

## SPROUTY GENES ARE REQUIRED FOR NORMAL MOUSE MAXILLARY INCISOR MORPHOGENESIS

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Tooth morphogenesis is classically divided into several phases, which are known as the placode, bud, cap, bell, and cytodifferentiation stages. Progression through these stages results from the interaction between two tissues, the ectodermally-derived epithelium and the neural crest-derived mesenchyme. The epithelium is of central importance throughout tooth development. It initiates tooth formation at the placode stage, and morphogenesis of the tooth at the cap stage is controlled by the epithelial enamel knot, which secretes a variety of growth factors. During the bell stage, the epithelium begins to differentiate into the enamel-secreting ameloblasts, as well as other tissues. The tooth serves as an excellent model for elucidation of the basic morphogenetic rules that govern epithelial bud development, because unlike the kidney, lung, and other organs that undergo budding of epithelium into mesenchyme, growth of the tooth bud is not complicated by multiple branching events. We have previously shown that Sprouty genes, which are inducible repressors of receptor-tyrosine kinase (RTK) signaling, are critical for maintaining normal tooth number in the molar region (Klein et al., 2006). In animals that are null for either *Spry2* or *Spry4*, supernumerary teeth develop in the molar region as a result of hypersensitivity to RTK signaling. In our current studies, we are examining incisor defects in Sprouty mutants, and we show here that mice heterozygous for *Spry2* and homozygous null for *Spry4* possess duplicated upper incisors. To analyze the morphogenetic mechanisms which underlie the incisor duplication, we have dissected epithelia from control and Sprouty mutant tooth germs and imaged the isolated epithelia using confocal microscopy. We show that the mutant tooth germs look grossly normal at the placode stage. However, at the early cap stage apparent duplications in the cervical loop precursors are detected. At the late cap stage, two enamel knots are detected in the mutant incisors, which promotes the continued development of the duplicated incisors. We propose that excess

RTK signaling causes incisor duplications by controlling cell death and proliferation, and that

the role of Sprouty genes is to ensure normal tooth number in the incisor region by antagonizing RTK signaling between epithelium and mesenchyme. Study of the genetic regulation of tooth bud morphogenesis will lead to a broader understanding of the mechanisms that control epithelial bud size and shape in general.

**REFERENCE:** Klein, O. D., Minowada, G., Peterkova, R., Kangas, A., Yu, B. D., Lesot, H., Peterka, M., Jernvall, J., and Martin, G. R. (2006). Sprouty Genes Control Diastema Tooth Development via Bidirectional Antagonism of Epithelial-Mesenchymal FGF Signaling. *Dev Cell* 11, 181-190.

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