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
Stephanie Gline, David Weisblat
MCB, U.C. Berkeley, Berkeley, CA USA

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
Aleksandar Popadic, Steven Hrycaj, Najmus Mahfooz, Nataliya Turchyn
Department of Biological Sciences, Wayne State University, Detroit, MI, USA

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 Sex-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
Jennifer Tenlen, Bob Goldstein
Department of Biology, UNC-Chapel Hill, Chapel Hill, NC, USA

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) embryos failed 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
Masaki Takeuchi, Maiko Takahashi, Shinichi Aizawa
VBP, CDB, RIKEN Kobe, Japan

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, agnathian 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 both basal ray-finned fish bichir and agnathian 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 meso-endoderm inducing activity. Furthermore, eomesodermin, but not