Part one

Genes, molecules and tooth initiation

1 Homeobox genes in initiation and shape of teeth during development in mammalian embryos

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1.1. Introduction

The past decade has seen remarkable advances in our understanding of the genetic control of embryonic development. We now know that developmental processes are initiated and controlled by interacting pathways of extracellular signalling molecules, receptors, intracellular signalling proteins and nuclear (transcription) factors. The different types of proteins (genes) that carry out these functions most often occur as members of families of related proteins characterised by possessing conserved amino acid motifs but which do not necessarily have similar functions in development. Thus for example, the transforming growth factor-beta (TGFβ) superfamily of secreted signalling proteins consists of a large family of proteins that share some homology with TGFβ, and in many cases share cell surface receptors (Kingsley, 1994). However, within this family different members have very different and specific functions in development. The bone morphogenetic protein Bmp-4, for example, probably has multiple functions as a signalling molecule in embryogenesis, including a role in lung morphogenesis, but targeted mutation of Bmp-4 (gene knock-out) shows a requirement for this protein for early mesoderm formation (Winnier *et al*., 1995; Bellusci *et al*.,1996). Bmp-7 on the other hand appears to have no direct role in mesoderm formation but is required for skeletal development (Luo *et al*., 1995). This illustrates a recurring theme in development where similar molecules have multiple functions, some of which overlap with other family members whereas others are unique. This almost certainly reflects the evolution of these gene families by gene duplication resulting in some shared and some unique functions.

The first of these families of developmental genes to be discovered and the one which has produced the greatest interest is that of the homeobox genes. The discovery of the homeobox as a small (180 bp) conserved region of DNA found in homeotic genes of flies (*Drosophila*) provided the springboard for all subsequent advances in this field (reviewed in Duboule, 1994). The discovery of the homeobox was an important milestone because it demonstrated that genes controlling fly development can also be present in vertebrates. This allowed progress in the understanding of vertebrate development to proceed at a far greater pace than was ever thought possible by cloning genes through nucleotide homology with fly genes.

Homeotic mutations in flies had long been suspected of holding important clues to understanding morphogenesis. Cells in an embryo differentiate into a limited number of specialised types, around 200 different cell types in mammals, and it is the arrangements of these cell types into defined structures with characteristic forms that is the main achievement of embryogenesis. The genetic control of morphogenesis of different structures was a mystery until molecular cloning became possible, followed by analysis of the expression, function and control of homeotic genes.

Homeotic mutations involve mutations in single genes that produce a phenotype where one body part of the fly is replaced by another. Thus the *Antennapedia* mutation is characterised by the development of legs in place of antennae on the fly's head. Eight of the homeotic genes identified in flies were cloned and found to be different except for one small 180 bp sequence towards the C terminus that was highly conserved. This sequence, or 'box', was named the homeobox and was also found to be present in other developmental genes in flies, most notably the segmentation genes *fushi tarazu* and *engrailed* (reviewed in Duboule, 1994).

The clues to the function of the homeobox came from structural analysis that showed that the homeodomain (60 amino acids) forms a helix-turn-helix structure which has DNA binding characteristics (Laughon and Scott, 1984, Sherperd *et al*., 1984). Homeoproteins are thus DNA-binding proteins that regulate gene transcription and as such may exert a hierarchical control function over the expression of genes required for morphogenesis of a particular structure.

The conservation of the homeobox sequence is not limited to flies, and since 1984 many vertebrate homeobox genes have been cloned by nucleotide homology. However, there is a clear and important distinction between homeobox genes that are most closely related to homeotic genes and others that are not. Homeotic genes have unique organisational and functional characteristics in flies that distinguish them from other homeobox genes. Most significantly eight of the homeotic genes are clustered forming a complex (HOMC) and their linear order in the clusters is reflected in their anterior–posterior expression domains in fly embryos, a feature known as colinearity. Similarly the mammalian homeobox genes that most closely resemble homeotic genes, called Hox genes, also show the same feature of colinearity. In mammals there are 39 Hox genes arranged as four clusters on different chromosomes. Hox genes and HOMC genes probably had a single common evolutionary ancestor in a segmented worm and the Hox genes were duplicated during evolution of vertebrates (reviewed in Manak and Scott, 1994).

Hox gene expression is first detected in ectoderm and mesoderm cells in mammalian embryos during gastrulation (Gaunt *et al*., 1986). During organogenesis expression is found in the developing central nervous system and hindbrain, in the developing prevertebrae (somites) and more 5′ genes are also expressed in limb buds. Targeted mutation analysis has confirmed that Hox genes play a role in development of the axial skeleton, mutation of individual genes results in abnormal development of the axial skeleton at particular anterior–posterior levels. Thus for example a null mutation of *Hoxb-4* results in partial homeotic transformation of the second cervical vertebra into a first cervical vertebra, whereas mutation of *Hoxc-8* produces a change in a more posterior vertebra (LeMouellic *et al*., 1992; Ramirez-Solis *et al*., 1993).

A potentially important feature of Hox gene expression for tooth development is the expression of the most 3′ Hox genes in cranial neural crest cells emanating from the developing hindbrain. Neural crest cells that form the branchial arches of the embryo migrate from distinct anterior–posterior positions in the hindbrain and caudal midbrain. Thus the cells of the first branchial arch which form the ectomesenchymal component of developing teeth are formed from neural crest cells that migrate from the rostral hindbrain (rhombomeres 1 and 2) and caudal midbrain. The anterior boundaries of expression of the most 3′ Hox genes have been shown to correspond to rhombomeric boundaries in the hindbrain and more significantly to be expressed in migrating neural crest cells at the appropriate axial level. *Hoxb-2* expression, for example, has an anterior boundary at rhombomere 3/4 and is also expressed in neural crest cells that migrate from rhombomere 4 to populate the second branchial arch (Prince and Lumsden, 1994). This feature has been suggested as a mechanism of patterning the mesenchymal cells of the branchial arches by positional specification of neural crest cells prior to their migration through the combination of Hox genes they express, referred to as the ‘branchial Hox code’.

Since teeth in the first branchial arch were first thought to develop from neural crest cells contributed from rhombomeres 1 and 2 that populate this arch, and the neuroepithelium of the second arch expresses *Hoxa-2*, it was considered possible that tooth morphogenesis is patterned by the same branchial Hox code. However, it appears that this is not so, since rhombomere 1 does not express a Hox gene and although *Hoxa-2* is expressed in rhombomere 2 it is unique in that this expression is not transferred to neural crest cells that migrate from this rhombomere. For both these reasons, expression of Hox genes has not been observed in the first branchial arch mesenchyme and so patterning of the first arch structures cannot directly involve Hox genes (see also Chapter 11). Also, we now know that, in the mouse it is the non-segmental posterior midbrain crest which forms the ectomesenchyme of the mandibular molars (Imai *et al*., 1996). In the chick, and also the equivalent bones to those bearing teeth in the mouse, are formed from mid brain crest (Koentges and Lumsden, 1996). There are however many non-Hox homeobox genes that are expressed in the first branchial arch and also during tooth development. This chapter describes these expression patterns and proposes how some of these genes might function to control tooth morphogenesis via an ‘odontogenic homeobox code’ (Sharpe, 1995).

1.2. Homeobox genes and tooth bud initiation

The first morphological sign of tooth development is a narrow band of thickened epithelium (primary epithelial band) on the developing mandible and maxilla that forms four zones, one in each quadrant. These bands specify the area of epithelium from which teeth are

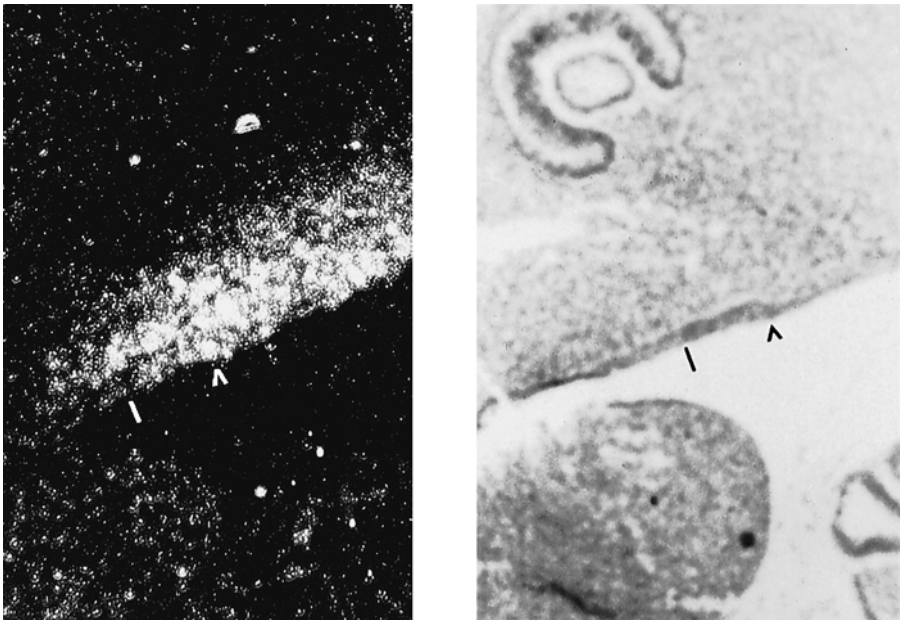

Figure 1.1. ***In situ*** **hybridisation of a section in a dorsoventral plane with anterior at the top, of a day 10.5 gestation mouse embryo head showing *Msx-2* gene expression in the ectomesenchyme and oral epithelium in relation to the epithelial thickening. Left, dark field, right, light field. Section is sagittal (parallel to A/P axis, anterior to the top). Line, middle extent of epithelial band; arrowhead, lingual extent of epithelial band. Magnification × 200. (Courtesy of Bethan Thomas.)**

capable of forming. The position of the bands thus determines and restricts the location within the mandible and maxilla of tooth development. Tooth buds form at discreet locations in these bands by secondary thickening of the epithelium and invagination into the underlying ectomesenchyme. The first important question in tooth development, therefore, is which cells provide the information that specifies the position of the oral epithelial thickenings? Although results from recombination experiments have largely supported the epithelium as the source of the initiation signals there is still some doubt as to whether it is the underlying neural crest-derived ectomesenchymal cells that are patterning the epithelial cells (Mina and Kollar, 1987; Lumsden, 1988; and reviewed by Ruch, 1995).

One of the interesting observations from the localisation of homeobox genes expressed in tooth development is that for many genes, expression is not restricted to either the dental epithelium or mesenchymal cells. This is particularly evident for the *Msx-2* homeobox gene, which shows spatially restricted expression in both epithelial and mesenchymal cells during tooth development (Figure 1.1; MacKenzie *et al*.,1992). More significantly, the early expression of *Msx-2* prior to tooth bud formation is also found in epithelium and mesenchyme. *Msx-2* is expressed in the distal tips of the mandibular and maxillary arch mesenchyme. *Msx-1*, a close relative of *Msx-2*, is expressed in a similar domain of ectomesenchyme as *Msx-2* but extends slightly more proximal then *Msx-2* (MacKenzie *et al*., 1992). *Msx-1* is not expressed in the oral epithelium (MacKenzie *et al*., 1991). These distinct early expression domains of *Msx-1* and *-2* in epithelium and mesenchyme suggest a possible role for these genes in initiation of the primary epithelial band (Sharpe, 1995).

Experimental evidence for a possible role of these genes in initiation of tooth development has come from *in vivo* experiments using targeted mutagenesis and also *in vitro* experiments involving explant cultures of early tooth germs. Targeted mutation of the *Msx-1* gene results in development of all teeth being arrested at the early bud stage (Satokata and Maas, 1994). Similarly mutations in MSX1 have been shown to be associated with tooth agenesis in humans (Vastardis *et al*., 1996). Since *Msx-1* is expressed at high levels in the condensing mesenchyme at the bud stage, this suggests that *Msx-1* is required for a signalling pathway from bud mesenchyme to dental epithelium in tooth histogenesis but not initiation of the tooth bud. The recombination experiments have shown that following an initial signal (possibly Bmp-4 or Shh), from the thickened epithelium to the underlying mesenchyme, the direction of communication is then reversed and signals pass from the condensing mesenchyme to the epithelial bud (Figure 1.2A). The toothless phenotype of the *Msx-1* mutants implies that *Msx-1* regulates the expression of these signalling molecules. A possible candidate signalling molecule is Bmp-4, and in fact, the levels of *Bmp-4* expression in tooth bud mesenchyme of *Msx-1* mutants is reduced. Moreover, tooth development in *Msx-1* mutants can be rescued by the addition of beads coated in Bmp-4; thus there is a strong case for Msx-1 regulating *Bmp-4* in condensing

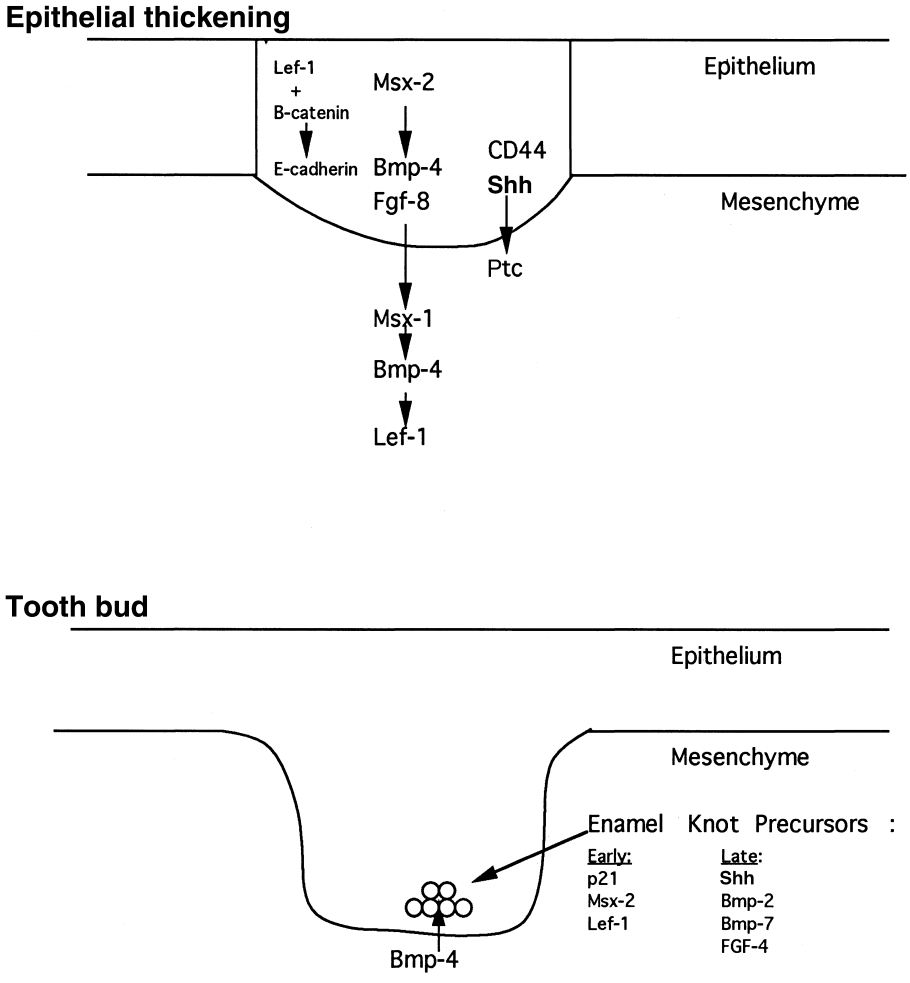

Figure 1.2. **A. Proposed signalling pathways involved in interactions between primary odontogenic epithelium thickening and ectomesenchyme at the epithelial band stage. B. Gene expression in the enamel knot precursor cells induced at the early bud stage by signals from the mesenchyme which may include Bmp-4.**

mesenchyme (Chen *et al.*, 1996). Although the *Msx-1* mutants give a clear tooth phenotype, the arrest does not occur at the initiation stage, i.e. tooth buds are produced. The earliest requirement for *Msx-1* would thus appear to be for mesenchymal to epithelial signalling at the bud stage. While it is possible that the targeted mutation is not a complete null (only the third helix is deleted in the mutant allele) it is more likely that *Msx-2* and/or *Dlx-2* may compensate for loss of *Msx-1* in tooth initiation. *Msx-2* targeted mutants have been generated and appear to have normal early tooth development. Significantly, however, tooth development in *Msx-1*/*Msx-2* double mutants is reported as being arrested earlier than the tooth bud stage and initiation may not occur at all in the absence of both genes (Maas *et al.*, 1996). Thus, there is strong *in vivo* data that supports the role of these genes in initiation. The possible role of *Dlx-2* in tooth development is discussed below.

Lef-1 is a transcription factor which is a member of the HMG-box family of DNA-binding proteins, of which the best known is the mammalian sex-determining gene SRY. Lef-1 is very closely related to another protein, Tcf-1, and these genes probably appeared by duplication of a single ancestor gene (Gastrop, *et al.*, 1992). *Lef-1* and *Tcf-1* show a very similar pattern of expression during tooth development, and both are expressed in T-lymphocytes. The function of both genes has been studied by targeted gene disruption where, surprisingly, the phenotypes produced are quite different. *Lef-1* mutant mice have tooth development arrested at the bud stage (similar to the *Msx-1* -/- mice) and show no major defects in T-lymphocytes (van Genderen *et al.*, 1994). *Tcf-1* mutants have normal tooth development but severely impaired T-cell function (Verbeek *et al.*, 1995). One possible hypothesis is that the archetypal gene is expressed and required for tooth development and that *Tcf-1*, duplicated from *Lef-1* and acquired a novel function in T-cells but which is not required for tooth development. *Lef-1* is expressed in

secondary epithelial thickenings and in condensing mesenchyme of the tooth bud in a similar domain to *Msx-1*. Because tooth development in *Lef-1* mutants is arrested at the bud stage, it was originally believed that *Lef-1* would be required for initiating the signalling pathway of molecules from condensing mesenchyme to the epithelium of the tooth bud similar to *Msx-1*. However, detailed recombination experiments using *Lef-1* mutant and wild-type epithelium and mesenchyme have shown that the *Lef-1* is required in the early thickened epithelium for tooth development (Kratochwil *et al*., 1996). Thus a recombination of *Lef-1* mutant epithelium with wild-type mesenchyme does not give tooth development, whereas the reciprocal recombination of mutant mesenchyme and wild-type oral epithelium allows normal tooth development *in vitro*. Moreover, the requirement for *Lef-1* in the thickened epithelium is transient, since mutant mesenchyme, cultured with wild-type epithelium and then dissociated and recultured with mutant epithelium, gives rise to normal tooth development, indicating that the signals regulated by *Lef-1* have been initiated in the mutant mesenchyme by the wild-type epithelium. Interestingly *Tcf-1* expression is barely detectable in epithelium but mesenchymal expression overlaps that of *Lef-1* indicating why *Tcf-1* may not be able to compensate for loss of *Lef-1* function in tooth bud development. The possibility that expression of Lef-1 in epithelium may be sufficient to induce tooth development has come from ectopic expression experiments in transgenic mice using a keratin 14 promoter which produced ectopic teeth (Zhou *et al*., 1995).

The genetic pathway in which *Lef-1* belongs is starting to be elucidated, providing important insights into the control of bud stage tooth development. Lef-1 interacts with β-catenin in the cell cytoplasm, resulting in transport of the Lef-1 protein into the nucleus. β-catenin is a component of the cell adhesion molecule E-cadherin, the expression of which is localised to tooth epithelial thickenings (Kemler, 1993, Behrens *et al*., 1996, Huber *et al*., 1996). β-catenin has been shown to be downstream of Wnt signalling in *Xenopus* and since some Wnt genes colocalise with Lef-1 and E-cadherin in tooth epithelial thickenings (A. McMahon, personal communication) a pathway involving Wnt regulation of *Lef-1* expression resulting in activation of E-cadherin with β-catenin as an intermediate may be important in early tooth development (Yost *et al*., 1996; Molenaar *et al*., 1996; Luning *et al*., 1994).

The localisation of other signalling molecules and transcription factors in tooth bud epithelial thickenings demonstrates that the Lef-1 pathway is not the only one of importance. The high levels of Shh gene expression in secondary tooth bud epithelial thickenings suggest an important yet undetermined role for this pathway in odontogenesis (Figure 1.3) (Bitgood and McMahon, 1995; Koyama *et al*., 1996; Sharpe, unpublished).

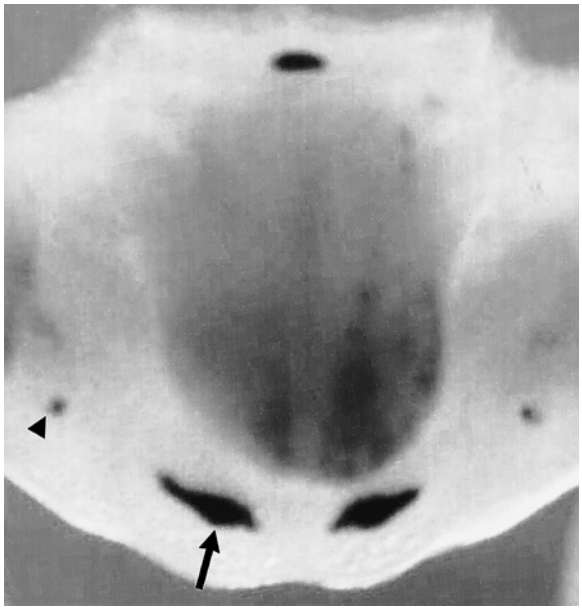

Figure 1.3. **Whole mount *in situ* hybridisation of a day-13.5 mandible showing expression of *Shh* in the epithelium of the developing incisor (arrow) and molar (arrowhead) tooth germs viewed from the oral aspect.**

1.3. Patterning of tooth position and shape

Mammalian teeth have characteristic shapes for each position in the jaws. The shape and position are important for dietary requirements and have evolved and diversified for particular specialised feeding functions. Incisors are conical, or chisel-shaped and located at the front of the jaws, where they are used not only for obtaining and cutting food, but also for grooming or defence functions. Molars are triangular, rectangular and multicuspid in shape and are located towards the back of the jaws aand are for processing food, either by cutting, grinding or crushing. Variations on these basic shapes have evolved for specialised diets such as carnassial teeth in carnivores which are a sectorial adaptation of tritubercular molars. The correct pattern of the dentition is thus essential for animal survival. The dentition of any animal species is as unique as its DNA and since patterns are inherited the developmental mechanisms that direct pattern formation must be genetically controlled.

The importance of Hox genes in development of the

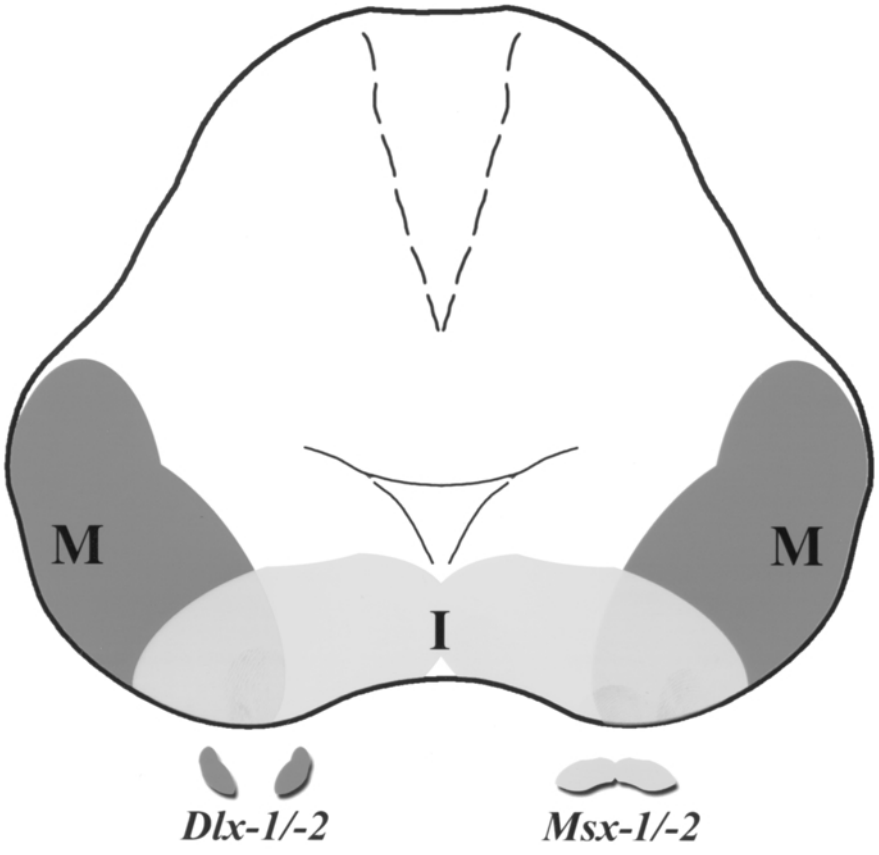

Figure 1.4. **Simplified model of the 'odontogenic homeobox gene code' of dental patterning based on the expression of *Msx-1, Msx-2, Dlx-1*and *Dlx-2* in the developing mandible. Overlapping domains of homeobox gene expression in the ectomesenchyme of the mandibular process can specify the shape of the tooth that develops at a particular position. Oral aspect, anterior to the top; I, incisor field; M, molar field.**

axial skeleton has been discussed. But it is possible that homeobox gene expression in the first branchial arch may be involved in patterning the development of first branchial arch structures such as teeth. A molecular model of patterning the dentition, based on the expression of several homeobox genes in neural crest-derived ectomesenchyme has been proposed and termed the 'odontogenic homeobox gene code' (Sharpe, 1995). A simplified form of this model is illustrated in Figure 1.4 where the expression of homeobox genes, such as *Dlx-1, Dlx-2*, *Msx-1* and *Msx-2* are proposed to specify the development of tooth germs into either molars or incisors. *Dlx-1* and *Dlx-2* are homeobox genes belonging to a family of seven or eight genes related to the *Drosophila Distalless* gene which is involved in appendage development. *Dlx-1* and *Dlx-2* are located within 10 kb of each other on mouse chromosome 2 and their expression in mandibular and maxillary mesenchyme is almost indistinguishable (Bulfone *et al*., 1993). Both genes are predominately expressed in the proximal ectomesenchyme of the mandible and maxilla in the area where molars will develop prior to the start of tooth development (E10). Expression is absent in more distal mesenchyme where incisors will develop. The expression of *Dlx-1* and *Dlx-2* is complemented by that of *Msx-1* and *Msx-2*, which are predominantly expressed in distal mesenchyme and not in proximal areas. In order to test this model, mice embryos with targeted disruption of the *Dlx-1* and *Dlx-2* genes using homologous recombination in ES cells have been analysed. Mice with null mutations in either gene have normal tooth development. It is only when double mutations are created, i.e. mice with null mutations in both genes, that tooth development is affected. *Dlx-1/-2 -/-* embryos have normal upper and lower incisors and lower molars but do not develop any upper molars (Qiu *et al*., 1997; Thomas *et al*., 1997).

Since the odontogenic mesenchyme expressing these homeobox genes is neural crest derived, this model predicts that populations of cranial neural crest cells are specified as odontogenic and further regionally specified as maxilla/mandible/molar/incisor. The failure of maxillary molar teeth to develop in the *Dlx-1/-2* double mutant embryos thus supports the odontogenic homeobox code model for patterning of maxillary molar tooth development and suggests that *Dlx-1* and *Dlx-2* are required for the specification of a subpopulation (maxillary molar) of odontogenic neural crest cells. The normal development of mandibular molars implies that tooth patterning in the lower and upper jaws is controlled independently, a

feature that was not originally predicted by the model but one that has interesting implications for mechanisms of evolution of dental patterns.

Goosecoid (Gsc) was originally envisaged as part of the odontogenic homeobox code but has since been shown to be important for mandibular skeletal, but not tooth, development. Targeted null mutations in the Gsc gene produce craniofacial defects that resemble first arch syndrome in humans, but the development of teeth in these mice is normal (Rivera-Perez *et al*., 1995; Yamada *et al*., 1995).

Gsc has been shown to be upregulated in *Xenopus* mesoderm formation by the secreted signalling protein activin. Activin is a member of the TGFβ superfamily of growth factor-like signalling molecules that have wide-ranging functions in embryogenesis. Targeted null mutation of the activin-βA gene in transgenic mice was predicted to result in defects in mesoderm formation. However, mesoderm formation in activin-βA mutants was found to be normal but significantly, the major phenotype was abnormalities in craniofacial development, particular tooth development. Activin-βA -/- mice develop no incisor teeth and no mandibular molars but development of maxillary molars is always normal. Thus null mutations in a molecule (activin-βA) produce a tooth patterning phenotype. In common with the Dlx-1/2 mutations, activin-βA is not required for development of all teeth but only for initiation of incisors and mandibular molars. Unlike the Dlx-1/2 mutations, activin-βA affects incisor tooth development to the same extent in the mandible and maxilla and this may either indicate that incisor development is controlled differently to molar development or that activin is involved in regulating different pathways in the mandible and maxilla (Matzuk *et al*., 1995).

Other homeobox genes are expressed during tooth development and some of these may also contribute to the odontogenic homeobox code. Other members of the Dlx family such as *Dlx-5* and *Dlx-6* are expressed in proximal regions of the developing mandible but not the maxilla and may form part of the code that is required for mandibular molar development. *Barx-1* is a homeobox gene that is expressed in the mesenchyme in areas where molars form but not incisor mesenchyme (Tissier-Seta *et al*., 1995).

1.4. Regulation of tooth shape

Once the spatial information is provided to specify development of a tooth germ into an incisor or molar, genes must be activated that control the shaping process to produce cusps (morphogenesis). The physical processes that direct cusp development have classically been suggested to involve the differentiation of the stellate reticulum and differential cessation of mitosis in the dental epithelium. The importance of differentiated transient epithelial structures, enamel knots, has recently gained significance. The enamel knot was originally identified in the 1920s in molar cap stage tooth germs as a transient, small group of epithelial cells immediately above the condensing mesenchyme forming the dental papilla. A role for the enamel knot in cusp formation was proposed by Orban (1928) and Butler (1956) who suggested that enamel knot cells act as a local restraint causing post-mitotic internal enamel epithelium to inflect at the site of the future first cusp and the external enamel epithelium to dimple as the swelling pressure of the developing stellate reticulum separated the external and internal enamel epithelia everywhere else in the tooth germ.

Msx-2 was the first gene whose expression was localised to enamel knot cells and it was proposed that *Msx-2* expression provided a molecular link between tooth initiation and shape (MacKenzie *et al*., 1992). Subsequently the expression of several more genes has been shown to be restricted to enamel knot epithelial cells in the tooth bud and the origin of enamel knot cells traced back to a few epithelial cells at the tip of molar tooth buds. Expression of the genes for the secreted factors Shh, Bmp-2, Bmp-4, Bmp-7 and Fgf-4 are all localised in enamel knot cells (Chapter 2, Figure 1.2B) suggesting that this structure acts as a signalling centre similar to both the AER and the zone of polarising activity (ZPA) in limb development (Vaahtokari *et al*., 1996). Expression of these genes has also identified the formation of secondary enamel knots in regions corresponding to the future cusp tips and it is proposed that the first (primary) enamel knot acts as a signalling centre to direct secondary knot formation which functions to control local epithelial cell proliferation rates. Enamel knot cells have a transient existence and rapidly undergo apoptosis which is probably controlled in part by early expression of p21 in the enamel knot cells (I. Thesleff, personal communication).

The specification of tooth patterning by the odontogenic homeobox code suggests that one way the code acts is by the odontogenic mesenchyme cells that condense at the base of the epithelial tooth bud communicating to the epithelial tooth bud cells to initiate enamel knot cell differentiation at specific positions (see Chapter 2).

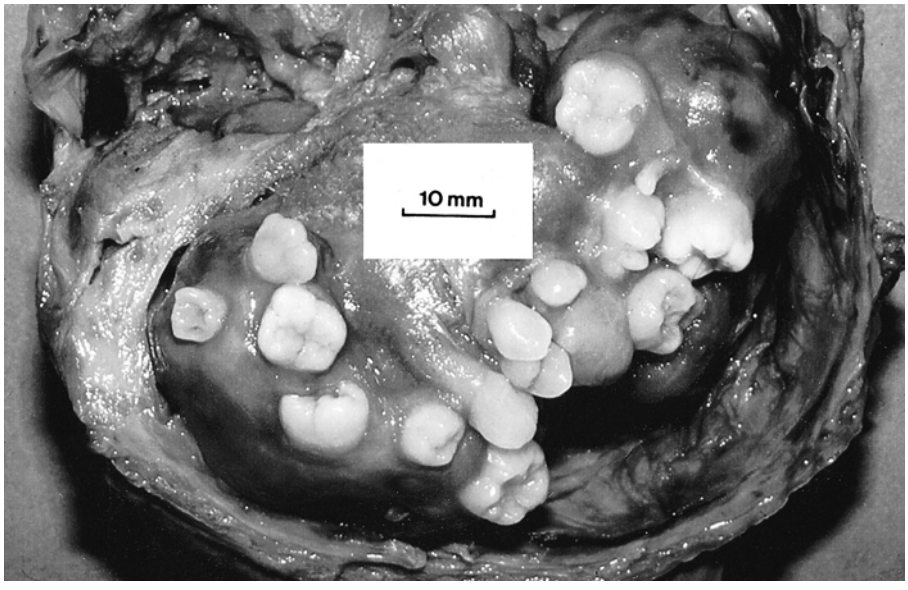

Figure 1.5. **Development of ectopic teeth in a human ovarian teratoma. (Courtesy of the Anatomy Museum, Guy's Hospital.)**

1.5. Ectopic tooth development

The correct development of teeth in the right place at the right time clearly involves many different interacting cellular and molecular processes. It comes as something of a surprise, therefore, to see perfectly formed teeth develop at ectopic sites. One of the most common sites of ectopic teeth formation is in ovarian teratomas (Figure 1.5). Teratomas are defined as germ cell tumours and since the normal development of ovaries and testes does not involve any contribution from cells of neural crest origin it is difficult to reconcile the development of teeth in these structures. Since other neural crest-derived organs such as pigmented hair also form in these teratomas it seemed likely that cells of neural crest origin are present. Alternatively since germ cells are pluripotent it is possible that in certain circumstances these may contribute to tooth mesenchyme, and that the neural crest cell phenotype, although only present normally in developmental stages, is strongly expressed in this abnormal development of these germ cells, neural crest being a vertebrate synapomorphy. Whatever the cellular origin of these teeth the fact remains that their shape, mineralisation, etc. is perfectly normal and clearly the mechanisms operating in normal tooth development in the oral cavity are working in this ectopic site. If it were only possible to study the formation of these ectopic teeth during the early developmental stages they could teach us a great deal about the molecular control of normal tooth development.

Summary

Homeobox genes are involved in the genetic control of many different developmental processes, including organogenesis, in invertebrate and vertebrate embryogenesis. Many different homeobox genes are expressed in the developing orofacial region and during tooth development in mammalian embryos and the roles of several of these genes is beginning to be elucidated. Homeobox genes were discovered in *Drosophila* where mutations in certain homeobox genes, namely homeotic genes, produced changes in embryonic patterning resulting in transformations of body parts. The potential role of homeobox genes in positional information of cells in organogenesis has led to investigation of the possible role of homeobox genes expressed in the developing orofacial region of mammalian embryos in patterning for tooth position and shape.

Acknowledgements

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References

Behrens, J., von Kries, J. P., Kühl, M., Bruhn, L., Wedlich, D., Grosschedl, R. and Birchmeier, W. (1996). Functional interaction of β-catenin with the transcription factor LEF-1. *Nature*, **382**, 638–642.

Bellusci, S., Henderson, R., Winnier, G., Oikawa, T. and Hogan, B. L. M. (1996). Evidence form normal expression and targeted misexpression that bone morphogenetic protein-4 (Bmp-4) plays a role in mouse embryonic lung morphogenesis. *Development*, **122**, 1693–1702.

Bitgood, M. J. and McMahon, A. P. (1995). *Hedgehog* and *Bmp* genes are coexpressed at many diverse sites of cell–cell interaction in the mouse embryo. *Developmental Biology*, **172**, 126–138.

Bulfone, A., Kim, H. J., Puelles, L., Porteus, M. H., Grippo, J. F. and Rubenstein, J. L. R. (1993). The mouse *Dlx-2 (Tes-1)* gene is expressed in spatially restricted domains of the forebrain, face and limbs in midgestation mouse embryos. *Mechanisms of Development*, **40**, 129–140.

Butler, P. M. (1956). The ontogney of molar pattern. *Biological Reviews*, **31**, 30–70.

Chen, Y., Bei, M., Woo, I., Satokata, I. and Maas, R. (1996). *Msx1* controls inductive signalling in mammalian tooth morphogenesis. *Development*, **122**, 3035–3044.

Duboule, D. (1994). *Guidebook to the Homeobox Genes*. Oxford: Oxford University Press.

Gastrop, J., Hoevenagel, R., Young, J. R. and Clevers, H. C. (1992). A common ancestor of the mammalian transcription factors RCF-1 and TCF-1 alpha/LEF-1 expressed in chicken T cells. *European Journal of Immunology*, **22**, 1327–1330.

Gaunt, S. J., Miller, J. R., Powell, D. J. and Duboule, D. (1986). Homeobox gene expression in mouse embryos varies with position by the primitive streak stage. *Nature*, **324**, 662–664.

Gehring, W. J. (1987). Homeo boxes and the study of development. *Science*, **236**, 1245–1252.

van Genderen, C., Okamura, R., Fariñas, I., Quo, R. G., Parslow, R. G., Bruhn, L. and Grosschedl, R. (1994). Development of several organs that require inductive epithelial–mesenchymal interactions is impaired in LEF-1 deficient mice. *Genes and Development*, **8**, 2691–2703.

Huber, O., Korn, R., McLaughlin, J., Ohsugi, M., Herrmann, B. G. and Kemler, R. (1996). Nuclear localization of β-catenin by interaction with transcription factor LEF-1. *Mechanisms of Development*, **59**, 3–10.

Imai, H., Osumi-Yamashita, N.,Ninomiya, N. and Eto, K. (1996). Contribution of early-emigrating midbrain crest cells to the dental mesenchyme of mandibular molar teeth in rat embryos. *Developmental Biology*, **176**, 151–165.

Kemler, R. (1993). From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. *Trends in Genetics*, **9**, 317–321.

Kingsley, D. M. (1994). The TGF-B superfamily: new members, new receptors, and new genetic tests of function in different organisms. *Genes and Development*, **8**, 133–146.

Koentges, G. and Lumsden, A. (1996). Rhombencephalic neural crest segmentation is preserved throughout craniofacial ontogeny. *Development*, **122**, 3229–3242.

Koyama, E., Yamaai, T., Iseki, S., Ohuchi, H., Nohno, T., Yoshioka, H., Hayashi, Y., Leatherman, J. L., Golden, E. B., Noji, S. and Pacifici, M. (1996). Polarizing activity, *Sonic Hedgehog*, and tooth development in embryonic and postnatal mouse. *Developmental Dynamics*, **206**, 59–72.

Kratochwil, K., Dull, M., Fariñas, I., Galceran, J. and Grosschedl, R. (1996). *Lef–1* expression is activated by Bmp-4 and regulates inductive tissue interactions in tooth and hair development. *Genes and Development*, **10**, 1382–1394.

Laughon, A. and Scott, M. P. (1984). Sequence of a *Drosophila* segmentation gene: protein structure homology with DNA-binding proteins. *Nature*, **310**, 25–31.

LeMouellic, H., Lallemand, Y., and Brûlet, P. (1992). Homeosis in the mouse induced by a null mutation in the *Hox-3.1* gene. *Cell*, **69**, 251–264.

Lumsden, A. G. S. (1988). Spatial organisation of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. *Development* (Supp.), **103**, 155–169.

Lüning, C., Rass, A., Rozell, B., Wroblewski, J. and Öbrink, B. (1994). Expression of E-cadherin during craniofacial development. *Journal of Craniofacial Genetics and Developmental Biology*, **14**, 207–216.

Luo, G., Hofmann, C., Bronckers, A. L. J. J., Sohocki, M., Bradley, A. and Karsenty, G. (1995). Bmp-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. *Genes and Development*, **9**, 2808–2820

Maas, R. L., Chen, Y., Bei, M., Woo, I. and Satokata, I. (1996). The role of Msx genes in mammalian development. *Annals of the New York Academy of Sciences*, **785**, 171–181.

MacKenzie, A., Leeming, G., Jowett, A., Ferguson, M. W. J. and Sharpe, P. T. (1991). The homeobox gene *Hox-7.1* has specific regional and temporal expression patterns during early murine craniofacial embryogenesis, especially tooth development *in vivo* and *in vitro*. *Development*, **111**, 269–285.

MacKenzie, A. L., Ferguson M. W. J. and Sharpe P. T. (1992). Expression patterns of the homeobox gene *Hox-8* in the mouse embryo suggest a role in specifying tooth initiation and shape. *Development*, **115**, 403–420.

Manak, J. R. and Scott, M. P. (1994). A class act: conservation of homeodomain protein functions. In *The Evolution of Developmental Mechanisms*, eds. M. Akam, P. Holland, P. Ingham and G. Wray pp. 61–77. Cambridge: The Company of Biologists.

Matzuk, M. M., Kumar, T. R., Vassaill, A., Bickenbach, J. R., Roop, D. R., Jaenisch, R. and Bradley, A. (1995). Functional analysis of activins during mammalian development. *Nature*, **374**, 354–356.

Mina M. and Kollar E. J. (1987). The induction of odontogenesis in non-dental mesenchyme combined with early murine mandibular arch epithelium. *Archives of Oral Biology*, **32**, 123–127.

Molenaar, M., van de Wetering, M., Oosterwegel, M., Peterson-Maduro, J., Godsave, S., Korinek, V., Roose, J., Destree, O. and Clevers, H. (1996). XTcf-3 transcription factor mediates beta-catenin-induced axis formation in *Xenopus* embryos. *Cell*, **86**, 391–399.

Orban, B. (1928). *Dental Histology and Embryology*. Chicago: Rogers Printing.

Prince, V. and Lumsden, A. (1994). *Hoxa-2* expression in normal and transposed rhombomeres: independent regulation in the neural tube and neural crest. *Development*, **120**, 911–923.

Qiu, M., Bulfone, A., Ghattas, I., Menses, J. J., Sharpe, P. T., Presley, R., Pedersen, R. A. and Rubenstein, J. L. R. (1997). Role of the *Dlx* homeobox genes in proximodistal patterning of the branchial arches: mutations of *Dlx-1*, *Dlx-2* and *Dlx-1* and *-2* alter