

# Neural Crest Cells and Patterning of the Mammalian Dentition

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**ABSTRACT** The mammalian dentition is composed of serial groups of teeth, each with a distinctive crown and root morphology, highly adapted to its particular masticatory function. In the embryo, generation of individual teeth within the jaws relies upon interactions between ectoderm of the first branchial arch and the neural crest-derived ectomesenchymal cells that migrate into this region from their site of origin along the neural axis. Classic tissue recombination experiments have provided evidence of an essential role of the ectoderm in initiating tooth development; however, the underlying ectomesenchyme rapidly acquires dominance in establishing shape. A key question is how these cells acquire this positional information. One theory suggests that ectomesenchymal cells are pre-patterned with respect to shape generation. Alternatively, this cell population acquires positional information within the first branchial arch itself, following migration. Recent molecular evidence suggests a high degree of plasticity within these ectomesenchymal cells. In particular, signalling molecules within the ectoderm exert a time-dependent influence upon the ectomesenchyme by establishing specific domains of homeobox gene expression. Initially, these ectomesenchymal cells are plastic and able to respond to signalling from the ectoderm, however, this plasticity is rapidly lost and pattern information becomes fixed. Therefore, in the first branchial arch, local regulation between the ectoderm and neural crest-derived ectomesenchyme is crucial in establishing the appropriate tooth shape in the correct region of the jaw. *J. Exp. Zool. (Mol. Dev. Evol.)* 306B:251–260, 2006. © 2005 Wiley-Liss, Inc.

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 (Trainor and Krumlauf, 2000a, 2001; Graham and Smith, 2001). In the craniofacial region, cranial neural crest cells (CNCC) provide the fundamental building blocks for generating much of the skeleton and connective tissue of the head, in addition to the cranial ganglia and peripheral nerves that innervate these skeletal structures. CNCC also make a significant contribution towards formation of the mature dentition.

A common finding in vertebrates is the highly organised and reproducible migration of distinct CNCC streams, from their site of origin along the neural axis into the branchial arches (Kulesa et al., 2004). In particular, organisation of the

hindbrain at both the morphological and molecular level provides an axial registration between the sites of origin of CNCC and their ultimate destination within the branchial arches (Hunt et al., '91a,b). Manipulation of the CNCC primordia axial level in the chick neural tube by transplantation leads to crest migration and generation of skeletal structures commensurate with the original axial level of the transplant, structures inappropriate for the new location (Noden, '83). These findings led to the idea that CNCC were pre-patterned, replete with the necessary genetic information prior to their migration from the neural tube, allowing production of the requisite skeletal structures in the correct position at their ultimate destination within the early

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cranium and branchial arches. More recently, progressively more elegant and technically demanding transplantation and ablation experiments carried out in a variety of species have suggested a degree of plasticity in CNCC behaviour. In particular, these cells appear to be influenced by local cell community effects (Trainor and Krumlauf, 2000b; Schilling et al., 2001) and their very presence even dispensable for normal branchial arch patterning (Veitch et al., '99; Gavalas et al., 2001). These findings have focused attention upon the role of other tissues in the branchial arch region as a source of pattern information, notably the endoderm (Begbie et al., '99; Veitch et al., '99; Couly et al., 2002), mesoderm (Trainor and Tam, '95; Trainor and Krumlauf, 2000b) and ectoderm (Tucker et al., '99; Ferguson et al., 2000). Certainly, first branchial arch ectoderm seems to play a crucial role in establishing pattern information within CNCC that migrate into the primitive jaws and ultimately contribute to formation of the dentition.

#### CONTRIBUTION OF CNCC TO TOOTH DEVELOPMENT

From the early part of the last century, embryologists have been studying the fate of CNCC as they migrate into the future head region of the embryo. Early studies in amphibians demonstrated that ablation could result in failure of some first branchial arch structures to develop (Adams, '24; Stone, '26; Raven, '31), whilst manipulation of CNCC in vivo distinguished separate regions of origin for maxillary and mandibular odontoblasts and provided the first indication that the mesenchymal component of tooth primordia might be of CNCC origin (Sellman, '46). Further dissemination of a role for CNCC during tooth development came from xenoplastic transplant experiments carried out between urodele and anuran larvae in vivo. Urodeles possess true teeth, anurans do not; generation of chimeric larvae demonstrated that anuran stomatodeal neural crest in combination with urodele stomatodeal ectoderm was capable of forming teeth. Although unrealised during normal development, anuran neural crest had the capacity for induction of a dental papilla and dentine (Wagner, '49, '55).

The pathways of CNCC migration have been extensively mapped in vertebrate embryos. These experiments have confirmed that this cell population makes a fundamental contribution to develop-

ment of the branchial arch apparatus in a number of species. (Tosney, '82; Lumsden et al., '91; Schilling and Kimmel, '94; Trainor and Tam, '95; Kontges and Lumsden, '96) However, it has been technically demanding to selectively label mammalian CNCC and follow their migration, particularly to the embryonic stages associated with odontogenesis. Mammalian neural folds are small and difficult to manipulate in vivo, whilst maintenance of whole embryos in culture for the necessary length of time is hard to achieve. Direct evidence of CNCC within mammalian dental mesenchyme was achieved following DiI injection into the midbrain and anterior hindbrain of E10 rat embryos subjected to rolling culture followed by isolated mandibular organ culture (Imai et al., '96). This allowed tooth development to reach the bud stage and so facilitated an analysis of labelled CNCC within odontogenic regions of the mandible. Labelled cells derived from the anterior midbrain were predominantly distributed in the frontonasal mass, with some limited spread into the maxilla and proximal mandible, whilst cells from the posterior midbrain were largely found in the maxillary and mandibular prominences and trigeminal ganglion; anterior hindbrain CNCC were distributed mainly in the trigeminal ganglion. Cells labelled from the posterior midbrain at the 3–5 somite stage were consistently distributed throughout the entire mandibular process, including the distal odontogenic region. Further analysis of these mandibular processes after 6 days of organ culture demonstrated that CNCC migrating from the posterior midbrain were present in the dental mesenchyme associated with bud stage molar tooth germs (Imai et al., '96).

Whilst cell-labelling studies have been useful in the identification of mammalian CNCC migratory pathways, they have not provided a comprehensive cell lineage analysis of these cells as they become terminally differentiated to a specific cell type. More recently, a genetic marker has been utilised to follow CNCC migration and differentiation in the mouse (Chai et al., 2000). The normal embryonic expression of *Wnt1* is such that all cranial and spinal ganglia and skeletogenic CNCC in the branchial arches are ultimately derived from *Wnt1*-expressing precursor cells in the central nervous system. Transgenic mice expressing *lacZ* under control of a *Wnt1* promoter therefore demonstrate staining in early migratory CNCC, but this is lost prior to differentiation. To circumvent this problem, a *Wnt1* promoter has been used to drive Cre expression and generate

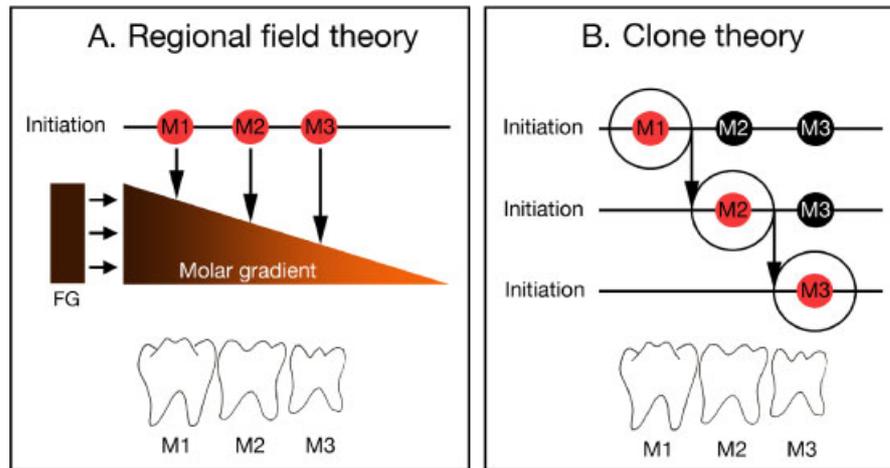


Fig. 1. Schematic representation of the regional field (A) and clone (B) theories as applied to the formation of a molar dentition (M1-M3). (A) According to the regional field theory, identical tooth primordia (red dots) are acted upon by a morphogenetic substance (orange shading) generated by a field generator (FG). The substance has a graded concentration in the field; in this example, each initiated tooth develops into a molar, each with a slightly different morphology. (B) According to the clone theory, final tooth form is related to the times at which the tooth primordia (black dots) are initiated (red dots). The circle represents the growing margin of the clone with an associated zone of inhibition around its periphery. Once adjacent tissues have reached the critical stage for initiation, a new molar tooth is formed within the series. Adapted from Lumsden ('79).

conditional mice that exhibit ubiquitous *lacZ* expression in *Wnt1*-expressing CNCC precursors. This allows cell staining and therefore identification at much later stages of development. These mice clearly demonstrate that CNCC-derived ectomesenchyme contributes to the formation of condensed dental mesenchyme at the initial bud stage and subsequently to the formation of the dental papilla and follicle in the developing tooth germ. In addition, analysis of 6-week-old mice demonstrated definitively a CNCC origin for the odontoblasts, dentine matrix, pulp tissue, cementum and periodontal ligament of teeth in the adult murine dentition (Chai et al., 2000).

### PATTERNING THE DENTAL AXIS

The first branchial arch gives rise to a number of axial-specific structures, notably the jaw skeleton and primary masticatory musculature. In addition, the mammalian first arch is also characterised by generation of the dentition. Within the dentition, teeth form a species-specific series of structures whose differences can be described in terms of changes in shape and size along the dental axis. The generation of a tooth in the embryo requires co-ordinated molecular signalling between the ectoderm of the first branchial arch and the underlying CNCC that migrate into these regions (Jernvall and Thesleff, 2000; Cobourne and Sharpe, 2003; Tucker and Sharpe, 2004). Given the essential contribution of these embry-

onic tissues to tooth development, fundamental questions exist as to where the necessary patterning information resides and how much relative plasticity there is in the interpretation of this information during the morphogenesis of teeth.

Historically, two theories have been proposed to account for the developmental mechanisms responsible for patterning dental shape along the axis. A regional field theory suggests that all tooth primordia are initially equivalent, the shapes that the teeth ultimately develop into being controlled by a morphogenetic field of substances or signals generated in the jaw primordia (Fig. 1A). These signals would have a graded concentration along the developing dental axis, such that each tooth develops differently according to its position relative to the source of the signal (Butler, '39, '67). In contrast, a clone model predicts that all the teeth develop from a single clone of mesenchymal cells (Fig. 1B). This mesenchymal clone grows to form the individual teeth in sequence (Osborne, '73, '78). The fundamental principle of the clone theory, unlike the regional field theory, is that the shape of the tooth is determined from the moment the primordium has been initiated.

### TESTING THE MEMORY OF SHAPE

The initiation of dental primordia is clearly a pre-requisite of both theories and this has been demonstrated to be a property of ectoderm in the first arch, at least prior to E12 in the mouse

(Lumsden, '88). Murine tissue recombination has been used to test some of the predictions within the regional field and clone hypotheses (Lumsden, '79; Lumsden and Buchanan, '86). Using the developing lower molar dentition of the mouse as a model, isolated presumptive first molar tissue of E12 and later embryos give rise to all three molar teeth in their normal sequence when grafted into the anterior chamber of the eye. These findings are consistent with a region of ectoderm specifying a zone of CNCC which could subsequently spread out in a proximal direction and lay down the pattern of the future molar dentition; evidence for a clone-type model. In the murine dentition there would be two such regions of ectoderm, one for the incisor field and the other for the molar (Lumsden, '79).

Considerable evidence exists from other recombination experiments to suggest the dominance of CNCC in the specification of tooth shape, once the tooth primordia have been initiated. In the mouse between E13 and E16, the recombination of molar ectoderm with incisor dental papilla results in the formation of an incisor tooth. Similarly, recombination of incisor ectoderm with molar dental papilla between the same stages produces a molar tooth (Kollar and Baird, '69). Cervical loop, vestibular lamina or coronal ectoderm at E13–E16, recombined with incisor or molar dental papilla from the same stages, also result in teeth whose shapes are dictated by the origin of the mesenchyme (Kollar and Baird, '70a). Together, these experiments support an inductive role for the CNCC-derived dental mesenchyme at these stages of development and demonstrate that the ultimate shape of a tooth is a product of this ectomesenchymal component of the transplant (Kollar and Baird, '70b). All the early recombination experiments have indicated that during the initial stages of odontogenesis, the inductive capacity for tooth shape resides within first arch ectoderm (Miller, '69; Mina and Kollar, '87; Lumsden, '88). However, at later stages (around E13, or corresponding to the bud stage of development in the mouse) this odontogenic potential switches, and the underlying CNCC-derived ectomesenchyme assumes the capacity to dictate shape (Kollar and Baird, '69; Kollar and Baird, '70a,b).

The original field and clone theories were based upon the analysis of adult dentitions and were fundamentally descriptive. They provided theoretical models for the mechanisms that might be involved in patterning the dentition. In recent years, experiments carried out at the genetic level

have begun to refine these theories and provide a molecular explanation for the results of transplantation experiments.

### A MOLECULAR BASIS FOR ODONTOGENIC PATTERN

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-basepair sequence originally discovered in the homeotic selector genes of *Drosophila*. Homeotic genes are a family of master regulatory genes that contain a homeobox, these genes being ultimately responsible for specifying segment identity in the developing fly. The vertebrate homologues are the Hox genes and these genes have been shown to be responsible for specifying the vertebrate embryonic body axis during development. Overlapping domains of Hox gene expression confer a positional code that specifies the morphogenesis of individual vertebrae at different axial levels (McGinnis, '92). In addition, segment-specific combinatorial Hox gene expression in migrating CNCC is responsible for generating regional diversity in the branchial arch system (Hunt et al., '91a,b).

A peculiarity of branchial arch patterning is the finding that Hox genes are not expressed in CNCC that migrate into the first branchial arch. Skeletal elements within the first and second branchial arches are profoundly sensitive to the expression domains of the rostral-most Hox gene *Hoxa2*; targeted mutation of *Hoxa2* produces homeotic transformation of second arch structures into elements derived from the first branchial arch (Gendron-Maguire et al., '93; Rijli et al., '93), whilst *Hoxa2* over-expression in the first arch leads to transformation of first arch skeletal elements into second (Grammatopoulos et al., 2000; Pasqualetti et al., 2000). However, over-expression of *Hoxa2* in the mandibular primordium does not affect tooth development, suggesting that patterning of the dentition and skeletal elements of the branchial arches are independent (James et al., 2002).

Whilst Hox genes are not involved in patterning the dentition, a number of subfamilies of homeobox-containing genes are. These include members of the *Barx*, *Dlx*, *Lhx*, *Pitx* and *Msx* classes. These genes demonstrate regionally restricted and highly specific domains of expression and it has been suggested that these gene expression patterns

may act to ultimately specify tooth shape (Sharpe, '95; Thomas and Sharpe, '98). This odontogenic homeobox code infers that for each tooth-forming region of the mandible, a specific combination of homeobox genes within the ectomesenchyme dictates the ultimate morphology that a tooth primordium will develop into following initiation (Sharpe, 2001) (Fig. 2). There are now examples of both gain and loss-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 (Qiu et al., '97; Thomas et al., '97). These genes are both expressed in the maxillary and mandibular molar ectomesenchyme and these findings suggest that for maxillary molar specification, both *Dlx-1* and *Dlx-2* are required in these regions. In the absence of *Dlx-1* and *Dlx-2*, the maxillary molar ectomesenchyme re-programmes to a chondrogenic fate. In these double mutant embryos, a small region of ectomesenchyme directly below the maxillary molar epithelial thickenings was found to have lost *Barx-1* and assumed *Sox-9* expression. *Sox-9* is expressed in early chondrogenic cells and these molecular findings were confirmed by the presence of ectopic cartilage in the regions of the maxillary molars in the mutants (Qiu et al., '97; Thomas et al., '97). Transformation of tooth type, from incisor to molar, is possible by manipulating homeobox gene expression in the future incisor-forming ectomesenchyme of the mandibular process (Tucker et al., '98). *Barx-1* is normally expressed specifically in the proximal molar-forming ectomesenchyme of the mandibular process, 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. By inhibiting Bmp-4 signalling in the distal region using the Bmp antagonist Noggin, these workers extended the normal expression domain of *Barx-1* and downregulated 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 (that had been exposed to an altered code of ectopic *Barx-1* and loss of *Msx-1*) into renal capsules resulted in the formation of multicusped molar teeth rather than incisors. Thus, an alteration of homeobox gene expression domains was shown to be capable of re-specifying the identity of developing teeth (Tucker et al., '98).

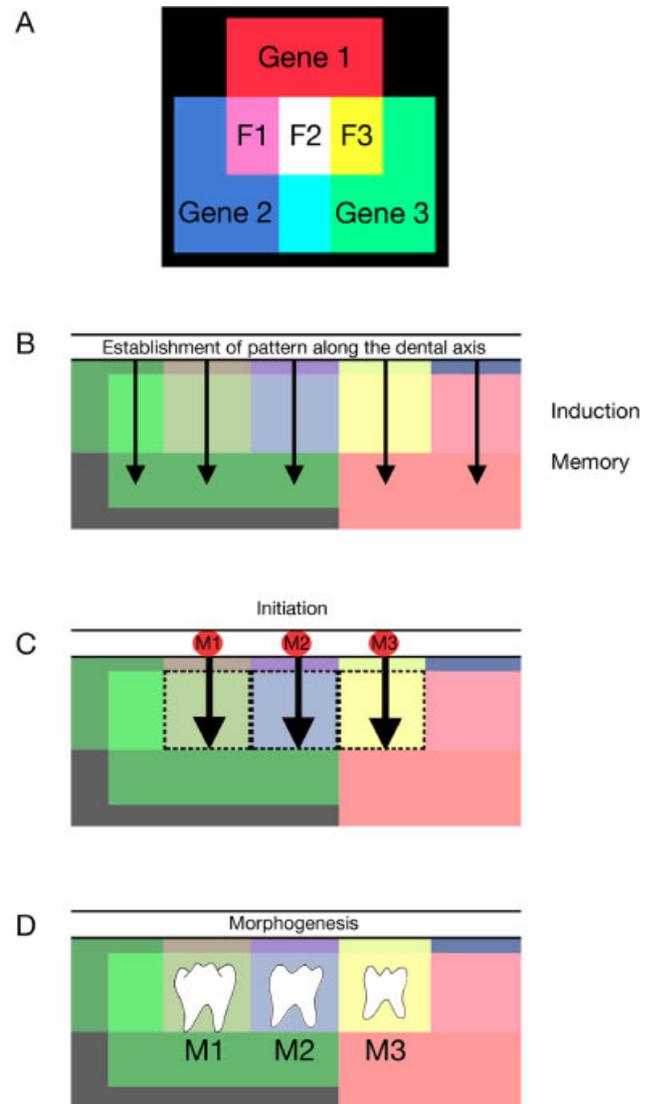


Fig. 2. Schematic representation of the dental patterning by an odontogenic homeobox code. (A) Gene patterns within the ectomesenchyme of the first branchial arch are analogous to the mixing of primary colours. Overlapping primary colour domains give rise to composite colours dependent upon the relative contributions. The final colour or genetic readout is dictated by both the presence and absence of constituent components. Thus, the inter-mixing of genes 1–3 results in three different morphogenetic fields (F1–3). (B) In the first branchial arch, pattern is established by signalling from the ectoderm-inducing specific domains of homeobox gene expression. Initially this pattern is plastic, but within a distinct timeframe the ectomesenchyme attains memory and these domains become fixed. These domains represent the molecular information necessary for the establishment of tooth shape. (C,D) Following the initiation of tooth development via ectodermal signalling, the production of a molar field is achieved via subtle differences in homeobox gene expression within the ectomesenchyme.

Co-ordinated growth and patterning of CNCC within the facial region also appears to be mediated by the secreted signalling molecule Sonic hedgehog (Shh). Early in facial development, *Shh* transcripts are present in the oral ectoderm and pharyngeal endoderm associated with the first branchial arch (Echelard et al., '93; Chang et al., '94) and loss of responsiveness to Hedgehog signalling in CNCC leads to extensive defects in the craniofacial skeleton (Jeong et al., 2004). This specification of CNCC-derived structures also extends to the dentition; mutant embryos lack mandibular incisors and have a single maxillary incisor, whilst the remaining teeth are malformed and arrested. These patterning defects appear to be mediated by Shh-induced expression of Forkhead (Fox) transcription factors, including *Foxc2*, *Foxd1*, *Foxd2*, *Foxf1* and *Foxf2*. The normal distribution of expression domains associated with these genes in ectomesenchyme of the early head has led to the suggestion that a Fox code may also exist, different combinations of Fox transcription factors providing instructive information specifying distinct cell fates in different regions of the early face. In particular, for the mandibular dentition, the region specifying lower incisors being represented by a *Foxd1+Foxd2+Foxf1+Foxf2* code, whilst the molar teeth lie outside the region of Fox influence (Jeong et al., 2004). A role for Shh in later growth and morphogenesis of the tooth germ has also been established from analysis of conditional knockout embryos either lacking *Shh* activity or *Smo* responsiveness in ectoderm of the developing tooth (Dassule et al., 2000; Gritli-Linde et al., 2002). Shh mediates proliferative activity in both ectodermal and ectomesenchymal compartments of the tooth and is important for normal cytodifferentiation of enamel-forming ameloblasts. Both the incisor and molar teeth of these embryos have abnormal growth and morphogenesis.

Another important component of the ectodermal signalling that regulates gene expression in the developing first arch is Fgf8. *Fgf-8* is expressed in the proximal ectoderm of the first branchial arch from E8.75, subsequently being present in the thickened ectoderm of presumptive tooth buds at E11-E11.5 (Crossley and Martin, '95; Kettunen and Thesleff, '98). Conditional inactivation of *Fgf8* function within the first arch demonstrates a dual role for this signalling molecule (Trumpp et al., '99). Fgf8 is important for promoting mesenchymal cell survival and outgrowth of the maxillary and mandibular primordia, but also induces a

developmental program of gene expression essential for normal morphogenesis of these regions; in its absence, most skeletal and dental structures derived from the first branchial arch are missing, except for those in the distal-most regions. Thus, the maxillary and mandibular incisors are able to form, but the remainder of the jaw skeleton and molar dentition is essentially absent. Consistent with the expression domains, proximal regions of the first branchial arch are Fgf8-dependent for growth and morphogenesis, whilst distal structures are Fgf8-independent (Trumpp et al., '99).

Therefore, a number of lines of evidence are now available to suggest that whilst CNCC-derived ectomesenchyme of the maxillary and mandibular processes is able to dictate tooth shape, migrating CNCC that populate the first branchial arch are not pre-specified with respect to proximal-distal position and ultimate odontogenic fate. This had first been hinted at by recombination experiments which demonstrated that non-first arch-derived CNCC could support tooth development (Mina and Kollar, '87; Lumsden, '88). Three other findings, namely (1) that prior to E10.5, epithelial signals are able to induce target gene expression irrespective of the position in the ectomesenchyme of the first arch (Ferguson et al., 2000), (2) that combinatorial expression patterns of ectomesenchymal genes are ultimately generated by signalling from the overlying ectoderm and (3) that by manipulating ectodermal signalling it is possible to change tooth identity via the alteration of ectomesenchymal gene expression patterns (Tucker et al., '98), have together confirmed that CNCC migrating into the first arch are not pre-specified with respect to tooth development.

### COMPETENCY OF CNCC DURING EARLY SIGNALLING INTERACTIONS

In a number of recombination studies, a role for presumptive oral ectoderm has been established for induction of regionally restricted gene expression in the underlying ectomesenchyme of the first branchial arch (Neubüser et al., '97; Tucker et al., '98; St Amand et al., 2000). Interestingly, these studies also suggested that competency of first arch ectomesenchyme to respond to these signals was closely related to the developmental stage of the mandibular process. The temporal regulation of gene expression has been investigated within the proximal and distal domains of the mandibular

arch ectomesenchyme (Ferguson et al., 2000). Using Fgf-8-induced expression of *Msx-1* and *Barx-1* (early markers of presumptive incisor and molar ectomesenchyme, respectively) as a model, this study demonstrated that the ectomesenchyme responded to ectodermal signalling in a time-dependent manner. Specifically, prior to E11.5, ectomesenchymal gene expression was dependent upon ectodermal signalling, whereas after E11.5 (the embryonic stage when the first morphological sign of tooth development occurs), expression became independent of the ectoderm. However, between E9.5 and E10.25, the ectomesenchymal cells were able to respond to ectodermal signals in an unrestricted manner, but by E10.5 this had changed dramatically and competence of the ectomesenchyme to express these genes had become regionally restricted. At E10.5, only those ectomesenchymal cells that had previously expressed these genes retained competence (Ferguson et al., 2000). This suggested that the determination of tooth type was specified by the regional expression of ectomesenchymal genes, but this expression was produced in response to ectodermal signals. This was consistent with the results of previous recombination experiments which demonstrated that prior to E10.5 the ectoderm determines tooth type (Miller, '69; Lumsden, '88; Kollar and Mina, '91) whereas after E11.5, CNCC-derived ectomesenchyme determines tooth type (Kollar and Baird, '69).

### HOMOGENEITY IN THE MAXILLA AND MANDIBLE?

Together, these recombination experiments provide strong evidence for a model whereby CNCC migrate into the first arch unspecified with regard to dental pattern information. This information is imparted upon these cells in a manner dependent upon time and position by secreted signals from the ectoderm (Fig. 3). Thus, at the bud stage of tooth development, a crucial time when morphology of the tooth germ will change in a manner dependent upon which type of tooth is to form, the pattern information resides in the ectomesenchyme and is fixed. However, a small caveat to this model is molecular evidence to suggest that the maxillary and mandibular ectomesenchyme respond differently to ectodermal signalling for certain genetic pathways (Ferguson et al., 2000). With regard to *Dlx-2* and *Dlx-5*, whilst Fgf-8 can induce the expression of these two genes in isolated mandibular ectomesenchyme, only *Dlx-2* can be induced in maxillary ectomesenchyme. These observations are consistent with normal expression domains of these two genes; *Dlx-2* is expressed in the proximal ectomesenchyme of the maxillary and mandibular processes, whereas *Dlx-5* is essentially only expressed in the proximal mandibular ectomesenchyme (Qiu et al., '97). Further recombination experiments have shown the capability of maxillary ectoderm in inducing *Dlx-5* expression in mandibular arch

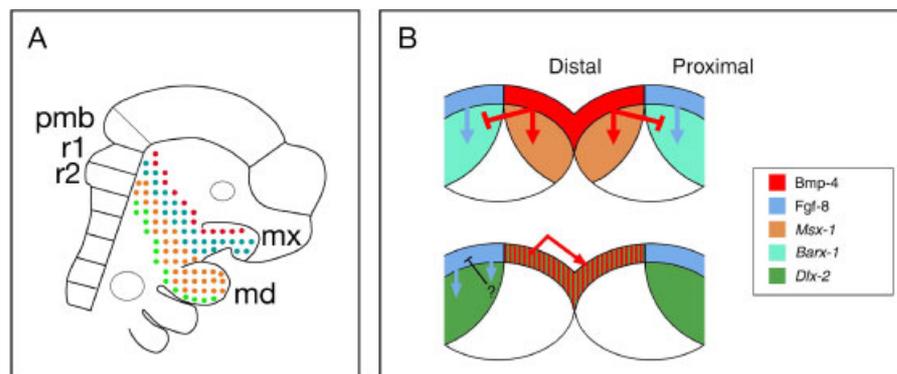


Fig. 3. Patterning the murine dentition. (A) Neural crest cells migrate into the maxillary (mx) and mandibular (md) processes of branchial arch 1 in distinct streams originating from the posterior midbrain (pmb) and rhombomeres (r) 1–2. (B) Signalling interactions establishing homeobox gene expression in the mandibular arch. The upper panel depicts regulation of *Barx-1* and *Msx-1* at E10.5. Complementary domains of Fgf-8 (blue) in the proximal and Bmp-4 (red) in the distal regions of the mandible result in reciprocal expression of *Barx-1* (light blue) and *Msx-1* (brown) in the proximal and distal mesenchyme, respectively. The lower panel represents regulation of *Dlx-2* by Fgf-8 and Bmp-4 at E10.5. Bmp-4 (red) induces *Dlx-2* (green) in the distal epithelium via an intra-epithelial signal. Fgf-8 (blue) in the proximal epithelium induces *Dlx-2* in the underlying mesenchyme. In addition, Fgf-8 also inhibits *Dlx-2* in the epithelium of this region by an unknown signal that passes via the mesenchyme. Adapted from Tucker et al. ('98) and Thomas et al. (2000).

ectomesenchyme (Ferguson et al., 2000). Thus, ectomesenchyme of the maxillary and mandibular primordia behave fundamentally different to each other. This has been further confirmed by reciprocal transplantations of isolated ectomesenchyme from the molar regions of the mandible or maxilla. Analysis of marker genes in these transplants demonstrates that between E9.5 and E10.25, the ectomesenchyme of the mandible and maxilla does not take on the expression characteristics of the host; they appear to be intrinsically different in their responses to ectodermal signalling. These findings provide some basis for explaining fundamental differences in skeletal structures and subtle differences between dentitions in the mandible and maxilla, and the observed differences in maxillary and mandibular molar phenotype in *Dlx-1*  $-/-$  /*Dlx-2*  $-/-$  and *Activin- $\beta$ A* mutant mice (Qiu et al., '97; Ferguson et al., '98). However, how a seemingly homogenous population of CNCC, which migrate into the maxillary and mandibular primordia, acquire subtle differences in their ability to respond to instructive signals from the ectoderm is not fully understood (Ferguson et al., 2000).

### CONCLUSIONS

A molecular model of time-dependent dental pattern generation within CNCC that migrates into the first branchial arch, via early signalling from the overlying ectoderm, is now established. Fixation of this pattern information allows reciprocal signalling from ectomesenchyme to the ectodermal components of the tooth germ during the shape-changing events that leads to the firm establishment of crown and root morphology. Less is known about the mechanisms that regulate different molecular responses within ectomesenchyme derived from the mandible and maxilla. The analysis of how CNCC streams are distributed to discreet areas of the first arch, in particular, the tooth-forming regions and the relative contribution of local cell community signalling within these cell groups, will allow further dissection of the complex molecular processes that give rise to mammalian tooth shape.

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