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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