conditionally removed Fgf8 from urethral epithelium. Surprisingly, Fgf8 is not necessary for initiation, outgrowth or normal patterning of the genitalia. Analysis of 22 Fgfs revealed no redundancy in the urethral epithelium, in contrast to the situation in the AER. To determine whether the Fgf8 pathway is activated during external genital initiation, we examined 4 downstream targets and show that these markers are either absent from early genital tubercles or are not regulated by Fgf8. Mapping of Fgf8 protein distribution indicates that Fgf8 is undetectable in the genital tubercle. Thus, Fgf8 is transcribed but not translated, and the pathway is not activated. Finally, a phylogenetic survey of Fgf8 expression in amniote genitalia reveals expression in eutherian and metatherian mammals, but absence from turtles and alligators, indicating that Fgf8 expression is neither a required nor a conserved feature of amniote external genital development. We propose that Fgf8 expression is an early readout of the genital initiation signal rather than the signal itself, and suggest that cloacal ectoderm may be the source of the initiation signal.

doi:10.1016/j.ydbio.2009.05.419

Program/Abstract # 390
Sonic hedgehog controls growth of external genitalia by regulating cell cycle kinetics
Ashley W. Seifert[a], Brandi K. Ormerod[b], Martin J. Cohn[a]
[a]*Department of Biol., Univ. of Florida, Gainesville, FL, USA*
[b]*Pruitt Family Department of Biomedical Engineering, Univ. of Florida, Gainesville, FL, USA*

Embryonic development requires tight coordination of pattern formation and growth. *Sonic hedgehog* (*Shh*) has been implicated in both of these processes, and recent work in the limb and brain has suggested that specification of positional identity and elaboration of pattern involves Shh-mediated expansion of progenitor cell pools. Shh has been shown to regulate expression of several cell cycle genes, although how these interactions influence the rate of growth is not understood. In this study we show that, in the developing genitalia, Shh signaling controls growth by regulating the length of the cell cycle. Conditional inactivation of Shh in the genital tubercle extends the cell cycle from 8.5h to 14.4h, and growth of the genitalia is reduced by 75. The early molecular pattern of the genital tubercle is surprisingly normal in the absence of *Shh*, however genes that regulate the G1/S transition are downregulated and the duration of G1 is extended. This leads to fewer cells entering S-phase, which reduces the number of progenitor cells and, ultimately, the size of the genitalia. The ability of Shh to regulate cell number by controlling the length of specific cell cycle phases identifies a novel mechanism by which Shh regulates growth during organ patterning. These results have additional implications for understanding hedgehog pathway-mediated tumor progression and heterochronic changes during morphological evolution.

doi:10.1016/j.ydbio.2009.05.420

Program/Abstract # 391
Functional redundancy of Smad1/5 signaling in BMP-mediated mouse limb interdigital programmed cell death
Yuk Lau Wong[a], Richard R. Behringer[b], Kin Ming Kwan[a]
[a]*Department of Biology, The Chinese University of Hong Kong, HKSAR, China*
[b]*Department of Genetics, UTMDACC, Houston, TX, USA*

Programmed cell death (PCD) is not only important in regulating development and homeostasis in multicellular organisms but also sculpting the shape and structure of developing limb. It is coordinated by reciprocal epithelialmesenchymal interactions between specialized regions of limb bud under the effects of different signaling factors. Bone morphogenetic proteins (BMPs) are secreted signals shown to regulate PCD in developing limb but the intracellular molecular components and pathways remain largely unknown. Smad1 and Smad5 are two of the logical candidates as they are intracellular mediators of BMPs signaling. Knock-out of BMPs and its signaling components results in early embryonic lethality that hinders their studies in limb formation. To circumvent the problem and allow studies of Smads in developing limb by loss-of-function approach, Cre/loxP system was employed to inactivate Smad1 and/or Smad5 in developing limb ventral ectoderm by the use of Smad1 and/or Smad5 floxed alleles and Engrailed1-Cre-recombinase transgene that express in ventral ectoderm and apical epidermal ridge. Our preliminary data showed that either inactivation of Smad1 or Smad5 resulted in phenotypic abnormalities in limb. However, Smad1/5 double conditional mutant has syndactyly. Thus, Smad1/5 are required and function redundantly in BMPs-mediated interdigital PCD. Experiments including cell proliferation, cell death assay and in situ hybridization are being performed to investigate possible molecular mechanisms of Smad1/5 in regulating interdigital PCD.

doi:10.1016/j.ydbio.2009.05.421

Program/Abstract # 392
Sall genes regulate region-specific morphogenesis in the mouse limb by modulating Hox activities
Yasuhiko Kawakami[a], Yukako Uchiyama[c], Concepcion Rodriguez Esteban[b], Toshiaki Inenaga[c], Naoko Koyano-Nakagawa[a], Merce Marti[d], Marie Kmita[e], Paula Monaghan-Nichols[f], Ryuichi Nishinakamura[c], Juan C. Izpisua Belmonte[b,d]
[a]*Stem Cell Ins., Univ. Minnesota, USA*
[b]*Gene Exp. Lab., Salk Institute, USA*
[c]*Kumamoto Univ., Japan*
[d]*CMR-Barcelona, Spain*
[e]*IRCM, Université de Montréal, Canada*
[f]*Department of Neurobiol., Univ. Pittsburgh, USA*

The genetic mechanisms that regulate the complex morphogenesis of generating cartilage elements in correct positions with precise shapes during organogenesis, a fundamental question in developmental biology, is still not well understood. By focusing on the developing mouse limb, we confirm the importance of transcription factors encoded by the Sall gene family in proper limb morphogenesis, and further show that they have overlapping activities in regulating regional morphogenesis in the autopod. Sall1/Sall3 double knockout (dKO) mutants exhibit defects in the autopod. We show that Sall activity affects the Shh signaling pathway and the Hox network. Shh signaling is partially impaired in the Sall mutant limbs. Additionally, our data suggest an antagonism between Sall1Sall3 and Hoxa13Hoxd13. We demonstrate that expression of Epha3 and Epha4 is downregulated in the Sall1/3 dKO mutants, and conversely, upregulated in Hoxa13 and Hoxd13 mutants. Moreover, the expression of Sall1 and Sall3 is upregulated in Hoxa13 and Hoxd13 mutants. Furthermore, we show that Sall and Hox compete for a target sequence in the Epha4 upstream region. In conjunction with the Shh pathway, the antagonistic interaction between Hoxa13Hoxd13 and Sall1Sall3 in the developing limb may contribute to fine-tuning local Hox activity that leads to proper morphogenesis of each cartilage element of the vertebrate autopod.

doi:10.1016/j.ydbio.2009.05.422