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How Do Genes Make Teeth to Order Through Development?

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ABSTRACT This introduction to new patterning theories for the vertebrate dentition outlines the historical concepts to explain graded sequences in tooth shape in mammals (incisors, canines, premolars, molars) which change in evolution in a linked manner, constant for each region. The classic developmental models for shape regulation, known as the 'regional field' and 'dental clone' models, were inspired by the human dentition, where it is known that the last tooth in each series is the one commonly absent. The mouse, as a valuable experimental model, has provided data to test these models and more recently, based on spatial-temporal gene expression data, the 'dental homeobox code' was proposed to specify regions and regulate tooth shape. We have attempted to combine these hypotheses in a new model of the combinatorial homeobox gene expression pattern with the clone and field theories in one of 'co-operative genetic interaction'. This also explains the genetic absence of teeth in humans ascribed to point mutations in mesenchymally expressed genes, which affect tooth number in each series. *J. Exp. Zool. (Mol. Dev. Evol.) 306B:177–182, 2006.* © 2006 Wiley-Liss, Inc.

The papers in this issue are those produced after the symposium held at the annual SICB meeting, January 2005, with an intention to stimulate an interdisciplinary dialogue on all aspects of patterning the vertebrate dentition. One between those immersed in molecular developmental research into tooth development in the mouse animal model (Cobourne and Mitsiadis, 2006, this issue) with others interested in the evolution of pattern in the vertebrate dentition (Smith, 2003). Part of this pattern may be the loss and gain of teeth throughout a phylogeny, so that recognition of vestigial and atavistic teeth in mammals, including humans, recorded as the ''phylogenetic memory'', will provide valuable data for this debate on patterning the dentition (Peterkova et al., 2006, this issue). Currently, there is only limited dialogue between those with genetic information on non-mammalian dentitions (e.g. the natural mutants of cichlid fish, which exhibit tremendous dental diversity of tooth shape and number) and those with tools for understanding molecular controls for mammalian tooth shape. Despite our understanding of the genetic networks that mediate mammalian cusp pattern (Jernvall and Thesleff, 2000), important questions remain regarding the genetic and developmental basis of differences in tooth shape. In the cichlids it is observed that evolution of novelty can be driven by only a few changes in the genes (Streelman and Albertson, 2006, this issue) and this would seem to be a very tractable model when gene expression data are applied to the temporal pattern of tooth development. Important data are now emerging on the non-mammalian members of the osteichthyans (Fraser et al., 2006a,b; Huysseune and Witten, 2006, this issue) with details of spatio-temporal patterning of the multiple sites of tooth production and the mechanisms for controlling their replacement, an activity not present in the mouse. With new insight, those without teeth such as the normally edentate chick, may allow us to discover how teeth could be recovered in evolution from such a genetic misfit as achieved experimentally with the chick and mouse chimeras (Mitsiadis et al., 2003b, 2006, this issue). Also, data on a group of fossil tetrapods, as the first amniotes to

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achieve occlusion between upper and lower teeth, could predict how shape control became important between upper and lower jaws, an innovation occurring 300 Myr ago (Reisz, 2006, this issue). Starting with the earliest mineralized tissues, in the fossil record of early agnathan vertebrates, debate is focused on the phylogenetic origins of dental skeletal tissues with their great diversity in the dermal armour and apparent developmental freedom to express all skeletal phenotypes (Donoghue et al., 2006, this issue). Evolution of genes controlling mineralization through secretory calcium-binding phosphoproteins are shown to originate from a common ancestral gene *SPARC*, with a tandem duplication history in tetrapods (Kawasaki and Weiss, 2006, this issue). There are perhaps no vertebrate organs that can be studied from so many different aspects as dermal denticles (odontodes) and teeth, including the stage in a phylogeny at which these diverged (Smith and Coates, '98; Smith, 2003). Their diversity of form, structure and arrangement in the different classes of vertebrates, together with their rich fossil record, have long drawn the attention of zoologists and palaeontologists, and more recently of developmental biologists and geneticists.

HISTORICAL PERSPECTIVES

The progress in embryology towards the end of the 19th century enabled evolutionary theories to be tested against observations of tooth development. Thus it was found that the first cusp to develop on mammalian upper molars is not the ''protocone'', as postulated by the tritubercular theory of molar evolution, but the ''paracone''. We now know that this is the site of the first enamel knot, a classical feature of tooth germ histology and a signalling centre, identified by the sequential expression of specific genes (Jernvall and Thesleff, 2000). With the inception of experimental embryology in the 20th century, development could be described in causal terms, such as induction. Experiments on amphibia (Platt, 1893, 1897) led to the recognition of dorsal ectoderm (the neural crest) rather than mesoderm, as the source of mesenchyme for the developing teeth (see Cobourne and Mitsiadis, 2006, this issue). By the use of organotypic culture techniques, experimental work became extended to mammalian teeth, particularly those of the mouse. Investigation of the inductive relations between epithelium and mesenchyme (Mina and Kollar, '87; Lumsden, '88) led, under the influence of molecular biology and developmental genetics, to a greater understanding of the role of molecules and embryonic germ layers in odontogenic patterning (Tucker and Sharpe, 2004; Mitsiadis et al., 2006, this issue).

Butler's field theory of 1939 was based on the observed discordance between tooth position and shape. He recognized that a series of tooth shapes occurred in a wide range of mammals and that this had shifted its position in the course of evolution. This ''regional field'' theory predicts that all tooth primordia are initially equivalent and that tooth shape is controlled by different concentrations of diffusible signalling molecules expressed in the first branchial arch. These signals could thus produce periodicity along the developing dental axes. The anterior–posterior dental axis is conceived of as three regions: the incisive, the caniniform and the molariform. Butler ('39) includes premolars with molars in the molariform region. In each region, there is a ''best copy'' of the group. First incisors, canines and first molars are very stable teeth and are seldom missing in the human permanent dentition (Larmour et al., 2005). The ''clone'' model (Osborn, '78) predicts that teeth develop from a single clone of cranial neural crest-derived mesenchymal cells. These cells are non-equivalent for each of the groups, thus giving rise to each different shaped dental series. However, there is no explanation of how the regional tooth shape differences are achieved. It had been proposed that these ectomesenchymal cell populations (clones) possess positional information, but it is now accepted that initially they may not have shape information (see Cobourne and Mitsiadis, 2006, this issue). We can also see from developmental data in non-mammalian vertebrates that the earliest teeth in the embryo have poorly developed shape, as do those in chondrichthyans (Reif, '76), osteichthyan fish (see Streelman and Albertson, 2006, this issue) and reptiles (Osborn, '71; see Smith 2003, for fuller discussion).

We have attempted to fit data from mammalian teeth on homeobox genes and signalling molecules in osteichthyan fish dentitions (Fraser et al., 2004, 2006a, this issue; Jackman et al., 2004) to a general model. Most papers in this symposium issue address the two opposing historical theories of regional specification, the ''field'' and ''clone'' theories. The clone theory of Osborn ('78) explained serial differences of tooth pattern by changes in time within cell lineages, rather than by gradation in an external field, each clone being

non-time equivalent. This theory was supported by Lumsden's ('79) finding that the prospective molar region of the mouse, when explanted, could produce all three molars in succession. Lumsden compared the formation of posterior molar teeth to the development of the limb and proposed that it is under the control of a progress zone. Currently, there is no evidence that progress zones are responsible for the graded sequence of premolar and incisor patterns of mammals, but this cannot be tested in the simplified dentition of the mouse. Studies comparing replacing teeth and their primary predecessors in fish suggest that they are successive derivatives of the same clone of cells with reiterative use of the same set of genes (Fraser et al., 2006a,b, this issue). However, the primary tooth sites (clones) are determined within a broad but restricted field of gene expression, whereas the secondary ones are not, as they arise instead from the side of the primary tooth. We have addressed these issues with genetic and molecular data from osteichthyan fish and mammals, and propose a new conceptual model.

DENTAL AXIS SPECIFICATION AND ODONTOGENIC PATTERN

The ''field'' and ''clone'' theories provided theoretical models for the mechanisms that might be involved in patterning the dentition and were based also upon the analysis of human dentitions (Butler, '39; Osborn, '78). Recently, much progress has been made in dissecting these mechanisms at the genetic level (Tucker and Sharpe, 2004). A number of subfamilies of homeobox-containing genes are involved in controlling neural crest specification. These genes code for transcription factors responsible for regulating the expression of downstream target genes. Cranial neural crest-derived cells carry a homeobox code defined patterning, thus specifying the region of the first branchial arch where teeth are developing (Sharpe, '95; Cobourne and Mitsiadis, 2006, this issue). Region-specific combinatorial homeobox gene expression in the branchial arch mesenchyme specifies each tooth identity. This ''homeobox code'' thus will set up regional diversity within the tooth-forming regions of the first branchial arch. It is plausible, therefore, that the specification and patterning of the dentition is controlled by a homeobox code (Sharpe, '95; Cobourne and Mitsiadis, 2006; this issue). Indeed, a number of homeobox-containing genes, such as members of the *Msx*, *Dlx*, *Barx*, *Lhx* and *Pitx* classes do show temporal and spatial patterns of expression within the first branchial arch (Tucker and Sharpe, 2004). These genes are expressed in spatially restricted regions of the first branchial arch during facial development. Prior to the initiation of odontogenesis, the *Msx* and *Isl1* genes exhibit highly specific domains of expression in the anterior regions of the first arch, where incisors will develop (Mitsiadis et al., 2003a; Tucker and Sharpe, 2004). In contrast, expression of several of the *Dlx*, *Barx* and *Pitx* genes is restricted to the posterior regions of the first branchial arch where the future molar teeth will develop. The analysis of mice with targeted mutations in the *Msx*, *Barx*, *Pitx* and *Dlx* genes provide some evidence for this. In *Msx*−/− mice, the incisors fail to develop and molar development is arrested at the late bud stage, while targeted null mutations in *Dlx*, *Barx* and *Pitx* result in either an alteration of the molar shape or complete absence of molars (Tucker and Sharpe, 2004; Sharpe, personal communication; Mitsiadis, unpublished results).

The formation of dental placodes (see Peterkova et al., 2006, this issue) is regulated by interactions between the oral epithelium (ectoderm/endoderm) and underlying mesenchyme, and several signalling molecules have been implicated as activators or inhibitors of placode formation (Mustonen et al., 2004; Tucker and Sharpe, 2004). Strong epithelial signals such as BMPs and FGFs are needed to create dental placodes. Ectodysplasin (EDA) is also required for placode formation: EDA overexpression leads to placode expansion, while suppression of the *EDA* gene is responsible for the creation of smaller placodes (Mustonen et al., 2003, 2004). However, the function of EDA appears to be downstream of the primary inductive signal required for placode initiation (Mustonen et al., 2004). Alteration of the epithelial signalling will affect tooth morphology. Transformation of a tooth type, from incisor to molar, is possible after suppression of BMP expression in the incisor field of the mandible (Tucker et al., 1998). Manipulation of epithelial signals may thus lead to a change of the tooth identity via the alteration of the homeobox-containing gene expression in the mesenchyme (Fig. 1).

While the two first theories were inspired from the human dentition, the dental homeobox code model is mainly based on findings on mouse teeth (Tucker and Sharpe, 2004). The dentition of rodents differs significantly from that of humans, since there is only one dentition and, furthermore, canines and premolars are missing. Mutations of

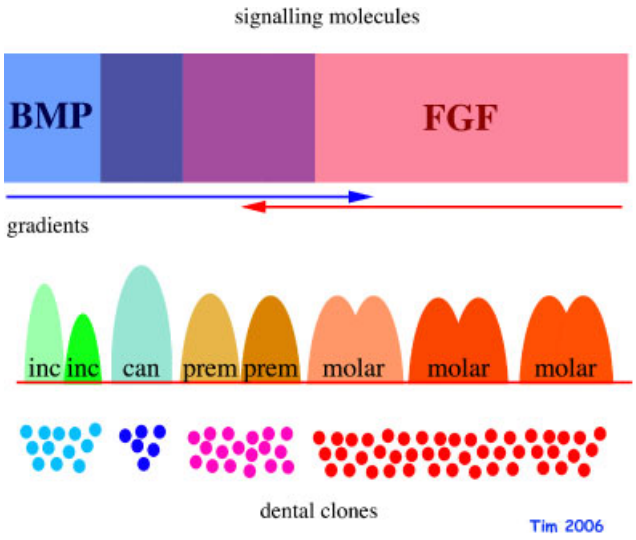

Fig. 1. Schematic representation showing the importance of epithelial signalling molecules and cranial neural crest-derived mesenchymal cells for the creation of tooth shape diversity in humans. Gradients of BMPs and FGFs along the anterio-prosterior axis will contribute to the formation of incisors (inc, green), canines (can, blue), premolars (prem, orange) and molars (red). Gradients of BMP signalling molecules are involved in the formation of the anterior teeth (e.g. incisors), while gradients of FGF signalling molecules are involved in posterior teeth formation (e.g. molars). Expression of homeobox genes in the neural crest-derived cells defines different dental cell populations (dental clones), which are contributing to the formation of incisors, canines, premolars and molars. Intense colours in teeth of the same shape indicate that they are often absent in humans.

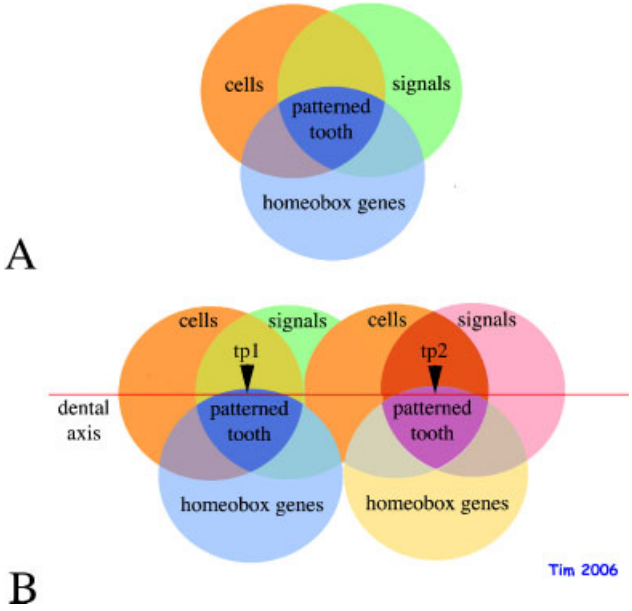

Fig. 2. A graphic model to incorporate the three previous models in a new one for creation of tooth patterning. Neural crest-derived cells contacting the oral epithelium are under the influence of epithelial signals that will activate the expression of homeobox-containing genes. All these elements (cells, signals and homeobox genes) will contribute equally to patterned tooth formation (**A**). Defects in cells (e.g. number), signals (e.g. EDA) or homeobox genes (e.g. MSX1) will be responsible for teeth with abnormal morphology and/or tooth agenesis. Disposal of the various teeth on the dental axis (tooth position one—tp1, tooth position two—tp2, etc.) is time-dependent, and different signals and combinations of homeobox genes will contribute to different tooth shapes (**B**).

tooth-specific genes in mice generally affect all teeth of the same type (i.e. molars). By contrast, point mutations in several mesenchymally expressed genes such as *PAX9* and *MSX1* in humans do not affect all teeth of the same class (Mostowska et al., 2003). Teeth that are commonly absent in humans are lateral incisors, second premolars and the second and third permanent molars (Fig. 1). These observations indicate the complexity of the tooth patterning in many mammalian vertebrates and perhaps indicate that tooth number in each clone (ICM shape series) is separately affected.

A NEW MODEL: CO-OPERATIVE GENETIC INTERACTION (CGI)

We could explain the species differences contributing to the absence of teeth by combining the clone and homeobox code hypotheses. Mutations affecting genes that are expressed in a mesenchymal dental clone may affect cell proliferation and thus their capability to produce the normal number of cells needed for the formation of the precise number of a tooth type. In a similar way, we could hypothesize that the protein, which is produced by the cells affected by the point mutation, has become less functional at a lower concentration. These factors together may contribute to either size reduction or complete loss of a tooth in the series. As a consequence, the number of a given tooth type will decrease in individuals having such mutations.

We have justified the need for a new genetic/developmental model ''CGI'' and propose one in which all the above-mentioned factors will contribute to tooth patterning: position, number and shape specification. Neural crest-derived cells, homeobox-containing genes and signalling molecules, all have an important role in tooth specification as this is illustrated by the schematic representation of our model (Fig. 2).

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