*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 accurred 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

Chanjae Lee, Minh-Phuong Le, David Cannatella, John Wallingford Department of Molecular Cell and Developmental Biology, University of Texas at Austin, Austin, TX, USA

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  $\gamma$ -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 Jennifer C. Kasemeier-Kulesa<sup>a,b</sup>, Rebecca McLennan<sup>b</sup>, Frances Lefcort<sup>b</sup>, Paul M. Kulesa<sup>a,c</sup> <sup>a</sup>Stowers Institute for Medical Research, Kansas City, MO, USA <sup>b</sup>Department of Cell Biology and Neuroscience, Montana State University, Bozeman, MT, USA <sup>c</sup>Department of Anatomy and Cell Biology,

University of Kansas School of Medicine, Kansas City, KS, USA

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 # 246 Late emerging trunk neural crest cells in the turtle *Trachemys scripta*

Judith A. Cebra-Thomas<sup>a</sup>, Anne Terrell<sup>a</sup>, Lin Gyi<sup>b</sup>, James Robinson<sup>b</sup>, Melinda Yin<sup>b</sup>, Scott F. Gilbert<sup>b</sup>

<sup>a</sup>Department of Biology, Millersville University, Millersville, PA, USA <sup>b</sup>Department of Biology, Swarthmore College, Swarthmore, PA, USA

Turtle plastron bones develop by intramembranous ossification from the condensation of cells that stain positively for HNK1, PDGFR $\alpha$  and p75, indicating that these bones are derived, like the facial bones, from neural crest cells. At Greenberg stage 17, comparable to H&H Stage 28 chick embryos and well after the initial wave of neural crest migration, cells that are positive for HNK1 and the early neural crest marker, FoxD3 begin accumulating in the thickened dermis of the carapace and migrating to the developing plastron. We have been able to demonstrate that these cells share the defining attribute of neural crest cells, that of emerging from the neural tube. We injected the lipophilic dye Dil into the lumen of the neural tube of St.17 turtle embryos. Within a day after injection, DiI-positive cells can be seen in the carapacial ridge "staging area" that contains the HNK1-positive cells. Moreover, these cells form migratory streams going away from the dorsum. In addition, we have cultured neural tubes from St.17 embryos, and observed HNK1+ cells migrating away from them. Currently, we are in the process of comparing the molecular and functional properties of these late trunk neural crest cells with those of cranial neural crest cells. These data support our hypothesis that the plastron bones of the turtle are formed by a late emerging population of neural crest cells that collect dorsally in the carapacial dermis and then migrate ventrally.

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## Program/Abstract # 247 Preplacodal region marked by Six1 in mice Keiko Ikeda, Kiyoshi Kawakami Div. Biology, Jichi Medical University, Tochigi, Japan

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 preor pan-placodal region (PPR) located around the anterior neural plate.