

vertebrates. In sea urchin, DA and its receptor DRD1 were detected from the period when the embryos acquire rotatory movement in the unhatched blastula stage, indicating dopaminergic system is a likely candidate that is involved in the ciliary beating in blastulae. Immunohistochemically DA and DRD1 were detected associated with a few micrometer diameter granules, and they were closely localized with tubulin at the base of cilia. Inhibition of DA synthesis or knockdown of *DRD1* gene resulted in severe decreasing of swimming activity in blastulae. During larval development, the ciliary band emerged evidently associated with local cell proliferation and the change of ciliary ectodermal cell shape on the larval arms and the anterior and posterior epaulets. The serotonin receptor cell network and serotonergic nervous fibers closely fringed the ciliary bands of the anterior and posterior epaulets and of the larval arms. The present observations provided the structural basis of our previous observation that larval swimming activity is less sensitive to serotonin deprivation in older larvae than younger blastulae.

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#### Program/Abstract # 118

##### Serotonin signaling initiates gastrulation in the sea urchin

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Gastrulation in the sea urchin begins with vegetal plate invagination. Pharmacological studies from our lab suggest that 5-HT triggers the gastrulation process. However, the rate limiting enzyme for 5-HT synthesis, tryptophan hydroxylase (TPH), has been reported to not be expressed until the late gastrula stage, and only in cells of the developing serotonergic nervous system; furthermore an inhibitor of TPH, p-chlorophenylalanine (p-CPA), did not block gastrulation. This calls into question the role of serotonin in the gastrulation process. In the present study we show that methyl-p-CPA blocks gastrulation *in vivo* and tryptophan hydroxylation *in vitro*. Serotonin was identified in vegetal plate and primary mesenchyme cells in mesenchyme blastula and early gastrula embryos prior to localization in serotonin neuron precursors. We demonstrate that preneural embryos utilize a different enzyme, phenylalanine hydroxylase/tryptophan hydroxylase (PAH/TPH), than that (TPH) used in developing larval serotonergic neurons to hydroxylate tryptophan, and PAH/TPH mRNA is present in blastula and gastrula stage embryos. Vegetal plate invagination blocked by methyl-p-CPA can be rescued by co-incubation with serotonin, as well as with agonists and downstream effectors of type 2 (DOI, PMA) and type 7 (8-OH, cyclic AMP) serotonin receptors. The direct activator of protein kinase A (PKA), cyclic AMP, demonstrated the greatest rescue effect, and methyl p-CPA inhibits phosphorylation of PKA and, to a lesser extent, protein kinase C. This study demonstrates that serotonin acts on type 2 and/or type 7 receptors to initiate gastrulation in the sea urchin.

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#### Program/Abstract # 119

##### Dissecting Tentacle Formation in Hydra Using Chemical Genetics

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In the basal metazoan *Hydra*, tissues are in a state of constant growth and replacement. Thus, developmental processes such as morphogenesis and differentiation are continuously active in the adult *Hydra* and are orchestrated by ongoing signal transduction. Classical genetic approaches

to dissect developmental pathways are difficult to carry out with *Hydra*. We have initiated a small molecule screen to better understand regeneration and maintenance of *Hydra's* simple body plan. We have identified a small molecule, DAC-2-25, that expands the existing tentacle zone in a progressive and polar fashion. Using phylogenetic profiling we have identified strains that respond to DAC-2-25 (e.g. *Hydra vulgaris* strain AEP), or that don't respond, (e.g. *Hydra vulgaris* strain Zurich). We have used chimeras of these strains to identify the responding tissue layer. Transgenic *Hydra* expressing fluorescent proteins under the control of relevant promoters are being used to examine how DAC-2-25 perturbs tentacle formation. Structure-activity relationship studies have identified the features of DAC-2-25 that are required for activity. Ultimately, we plan to identify the protein target of DAC-2-25 by using a combination of affinity chromatography and bulk segregant analysis of progeny from a cross between responding and non-responding strains.

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#### Program/Abstract # 120

##### Nodal signaling is involved in left-right asymmetric ocellus formation in *Ciona intestinalis*

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Nodal signaling plays an essential role in establishment of left-right asymmetry in a variety of animals. In the ascidian, *Ciona intestinalis*, left-right asymmetry can be observed e.g. in the coiling of the elongating tail of tailbud embryos in the chorion, positioning of the sensory pigment cells in the larva and gut formation in the juvenile, and *Nodal* is expressed on the left side of the sensory vesicle (SV) and epidermis in the developing tailbud embryo. However, it is largely unknown how Nodal signaling is involved in establishment of left-right asymmetric morphology. To address the involvement of the left-sided Nodal signaling in the establishment of asymmetric morphology in the development of *C. intestinalis*, we analyzed effect of the inhibition of Nodal signaling on the formation of the ocellus pigment cell, located on the right side of the SV of the larva in normal development. Upon the inhibition of Nodal signaling with the inhibitor, SB431542, the ocellus pigment cell was located on the midline, and melanin granules in the cell were separated by the midline. Moreover, *Ci-opsin1*, a marker gene of the ocellus photoreceptor cells expressed on the right side of the SV in normal development, was ectopically expressed on the left side as well as on the right side of the SV. Likewise, *Ci-Rx* that is required for ocellus differentiation and is expressed on the right side of the SV in normal development was expressed bilaterally upon the inhibition of Nodal signaling. These results suggest that the left-sided Nodal signaling controls the asymmetric ocellus formation in the larval development of *C. intestinalis*.

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#### Program/Abstract # 121

##### Post-intercalation elongation and narrowing of the ascidian notochord requires actomyosin contractility and endocytosis

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The control of cell shape is of central importance in morphogenesis, but issues of scale and resolution make it challenging to characterize these shapes across entire tissues and organs. We have developed a computer-assisted method to reconstruct the three-dimensional shape of every cell in the developing notochord of the simple chordate *Ciona savignyi* based on confocal microscopy of phalloidin-stained embryos.