(*Dlx5/6*). *Dlx5/6* induce expression of *Hand2*, a basic helix-loop-helix transcription factor. This pathway places *Hand2* at the center of a complex signaling cascade, but little is known of its function in mammalian craniofacial development since *Hand2*$^{-/-}$ embryos die around embryonic day (E) 10.5 from vascular failure. To bypass these defects, we created a conditional deletion of *Hand2* using a traditional Cre-*lox*P system. Using the *Wnt1-Cre* mouse line, we delete *Hand2* within all migrating NCCs. Mutant mice exhibit severe craniofacial defects including mandibular hypoplasia, a single incisor, aglossia, and loss of tympanic rings and Meckel's cartilage. These changes are preceded by aberrant maintenance of *Dlx5/6* expression in the distal mandibular arch and subsequent upregulation of *Runx2* expression. In vitro studies show that Hand2 is able to repress the Dlx5/6 enhancer, I56i. This suggests that Hand2 functions by repressing *Dlx5/6* expression within the distal midline. In its absence, *Dlx5/6* expression is maintained and results in expression of *Runx2* followed by the repatterning of distal tongue mesenchyme to bone.

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Program/Abstract # 91
Gain-of-function in Ras signaling perturbs dental development in mouse and human
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Ras/MAPK signaling is critical in animal development, and RTK signaling, which activates Ras signaling, is known to play an important role in tooth development. Our previous work has shown that increasing Ras/MAPK signaling by inactivating Sprouty genes adversely affects tooth morphogenesis. Here, we directly examined the effects of activating Ras/MAPK signaling in both humans and mice. Costello Syndrome (CS) is caused by a heterozygous de novo germline mutation in HRAS that results in a constitutively active Ras protein. We examined a cohort of CS patients and identified a number of craniofacial and dental anomalies. We found that a large majority of patients presented with pronounced enamel hypoplasia. Microcomputed tomography of exfoliated primary teeth from CS patients showed a significant decrease in enamel thickness compared to controls. We next examined the CS mouse model and found that the mice also had an enamel defect. Further inspection revealed disorganization of the ameloblasts in the mouse incisor. We are currently studying cell proliferation and polarity of the amelobasts in the mutant mouse incisors. In addition, we are using an ameloblast-like cell line to determine the effects of increased Ras/MAPK signaling on the behavior of the cells.

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Program/Abstract # 92
The control of inner ear morphogenesis by Sprouty and Tbx1 genes in mouse models of 22q11.2 deletion syndrome
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DiGeorge/Velo-caldio-facial/22q11 deletion syndrome (22q11DS) is one of the most common microdeletion disorders, characterized by severe malformations of many organ systems. Most of the patients have some form of hearing loss and about 10% have defects in the structure of their inner ears. However, while for some people the condition is severe, for others it is not, and it is not yet known what causes the differences between individuals. Haploinsufficiency for the *TBX1* gene has been linked to 22q11DS defects, including inner ear defects. We have recently identified another group of genes, the Sprouty (*Spry*) genes, that regulate inner ear development. Mice with loss of function mutations in the *Spry1* and *Spry2* genes have abnormally shaped cochleas and semi-circular canals. To determine whether these genes interact with *Tbx1* during ear development, we generated *Spry1-/-;Spry2-/-;Tbx1* embryos and examined their inner ear structures by paint-fill metod. *Spry1-/-;Spry2-/-;Tbx1* embryos have a more severe phenotype than *Spry1-/-;Spry2-/-* or *Tbx1* mutants. We found that the several signaling pathways implicated in inner ear development are deregulated in the *Spry1-/-;Spry2-/-* otic vesicle and that these effects are further enhanced by *Tbx1* haploinsufficiency. These data suggest that Sprouty genes have the potential to modify inner ear development in patients with 22q11DS.

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Program/Abstract # 93
The Role of FGF Gradients in the Regulation of Early Limb Growth
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While molecular biology can identify the molecular components regulating tissue growth, by itself it can not explain how embryos determine their shape and size. FGFs, which control cell proliferation, differentiation, migration and survival, are key molecules in embryonic morphogenesis. In this paper, we use a reaction-diffusion model for morphogen diffusion and a Glazier-Graner-Hogeweg multi-cell model to simulate numerically the role of FGF4 and FGF8 in regulating the early growth of the vertebrate limb. FGF diffusion, decay and secretion, and cell growth in response to FGF concentrations, determine the shape and size of limbs, and hence more generally, of tissues and organs during embryonic development. Physiologically reasonable values for FGF secretion, diffusion and decay grow a simulated limb with correct shape, size and antero-posterior asymmetry. We show that the limb mainly expands by growth of the distal domain which has high FGF concentrations and that the distalized expansion locks the region of high FGF concentration into the distal tip. We conclude that the interaction between growth and FGF gradients dominates regulation of the proximo-distal and antero-posterior outgrowth of the limb and the FGF distribution.

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Program/Abstract # 94
The Limb Mesenchyme Recruitment Model for Patterning the Vertebrate Limb
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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 AER 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
Does 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 willdifferentiate 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 *Tbx18*- and *Bmp4*-expressing mesenchyme for its differentiation into ureter. We find that the expression of the extra-cellular BMP antagonist *Grem1* 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 acritical upstream regulator of Gremlin-mediated BMP4 signaling during urinary tract morphogenesis.

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