How to build a tooth? Developmental biology is revealing the instructions

Developmental biology is at present one of the most rapidly progressing fields in biology and biomedicine. The advances of gene technology have led to a rapid explosion in the understanding of the molecular mechanisms regulating embryonic development. New genes and their functions are continuously being discovered in experimental studies using animal embryos, and molecular genetic studies in humans are unravelling gene mutations causing congenital defects. Today animal models can be created for human diseases and new possibilities are opening up for prevention, diagnosis and treatment of congenital defects. In addition there is now great hope that the knowledge on the molecules driving tissue and organ development and cell differentiation will lead to tools for tissue regeneration and stem cell therapies in the future. It may even be possible to grow whole new organs such as teeth.

I entered the exciting field of developmental biology 30 years ago as an undergraduate dental student and have been fortunate to be part of its dramatic progress, which really can be called a revolution. I received excellent training from my thesis supervisor Lauri Saxén in experimental embryology. In the 1980’s it became possible to study development at the level of genes, and with excellent students in my group we combined gene technology with classical methods of experimental embryology. We dissected dental tissues from mouse embryos and cultured them in various conditions, and studied the expression of genes. Over the years we have analysed the functions of many different genes by various approaches. Recently we have used transgenic mouse technology to examine the functions of some genes, in particular genes which affect the number of teeth in humans (see below).

The fascinating concept of a common molecular tool-kit regulating development evolved during the 1980’s and 1990’s. It was realized that the same genes regulate developmental processes in all animals and in all different organs and tissues. Our group contributed to this concept by unraveling the roles that many important molecules belonging to the conserved developmental tool-kit have in teeth (Jernvall and Thesleff, 2000, Thesleff, 2000, Thesleff and Mikkola, 2002). This indicated that the teeth are no exception of the rule; on the contrary, it is noteworthy that no «tooth-specific» regulatory molecules have so far been identified (besides some which are involved in the formation of dentin and enamel). In other words, all currently known genes that affect the posi-

Dyrking av kunstige tenner: Science eller fiction?


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tion, shape or number of teeth have developmental regulatory functions also in other tissues.

**Signaling molecules: Key regulators of tooth development**

The special aim of our research has been to elucidate the mechanisms whereby cells communicate during development. It has been known for more than a century that cells signal to each other and thereby direct cells to new pathways and thereby controlled the advancement of development. This has been called embryonic induction and today this is believed to be the single most important mechanism of developmental regulation. Many embryologists showed already in the 1950’s and 1960’s that intercellular signaling is an important feature of tooth development, and that in teeth the signaling events take place mainly between the epithelial and mesenchymal tissue components (Fig 1, Kollar and Baird, 1969, Ruch et al., 1983, Thesleff and Nieminen, 2001).

The developmental signal molecules are small proteins which usually act by binding on specific receptors at the surface of the responding cell (Fig 2). A multistep intracellular cascade leads to regulation of gene expression in the nucleus and the cell then changes its behaviour. Our work has pinpointed the roles of several signal molecules and their targets during the early steps of tooth development when teeth are initiated and their shapes are determined. We have proposed a model on how conserved signaling molecules mediate sequential and reciprocal interactions between dental epithelium and mesenchyme and thereby regulate advancing tooth morphogenesis (Fig 3). The model is based on results from many laboratories including our own. The most studied signals belong to four different families including fibroblast growth factor (FGF), bone morphogenetic proteins (BMP), hedgehog and WNT. In addition to signals, the model in Fig 3 also includes several genes which are regulated by the signals in the responding tissues. Mutations in many of these genes have already been shown to cause dental defects in mice as well as in humans (see below).

A breakthrough in our research was the discovery that BMP is an important signal during the initiation of tooth development. BMP is synthesized by the early dental epithelium, and it induces...
in the mesenchyme the expression of many genes including the transcription factor Msx1 (Vainio et al., 1993). For this study an organ culture method was designed in which the local effects of signal molecules are analysed by introducing them with small beads on developing dental tissues (Fig 4). Tooth germs were dissected from mouse embryos and epithelial and mesenchymal tissues were separated and placed on metal grids covered with culture medium. The beads were incubated in a high concentration of BMP protein and placed on dental mesenchyme and the explant was cultured for one day in an incubator. Thereafter the expression of candidate target genes was examined by in situ hybridization from whole mounts (Fig 5). Later it was shown by others that Msx1 is, in fact, a necessary gene for tooth development both in mice and humans. No teeth developed in so-called knockout mice lacking the function of Msx1. Their tooth development was arrested at the bud stage (Sato-kata and Maas, 1994). In humans mutations in the MSX1 gene cause severe oligodontia (eight or more missing teeth) (Vastardis et al., 1996).

**Enamel knots: Signalling centers governing tooth shape**

An important leap forward in our studies was the discovery of signalling centers, called enamel knots in the tooth germ epithelium (Jernvall et al., 1994). Although the enamel knots had been descri-

**Gene expression in teeth: A database at www.bite-it.helsinki.fi**

During the years we and others have reported the expression patterns of numerous genes during tooth development. Most data were obtained by in situ hybridization, a method detecting the sites of mRNA expression in tissues. As the information of gene expression patterns was accumulating with increasing speed, Pekka Nieminen in our group constructed in 1995 an Internet database to help ourselves as well as the whole scientific community to keep track on genes expressed in teeth (Nieminen et al., 1998, www.bite-it.helsinki.fi). This graphical database shows gene expression patterns at va-
rious stages of tooth development and in addition it contains supplementary data on important genes. At present the database includes information of about 350 genes. As an example, the page on Msx1 expression is shown in Fig 7. The database is now widely used internationally and members of our laboratory keep it updated.

Although the information on the patterns of gene expression do not directly tell about gene function, they give important insights. For example, the co-expression of several signal molecule genes in the enamel knots actually led to the unraveling of the function of the enamel knot as a signaling center of teeth. Today, our gene expression database is widely used as a tool to detect co-expression of genes and to discover molecular interactions between genes.

The cleidocranial dysplasia gene

**RUNX2: A promise for the induction of new teeth**

Cleidocranial dysplasia is a syndrome affecting bone and tooth development and it is caused by mutations in **RUNX2** (earlier called **CBFA1**), a gene encoding a transcription factor. The symptoms include hypoplastic bone, in particular deficient formation of calvarial bones and clavicles. The tooth phenotype is particularly interesting as the patients have supernumerary teeth, and sometimes an almost complete third dentition develops (Jensen et al., 1990). This indicates, remarkably, that all of us have the potential to develop a third dentition and that this is normally inhibited by the **RUNX2** gene.

**RUNX2** is a «master gene» of bone development and it is needed for osteoblast differentiation. **RUNX2** knockout mice have no bone at all and their skeleton is composed of only cartilage. We studied the tooth phenotype of these mice and quite unexpectedly observed that their teeth failed to develop (Fig 8). Teeth were initiated but their development was arrested after the bud stage indicating that **RUNX2** function is necessary for bud to cap stage transition (D’Souza et al., 1999).

Cleidocranial dysplasia is caused by reduced function of **RUNX2**. That supernumerary teeth develop in these patients whereas the complete loss of **RUNX2** function in mice inhibits tooth formation appears controversial, but it is a challenging problem. Unfortunately, the mice do not normally develop a secondary denti-
tion at all and therefore they cannot be used as model animals for studies on secondary tooth formation. However, we hope that by clarifying the function of RUNX2 in primary tooth development in mice we can shed light to the mechanisms whereby the secondary dentition develops in humans, and that this will also explain why the third dentition develops in the human cleidocranial dysplasia patients.

We are now analysing RUNX2 knockout mice and aim to clarify the cause of arrested tooth development. We are searching for genes which are regulated by RUNX2 by comparing gene expression between the mutants and wildtype mice. We use microarray technology and DNA chips which allows the simultaneous analysis of thousands of genes including also presently unknown genes. Could this information be used for inducing a new set of teeth in the future?

**Ectodysplasin, the molecule lacking in ectodermal dysplasia: A stimulator of tooth formation**

Ectodermal dysplasia syndromes are characterized by absence or hypoplastic development of several ectodermal organs. In addition to these organs include hairs, nails and a variety of exocrine glands such as sweat glands, salivary glands and mammary glands. The development of all these organs are regulated by epithelial-mesenchymal interactions which are mediated by the same signals as tooth development (Pispa and Thesleff, 2003). The most common form is the hypohidrotic or anhidrotic ectodermal dysplasia (HED). The tooth phenotype of these patients includes severe hypodontia, sometimes complete anodontia (Fig 8). Several gene mutations causing HED have been identified recently by positional cloning of the human and corresponding mouse mutants (Thesleff and Mikkola, 2002). Interestingly these genes function in the tumor necrosis factor (TNF) signal pathway. X-chromosomal HED is caused by mutations in the actual TNF signal, called ectodysplasin, and other forms result from mutations in the ectodysplasin receptor (Edar) and other components in the same signal pathway.

We have shown that ectodysplasin regulates the functions of the enamel knots and hair placodes (which are signaling centers for hair formation) (Pispa et al., 1999, Laurikkala et al., 2000, 2001). We have also used several mouse mutants in these studies including the natural occurring ectodysplasin knockout called the Tabby mouse. In addition we have produced transgenic mice over-expressing either ectodysplasin or its receptor Edar in the ectoderm. Interestingly, while the third molars are mostly missing in the Tabby mice, the ectodysplasin overexpressing mice have extra teeth (Fig 10, Mustonen et al., 2003). Hence, ectodysplasin is an important signal regulating tooth number and shape. Since ectodysplasin is a secreted protein it is tempting to imagine that it could be used in the future to induce new teeth and hairs in ectodermal dysplasia patients. Could it be used to grow new teeth in other patients too?
Prospects for growing new teeth: Dental stem cells and signal molecules

A frequent question asked after my lectures and seminars over the years has been: «When can you grow new teeth for us?», Earlier I used to say «never», but more recently, due to the fantastic progress in the fields of developmental biology and stem cell biology, I have started to be more optimistic. This is first of all due to the rapid increase in our understanding of the molecular regulation of tooth development. Of particular importance here is the identification of the signal molecules such as ectodysplasin and many others guiding tooth development at different stages (Fig 3). Secondly, the recent scientific breakthroughs in stem cell research have indicated that adult cells may be much more plastic in their behavior than previously thought (Donovan and Gearhart, 2001; Jiang et al., 2002). In other words, cells may change the directions of differentiation according to environmental signals. The interest in tissue regeneration in general has increased tremendously and data is accumulating rapidly on the competence of various cell types to be programmed to differentiate in order to find ways to identify and harvest potential stem cells and to induce their differentiation to specific directions. In addition, the practical designs for transplantation and culture of cells and tissues present great challenges. However, the dreams about tooth regeneration may after all come true some day in the future.

References


Søkeord for nettversjon: www.tannlegetidende.no: Biologi; Emalje; Dentin; Forskning; Genteknologi; Molekylærbiologi

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