

inner ear, trigeminal and distal cranial ganglia, as well as in somites and nephrogenous mesenchyme in mice. Cranial sensory organs and ganglia are derived from thickened ectoderm termed cranial placodes, which are derived from pre-placodal region (PPR), a continuous ectodermal region surrounding the neural plate. *Six1* is also known as a marker for the PPR and placodes. Analyses of *Six1*<sup>-/-</sup> mice revealed the essential roles of *Six1* in the development and morphogenesis of the organs where *Six1* is expressed. To identify the enhancers responsible for the expression of *Six1* during embryogenesis, we compared genome sequences around *Six1* loci among vertebrates and found out 16 conserved non-coding sequences (CNSs). The identified CNSs were hooked onto a minimal promoter with EGFP reporter and electroporated into chick embryos to monitor enhancer activities. We identified eight independent enhancers that showed specific expression similar to the endogenous *Six1* expression domains. The enhancer activities were confirmed in mice harboring the CNS upstream of minimal promoter with lacZ reporter. Elements for the CNS that showed expression in the PPR were analyzed by mutagenesis, and homodomain protein binding sites in the CNS were identified as essential for the enhancer activity in the PPR. The involvement of *Dlx5* and *Msx1* was suggested by overexpression and RNAi experiments in chick embryo. The evolution of *Six1* enhancers will be also discussed.

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#### Program/Abstract # 471

##### Dual functions of the miR-10 locus miRNAs in refinement of Hox gene expression

Derek Lemons, William McGinnis

Department of Biol., UCSD, La Jolla, CA, USA

Controlled regulation of gene expression is essential to proper development. This control can be imposed at nearly every step between initiation of transcription and the eventual degradation of a protein. The discovery of miRNAs demonstrated pervasive post-transcriptional regulation by an ever expanding group of small RNAs which can be expressed in temporally and spatially restricted patterns similar to protein coding genes. The miR-10 locus, which resides in between the *Hox4* and *Hox5* orthologs in most bilaterian animals, encodes two functional miRNAs miR-10 and miR-10\*, which have highly conserved complementary sequences in the 3'UTRs of insect *Scr* and *Abd-B* orthologs respectively. These miRNAs and *Hox* genes in *Drosophila* are expressed in highly complementary and largely non-overlapping domains, suggesting that while the miR-10 miRNAs do not contribute to the gross pattern of *Hox* gene expression, they are responsible for maintaining precise and developmentally robust expression patterns.

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#### Program/Abstract # 472

##### Segmental origin and Hox dependence of neural crest-derived otic ganglion

Yi-Ju Chen, Masumi Takano-Maruyama, Gary O. Gaudio

Department of Biol., Univ. of Texas at San Antonio, San Antonio, TX, USA

Classic studies in chick-quail chimeric embryos show that parasympathetic motor ganglia arise from preotic and postotic segments of the developing hindbrain. Although this observation provides a broad view of the segmental origin of parasympathetic ganglia, it suggests that individual ganglion may arise from rhombomere (r)-specific neural crest cells (NCCs). In turn, the NCCs may be controlled by the

determinants of rhombomere identity, the *Hox* genes. To address these issues, we performed genetic fate maps of *Hox* gene-expressing Cre and ROSA-EYFP lineage reporter mouse lines to label NCCs originating from specific rhombomeres along the rostrocaudal axis. The identification of individual parasympathetic motor ganglion derived from specific *Hox* lineage reporter lines was subsequently matched with corresponding *Hox* knockout mice to determine its dependency on *Hox* gene function. Using a *Hoxa3* lineage reporter line, we show that the otic ganglion, whose fate had not been previously mapped, originates from r6. We found that r6 NCC-derived otic ganglion is independent of *Hoxa3* and *Hoxb3*, the *Hox3* paralogous (P) genes known to synergize in r6, but instead require the *Hox1P* genes, *Hoxa1* and *Hoxb1*. In the absence of the *Hox1P* genes, the otic ganglion is almost eliminated. This defect is associated with increased apoptosis and loss of dorsal rhombomere identity, as indicated by the absence of *Kreissler/Mabf* protein expression, which normally labels r5 and r6. These findings suggest that individual parasympathetic motor ganglion originates exclusively from a single rhombomere and depends on the combined function of *Hox* paralogous genes.

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#### Program/Abstract # 473

##### Pax7-SUMOylation and neural crest development

Zhidong Luan, Martin I. Garcia-Castro

Department of Mol. Cell. and Dev. Biol., Yale University, New Haven, CT, USA

The paired-box transcription factor Pax7 is expressed in the dorsal neural tube, neural crest cells (NCCs) and somite tissues during vertebrate development. Pax7 is also expressed in muscle satellite cells during adulthood and has been shown to be critical for muscle homeostasis. Recently Pax7 was shown to be a required early marker of NCC precursors. Despite its apparent relevance, little is known about how Pax7 operates, whether it plays a similar role in all these cells or if it provides specific traits to all or any of them. In an effort to further our understanding of the distinct role(s) played by Pax7 during NCC development, we performed a yeast two hybrid screen and identified the SUMOylase enzyme Ubc9 as a novel Pax7 partner. We have verified the interaction of Pax7 with Ubc9 through GST pull down assays and present *in situ* hybridization and immunostaining expression data suggesting their co-expression. Furthermore, *in vitro* and *in vivo* experiments demonstrate the SUMOylation of Pax7, and suggest an early role during neural crest development. Additionally this study unveils an unexpected enrichment of SUMOylation machinery in the neural plate border where prospective NCCs reside. We further provide evidence of the requirement of the SUMO pathway, during early neural crest development.

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#### Program/Abstract # 474

##### The role of zebrafish zic genes in neural crest development

Jessica Pierson<sup>a</sup>, Molly Nyholm<sup>b</sup>, Yevgenya Grinblat<sup>b</sup>

<sup>a</sup>CMB Program, UW-Madison, Madison, WI, USA

<sup>b</sup>Department of Zool. and Anat., UW-Madison, Madison, WI, USA

Zic genes encode a conserved family of zinc finger transcription factors. We are focused on *zic2a* and *zic5*, which are closely linked and similarly expressed at the neural plate border and throughout the dorsal neural tube during neurula stages. Studies in mouse and *Xenopus* have identified *zic2a* and *zic5* as important regulators of neural crest (NC) induction and perhaps migration, but have not explored these roles in detail. We have observed a severe reduction in jaw cartilage formation in embryos injected with morpholinos that