fore allows me to study how the segmentation gene network has evolved in the insects. I am currently focusing on the roles of caudal and giant and have found that caudal is required for proper development of nearly the entire body, while giant seems to give a canonical gap phenotype.

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Program/Abstract # 240
Characterization of non-segmental progeny of the mesodermal lineage in the leech Helobdella
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The clitellate annelid Helobdella is a member of Spiralia, a group of protostomes defined by their conserved spiral cleavage pattern. One example of conservation in spiralian development is the cell 4d, known as the mesodermal precursor in all spiralian species and shown to be associated with the embryonic organizer in some. In clitellates, 4d divides bilaterally to give rise to a pair of mesodermal (M) teloblasts. Each M teloblast undergoes iterated divisions to produce a column of blast cells, the precursors of segmental mesoderm. It is generally believed that development of the M lineage is conserved across clitellate species, but comparative embryology suggested that inter-species variation in the mesodermal lineage may exist. To begin to tackle this issue, we improved the resolution and accuracy in tracing the mesodermal lineage by using a plasmid expressing a histone 2B:GFP fusion protein as a tracer in Helobdella. We revealed that the M teloblasts undergo several rounds of division prior to the production of segmental blast cells. The first two divisions of each M teloblast give rise to precursors of migratory ‘freckle’ cells, which populate the inner surface of the micromere cap during gastrulation. Further, we show that the 3rd through 6th divisions of each M teloblast produce cells giving rise to non-segmental prostomial tissue. This technique has allowed us to observe a new level of detail in the 4d lineage of a clitellate and opens the door for comparisons in other spiralians, which should contribute to the understanding of spiralian development and its evolution.

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Program/Abstract # 241
Molecular mechanisms governing the establishment of species-specific morphologies — Emerging views
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The origin of macro- and microevolutionary differences is a long standing topic in evolutionary biology. To address this question, we focus on hemimetabolous insects and discuss how their mode of development may be particularly suitable for generating phenotypic variation. More specifically, two hox genes, Ultrabithorax and Sex combs reduced, are used to illustrate how the differences in their functions during embryonic and post-embryonic development may have a significant impact on morphological evolution. By using insect hind legs as a model, it is possible to visualize and understand how small, population-level differences in the expression of Ubx could lead to the large morphological differences over time. In the same way, a common Scr-triggered mechanism may account for some of the diversity observed in the insect prothorax. These model studies indicate that in addition to their early embryonic function in establishing segmental identity, hox genes may also play a large role in generating species-specific morphologies during post-embryonic development.

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Program/Abstract # 242
Conservation of Mago nashi function in the tardigrade Hypsibius dujardini
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A key question in evolutionary biology is how morphological diversity arises. We have developed the tardigrade Hypsibius dujardini, which is closely related to the well-studied arthropod and nematode phyla, for comparative studies of embryogenesis and germline development. Mago nashi is a highly-conserved protein with diverse roles in development. In D. melanogaster, Mago is necessary during oogenesis to specify both the body plan and germline. In C. elegans, MAG-1 is necessary for embryo elongation and sex determination. From a H. dujardini sequence database, we identified a sequence (Hd-mago) with significant homology to Mago/MAG-1. To assess the role of Hd-mago in H. dujardini development, we adapted protocols used in C. elegans for RNA interference by injection of dsRNA. Embryos from mock-injected adults began to elongate along the anterior–posterior axis after 30 h, and hatched after 4.5 days. Conversely, Hd-mago(RNAi) embryosfailed to elongate, even after 5 days, and failed to hatch. However, Hd-mago(RNAi) embryos expressed appropriate markers of tissue differentiation. Therefore, loss of Hd-mago does not prevent differentiation, but does affect morphogenesis. This result is not due to nonspecific effects of dsRNA itself since Hd-actin(RNAi) embryos arrest much earlier in development, with multinucleated cells. Thus, the role of Hd-mago in embryogenesis appears to be conserved with MAG-1 in C. elegans. Furthermore, these results provide the first evidence that RNAi can be used to study gene function in phylum Tardigrada. We are currently working to characterize Hd-mago expression and possible function in the H. dujardini germline.

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Program/Abstract # 243
Germ layer patterning in bichir and lamprey: an insight into its evolution in vertebrates
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Vertebrate ancestors would have increased their egg size to store yolk, and the increase is considered to have altered the cleavage pattern and germ layer formation. Amphibian holoblastic cleavage in which all blastomeres contribute to any one of the three primary germ layers has been widely thought to be a developmental pattern in the stem lineage of vertebrates, and meroblastic cleavage to have evolved independently in each vertebrate lineage. In extant primitive vertebrates, agnathan lamprey and basal bony fishes also undergo holoblastic cleavage, and their vegetal blastomeres have been generally thought to contribute to embryonic endoderm. However, the identification of their primary germ layers based on molecular evidence was not reported. We performed the marker analyses in most basal ray-finned fish bichir and agnathan lamprey embryos, resulting that their mesoderm and endoderm develop in the equatorial marginal zone, and their vegetal cell mass is extraembryonic nutritive yolk cells, having non-cell autonomous mesoendoderm inducing activity. Furthermore, eomesoderm, but not
VegT, ortholog is expressed maternally in these animals as well as zebrafish, mouse and protochordates, suggesting that VegT is a maternal factor for endoderm differentiation only in amphibian. The study raises the viewpoint that the lamprey/bichir type holoblastic development would have been ancestral to extant vertebrates and retained in their stem lineage as a preliminary state toward the meroblastic development; amphibian-type holoblastic development would have been acquired secondarily, accompanied by the exploitation of new molecular machinery such as maternal VegT.

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Program/Abstract # 244

Changes in localization and expression levels of Shroom2 and spectrins contribute to variation in amphibian egg pigmentation patterns

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One contributing factor in the worldwide decline in amphibian populations is thought to be exposure of eggs to UV light. Enrichment of pigment in the animal hemisphere of eggs laid in the sunlight defends against UV exposure, but less is known about how such mechanisms were modified during evolution to achieve the wide diversity of amphibian egg pigment patterns. Here, we show that ectopic expression of the γ-tubulin regulator, Shroom2, is sufficient to induce co-accumulation of pigment granules, spectrin, and dynactin in Xenopus blastomeres. Moreover, Shroom2 and spectrin are enriched and co-localize specifically in the pigmented animal hemisphere of Xenopus eggs and blastulae. Moreover, Shroom2 mRNA is expressed maternally at high levels in Xenopus. By contrast to Xenopus, eggs and blastulae of Physalaemus pustulosus have very little surface pigmentation. Rather, we find that pigment is enriched in the perinuclear region of these embryos, where it co-localizes with spectrin. Moreover, maternal Shroom2 mRNA was barely detectable in Physalaemus, though zygotic levels were comparable to Xenopus. We therefore suggest that a Shroom2/spectrin/dynactin-based mechanism controls pigment localization in amphibian eggs, and that variation in maternal Shroom2 mRNA levels accounts in part for variation in amphibian egg pigment patterns during evolution. Localization and expression levels of Shroom2 and spectrins govern animal hemisphere pigmentation in amphibian eggs.

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Program/Abstract # 245

CXCR4 drives neural crest cells to the sympathetic ganglia

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The proper guidance of neural crest progenitor cells is critical to the development of the vertebrate body plan, including formation of the dorsal root ganglia (DRG) and sympathetic ganglia (SG) of the peripheral nervous system. Trunk neural crest cells (NCCs) are sculpted into discrete migratory streams through rostral somite halves, however it is unclear what molecular mechanisms drive NCCs over long distances to ventral locations within the embryo. Here, we determined a role for chemokine signaling to modulate trunk NCC migration along the ventromedial pathway to the dorsal aorta. Expression analysis by RT-PCR and in situ hybridization revealed that a subset of trunk NCCs expressed CXCR4 and the tissue dorsal to the dorsal aorta expressed SDF-1. In vitro time-lapse confocal imaging and in vivo bead transplantation experiments showed attraction and gathering of NCCs around SDF-1 soaked beads, respectively. Knock down of NCC CXCR4 expression using shRNA revealed disruption of long distance NCC migration and differentiation of sympathetic neurons. Significantly fewer CXCR4-shRNA+ cells reached ventral SG target sites and located to the inner core of SG, a site of neuronal differentiation Thus, CXCR4/SDF-1 signaling plays a vital role in trunk NCC navigation and may be part of a signaling network to sort a common pool of trunk NCCs into the SG and DRG.

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Program/Abstract # 247

Preplacodal region marked by Six1 in mice

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The border between neural and non-neural ectoderm gives rise to paired placodes and neural crest. The sensory placodes, transient thickenings of ectodermal epithelium, give rise to cranial sense organs such as the nose and ear, and represent an important source of neural tissue for the ganglia of the cranial nerves and for the lateral lines. Placodes arise from either neural folds themselves or adjacent to the neural crest in the presumptive head. During late gastrulation and early segmentation stages, all placodes develop from contiguous pre- or pan-placodal region (PPR) located around the anterior neural plate.