

We have previously described a signaling center in birds, the Frontonasal Ectodermal Zone (FEZ) that regulates growth and patterning of the frontonasal process (FNP). The FEZ is comprised of ectoderm flanking a boundary between *Sonic hedgehog* (*Shh*) and *Fibroblast growth factor 8* (*Fgf8*) expression domains within the FNP ectoderm. Mechanisms that govern formation of the FEZ are unknown, but the expression patterns of *Bmps* and their receptors suggest that BMP signaling could play a role. Our objective was to assess the role of BMPs during FEZ formation. We blocked BMP signaling in the FNP prior to FEZ formation by injecting a replication competent avian retrovirus encoding the BMP antagonist *Noggin* into the mesenchyme beside the forebrain at HH stage 10. We assessed FEZ formation 72 h after infection ( $\sim\beta$ HH22) and observed that *Shh* expression was reduced or absent and *Fgf8* expression was not affected. We also observed changes in gene expression in neural crest cells. *Bmp2* expression was absent and *Bmp4* expression was expanded proximally. Embryos developing for 72 and 96 h after infection exhibited malformations of the FNP that indicated the FEZ did not form. Electroporating a constitutively active BMP receptor into the ectoderm 24 h after infection ( $\sim\beta$ HH14) partially rescued this phenotype. These data indicate that BMP signaling mediates interactions between the neural crest mesenchyme and the ectoderm of the FNP to regulate FEZ formation. We are currently assessing the signaling and patterning properties of the FEZ that forms after blocking BMP signaling in the FNP.

doi:10.1016/j.ydbio.2007.03.605

#### Program/Abstract # 305

##### Bmp signalling in the epibranchial placodes

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A number of lines of evidence have highlighted a role for bone morphogenetic proteins (BMPs) in the formation of the epibranchial placodes. It has been shown that the pharyngeal endoderm induces the formation of the epibranchial placodes via the action of BMP7. More recently, we have found that *Bmp4* is expressed in the placodes themselves. We have also analyzed the expression profiles of members of the Cerberus/Dan family of BMP antagonists, and we find that the location and timing of PRDC expression within the endoderm of the arches coincides with epibranchial placode formation. These observations strengthen the idea that BMP signaling has an important and ongoing function in the formation of the epibranchial placodes. It is likely that BMP7 has a role in inducing the placodes and BMP4 a role in maintaining them, and that the activity of these two factors is modulated by PRDC. We are currently investigating the precise roles of BMP signaling, and the importance of the levels of BMP activity, for placode development using both gain of function and loss of function approaches in the chick embryo.

doi:10.1016/j.ydbio.2007.03.606

#### Program/Abstract # 306

##### Study of *Xenopus* orthologs of novel genes expressed in the mouse AVE

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The classical transplantation experiments of Spemann and Mangold and the molecular characterization of Spemann organizer have established that the amphibian organizer could be subdivided into head and trunk inducing regions. Organizer genes also exhibit different patterning properties. Cerberus, dickkopf1 or frzb can induce anterior neural structures, whereas chordin or noggin can only induce more caudal structures. In the peri-gastrulation mouse embryo, the orthologs of the organizer genes are found mainly in the anterior end of the primitive streak, its derived axial mesendoderm and in the extra-embryonic anterior visceral endoderm (AVE). Together with the orthologs of secreted head inducers of the organizer, several transcription factors with known roles in forebrain specification are also expressed in the AVE before gastrulation. Hence, the AVE was proposed to be the mouse head-organizer. In order to further characterize the molecular mechanisms involved in the early forebrain induction, we have carried out a screen for genes differentially expressed in the AVE. Interestingly most of the *Xenopus* orthologs of novel genes expressed in AVE were detected in the dorsoanterior endoderm, a region considered to be the topological equivalent of the AVE. The combined results of the studies conducted in these two species will further contribute to the understanding of how the vertebrate head is induced and patterned.

doi:10.1016/j.ydbio.2007.03.607

#### Program/Abstract # 307

##### Autonomous sorting & surface segregation of primitive endoderm in mouse embryoid bodies

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The genesis of the primitive endoderm can be mimicked in vitro by the aggregation of either dissociated embryonic stem cells or F9 embryonal carcinoma cells in suspension to form embryoid bodies (EBs). We present morphological evidence that nascent primitive endoderm cells are first born within the interior of EBs and translocate concomitantly to the surface exterior. Furthermore, we have generated heterotypic EBs by mixing undifferentiated F9 cells and F9 cells differentiated into endoderm by prior exposure to retinoic acid. Within composite EBs the