The dermis promotes the development and supports functional components of skin such as hair follicles, sweat glands, nerves, and blood vessels. In the chick, the dermis originates from the somites, the lateral plate mesoderm, and cranial neural crest. Despite the importance of dermis in the structural and functional integrity of the skin, genetic analysis of dermal development in different parts of the embryo is incomplete. First, we show that mouse ventral dermis originates from the lateral plate mesoderm. Next, we demonstrate that Wnt/β-catenin signaling is active and necessary during the development of ventral dermal cells. Loss of β-catenin function in the flank mesenchyme leads to the absence of ventral dermis in the mouse embryo. Wnt/β-catenin signaling is required for cell survival and is sufficient for mouse ventral dermal cell specification. Despite the different origins of dorsal and ventral dermal cells, this study reveals new roles for β-catenin/Wnt signaling during early dermal cell development. This is the first study to define the origin and signaling requirement of mammalian ventral dermis.

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Program/Abstract # 134
Role of nectins in the development of epithelial appendages
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Nectins are immunoglobulin-like cell adhesion proteins which function in cell–cell junctions and cell–cell contacts. Mutations in the nectin-1 gene are responsible for a rare human syndrome characterized by ectodermal dysplasia (ED), cleft lip and palate, and limb defects. The nectin-1 null mutant mice were reported to have a mild defect in epidermal stratification [Wakamatsu et al. J. Biol. Chem. 2007 282:18173–81] and our analysis revealed additionally a subtle defect in the formation of dental enamel but we did not detect other obvious phenotypes. In order to explore the possibility that the function of nectin-1 is compensated by nectin-3, we first compared their expression patterns. In E15 mouse embryos nectin-1 was expressed in the suprabasal layer of epidermis. Nectin-1 and nectin-3 were coexpressed in the inner root sheath of hair follicles and in the stellate reticulum cells of the tooth buds. We then generated nectin-1 and nectin-3 double null mice. They exhibited characteristics of human ED patients, including skin, tooth, hair and limb abnormalities. I will present the phenotypic analysis of the nectin-1−/−; nectin-3−/− mice. These results suggest that nectin-1 and nectin-3 are required for the development of epidermis and epithelial appendages, and that the necessary function of nectin-1 in humans is partially compensated by nectin-3 in the mouse.

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Program/Abstract # 135
Fgf2b signaling integrates tooth morphogenesis and dental axon patterning
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Dental trigeminal nerve fiber growth and patterning are strictly integrated with tooth morphogenesis, but it is still unknown, how these two developmental processes are coordinated. We show that targeted inactivation of the dental epithelium expressed Fgf2b results in cessation of the mouse molar tooth development at the degenerated cap stage and the failure of the trigeminal molar nerve to establish the lingual branch at while the buccal branch develops properly. This axon patterning defect correlates to the histological absence of the mesenchymal dental follicle and adjacent Semaphorin3A-free dental follicle target field as well as appearance of ectopic Sem3A expression domain in the lingual side of the tooth. Tgfbeta1, which controls Sem3A, and Fgf4, which induced Tgfbeta1, were absent from the Fgf2b−/− tooth. Fgf4 beads rescued Tgfbeta1 in the Fgf2b−/− and Tgfbeta1 induced de novo Sem3A in the dental mesenchyme. Collectively these results demonstrate that epithelial Fgf2b, by mediating local epithelial–mesenchymal interactions, integrates tooth morphogenesis and dental axon patterning during odontogenesis.

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Program/Abstract # 136
Development of successional teeth
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The epithelial thickening in the oral epithelium is the first stage of tooth development. This thickening grows deeper into the mesenchyme and forms the dental lamina. Here, we focus on differences in dental lamina development between monophyodont, diphyodont and polyphodont species. Mouse, as the main model for tooth developmental study, forms only one tooth generation. The dental lamina stage is reduced in time and shortly after lamina establishing, individual epithelial anlagen bud off to form distinctive teeth. In the contrast, pig with two generations of teeth, develops well-established dental lamina that protrude deeply into mesenchyme. In the middle of prenatal development, the dental lamina loses the connection to oral epithelium after initiation of the second generation. In python, the ribbon-like lamina forms up to four generations of successional teeth and maintains the connection to oral epithelium in the pre-hatching period. Our previous study on snakes provided evidence that expression of Shh is first confined to the odontogenic band and defines the position of the future dental lamina (Buchtova et al., in press). Based on studies in snakes as well as work done by others on shrews and mice, we predict that Shh expression in pigs will first be detected in the odontogenic band. However unlike mice but similar to the snake, we expect to see prolonged expression in the oral–dental interface. Because pig fetus has a second generation of teeth developing just as humans, they represent an important mammalian model system for identifying the key signals needed to initiate the successional teeth.

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Program/Abstract # 137
Characterization of Tmem16f in vertebrate development
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TMEM16F is one of the ten homologues in the mouse and human TMEM16 family of proteins, a structurally related group of proteins
obtained a gene trapped allele of mouse TMEM16E and TMEM16G, which have roles in determining cell morphology. The widespread embryonic expression and evolutionary conservation of this gene family suggest that the TMEM16 proteins may play important roles during vertebrate development. We have recently expressed in cancerous tissue and several, including TMEM16A, TMEM16E and TMEM16G, may have roles in determining cell morphology. The widespread embryonic expression and evolutionary conservation of this gene family suggest that the TMEM16 proteins may play important roles during vertebrate development. We have recently obtained a gene trapped allele of mouse Tmem16f (Tmem16f^{fl/fl}) in which a β-galactosidase/neomycin resistance construct integrated into an intron of Tmem16f. RNA in situ hybridization and X-gal staining in our lab demonstrated expression of Tmem16f in a variety of developing tissues including the developing bones. We are presently characterizing the expression pattern and function of this gene in the mouse model. In addition, we are determining its functional relationship to other members of the TMEM16 family.

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Program/Abstract # 138
Emx2 in limb dorsalization
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Lmx1b is a homeodomain transcription factor known for its role in limb dorsalization, i.e. knockout (KO) mice have a ventral–ventral limb phenotype. Yet, little is known about downstream targets or the underlying mechanisms of limb dorsalization. Emx2 is expressed in the prospective scapula, a dorsal limb girdle component. Early Emx2 expression in the proximal dorsal region of the limb has been reported which partially overlaps Lmx1b expression. To demonstrate a potential relationship between Emx2 and Lmx1b, we compared Emx2 gene expression data from gene arrays of Lmx1b KO and wild type mouse limbs during joint and tendon formation (i.e., 11.5–13.5 day post coitum (dpc)). Limbs with shoulder girdles were harvested at 11.5/12.5, while at 13.5 dpc only the distal limbs were used. RNA was extracted and gene expression was determined using Affymetrix 430 2.0 mouse genome arrays. Whole-mount in situ hybridization was performed using Lmx1b and Emx2 dig-labeled RNA probes. Differential expression of Emx2 was 2.6 fold at 11.5 dpc and 5.3 fold at 12.5 dpc in the presence of Lmx1b. Unexpectedly, the distal limb bud at 13.5 dpc exhibited an Lmx1b-related differential increase in Emx2 (2.2 fold). Emx2 expression was localized to the proximal dorsal limb and along the adjoining flank mesenchyme in 11.5 dpc mice. By 13.5 dpc, expression could be observed in the shoulder girdle and additionally in the dorsal autopod region, especially within the predicted extensor tendons. This expression was absent in Lmx1b KO mice. These data suggest that Emx2 is a target of Lmx1b regulation during limb dorsalization and support a role for Emx2 in extensor tendon specificationdevelopment.

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Program/Abstract # 139
The role of FGF4 and FGF8 in posterior development of the mouse embryo
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FGF signaling has been shown to be required for proper posterior development of the mouse embryo, both during the process of gastrulation and during elongation and segmentation. Although FGF Receptor I (FGFR1) has been identified as a critical player in embryo elongation and segmentation, the FGF ligands involved in this process have not been conclusively identified. Using conditional alleles of Fgf4 and Fgf8 and the HoxB1 cre driver, we show that expression of these FGFs is absolutely critical for the continued generation of mesoderm during posterior elongation. A reduction in Fgf8 mRNA can be detected by E8.5, and examination of pea3 expression suggests that FGF signaling is lost by E9.5. Although paraxial mesoderm production and somite formation cease by E9.5, embryos survive until birth with severe axial truncations. While abnormal thoracic vertebrae and ribs are formed, only fragments of lumbar and sacral vertebrae are present and no caudal vertebrae are observed. The failure to produce somitic mesoderm after about E9 does not appear to be due to a dramatic reduction in cell proliferation of epithelial cells or an increase in apoptosis at the primitive streak. We have examined the expression of genes involved in posterior development and segmentation in the mouse and components of the retinoic acid signaling pathway to further characterize the role of FGF signaling. Our results identify FGF4 and FGF8 as critical components of the processes controlling posterior development of the mouse embryo, both in continued generation of new mesodermal cells and in regulation of somite formation.

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