Title
A follistatin-BMP7 feedback circuit controls taste papillae development and patterning in mouse tongue

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occurred. Overexpression of *TBX22* caused a striking decrease in proliferation but did not change the level of apoptosis. Furthermore we identified two targets of *TBX22* that could be mediating the phenotype, *DLX5* and *MSX2*. We have therefore demonstrated novel functions for *TBX22*, a gene that causes some forms of human orofacial clefting.

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Program/Abstract # 494
A point mutation in Arid1a reveals an essential role for this SWI/SNF subunit in extraembryonic blood vessel and trophoblast development

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Arid1a is a signature subunit of the mammalian SWI/SNF ATP-dependent chromatin remodeling complex. We performed an ENU mutagenesis screen for Arid1a coding mutations and generated mice carrying a valine-to-glycine point mutation in the ARID DNA binding domain of Arid1a. Although mutant protein is expressed near wild-type levels and capable of interacting with its catalytic subunit, Brg1, it appears to display reduced DNA binding capacities in vitro. Homozygous mutant embryos undergo development arrest by E10.5 and exhibit defects in the trophoblast placenta and extraembryonic vasculature, including a compacted labyrinth layer and reduced vascular branching. These data suggest the ARID domain of Arid1a is essential for development.

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Program/Abstract # 495
The role of Friend of GATA in primitive red blood cell development

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The transcription factor GATA-1 and its cofactor Friend of GATA (FOG) are required to promote embryonic red blood cell (RBC) development in mice. In contrast, the current model in *Xenopus*, based on overexpression studies, predicts that FOG inhibits RBC development by recruiting the transcriptional co-repressor C-terminal Binding Protein (CtBP). To resolve these seemingly contradictory findings, we have used morpholinos to perform a loss-of-function study in frogs. We find that in *Xenopus*, as in mice, FOG is in fact required for RBC development. Specifically, targeted injection of FOG morpholinos into the ventral blood-forming mesoderm of 8-cell *Xenopus* embryos results in a dose-dependent loss of *globin* expression at the tailbud stage. In addition, we find that over-expression of both wildtype FOG and mutant FOG isoforms that either lack known repressor binding domains, or harbor mutations at key GATA-interaction residues, also result in loss of blood. Together, these studies suggest that FOG is required for RBC development and that loss of blood seen with FOG overexpression is likely due to a dominant-interfering effect by which excess FOG sequesters other co-factors required for RBC development away from endogenous target promoters. Specific domains of FOG required for RBC development *in vivo* have not yet been elucidated. We are currently asking whether various FOG mutant constructs can rescue RBC formation in FOG morphants. This will allow us to determine which functional domains of FOG are required for normal erythropoiesis, and may suggest novel binding partners that are important for FOG's role during RBC development.

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Program/Abstract # 496
Stacked expression of Hand2 and Dlx mediates signaling from Edn1 to produce discrete pharyngeal arch patterning domains

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Recent studies have suggested that Endothelin1 (Edn1) acts in a dose dependent fashion to pattern skeleton from the first two pharyngeal arches into dorsal, intermediate, and ventral domains via its targets *hand2* and Dlx. We hypothesized that *hand2* expression defines the ventral domain, in part by repressing intermediate domain genes. We further hypothesized that Dlx genes pattern the intermediate domain. Third, we propose that the combined patterning from *hand2* and Dlx delineates ventral/intermediate domains from dorsal. Here we demonstrate that *hand2* is expressed next to *edn1*, and the expression of all Dlx genes extends dorsal to *hand2*. We provide evidence that *dlx3b*, *dlx4b*, and *dlx5a* are redundantly required for intermediate domain patterning. Furthermore we show that by 36 hpf, *dlx3b*, *dlx4a*, and *dlx4b* are specifically expressed in intermediate arch mesenchyme. Previous work demonstrated that *hand2* is required for ventral cartilage formation. We confirmed this with two alleles of *hand2*. We further show that in *hand2* mutants, *dlx3b*, *dlx4a*, and *dlx4b* expression expands into the ventral domain at 36hpf. Finally, when Dlx-MO is injected into *hand2* mutants, both ventral and intermediate defects are seen, and the ventral-most structures may acquire dorsal shape. Collectively our work suggests that the stacked expression of *hand2* and Dlx mediates signaling from Edn1 to generate ventral and intermediate domains with distinct identities separate from dorsal.

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Program/Abstract # 497
A follistatin-BMP7 feedback circuit controls taste papillae development and patterning in mouse tongue

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Interactions between epithelium and mesenchyme are thought to drive development and patterning of taste papillae, but the identities of the mesenchymal signals are unknown. Using mouse genetics, we show that *Fst*, which is expressed in tongue mesenchyme during development, controls these processes in both anterior (normally gustatory) and posterior (normally non-gustatory) lingual epithelium. In anterior $Fst^{-/-}$ tongue there are increased numbers of *Sox2*+ taste progenitors, with fungiform papillae of abnormal size and spacing. In posterior $Fst^{-/-}$ tongue, ectopic *Sox2*+ epithelial domains develop and non-gustatory filiform papillae are absent. Increased *Bmp7* expression is evident in regions of ectopic *Sox2*+ progenitors, and

further experiments indicate that the phenotypes in *Fst*$^{-/-}$ tongue result from loss of FST-mediated antagonism of a BMP7 positive autoregulatory loop in lingual epithelium. Incorporation of these findings with information about other molecular interactions within the epithelium leads us to propose a model in which *Wnt* and *Shh* serve as an activator/inhibitor pair to pattern taste papillae along the tongue dorsum through diffusion-driven instability, and the FST-BMP7 loop functions to suppress spatial noise within this circuit. Computational experiments lend support to such a model.

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Program/Abstract # 498
Cdx2 regulates patterning of the intestinal epithelium
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The *Cdx* genes encode homeodomain transcription factors related to *caudal* in *Drosophila*. The three mouse homologues, *Cdx1*, *Cdx2* and *Cdx4*, are essential for proper vertebral anterior–posterior patterning in all vertebrate model systems examined to date. *Cdx1* and *Cdx2* (but not *Cdx4*) are also expressed in the intestinal epithelium during development and expression persists throughout the lifespan of the mouse. *Cdx1* null mice exhibit homeotic transformations of the axial skeleton, but no phenotype of the intestine has been described. *Cdx2* null mice die at embryonic day 3.5 (E3.5) due to a failure of implantation and preventing assessment of the null phenotype at later stages. *Cdx2* heterozygous mice display vertebral homeoses and occasional polyps in the colon and small intestine. Areas of metaplasia within these lesions exhibit an esophageal-like keratinized epithelium, suggestive of a transformation of the intestinal epithelium to a more anterior (stomach) character, and supporting a critical role for Cdx2 in the patterning of the intestinal endoderm. To more fully address the role of Cdx2 in the intestine, we are using a tamoxifen-inducible villin-Cre transgenic, which is expressed in the definitive intestinal endoderm commencing at E9.5 to inactivate a floxed *Cdx2* allele, thus circumventing the early lethality inherent to Cdx2 null embryos. Results from this conditional mutant are consistent with a role for Cdx2 in patterning the small intestine.

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Program/Abstract # 499
Reciprocal repression between the transcription factors Nkx6.1 and Ptf1a determines cell fate choice of multipotential pancreatic progenitors
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Limited knowledge of the molecular mechanisms that regulate endocrine b-cell differentiation in the pancreas of has thus far precluded the development of a viable, cell-based therapy for diabetes mellitus. A key unanswered question is how multipotential pancreatic progenitors become specified to adopt an endocrine or an exocrine fate. By combining genetic lineage tracing, loss of function, and misexpression experiments in mice, we demonstrate that reciprocal repression between the transcription factors Nkx6.1 and Ptf1a patterns the undifferentiated pancreatic progenitor epithelium into a pre-endocrine and pre-exocrine domain. Our data shows that Nkx6.1 establishes a pre-endocrine domain by directly repressing the exocrine fate determinant Ptf1a. Compared to wild type embryos, Nkx6.1 mutants display an expansion of the pre-exocrine domain, as marked by Ptf1a, as well as a loss of endocrine cells. Furthermore, constitutive and heritable expression of Nkx6.1 in multipotential progenitors is sufficient to block exocrine differentiation and directs progenitors into an endocrine fate. Conversely, misexpression of Ptf1a in pancreatic progenitors suppresses Nkx6.1 expression and prevents endocrine cell differentiation. Overall, these results suggest that pancreatic endocrine and exocrine cell fate are established in undifferentiated, multipotential progenitors by a cross-inhibitory loop between Nkx6.1 and Ptf1a.

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Program/Abstract # 500
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Abstract #500 will be presented as scheduled, but will not be published due to lack of license agreement between authors and publisher.

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Program/Abstract # 501
Foxa transcription factors and the intervertebral disk
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The intervertebral disk (IVD) is composed primarily of two parts: an outer annulus fibrosus, composed mainly of collagen and an inner gel-like nucleus pulposus. We have shown that the nucleus pulposus is derived from the embryonic notochord in mice. Herniation of the nucleus pulposus results in back pain. Though this affects millions of people, there are few effective treatments for chronic back pain. Insight into the mechanisms of IVD development and degeneration could lead to better treatment of back pain. The forkhead box (Fox) family of transcription factors is required both for embryonic development and post-natal life. The Foxa1 and Foxa2 genes are expressed in the endoderm, notochord, and floorplate of the developing embryo. Their roles have been extensively characterized in endodermally-derived organs, but relatively little is known about their role in the notochord; a Foxa2 null mouse dies *in utero* lacking this structure. The Foxa1 null allele and a Foxa2 conditional allele under the control of various Cre recombinases have been used to study the development of the lung and liver. Using these alleles with a tamoxifen-inducible Sonic hedgehog (Shh) Cre recombinase, we are examining the role of Foxa genes in the formation of the nucleus pulposus. Preliminary results in newborn mice null for Foxa1 and lacking Foxa2 in all Shh-expressing cells have a severely deformed nucleus pulposus. Further study of the role of Fox genes in the formation of