Program/Abstract #510
How somitic cells migrate into the axolotl limb bud and vertebrate appendicular muscle evolution
Elizabeth Sefton, Nadine Piekarski, James Hanken
Harvard University, Cambridge, MA, USA

Skeletal muscles of the vertebrate trunk and limbs are derived from somites. There are different mechanisms proposed for how somitic cells reach the limb bud. It is presumed that the ancestral mode involves an epithelial extension of the somites, as represented by chondrichthyans and some teleost fishes. The derived mode, where individual muscle progenitor cells migrate from the somites into the fin/limb bud, has been reported in chickens, mice and zebrafish. This finding in zebrafish led to the conclusion that the genetic mechanism for tetrapod limb muscle development evolved prior to the radiation of sarcopterygians. However, in studies of non-avian reptiles, the ancestral mode of epithelial extensions of the somites has been reported. Amphibians represent a key group to further unravel the evolutionary history of limb muscle development. Previous studies in amphibians have been complicated due to the delayed development of limb buds relative to the somites. We use transgenic fate mapping techniques in the axolotl to analyze the mode of limb muscle formation. Furthermore, we characterize the somitic cells contributing to the limb by examining the expression of migrating muscle precursor markers, including lbx1 and mox2. The results obtained from the axolotl will provide further insights into the evolution of appendicular muscle development.

doi:10.1016/j.ydbio.2011.05.572

Program/Abstract #511
The embryonic origin of the axolotl skull (*Ambystoma mexicanum*)
Nadine Piekarski, James Hanken
Harvard University Organismic & Evolutionary Biology, Cambridge, MA, USA

The vertebrate skull is derived from two different embryonic cell populations, neural crest and mesoderm. The fates of these cell populations and their respective contributions to cranial cartilages and bones have been studied in great detail using the quail-chick chimeric system. Enabled by technical advances, such studies have been extended recently to vertebrates other than birds, such as mouse and the African clawed frog, *Xenopus laevis*. This work has facilitated a comparative consideration of the embryonic origin of the vertebrate skull based on experimental data. They are important topics that remain to be elucidated, such as the extent of interspecific differences in skull segmentation and variability in the embryonic origin of specific bones, e.g., frontal and parietal. We are trying to address these issues by extending the fate-mapping approach to a urodele, the Mexican axolotl (*Ambystoma mexicanum*). Unlike *Xenopus*, axolotls are obligately neotenic, and thus do not metamorphose. The skull is initially cartilaginous, but develops bones in later stages of development. We performed long-term fate mapping experiments of cranial neural crest using GFP-transgenic axolotls, to assess its contributions to the skull. These experiments may reveal patterns of derivation unique to anurans and salamanders, or even to amphibians. In a broader context, new data obtained will enhance our knowledge of constrained and variable features of skull derivation, and of the evolution of the vertebrate head in general.

doi:10.1016/j.ydbio.2011.05.573

Program/Abstract #512
Major shifts in the evolution of somitogenesis: The reptile *Anolis carolinensis* represents a fourth type of segmentation clock among vertebrates
Walter Eckalbar[a], Eris Lasku[a], Carlos Infante[b], Dale DeNardo[a], Jonathan Losos[c], Alan Rawls[a], Jeanne Wilson-Rawls[a], Kenro Kusumi[a]
[a]*Arizona State University, Tempe, AZ, USA*
[b]*University of Georgia, Athens, GA, USA*
[c]*Harvard University, Cambridge, MA, USA*

In vertebrates, while the hairy-enhancer of split (hes) genes are the core oscillators of somitogenesis, the mechanism by which hes genes drive Notch receptor activation has evolved divergently. In teleosts, Notch is cyclically activated by deltaC ligand, but in mammals and birds, delta ligands do not display oscillatory expression and Notch is cyclically inhibited by Lunatic fringe. In mammals, Notch is further inhibited by Dll3, a non-cycling, divergent ortholog of deltaC, which is absent from avian genomes. To better understand the evolution of somitogenesis, we identified key regulators of somitogenesis in the *Anolis carolinensis* lizard model, which is the first sequenced non-avian reptile, and found a surprising divergence from other vertebrates. In *Anolis*, lunatic fringe is not a cycling gene; it is expressed in somites but absent from the presomitic mesoderm (PSM). Intriguingly, unlike any other vertebrate, the *Anolis* dll1 orthologue displayed cycling expression in the PSM. The dll2 *Anolis* gene, a divergent ortholog of X-Delta-2, deltaC, and Dll3, is dynamic in expression level. Other components of the segmentation clock are conserved with other vertebrates, including hes7 cycling and the expression of tbx6, fgf8, and mesp2. No components of the Wnt or FGF pathway, which are oscillatory in mammals, were found to cycle in *Anolis*. These findings suggest there have been at least four major switches in the evolution of somitogenesis. Further molecular analysis of unstudied groups, such as chelonian and crocodilian reptiles and caudate amphibians, will help address this hypothesis. Acknowledgments: The Broad Institute for pre-publication release of Anolis genomic sequence; ASU School of Life Sciences.

doi:10.1016/j.ydbio.2011.05.574

Program/Abstract #513
Morphology and regression of the dental lamina
Marcela Buchtova[a], Oldrich Zahradnícek[b], Eva Janecková[c], Eva Matalova[c], Abigail S. Tucker[d]
[a]*Academy of Sciences Instit of Animal Physiology & Genetics, Brno, Czech Republic*
[b]*Charles University, Prague, Czech Republic*
[c]*Brno, Czech Republic*
[d]*King´s College London Dental Institute, London, UK*

Tooth development initiates with the formation of an oral epithelial thickening. This thickening grows deeper into the mesenchyme and forms the dental lamina. Here, we focus on differences in the development of the dental lamina among mono- (chameleon) and polyphyodont (python, pit-viper, gecko) reptiles and mono- (mouse) and diphyodont (pig) mammals. We aim to compare the timing of dental lamina development, with specific focus on the initiation of replacement teeth, comparing the structure and cell dynamics of the dental lamina in different species. Dental lamina growth was angled in the lingual direction for all investigated species. The formation of tooth germs in the monophyodont species was initiated in close proximity to the oral epithelium, however chameleon tooth germs in contrast to mouse developed as asymmetrical structures with a large cervical loop on the lingual side. The replacement lamina formed in diphyodont species as the primary dentition reached the late bell stage. As the replacement generation