families are among the earliest markers of the presumptive neural crest and their expression is dependent on Wnt, FGF and BMP signaling. The pax3/7 and zic genes have undergone multiple duplications in the evolution of teleost fish. While many pax3/7 and zic paralogs are expressed during neural crest development, their expression is initiated at different times and is seen in different areas along the anterior-posterior axis. These expression differences could result from the gain or loss of regulatory elements in particular pax3/7 and zic genes or from modification of existing elements. We identified multiple paralogous enhancer families for the pax3/7 and zic genes and characterized their activity using transgenic zebrafish. The differences in activity of the enhancer paralogs mirror the differences in expression pattern among the genes they regulate. This indicates that the divergent expression patterns of genes in these families are partly the result of enhancer modification. To determine the nature of this enhancer paralog divergence we used small molecule inhibitors and heat shock-induced gene expression to knock down or upregulate components of the Wnt, FGF and BMP pathways in zebrafish lines containing reporter constructs with the enhancers. We found that pax3/7 and zic regulation involves the integration of multiple signals through multiple enhancers. The differences in activity among paralogous enhancers are partly the result of differences in response to these signaling pathways.

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Program/Abstract #484
Conserved functions of PAX3/7 during evolution
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Pax genes encode evolutionarily conserved transcription factors that play critical roles in development. Among these, the Pax3 and Pax7 genes arose by duplication from a unique ancestral Pax3/7 gene, and have similarities in their protein sequence and expression. Previously, we replaced Pax3 with Pax7 followed by an IRES-nlacZ reporter, using gene targeting in the mouse. Pax7 can substitute for Pax3 function in dorsal neural tube, neural crest cell (NCC), and somite development, but only partially in the formation of muscles involving long-range migration of muscle progenitor cells. We have now generated Pax3LampreyPax37-IRES-nlacZ/+ and Pax3AmphioxusPax37-IRES-nLacZ alleles in which mouse Pax3 was replaced by Lamprey or Amphioxus Pax3/7. Analysis of Pax3LampreyPax37-IRES-nlacZ/- and Pax3AmphioxusPax37-IRES-nLacZ/- embryos reveal that Lamprey and Amphioxus Pax3/7, similar to mouse Pax7, can compensate for Pax3 deficiency in dorsal neural tube, and somite development. Surprisingly, muscle progenitor cells migrate to hind limb buds, though Lamprey and Amphioxus do not form limbs. Moreover, we found that Amphioxus Pax3/7 can rescue the NCC migration despite Amphioxus not having NCCs. Our results suggest that the current functions of these factors were already present in the Pax3/7 protein before gene duplication at the onset of vertebrate evolution.

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Program/Abstract #485
Nourish and perish: Characterizing the nutritional endoderm in *Eleutherodactylus coqui*
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In *Xenopus laevis*, VegT induces nodal related TGFβ signaling, culminating with expression of Sox17, the endoderm specifier. *Eleutherodactylus coqui* is a direct developing frog and lacks a tadpole stage. In *E. coqui*, unlike *X. laevis*, the yolk-rich vegetal region lacking in EcVegT and EcVg1 RNAs functions as a nutritive tissue (NE: Nutritional Endoderm) only. The definitive endoderm (DE) comes from cells closer to the animal pole (Buchholz et al., 2007 Dev Dyn 236:1259–1272). We explored differences between NE and DE and asked whether lack of TGFβ signaling is involved in the formation of NE. An attempt to block nodal signaling in prospective DE cells in *X. laevis*, did not transform them into NE like cells but resulted in gross morphological changes and suppression to the contribution of mesodermal lineage. Presumptive NE cells in *E. coqui* become binucleated at blastula. Multinucleation starts at gastrula, and increases gradually until the frogs hatch. Similar to *X. laevis*, β catenin, a transcriptional cofactor of Sox17, shows nuclear localization in prospective DE at gastrula, but intriguingly in the NE as well. Accumulation of β catenin in NE continues in advanced stages of development. *E.coqui* oocytes have a stockpile of EcSox17 RNA which decreases sharply after gastrulation. Interestingly, the TGFβ EcActivin B and nodal EcDerriere are expressed in the presumptive NE cells despite their lack of mesoderm inducing activity. Given the presence of EcSox17, nuclear β catenin, EcActivin B, and EcDerriere in the yolk-rich cells, overexpression of these pathways rather than underexpression may play a role in the diverged fate of NE cells in *E. coqui*.

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Program/Abstract #486
Differential EcSmad2 expression in early development of the direct developing frog *Eleutherodactylus coqui*
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In *Xenopus laevis*, Nodal mediated phosphorylation of Smad2 leads to heterodimerization with Smad4 and translocation into the nucleus to turn on endoderm/mesoderm specific genes. Coqui frogs develop a novel tissue, nutritional endoderm, which consists of large yolky cells that provide nutrition to the embryo but disappear after yolk utilization (Buchholz et al., 2007 Dev Dyn 236:1259–1272). We hypothesized that the absence of Nodal signaling was responsible for such a situation. We investigated whether EcSmad2 expression is restricted to the prospective definitive endoderm and absent in the prospective nutritional endoderm by qPCR. There is a maternal contribution of EcSmad2 RNA and higher expression during early gastrulation stages followed by a steady decline. Both prospective definitive endoderm and nutritional endoderm have EcSmad2 RNA in early stages and expression is higher in definitive endoderm in post-gastrulation stages. Western blot analysis was performed to test the temporal and spatial expression of Smad2 and its activated form PSmad2. There is a maternal contribution of the protein and expression of both native and active protein increases as development progresses. There is an equal abundance of native and PSmad2 in both prospective definitive and nutritional endodermal tissues pre-gastrulation. After gastrulation, both native and PSmad2 persists in nutritional endoderm, although in a smaller amounts. The presence of EcSmad2 RNA and the native and active forms of the protein in nutritional endoderm suggests functions for this tissue other than just providing nourishment to the growing embryo.

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