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REVIEW

Cell Fate Determination During Tooth Development and Regeneration

Thimios A. Mitsiadis* and Daniel Graf

Teeth arise from sequential and reciprocal interactions between the oral epithelium and the underlying cranial neural crest-derived mesenchyme. Their formation involves a precisely orchestrated series of molecular and morphogenetic events, and gives us the opportunity to discover and understand the nature of the signals that direct cell fates and patterning. For that reason, it is important to elucidate how signaling factors work together in a defined number of cells to generate the diverse and precise patterned structures of the mature functional teeth. Over the last decade, substantial research efforts have been directed toward elucidating the molecular mechanisms that control cell fate decisions during tooth development. These efforts have contributed toward the increased knowledge on dental stem cells, and observation of the molecular similarities that exist between tooth development and regeneration. **Birth Defects Research (Part C) 87:199–211, 2009.** © 2009 Wiley-Liss, Inc.

Key words: tooth; incisor; cell fates; ameloblasts; odontoblasts; notch signaling; bone morphogenetic protein (BMP); craniofacial development; dental defects; islet1; Tbx1; Pitx1

INTRODUCTION

Mammalian tooth development proceeds through a series of well-defined morphological stages that necessitate sequential and reciprocal interactions between the epithelium and mesenchyme (see Fig. 1). The mesenchyme is derived from cranial neural crest cells that form a uniform pool of multipotent progenitors. These cells migrate from the dorsal part of the neural tube and subsequently acquire diverse cell fates to generate structures of unique morphology and function, such as bone, cartilage, smooth muscle, neurons, and teeth (Anderson, 1993; Weissman et al., 2001). In mice, the first sign of tooth development is seen as a thickening of the oral epithelium at embryonic day 10.5 (E10.5), which invaginates the underlying mesenchyme and progressively forms the tooth bud (E12.5–E13.5). The epithelium continues to grow and adopt the cap (E14.5–E15.5) and bell configurations (E16.5–E18.5). At the late bell stage (E18.5), the mesenchymal cells form the dental follicle and dental pulp. Pulp cells adjacent to the dental epithelium differentiate into odontoblasts and the epithelial cells next to dental pulp differentiate into ameloblasts (Bluteau et al., 2008). Odontoblasts are columnar postmitotic cells that form a layer in contact with the dentin. Odontoblastic processes are formed at their distal part, penetrate the dentin, and participate in the secretion of dentin matrix and minerals. The matrix is composed of collagens (90%) and noncollagenous proteins, such as dentin sialophosphoprotein and dentin matrix protein 1. The deposition of apatite minerals on this matrix gives rise to the mature calcified dentin. Enamel is secreted by ameloblasts along the dentinoenamel junction. Enamel is mainly composed of hydrophobic proteins, such as amelogenin, ameloblastin, enamelin, amelotin, tuftelin, and odontogenic ameloblast-associated proteins (Sire et al., 2007).

Tissue recombination experiments have shown that a molecular dialogue starts once the neural crest-derived cells contact the oral epithelium (Fig. 2). The same experiments have also demonstrated that the inductive capacity for mouse tooth formation resides in the epithelium until E12, after which it shifts to the condensing mesenchyme (Mina and Kollar, 1987). The importance of cranial neural crest-derived cells in odontogenesis has been shown in experiments where transplantation of mouse neural crest cells into chick embryos allowed growth of tooth germs (Mitsiadis et al.,

Thimios A. Mitsiadis and **Daniel Graf** are from the Institute of Oral Biology, ZZMK, Faculty of Medicine, University of Zurich, 8032 Zurich, Switzerland

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*Correspondence to: Thimios A. Mitsiadis, Institute of Oral Biology, ZZMK, Faculty of Medicine, University of Zurich, Plattenstrasse 11, 8032 Zurich, Switzerland. E-mail: thimios.mitsiadis@zzmk.uzh.ch

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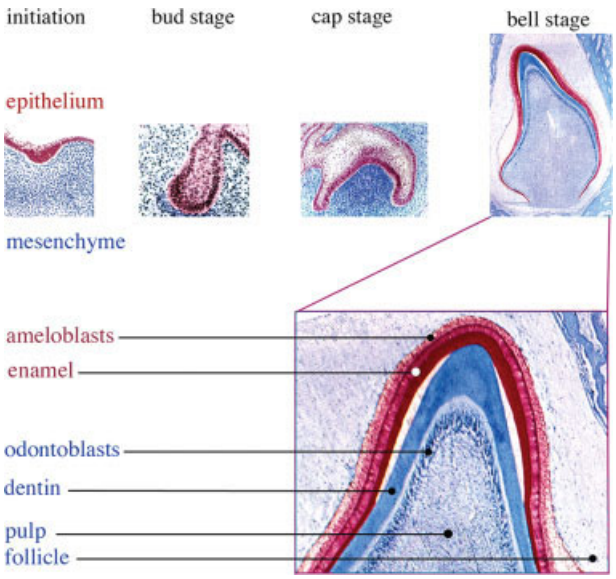

Figure 1. The different stages of human embryonic tooth development. In red the epithelium and its derivatives, in blue the mesenchyme and its derivatives. This figure was obtained from an article by Bluteau et al., 2008.

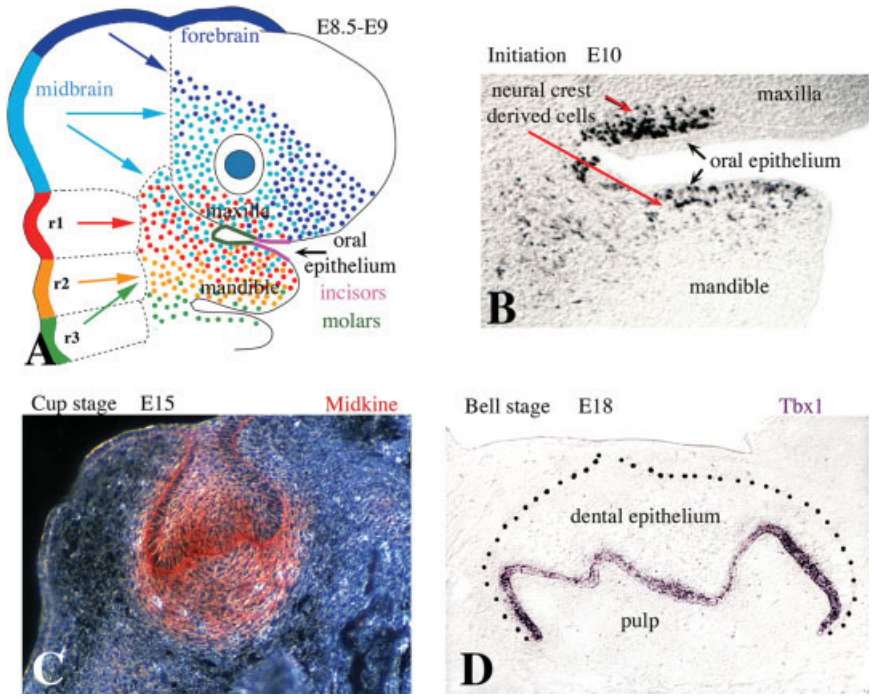

Figure 2. (**A**) Schematic representation of the migration of cranial neural crest cells toward the facial region and the oral cavity. (**B**) Section showing neural crest-derived cells (red arrows) in contact with the oral epithelium. (**C**) Section showing exclusive expression of the midkine protein in dental tissues (red color). (**D**) Section demonstrating expression of Tbx1 in cells of the inner dental epithelium (violet color). r1, rhombomere 1; r2, rhombomere 2; r3, rhombomere 3.

2003b, 2006). Molecular and cellular interactions control all steps of tooth formation by coordinating the diverse cellular processes, such as cell proliferation, apoptosis, cytoskeleton modifications, terminal differentiation, and extracellular matrix synthesis. The same signals are repetitively used during the different stages of odontogenesis. These signals emerge in various developmental contexts: for example, the same signal that activates proliferation in a cell type elsewhere will lead another cell type to differentiate and in a third cell type will trigger apoptosis. The result is determined by the developmental state of a cluster of cells and depends on various cellular targets, such as transcription factors and cytoskeleton proteins.

TIMING IN TOOTH DEVELOPMENT

These developmental signaling events must be precisely regulated. A signal that is produced at the wrong time will lead to inappropriate developmental responses. Cell division, apoptosis, cell differentiation, tissue growth and patterns, and organ formation require proper timing. A molecular timing mechanism exists, and its function is to specify cell fates in all tissues and organs of the developing embryo (Dorsky et al., 2000; Moss, 2007). Timing could be considered as the fourth dimension of embryonic development, but it is very difficult to observe in vertebrates because their developmental stages are not really disconnected.

Timing is thus an important issue in tooth development and its deregulation may have remarkable consequences with a range of effects, from the generation of pathologies (e.g., dental defects) to evolution. Changes in timing could be one of the most important reasons in the evolution of tooth morphology. Although differences in size and shape can be precisely measured in teeth of various species, the causal molecular mechanisms are complex and difficult to define.

DENTAL CELL DIVERSITY—CELL FATE SPECIFICATION

The generation of cell diversity is a central issue in developmental biology. This process requires numerous cellular and genetic interactions that result in the pro-

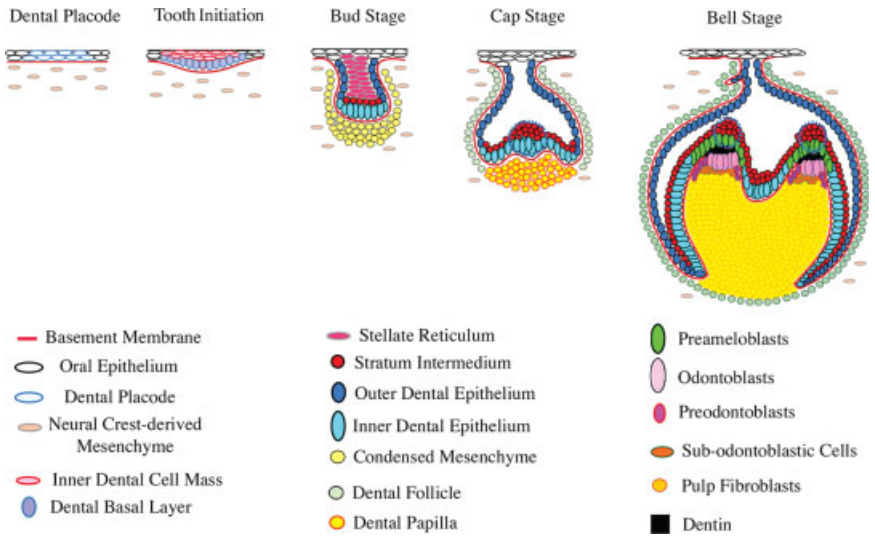

Figure 3. Schematic representation of the acquisition of diverse cell fates during tooth development.

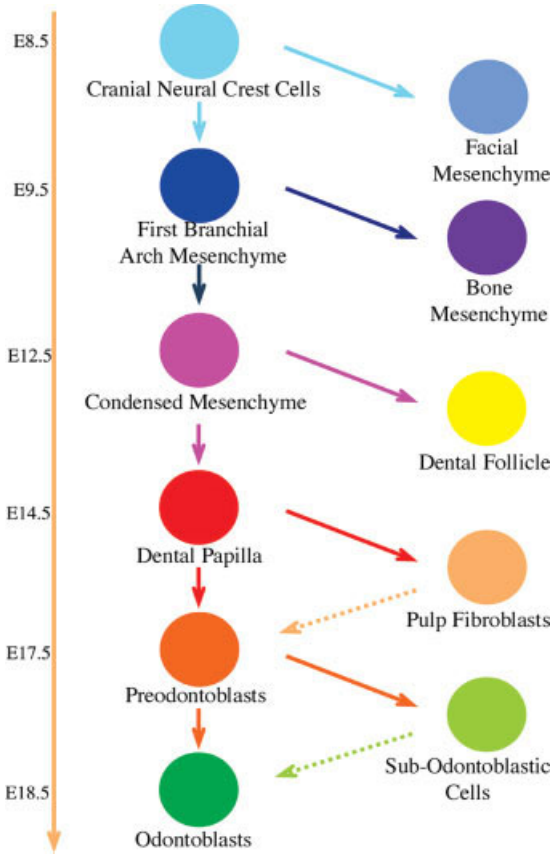

Figure 4. Mesenchymal cell fates during odontogenesis (from cranial neural crest cells to odontoblasts). Factors expressed during odontogenesis influence lineage commitment and instruct them to choose a particular fate, at the expense of others.

gressive restriction of cells to particular cell phenotypes. Technical progress in biology, during recent years, has allowed the analysis of cell lineage relationships in the developing teeth. These techniques include the genetic marking of cell fates (Trainor et al., 1999; Chai et al., 2000) and lineage tracers for microinjection of dental progenitor cells (Mitsiadis et al., 2008b; Diep et al., 2009). The results have shown that dental precursor cells cause a variety of cell types (Fig. 3). However, it is important to understand how the fate of uncommitted dental progenitor cells is influenced by factors in their local environment. Factors expressed in early stages of odontogenesis, such as bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), and Wnt molecules, may influence lineage commitment in two different ways: precursor cells are either instructed to choose a particular fate, at the expense of others, in response to a given signal, or choose their fate stochastically, and factors simply support their survival and/or proliferation. Growth factor concentrations vary during development and the response of the progenitors differs according to the concentration of a given factor (Lillien and Gulacsi, 2006). Tissue recombination and transplantation experiments have indicated that signals from the oral epithelium influence neural crest-derived mesenchyme cells to adopt a dental fate (Mina and Kollar, 1987; Lumsden, 1988; Mitsiadis et al., 2003b). Indeed, in many systems, developmental decisions involve one fate that is promoted by a specific signal and an alternative fate that is assumed in the absence of that signal (Kelly and Melton, 1995) (Fig. 4).

THE FATE OF DENTAL EPITHELIUM: GRADIENTS AND SHAPES

The fate of the presumptive dental epithelium is defined early during embryonic development (E8.5) by the bicoid-related transcription factor, Pitx2 (Mucchielli et al., 1997; Mitsiadis et al., 1998b). At more advanced stages, *Pitx2* expression becomes restricted to the epithelium of the tooth germs (Mucchielli et al., 1997). The pattern of the mammalian dentition is also defined early, before any

obvious sign of tooth development. Parallel independent genetic pathways control development of the various groups of teeth: incisors, canines, premolars, and molars. Dental diversity may be the result of prepatterned cranial neural crest cell populations that contribute to the formation of precise and specific tooth shapes. Alternatively, the morphological variety may reflect the different responses of the neural crest-derived cells that colonize the mandible and maxilla to signals originated by the oral epithelium. Thus, rodent dentition pattern must be determined by a prepatterning of either the oral epithelium into molar and incisor fields or the cranial neural crest-derived cells as incisor and molar populations.

It is widely accepted that the dental fields that define the number and shape of teeth in the oral cavity are established by signals originating from the oral epithelium. Their degree of concentration determines the arrangement and fate of the responding cells. Signals emanating from a localized source may form morphogenic gradients that provide positional information to populations of uncommitted cells to decide their developmental fate accordingly (Green, 2002). This mechanism leads to the formation of distinctive cell types and consequently to the generation of complex patterns and structures. Members of the transforming growth factor-β (TGFβ), hedgehog (Hh), and Wnt families function as morphogens (Charron and Tessier-Lavigne, 2007). In contrast, members of the FGF family have permissive rather than concentration-related effects.

Numerous signaling factors are involved in different stages of embryonic tooth development. For example, BMP2, BMP4, and Midkine (MK) regulate epithelial–mesenchymal interactions (Vainio et al., 1993; Kratochwil et al., 1996; Nadiri et al., 2004; Bluteau et al., 2008), FGF3, FGF4, FGF8, FGF9, and FGF10 are involved in cell proliferation and regulate expression of specific target genes (Bei and Maas, 1998; Kettunen et al., 1998, 2000). Wnt3, Wnt7b, Wnt10a, and Wnt10b are regulators of cell proliferation, migration, and differentiation during tooth initiation and morphogenesis (Dassule and McMahon, 1998), and sonic hedgehog (shh) contributes to tooth initiation and morphogenesis (Khan et al., 2007). From all these molecules, BMP4 and FGF8 constitute essential early oral epithelial signals that have a crucial role in activating specific homeobox genes in the underlying mesenchyme (Mitsiadis, 2001). It has been proposed that these two signals could control tooth patterning in rodents: BMP4 directs the shape of incisors and FGF8 the shape of molars (Mitsiadis and Smith, 2006). The mesenchyme of the developing incisors expresses a specific complement of genes (*Msx1*, *Msx2*), whereas the mesenchyme of the molars possesses a different complement of genes (*Dlx1*, *Dlx2*, *Barx1*). The specific complement of these transcription factors dictates the development of the tooth germs toward an incisorform or molariform shape (Cobourne and Mitsiadis, 2006). Based on the restricted expression domains of signaling molecules and homeobox genes in the cranial neural crest cell-derived mesenchyme of the maxilla and mandible, a ''cooperative genetic interaction'' model has been proposed (Mitsiadis and Smith, 2006).

Expression of *BMP4* in oral epithelium is required for the expression of *Msx1* and *Msx2* in the underlying mesenchyme. Msx1 has an important role in directing the development of incisor morphogenesis and forms part of few homeobox genes that provide the spatial information for dental patterning (Cobourne and Mitsiadis, 2006; Mitsiadis and Smith, 2006). On the other hand, FGF8 positively regulates the mesenchymal expression of *Barx1* and *Dlx2* that are essential molecules for molar morphogenesis (Cobourne and Mitsiadis, 2006). Previous studies have suggested that Barx1 is involved in determining the identity of neural crest-derived mesenchymal cells in both maxilla and mandible (Tissier-Seta et al., 1995; Mucchielli et al., 1997; Mitsiadis et al., 1998b). *Pax9* is also expressed in the mesenchyme that underlies the locations of the epithelial invaginations (Neubuser et al., 1997). The presence of all these transcription factors appears to be required for a transcriptional program responsible for the characteristic growth and morphology of teeth (Cobourne and Mitsiadis, 2006).

BMP Signaling Network

The reiterated requirement of BMP signaling indicates the continuous need to probe cellular and molecular interactions to direct appropriate tooth development. BMP molecules encompass a large subgroup of phylogenetically conserved signaling proteins belonging to the TGFβ superfamily. More than 20 BMP-like molecules have been identified in vertebrates and invertebrates. Based on the degree of amino acid sequence homology, they can be classified into several subgroups. Two important clusters are formed by BMP2/BMP4 and BMP5/BMP6/BMP7/BMP8. Although BMP molecules were originally identified as molecules that induce bone and cartilage formation, they exert a wide range of biological functions (e.g. cell differentiation, apoptosis) in a variety of cell types during embryonic development. In fact, BMP signaling plays a pivotal role in morphogenesis of most tissues and organs (Hogan, 1996; Zhao, 2003; Chen et al., 2004; Kishigami and Mishina, 2005).

BMP molecules signal through two different types of serine–threonine kinase receptors, termed BMPR type I and type II. Type I receptors encompass BMPRIA (Alk3), BMPRIB (Alk6), Activin receptor type IA (ActRIA or Alk2), and Alk1 (Koenig et al., 1994; ten Dijke et al., 1994b,c; Macias-Silva et al., 1998; David et al., 2007), whereas BMPRII, ActRII, and

ActRIIB are type II receptors (Kawabata et al., 1995; Rosenzweig et al., 1995; Yamashita et al., 1995). Individual BMP molecules have different affinities for the various type I receptors (ten Dijke et al., 1994a; Ebisawa et al., 1999), which are also influenced by the type II receptors (Yu et al., 2005). For this reason, the various BMP proteins are not redundant, although it is not well understood how they achieve their distinct activities in vivo.

The BMP-associated signaling cascades are divided into Smad-dependent and Smad-independent signaling. Binding of BMP molecules to a preformed type I/type II receptor heterodimer complex induces Smad-dependent signaling. Smad-independent signaling is induced when the BMP first binds to an isolated type I receptor, with subsequent recruitment of the type II receptor to the BMP/BMPRI complex (Nohe et al., 2002; Hassel et al., 2003; Hartung et al., 2006).

The fine-tuning of BMP signaling is in part achieved by interaction of BMP molecules with a variety of secreted proteins, mostly antagonists, which are located in the extracellular space. The importance of this extracellular regulation is illustrated by the severe and complex phenotypes observed in the absence of those antagonists (Zhao, 2003).

BMP antagonists can be classified into three subfamilies: the DAN family (cerberus, coco, gremlin, ectodin, etc.), twisted gastrulation, and the chordin and noggin subgroup (Avsian-Kretchmer and Hsueh, 2004). The different types of BMP antagonists can bind to BMP molecules in diverse ways. For example, noggin binds BMP7 in a clamp-like fashion and blocks binding to both the BMPRI and BMPRII epitopes (Groppe et al., 2002), whereas chordin binds synergistically with twisted gastrulation to form a ternary complex for efficient blocking (Graf et al., 2002; Yamamoto and Oelgeschlager, 2004). As the BMP network is very complex, our understanding of its involvement in tooth development is not well known and yet incomplete.

Functional involvement of BMP signaling in tooth development could be studied in gene deletion mutants. Conditional deletion of Alk3 (BMPR1A) in the epithelium arrests tooth development at the bud stage, thus indicating the importance of mesenchyme-derived BMP signals for the further development of tooth epithelium (Andl et al., 2004). This early arrest, however, obscures insight of Alk3 function in the development of later structures, such as the root, crown, enamel, or dentin. Bmp7-deficient mouse embryos often lack incisors, and occasionally molars (Zouvelou et al., 2009). In contrast, mutants deficient for the BMP antagonist, ectodin, exhibit supernumerary teeth in their oral cavity (Murashima-Suginami et al., 2007). Overexpression of noggin in the epithelium results in various phenotypic alterations including lack of mandibular molars, reduced number of maxillary molars, disrupted root size and pattern, and poorly mineralized enamel (Plikus et al., 2005).

These studies reveal the involvement of BMP signaling in the formation of the various dental structures during the different stages of odontogenesis. However, there is a clear lack of information concerning the roles of BMP signaling during the establishment of cusp shapes, root formation, and generation and maintenance of stem cells and/or progenitors in dental tissues. A promising approach to address these issues is offered by studies combining gene reporting with cell-specific gene deletion (Graf and Economides, 2008; Zouvelou et al., 2009).

Islet1

Islet1 is a transcriptional regulator that plays critical roles in the control of pattern formation and cell fate decisions of many various tissues (Laugwitz et al., 2008). *Islet1* is expressed only in the incisor field of the oral epithelium, before the initiation of tooth formation (Mitsiadis et al., 2003a). Expression of *Islet1* during odontogenesis is detected only in the epithelium of the incisors. *Islet1* expression in both proliferating and differentiating epithelial cells of the incisor indicates a potential role of Islet1 in the progression of progenitor cells from the dividing, undifferentiated state, to that of postmitotic ameloblasts. The induction of *Islet1* in epithelium is intrinsic and not induced by the underlying mesenchyme. Islet1 and Bmp4 appear to have a positive autoregulatory relationship (Mitsiadis et al., 2003a). Forced expression of *Islet1* in the molar field of the oral epithelium results in the loss of *Barx1* expression in mesenchyme, leading to inhibition of molar development (Mitsiadis et al., 2003a). Thus, Islet1 is part of the genetic program that defines territories associated with incisor formation in the oral epithelium.

THE FATE OF TOOTH NUMBER

The size of the dental fields in the oral epithelium, and thus the proportion of the number of teeth, is established at the very early stages of embryonic development. Ectodysplasin (Eda) signaling molecules have been shown to be involved in the determination of the dental fields (reviewed in Mikkola, 2008). Indeed, disruption of genes encoding for the Eda, Edar (Eda receptor), and Edaradd (an intracellular adaptor protein) proteins leads to mice with an irregular number of teeth. Increased Eda signaling has as a consequence the formation of supernumerary teeth. In contrast, loss of function of *Eda* or *Edar* quite often leads to a reduction in tooth number.

SIGNALS SPECIFYING DENTAL CELL FATES

Signaling molecules and transcription factors are involved in the determination of specific cell populations within dental tissues (Mitsiadis, 2001). During embryogenesis, a subpopulation of oral

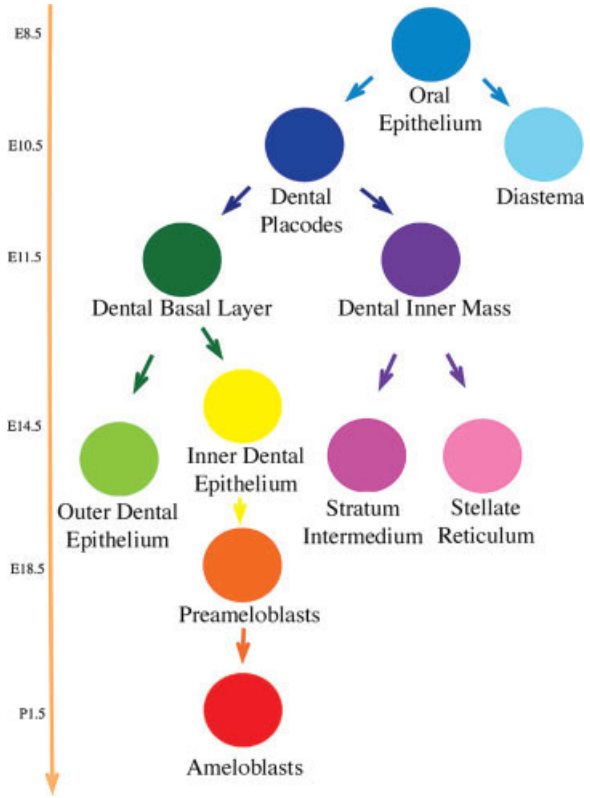

Figure 5. Epithelial cell fates during odontogenesis (from cells of the oral epithelium to ameloblasts).

epithelial cells acquires odontogenic potential and progressively forms a structure of four distinct cell populations (i.e., stratum intermedium, stellate reticulum, outer, and inner dental epithelial cells), known collectively as the enamel organ (see Fig. 5). DiI labeling showed that there is no cellular continuity between the different precursors that cause the four dental epithelial cell layers (Mitsiadis et al., 2008b). Inner dental epithelium contains precursor cells undergoing a precise developmental program that differentiate into cells forming tooth-specific hard tissues. Depending on their position (e.g., labial/lingual in incisors, crown/root in molars), these precursors cause either ameloblasts or epithelial remnant cells (epithelial rests of Malassez). The specification of these dental cell types involves genes with restricted expression patterns to one or another cell type during odontogenesis. Although several genes are differentially expressed in dental cell populations (Mitsiadis, 2001), they are unlikely to play a formative role in cell fate specification because of their relatively late onset of expression. Molecules of the Notch pathway and the Tbx1 transcription regulator constitute an exception.

Notch Signaling Pathway

Previous studies suggested that molecules of the Notch signaling pathway may play a role in specifying dental cell-type identity (Mitsiadis et al., 1995, 1997, 1998a, 2005). The Notch signaling pathway is known as a key player in cell fate determination (Greenwald, 1998; Artavanis-Tsakonas et al., 1999). Notch genes encode transmembrane receptors that participate in communication between neighboring cells. Four mammalian Notch receptors are known: Notch1, Notch2, Notch3, and Notch4. These receptors differ in the number of EGF-like repeats of the extracellular domain and the length of the intracellular domain, whereas the ankyrin repeats are most conserved (Maine et al., 1995; Kortschak et al., 2001). Notch receptors are activated by transmembrane ligands belonging to the Delta or Jagged (Serrate) families. These ligands are known as "DSL" from Delta (*Drosophila*), Serrate (*Drosophila*), and Lag-1 (*C. elegans*). The affinity of Notch receptors for different Notch ligands is controlled by glycosylation. Ligand binding dissociates the extracellular domain from the transmembrane subunit of the Notch receptor. An intracellular domain of the Notch receptor after cleavage translocates to the nucleus (Lewis, 1998), where it modulates gene expression by binding to a ubiquitous transcription factor, CBF-1 (RBP-JK) (Artavanis-Tsakonas et al., 1999). Deltex protein has been proposed to participate in the signaling mediated by Notch (Matsuno et al., 1998).

Notch signaling has diverse and multiple functions on cell differentiation, proliferation, and survival. Notch activation can affect every aspect of cell fate determination in a context-dependent manner. In invertebrates, Notch activation directs accurate cell fate choices by restricting differentiation toward alternative fates and permits the self-renewal and survival of multipotent cells (Artavanis-Tsakonas et al., 1999). Thus, Notch restricts differentiation toward a certain cell fate and permits differentiation toward a different cell fate by controlling the effects and timing of differentiation signals. Diverse cell types express the numerous Notch receptors and ligands in vertebrates, thus indicating that the Notch signaling pathway has a more complicated function. It is well established that Notch signaling plays an important role in the processes of both lateral specification inhibition (equivalent cells) and inductive signaling (different cell types).

Previous studies have shown that in the developing teeth, *Notch1* is expressed in stratum intermedium, whereas *Jagged2* and *Delta1* are expressed in the

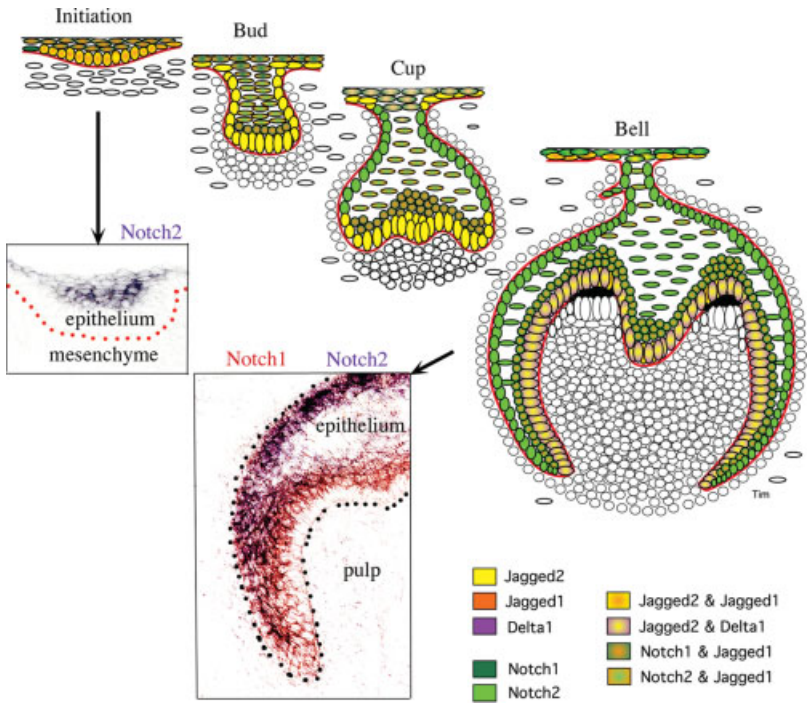

Figure 6. Notch signaling for the acquisition of the different epithelial fates in the developing teeth. The combinations of colors represent the combinations between Notch receptors and ligands in each cell layer. The photos in the boxes show expression of the Notch receptors in the various stages of odontogenesis.

adjacent cell layer of inner enamel epithelium, suggesting that the Jagged2 and Delta1 proteins may function as the ligands for the Notch1 protein during tooth development (Mitsiadis et al., 1995, 1997, 1998a, 2005). It has been shown that these ligands activate the Notch1 receptor in other mammalian cells (Lindsell et al., 1995).

It is possible that the determination of cell fates in the enamel organ occurs via inhibitory interactions between adjacent dental epithelial cells. During the first stages of tooth development (i.e., bud stage), the dental epithelium appears to constitute a developmental equivalence group of cells in which inner dental epithelium cells suppress differentiation in their immediate neighbors through lateral inhibition (Fig. 6). These interactions seem to be mediated through the Notch signaling pathway.

To influence developmental decisions, molecules of the Notch signaling pathway must obviously interact with other signaling pathways. Notch-dependent cell fate acquisition between nonequivalent dental precursor cells is influenced by extrinsic signals, such as BMP and FGF molecules. BMP and FGF molecules have opposite effects on the expression of Notch receptors and ligands in dental tissues (Mitsiadis et al., 1997, 1998a), indicating that cell fate choices during odontogenesis are under the concomitant control of the Notch and BMP/FGF signaling pathways. Regulation of the Notch pathway by these and/or other signaling molecules is essential for maintaining the correct balance among cell proliferation, differentiation, and apoptosis during embryonic tooth development. Notch-mediated lateral inhibition has a pivotal role in the establishment of the tooth morphology, as shown in *Jagged2* mutant mice, where the overall development and structure of their teeth is severely affected (Mitsiadis et al., 2005).

Tbx1

Numerous studies have shown that Tbx1 plays an important role in the specification of many different cell populations during embryonic development (Papaioannou and Silver, 1998; Naiche et al., 2005). Several clinical and genetic findings have shown that Tbx1 also plays a significant role for the early determination of epithelial cells to adopt the ameloblast fate. First, subjects afflicted by the DiGeorge Syndrome (DGS, a TBX1 dependent disorder) exhibit hypodontia and enamel defects (Borglum Jensen et al., 1983; Fukui et al., 2000). Second, hypoplastic incisors that lack enamel are observed in mice lacking the *Tbx1* gene (Jerome and Papaioannou, 2001; Caton et al., 2009).

DiI labeling experiments demonstrated that cells expressing *Tbx1* are the progenitors for cells of the inner dental epithelium (see Fig. 2). Expression of *Tbx1* in dental epithelium is activated/maintained by signals originated from the mesenchyme. Mesenchyme-derived FGF molecules (Kettunen et al., 2000; Harada et al., 2002) operate in a paracrine manner to affect both *Tbx1* expression and proliferation/maintenance of ameloblast progenitors in dental epithelium. *FGF3*−/− mice have defective enamel and compound *FGF3*−/− and *FGF10*+/− mutant mice have very thin or no enamel, supporting the idea that these genes control the proliferation of ameloblast precursors (Harada et al., 2002; Wang et al., 2007). FGF molecules form with Tbx1 a regulatory loop in teeth (Mitsiadis et al., 2008b). Malfunction in this loop results in a failure to form the necessary number of ameloblast precursors.

DEFINITION OF THE MAXILLA–MANDIBLE FATE

The organization of the maxillary and mandible dentition exhibits an intriguing mirror image patterning. Although these dentitions present striking histological and morphological similarities, this does not necessarily mean that identical cellular and genetic pathways operate in both. The maxillary teeth are formed by cells originated

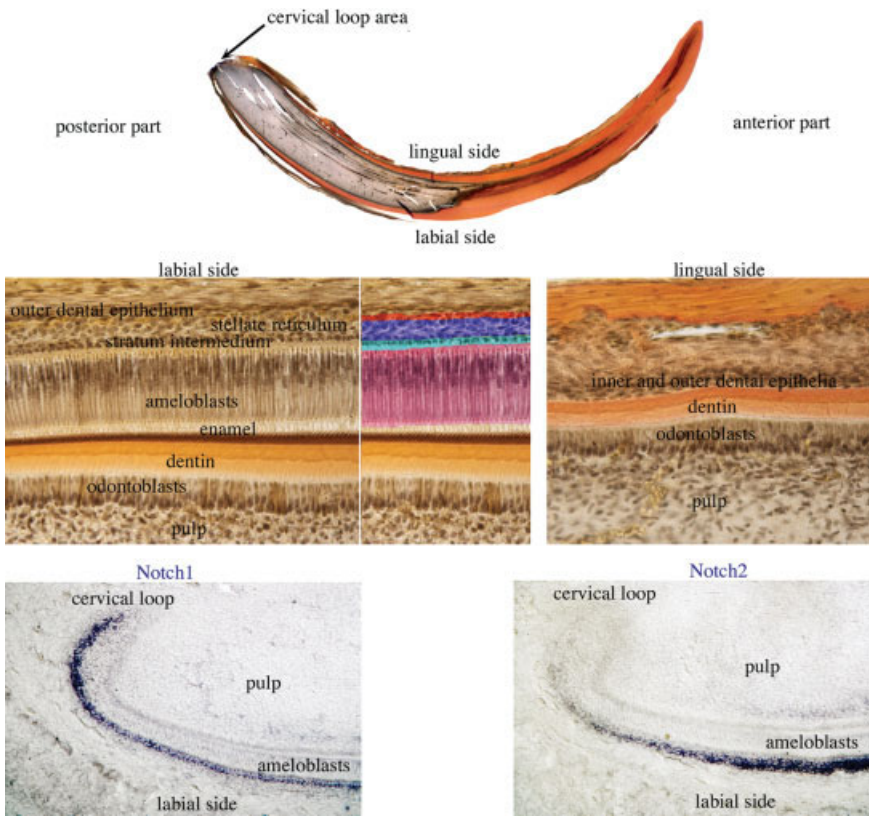

Figure 7. Sections through mouse incisors show the creation of different cell layers in epithelium (pseudocolors) and the expression of the Notch receptors in distinct cell populations (violet color).

from midbrain and forebrain neural crest, whereas the mandible teeth receive neural crest cells derived from hindbrain (rhombomeres 1 and 2) and midbrain (Osumi-Yamashita et al., 1994; Trainor and Tam, 1995; Imai et al., 1996; Cobourne and Mitsiadis, 2006) (Fig. 2). Mutations in transcription factors, such as Lef1, Msx1, Pitx2, and Pax9, result in developmental arrest of all teeth, indicating that these genes participate in processes common to development of all teeth (reviewed in Mitsiadis, 2001). In contrast, several transcription factors, such as Dlx, are differentially expressed in the maxillary and mandible processes (Zhao et al., 2000), thus indicating a basic genetic difference between upper and lower molar specification. The failure of maxillary molar development in double mutants that lack both *Dlx1* and *Dlx2* (*Dlx1/2*) genes suggests a specific role for these genes in regional specification of the odontogenic mesenchyme (Qiu et al., 1997).

Pitx1 is another gene controlling tooth identity in the mandible and maxilla. *Pitx1* is initially expressed in the proximal mesenchyme of the developing mandible where molars will develop. During odontogenesis, *Pitx1* is exclusively expressed in dental epithelium (Mitsiadis and Drouin, 2008). The expression of *Pitx1* in both mandibular mesenchyme and dental epithelium is negatively regulated by BMP4 (St Amand et al., 2000; Mitsiadis and Drouin, 2008). In *Pitx1* mutant mice, mandible molars are smaller than in wild-type mice, their number of cusps is reduced, and first and second molars fuse, most probably because of the delayed development of the second molars (Mitsiadis and Drouin, 2008). The morphological change in the *Pitx1*−/− mandible molars could be attributed to the shortening of the mandible and the consequent narrowing of the field where molars develop. Disruption in expansion and growth of the tooth primordia could reflect an altered patterning, resulting in a new shape and size of the teeth.

Although a part of the genetic code controlling tooth development (i.e., Fgf8, Bmp4, Shh, Msx1 and Msx2) is unaltered in *Pitx1*−/− mice (St Amand et al., 2000), *Tbx1* expression is completely suppressed in the epithelium of the mutant teeth (Mitsiadis and Drouin, 2008). Pitx1 seems to play a role in specifying a subpopulation of neural crest-derived mesenchymal cells in the mandible. In *Pitx1* mutants, *Barx1* expression is only downregulated in the mesenchyme of the mandibular molars (Mitsiadis and Drouin, 2008). Thus, the odontogenic specification of neural crest-derived mesenchymal cells may be partly controlled by Pitx1 in the mandible.

Activin is one of the earliest mesenchymal signals that confer positional information for the exact location where the teeth will develop. *Activin ßA*, activin receptor *ActR*[*IIA*+/−;*IIB*−/−], and *Smad2*+/− mice also exhibit a tooth phenotype, in which the incisors and mandibular molars are missing but the maxillary molars develop normally (Ferguson et al., 1998, 2001).

FATES OF DENTAL STEM CELLS

The rodent incisor has a cylindrical shape and differs from other teeth in that it is a continuously growing organ that erupts throughout the life of the animal (Mitsiadis et al., 2007). The incisor is formed by two distinct epithelia, the lingual and labial epithelia, which enclose the mesenchyme of the pulp (see Fig. 7). The lingual side of the incisor is composed of two epithelial cell layers, the inner and the outer dental epithelia. Cementum is deposited on this side of the incisor that is known as the root analogue of the molar. The labial side of the incisor is comprised of four epithelial cell layers: the inner and outer

dental epithelia that surround the core of stellate reticulum and stratum intermedium. The inner and outer enamel epithelia are in contact with the mesenchyme of the pulp and the follicle, respectively. Cells of the stratum intermedium are compressed, flattened cuboidal cells in contact with the inner dental epithelium. Enamel is deposited on the labial side of the incisor, which is thus analogous to the crown of a molar. The cervical loop is located at the posterior end of the epithelium and consists of a core of undifferentiated cells surrounded by the inner and outer dental epithelia. Cells of the core of this area proliferate and generate the transit amplifying progenitor cells, which then differentiate into all of the cells of the incisor, including the terminally differentiated ameloblasts (Mitsiadis et al., 2007; Bluteau et al., 2008).

Cells responsible for the continual growth of the incisor are located in the cervical loop, suggesting that this area is the putative stem cell niche (Mitsiadis et al., 2007). Cell tracking experiments have demonstrated that the cells from the cervical loop are maintained in this area, whereas others migrate toward the anterior part of the incisor where they differentiate into ameloblasts (Harada et al., 1999). A characteristic feature of stem cells is their slow cycling nature. BrdU incorporation studies have demonstrated that the cells of the cervical loop region are cycling slowly. Short periods of BrdU incubation have shown that the cells of the inner dental epithelium are highly proliferative and cycling very quickly. In contrast, other cells of the cervical loop are labeled only very sparsely and divide slowly. Pulse-chase experiments have demonstrated that BrdU labeling still remains in cells located in the internal part of the cervical loop epithelium. These cells divide slowly, remain in the cervical loop, and maintain the stem cell population through self-renewal. Cells originated from the cervical loop region exhibit a clonal potential and have the ability to differentiate into cells expressing markers of the inner dental epithelium and ameloblasts, such as the low-affinity neurotrophin receptor, P75NTFR, and amelogenin.

In incisors, Notch receptors and ligands are expressed in adjacent cell populations: *Notch1* is expressed in the stratum intermedium, whereas *Delta1*, *Jagged1*, and *Jagged2* are expressed in cells of the inner dental epithelium and ameloblasts (Mitsiadis et al., 1998a, 2005, 2007; Harada et al., 1999). Notch receptors are also expressed in the cells of the cervical loop area, but their expression remains absent in cells of the inner dental epithelium and ameloblasts (see Fig. 7). Notch2 and lunatic fringe (Lnf), a modulator of Notch signaling, have been implicated in the rotation of the incisor that determines the lingual/labial asymmetry (Mucchielli and Mitsiadis, 2000; Pouyet and Mitsiadis, 2000). These patterns of expression indicate that the Notch signaling plays a role in the maintenance and determination of the dental stem cell fates.

FGF molecules, such as FGF3 and FGF10, are important mesenchymal signals involved in the maintenance of epithelial cell populations within the cervical loop (Harada et al., 2002). *FGF10* is expressed in the mesenchyme that surrounds the cervical loop epithelium and *FGF3* expression is restricted to the mesenchyme that underlies the inner dental epithelium. FGF10 seems to be involved in the creation of the adult stem cell compartment in the cervical loop region. Indeed, incisors from the *FGF10* null mice are smaller and the cervical loop is missing. The reduced size of the mutant incisor is due to the lack of the stem cell compartment in the cervical loop, rather than an overall decrease of cell proliferation in the incisor. FGF10 has been shown to prevent apoptosis in the cervical loop epithelium and induces differentiation of progenitor cells into cells of the stratum intermedium. Bead implantation experiments have shown that FGF10 stimulates *Lnf* expression, thereby modulating Notch signaling in the cervical loop region (Harada et al., 2002). These findings suggest interactions between the FGF and Notch signaling pathways for the maintenance of stem cells of the cervical loop in an undifferentiated state (reviewed in Mitsiadis et al., 2007).

STEM CELL FATES DURING TOOTH REPAIR AND REGENERATION

Stem cells play a critical role in tissue homeostasis and repair throughout life. Their fate between self-renewal and differentiation is regulated by both cell intrinsic determinants and signals from a specialized microenvironment (Moore and Lemischka, 2006). Physical interactions and molecular cross talk with stem cells, as well as the orientation of the cleavage plane during their mitosis, determine the fate of these cells. Molecules of the Wnt, Notch, and BMP pathways have been shown to control the balance between symmetric and asymmetric division of stem cells and related cell fate specification outcomes (Betschinger and Knoblich, 2004; Lechler and Fuchs, 2005). A deregulation in the control of these events could lead to an abnormal tissue formation and a reduced tissue repair.

The growing interest in the molecular regulation of stem cells arises from the potential to influence their fate and consequently their functions during tissue repair and/or regeneration. The reparative mechanisms that operate following dental injuries involve a series of highly conserved processes that share genetic programs that occur throughout embryogenesis (reviewed in Mitsiadis and Rahiotis, 2004). In a severe wound, necrotic odontoblasts are replaced through a complex process by cells having stem cell properties, to differentiate into a new generation of odontoblasts that produce a novel dentin matrix, called reparative dentin (Mitsiadis and Rahiotis, 2004; Mitsiadis et al., 2008a).

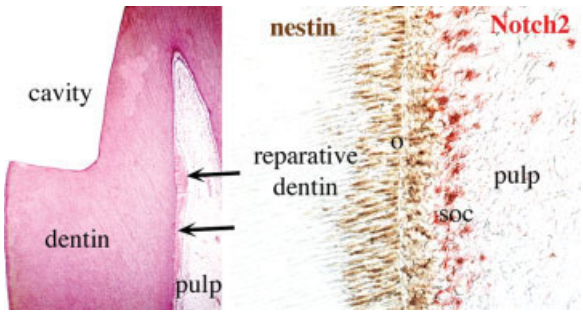

Figure 8. Sections of an injured area of the tooth show the formation of a reparative dentin (arrows), and reexpression of nestin (brown color) in the newly formed odontoblasts (o) and Notch2 (red color) in cells of the subodontoblastic layer (soc).

Signaling molecules that are expressed by pulp cells play a role in both tooth homeostasis and repair (reviewed in Smith and Lesot, 2001). Signals present at the injury site may act both as mitogens or chemotactic factors for pulp cells and initiate the healing process. Notch, nestin, and cadherins are also involved in the dynamic processes triggered by pulp injury and contribute to the signaling cascade by coordinating cell fate decisions, proliferative, migratory, and differentiation activities (Mitsiadis et al., 1999; Mitsiadis and Rahiotis, 2004). In injured teeth, Notch receptors are expressed by progenitors and undifferentiated cells of the subodontoblastic layer, but not by newly formed odontoblasts (see Fig. 8). Notch signaling reactivation during pulp healing might enhance survival of uncommitted precursors, while preserving multilineage potential. Notch expression is activated in cells close to the injury site, as well as in cells located at the apex of the roots, suggesting that these sites represent important pools of cells from which different cell types could derive after injury (Mitsiadis et al., 1999). Bone marrow stem cells express Notch receptors (Calvi et al., 2003), suggesting that the Notch-positive cells of the pulp have at least some stem cell properties. Activation of the Notch molecules in endothelial cells after injury may reflect another pool of stem cells (Lovschall et al., 2007). Activation of Notch signaling during tooth regeneration may ensure a continuous balance between odontoblasts and dental pulp progenitor cells. Aggregates of Notch-positive cell populations could be used for transplantation in the injured teeth. Local transplantation of these cells for therapeutic applications may permit a more extensive reconstruction than in those injuries that would spontaneously heal.

CONCLUSIONS

To understand the control of cell fate decisions, cytodifferentiation, and morphogenesis is a challenge that necessitates a thorough elucidation of the cellular and molecular events involved in initiation, development, repair, and regeneration of teeth. Understanding when and how signaling molecules control cell fate outcomes will provide new tools with which to direct the differentiation of cells into particular cell types. The identification of dental epithelial and mesenchymal stem cells and the knowledge of molecules involved in their fate determination is a considerable accomplishment. The engineering of tridimensional scaffolds with a composition and shape more or less similar to that of the teeth to reconstruct and the addition of signaling molecules might facilitate the transplantation and the differentiation of stem cells toward the desired fate. This scientific knowledge is likely to instruct development of novel therapies in the near future. However, the main challenge in cell therapy is to find a compromise between the benefits to the patients, ethical issues, and state regulations.

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REFERENCES

Anderson DJ. 1993. Molecular control of cell fate in the neural crest: the sympathoadrenal lineage. Annu Rev Neurosci 16:129–158.

Andl T, Ahn K, Kairo A, et al. 2004. Epithelial Bmpr1a regulates differentiation and proliferation in postnatal hair follicles and is essential for tooth development. Development 131: 2257–2268.

Artavanis-Tsakonas S, Rand MD, Lake RJ. 1999. Notch signaling: cell fate control and signal integration in development. Science 284:770–776.

Avsian-Kretchmer O, Hsueh AJ. 2004. Comparative genomic analysis of the eight-membered ring cystine knot-containing bone morphogenetic protein antagonists. Mol Endocrinol 18: 1–12.

Bei M, Maas R. 1998. FGFs and BMP4 induce both Msx1-independent and Msx1-dependent signaling pathways in early tooth development. Development 125:4325–4333.

Betschinger J, Knoblich JA. 2004. Dare to be different: asymmetric cell division in *Drosophila*, *C. elegans* and vertebrates. Curr Biol 14:R674–R685.

Bluteau G, Luder HU, De Bari C, Mitsiadis TA. 2008. Stem cells for tooth engineering. Eur Cell Mater 16:1–9.

Borglum Jensen S, Jacobsen P, Rotne L, et al. 1983. Oral findings in DiGeorge syndrome. Int J Oral Surg 12:250–254.

Calvi LM, Adams GB, Weibrecht KW, et al. 2003. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature 425:841–846.

Caton J, Luder HU, Zoupa M, et al. 2009. Enamel-free teeth: Tbx1 deletion affects amelogenesis in rodent incisors. Dev Biol 328:493–505.

Chai Y, Jiang X, Ito Y, et al. 2000. Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. Development 127: 1671–1679.

Charron F, Tessier-Lavigne M. 2007. The Hedgehog, TGF-beta/BMP and Wnt families of morphogens in axon guidance. Adv Exp Med Biol 621:116–133.

Chen D, Zhao M, Mundy GR. 2004. Bone morphogenetic proteins. Growth Factors 22:233–241.

Cobourne MT, Mitsiadis T. 2006. Neural crest cells and patterning of the

mammalian dentition. J Exp Zoolog B Mol Dev Evol 306:251–260.
Dassule HR, McMahon AP. 1998. Analysis of epithelial-mesenchymal interactions in the initial morphogenesis of the mammalian tooth. Dev Biol 202:215–227.
David L, Mallet C, Mazerbourg S, et al. 2007. Identification of BMP9 and BMP10 as functional activators of the orphan activin receptor-like kinase 1 (ALK1) in endothelial cells. Blood 109:1953–1961.
Diep L, Matalova E, Mitsiadis TA, Tucker AS. 2009. Contribution of the tooth bud mesenchyme to alveolar bone. J Exp Zoolog B Mol Dev Evol 312:510–517.
Dorsky RI, Moon RT, Raible DW. 2000. Environmental signals and cell fate specification in premigratory neural crest. Bioessays 22:708–716.
Ebisawa T, Tada K, Kitajima I, et al. 1999. Characterization of bone morphogenetic protein-6 signaling pathways in osteoblast differentiation. J Cell Sci 112 (Part 20):3519–3527.
Ferguson CA, Tucker AS, Christensen L, et al. 1998. Activin is an essential early mesenchymal signal in tooth development that is required for patterning of the murine dentition. Genes Dev 12:2636–2649.
Ferguson CA, Tucker AS, Heikinheimo K, et al. 2001. The role of effectors of the activin signaling pathway, activin receptors IIA and IIB, and Smad2, in patterning of tooth development. Development 128:4605–4613.
Fukui N, Amano A, Akiyama S, et al. 2000. Oral findings in DiGeorge syndrome: clinical features and histologic study of primary teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 89:208–215.
Graf D, Economides AN. 2008. Dissection of BMP signaling using genome engineering tools. In: Vukicevic S, Sampath KT, editors. Bone morphogenetic proteins: from local to systemic therapeutics. Basel/Switzerland: Birkhauser. pp. 115–139.
Graf D, Nethisinghe S, Palmer DB, et al. 2002. The developmentally regulated expression of twisted gastrulation reveals a role for bone morphogenetic proteins in the control of T cell development. J Exp Med 196: 163–171.
Green J. 2002. Morphogen gradients, positional information, and Xenopus: interplay of theory and experiment. Dev Dyn 225:392–408.
Greenwald I. 1998. LIN-12/Notch signaling: lessons from worms and flies. Genes Dev 12:1751–1762.
Groppe J, Greenwald J, Wiater E, et al. 2002. Structural basis of BMP signaling inhibition by the cystine knot protein Noggin. Nature 420:636–642.
Harada H, Kettunen P, Jung HS, et al. 1999. Localization of putative stem cells in dental epithelium and their association with Notch and FGF signaling. J Cell Biol 147:105–120.
Harada H, Toyono T, Toyoshima K, et al. 2002. FGF10 maintains stem cell compartment in developing mouse incisors. Development 129: 1533–1541.
Hartung A, Bitton-Worms K, Rechtman MM, et al. 2006. Different routes of bone morphogenic protein (BMP) receptor endocytosis influence BMP signaling. Mol Cell Biol 26:7791–7805.
Hassel S, Schmitt S, Hartung A, et al. 2003. Initiation of Smad-dependent and Smad-independent signaling via distinct BMP-receptor complexes. J Bone Joint Surg Am 85 (Suppl 3): 44–51.
Hogan BL. 1996. Bone morphogenetic proteins: multifunctional regulators of vertebrate development. Genes Dev 10:1580–1594.
Imai H, Osumi-Yamashita N, Ninomiya Y, Eto K. 1996. Contribution of early-emigrating midbrain crest cells to the dental mesenchyme of mandibular molar teeth in rat embryos. Dev Biol 176:151–165.
Jerome LA, Papaioannou VE. 2001. DiGeorge syndrome phenotype in mice mutant for the T-box gene, Tbx1. Nat Genet 27:286–291.
Kawabata M, Chytil A, Moses HL. 1995. Cloning of a novel type II serine/threonine kinase receptor through interaction with the type I transforming growth factor-beta receptor. J Biol Chem 270:5625–5630.
Kelly OG, Melton DA. 1995. Induction and patterning of the vertebrate nervous system. Trends Genet 11: 273–278.
Kettunen P, Karavanova I, Thesleff I. 1998. 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 22:374–385.
Kettunen P, Laurikkala J, Itaranta P, et al. 2000. Associations of FGF-3 and FGF-10 with signaling networks regulating tooth morphogenesis. Dev Dyn 219:322–332.
Khan M, Seppala M, Zoupa M, Cobourne MT. 2007. Hedgehog pathway gene expression during early development of the molar tooth root in the mouse. Gene Expr Patterns 7: 239–243.
Kishigami S, Mishina Y. 2005. BMP signaling and early embryonic patterning. Cytokine Growth Factor Rev 16: 265–278.
Koenig BB, Cook JS, Wolsing DH, et al. 1994. Characterization and cloning of a receptor for BMP-2 and BMP-4 from NIH 3T3 cells. Mol Cell Biol 14:5961–5974.
Kortschak RD, Tamme R, Lardelli M. 2001. Evolutionary analysis of vertebrate Notch genes. Dev Genes Evol 211:350–354.
Kratochwil K, Dull M, Farinas I, et al. 1996. Lef1 expression is activated by BMP-4 and regulates inductive tissue interactions in tooth and hair development. Genes Dev 10:1382–1394.
Laugwitz KL, Moretti A, Caron L, et al. 2008. Islet1 cardiovascular progenitors: a single source for heart lineages? Development 135:193–205.
Lechler T, Fuchs E. 2005. Asymmetric cell divisions promote stratification and differentiation of mammalian skin. Nature 437:275–280.
Lewis J. 1998. Notch signaling and the control of cell fate choices in vertebrates. Semin Cell Dev Biol 9:583–589.
Lillien L, Gulacsi A. 2006. Environmental signals elicit multiple responses in dorsal telencephalic progenitors by threshold-dependent mechanisms. Cereb Cortex 16(Suppl 1):i74–i81.
Lindsell CE, Shawber CJ, Boulter J, Weinmaster G. 1995. Jagged: a mammalian ligand that activates Notch1. Cell 80:909–917.
Lovschall H, Mitsiadis TA, Poulsen K, et al. 2007. Coexpression of Notch3 and Rgs5 in the pericyte-vascular smooth muscle cell axis in response to pulp injury. Int J Dev Biol 51:715–721.
Lumsden AG. 1988. Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. Development 103(Suppl):155–169.
Macias-Silva M, Hoodless PA, Tang SJ, et al. 1998. Specific activation of Smad1 signaling pathways by the BMP7 type I receptor, ALK2. J Biol Chem 273:25628–25636.
Maine EM, Lissemore JL, Starmer WT. 1995. A phylogenetic analysis of vertebrate and invertebrate Notch-related genes. Mol Phylogenet Evol 4:139–149.
Matsuno K, Eastman D, Mitsiades T, et al. 1998. Human deltex is a conserved regulator of Notch signaling. Nat Genet 19:74–78.
Mikkola ML. 2008. TNF superfamily in skin appendage development. Cytokine Growth Factor Rev 19:219–230.
Mina M, Kollar EJ. 1987. The induction of odontogenesis in non-dental mesenchyme combined with early murine mandibular arch epithelium. Arch Oral Biol 32:123–127.
Mitsiadis T. 2001. Bases moléculaires du développement dentaire. In: Piette E, Goldberg M, editors. La dent normale et pathologique. De Boeck-Université Press: Brussels. pp. 19–38.
Mitsiadis TA, Drouin J. 2008. Deletion of the Pitx1 genomic locus affects mandibular tooth morphogenesis and expression of the Barx1 and Tbx1 genes. Dev Biol 313:887–896.
Mitsiadis TA, Rahiotis C. 2004. Parallels between tooth development and repair: conserved molecular mechanisms following carious and dental injury. J Dent Res 83:896–902.

Mitsiadis TA, Smith MM. 2006. How do genes make teeth to order through development? J Exp Zoolog B Mol Dev Evol 306:177–182.

Mitsiadis TA, Lardelli M, Lendahl U, Thesleff I. 1995. Expression of Notch 1, 2, and 3 is regulated by epithelial-mesenchymal interactions and retinoic acid in the developing mouse tooth and associated with determination of ameloblast cell fate. J Cell Biol 130:407–418.

Mitsiadis TA, Henrique D, Thesleff I, Lendahl U. 1997. Mouse Serrate-1 (Jagged-1): expression in the developing tooth is regulated by epithelial-mesenchymal interactions and fibroblast growth factor-4. Development 124:1473–1483.

Mitsiadis TA, Hirsinger E, Lendahl U, Goridis C. 1998a. Delta-notch signaling in odontogenesis: correlation with cytodifferentiation and evidence for feedback regulation. Dev Biol 204:420–431.

Mitsiadis TA, Mucchielli ML, Raffo S, et al. 1998b. Expression of the transcription factors Otlx2, Barx1 and Sox9 during mouse odontogenesis. Eur J Oral Sci 106 (Suppl 1):112–116.

Mitsiadis TA, Fried K, Goridis C. 1999. Reactivation of Delta-Notch signaling after injury: complementary expression patterns of ligand and receptor in dental pulp. Exp Cell Res 246: 312–318.

Mitsiadis TA, Angeli I, James C, et al. 2003a. Role of Islet1 in the patterning of murine dentition. Development 130:4451–4460.

Mitsiadis TA, Cheraud Y, Sharpe P, Fontaine-Perus J. 2003b. Development of teeth in chick embryos after mouse neural crest transplantations. Proc Natl Acad Sci USA 100:6541–6545.

Mitsiadis TA, Regaudiat L, Gridley T. 2005. Role of the Notch signaling pathway in tooth morphogenesis. Arch Oral Biol 50:137–140.

Mitsiadis TA, Caton J, Cobourne M. 2006. Waking-up the sleeping beauty: recovery of the ancestral bird odontogenic program. J Exp Zoolog B Mol Dev Evol 306:227–233.

Mitsiadis TA, Barrandon O, Rochat A, et al. 2007. Stem cell niches in mammals. Exp Cell Res 313:3377–3385.

Mitsiadis TA, De Bari C, About I. 2008a. Apoptosis in developmental and repair-related human tooth remodeling: a view from the inside. Exp Cell Res 314:869–877.

Mitsiadis TA, Tucker AS, De Bari C, et al. 2008b. A regulatory relationship between Tbx1 and FGF signaling during tooth morphogenesis and ameloblast lineage determination. Dev Biol 320:39–48.

Moore KA, Lemischka IR. 2006. Stem cells and their niches. Science 311: 1880–1885.

Moss EG. 2007. Heterochronic genes and the nature of developmental time. Curr Biol 17:R425–R434.

Mucchielli ML, Mitsiadis TA. 2000. Correlation of asymmetric Notch2 expression and mouse incisor rotation. Mech Dev 91:379–382.

Mucchielli ML, Mitsiadis TA, Raffo S, et al. 1997. Mouse Otlx2/RIEG expression in the odontogenic epithelium precedes tooth initiation and requires mesenchyme-derived signals for its maintenance. Dev Biol 189:275–284.

Murashima-Suginami A, Takahashi K, Kawabata T, et al. 2007. Rudiment incisors survive and erupt as supernumerary teeth as a result of USAG-1 abrogation. Biochem Biophys Res Commun 359:549–555.

Nadiri A, Kuchler-Bopp S, Haikel Y, Lesot H. 2004. Immunolocalization of BMP-2/-4, FGF-4, and WNT10b in the developing mouse first lower molar. J Histochem Cytochem 52: 103–112.

Naiche LA, Harrelson Z, Kelly RG, Papaioannou VE. 2005. T-box genes in vertebrate development. Annu Rev Genet 39:219–239.

Neubuser A, Peters H, Balling R, Martin GR. 1997. Antagonistic interactions between FGF and BMP signaling pathways: a mechanism for positioning the sites of tooth formation. Cell 90:247–255.

Nohe A, Hassel S, Ehrlich M, et al. 2002. The mode of bone morphogenetic protein (BMP) receptor oligomerization determines different BMP-2 signaling pathways. J Biol Chem 277:5330–5338.

Osumi-Yamashita N, Ninomiya Y, Doi H, Eto K. 1994. The contribution of both forebrain and midbrain crest cells to the mesenchyme in the frontonasal mass of mouse embryos. Dev Biol 164:409–419.

Papaioannou VE, Silver LM. 1998. The T-box gene family. Bioessays 20:9–19.

Plikus MV, Zeichner-David M, Mayer JA, et al. 2005. Morphoregulation of teeth: modulating the number, size, shape and differentiation by tuning Bmp activity. Evol Dev 7:440–457.

Pouyet L, Mitsiadis TA. 2000. Dynamic Lunatic fringe expression is correlated with boundaries formation in developing mouse teeth. Mech Dev 91:399–402.

Qiu M, Bulfone A, Ghattas I, et al. 1997. Role of the Dlx homeobox genes in proximodistal patterning of the branchial arches: mutations of Dlx-1, Dlx-2, and Dlx-1 and -2 alter morphogenesis of proximal skeletal and soft tissue structures derived from the first and second arches. Dev Biol 185:165–184.

Rosenzweig BL, Imamura T, Okadome T, et al. 1995. Cloning and characterization of a human type II receptor for bone morphogenetic proteins. Proc Natl Acad Sci USA 92:7632–7636.

Sire JY, Davit-Beal T, Delgado S, Gu X. 2007. The origin and evolution of enamel mineralization genes. Cells Tissues Organs 186:25–48.

Smith AJ, Lesot H. 2001. Induction and regulation of crown dentinogenesis: embryonic events as a template for dental tissue repair? Crit Rev Oral Biol Med 12:425–437.

St Amand TR, Zhang Y, Semina EV, et al. 2000. Antagonistic signals between BMP4 and FGF8 define the expression of Pitx1 and Pitx2 in mouse tooth-forming anlage. Dev Biol 217:323–332.

ten Dijke P, Franzen P, Yamashita H, et al. 1994a. Serine/threonine kinase receptors. Prog Growth Factor Res 5:55–72.

ten Dijke P, Yamashita H, Ichijo H, et al. 1994b. Characterization of type I receptors for transforming growth factor-beta and activin. Science 264:101–104.

ten Dijke P, Yamashita H, Sampath TK, et al. 1994c. Identification of type I receptors for osteogenic protein-1 and bone morphogenetic protein-4. J Biol Chem 269:16985–16988.

Tissier-Seta JP, Mucchielli ML, Mark M, et al. 1995. Barx1, a new mouse homeodomain transcription factor expressed in cranio-facial ectomesenchyme and the stomach. Mech Dev 51:3–15.

Trainor PA, Tam PP. 1995. Cranial paraxial mesoderm and neural crest cells of the mouse embryo: co-distribution in the craniofacial mesenchyme but distinct segregation in branchial arches. Development 121: 2569–2582.

Trainor PA, Zhou SX, Parameswaran M, et al. 1999. Application of lacZ transgenic mice to cell lineage studies. Methods Mol Biol 97:183–200.

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

Wang XP, Suomalainen M, Felszeghy S, et al. 2007. An integrated gene regulatory network controls stem cell proliferation in teeth. PLoS Biol 5: e159.

Weissman IL, Anderson DJ, Gage F. 2001. Stem and progenitor cells: origins, phenotypes, lineage commitments, and transdifferentiations. Annu Rev Cell Dev Biol 17:387–403.

Yamamoto Y, Oelgeschlager M. 2004. Regulation of bone morphogenetic proteins in early embryonic development. Naturwissenschaften 91:519–534.

Yamashita H, ten Dijke P, Huylebroeck D, et al. 1995. Osteogenic protein-1 binds to activin type II receptors and induces certain activin-like effects. J Cell Biol 130:217–226.

Yu PB, Beppu H, Kawai N, et al. 2005. Bone morphogenetic protein (BMP) type II receptor deletion reveals BMP ligand-specific gain of signaling in pulmonary artery smooth muscle cells. J Biol Chem 280: 24443–24450.

Zhao GQ. 2003. Consequences of knocking out BMP signaling in the mouse. Genesis 35:43–56.

Zhao Z, Stock D, Buchanan A, Weiss K. 2000. Expression of Dlx genes during the development of the murine dentition. Dev Genes Evol 210: 270–275.

Zouvelou V, Luder HU, Mitsiadis TA, Graf D. 2009. Deletion of BMP7 affects the development of bones, teeth, and other ectodermal appendages of the orofacial complex. J Exp Zoolog B Mol Dev Evol 312: 361–374.