embryos often display abnormal cell division with a skewed division plane. As a result, elongated but arrested early embryos were observed. In the same screen, a temperature sensitive allele, ram(wx72ts), was isolated. ram(wx72ts) does not complement ram-6(wx66) and was mapped to same narrow overlapping region of ram-6(wx66), suggesting that they are allelic. Temperature shift experiment with ram(wx72ts) defines the functional requirement at an overlapping developmental window as the other ram genes. Genetic mapping and SNP mapping located ram-6 on the right arm of chromosome V leading to our successful rescue of ram-6 mutants by YAC microinjection. Experiments are currently underway to molecularly characterize ram-6 and to dissect its role in sensory rays morphogenesis. (This study is funded by the Research Grants Council, Hong Kong.)

doi:10.1016/j.ydbio.2006.04.222

200 Enhanced BMP signaling through a type I BMP receptor ALK2 shows ectopic cartilage formation in mouse craniofacial portion
Yoshihiro Komatsu 1, Tomokazu Fukuda 1, Gregory Scott 1, Nobuhiko Kamiya 1, Kenichi Yamamura 2, Yuji Mishina 1
1 Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences/NIH, Research Triangle Park, USA
2 Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto, Japan

ALK2 (also known as AVCR1 or ActR1) is one of the BMP type I receptor and has important roles during mouse embryogenesis as shown by loss of function studies. However, gain of function studies for ALK2 signals have not been carried out in the mouse to address its sufficiency among developmental procedures. To avoid the potential lethality caused by overdose of BMP signaling, we employed the Cre-loxP system out in the mouse to address its sufficiency among develop-
ment. Objective: To examine the role of BMP signaling by bone morphogenetic proteins (BMPs) in mouse craniofacial portion. ALK2 shows ectopic cartilage formation in mouse craniofacial portion. ALK2 activated by P0-Cre (caALK2) in a neural crest specific manner. CaALK2 activates BMP signaling pathway in cranial neural crest cell lineages.

doi:10.1016/j.ydbio.2006.04.223

201 Characterization of cuticular collagen gene ram-2 in sensory ray morphogenesis
C.N. Tam, W.S. Hui, K.L. Chow
Hong Kong University of Science and Technology, Hong Kong

Cuticular collagens make up a large protein family in C. elegans. Recently, we have identified three cuticular collagen genes, mutations of which display only abnormal morphology of the sensory rays. We report here the characterization of ram-2, its possible role and transcription regulation in sensory ray morphogenesis. Ram-2 is required for the anterior dorsal migration of the ray cells as shown in the mutants of 3 available alleles. Based on the RNAi phenotype and Df mapping, bx32 allele behaves as a temperature sensitive dominant negative allele. Since different collagen encoding ram genes display non-allelic non-complementation property, it is possible that they may be partners constituting the same collagen triplex. ram-2 expression is initially observed in 6 hypodermal nuclei ventral to the male tail seam. The expression declines upon reaching adulthood. Since the temporal expression of ram-2 overlaps with the ray formation period, we delineate its transcriptional regulation and screen for transcriptional regulator controlling the initiation of ray morphogenesis. Promoter deletion analysis of using reporter marker and cDNA rescue assays identified two essential cis elements within 50 bp consisting of a GATA binding site. Subsequently, three potential candidate genes regulating the ram-2 transcription were identified in a genetic screen. The biological significance will be discussed. (The research is funded by Research Grants Council, Hong Kong.)

doi:10.1016/j.ydbio.2006.04.224

202 Twisted gastrulation (Twsg1) is critical for the morphogenesis of the medial region of the mandibular arch
Anna Petryk 1, Michael P. Jarcho 1, Nick Lowe 1, Mina Mina 2, Rajaram Gopalakrishnan 1
1 Univ. of Minnesota, Minneapolis, USA
2 Univ. of Connecticut Health Center, Farmington, USA

Background: The mandibular component of the first branchial arch (mdBA1) gives rise to the mandible. Growth of the medial region of mdBA1 during embryonic life is largely dependent on signaling by bone morphogenetic proteins (BMPs). Twsg1 is a secreted protein that binds to BMPs and modulates their activities. Twsg1 is expressed in the developing BA1 and its deficiency in mice results in agnathia with variable degrees of accompanying craniofacial defects. Our hypothesis is that agnathia in Twsg1 null mice results from excessive BMP signaling in the medial region. Objective: To examine the role of Twsg1 in morphogenesis of BA1. Design/Methods: Twsg1+/− mice were generated by gene targeting using Cre-loxP technology. Histological evaluation and skeletal preparations were performed at birth and in situ hybridization at
embryonic stages E9.5 and E10.5. Results: mdBA1 was medially fused in the mutants at E9.5. At birth, the mandible and anterior two-thirds of the tongue were missing. The BA1-derived malleus and incus were variably affected. Maxillary incisors were missing, but the molars were preserved. Expression of the medial markers eHAND and dHAND was significantly diminished, while expression of the lateral marker Barx1 was preserved. The expression of Msx2, which is downstream from BMP signaling and mediates BMP-induced apoptosis was expanded. Studies of cell death and proliferation in the medial region are ongoing. Conclusions: Twsq1 is involved in morphogenesis of the medial region of BA1. Funded by NIH HD33692 to A.P and AHC Seed grant # 03-14 to R.G.

doi:10.1016/j.ydbio.2006.04.225

203 BMP signaling and the temporal control of mandibular osteogenesis
Amy E. Merrill, Brian F. Eames, Scott J. Weston, Thayer Heath, Richard A. Schneider
Univ. of California, San Francisco, CA, USA

Identifying mechanisms that control the timing of skeletal differentiation is a prerequisite for understanding normal and abnormal craniofacial development. Toward this goal, we investigate molecular signaling interactions that establish when bone forms in the mandible. We employ an avian chimeric system that allows us to manipulate time-dependent signaling events by exploiting the divergent maturation rates of quail and duck embryos. We transplant populations of neural crest mesenchyme, which are the osteogenic precursors of the mandible, from quail to duck embryos, and find that quail donor mesenchyme maintains its faster timetable for osteogenesis within the slower environment of duck hosts. We hypothesize that bone forms prematurely in these chimeras as a result of donor mesenchyme altering the timing of signaling interactions, which are necessary for bone formation. Because such signaling interactions are known to be mediated by members of the Bone Morphogenetic Protein (BMP) family, we first defined the precise stages during which signaling interactions are required for mandibular osteogenesis, and then identified those BMPs that appear to mediate these signaling interactions based on donor-induced changes to their spatiotemporal expression patterns. To compliment these experiments, we also ascertained the potential of exogenous BMPs to regulate the timing of skeletal differentiation in an organ culture system. Together, our results demonstrate that osteogenic mesenchyme controls the timing of osteogenesis by regulating BMP signaling, and that BMPs are sufficient to induce the premature differentiation of bone.


204 Spatiotemporal regulation of mandibular neuromusculoskeletal integration
Christian Mitgutsch, Brian F. Eames, Katie Au, Noel Sinth, Angelo Kaplan, Richard A. Schneider
Univ. of California, San Francisco, CA, USA

During embryogenesis, neural, muscular, skeletal, vascular, and connective tissues form structurally and functionally integrated anatomical complexes. Our goal is to identify developmental mechanisms that control and synchronize the formation of these complexes. To do so, we exploit the divergent developmental programs of quail and duck, capitalizing on the fact that these birds have considerably different morphologies and rates of maturation. We compare the morphogenesis of the neuromusculoskeletal system within the mandible of quail and duck using molecular, immunocytochemical, and histological methods. Our results reveal the precise order in which jaw structures differentiate and become highly integrated. Moreover, by creating quail–duck chimeras, we gather evidence that neural crest cells play an essential role in mediating these processes. Neural crest cells appear to control the rate at which bone and cartilage differentiate and determine the size and shape of individual elements. These cells also influence the course of cranial nerve development and affect the differentiation and morphology of the associated jaw muscles. Thus, our analyses suggest that the spatiotemporal integration of elements in the craniofacial complex results from neural-crest-mediated hierarchical organization and modularization, developmental properties that have likely played an important role during the evolution of the vertebrate head.

doi:10.1016/j.ydbio.2006.04.227

205 Local and systemic mechanisms controlling osteogenesis
Andrew H. Jhee, Brian F. Eames, Richard A. Schneider
Univ. of California, San Francisco, CA, USA

Discovering molecular mechanisms that control the development of craniofacial bones, which are derived from the cranial neural crest (CNC), is necessary for preventing birth defects, eliminating invasive surgeries, and augmenting hard tissue wound healing. To achieve this end, we employ a powerful avian transplant system that exploits the significant difference between quail and duck in the rates of their maturation (quail hatch in 17 days, whereas duck hatch in 28 days). Unilateral transplantation of premigratory CNC cells from a quail donor into a stage-matched duck host results in the generation of chimeric quck. In these quck, quail donor cells differentiate according to their own timetable and secrete bone matrix (osteoid) earlier in comparison to duck host cells. Furthermore, cartilage hypertrophy, angiogenesis, and osteoclast recruitment all occur earlier in regions derived from quail. Thus, CNC regulates the differentiation of its deriva-