Epithelial-mesenchymal signalling regulating tooth morphogenesis

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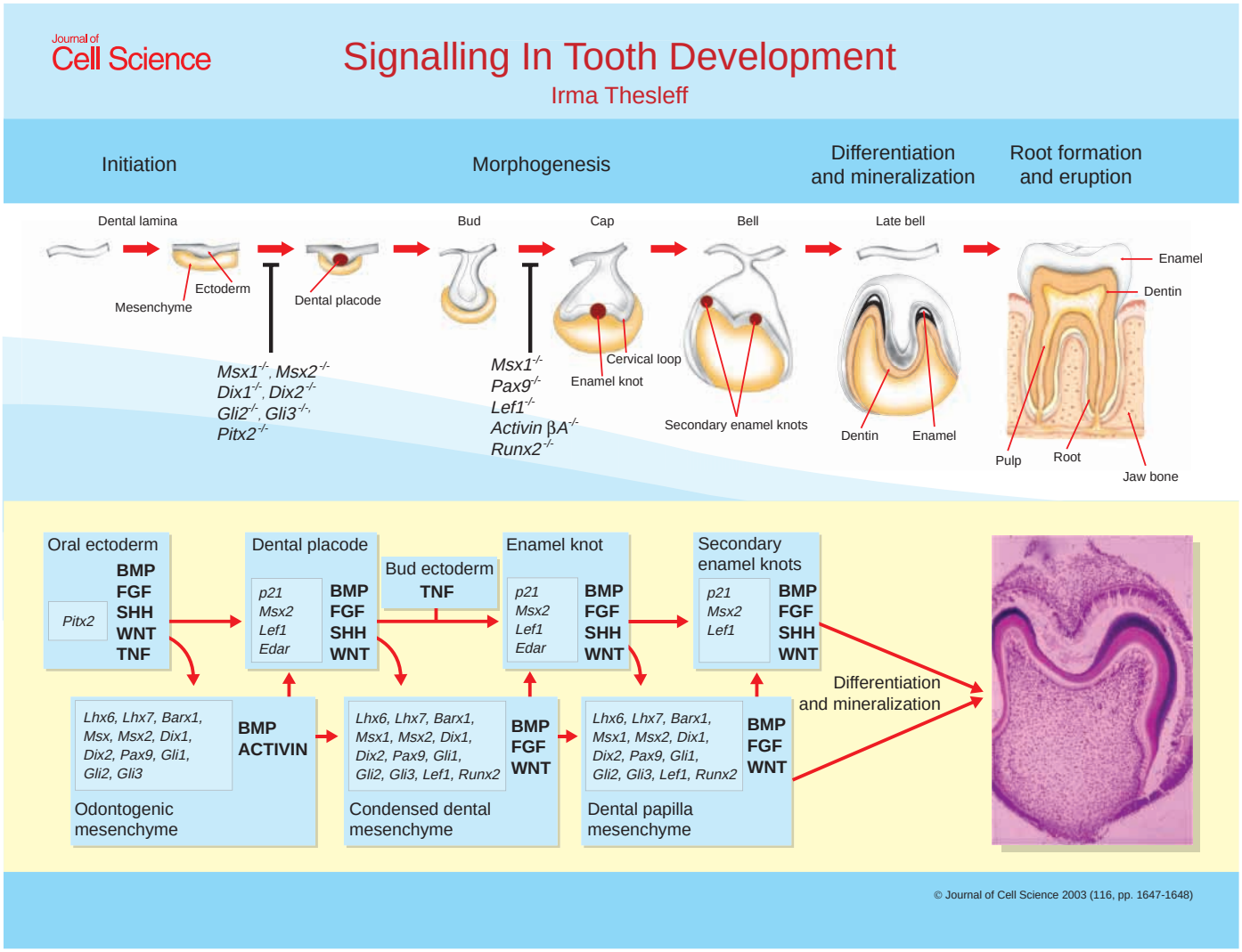
Teeth develop as ectodermal appendages in vertebrate embryos, and their early development resembles morphologically as well as molecularly other organs such as hairs and glands. Interactions between the ectoderm and underlying mesenchyme constitute a central mechanism regulating the morphogenesis of all these organs. Tooth morphogenesis is an advancing process that is regulated by sequential and reciprocal interactions between the epithelial and mesenchymal tissues and, during which the simple oral ectoderm thickens, buds, grows and folds to form the complex shape of the tooth crown.

During tooth initiation the ectoderm (white) thickens and forms a placode that buds to the underlying neural-crest-derived mesenchyme (yellow). The epithelium signals to the mesenchyme, which then condenses around the epithelial bud. During subsequent morphogenesis the epithelium folds and grows to surround the dental papilla mesenchyme (cap stage). The final shape of the tooth crown becomes fixed during the bell stage, when the hard tissue-forming cells of the tooth (odontoblasts and amelobasts) differentiate at the interface of the epithelium and mesenchyme and deposit the dentin and enamel matrices, respectively.

Paracrine signal molecules of several conserved families mediate cell communication during tooth development. Most of them belong to the transforming growth factor β (TGFβ), fibroblast growth factor (FGF), Hedgehog and Wnt families. Although the signals mostly regulate interactions between the ectoderm and mesenchyme, they also mediate communication within one tissue layer. Ectodysplasin, a recently identified signal molecule in the tumor necrosis factor (TNF) family, and its receptor Edar mediate signalling between ectodermal compartments in tooth germs. The genes regulated by the different signals include transcription factors and signal receptors that regulate



(See poster insert)

the competence of the cells to respond to the next signals, as well as new signals that act reciprocally and thereby continue the communication between cells and tissues (reviewed by Jernvall and Thesleff, 2000; Thesleff and Mikkola, 2002).

The same signals are used sequentially throughout morphogenesis, and many signals often show co-expression. A characteristic feature of tooth development is the reiterated appearance of transient signalling centers in the epithelium during key morphogenetic steps. These signalling centers (red) express more than ten different signal molecules including SHH (sonic hedgehog) and several BMPs (bone morphogenetic proteins, belonging to the TGFβ superfamily), FGFs and Wnts. The first signalling centers appear in the dental placodes when epithelial budding begins. Subsequently, at the bud-to-cap transition, the enamel knot signalling centers appear. These regulate the advancing morphogenesis of the tooth crown and control the initiation of the secondary enamel knots at the sites of epithelial foldings that mark cusp formation.

An early signalling event in tooth development is the induction of the odontogenic mesenchyme by BMPs and FGFs from the epithelium. Tissue recombination studies have shown that epithelial signals induce in the mesenchyme the competence to instruct subsequent tooth morphogenesis. BMPs and FGFs induce the expression of several mesenchymal transcription factors, many of which are necessary for the continuation of tooth development. For example, teeth are missing in double mutants of *Msx1* and *Msx2*, *Dlx1* and *Dlx2*, as well as *Pax9* null mice. The functions of the genes in the upper panel of the poster are necessary for normal tooth development in mice, and some of them also in humans.

The first epithelial signals induce in the mesenchyme the expression of reciprocal signal molecules, including activin, FGF and BMP4, which act back on the epithelium and regulate the formation of the dental placode. In addition, Wnts and the TNF signal ectodysplasin, secreted by ectodermal cells, regulate placode development. The placodal signals then regulate budding of the epithelium and condensation of the mesenchymal cells. They maintain the expression of earlier induced transcription factors in the mesenchyme and induce the expression of new genes such as the transcription factor *Runx2* and the signal *Fgf3*, which regulate epithelial morphogenesis from bud to cap stage. At this time mesenchymal BMP4 is required for the formation of the enamel knot at the tip of the bud. It induces the expression of *p21*, which is associated with the exit of the knot cells from the cell cycle. The Edar receptor is also induced in the enamel knot, making the cells responsive to the TNF signal ectodysplasin, which is expressed in the flanking epithelium of the tooth bud. Ectodysplasin-edar signalling regulates the formation and, perhaps, the signalling activity of the enamel knot.

The enamel knot cells express in nested patterns several signal molecules including *Shh*, *Bmp-2*, *Bmp-4* and *Bmp-7*, *Fgf-3*, *Fgf-4*, *Fgf-9* and *Fgf-20*, and *Wnt-3*, *Wnt-10a* and *Wnt-10b*. Signals from the enamel knot affect both epithelial and mesenchymal cells, and subsequent reciprocal interactions between the mesenchyme and epithelium are responsible for the maintenance of the enamel knot as well as for the subsequent morphogenesis of the epithelium. An SHH signal from the enamel knot is needed for the growth of the epithelial cervical loops flanking the enamel knots. The enamel knot signals also regulate the patterning of the tooth crown by influencing the initiation of the secondary enamel knots that express most of the same signal molecules as the primary enamel knots. They form in an exact sequence and determine the sites where the epithelial sheet folds and cusp development starts. Their development is regulated by signals from earlier formed primary and secondary enamel knots together with mesenchymal signals. Conceivably this involves mechanisms of lateral inhibition and activators and inhibitors. Recently a gene network model was presented that can reproduce both the reiteration of the epithelial signalling centers and their gene expression patterns as well as the resulting tooth morphologies of different mammalian species (Salazar-Ciudad and Jernvall, 2002).

It is obvious that the signalling networks regulating tooth morphogenesis are much more complex than presented in this schematic illustration. For example, there are numerous specific inhibitors of signals that have central roles in modulating locally the signalling activities. Also, the different signalling pathways are integrated at various levels and have synergistic as well as counteractive effects. Nevertheless, the model illustrates the general principle of the development of multicellular organisms, namely that the cells and tissues communicate via conserved signal molecules which are used reiteratively during advancing morphogenesis. The variation in cellular responses to the same signals in different tissues and at different times is caused by the different histories of the cells determining their competence to receive and respond to the signals.

Expression patterns of signal molecules and other genes during tooth development can be found in a graphical database Gene Expression in Tooth (http://bite-it.helsinki.fi).

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