John Saunders’ classic apical ectodermal ridge (AER) removal experiments performed over 60 years ago demonstrated that the AER is required for proximodistal (PD) outgrowth and patterning of the limb. Despite intense investigation, the mechanisms whereby the AER regulates these processes remain poorly understood. We propose that one of the primary roles of the AER is to regulate directional growth of the adjacent limb mesenchyme. First, we show that Fgf signaling is necessary and sufficient to activate Wnt5a expression in gradient fashion in the limb mesenchyme. We also demonstrate that Wnt5a/Ror2 signaling is necessary and sufficient for directional growth of the limb mesenchyme. Taken together, we propose that the AER directs polarized growth of the adjacent mesenchyme through establishment of a Wnt5a gradient. Because the AER, through Wnt5a, regulates directional growth of the mesenchyme, it follows that its shape will in turn play a crucial role in shaping the mesenchyme it recruits. We report that the AER is almost circular at the time of its induction which would be predicted to recruit a cylindrical population of mesenchyme which condenses to form the stylopod. Over time, the AER extends along the anteroposterior (AP) and thins along the dorsoventral (DV) axes in a manner consistent with the formation of the wider (AP) and thinner (DV) zeugopod and autopod elements. These results highlight a novel morphogenetic paradigm: the dimensions of recruitment signaling centers ultimately shape organs. This model also provides mechanistic insight for evolutionary change.

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Program/Abstract # 95
Twist Function in Limb Morphogenesis
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Twist1 has been demonstrated to play critical roles in the early development of neural crest and mesodermally derived tissues. Twist2 has been less well characterised but its relatively late onset of expression suggests specific roles in the development of a number of sites. We have used RCAS-mediated overexpression to investigate the function of Twist2 in limb development. Expression of Twist2 within the developing limbs begins prior to formation of the limb bud and persists within the peripheral mesenchyme until digital rays condense when Twist2 expression becomes restricted to the interdigital mesenchyme. Viral misexpression following injection into the lateral plate mesoderm results in a spectrum of hypoplastic limb phenotypes. These include generalized shortening of the entire limb, fusion of the autopod skeletal elements, loss of individual digits or distal truncation resulting in complete loss of the autopod. These phenotypes appear to result from a premature termination of limb outgrowth. In situ hybridisation analysis demonstrates that many components of the Shh/Fgf/Gremlin regulatory loop that controls early limb outgrowth are downregulated by Twist2 overexpression. However, despite loss of Twist2 Fgf8 and other regulatory loop factors such as Gre1, Shh expression is sustained at normal levels. Twist2 is endogenously co-expressed with Shh but is complementary to Gre1. This suggests that Shh and Gre may be transcriptional targets of Twist2. These data indicate that Twist2 regulates limb morphogenesis through control of the Shh/Fgf/Gre autoregulatory loop.

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Program/Abstract # 96
Dos Lunatic fringe play a distinct role in tail development?
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During vertebrate segmentation, paired somites bud from the presomitic mesoderm (PSM). The Notch pathway, and a key Notch modulator Lunatic fringe (Lfng), play multiple roles during segmentation. In the posterior PSM, cyclic Notch activity and Lfng expression function in the segmentation clock, timing somitogenesis. In the anterior PSM, stable Lfng expression is involved in pre-somite patterning. Recent results from our lab indicate that oscillatory Lfng expression in the clock is required during formation of the anterior skeleton (primary body formation), but is largely dispensable during tail development (secondary body formation). In contrast, we find that Lfng expression in the anterior PSM, during R/C somite patterning, is required during tail development. Specifically, we find that mice that retain this expression form largely normal tails, while tail truncation is observed in Lfng null animals. This suggests the possibility that Lfng activity in the anterior PSM may regulate long-range signals that are required for tail outgrowth. To address the functional requirements for Lfng during tail development, we are examining secondary body development in embryos that lack Lfng, as well as embryos that retain Lfng expression only in the anterior PSM. In addition, we are examining gene expression in embryos that retain Lfng expression only in the anterior PSM to identify genes and pathways that may be critical for tail extension. These studies will elucidate the role of Lfng during tail outgrowth and further test the hypothesis that the Notch pathway plays distinct roles during primary and secondary body formation.

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Program/Abstract # 97
SIX1 initiates branching morphogenesis by regulating gremlin 1 expression in the metanephric mesenchyme, which acts to locally restrict BMP4 activity
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Urinary tract morphogenesis requires subdivision of the ureteric bud (UB) into tip- and trunk-specific domains, which will differentiate into intra-renal collecting system and the extra-renal ureter, respectively. We report here that Six1-/- UB tip cells failed to form a tip-specific domain. Failure of invasion into the metanephric mesenchyme (MM), the mutant UB elongates within the peripheral mesenchyme which acts to locally antagonize BMP4 activity to induce UB outgrowth and branching morphogenesis. We find that the expression of the extra-cellular BMP antagonist Gremlin1 in the MM is SIX1-dependent. Treatment of mutant kidney rudiments in culture with recombinant GREM1 protein restores ampulla formation and its subsequent branching morphogenesis. As GREM1 acts as an antagonist of BMP4 to induce UB outgrowth and branching morphogenesis, we tested whether genetically lowering BMP4 activity could restore kidney organogenesis. Indeed, genetic reduction of BMP4 levels in Six1-/- embryos restores urinary tract morphogenesis and kidney formation. This study provides the first direct evidence for the requirement of Six1 in spatially restricting BMP4 signaling in the mesenchyme during the initiation of UB patterning and branching morphogenesis. Our results indicate that Six1 acts by regulating Grem1 in the MM to locally antagonizing BMP4 activity to ensure that the UB tip cells are induced for ampulla formation and its subsequent branching. This finding uncovers an essential function for SIX1 as an critical upstream regulator of Grem1-mediated BMP4 signaling during urinary tract morphogenesis.