British Journal of Orthodontics/Vol. 26/1999/21–28

Scientific Section

REVIEW ARTICLE*

The Genetic Control of Early Odontogenesis

MARTYN. T. COBOURNE, B.D.S. (HONS), F.D.S.R.C.S. (ENG), M.SC. (U.LOND), M.ORTH. R.C.S. (ENG).
Department of Craniofacial Development, G. K. T. Dental Institute, King's College London, London Bridge, London SE1 9RT, U.K.

Refereed Paper

Introduction

Odontogenesis is a highly co-ordinated and complex process which relies upon cell-to-cell interactions that result in the initiation and generation of the tooth. Whilst the gross histological processes are well documented (Ferguson, 1990), the mechanisms that are involved at a molecular level are only now beginning to be elucidated (Maas and Bei, 1997). This is largely due to the revolution in molecular biological techniques that has occurred over the last decade and their continued application in developmental biology.

It is the mouse that has become the principle organism used to study mammalian development because of its suitability for both genetic and embryological manipulation (Ignelzi *et al*., 1995). Engineered genes can be permanently inserted into the germline to produce transgenic mice which allows a direct method of studying the function of a gene during development. Similarly, gene targeting experiments can produce selective gene knockout and transgenic mice that are missing the expression of specific genes. As these techniques of introducing DNA into the cell have become more sophisticated, the generation of transgenic mice using reporter gene constructs are now beginning to provide information on the regulatory sequences that are involved in controlling the transcription of particular genes.

Fundamental to the study of tooth development is the manipulation of tooth germ explants from wild type and mutant mice. These explants can be cultured *in vitro* as far as odontoblast and ameloblast differentiation with the early stages of both dentine and enamel secretion beginning to occur (Figure 1). The technique of *in situ* hybridization, using labelled mRNA probes allows the domains of expression of specific genes to be visualized in these developing tooth germs. Cultured tooth germs can now be transferred into the kidney capsules of adult male mice, allowing tooth development to proceed to full crown formation and localized alveolar bone differentiation (Figure 2). Further adaptations to the culture technique have provided great insight into the signalling mechanisms that occur during odontogenesis. Dental epithelium and mesenchyme can be separated, and recombined with tissues of different origins, developmental stages, and altered genetic constitutions. Agarose or heparin acrylic beads expressing protein signalling molecules, and growth factors can be implanted into cultured epithelium and mesenchyme. The resulting effects of these techniques on downstream gene expression and odontogenic phenotype can be then be evaluated. This article aims to give an overview of the more established theories on the molecular regulation of tooth development and to introduce some recent advances that have occurred in this ever expanding field.

An Overview of Odontogenesis

The first morphological evidence of odontogenesis is the formation of a primary thickening of the oral epithelium. This primary epithelial band forms a continuous horseshoe-shaped sheet of epithelium around the lateral margins of the developing oral cavity. The free margin of this band gives rise to two processes which invaginate into the underlying mesenchyme. The outer process, the vestibular lamina, will form the vestibule that demarcates the cheeks and lips from the tooth-bearing regions. The inner process is the dental lamina and it is from the dental lamina that the tooth buds form. Discreet swellings of the dental lamina form the enamel organs of the future developing teeth. As these enamel organs differentiate through the characteristic bud, cap and bell stages, localized condensations of neural crest-derived ectomesenchymal cells become engulfed, forming the dental papilla. More peripherally, the condensing mesenchymal cells extend around the enamel organ as the dental follicle. Together, all of these developing dental tissues are known as the tooth germ.

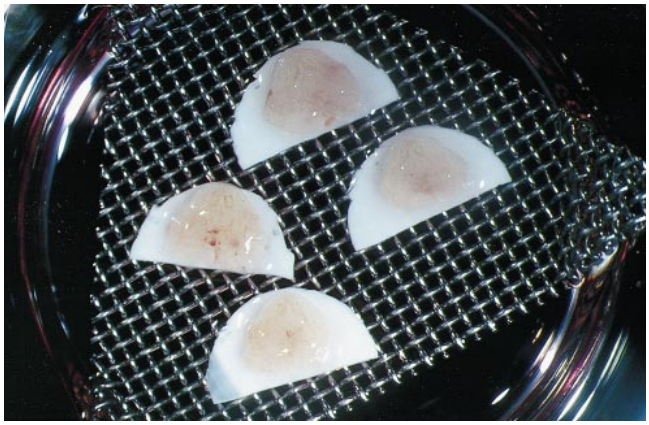

FIG. 1 E13·5-day-old murine mandibular processes being cultured. The mandibles are placed on a 0·1 μm Millipore filter supported by a 0·25-mm diameter wire mesh in an organ culture dish containing culture media.

* This review series is coordinated by sub-editor Dr. Jonathan Sandy.

0301-228X/99/010000+00$02.00

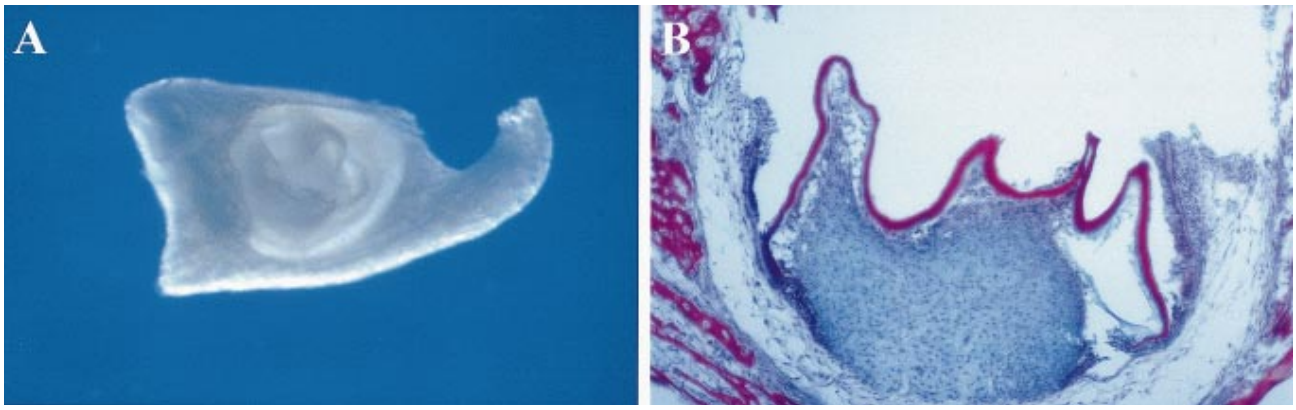

FIG. 2 Murine first mandibular molar developing in its crypt of alveolar bone. An E12·5 mandibular tooth germ was cultured for 3 days and then transferred to the kidney capsule of an adult male mouse for a further 10 days. (A) Appearance following harvest; (B) Histology (Alcian blue/chlorontaine ×10).

At the late bell stage of development, the innermost layer of cells of the enamel organ, the inner enamel epithelium, induce those adjacent cells of the dental papilla to differentiate into odontoblasts. Odontoblasts are responsible for the formation and mineralization of the dentine matrix. Dentine formation is preceded by the formation of predentine. The first layer of predentine acts as a signal to the overlying inner enamel epithelial cells to differentiate into ameloblasts and begin secreting the enamel matrix. At the margins of the enamel organ the cells of the inner enamel epithelium are confluent with the outer enamel epithelial cells at the cervical loop. Growth of these cells in an apical direction forms a skirt-like sheet of cells, Hertwig's epithelial root sheath, which maps out the future root morphology of the developing tooth and induces the further differentiation of odontoblasts. Degeneration of this root sheath leads to the exposure of the cells of the dental follicle to the newly-formed root dentine. This induces the differentiation of these mesenchymal cells into cementoblasts which begin to deposit cementum onto the root surface. Surrounding the enamel organ, the cells of the dental follicle produce the alveolar bone and collagen fibres of the periodontium. The developing tooth remains housed in this cavity of alveolar bone until the process of eruption begins.

Patterning of the Dentition

In all mammalian dentitions the teeth form a meristic series with the members of each group (incisors, canines, premolars, and molars) having graded characteristic shapes and sizes according to their position along the dental arch. This patterning is tightly controlled: transpositions are occasionally seen, but they usually involve teeth at the border of a particular series (i.e. canines and premolars); more severe anomalies of patterning (i.e. molars developing at the front of the arch) do not occur. Classically, two theories have been proposed to account for this. The field theory (Butler, 1939) suggests that all tooth primordia are initially equivalent, with the individual shapes that they subsequently develop into being controlled by varying concentrations of morphogens in the local environment. A number of diffusible signalling molecules have been identified that may be involved in concentration-dependant, threshold response mechanisms which could produce periodicity along the developing dental axes. However, if these mechanisms are responsible for patterning in both dentitions then they must act very early on in the developmental process. Unlike the mandibular dental axis the developing maxillary dentition is not continuous. The maxillary incisors develop in the medial nasal processes, whilst the remainder of the dentition develops in the maxillary processes of the first arch (Weiss *et al.*, 1998).

In the clonal model (Osborn, 1978), the tooth primordia are said to be prespecified with each migrating cell population being equipped with the necessary positional information to produce the different classes of teeth from inception. Much attention has been given to neural crest cells, a transient embryonic cell population that arises from the lateral margins of the neural plate during neurulation. Migration of these cells from the region of the developing hindbrain provides much of the mesenchyme (ectomesenchyme) of the developing oro-facial region, including that contributing to odontogenesis (Noden, 1984). A key question in understanding how the developing teeth are initiated and patterned is to know the extent to which neural crest cells are prespecified prior to their migration. A number of different genes have emerged that are strong candidates for controlling neural crest prespecification.

Homeobox Genes

Homeobox genes are a large group of genes that code for transcription factors responsible for regulating the expression of downstream target genes. The homeobox is a highly conserved 180-basepair sequence that codes for the homeodomain, a 60-amino acid helix-turn-helix DNA-binding motif within the encoded transcription factor (McGinnis *et al.*, 1984a). The homeobox was originally discovered in the homeotic selector genes of the fruitfly *Drosophila melanogaster*, where they are responsible for specifying segment identity in the developing fly. Homeotic genes exhibit a feature known as co-linearity, their spatial arrangement along the chromosome is in the same order as their patterns of expression along the anteroposterior axis of the fly embryo (Lewis, 1978). Genes expressed at the 3′ end of the complex are expressed anteriorly, whereas those at the 5′ end are expressed posteriorly. Thus, each segment

of the fly has a different combination of homeotic gene expression that specifies the individuality of that segment. Mutations in these genes can lead to bizarre homeotic transformations where one segment of the fly can be transformed into another segment. As an example, the antennapedia gene specifies identity of the second thoracic segment, in the dominant mutation of antennapedia this gene is also expressed in the head. The result of this is the growth of legs from the head sockets instead of antennae. Cross-hybridization studies have shown that the homeobox is not just confined to insects, it has been conserved during evolution and is also found in vertebrates (McGinnis *et al*., 1984b). The equivalent family of vertebrate genes are the Hox genes and these, too, express co-linearity. Both in mice and man, four Hox gene clusters are found on four different chromosomes.

The hindbrain region of the developing neural tube from which the neural crest migrates is segmented into eight rhombomeres. Segment specific combinatorial Hox gene expression specifies each rhombomeres identity. The migrating neural crest carries this Hox code defined patterning which is transferred to the branchial arches (Lumsden *et al*., 1991). The Hox code thus sets up regional diversity within the branchial arch system. It is plausible, therefore, that the Hox code of those cells migrating to the tooth forming regions is responsible for specifying and patterning the dentition. However, the Hox genes are not expressed in regions rostral to rhombomere 2 which means that no Hox gene expression is seen in the neural crest that migrates to the craniofacial region, including the first branchial arch (Hunt *et al*., 1991a,b). In terms of patterning tooth development, we have to look at a subfamily of homeobox genes that do show temporal and spatial patterns of expression within the first branchial arch.

Msx and Dlx Homeobox Genes

Two classes of homeobox genes, the Msx and Dlx genes are expressed in both migrating neural crest cells and in spatially restricted regions of the first branchial arch during murine development. These genes also contain a highly conserved homeobox, but it is more divergent than the Hox and homeotic genes. The vertebrate Msx genes are a three-gene family related to the *Drosophila* gene muscle-segment homeobox (*msh*) (Bell *et al*., 1993). The *Dlx* genes have also been conserved during evolution and bear homology to the distal-less gene of *Drosophila* (Porteus *et al*., 1991).

Prior to the initiation of odontogenesis both Msx-1 and Msx-2 exhibit very specific horseshoe-shaped fields of corresponding mesenchymal expression in the anterior regions of the first arch (MacKenzie *et al*., 1992). These expression patterns are coincident except along their posterior border where the expression of Msx-1 extends further than Msx-2. This region of isolated mesenchymal Msx-1 expression corresponds to the position of the future primary epithelial thickening. As tooth development progresses the expression of Msx-1 becomes localized in the mesenchymal cells of the dental follicle and papilla. The domains of expression of Msx-2 also become more restricted to the dental follicle and papilla, but unlike Msx-1, Msx-2 is also expressed strongly in the enamel organ (Figure 3). In contrast, the expression of Dlx-1 and Dlx-2 in the maxillary and mandibular arch mesenchyme is restricted to the proximal regions where the future molar teeth will develop.

Barx-1

Barx-1 is another homeobox containing transcription factor that exhibits regionalized expression within the ectomesenchyme of the first branchial arch (Tissier-Seta *et al.*, 1995). Prior to the appearance of the primary epithelial thickening Barx-1 (along with Dlx-2) is expressed in the posterior regions of the first branchial arch mesenchyme, the region of future molar development. There is no Barx-1 expression in the anterior regions. As tooth development proceeds, Barx-1 expression becomes localized exclusively to the mesenchymal regions around the developing molars (Tissier-Seta *et al.*, 1995; Thomas and Sharpe, 1998).

An Odontogenic Homeobox Code

Based upon such highly specific domains of expression, it has been suggested that these genes provide a homeobox code that specifies regions of the developing jaws to assume

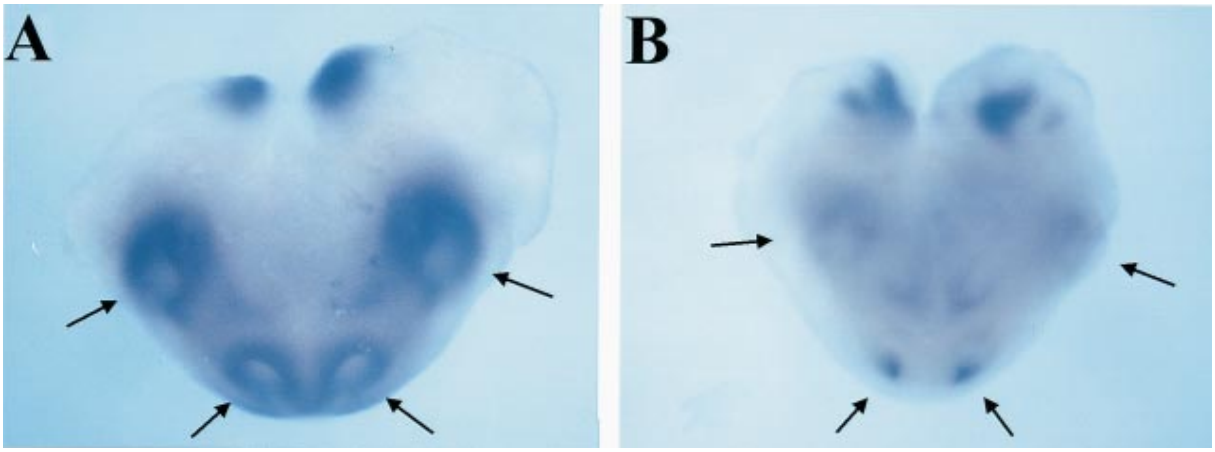

FIG. 3 Digoxygenin-labelled *in situ* hybridization of E13·5 murine mandibular processes showing localized expression (arrowed) of (A) Msx-1 and (B) Msx-2 in the regions of the developing tooth germs.

odontogenic potential (Sharpe, 1995). Each particular region expresses a unique combination of homeobox genes which then gives rise to teeth of a particular class. The molecular basis of this patterning is the differential expression of the coded homeobox nuclear proteins which regulate downstream gene transcription. The analysis of mice with targeted mutations in the expression of Msx-1 provide some evidence for this (Satokata and Maas, 1994). In these mice, the incisors fail to develop and molar development is arrested at the late bud stage. Mice with targeted mutations in either the Dlx-1 or Dlx-2 genes have normal tooth development, despite having a number of defects in skeletal elements derived from proximal first arch mesenchyme. However, targeted null mutations in both Dlx-1 and Dlx-2 results in mice with a phenotype expressing absent maxillary molars (Qiu *et al*., 1997). Heterologous recombination experiments between mutant and wild-type maxillary epithelium and mesenchyme indicate that the mutant mesenchyme in these double homozygous mice has lost its odontogenic potential. The neural crest derived mesenchyme in the molar region of these mutants appears to have an altered fate, becoming chondrogenic instead of odontogenic (Thomas *et al*., 1997).

Epithelial-mesenchymal Interactions

Once the process of odontogenesis has been initiated, complex interactions between epithelium and mesenchyme are responsible for generating the tooth (Thesleff and Sharpe, 1997). Recombination experiments have confirmed that prior to the bud stage of development the potential to induce tooth morphogenesis resides in the epithelium, only mandibular arch epithelium is capable of inducing the differentiation of a dental papilla in first arch mesenchyme prior to the bud stage (Mina and Kollar, 1987). However, the mandibular epithelium can only specify tooth development in mesenchyme that is neural crest derived, be it cranial or trunk (Lumsden, 1988). After the bud stage, this inductive potential shifts to the mesenchyme, with odontogenic mesenchyme being capable of inducing tooth formation when recombined with non-odontogenic epithelium (Kollar and Baird, 1970). The molecular basis for these experimental observations is dependant upon many of the diffusible protein signalling molecules and growth factors that are known to mediate reciprocal signalling between cell groups in epithelium and mesenchyme during development (Table 1). A number of members of these groups have been identified in the developing tooth germ at various stages of development.

Bone Morphogenetic Proteins

The bone morphogenetic proteins (Bmp's) are a large family of dimeric proteins within the Transforming Growth Factor β superfamily of cytokines. Originally identified as the active components within osteo-inductive extracts derived from bone they are now known to be involved in a wide range of signalling functions that mediate tissue interactions during development (Kingsley, 1994). Vertebrate Bmp-2 and Bmp-4 are homologous with the *Drosophila* decapentaplegic (*Dpp*) gene which has an important role in ectodermal-mesodermal signalling in the fly. The expression of Bmp's has been associated with epithelial-mesenchymal interactions involved in the development of a number of organs, including the teeth (Vainio *et al*., 1993; Heikinheimo *et al*., 1994). Bmp-2, 4, and 7 are all expressed in the presumptive dental epithelium during early tooth morphogenesis. The expression pattern of Bmp-4, however, shifts from the epithelium to the condensing dental mesenchyme at the same time that the inductive potential for odontogenesis shifts from epithelium to mesenchyme. This suggests that Bmp-4 may be a principle component of the signal responsible for inducing odontogenic potential in the mesenchyme. Beads expressing Bmp-4 protein are also capable of inducing the expression of Msx-1, Msx-2 and Bmp-4 in cultured mesenchyme from the tooth bearing regions (Vainio *et al*., 1993). Mice which do not express Msx-1 have molar development that arrests at the late bud stage and mesenchymal expression of Bmp-4 which is down-regulated. In the presence of Bmp-4 however, these tooth germs are capable of reaching the cap stage. It is evident that Msx-1 is required to mediate this shift of Bmp-4 expression from epithelium to mesenchyme, possibly acting as an amplifier of the Bmp-4 signal. Recent *in vitro* experiments involving the use of noggin (an antagonist of Bmp-4) have verified the role of epithelial Bmp-4 as providing a positive feedback loop which maintains the expression of Msx-1 and Bmp-4 in the mesenchyme. This has the effect of restricting the expression of these genes to the future tooth forming regions (Tucker *et al*., 1998).

Fibroblast Growth Factors

The fibroblast growth factors (FGFs) are a large family of heparin binding proteins that are known to mediate the growth and differentiation of cells from a wide variety of developmental origins (Wilkie *et al*., 1995). Comprehensive *in situ* hybridization studies have shown that Fgf-4, Fgf-8, and Fgf-9 are all expressed in epithelial cells of the developing tooth germ at times when epithelial-mesenchymal interactions are known to be regulating odontogenic morphogenesis (Kettunen and Thesleff, 1998). Expression of Fgf-8 and Fgf-9 is seen initially in the primitive oral epi-

TABLE 1 *Intercellular protein signalling molecules*

Fibroblast growth factors (FGF's)
FgF-1 to FgF-10
eFgF
Transforming growth factor β (TGF-β) superfamily
Activin
Vg-1
Bone morphogenetic proteins
Decapentaplegic (*Drosophila*)
Hedgehog
Hedgehog (insects)
Sonic hedgehog (vertebrates)
Indian hedgehog (vertebrates)
Desert hedgehog (vertebrates)
Tiggywinkle hedgehog (vertebrates)
Wingless
Wingless (insects)
Wnt family proteins (vertebrates)
Delta and serrate
Ephrins

Adapted from Wolpert, L. (1998).

thelium, at the time of odontogenic initiation this expression becomes restricted to the area of the presumptive dental epithelium and persists until the beginning of the bud stage. Both Fgf-4 and Fgf-8 expression then becomes up-regulated later on at the cap stage of development in the enamel knot, and later again in the secondary enamel knots that form at the sites of future cuspal morphogenesis. These findings suggest roles for Fgf-8 and Fgf-9 in mediating the initiation of tooth development, and for Fgf-4 and Fgf-9 in determining coronal morphology.

Sonic Hedgehog

Sonic hedgehog (Shh) is the vertebrate homologue of the *Drosophila* hedgehog (*hh*) segment polarity gene which is involved in defining the identity of parasegment borders in the developing fly embryo. In the vertebrate, Shh encodes a signal peptide that mediates both long and short range patterning in a number of well known developmental signalling centres (Hammerschmidt *et al*., 1997). During odontogenesis, Shh is expressed strongly in the epithelial thickenings of the future tooth forming regions (Bitgood and McMahon, 1995) and also at a later stage of development in the enamel knot (Vaahtokari *et al*., 1996a). This expression pattern has led to speculation that Shh is involved in epithelial signalling both during the initiation of tooth development and at a later stage during cuspal morphogenesis. Targeted disruption of Shh in knockout mice results in severe defects of the central nervous system, the axial skeleton, and the limbs (Chiang *et al*., 1996). These mice are cyclopic and have holoprosencephaly, a failure of midline cleavage of the developing forebrain and they die before birth. As Sonic hedgehog is required for embryonic viability prior to the onset of odontogenesis in these mutant mice they reveal little regarding its role in tooth development. However, the role of some downstream target genes in the Shh signalling pathway has recently been investigated in relation to odontogenesis (Hardcastle *et al*., 1998). The Gli zinc finger transcription factors (Gli-1, –2, –3) are known to act downstream of Shh. Analysis of mice with the expression of Gli-2 knocked out revealed abnormal development of the maxillary incisors, possibly due to a mild form of holoprosencephaly. However, Gli-3 mutants showed normal development of their dentitions. Double homozygous knockout mice for Gli-2/Gli-3 had no teeth that developed normally, whereas in double homozygous/heterozygous mutants (Gli-2 –/–, Gli-3 +/–) maxillary incisor development arrested, and all molars and the mandibular incisors were microdont. These results have confirmed an essential role for Shh signalling in odontogenesis and suggest a degree of functional redundancy between some members of the downstream target genes.

The Role of the Enamel Knot

The enamel knot is composed of a transient population of non-dividing epithelial cells that appear during the late bud stage of development at the site of the primary tooth cusps. Initially, the enamel knot expresses the Bmp-2, Bmp-7, and Shh signalling molecules, but later, during the cap stage, it also expresses Bmp-4 and Fgf-4. It is thought that the enamel knot acts as a signalling centre, being responsible for directing cell proliferation and subsequent cuspal morphogenesis in the developing enamel organ (Vaahtokari *et al*., 1996a). In molar teeth, secondary enamel knots also appear at the sites of the future secondary cusps, almost certainly under the influence of the primary knot. Both the primary and secondary knot cells express Fgf-4 and are non-dividing; Fgf-4 is known to stimulate proliferation of both dental epithelium and mesenchyme. It has been proposed that this induced cell proliferation of the enamel organ in conjunction with the lack of cell division in the enamel knot allows the growth and folding of the developing cusps (Jernvall *et al*., 1994). At the cap stage of development, the cells of the enamel knot undergo apoptosis and disappear, presumably switching off its signalling function (Vaahtokari *et al*., 1996b). The enamel knot is formed during the late bud stage of tooth development when the capacity to induce tooth morphogenesis is known to reside in the mesenchyme. The proposed signalling function of the enamel knot implies that an epithelial derived structure does have a regulatory role to play in the later stages of odontogenesis. The enamel knot is seemingly necessary for morphogenesis of the tooth germ to progress from the bud to the cap stage.

Clinical Perspectives

Targeted mutations in transgenic mice have pinpointed genes that can produce phenotypically expressed disruption in murine tooth development. Attempts are now being made to isolate and clone genes in human populations that may be responsible for hypodontia (Thesleff, 1996). Family studies have established that incisor and premolar hypodontia is inherited via an autosomal dominant gene which demonstrates incomplete penetrance (Burzynski and Escobar, 1983). This form of hypodontia, affecting one or a few teeth (upper lateral incisors and lower second premolars most commonly) has recently been investigated in relation to the expression of the human MSX-1 and MSX-2 genes (Nieminen *et al.*, 1995). Five Finnish families were studied that included 20 individuals affected with the congenital absence of from one to four teeth, but linkage analysis excluded these genes as causative loci for this form of hypodontia. However, genetic linkage analysis in a family affected with a rather more severe form of hypodontia (oligodontia) involving the absence of all second premolars and third molars has identified a causative locus on chromosome 4p where the MSX-1 gene resides. Sequence analysis of this region revealed an Arg31Pro mutation in the homeodomain of the MSX-1 gene in all the affected family members. It was proposed that this simple mutation prevented the normal integration of the MSX-1 transcription factor with target DNA and other transcription factors. These compromised MSX-1 interactions being critical for the normal development of specific teeth (Vastardis *et al*., 1996).

Many of the genes that are known to be key players in odontogenesis also have widespread roles during development of the craniofacial complex. Mutations in some of these genes have now been identified in a number of human craniofacial anomalies. A locus for one rare autosomal dominant form of craniosynostosis (Boston type) has been mapped to chromosome 5qter. The human MSX-2 gene is

localized here and affected individuals from one family had a simple Hist7Pro mutation in the MSX-2 homeodomain (Jabs *et al*., 1993). No mutations were found in the MSX-2 gene of individuals affected with the more common Crouzon or Apert craniosynostotic syndromes. However, analysis of the function of MSX-2 in the development of the Boston form of this disease may well help in our understanding of these more common forms. The tricho-dento-osseous syndrome (TDO) is an autosomal dominant disorder characterized by abnormal hair, enamel hypoplasia and taurodontism, and cranial thickening associated with the frontal/mastoid air sinuses. The TDO locus has been mapped to chromosome 17q21, a region that includes the human DLX-3 and DLX-7 genes. A 4-basepair deletion in DLX-3 has very recently been identified which correlated with the TDO phenotype in six families. This mutation produced a frameshift and premature termination codon in the transcribed DLX-3 transcription factor (Price *et al.*, 1998).

The human *SHH* gene has been identified as the *HPE3* gene on chromosome 7q36, the first known gene to cause holoprosencephaly. Five affected families have recently been identified that carry mutations in the *SHH* gene. Two of these mutations truncate the gene, whereas the others replace critical residues in the transcribed SHH protein (Roessler *et al*., 1996). This study confirms that alterations in *SHH* can lead to dominant effects on human development. Patched (Ptc) is a putative 12-transmembrane domain protein that acts as a receptor for the Shh ligand (Stone *et al.*, 1996). Patched is an unusual receptor in that it represses the Shh signal unless bound by the ligand. Mutation of the human Patched (*PTCH*) gene occurs in the autosomal dominant basal cell naevus (Gorlins) syndrome (Hahn *et al.*, 1996; Johnson *et al.*, 1996). This syndrome is characterized by multiple basal cell carcinomas of the skin, medulloblastomas, and multiple keratocysts of the jaws. These patients can also develop anomalies found in holoprosencephaly including skeletal abnormalities and cleft lip/palate. As a suppressor of Shh it would seem that a loss of function *PTCH* mutation can cause abnormal growth in certain cell types (Dean, 1996).

Conclusions

The study of odontogenesis is providing insight into the developmental control mechanisms that operate at a molecular level during embryogenesis. The developing tooth provides one of the most useful experimental models for the study of induction and patterning mechanisms that are involved during organ morphogenesis. As our knowledge of these tissue interactions is extended, the murine model is likely to continue providing valuable information into the processes involved during human odontogenesis and craniofacial development. This will undoubtedly lead to advances in our understanding of the mechanisms that are involved in producing anomalies in the development of the craniofacial complex.

Acknowledgements

I am grateful to Professor Paul Sharpe, for the valuable comments he made during the preparation of this manuscript, and to Stalyn Kariyawasam, for the histology in Figure 2B. I would also like to acknowledge the support of the Wellcome Trust for providing my funding.

References

Bell, J. R., Noveen, A., Liu, Y. H., Ma, L., Dobias, S., Kundu, R., Luo, W., Xia, Y., Lusis, A. J. and Snead, M. L. (1993)
Genomic structure, chromosomal location, and evolution of the mouse Hox-8 gene,
Genomics, **16,** 123–131.

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

Burzynski, N. and Escobar, V. (1983)
Classification genetics of numeric anomalies of the dentition,
Birth Defects, **13,** 95–106.

Butler, P. M. (1939)
Studies of the mammalian dentition. Differentiation of the postcanine dentition,
Proceedings of the Zoological Society of London, **109B,** 329–356.

Chiang, C., Litingtung, Y., Lee, E., Young, K. E., Corden, J. L., Westphal, H. and Beachy, P. A. (1996)
Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function,
Nature, **383,** 407–413.

Dean, M. (1996)
Polarity, proliferation and the hedgehog pathway,
Nature Genetics, **14,** 245–247.

Ferguson, M. W. J. (1990)
The dentition through life,
In: R. J. Elderton (ed.) *The Dentition and Dental Care*, Dental Series 3,
Heinemann, Oxford, pp. 1–29.

Hahn, H., Wicking, C., Zaphiropoulos, P. G., Gailani, M. R., Shanley, S., Chidambaram, A., Volechovsky, I., Holmberg, E., Unden, A. B., Gillies, S., Negus, K., Smyth, I., Pressman, C., Leffell, D. J., Gerrard, B., Goldstein, A. M., Dean, M., Toftgard, R., Chenevix-Trench, G., Wainwright, B. and Bale, A. E. (1996)
Mutations of the human homolog of *Drosophila* patched in the nevoid basal cell carcinoma syndrome,
Cell, **85,** 841–851.

Hammerschmidt, M., Brook, A. and McMahon, A. P. (1997)
The world according to hedgehog,
Trends in Genetics, **13,** 14–21.

Hardcastle, Z., Mo, R. and Sharpe, P. T. (1998)
The Shh signalling pathway in tooth development: defects in Gli-2 and Gli-3 mutants,
Development, **125,** 2803–2811.

Heikinheimo, K. (1994)
Stage-specific expression of decapentaplegic-Vg-related genes 2, 4 and 6 (bone morphogenetic proteins 2, 4 and 6) during human tooth morphogenesis,
Journal of Dental Research, **73,** 590–597.

Hunt, P., Gulisano, M., Cook, M., Sham, M., Faiella, A., Wilkinson, D., Boncinelli, E. and Krumlauf, R. (1991a)
A distinct hox code for the branchial region of the vertebrate head,
Nature, **353,** 861–864.

Hunt, P., Wilkinson, D. and Krumlauf, R. (1991b)
Patterning the vertebrate head: murine Hox 2 genes mark distinct subpopulations of premigratory and migratory neural crest,
Development, **112,** 43–51.

Ignelzi, M. A., Liu, Y., Maxson, R. E. and Snead, M. L. (1995)
Genetically engineered mice: tools to understand craniofacial development,
Critical Reviews in Oral Biology and Medicine, **6,** 181–201.

Jabs, E. W., Muller, U., Xiang, L., Liang, M., Luo, W., Haworth, I. S., Klisak, I., Sparkes, R., Warman, M. L., Mulliken, J. B., Snead, M. L. and Maxson, R. (1993)
A mutation in the homeodomain of the human MSX-2 gene in a family affected with autosomal dominant craniosynostosis,
Cell, **75,** 443–450.

Jernvall, J., Kettunen, P., Karavanova, I., Martin, I. B. and Thesleff, I. (1994)
Evidence for the role of the enamel knot as a control center in mammalian tooth cusp formation: non-dividing cells express growth stimulating Fgf-4 gene,
International Journal of Developmental Biology, **38,** 463–469.

Johnson, R. L., Rothman, A. L., Xie, J., Goodrich, L. V., Bare, J. W., Bonifas, J. M., Quinn, A. G., Myers, R. M., Cox, D. R., Epstein Jr, E. H. and Scott, M. P. (1996)
Human homolog of patched, a candidate gene for the basal cell nevus syndrome,
Science, **272,** 1668–1671.

Kettunen, P. and Thesleff, I. (1998)
Expression and function of FGF's-4, –8, and –9 suggest functional redundancy and repetitive use as epithelial signals during tooth morphogenesis,
Developmental Dynamics, **211,** 256–268.

Kingsley, D. M. (1994)
What do BMP's do in mammals? Clues from the mouse short ear mutation,
Trends in Genetics, **10,** 16–21.

Kollar, E. J. and Baird, G. R. (1970)
Tissue interactions in developing mouse tooth germs: II. The inductive role of the dental papilla,
Journal of Embryology and Experimental Morphology, **24,** 173–186.

Lewis, E. B. (1978)
A gene complex controlling segmentation in *Drosophila*,
Nature, **276,** 565–570.

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

Lumsden, A. G. S., Sprawson, N. and Graham, A. (1991)
Segmental origin and migration of neural crest cells in the hindbrain region of the chick embryo,
Development, **113,** 1281–1291.

Maas, R. and Bei, M. (1997)
The genetic control of early tooth development,
Critical Reviews in Oral Biology and Medicine, **8,** 4–39.

MacKenzie, A., 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.

McGinnis, W., Levine, M. S., Hafen, E., Kuroiwa, A. and Gehring, W. J. (1984a)
A conserved DNA sequence in homeotic genes of the *Drosophila* antennapedia and bithorax complexes,
Nature, **308,** 428–433.

McGinnis, W., Garber, R. L., Wirz, J., Kuroiwa, A. and Gehring, W. J. (1984b)
A homologous protein-coding sequence in *Drosophila* homeotic genes and its conservation in other metazoans,
Cell, **37,** 403–408.

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.

Nieminen, P., Arte, S., Pirinen, S., Peltonen, L. and Thesleff, I. (1995)
Gene defect in hypodontia: exclusion of MSX-1 and MSX-2 as candidate genes,
Human Genetics, **96,** 305–308.

Noden, D. M. (1984)
Craniofacial development: new views on old problems,
Anatomical Record, **208,** 1–13.

Osborn, J. W. (1978)
Morphogenetic gradients: fields versus clones,
In: Butler, P. M. and Joysey, K. A. (eds) *Development, Function and Evolution of the Teeth*,
Academic Press, New York, pp. 171–201.

Porteus, M. H., Bulfone, A., Ciaranello, R. D. and Rubenstein, J. L. R. (1991)
Isolation and characterization of a novel cDNA clone encoding a homeodomain that is developmentally regulated in the ventral forebrain,
Neuron, **7,** 221–229.

Price, J. A., Bowden, D. W., Wright, J. T., Pettenati, M. J. and Hart, J. T. (1998)
Identification of a mutation in DLX-3 associated with tricho-dento-osseous (TDO) syndrome,
Human Molecular Genetics, **7,** 563–569.

Qiu, M., Bulfone, A., Ghattas, I., Meneses, J. J., Christensen, L., Sharpe, P. T., Presley, R., Penderson, 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 morphogenesis of proximal skeletal and soft tissue structures derived from the first and second arches,
Archives of Developmental Biology, **185,** 165–184.

Roessler, E., Belloni, E., Gaudenz, K., Jay, P., Berta, P., Scherer, S. W., Tsui, L. and Muenke, M. (1996)
Mutations in the human Sonic hedgehog gene cause holoprosencephaly,
Nature Genetics, **14,** 357–360.

Satokata, I. and Maas, R. (1994)
Msx-1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development,
Nature Genetics, **6,** 348–356.

Sharpe, P. T. (1995)
Homeobox genes and orofacial development,
Connective Tissue Research, **32,** 17–25.

Stone, D. M., Hynes, M., Armanini, M., Swanson, T. A., Gu, Q., Johnson, R. L., Scott, M. P., Pennica, D., Goddard, A., Phillips, H., Noll, M., Hooper, J. E., de Sauvage, F. and Rosenthal, A. (1996)
The tumor-suppressor gene patched encodes a candidate receptor for Sonic hedgehog,
Nature, **384,** 129–134.

Thesleff, I. (1996)
Two genes for missing teeth,
Nature Genetics, **13,** 379–380.

Thesleff, I. and Sharpe, P. T. (1997)
Signalling networks regulating dental development,
Mechanisms of Development, **67,** 111–123.

Thomas, B. L. and Sharpe, P. T. (1998)
Patterning of the murine dentition by homeobox genes,
European Journal of Oral Sciences, **106** (Supplement 1), 48–54.

Thomas, B. L., Tucker, A. S., Qiu, M., Ferguson, C. A., Hardcastle, Z., Rubenstein, J. L. R. and Sharpe, P. T. (1997)
Role of the Dlx-1 and Dlx-2 genes in patterning of the murine dentition,
Development, **124,** 4811–4818.

Tissier-Seta, J. P., Mucchielti, M. L., Mark, M., Mattei, M. G., Goridis, C. and Bruney, J. F. (1995)
Barx-1, a new mouse homeodomain transcription factor expressed in craniofacial ectomesenchyme and the stomach,
Mechanisms of Development, **51,** 3–15.

Tucker, A. S., Al Khamis, A. and Sharpe, P. T. (1998)
Interactions between Bmp-4 and Msx-1 act to restrict gene expression to odontogenic mesenchyme,
Developmental Dynamics, **212,** 533–539.

Vaahtokari, A., Aberg, T., Jervvall, J., Keranen, S. and Thesleff, I. (1996a)
The enamel knot as a signalling center in the developing mouse tooth, *Mechanisms of Development*, **54,** 39–43.

Vaahtokari, A., Aberg, T. and Thesleff, I. (1996b)
Apoptosis in the developing tooth: association with an embryonic signaling centre and suppression by EGF and FGF-4, *Development*, **122,** 121–129.

Vainio, S., Karavanova, I., Jowett, A. and Thesleff, I. (1993)
Identification of Bmp-4 as a signal mediating secondary induction between epithelial and mesenchymal tissues during early tooth development, *Cell*, **75,** 45–58.

Vastardis, H., Karimbux, N., Guthua, S. W., Seidman, J. G. and Seidman, C. E. (1996)
A human MSX-1 homeodomain missense mutation causes selective tooth agenesis, *Nature Genetics*, **13,** 417–421.

Weiss, K., Stock, D., Zhao, Z., Buchahan, A., Ruddle, F. and Shashikant, C. (1998)
Perspectives on genetic aspects of dental patterning, *European Journal of Oral Sciences*, **106** (Supplement 1), 55–63.

Wilkie, A. O. M., Morriss-Kay, G. M., Jones, E. Y. and Heath, J. K. (1995)
Functions of fibroblast growth factors and their receptors, *Current Biology*, **5,** 500–507.

Wolpert, L. (1998)
*Principles of Developmen*t, Current Biology, Oxford University Press, Oxford.