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Prior studies in the developing chick embryo indicate that *Hoxd10* and *Hoxd11* have opposing effects on the specification of motoneuron subtypes within the lateral motor column (LMC) of the lumbosacral (LS) spinal cord. *Hoxd10* is initially expressed by newly differentiated motoneurons in all LS segments but later restricted to rostral LS motoneurons (~LS1–5). *Hoxd11*, in contrast, is expressed only in caudal LS segments (~LS4–8). When overexpressed in LS segments, *Hoxd10* promotes the development of motoneurons bearing molecular markers and projection patterns characteristic of lateral LMC (LMCI), while *Hoxd11* suppresses LMCI development. These effects mirror normal rostro-caudal differences in subtype distribution. *Hoxd11* also appears to regulate the extent of the LS LMC as a whole by direct or indirect downregulation of *Foxp1*, a transcription factor critical for LMC development, and upregulation of two factors that define the medial motor column (MMC), *Lim3* and *Scip*. To elucidate mechanisms of Hox action, we created a hybrid protein in which the DNA-binding homeodomain of *Hoxd10* was replaced with that of *Hoxd11* (*Hoxd10<sup>d11HD</sup>*). *Hoxd10<sup>d11HD</sup>*, when expressed in rostral LS segments, behaves in a manner similar to *Hoxd11*, and in direct opposition to *Hoxd10*, by suppressing development of the LMCI. However, it does not appear to affect total LMC size. We therefore propose that the repressive effects of *Hoxd11* on LMCI formation are mediated primarily by its homeodomain, and that the homeodomain is sufficient to direct some but not all *Hoxd11* actions.

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#### Program/Abstract # 487

##### Sensory neurons are required for migration and axon pathfinding of relay motor neurons

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Wiring the billions of neurons in the vertebrate central and peripheral nervous systems into functional circuits is one of the most complex processes in developmental neurobiology. A major challenge is to understand the logic underlying the assembly of neurons into functional circuits. Cell migration and axon pathfinding are critical patterning events that contribute to the assembly of neural circuits, but how these events are coordinated remains unclear. In the vertebrate head, we show that epibranchial placode-derived sensory neurons act as an intermediate target that coordinates the migration and axon pathfinding of parasympathetic relay motor neurons along the rostrocaudal axis of the body. In the absence of placodal sensory neurons, migratory neural crest destined for the postganglionic motor neuron fate undergo programmed cell death and axons of preganglionic motor neurons terminate abruptly in the area normally occupied by placodal sensory neurons, thereby failing to reach their distant target sites. Placodal sensory neurons are thus required for patterning the stereotypic relationship of relay motor neurons, presaging their ultimate integration into the sensory pathway of the parasympathetic reflex circuit.

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#### Program/Abstract # 488

##### The role of *Tgif* and *Tgif2* during head development

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Holoprosencephaly (HPE) is the most common forebrain malformation in humans. *Tgif* (TG-interacting factor) and *Tgif2* encode transcriptional repressors that regulate the TGF $\beta$  pathway via direct association with Smad proteins. In humans, loss-of-function mutations in the TGF $\beta$  gene cause HPE. During mouse embryonic development, both *Tgif* and *Tgif2* are widely expressed suggesting possible functional redundancy. *Tgif*;*Tgif2* double knock-out mouse embryos fail to gastrulate due to the ectopic upregulation of Nodal pathway. To dissect the possible role of *Tgif* and *Tgif2* during the development of mouse embryo proper, we generated mice with epiblast specific deletion of *Tgif* and null alleles of *Tgif2*. *Sox2Cre*;*Tgif<sup>f/r</sup>*;*Tgif2<sup>-/-</sup>* (*Tgif*;*Tgif2*cdko) embryos have defects of left-right patterning and anterior head structure. In the mutant embryos, the situs specific molecular markers, such as *Nodal* and *Pitx2*, are expressed bilaterally. Intriguingly, the phenotype was partially rescued in *Nodal<sup>lacZ/+</sup>*;*Tgif*;*Tgif2*cdko embryos when the dose of *Nodal* was genetically reduced. Importantly, *Tgif*;*Tgif2*cdko mutant embryos have HPE. Scanning EM analysis shows that *Tgif*;*Tgif2*cdko embryos lack the separation of rostroventral neural tissue at E9.25. Molecular analysis for *Shh* and *Fgf8* mRNA shows that rostroventral forebrain tissue formation is defective. These results suggest that the patterning of rostroventral brain tissue is impaired. Taken together, these results indicate that *Tgif* and *Tgif2* have significant roles during the patterning of neural tissue, presumably by regulating a TGF $\beta$  signaling pathway.

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#### Program/Abstract # 489

##### Mesodermal *Wnt4a* signaling regulates segmentation of head mesoderm and pharyngeal endoderm in zebrafish

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All vertebrates have a unique segmented structure in the developing head – the pharyngeal arches. The pharyngeal arches are composed of all embryonic germ layers. Whereas pharyngeal endoderm (PE) segmentation is essential for segmentation of other tissues in the pharyngeal arches, little is known about the tissue interactions and molecular pathways that drive PE segmentation. Using transgenic imaging approaches in zebrafish, we show intimate interactions of the lateral plate mesoderm (LPM) and PE during head segmentation. Next we show that *Wnt4a* controls segmentation of both the LPM and PE. *wnt4a* is expressed in the LPM directly adjacent to the PE just before and during PE segmentation. Reducing *Wnt4a* levels using a *Wnt4a*-morpholino (MO) blocks segmentation of the LPM and PE. Moreover, we use the UAS/*Gal4* system to manipulate *Wnt* signaling in specific tissues and show that inhibition of canonical *Wnt* signaling in LPM, but not PE, phenocopies the segmentation defects of *Wnt4a*-MO animals. In addition, transgenic expression of *Wnt4a* in LPM, but not PE, partially rescues the LPM and PE segmentation defects of *Wnt4a*-MO animals. Finally, nitroreductase-mediated LPM ablation also causes defects of PE segmentation. All together, these data show that 1.) mesodermal *Wnt4a* signaling is required autonomously for LPM segmentation and 2.) segmentation of LPM is essential for PE segmentation. In conclusion, our study reveals an unappreciated role of LPM in the initial establishment of vertebrate head segmentation.

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#### Program/Abstract # 490

##### Notch and *Fgf* signaling patterns the vertebrate dorsal face

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