<Barresi13e, Further Development_c26_07>

How Do the Correct Number of Cusps Form in a Tooth?

One set of rules constraining limb development may be the mathematics of the reaction-diffusion mechanism (“the Turing model”), a model that can be extended throughout development. Stephen J. Gould (1989) joked that paleontologists believe mammalian evolution occurs when two teeth mate to produce slightly altered descendant teeth. Because tooth enamel is more durable than ordinary bone, teeth often remain after all the other bones have decayed, and the study of tooth morphology has been critical to mammalian systematics and ecology. Changes in the cusp pattern of molars are regarded as especially important in allowing the evolutionary radiation of mammals into new ecological niches. What mechanism allows molars to change their form so rapidly?

Jukka Jernvall and colleagues (Jernvall et al. 2000; Salazar-Ciudad and Jernvall 2002, 2004; Salazar-Ciudad 2008) pioneered a computer-based approach to phenotype production using geographic information systems (GIS) to map gene expression patterns in incipient tooth buds. Their studies showed that gene expression patterns forecast the exact location of the tooth cusps in mice and voles based on differences in gene expression patterns (Figure 1). They also showed how reaction-diffusion mechanisms can explain the differences in gene expression between mice and voles (Salazar-Ciudad and Jernvall 2004, 2010).

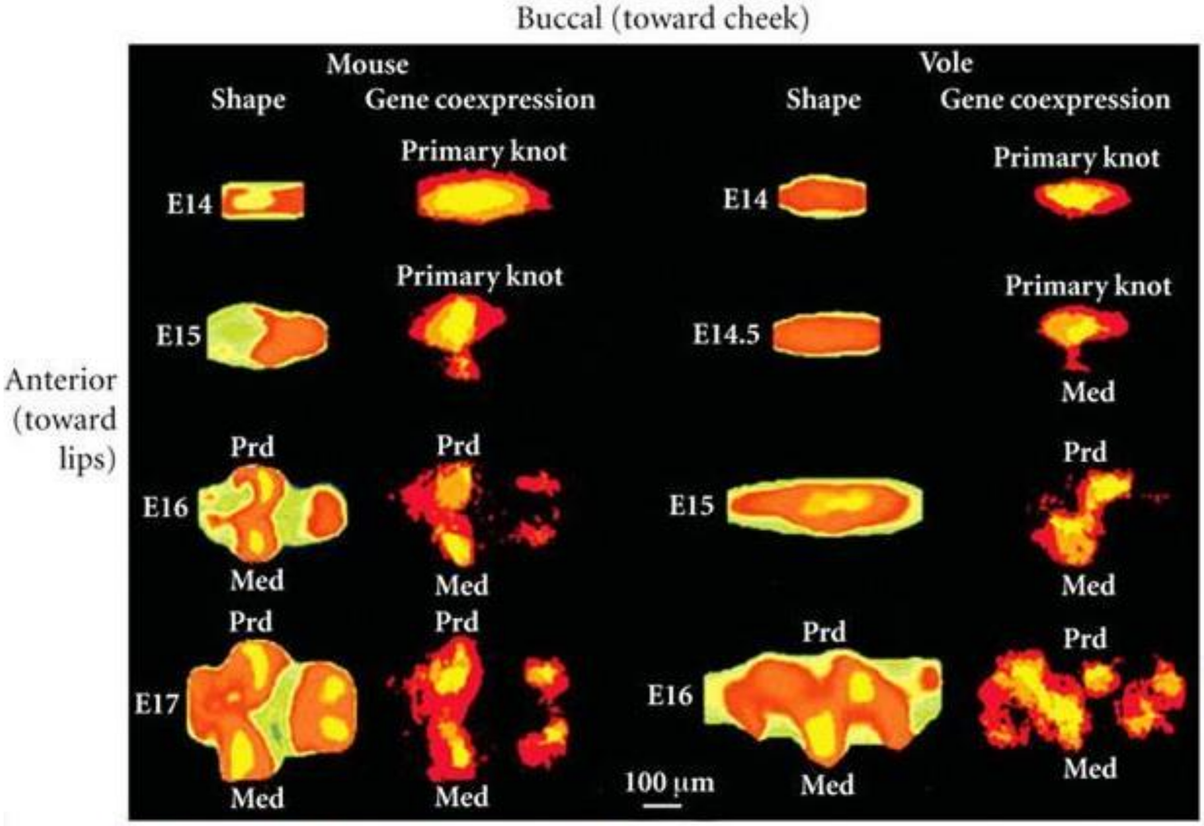

Figure 1 GIS analysis of gene activity in the formation of the first set of cusps in mouse and vole molars. (The first two cusps are the proconid, labeled Prd, and the metaconid, labeled Med). For both species, GIS mapping of molar shape is shown on the left, and the expression of Fgf4 and Shh (two genes expressed from the enamel knots) is shown at the right. In the mouse, the gene expression pattern on embryonic day 15 predicts the formation of the new cusps seen on day 16; the gene expression pattern on day 16 predicts the formation of cusps in those areas on day 17. Similarly, in the vole molar, whose cusps are diagonal to one another, gene expression predicts cusp formation. Jernvall, J., Soile V.e. Keranen, Irma Thesleff. 2000. Evolutionary modification of development in mammalian teeth: Quantifying gene expression patterns and topography. *Proc Natl Acad Sci USA.* 97(26:1444-8).

The signaling center of the tooth is the enamel knot, which secretes BMPs, FGFs, and Shh. Shh and FGFs inhibit BMP production, while BMPs stimulate both the production of more BMPs and the synthesis of their own inhibitors. BMPs also induce epithelial differentiation, whereas FGFs induce epithelial growth. For the studies described here, two additions to the classic reaction-diffusion equation involved (1) changing the “constants” of diffusion as development progresses and (2) changing the amount of elasticity associated with the extracellular matrix as development progresses. (This is because the extracellular matrix changes as the cells differentiate, altering tissue shape and the diffusion of paracrine factors.) The result is a pattern of gene activity that changes as the shape of the tooth changes, and vice versa (Figure 2). Under this model, the large differences between mouse and vole molars can be generated by small changes in the binding constants and diffusion rates of the BMP and Shh proteins. A small increase in the diffusion rate of BMP4 and a stronger binding constant of its inhibitor are sufficient to change the vole pattern of tooth growth into that of the mouse.

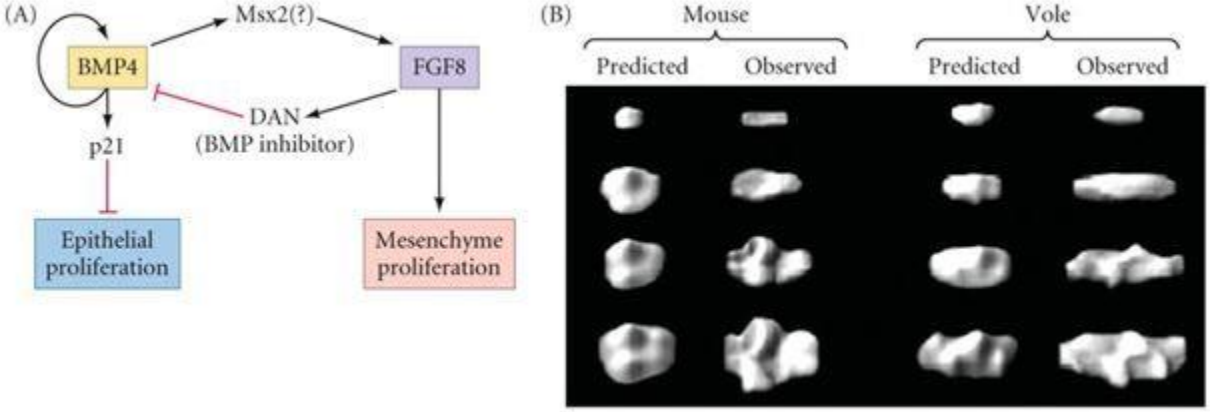

Figure 2 Basic model for cusp development in mice and voles. (A) Experimentally derived gene network wherein BMPs activate their own production as well as the production of their inhibitors, Shh and FGFs. The FGFs and Shh stimulate cell proliferation; the BMPs inhibit it. (B) Predicted and observed results from this model. The model can generate the final and intermediate forms of molar development in mice and voles, and the difference between mouse and vole molars can be reproduced by slight alterations in the rate of BMP diffusion and binding to inhibitors. (Salazar-Cuidad, I., J. Jernvall. 2002.) A gene network model accounting for development and evolution of mammalian teeth. *Proc Natl Acad Sci USA*. 99(12): 8816-8120.)

The work on mouse and vole molars showed how large morphological changes can result from very small changes in initial conditions. The set of equations that emerged also modeled the observed tooth shape variation seen in natural populations of seals, thereby relating small changes in development with microevolutionary variation within a species. Not only did the “virtual” teeth resemble real seals’ teeth, but the progression of cusps in the computer-modeled teeth followed the developmental pattern seen in the actual teeth. Moreover, by altering the parameter of epithelial growth, the equations modeled interspecies variation of jaw dentition. Another conclusion is that all the cells can start off with the same basic set of instructions, and specific instructions will emerge as the cells interact.

The Turing reaction-diffusion model also predicts that some types of teeth are much more likely to evolve in certain ways than in others (Figure 3). Moreover, the ecological context of tooth use (herbivory versus carnivory, for instance) would select certain variants and not others, and this model can predict both the number and size of molars under different ecological conditions (Kavanagh et al. 2007; Polly 2007). The predictions conform to what paleontologists have observed about mammalian evolution. These studies show the power of mathematical modeling to integrate development, cell biology, and genetics into a predictive model for evolution.

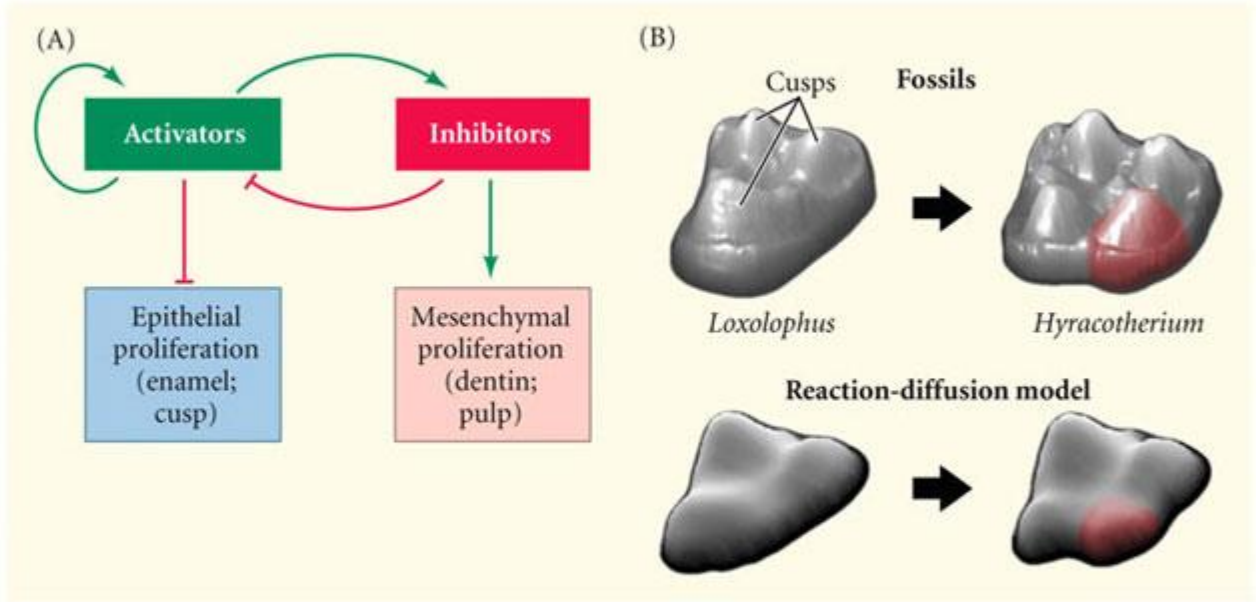

Figure 3 Mammalian tooth cusp pattern modeled by reaction-diffusion equations. (A) The reactiondiffusion mechanism serves as a motor regulating the genes responsible for slowing the growth of the enamelforming cells and promoting the proliferation of the dentin pulp-forming cells. (B) Possible formation of the four-cusped tooth of Hyracotherium (a fossil horse from 55 million years ago) from the three-cusped tooth of Loxolophus, a mammal that may have been an earlier member of the horse lineage. This transition in tooth shape can be achieved by modifying a single parameter of the reaction-diffusion equation. (Courtesy of J. Jernvall.)