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REVIEW

Tooth and jaw: molecular mechanisms of patterning in the first branchial arch

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Summary The mammalian jaw apparatus is ultimately derived from the first branchial arch derivatives, the maxillary and mandibular processes, and composed of a highly specialised group of structures. Principle amongst these are the skeletal components of the mandible and maxilla and the teeth of the mature dentition. Integral to the development of these structures are signalling interactions between the stomodeal ectoderm and underlying neural crest-derived ectomesenchymal cells that populate this region. Recent evidence suggests that in the early mouse embryo, regionally restricted expression of homeobox-containing genes, such as members of the Dlx, Lhx and Gsc classes, are responsible for generating early polarity in the first branchial arch and establishing the molecular foundations for patterning of the skeletal elements. Teeth also develop on the first branchial arch and are derived from both ectoderm and the underlying ectomesenchyme. Reciprocal signalling interactions between these cell populations also control the odontogenic developmental programme, from early patterning of the future dental axis to the initiation of tooth development at specific sites within the ectoderm. In particular, members of the Fibroblast growth factor (Fgf), Bmp, Hedgehog and Wnt families of signalling molecules induce regionally restricted expression of downstream target genes in the odontogenic ectomesenchyme. Finally, the processes of morphogenesis and cellular differentiation ultimately generate a tooth of specific class. Many of the same genetic interactions that are involved in early tooth development mediate these effects through the activity of localised signalling centres within the developing tooth germ.

Introduction

In mammals, teeth form on the oral surface of the fronto-nasal process and first branchial arch derivatives, the maxillary and mandibular processes and are derived from two principle cell types, ectoderm and cranial neural crest-derived ectomesenchyme. The ectodermal component gives rise only to the ameloblasts that form the enamel of the tooth crown. The remaining structures of the tooth, including the dentine, pulp and periodontal tissues, are derived from ectomesenchyme. In the early embryo, cranial neural crest cells migrate from the forebrain, midbrain and hindbrain regions to populate the fronto-nasal, maxillary and mandibular processes. The generation of a tooth relies upon a sequence of tightly regulated and reciprocal

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signalling interactions between the ectoderm lining the future oral cavity and these neural crest-derived ectomesenchymal cells.[1–3] Over 200 genes have now been demonstrated to be active in the developing tooth, many of which can be viewed within a comprehensive graphical database of gene expression profiles, available at http://bite-it.helsinki.fi.[4]

Embryological origins of the dental tissues

There have been no detailed fate mapping studies of the ectodermal origins of developing tooth germs. Indeed, there has also been the suggestion that foregut endoderm may have an early role in establishing the sites of molar tooth development.[5] In contrast, the migratory pathways of cranial neural crest cells have been extensively studied in a variety of species.[6–10] The vertebrate neural crest is a pluripotent cell population, derived from the lateral ridges of the neural plate during the early stages of embryogenesis. Neural crest cells disperse from the dorsal surface of the neural tube and migrate extensively throughout the embryo, giving rise to a wide variety of differentiated cell types.[11] Cell-labelling studies in the mouse have demonstrated that the fronto-nasal process is formed by neural crest cells derived from the mid and forebrain regions[12] and the first branchial arch is populated by crest cells from more caudal regions of the midbrain and the hindbrain.[13]

Whilst these cell-labelling studies have been useful in the identification of neural crest migratory pathways in mammals, they have not provided a comprehensive cell lineage analysis of these cells as they become terminally differentiated. Recently, a genetic marker has been utilised to follow neural crest migration and differentiation in the mouse.[14] Transgenic mice, generated by conditional gene knockout and exhibiting ubiquitous *lacZ* reporter gene expression in neural crest precursors, have allowed a method of staining and therefore identification of these cells at later stages of development. This has clearly demonstrated that in the developing tooth germ, cranial neural crest-derived ectomesenchyme contributes to the formation of condensed dental ectomesenchyme at the initial bud stage and subsequently to the formation of the dental papilla and follicle. In addition, the analysis of 6-week-old mice demonstrated definitively a cranial neural crest origin for the odontoblasts, dentine matrix, pulp tissue, cementum and periodontal ligaments of teeth in the adult murine dentition.[14]

Early generation of polarity in the first branchial arch

Teeth are highly specialised structures found nowhere else in the body; therefore, the evolution of jawed vertebrates and concomitant development of a species-specific dentition has provided a unique identity to the structures derived from the first branchial arch. A fundamental question is how the first arch initially becomes patterned in the developing embryo to ultimately give rise to these characteristic dental and skeletal structures. During early development the maxillary and mandibular primordia are broadly divided into an oral region, which gives rise to the dentition and an aboral region, which forms the skeletal elements. In the mandibular arch the oral region lies cranially and the aboral region more caudally, whilst a converse arrangement exists in the maxilla. Recently, some progress has been made in elucidating the molecular mechanisms that are responsible for patterning these regions.

Homeobox genes are a large group of genes that encode transcription factors responsible for regulating the expression of downstream target genes. The homeobox is a highly conserved 180 base pair sequence originally discovered in the homeotic selector genes of the fruitfly *Drosophila melanogaster*. Homeotic genes are a family of master regulatory homeobox genes, ultimately responsible for specifying segment identity along the anterior–posterior axis of the developing fly. The vertebrate homologues are the Hox genes and these genes specify the vertebrate embryonic body axis during development.[15] In addition, segment-specific combinatorial Hox gene expression in migrating neural crest cells is also responsible for generating diversity in the branchial arch system.[16] However, Hox genes are not expressed in the first branchial arch and it has been suggested that this loss of Hox gene expression has been essential for the skeletal rearrangements to occur that are necessary for the development of a jaw.[17] Indeed, overexpression of Hox genes in the first branchial arch neural crest leads to a failure of differentiation into cartilage and bone.[18,19] If Hox genes are not directly responsible for patterning the first arch, then what mechanisms exist to establish the positional fate of these ectomesenchymal cells?

The ectoderm and endoderm that covers the first arch derivatives is characterised by distinct tempero-spatial regions of gene expression. Many of these genes encode secreted molecules and one candidate for playing an early role in patterning the first branchial arch is the signalling peptide encoded by *Fgf-8*. Fgf-8 is a member of the Fibroblast growth factor (Fgf) family of signalling molecules and is expressed in the oral ectoderm of the murine first

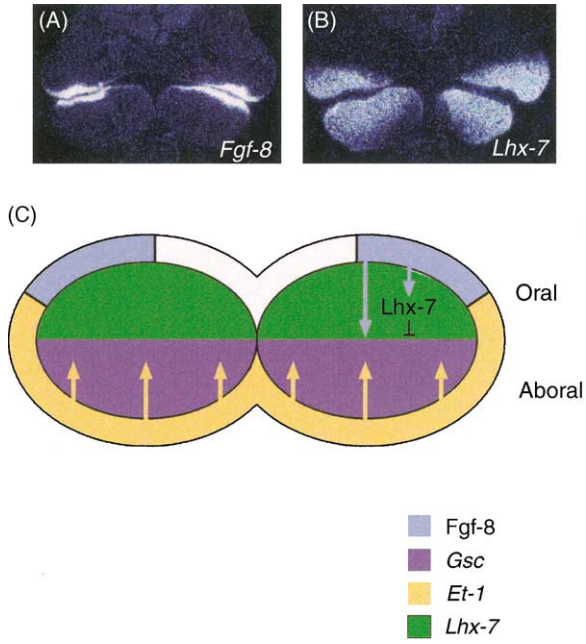

Figure 1 Early generation of polarity in the first branchial arch. (A) Expression of *Fgf-8* in the proximal oral ectoderm of the maxillary and mandibular processes at E10.5. (B) Expression of *Lhx-7* in the tooth-forming ectomesenchyme of the maxillary and mandibular processes at E10.5. (C) Schematic diagram representing the establishment of rostral-caudal polarity in the mandibular process at E10. Fgf-8 (light blue) in the rostral ectoderm induces *Lhx-7* (green) in the underlying ectomesenchyme. Within this rostral ectomesenchyme *Lhx-7* inhibits *Gsc* (purple) expression, with the result that *Gsc* is limited to the caudal regions. *Lhx-7* expression is maintained by Fgf-8 from the oral ectoderm, whilst *Gsc* is maintained by Et-1 (yellow) in the aboral ectoderm. Note that *Et-1* is expressed throughout the branchial arch ectoderm, but is only shown in the caudal regions for simplicity. Diagram adapted from Tucker et al.[22]

branchial arch from around embryonic day (E) 9[20,21] (Fig. 1A). It has been proposed that this specific expression of *Fgf-8* is involved in the early determination of polarity in the first branchial arch.[22] *Lhx-6* and *Lhx-7* are two LIM homeobox domain genes that are characteristically restricted to the ectomesenchyme within the oral half of the first arch[22,23] (Fig. 1B). However, this restricted expression domain is not an inherent property of these cells; ectomesenchyme derived from the second branchial arch, which does not normally express either of these genes, can be induced to express both *Lhx-6* and *Lhx-7* when recombined with ectoderm from the oral surface of the first branchial arch.[22] Fgf-8 is the most likely candidate as the endogenous inducer, its epithelial expression domain complements the ectomesenchymal domains of both genes and beads soaked in recombinant Fgf-8 protein can induce both *Lhx-6* and *Lhx-7* expression in a concentration-dependent manner in isolated mandibular ectomesenchymal cultures.[22] *Goosecoid* (*Gsc*) is another homeobox-containing gene expressed within the ectomesenchyme of the first branchial arch, however, in contrast to *Lhx-6* and *Lhx-7*, *Gsc* expression is restricted to the aboral regions[24] (Fig. 1C). Consistent with this restricted expression, mice with targeted mutations in *Gsc* have skeletal abnormalities of the mandible, including hypoplasia and malformations in Meckel's cartilage.[25,26] Interestingly, Fgf-8 also has an indirect role in defining the expression of *Gsc* in the first arch via the induction of *Lhx-6* and *Lhx-7*, which restricts *Gsc* expression to the aboral regions.[22] *Gsc* expression, in turn, is dependent upon signalling from Endothelin-1 (ET-1) in the aboral ectoderm of the first arch.[22,27] *ET-1* encodes a signalling peptide, which is expressed throughout the mandibular arch ectoderm. ET-1 seems to mediate these effects on mandibular development through binding of the ETA receptor, which is expressed throughout the ectomesenchyme of the mandibular arch. Targeted mutation of ET-1 or ETA both result in mandibular skeletal phenotypes similar to those of *Gsc*−/− embryos.[27,28] The generation of mice exhibiting a conditional loss of *Fgf-8* function in the ectoderm of the first branchial arch has further defined the important role of this signalling peptide during early patterning.[29] These mice exhibit an almost complete loss of first arch-derived skeletal structures. Pertinently, the only structures to develop are the malleus posteriorly and the mandibular midline anteriorly, including cartilage, bone and the incisor teeth. In the conditional mutants, the only region where functional *Fgf-8* remains is a small patch of ectoderm posteriorly. Therefore, whilst *Fgf-8* is essential for normal patterning of the majority of the axis of the first arch, distally in the future incisor regions an alternative regulatory cascade seems to be important.[29]

Distal-less genes incorporate a six-gene family of mammalian homeobox genes (*Dlx-1*, *-2*, *-3*, *-5*, *-6* and *-7*) that also exhibit highly nested domains of expression in the branchial arches during early development.[30–32] Within the mammalian genome, these genes are arranged in convergent pairs, with each pair having similar domains of expression (*Dlx-1/-2*; *Dlx-3/-7*; *Dlx-5/-6*).[32] In particular, along the rostral-caudal axis of the branchial arches *Dlx-1* and -2 are expressed more-or-less continuously, whilst the expression domains of *Dlx-5/-6* and *Dlx-3/-7* are found to be progressively more restricted in a caudal direction.[31,32] The study of mice with targeted mutations in *Dlx* genes has suggested that a *Dlx* code of expression might be important in establishing inter-arch identity within the branchial region. Certainly, mice with loss of either *Dlx-1*, *Dlx-2* or

Dlx-1/-2 function exhibit progressively more severe anomalies in structures derived from the more rostral regions of the branchial arches, in particular the maxillary process of the first branchial arch.[32,33] Even though they are expressed in caudal structures, the loss of *Dlx-1/-2* does not seem to affect the patterning of these regions because of compensatory action by other *Dlx* genes, a finding confirmed by the presence of defects in regions of the mandibular arch of *Dlx-5−/−* mice.[31,34] Further, in mice lacking the function of both *Dlx-5* and *-6*, genes that are only expressed in more caudal regions of the branchial arches, a homeotic transformation is found to occur; these mice have a conversion of mandibular arch structures to maxillary[35] (Fig. 2). Thus, nested *Dlx* gene expression appears to play a fundamental role in establishing both the identity of different branchial arches and the identity of the maxillary and mandibular processes of the first branchial arch.

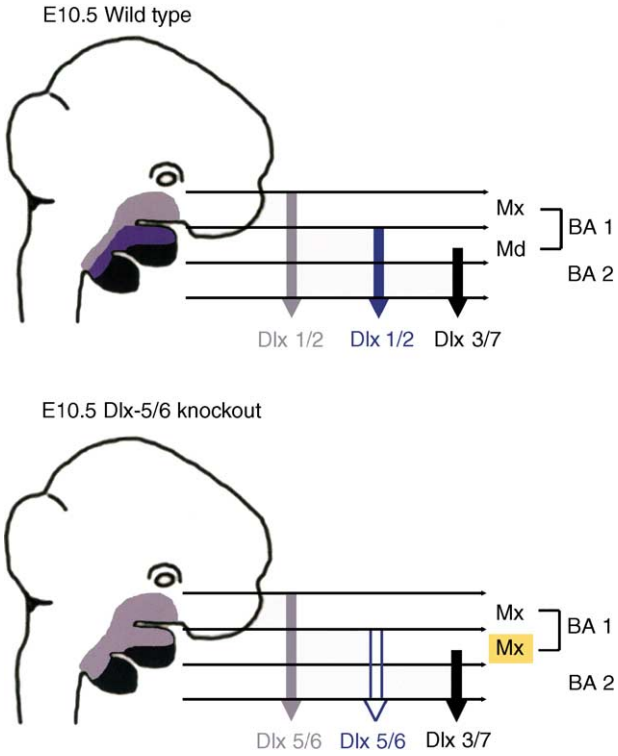

Figure 2 Patterning of the branchial arches by *Dlx* genes. Schematic diagram representing the restricted fields of *Dlx* gene expression within ectomesenchyme of the branchial arches in the developing mouse embryo. The upper panel represents a wild type embryo at E10.5. *Dlx-1/-2* expression (violet) is present within the ectomesenchyme throughout most of the rostral-caudal axis of the branchial arches, whilst the domains of *Dlx-5/-6* (purple) and *Dlx-3/-7* (dark blue) expression are progressively more restricted in a caudal direction. These expression domains result in normal patterning of the maxillary (Mx) and mandibular (Md) processes of the first arch (BA 1) and second arch (BA 2) structures. In the lower panel a *Dlx-5/-6* mutant embryo is shown. In the mutant, a loss of functional *Dlx-5/-6* results in re-programming of the mandibular process into an ectopic maxilla (Mx highlighted in yellow). Alteration of the *Dlx* code within the branchial arch system can thus produce a homeotic transformation. Diagram adapted from Depew et al.[35]

Patterning the dental axis

The mammalian dentition itself comprises a group of serially homologous structures whose differences along the proximal-distal axis of the jaw can be described in terms of changes in both shape and size. In simple terms, this means that along the dental axis incisors will develop in the distal regions and molars more proximally. Mechanisms are therefore in place that are responsible for patterning different regions of the future dentition along this proximal-distal axis. It is clear from studies of gene expression that these events occur at the molecular level within the ectomesenchyme of the mandibular arch prior to any morphological evidence of the initiation of tooth development. In recent years, considerable progress has been made in understanding how positional fate is ascribed to the neural crest cells that make up the tooth-forming regions of the first branchial arch.

An odontogenic homeobox code

Hox gene expression is not found in the ectomesenchyme of the first branchial arch and teeth can develop in a *Hoxa-2* positive environment, suggesting that these genes are not involved in patterning the dentition and that the evolution of a dentition has been independent of the loss of Hox gene expression necessary for the development of a jaw.[36] However, a number of subfamilies of homeobox-containing genes are expressed in the ectomesenchyme of the first branchial arch, including members of the Alx, Barx, Dlx, Lhx, Pitx, Msx and Gsc classes. These genes do not exhibit the genomic colinearity of Hox genes but they do demonstrate regionally restricted and highly specific domains of expression within the ectomesenchyme of the first branchial arch and it has been suggested that these gene expression patterns may act to ultimately specify tooth shape[37,38] (Fig. 3). This 'odontogenic homeobox code' infers that for each tooth-forming region, a specific combination of homeobox genes within the ectomesenchyme dictates the ultimate morphology that a tooth primordium will develop into following initiation.[37] There are now examples

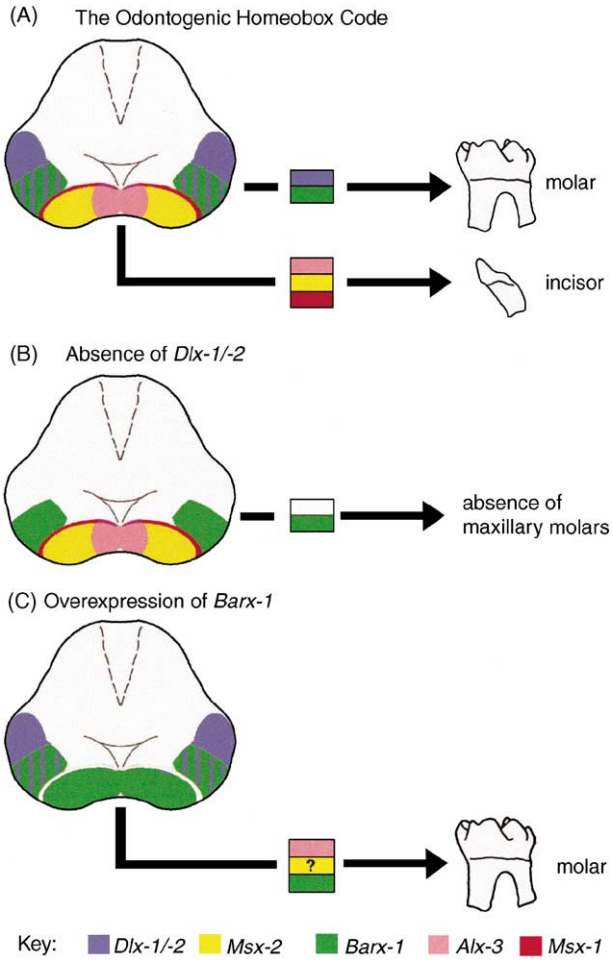

Figure 3 The odontogenic homeobox code. Schematic diagram illustrating homeobox gene expression in the ectomesenchyme of the maxillary and mandibular processes. (A) The restricted ectomesenchymal expression of several homeobox genes is thought to provide the necessary spatial information to determine tooth shape. In the proximal molar-forming regions *Dlx-1/-2* and *Barx-1* positive ectomesenchyme results in the formation of molar teeth. In the distal incisor-forming regions *Msx-1/-2* and *Alx-3* positive ectomesenchyme results in the formation of incisor teeth. (B) In mice lacking both *Dlx-1* and *Dlx-2* function, therefore having *Dlx-1/-2* negative and *Barx-1* positive ectomesenchyme in the molar-forming regions, maxillary molars fail to develop. Thus, whilst *Dlx-1/-2* are dispensable for mandibular molar development, they are essential for development of the maxillary molars. It should be noted that in the maxilla, incisors do not form instead of molars because *Alx-3* and *Msx-1* expression is also required for incisor specification. (C) Manipulation of homeobox gene expression, resulting in ectopic expression of *Barx-1* and loss of *Msx-1* (and possibly *Msx-2*) in the distal incisor-forming ectomesenchyme produces a transformation of tooth type; molar teeth form instead of incisors. Thus, a gain of a molar-patterning gene (*Barx-1*) and loss of an incisor-patterning gene (*Msx-1*) re-directs incisor morphogenesis into molar morphogenesis.

of both loss- and gain-of-function experiments that go some way to supporting this theory. Targeted disruption of both *Dlx-1* and *Dlx-2* in transgenic mice results in an absence of maxillary molar teeth.[32,39] These genes are both expressed in the maxillary and mandibular molar ectomesenchyme suggesting that for maxillary molar specification, both *Dlx-1* and *-2* are required. In the absence of *Dlx-1* and *-2*, maxillary molar ectomesenchyme is re-programmed to a chondrogenic fate.[32,39] Recently, a transformation of tooth type has been demonstrated by manipulating homeobox gene expression in the future incisor-forming ectomesenchyme of the mandibular process.[40] *Barx-1* (*BarH-like* homeobox-1) encodes another homeobox-containing transcription factor and is homologous to the *Drosophila BarH-1* and *-2* genes.[41] *Barx-1* is normally expressed specifically in the proximal molar-forming ectomesenchyme where its expression is induced by Fgf-8 in the overlying ectoderm. This expression domain is restricted to the proximal regions by antagonistic signalling from Bmp-4 in the distal incisor-forming ectoderm. In vitro inhibition of Bmp-4 signalling in the distal region of the mandibular arch extends the normal expression domain of *Barx-1* and downregulates the normal endogenous expression of *Msx-1*, which is normally induced in the distal ectomesenchyme by Bmp-4 in the overlying ectoderm. Transplantation of these early incisor regions (exposed to an altered code of ectopic *Barx-1* and loss of *Msx-1*) in vivo results in the formation of multicusped molar teeth rather than incisors. Thus, an alteration of homeobox gene expression domains is capable of re-specifying the identity of developing incisor teeth.[40]

There are several important points to note with regard to the homeobox model. Firstly, there is not one specific gene responsible for each tooth shape; secondly, the absence of a gene is as important as the presence in terms of reading the code; and thirdly, because the code is overlapping it can specify a wide range of subtle differences in tooth shape.[42] This third point is important because the peripheral regions of overlap between teeth of different classes appears to be particularly vulnerable with regard to human hypodontia.[43] In these cases, teeth at the end of a series (upper lateral incisors, lower second premolars, third molars) are those that are most frequently congenitally absent.[44]

Induction of the patterning process

The evidence to suggest that restricted patterns of homeobox gene expression in the ectomesenchyme

of the first branchial arch derivatives are responsible for patterning the dentition leads to the question of how these domains are established. Either the neural crest cells are pre-patterned prior to their arrival in the first arch or these domains result from the interaction of neural crest cells with the oral ectoderm following migration. Early recombination experiments of embryonic tissues have hinted at the latter hypothesis, principally because they demonstrate that non-first arch neural crest cells can also support tooth development; the ability to participate in odontogenesis is not an exclusive feature of the neural crest cells that migrate into the first branchial arch.[45,46] The concept that a relative plasticity of neural crest cells exists has now found some support at the molecular level. A role has been established for the presumptive oral ectoderm in inducing the regionally restricted homeobox gene expression that occurs in the underlying ectomesenchyme of the mandibular process.[40,47,48]

In the early murine mandibular process prior to E10, approximately 24 h before any morphological sign of tooth development has occurred, removal of the oral ectoderm leads to a rapid loss of almost all ectomesenchymal homeobox gene expression. Importantly, the addition of exogenous Fgf-8 onto these explants is able to restore the expression of many of these genes, consistent with the idea that this signalling protein is one source of the signals responsible for patterning the early maudible.[49] However, this response of mandibular ectomesenchyme to Fgf-8 is highly dynamic (Fig. 4). Prior to E10, all mandibular ectomesenchymal cells are equally competent to respond, homeobox genes can be induced in regions outside their normal restricted expression domains but only in close proximity to the source of Fgf-8. By E10.5, whilst removal of the oral ectoderm still results in downregulation of homeobox gene expression in isolated ectomesenchyme, addition of exogenous Fgf-8 can only restore gene expression in the original domains. However, removal at E11 or after does not effect gene expression, at this stage the ectomesenchymal expression domains are established and importantly, independent of epithelial signals.[49] These findings have ultimately provided a molecular explanation for the results of previous recombination experiments. Thus, in the mouse prior to the bud stage the oral ectoderm is able to induce odontogenesis and determine tooth type.[45,50,51] However, the recombination of dental tissues taken from later stages of development has demonstrated that after the bud stage, the necessary information required for determination of tooth shape resides in the ectomesenchyme of the dental papilla.[52]

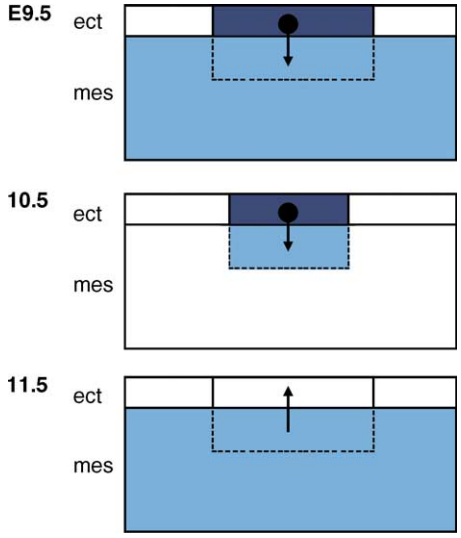

Figure 4 Responses of mandibular ectomesenchyme to ectodermal signalling. Schematic diagram illustrating the time-dependent response of ectomesenchymal homeobox gene expression to ectodermal signalling in the mandibular process. Light blue shading in the ectomesenchyme indicates competence to express homeobox-containing genes, such as *Msx-1*, *Barx-1* and *Lhx-7*. Dark blue shading in the ectoderm indicates the position of the active signal (i.e. Fgf-8). Arrows indicate the direction of signalling between ectoderm and underlying ectomesenchyme. At E9.5, ectomesenchymal gene expression is dependent upon the overlying ectoderm and all regions of the ectomesenchyme are equally competent to respond. At E10.5, ectomesenchymal gene expression is still dependent upon the ectoderm, but these expression domains have now become regionally restricted. At E11.5, the ectomesenchymal gene expression domains have become fixed and are no longer dependent upon the overlying ectoderm. At this stage, the ectomesenchyme is able to signal back to the ectoderm. Diagram adapted from Ferguson et al.[49]

These findings not withstanding, the maxillary and mandibular ectomesenchyme does seem to respond differently to ectodermal signalling for certain genetic pathways.[49] Fgf-8 can induce the expression of *Dlx-2* and *Dlx-5* in isolated mandibular ectomesenchyme, but only *Dlx-2* can be induced in maxillary ectomesenchyme. These observations are consistent with the observed expression patterns of these two genes; *Dlx-2* is expressed in the ectomesenchyme of the maxillary and mandibular processes, whereas *Dlx-5* is essentially only expressed in mandibular ectomesenchyme.[32] However, maxillary ectoderm is capable of inducing *Dlx-5* expression in mandibular arch ectomesenchyme, implying that the ectomesenchyme of the maxillary and mandibular primordia behave fundamentally differently to each other. Further, reciprocal transplantations of isolated ectomesenchyme from the molar regions of the mandible

or maxilla demonstrate that between E9.5 and E10.25 the ectomesenchyme of the mandible and maxilla do not take on the expression characteristics of their host, they appear to be intrinsically different in their responses to ectodermal signalling. These findings invite speculation as to how a seemingly homogenous population of cranial neural crest cells that migrate into the maxillary and mandibular primordia acquire subtle differences in their ability to respond to instructive signals from the ectoderm.[49] Clearly, some degree of pre-patterning may be present in these cells.

Initiation of tooth development

Having established discreet and fixed domains of ectomesenchymal cells that can dictate future pattern in the tooth-forming ectomesenchyme of the first branchial arch, the next stage is the actual generation of a tooth. In the mouse, the first morphological sign of tooth development occurs at approximately E11, with the formation of localised thickenings at specific locations in the ectoderm of the maxillary and mandibular processes. These ectodermal thickenings undergo localised proliferation to form the tooth buds, and in conjunction with the condensation of neural crest-derived ectomesenchymal cells around the bud tips, the tooth germs are formed. The oral ectoderm is crucial for this process to occur; only the ectoderm of the first branchial arch derivatives is able to induce odontogenesis.[45,46,51]

Early interactions between odontogenic ectoderm and ectomesenchyme

Some of the first molecular data regarding odontogenic initiation has come from studies of *Bmp-4* expression in the first branchial arch.[53] Bmp-4 is one member of a large group of signalling molecules known as Bone morphogenetic proteins. The expression domains of *Bmp-4* in the maxillary and mandibular processes are suggestive of a role in early tooth development.[53] In particular, in the mandibular process from E10, *Bmp-4* demonstrates a spatially restricted expression pattern in the presumptive incisor and, approximately 12 h later, presumptive molar ectoderm prior to the initiation of tooth development. However, by the time of tooth initiation this ectodermal expression is lost and *Bmp-4* begins to upregulate in the underlying ectomesenchyme in regions corresponding to the ectomesenchymal condensations beneath the localised thickenings of the developing tooth germs. At later stages, *Bmp-4* is clearly restricted to the ectomesenchyme of the bud and cap stage tooth germs.[53–55] Tissue recombination experiments have further demonstrated that Bmp-4 is able to autoregulate its own expression in odontogenic ectomesenchyme, but this induction is dependent upon *Msx-1*.[56] *Msx-1* (formerly *Hox-7*) is a member of a distinct subfamily of homeobox genes which is widely expressed in craniofacial ectomesenchyme[57–60] and interestingly, Bmp-4 is also able to induce the expression of *Msx-1* in oral ectomesenchyme.[53,55,56] A feedback loop seems to exist between *Bmp-4* and *Msx-1*; once the expression of *Bmp-4* has shifted to the ectomesenchyme, the continued expression of these two genes becomes independent of the ectoderm. The subsequent progressive localisation of *Msx-1* expression to the developing ectomesenchyme of the tooth buds is a direct result of this interaction.[55] Of particular interest is the finding that the timing of *Msx-1* localisation to the ectomesenchyme around the tooth buds coincides with the shift in odontogenic potential that occurs from ectoderm to the ectomesenchyme. Further, the analysis of mice with targeted mutations in *Msx-1* reveals that in the absence of functional *Msx-1*, all tooth development arrests at the bud stage.[61] These findings demonstrate that *Msx-1* is essential for normal tooth development to occur.

Bmp-4 can also induce the expression of Lymphoid enhancer-binding factor 1 (*Lef-1*) in dental ectomesenchyme in a manner independent of *Lef-1* transcription itself.[62] Lef-1, a member of the high mobility group (HMG) of proteins, is a cell type-specific transcription factor found in a number of tissues in the developing mouse embryo.[62–64] Within these cells, Lef-1 is known to be involved in mediating transcription of downstream targets during Wnt signalling. The Wnt gene family incorporates a large group of developmental genes that encode secreted cysteine-rich glycosylated proteins.[65] Early in tooth development *Lef-1* is initially expressed in the ectodermal thickenings, but during the bud stage this expression shifts to the condensing ectomesenchyme. At later stages of development, *Lef-1* transcripts continue to be localised to the ectomesenchymal component of the tooth germ but also upregulate in ectodermal cells of the enamel knot.[62] In *Lef-1*−/− mice, whilst the initiation of tooth development occurs, the dental papilla fails to form and in all the teeth a simple rudimentary bud remains as the only evidence of any odontogenesis.[66] Recombination experiments between tissues derived from normal and mutant embryos have demonstrated that in the tooth, *Lef-1* in the dental ectoderm is both necessary and sufficient to overcome the developmental arrest in tooth development. Once the dental papilla had

formed, *Lef-1* is no longer required for morphogenesis. Therefore, *Lef-1* in the dental ectoderm appears to be essential for the induction of the ectomesenchyme to form a dental papilla, but is dispensable for both initiation and later cytodifferentiation.[62]

Sonic hedgehog

Another important component of the odontogenic initiation process is the signalling peptide encoded by the Sonic hedgehog (*Shh*) gene. In the murine first branchial arch at E11.5, the expression of *Shh* is highly localised to the ectodermal thickenings of the future tooth germs.[67–71] There is evidence to suggest that this restricted domain of *Shh* is responsible for inducing localised proliferation of the dental ectoderm as it invaginates into the underlying ectomesenchyme to form a tooth bud. The addition of Shh protein, ectopically to tooth germs or non-dental oral ectoderm, has been shown to alter the morphology of tooth germs and to create ectodermal thickenings or invaginations in the non-dental ectoderm.[70] In addition, pharmacological inhibition of Shh signalling in E10.5 murine mandibular processes in vitro results in an arrest of tooth development associated specifically with reduced levels of ectodermal proliferation.[68] Further, conditional inactivation of *Shh* in developing tooth germs from slightly later stages of tooth development results in a reduction in size of the tooth bud.[72]

A number of proteins are known to be involved in the Shh signalling pathway, including the receptor complex formed by the transmembrane domain proteins Patched (Ptc) and Smoothened (Smo) and the Gli family of zinc finger transcription factors.[73,74] Unusually for a signalling pathway, binding of Shh ligand to the Ptc receptor relieves Ptc-mediated repression of Smo, which then activates the pathway.[75] Gli transcription factors have been demonstrated to have both activating and inhibitory roles in downstream signalling within the cell.[76,77] These components of the pathway are widely expressed in both the ectoderm and ectomesenchyme of the developing tooth.[67–71] The Shh pathway is known to be a powerful signalling cascade in both embryonic and adult tissues.[73,74] Mutations in the human *PTC* gene are associated with the autosomal dominant Nevoid Basal Cell Carcinoma syndrome (NBCCS or Gorlin syndrome).[78] Patients with NBCCS exhibit a wide range of phenotypes including multiple basal cell carcinomas (BCC), recurring keratocysts of the jaws and a predisposition to medulloblastoma and meningioma. *PTC* mutations have also been described in sporadic BCC, the most common form of Caucasian human cancer.[79–81] It is clearly important that this signalling cascade is appropriately restricted given its potent regulatory effects upon the cell cycle and this is reflected in the fact that many of the constituent components are intimately involved in inhibition of the signal. The demonstration that Shh protein has a direct long-range mode of action during odontogenesis suggests that it is also important to restrict Shh signalling in the tooth-forming regions of the first branchial arch.[82] Several recently discovered inhibitors of this pathway are expressed in the peripheral regions of odontogenic ectomesenchyme, providing some insight into how this restriction occurs. These include Gas-1, a glycosylphosphatidylinositol-linked membrane glycoprotein[83] (MTC, PTS unpublished observations) and Hedgehog-interacting protein (Hip). *Hip* encodes a membrane-associated glycoprotein that can bind Shh directly and attenuate the signal.[84] During odontogenesis, *Hip* expression is not found immediately adjacent to *Shh*-expressing cells, but rather at a distance and separated by cells expressing *Ptc*. Hip therefore seems to function in preventing the spread of excess Shh ligand beyond an immediate Ptc-induced zone in odontogenic ectomesenchyme.[85]

This highly localised role of Shh, inducing proliferation of dental ectoderm at specific tooth-forming sites along the future dental axis, makes the restriction of *Shh* transcription crucial in ensuring that tooth buds form in the correct place. Recent evidence in the mouse has demonstrated a mechanism whereby *Shh* transcription is restricted to the tooth-forming regions of the first branchial arch.[71] At E11.5, whilst *Shh* is localised to the ectodermal thickenings of the future incisor and molar teeth, the expression of *Wnt-7b*, a member of the Wnt gene family, is continuous throughout the oral ectoderm but noticeably absent from the tooth-forming regions that express *Shh* (Fig. 5). This expression pattern implies a possible relationship between these two genes in restricting *Shh* to the future dental ectoderm. The overexpression of a Wnt-7b murine retrovirus throughout the ectoderm of mandibular explants in vitro results in downregulation of *Shh* transcription in dental ectoderm and an arrest of tooth development at the ectodermal thickening stage. Further, this odontogenic phenotype is a direct result of the absence of *Shh* transcription in the dental ectoderm; exogenous Shh protein delivered to the tooth-forming regions via agarose beads is able to rescue tooth development when these explants are cultured in vivo.[71] These results are of particular significance for two reasons: firstly, they are some of the first to demonstrate how early regionalisation of first branchial arch ectoderm is controlled and secondly, they suggest

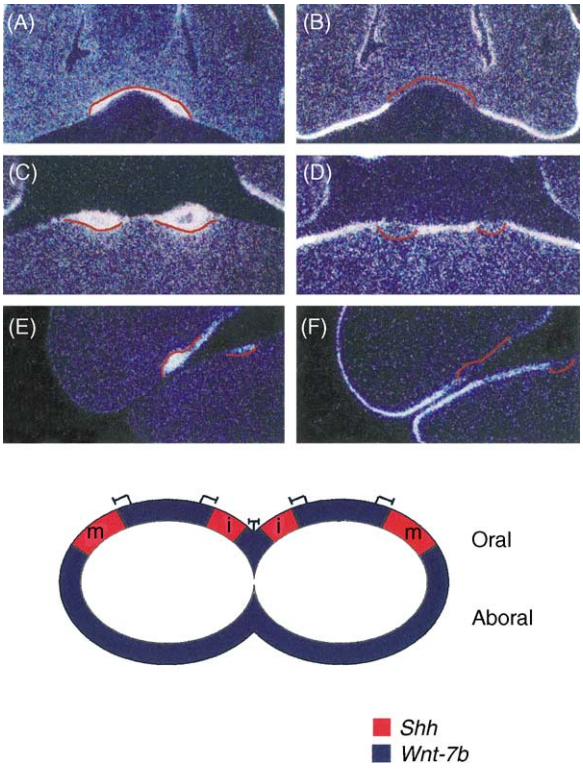

Figure 5 Expression of *Shh* and *Wnt-7b* in first branchial arch ectoderm. Coronal sections through the incisor and molar tooth-forming regions of the mouse embryo at E11.5, demonstrating *Shh* expression restricted to the ectodermal thickenings of the developing teeth (A, C, E) and *Wnt-7b* expression throughout the oral ectoderm, but noticeably absent from the tooth-forming regions (B, D, F). The developing teeth are outlined in red. (A and B) Maxillary incisors; (C and D) Mandibular incisors; (E and F) Maxillary and mandibular molars. The schematic diagram below represents the restricted *Shh* expression in the future incisor and molar ectoderm of the first arch and expression of *Wnt-7b* throughout the oral ectoderm. Note that *Wnt-7b* is not expressed in dental ectoderm and it inhibits *Shh* expression in these regions.

considerable conservation in the mechanisms involved in establishing ectodermal boundaries during development. A similar interaction exists between hedgehog and wingless signalling in the ectoderm of *Drosophila*.[86,87] These genes are the homologues of *Shh* and *Wnt-7b* in the fruitfly and they interact to establish boundaries in the ectoderm of the developing *Drosophila* larvae.

Tooth morphogenesis

Morphogenesis is the process whereby the dental lamina generates a tooth with a characteristic morphology. Initiation essentially links patterning with morphogenesis, but integral to all of these mechanisms is differentiation, whereby the constituent epithelial and mesenchymal cells of the tooth germ ultimately form the specific structures of the adult tooth.[2,3] The transition from bud to cap stage is a critical step in tooth morphogenesis, marking the onset of development of the tooth crown. Integral to this process is the primary enamel knot, a discreet, non-proliferating and transient signalling centre, which is formed by ectodermal cells situated at the tip of the tooth bud.[88–91]

Initially, the primary enamel knot is thought to be involved in directing the formation of the cap stage tooth germ. Tooth development arrests at the bud stage in a number of mouse mutants, including *Lef-1*, *Msx-1* and *Pax-9* and no enamel knots develop in these arrested tooth buds.[61,62,92] As a proposed signalling centre, the primary enamel knot expresses many of the signals seen in early odontogenic ectoderm.[1] In the developing murine molar tooth, the first site of enamel knot cells occurs in the mesial part of the bud ectoderm, marked by the localised expression of *Shh*, *Bmp-2*, *Bmp-7* and *Fgf-9*.[21,90] These cells also express *p-21* (a cyclin-dependent kinase inhibitor of cell proliferation) just prior to their withdrawal from the cell cycle.[89] This induction of the primary enamel knot is thought to be a prerequisite for tooth development to proceed to the cap stage[1] and there is evidence to suggest that Bmp signalling is involved in this induction. Both Bmp-2 and Bmp-4 are able to induce *p-21* and *Msx-2* expression in explants of oral ectoderm; thus, ectomesenchymal Bmp-4 may serve this function in vivo.[89] In addition, in several of the mouse mutants where tooth development fails to progress beyond the bud stage, *Bmp-4* expression is downregulated in the odontogenic ectomesenchyme.[61,62,92]

By the cap stage, the primary enamel knot forms a recognisable histological structure within the internal enamel epithelium and is clearly demarcated by the continued expression of *Shh*, *Bmp-2*, *Bmp-7* and *Fgf-9*, with additional upregulation of *Bmp-4*, *Fgf-4* and members of the Wnt signalling family.[21,69,88,90,93] The localised expression of *Shh* in the enamel knot indicates a further role for this signalling pathway during tooth morphogenesis and the generation of mice lacking *Shh* function specifically in the tooth germ has confirmed this.[72] Whilst the incisor and molar teeth are present in these mutant mice, they are generally small and abnormally shaped. In addition, the dental cords are absent and the teeth fuse to the oral ectoderm, with an associated failure of alveolar bone formation on the oral side of the tooth crypts. Whilst a normal enamel knot is present in the mutant tooth germs (identified by an absence of proliferation, high incidence of programmed cell death and the

normal restricted expression of enamel knot marker genes), in cells of the lingual epithelium both *Wnt-10b* and *Lef-1* are inappropriately expressed. These genes are normally restricted to the enamel knot and it would appear that in the absence of *Shh*, cells of the lingual epithelium undergo a partial fate change, assuming some characteristics of the less proliferative enamel knot population. *Shh* would seem to be required for asymmetrical development of the dental cap, which is an essential part of normal odontogenesis being responsible for the production of normal cuspal architecture that differentiates teeth of different classes.[72] Fgfs are also candidates for being key signals in regulating the growth and folding of the enamel organ after the bud stage. In transgenic mice overexpressing a soluble negative Fgf receptor[94] and lacking a functional Fgfr-2 receptor IIIb isoform[95] tooth morphogenesis fails to progress beyond the bud stage. Further, Fgf peptides have been shown to act as potent mitogens in both dental ectoderm and ectomesenchyme.[88,96] These findings present something of a paradox in that the primary enamel knot expresses a number of growth stimulatory signals, whilst its own cells remain non-proliferative.[1] However, a region of non-dividing cells within an area of active proliferation within the internal enamel epithelium may well explain the basic mechanism of folding of the enamel organ. The importance of the primary enamel knot has been demonstrated in the analysis of Downless mutant mice. In these mice the primary enamel knot is absent, the cells being distributed in a different shape with altered expression of signalling molecules. The molar teeth of these mice are considerably reduced in size and have flattened cusps that are reduced in number.[97] Between E14.5 and E15 in the developing mouse molar, the primary enamel knot progressively disappears in a mesial direction with the cells being removed by apoptosis.[89,91] The mechanism by which this apoptosis is induced is not fully understood, however, Bmp-4 is a candidate for regulating this activity. *Bmp-4* expression is closely associated with cells undergoing apoptosis in the cap stage primary enamel knot and recombination experiments have demonstrated that Bmp-4 can induce apoptosis in mandibular explants.[89]

In teeth with many cusps, after the disappearance of the primary enamel knot at the late cap stage, secondary enamel knots form at the sites of the future cusp tips within the internal enamel epithelium.[88] These secondary knots are intimately involved with regulating formation of the bell stage tooth germ. In common with the primary enamel knots, the secondary enamel knots are non-proliferative, show localised expression of *Fgf-4* and are also ultimately removed by apoptosis.[88,91] Apoptosis in the secondary knots is also closely associated with the expression domains of *Bmp-4*.[54] The secondary enamel knots mark the first signs of a species-specific cusp pattern and their normal positioning is clearly important for this process to occur. In the Tabby mouse mutant, molar cusp patterns are compressed and the tips of the cusps are close to each other or completely absent. In these mice, deficient growth of the internal enamel epithelium leads to inappropriate approximation of the secondary enamel knots, resulting in these cuspal defects.[98] This control of secondary enamel knot spacing may well be dictated by the highly localised expression of *Fgf-4* within the knots themselves, accompanied by more diffuse expression domains of other signals such as *Shh* in the ectoderm and *Bmp-4* in the ectomesenchyme.[1] The exact mechanisms involved in controlling this differential growth are not, however, well understood.

Human tooth agenesis

The absence of one or more teeth is a common developmental anomaly in man and whilst this condition is not life threatening, it can represent a significant clinical problem. The incidence of missing teeth or hypodontia has been reported to vary from 1.6 to 9.6%, excluding third molars, which are absent in around 20% of the population.[44] Interestingly, the incidence of missing teeth in the primary dentition is considerably lower, reported to be between 0.5 and 0.9% in a sample of Finnish children.[99] Several types of hypodontia are recognised in humans; the absence of one or a few or many teeth can occur as an isolated condition and is essentially a reflection of normal variation. Alternatively, hypodontia can be associated with one of the number of clinically recognised syndromes.

Recent advances in our understanding of the molecular mechanisms involved in murine tooth development have led to the identification of many candidate genes that might be involved in human hypodontia. A study by Nieminen and colleagues analysed the relationship of *MSX-1* and *MSX-2* to familial incisor-premolar hypodontia in five Finnish families with a total of 20 affected individuals. However, linkage analysis excluded these genes as loci for this form of hypodontia.[100] Nevertheless, these findings did not rule out a defect in either of these genes being associated with other forms of hypodontia and genetic linkage analysis of a family affected with a rather more severe form, involving the absence of all second premolars and third molars, has identified a causative locus on

chromosome 4p where the *MSX-1* gene resides.[101] Sequence analysis demonstrated a missense mutation within the homeodomain of the MSX-1 protein in all affected family members. This protein was subsequently found to be inactive in vivo and haploinsufficiency concluded to be the probable cause of the phenotype.[102] Interestingly, a mutation in *PAX-9* has also been identified in association with severe hypodontia in a family affected with agenesis of most permanent molars and a variable absence of second premolars and mandibular incisors. The insertion of a Guanine at position 219 produced a frameshift and premature termination of the protein. In addition, the mutation also altered the amino acid sequence within the highly conserved paired-domain.[103]

Whilst several other genes have been excluded as candidate genes for familial incisor-premolar hypodontia, notably *EGF*, *EGFR* and *FGF-3*,[104] progress has been made in the elucidation of genes mutated in several forms of syndromic hypodontia. A Dutch family showing various combinations of cleft lip, cleft palate and tooth agenesis were demonstrated to have a nonsense mutation in exon 1 of *MSX-1*.[105] Further, Kere, Srivastava and co-workers[106] succeeded in cloning the *EDA* (or *EDA-1*) gene, responsible for X-linked anhidrotic ectodermal dysplasia (EDA), a disorder associated with defects in the hair, skin, nails and teeth. The tooth defect manifests as severe hypodontia with abnormalities in the size and shape of the remaining dentition. Interestingly, the gene responsible for the spontaneous X-linked mouse mutation Tabby, which has an identical phenotype to EDA, has also been cloned.[107,108] The products of these genes are a group of alternatively spliced novel transmembrane proteins called Ectodysplasins, with some homology to tumour necrosis factor (TNF)-like proteins.[108–111] The analysis of Tabby mice has suggested that the tooth phenotype is essentially due to deficiencies in growth of the dental ectoderm.[98] Further, the Downless mouse mutant, which has an indistinguishable phenotype from Tabby, has been demonstrated to have mutations in the ectodermal dysplasia receptor (Edar) gene, which encodes a novel member of the TNF receptor family.[112] Mutations in the human homologue of mouse Downless cause autosomal recessive and autosomal dominant EDA, both clinically indistinguishable from the more common X-linked EDA.[113] Whilst the precise developmental mechanisms responsible for these various forms of hypodontia are not fully understood, the findings that premolars, lateral incisors and third molars are the teeth most frequently affected invites speculation that the timing of their development, being the last teeth within each series to develop, may make them more susceptible to falling below a developmental threshold.[43]

Conclusions

As this review has demonstrated, considerable progress has been made over the last decade in disseminating some of the molecular mechanisms that are involved during early development of the jaws and dental tissues, but areas do still remain where relatively little is known. In particular, how the early domains of signalling peptides are established in first branchial arch ectoderm prior to odontogenic initiation. Nonetheless, with advances in our present knowledge likely to increase with the pace that has occurred over the last decade, a much more thorough understanding can be expected in the not too distant future. This may well lead to the application of genetic engineering in the treatment of many craniofacial anomalies, including hypodontia.

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References

1. Jernvall J, Thesleff I. Reiterative signaling and patterning during mammalian tooth morphogenesis. *Mech Dev* 2000; **92**:19–29.
2. Peters H, Balling RT. Teeth. Where and how to make them. *Trends Genet* 1999;**15**:59–65.
3. Tucker AS, Sharpe PT. Molecular genetics of tooth morphogenesis and patterning: the right shape in the right place. *J Dent Res* 1999;**78**:826–834.
4. Nieminen P, Pekkanen M, Åberg T, Thesleff I. A graphical WWW-database on gene expression in tooth. *Eur J Oral Sci* 1998;**1**:7–11.
5. Imai H, Osumi-Yamashita N, Nimomya Y, Eto K. Contribution of foregut endoderm to tooth initiation of mandibular incisor in rat embryos. *Eur J Oral Sci* 1998;**106(Suppl 1)**: 19–23.
6. Couly GF, Le Douarin NM. Mapping of the early neural primordium in quail-chick chimeras. I. Developmental relationships between placodes, facial ectoderm, and prosencephalon. *Dev Biol* 1985;**110**:422–439.
7. Couly GF, Le Douarin NM. Mapping of the early neural primordium in quail-chick chimeras. II. The prosencephalic neural plate and neural folds: implications for the genesis of cephalic human congenital abnormalities. *Dev Biol* 1987;**120**:198–214.
8. Johnston MC. A radioautographic study of the migration and fate of cranial neural crest cells in the chick embryo. *Anat Rec* 1966;**156**:143–156.

9. Köntges G, Lumsden A. Rhombencephalic neural crest segmentation is preserved throughout craniofacial ontogeny. *Development* 1996;**122**:3229–3242.
10. Tan SS, Morriss-Kay GM. Analysis of cranial neural crest cell migration and early fates in postimplantation rat chimaeras. *J Embryol Exp Morphol* 1986;**98**:21–58.
11. LaBonne C, Bronner-Fraser M. Molecular mechanisms of neural crest formation. *Annu Rev Cell Dev Biol* 1999;**15**: 81–112.
12. Osumi-Yamashita N, Ninomiya Y, Doi R, Eto K. The contribution forebrain and midbrain crest cells to the mesenchyme in the frontonasal mouse embryos. *Dev Biol* 1994;**164**:409–419.
13. Imai H, Osumi-Yamashita N, Ninomiya Y, Eto K. Contribution of early-emigrating midbrain crest cells to the dental mesenchyme of mandibular teeth in rat embryos. *Dev Biol* 1996;**176**:151–165.
14. Chai Y, Jiang X, Ito Y. Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. *Development* 2000;**127**:1671–1679.
15. Akam M. Hox and HOM: homologous gene clusters in insects and vertebrates. *Cell* 1989;**57**:347–349.
16. Hunt P, Krumlauf R. Deciphering the Hox code: clues to patterning branchial regions of the head. *Cell* 1991;**66**:1075–1078.
17. Cohn MJ. Evolutionary biology: lamprey Hox genes and the origin of jaws. *Nature* 2002;**416**:386–387.
18. Couly G, Grapin-Botton A, Coltey P, Ruhin B, Le Douarin NM. Determination of the identity of the derivatives of the cephalic neural crest: incompatibility between Hox gene expression and lower jaw development. *Development* 1998;**125**:3445–3459.
19. Grammatopoulos GA, Bell E, Toole L, Lumsden A, Tucker AS. Homeotic transformation of branchial arch identity after Hoxa2 overexpression. *Development* 2000;**127**:5355–5365.
20. Crossley PH, Martin GR. The mouse Fgf8 gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo. *Development* 1995;**121**:439–451.
21. Kettunen P, Thesleff I. Expression and function of FGFs-4, -8, and -9 suggest functional redundancy and repetitive use as epithelial signals during tooth morphogenesis. *Dev Dyn* 1998;**211**:256–268.
22. Tucker AS, Yamada G, Grigoriou M, Pachnis V, Sharpe PT. Fgf-8 determines rostral-caudal polarity in the first branchial arch. *Development* 1999;**126**:51–61.
23. Grigoriou M, Tucker AS, Sharpe PT, Pachnis V. Expression and regulation of Lhx6 and Lhx7, a novel subfamily of LIM homeodomain encoding genes, suggests a role in mammalian head development. *Development* 1998;**125**:2063–2074.
24. Gaunt SJ, Blum M, De Robertis EM. Expression of the mouse goosecoid gene during mid-embryogenesis may mark mesenchymal cell lineages in the developing head, limbs and body wall. *Development* 1993;**117**:769–778.
25. Rivera-Perez JA, Mallo M, Gendron-Maguire M, Gridley T, Behringer RR. Goosecoid is not an essential component of the mouse gastrula organizer but is required for craniofacial and rib development. *Development* 1995;**121**:3005–3012.
26. Yamada G, Mansouri A, Torres M. Targeted mutation of the murine goosecoid gene results in craniofacial defects and neonatal death. *Development* 1995;**121**:2917–2922.
27. Clouthier DE, Hosoda K, Richardson JA. Cranial and cardiac neural crest defects in endothelin-A receptor-deficient mice. *Development* 1998;**125**:813–824.
28. Kurihara Y, Kurihara H, Suzuki H. Elevated blood pressure and craniofacial abnormalities in mice deficient in endothelin-1. *Nature* 1994;**368**:703–710.
29. Trumpp A, Depew MJ, Rubenstein JL, Bishop JM, Martin GR. Cre-mediated gene inactivation demonstrates that FGF8 is required for cell survival and patterning of the first branchial arch. *Genes Dev* 1999;**13**:3136–3148.
30. Bulfone A, Kim HJ, Puelles L, Porteus MH, Grippo JF, Rubenstein JL. The mouse Dlx-2 (Tes-1) gene is expressed in spatially restricted domains of the forebrain, face and limbs in midgestation mouse embryos. *Mech Dev* 1993;**40**: 129–140.
31. Depew MJ, Liu JK, Long JE. Dlx5 regulates regional development of the branchial arches and sensory capsules. *Development* 1999;**126**:3831–3846.
32. Qiu M, Bulfone A, Ghattas I. 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. *Dev Biol* 1997;**185**:165–184.
33. Qiu M, Bulfone A, Martinez S. Null mutation of Dlx-2 results in abnormal morphogenesis of proximal first and second branchial arch derivatives and abnormal differentiation in the forebrain. *Genes Dev* 1995;**9**:2523–2538.
34. Acampora D, Merlo GR, Paleari L. Craniofacial, vestibular and bone defects in mice lacking the Distal-less-related gene Dlx5. *Development* 1999;**126**:3795–3809.
35. Depew MJ, Lufkin T, Rubenstein JL. Specification of jaw subdivisions by Dlx genes. *Science* 2002;**298**:381–385.
36. James CT, Ohazama A, Tucker AS, Sharpe PT. Tooth development is independent of a Hox patterning programme. *Dev Dyn* 2002;**225**:332–335.
37. Sharpe PT. Homeobox genes and orofacial development. *Connect Tissue Res* 1995;**32**:17–25.
38. Thomas BL, Sharpe PT. Patterning of the murine dentition by homeobox genes. *Eur J Oral Sci* 1998;**106(Suppl 1)**: 48–54.
39. Thomas BL, Tucker AS, Qui M. Role of Dlx-1 and Dlx-2 genes in patterning of the murine dentition. *Development* 1997;**124**:4811–4818.
40. Tucker AS, Matthews KL, Sharpe PT. Transformation of tooth type induced by inhibition of BMP signaling. *Science* 1998;**282**:1136–1138.
41. Tissier-Seta JP, Mucchielli ML, Mark M, Mattei MG, Goridis C, Brunet JF. Barx1, a new mouse homeodomain transcription factor expressed in cranio-facial ectomesenchyme and the stomach. *Mech Dev* 1995;**51**:3–15.
42. Sharpe PT. Neural crest and tooth morphogenesis. *Adv Dent Res* 2001;**15**:4–7.
43. Thesleff I. Two genes for missing teeth. *Nat Genet* 1996;**13**:379–380.
44. Graber LW. Congenital absence of teeth: a review with emphasis on inheritance patterns. *J Am Dent Assoc* 1978;**96**: 266–275.
45. Lumsden AG. Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. *Development* 1988;**103**: 155–169.
46. Mina M, Kollar EJ. The induction of odontogenesis in non-dental mesenchyme combined with early murine mandibular arch epithelium. *Arch Oral Biol* 1987;**32**:123–127.
47. Neubüser A, Peters H, Balling R, Martin GR. Antagonistic interactions between FOF and BMP signaling pathways: a mechanism for positioning the sites of tooth formation. *Cell* 1997;**90**:247–255.
48. St Amand TR, Zhang Y, Semina EV. Antagonistic signals between BMP4 and FGF8 define the expression of Pitx1 and Pitx2 in mouse tooth-forming anlage. *Dev Biol* 2000;**217**: 323–332.

49. Ferguson CA, Tucker AS, Sharpe PT. Temporospatial cell interactions regulating mandibular and maxillary arch patterning. *Development* 2000;**127**:403–412.
50. Kollar EJ, Mina M. Role of the early epithelium in the patterning of the teeth and Meckel's cartilage. *J Craniofac Genet Dev Biol* 1991;**11**:223–228 [Comment].
51. Miller WA. Inductive changes in early tooth development. I. A study of mouse tooth development on the chick chorioallantosis. *J Dent Res* 1969;**48**:719–725.
52. Kollar EJ, Baird GR. The influence of the dental papilla on the development of tooth shape in embryonic mouse tooth germs. *J Embryol Exp Morphol* 1969;**21**:131–148.
53. Vainio S, Karavanova I, Jowett A, Thesleff I. Identification of BMP-4 as a signal mediating secondary induction between epithelial and mesenchymal tissues during early tooth development. *Cell* 1993;**75**:45–58.
54. Åberg T, Wozney J, Thesleff I. Expression patterns of bone proteins (Bmps) in the developing mouse tooth suggest roles in morphogenesis and cell differentiation. *Dev Dyn* 1997;**210**:383–396.
55. Tucker AS, Al Khamis A, Sharpe PT. Interactions between Bmp-4 and Msx-l act to restrict gene expression to odontogenic mesenchyme. *Dev Dyn* 1998;**212**:533–539.
56. Chen Y, Bei M, Woo I, Satokata I, Maas R. Msxl controls inductive signaling in mammalian tooth morphogenesis. *Development* 1996;**122**:3035–3044.
57. Hill RE, Jones PF, Rees AR. A new family of mouse homeo box-containing genes: molecular structure, chromosomal location, and developmental expression of Hox-7.1. *Genes Dev* 1989;**3**:26–37.
58. MacKenzie A, Leeming GL, Jowett AK, Ferguson MW, Sharpe PT. 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* 1991;**111**:269–285.
59. MacKenzie A, Ferguson MW, Sharpe PT. Hox-7 expression during murine craniofacial development. *Development* 1991;**113**:601–611.
60. Robert B, Sassoon D, Jacq B, Gehring W, Buckingham M. Hox-7, a mouse homeobox gene with a novel pattern of expression during embryogenesis. *EMBO J* 1989;**8**:91–100.
61. Satokata I, Maas R. Msx1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. *Nat Genet* 1994;**6**:348–356.
62. Kratochwil K, Dull M, Farinas I, Galceran J, Grosschedl R. Lef1 expression is activated by BMP-4 and regulates inductive tissue interactions in tooth and hair development. *Genes Dev* 1996;**10**:1382–1394.
63. Oosterwegel M, van de Wetering M, Timmerman J. Differential expression of the HMG box factors TCF-1 and LEF-1 during murine embryogenesis. *Development* 1993; **118**:439–448.
64. Zhou P, Byrne C, Jacobs J, Fuchs E. Lymphoid enhancer factor 1 directs hair follicle patterning and epithelial cell fate. *Genes Dev* 1995;**9**:700–713.
65. Cadigan KM, Nusse R. Wnt signaling: a common theme in animal development. *Genes Dev* 1997;**11**:3286–3305.
66. van Genderen C, Okamura RM, Farinas I. Development of several organs that require inductive epithelial–mesenchymal interactions is impaired in LEF-1-deficient mice. *Genes Dev* 1994;**8**:2691–2703.
67. Bitgood MJ, McMahon AP. Hedgehog and Bmp genes are coexpressed at many diverse sites of cell–cell interaction in the mouse embryo. *Dev Biol* 1995;**172**:126–138.
68. Cobourne MT, Hardcastle Z, Sharpe PT. Sonic hedgehog regulates epithelial proliferation and cell survival in the developing tooth germ. *J Dent Res* 2001;**80**:1974–1979.
69. Dassule HR, McMahon AP. Analysis of epithelial–mesenchymal interactions in the initial morphogenesis of the mammalian tooth. *Dev Biol* 1998;**202**:215–227.
70. Hardcastle Z, Mo R, Hui CC, Sharpe PT. The Shh signalling pathway in tooth development: defects in Gli2 and Gli3 mutants. *Development* 1998;**125**:2803–2811.
71. Sarkar L, Cobourne MT, Naylor S, Smalley M, Dale T, Sharpe PT. Wnt/Shh interactions regulate ectodermal boundary formation during mammalian tooth development. *Proc Natl Acad Sci USA* 2000;**97**:4520–4524.
72. Dassule HR, Lewis P, Bei M, Maas R, McMahon AP. Sonic hedgehog regulates growth and morphogenesis of the tooth. *Development* 2000;**127**:4775–4785.
73. Hammerschmidt M, Brook A, McMahon AP. The world according to hedgehog. *Trends Genet* 1997;**13**:14–21.
74. Ingham PW, McMahon AP. Hedgehog signaling in animal development: paradigms and principles. *Genes Dev* 2001;**15**:3059–3087.
75. Chen Y, Struhl G. Dual roles for patched in sequestering and transducing Hedgehog. *Cell* 1996;**87**:553–563.
76. Ruiz i Altaba A. Catching a glimpse of Hedgehog. *Cell* 1997;**90**:193–196.
77. Ruiz i Altaba A. The works of GLI and the power of hedgehog. *Nat Cell Biol* 1999;**1**:E147–E148.
78. Gorlin RJ, Cohen MM, Levin LS. Syndromes of the head and neck. 3rd ed. New York: Oxford University Press; 1989.
79. Gailani MR, Stahle-Backdahl M, Leffell DJ. The role of the human homologue of Drosophila patched in sporadic basal cell carcinomas. *Nat Genet* 1996;**14**:78–81.
80. Hahn H, Wicking C, Zaphiropoulous PG. Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. *Cell* 1996;**85**:841–851.
81. Johnson RL, Rothman AL, Xie J. Human homolog of patched, a candidate gene for the basal cell nevus syndrome. *Science* 1996;**272**:1668–1671.
82. Gritli-Linde A, Lewis P, McMahon AP, Linde A. The whereabouts of a morphogen: direct evidence for short- and graded long-range activity of hedgehog signaling peptides. *Dev Biol* 2001;**236**:364–386.
83. Del Sal G, Ruaro ME, Philipson L, Schneider C. The growth arrest-specific gene, gas1, is involved in growth suppression. *Cell* 1992;**70**:595–607.
84. Chuang PT, McMahon AP. Vertebrate Hedgehog signalling modulated by induction of a Hedgehog-binding protein. *Nature* 1999;**397**:617–621.
85. Cobourne MT, Sharpe PT. Expression and regulation of Hedgehog-interacting protein during early tooth development. *Conn Tiss Res* 2002;**43**:143–147.
86. DiNardo S, Heemskerk J, Dougan S, O'Farrell PH. The making of a maggot: patterning the Drosophila embryonic epidermis. *Curr Opin Genet Dev* 1994;**4**:529–534.
87. Martinez Arias A, Baker NE, Ingham PW. Role of segment polarity genes in the definition and maintenance of cell states in the Drosophila embryo. *Development* 1988;**103**: 157–170.
88. Jernvall J, Kettunen P, Karavanova I, Martin LB, Thesleff I. 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. *Int J Dev Biol* 1994;**38**:463–469.
89. Jernvall J, Åberg T, Kettunen P, Keranen S, Thesleff I. The life history of an embryonic signaling center: BMP-4 induces p21 and is associated with apoptosis in the mouse tooth enamel knot. *Development* 1998;**125**:161–169.
90. Vaahtokari A, Åberg T, Jernvall J, Keranen S, Thesleff I. The enamel knot as a signaling center in the developing mouse tooth. *Mech Dev* 1996;**54**:39–43.

91. Vaahtokari A, Åberg T, Thesleff I. Apoptosis in the developing tooth: association with an embryonic signaling center and suppression by EGF and FGF-4. *Development* 1996;**122**:121–129.
92. Peters H, Neubuser A, Kratochwil K, Balling R. Pax9-deficient mice lack pharyngeal pouch derivatives and teeth and exhibit craniofacial and limb abnormalities. *Genes Dev* 1998;**12**:2735–2747.
93. Sarkar L, Sharpe PT. Expression of Wnt signalling pathway genes during tooth development. *Mech Dev* 1999;**85**: 197–200.
94. Celli G, LaRochelle WJ, Mackem S, Sharp R, Merlino G. Soluble dominant-negative receptor uncovers essential roles for fibroblast growth factors in multi-organ induction and patterning. *EMBO J* 1998;**17**:1642–1655.
95. De Moerlooze L, Spencer-Dene B, Revest J, Hajihosseini M, Rosewell I, Dickson C. An important role for the IIIb isoform of fibroblast growth factor receptor 2 (FGFR2) in mesenchymal-epithelial signalling during mouse organogenesis. *Development* 2000;**127**:483–492.
96. Kettunen P, Karavanova I, Thesleff I. Responsiveness of developing dental tissues to fibroblast growth factors: expression of splicing alternatives of FGFR1, -2, -3, and of FGFR4; and stimulation of cell proliferation by FGF-2, -4, -8, and -9. *Dev Genet* 1998;**22**:374–385.
97. Tucker AS, Headon DJ, Schneider P. Edar/Eda interactions regulate enamel knot formation in tooth morphogenesis. *Development* 2000;**127**:4691–4700.
98. Pispa J, Jung HS, Jernvall J. Cusp patterning defect in Tabby mouse teeth and its partial rescue by FGF. *Dev Biol* 1999;**216**:521–534.
99. Jarvinen S, Lehtinen L. Supernumerary and congenitally missing primary teeth in Finnish children: an epidemiological study. *Acta Odontol Scand* 1981;**39**:83–86.
100. Nieminen P, Arte S, Pirinen S, Peltonen L, Thesleff I. Gene defect in hypodontia: exclusion of MSX1 and MSX2 as candidate genes. *Hum Genet* 1995;**96**:305–308.
101. Vastardis H, Karimbux N, Guthua SW, Seidman JG, Seidman CE. A human MSX1 homeodomain missense mutation causes selective tooth agenesis. *Nat Genet* 1996;**13**:417–421.
102. Hu G, Vastardis H, Bendall AJ. Haploinsufficiency of MSX1: a mechanism for selective tooth agenesis. *Mol Cell Biol* 1998;**18**:6044–6051.
103. Stockton DW, Das P, Goldenberg M, D'Souza RN, Patel PI. Mutation of PAX9 is associated with oligodontia. *Nat Genet* 2000;**24**:18–19.
104. Arte S, Nieminen P, Pirinen S, Thesleff I, Peltonen L. Gene defect in hypodontia: exclusion of EGF, EGFR, and FGF-3 as candidate genes. *J Dent Res* 1996;**75**:1346–1352.
105. van den Boogaard MJ, Dorland M, Beemer F, Avan Amstel HK. MSX1 mutation is associated with orofacial clefting and tooth agenesis in humans. *Nat Genet* 2000;**24**: 342–343.
106. Kere J, Srivastava AK, Montonen O. X-linked anhidrotic (hypohidrotic) ectodermal dysplasia is caused by mutation in a novel transmembrane protein. *Nat Genet* 1996;**13**: 409–416.
107. Ferguson BM, Brockdorff N, Formstone E, Ngyuen T, Kronmiller JE, Zonana J. Cloning of Tabby, the murine homolog of the human EDA gene: evidence for a membrane-associated protein with a short collagenous domain. *Hum Mol Genet* 1997;**6**:1589–1594.
108. Srivastava AK, Pispa J, Hartung AJ. The Tabby phenotype is caused by mutation in a mouse homologue of the EDA gene that reveals novel mouse and human exons and encodes a protein (ectodysplasin-A) with collagenous domains. *Proc Natl Acad Sci USA* 1997;**94**:13069–13074.
109. Bayes M, Hartung AJ, Ezer S. The anhidrotic ectodermal dysplasia gene (EDA) undergoes alternative splicing and encodes ectodysplasin-A with deletion mutations in collagenous repeats. *Hum Mol Genet* 1998;**7**:1661–1669.
110. Mikkola ML, Pispa J, Pekkanen M. Ectodysplasin, a protein required for epithelial morphogenesis, is a novel TNF homologue and promotes cell-matrix adhesion. *Mech Dev* 1999;**88**:133–146.
111. Monreal AW, Zonana J, Ferguson B. Identification of a new splice form of the EDA1 gene permits detection of nearly all X-linked hypohidrotic ectodermal dysplasia mutations. *Am J Hum Genet* 1998;**63**:380–389.
112. Headon DJ, Overbeek PA. Involvement of a novel Tnf receptor homologue in hair follicle induction. *Nat Genet* 1999;**22**:370–374.
113. Monreal AW, Ferguson BM, Headon DJ, Street SL, Overbeek PA, Zonana J. Mutations in the human homologue of mouse dl cause autosomal recessive and dominant hypohidrotic ectodermal dysplasia. *Nat Genet* 1999;**22**:366–369.