at the node and lateral plate mesoderm and exhibit right isomerism of the lungs. Node cilia are morphologically normal and demonstrate typical motility, consistent with Pkd1l1 and Pkd2 acting downstream of leftward flow. We show that Pkd1l1 is strikingly enriched in the embryonic node, and that its protein product co-localises with Pkd2 in primary cilia. Immunoprecipitation experiments demonstrate that Pkd1l1 and Pkd2 physically interact, an association mediated by Pkd1l1's intracellular coiled coil domain. These data argue that Pkd1l1 is the long-undiscovered mechanosensitive partner of Pkd2 required for the sensation of leftward flow. Our future experiments aim to uncover the molecular and cellular bases of Pkd1l1's apparent mechanosensitive role during L–R specification.

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Program/Abstract # 353
FGF signaling controls brain asymmetry in Zebrafish

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FGF signaling controls a diverse range of developmental functions, including cellular differentiation, migration and proliferation. Recently, it has been shown that FGF signaling regulates cilia length and function in multiple epithelia during Zebrafish and *Xenopus* development (Neugebauer et al., Nature 2009, Hong and Dawid, PNAS 2009). Here, we find that inhibition of FGF signaling at defined developmental stages shows distinct functions in the left–right pathway, including functions downstream of cilia. Strikingly, embryos in which FGF signaling is inhibited after midsomitogenesis have isolated perturbation of brain asymmetry, with bilateral expression of normally left-sided markers lefty1 and cyclops (TGF-β family members), while keeping expression of left-sided markers in the lateral plate mesoderm (LPM) normal, including southpaw and lefty2. Previously it was proposed that southpaw in the LPM

induced brain expression of lefty1 and cyclops (Long et al., Development 2003). In contrast to this model, when FGF signaling is inhibited, cyclops and lefty1 are bilaterally expressed in the brain even in the absence of southpaw. Two transcription factors, six3b and six7, are required for repression of asymmetric lefty1 expression in the brain (Inbal et al., Neuron 2007). We have found that FGF signaling regulates expression of these transcription factors. From our results, we propose a model for brain laterality, where FGF signaling activates six3b and six7, which in turn inhibits lefty1 expression in the brain. Here, southpaw, rather than initiating lefty1 and cyclops, inhibits the repressive activity of six3b and six7 in the left-side of the brain, acting as a permissive factor for normal lefty1 and cyclops expression.

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Program/Abstract # 354
Nipbl regulates organ laterality and Kupffer's vesicle development in Zebrafish

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The cohesin-associated protein Nipbl is known to be required for sister chromatid cohesion, but recent studies have revealed that it also influences gene expression, which may explain why partial reduction in Nipbl expression causes the multisystem developmental disorder Cornelia de Lange Syndrome. To gain insight into the origins of embryonic defects caused by mutations in Nipbl, we established a Zebrafish model in which Nipbl expression is reduced, to different extents, using morpholinos. Nipbl-morphants exhibited a range of heart and gut defects from abnormal looping to organ duplications; these changes were preceded by small but significant alterations in the early expression of key developmental regulatory genes. Restoration of expression of either of two of these, gata5 and sox32, partially rescued organ duplications, but not looping, suggesting that distinct mechanisms underlie looping and midline organ fusion defects. Here we show that Nipbl-morphants display aberrant expression of genes involved in left–right patterning, such as lefty2 and southpaw. Left–right patterning is known to be required for heart/gut looping and, in Zebrafish, is initiated by Kupffer's vesicle (KV), through activities dependent upon the motility of monocilia of KV cells. In Nipbl-morphants, we found that KV morphology was normal, but monocilia were shortened. Moreover, in dorsal forerunner cells, which are the precursors of KV, we observed reduced expression of both foxj1a, a transcription factor implicated in ciliogenesis, and dnah9, a gene required for cilia motility. These findings suggest that Nipbl regulates organ laterality by controlling cilia formation and function within KV. (Supported by NIH P01-HD052860).

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Program/Abstract # 355
Serotonin signaling is required for Wnt-dependent development of the ciliated gastrocoel roof plate and leftward flow in *Xenopus*

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Symmetry breakage and laterality specification in fish, amphibian and mammalian embryos depends on cilia-driven leftward flow during neurulation. In *Xenopus* a functionally relevant asymmetry of serotonin localization was described at the 32-cell stage. Here we report a role of serotonin signaling in the specification of the superficial mesoderm (SM) during gastrulation. The SM develops into the ciliated gastrocoel roof plate (GRP) epithelium, which drives leftward flow. Flow, and consequently asymmetry, were lost in embryos in which serotonin signaling through receptor type 3 was down-regulated, either through morpholino oligonucleotide-mediated gene knockdown or upon over-expression of a secreted frog or human serotonin-binding domain derived from receptor type 3. Serotonin, which we found to be distributed uniformly along the main body axes in the early embryo, was required for canonical Wnt signaling, which provides the instructive signal to specify the GRP. Serotonin was required for Wnt-induced double axis formation as well, suggesting a more general role of serotonin as competence factor for Wnt signaling.

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Program/Abstract # 356
Gastric H+/K+ATPase-dependent Wnt-signaling is required for FoxJ1 expression and cilia polarization in *Xenopus* left–right axis formation

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Cilia-driven leftward flow of extracellular fluid at neurula stages is essential for symmetry breakage in most vertebrate embryos. In the frog *Xenopus* asymmetric localization of the P-type ion pump gastric H+/K+ATPase a (ATP4a) was described at the 4-cell stage. This asymmetry presents the corner stone of the 'ion flux hypothesis', which postulates symmetry breakage at cleavage stages through asymmetric activities of ion channels and pumps. We have investigated the role of ATP4a in the context of leftward flow in *Xenopus*. No asymmetries of ATP4a mRNA expression were found along the dorso–ventral or left–right (LR) axis. Morpholino oligonucleotide meditated knockdown of ATP4a resulted in LR defects only when cells of the gastrocoel roof plate (GRP) were targeted, i.e. the site of leftward flow. Number and length of cilia were reduced at the GRP in ATP4a morphants and remaining cilia were mispolarized. Moreover, the master control gene of motile cilia, FoxJ1, was down-regulated. As FoxJ1 expression requires canonical Wnt signaling (our unpublished results) we explored a possible link between ATP4a and Wnt. Induction of secondary body axes by ventral expression of XWnt8 or Xdsh was inhibited in ATP4a morphants, implicating ATP4a in canonical Wnt signaling. Non-canonical signaling was affected in ATP4a morphants as well, as Wnt–PCP dependent convergent extention in activin-induced animal caps was inhibited. In summary, we demonstrate a role for ATP4a in ciliogenesis and leftward flow during LR axis specification. Our data are consistent with a model, in which ATP4a contributes to acidification of Wnt-signalosome vesicles, which is a prerequisite of both canonical and non-canonical Wnt signaling.

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Program/Abstract # 357
Asymmetric expression of Claudin-10 is required for correct left–right patterning

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