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Program/Abstract # 437
Processing of Lunatic fringe protein by subtilisin/furin-like proprotein convertases contributes to its short intracellular half-life
Emily T. Shifley, Susan E. Cole
Department of Molecular Genetics, The Ohio State University, Columbus, OH, USA

During vertebrate segmentation, oscillatory activation of Notch1 signaling is important in the clock that regulates the timing of somitogenesis. In mice, the cyclic activation of Notch1 requires the periodic expression of Lunatic fringe (*Lfng*). For LFNG to play a role in the segmentation clock, its cyclic transcription must be coupled with post-translational mechanisms that confer a short protein half-life. LFNG protein is cleaved and released into the extracellular space. We hypothesize that this secretion contributes to a short LFNG intracellular half-life, facilitating rapid oscillations within the segmentation clock. To test this hypothesis, we localized N-terminal protein sequences that control the secretory behavior of fringe proteins. We find that LFNG processing is promoted by specific pro-protein convertases including furin and SPC6. Mutations that alter LFNG processing do not prevent its secretion, but do alter its intracellular half-life. These mutations do not affect LFNG function in the Notch pathway, thus protein half-life affects the duration, but not the specificity of LFNG activity. Targeted mutation has been used to express Golgi-tethered LFNG from the endogenous locus allowing us to examine the *in vivo* effects of altered LFNG processing on oscillatory Notch signaling in the segmentation clock. These results have important implications for the mechanisms that contribute to the tight control of Notch signaling during vertebrate segmentation.

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Program/Abstract # 438
Evidence for *Hox*-specified positional identities in adult vasculature
Nathanael D. Pruett [a], Richard Visconti [b], Dimitri Scholz [a], Alexander Awgulewitsch [a]
[a] *Department of Medicine, MUSC, Charleston, SC, USA*
[b] *Department of Cell Biology, MUSC, Charleston, SC, USA*

The role of *Hox* transcriptional regulators in establishing positional identities during embryonic patterning is well documented; however, the activity of this conserved gene family in adult tissues is only recently being elucidated. The existence of positional information within the cardiovascular system in particular remains poorly studied. The paucity of information regarding *Hox* gene activity in vascular tissues, including in vivo expression data—a prerequisite for defining functional roles, leaves much to be discovered and offers great potential for significant advancements in our understanding of cardiovascular disease mechanisms. To gain insight into the global vascular activity of *Hox* proteins we selected several genes for *lacZ*-based transgenic mouse reporter gene analysis: *Hoxc10* and *Hoxc11*, which harbor posteriorly restricted embryonic expression patterns, and *Hoxa3*, whose expression is more anterior. In this study we show that these genes exhibit zonal vascular patterns that are reminiscent of the distinct A–P activity domains found during embryonic patterning. Medial, vascular smooth muscle cells (VSMCs) that originate from the same site have the potential for differential responses to various stimuli (hemodynamic stress, hypoxia, cell signaling, etc.); the molecular determinants specifying these cell phenotypes are currently obscure. *Hox* activity profiles (*Hox* code) might pre-determine these response options—an idea consistent with the original concept of *Hox* genes acting as selector genes that establish compartment-specific differentiation pathways in the embryo.

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Program/Abstract # 439
Only posterior interdigit provides positional information to its anterior PFR to specify each digit identity
Takayuki Suzuki [a,b], Sean M. Hasso [a], Toshihiko Ogura [b], John F. Fallon [a]
[a] *Department of Anatomy, University of Wisconsin, Madison, WI, USA*
[b] *Department of Developmental Neurobiology, IDAC, Tohoku University, Sendai, Japan*

The zone of polarizing activity is the primary signaling center controlling anterior posterior patterning of the amniote limb bud. The autopodial interdigits (IDs) are secondary signaling centers proposed to specify digit identity, through an as yet unidentified signal or signals. Here we focus on the digit and define a region of the digital ray that we name the phalanx-forming region (PFR) that expresses Sox9, Bmpr1b, and is phosphorylated-SMAD1/5/8 positive. The PFR cells are committed to the cartilage lineage, then respond unidirectionally to ID signals and finally are incorporated into the digit primordium. Using a novel in vivo reporter assay, we establish that each PFR has a unique SMAD1/5/8 activity, developing in a spatially- and temporally-restricted manner; this activity correlates with digit identity. Using our data, we propose a model that incorporates data from human, mouse, and chick, and provides a mechanism for understanding formation and variation of digits (number, size, and shape of phalanges) among amniotes, as well as a mechanistic explanation for human defects such as brachydactyly type A2. In this conference, we will show detail in which how only posterior interdigit provides positional information to its anterior PFR to specify each digit identity.

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Program/Abstract # 440
Sonic Hedgehog signaling in the apical ectodermal ridge is essential for proper patterning of the vertebrate limb
Cortney M. Bouldin [a], William J. Scott [b], Brian D. Harfe [a]
[a] *Department of Molecular Genetics and Microbiology, Genetics Institute, University of Florida, Gainesville, FL 32610, USA*
[b] *Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio 45229, USA*

Sonic Hedgehog (*Shh*) in the developing limb has been shown to mediate the action of the Zone of Polarizing Activity (ZPA). Through the analysis of *Shh* target genes, for example *Ptc1* and *Gli1*, it is known that the *Shh* signaling cascade is active in the limb mesoderm. Recently, array-based experiments have found that a number of target genes of the *Shh* signaling pathway were also present in the limb apical ectodermal ridge (AER). To investigate the possibility of *Shh* signaling in the AER, SHH protein was immunolocalized in the limb bud ectoderm including the apex. A *Ptc1LacZ* knock-in mouse was used to detect *Ptc1* in the posterior AER of the developing limb. To determine if *Shh* signaling in the AER plays a role in limb patterning,

we used a conditional knockout approach. *Smo*, a component of the Hedgehog signaling pathway, was removed from the limb AER using the *Msx2Cre* transgene. Removal of *Smo* from the AER resulted in the production of a post-axial extra digit. Quantitative analysis of the *Smo*-AER knockout mouse revealed that the length of the AER was increased after *Smo* loss. Our data suggest that Shh signaling, in addition to its known role in the limb mesenchyme, is also required in the AER for proper limb patterning.

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Program/Abstract # 441
Dual, separable roles of Sonic Hedgehog in limb bud patterning and expansion
Jianjian Zhu, Eiichiro Nakamura, Minh-Thanh Nguyen, Xiaozhong Bao, Susan Mackem
Laboratory of Pathology, NCI, NIH, Bethesda, MD, USA

Sonic hedgehog (Shh) functions as a limb morphogen to specify a distinct pattern of digits. Current models predict that the highest levels, or longest duration of Shh signals specify the most posterior digit type. Although Shh regulates proliferation in many contexts, it is unclear how effects on cell number in the limb bud relate to Shh's role in specifying digit identity. Deleting the mouse Shh gene at different times using tamoxifen-activated recombination of a conditional mutant allele, we find that Shh functions to control limb development in two phases: a very transient, early patterning phase regulating digit identity, and an extended growth-promoting phase during which the digit precursor mesenchyme expands and becomes recruited into condensing digit primordia. Our analysis reveals an unexpected alternating posterior-anterior sequence of normal mammalian digit formation (4-2-5-3), rather than an overall posterior-to-anterior progression as previously proposed. The progressive loss of digits upon sequentially earlier removal of Shh activity mirrors this alternating sequence, highlighting the role of Shh in limb expansion to produce the normal complement of digits, which can be temporally uncoupled from patterning. Such uncoupling may serve as one avenue for evolutionary adaptations in digit number without disturbing normal morphology. Ongoing work is aimed at evaluating the contribution of Shh effects on proliferation for digit formation and examining the conservation of digit condensation sequence in different vertebrates.

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Program/Abstract # 442
Regulation of differential gene expression in the neural tube by the morphogen Sonic Hedgehog
Nikos Balaskas [1], Kate Hill, Vanessa Ribes [1], James Briscoe *
Developmental Neurobiology, National Institute for Medical Research, London, NW7 1AA, UK
[1] *These authors contribute equally to this work.*

Shh patterns the dorso-ventral (D/V) axis of the neural tube by controlling the expression of transcription factors in progenitor cells. One such factor is Nkx2.2, which is expressed in response to high and prolonged levels of Shh signalling. Analysis of the regulatory elements controlling Nkx2.2 has revealed a 250 bp conserved non-coding element, sufficient to drive Nkx2.2 expression. Among other transcription factor binding sites, this element contains a Gli-binding site (GBS). Mutational analysis suggests that the GBS is necessary but not sufficient for Nkx2.2 expression. In vivo assays suggest that correct Nkx2.2 expression requires both positive and negative inputs from other transcription factors. Using mutational analysis and BAC recombineering our aim is to identify these factors and to dissect the mechanisms that control Nkx2.2 expression. In addition, by comparing the regulatory control of Nkx2.2 with other progenitor expressed transcription factors, we aim to understand the transcriptional network that underlies D/V patterning of the neural tube.

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