Despite the fact that skeletogenic cells immerse themselves in abundant extracellular matrix, remarkably little is known about the influence of proteoglycans on skeletal cell differentiation *in vivo*. Here, we reveal the effects of a loss-of-function mutation in *xylosyltransferase1* (*xylt1*) on cartilage and bone development during zebrafish endochondral ossification. Xylosyltransferases initiate glycosaminoglycan side chain addition to core proteins during the formation of proteoglycans, such as *aggrecan*, which is expressed highly in cartilage. Accordingly, *xylt1*$^{-/-}$ zebrafish demonstrate decreased Alcian blue staining in cartilage elements undergoing endochondral ossification, although the patterning of these elements is roughly the same as wild-type siblings. At early stages of overt chondrogenesis in *xylt1*$^{-/-}$ mutants, the cartilage markers *sox9b* and *col2a1b* are expressed in similar domains as wild-type, but at decreased levels. Interestingly, *xylt1*$^{-/-}$ zebrafish also have increased and premature Alizarin red staining in perichondral bone, even though *xylt1* does not appear to be expressed highly in developing perichondrium. Osteoinductive factors, such as Indian hedgehog (Ihh), are expressed in maturing chondrocytes and are known to signal to osteoblast precursors in the overlying perichondrium. In support of a hypothesis that mutant cartilages prematurely initiate chondrocyte maturation, *ihh* genes are expressed in *xylt1*$^{-/-}$ cartilage earlier than in wild type. In total, these data suggest that proteoglycans play a positive role during initial stages of overt chondrocyte differentiation and a negative role during chondrocyte maturation.

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Program/Abstract # 452
Network topologies controlling the progressive process of endoderm specification in sea urchin embryos
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The molecular program controlling endoderm development ensures that cells deriving from specific embryonic cell lineages differentiate to contribute to one of the three compartments of the larval gut, the fore-, mid- or hindgut. In foregut progenitor cells, this process initiates in a way common to both mesoderm and endoderm cell lineages, which become differentially specified based on the presence or absence of Delta/Notch signaling, respectively. Analysis of the gene regulatory network driving endoderm specification in pre-gastrula stage embryos shows that shortly after the separation of mesodermal and endodermal fates in precursor cells of the larval foregut, a second endoderm specification program becomes activated in cells which will give rise to the larval mid- and hindgut. The two endodermal domains are marked by the expression of FoxA in the future foregut domain and Even-skipped in the future hindgut domain. A central component of both endoderm programs is encoded by the posterior Hox gene *Hox11/13b*, which drives the expression of Brachyury first in foregut progenitor cells and later in cells that give rise to more posterior parts of the gut. Even though the two endoderm networks involve an overlapping set of regulatory factors, the sequence and combination of regulatory gene deployment occurs differently. As a result of this, the anteriorposterior polarity of the later gut which ensures the proper patterning of fore-, mid- and hindgut, is established even before the onset of gastrulation.

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Program/Abstract # 453
The role of *twist1* and tissue interactions in ectomesenchyme specification
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In vertebrates, cranial neural crest cells have the remarkable ability to not only differentiate into neuroglial derivatives but also into ectomesenchymal derivatives such as the craniofacial skeleton. Whereas previous studies have made significant progress in understanding neuroglial lineage specification, less is known about the tissue interactions and signaling pathways that specify ectomesenchymal fates. Using zebrafish, we are testing possible specification mechanisms by several techniques. First by birthdating these cells in transgenic zebrafish, we find that the earliest born contribute to ectomesenchymal derivatives, suggesting a temporal model for specification. To identify early players, we first performed ablations of the neighboring head mesoderm and pharyngeal endoderm. Contrary to other reports, our data indicate that signals from the head mesoderm and pharyngeal endoderm are not necessary for ectomesenchyme specification. Thus, we infer that either cell intrinsic factors or ectodermal signals specify ectomesenchyme. Further, we looked at *twist1* as a possible cell intrinsic factor in regulating ectomesenchyme fates. *Twist1*−/− mice have been reported to have defects in the expression of ectomesenchyme markers. Similarly in zebrafish, we find that knockdown of both *twist1a* and *1b* results in loss of ectomesenchyme gene expression *fli1a* and in abnormal persistence of expression of early crest gene *sox10*. Surprisingly, expression of other ectomesenchyme genes like *dlx2a* are normal in these embryos. This suggests the presence of a parallel pathway to *twist1*. Currently, we are using transgenic approaches to identify other regulators of ectomesenchyme and a genomic set regulated by *twist1*.

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Program/Abstract # 454
Cooperative activity of Noggin and Gremlin in sclerotome specification
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The specification of the somite derivatives dermomyotome and sclerotome requires inductive signals from adjacent tissues. Briefly, lateral plate mesoderm-derived BMPs induce presumptive hypaxial musculature, Wnts secreted from the neural tube function with Shh to trigger epaxial musculature development, and Shh and Noggin function to specify sclerotome. While this general model has satisfactorily stood the test of time, it is clear that the differentiation and elaboration of somite-derived cell types involves a much more complex and dynamic balance of activating and restricting factors. In this study, we examined the roles of the BMP antagonists Noggin and Gremlin in somite patterning. We crossed homozygous conditional Noggin and Gremlin mutants to Noggin/− Gremlin/− beta-actin:cre/beta-actin:cre males to produce one-quarter double mutant embryos. We have analyzed cell type-specific markers in these embryos and have confirmed the loss of *pax1* and *pax9* expression in early somites, consistent with the loss of sclerotome. In contrast, although dermomyotomal marker expression is reduced, myogenesis is not eliminated in the double mutant animals, as indicated by expression of *pax3*, *myf5* and *lbx1*. We conclude that, despite being

expressed in different locations (Noggin in the midline, Gremlin in the somite), these antagonists cooperate in somite patterning. Based on the loss of somitic *patched*1 and *hhip* expression in double mutants, we hypothesize that Noggin and Gremlin-mediated inhibition of BMP is required for Hh signal transduction that leads to sclerotome specification.

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Program/Abstract # 455
The developmental changes in the competency of mesodermal cells to give rise to myotome fibers in *Xenopus laevis*
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Somitogenesis involves the periodic formation of somites along the anteroposterior (AP) axis. The movements that position cells within the somite and along the axis are not well understood. Using a fate mapping approach, we show that during gastrulation, mesodermal cells surrounding the blastopore lip undergo cell movements that lead to the formation of myotome fibers positioned in discrete locations within the somite and along the AP axis. In particular, presomitic mesoderm (PSM) cells positioned lateral to the prospective notochord give rise to myotome fibers found within the central domain of the somite and along the entire trunk axis whereas PSM cells from the lower blastopore lip form myotome fibers located in the dorsal and ventral regions of the somite and are restricted to the posterior trunk and tail. We propose that the regional differences in the final position of the myotome fibers are driven by the convergent and extension movements executed by the developing notochord. In addition, cell transplantations show that PSM cells grafted from the tailbud to the gastrula can delay their differentiation, whereas PSM cells grafted from the gastrula to the tailbud can accelerate their differentiation into myotome fibers. Although these experiments suggest that cells in the PSM of the gastrula and tailbud are similar, additional experiments show that PSM cells from the gastrula are not competent to form myotome fibers when grafted to a mature somite, whereas PSM cells from the tailbud are. These experiments suggest that signals present in the tailbud embryo are important for instructing PSM cells in becoming myotome fibers.

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