Odontoblast differentiation

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Introduction

Histological and cytological organization as well as functional aspects confer specificity to the odontoblasts. These post-mitotic polarized cells, which should be named odontocytes, are aligned in a single layer at the periphery of the dental pulp, extending one or more cytoplasmic process(es) into the predentin-dentin. Odontoblasts are connected by junctional complexes, however these probably do not completely seal the inter-odontoblastic spaces. Odontoblasts also undergo age changes.

Smith and Hall (1980) recently discussed the evolutionary origin of vertebrate skeletogenic and odontogenic tissues and strongly supported the phylogenetic emergence of distinct osteogenic and dentinogenic lineages. They postulated that the dentin of the earliest agnathan vertebrates was provided by cells of neural crest origin and stressed the neural crest origin of cranial skeletogenic and odontogenic tissues in extant vertebrates. The developmental history of odontoblasts implies several successive steps, including acquisition of “tooth competence” by neural crest (progenitor) cells, their patterning in the developing jaws, commitment and tooth specific spatial distribution of a finite number of post-mitotic odontoblasts able to overtly differentiate.

In this review we shall summarize some facts and questions concerning preodontoblasts and then analyze with more detail the epigenesis of terminal differentiation.

From neural crest to the initial bell stage: the black box

The embryonic origin of dental cells is well established in amphibians and mouse (see Smith and Hall, 1990, for review). Neural crest cells from midbrain and rostral hindbrain will occupy the first visceral arch and provide dental papilla cells (including odontoblasts), cells of the periodontium and some bone cells (bone of attachment). The oral ectoderm gives rise to the enamel organ from which ameloblasts will emerge. The complete, heterodont, mammalian dentition comprises incisors, canines and molars. Species-specific variations exist. For example adult rodents miss the canines (see P.M. Butler in this issue). The main unsolved problems concern the mechanisms of acquisition of specific “tooth” competence by neural crest cells and its molecular definition, the control of the patterned distribution of tooth class specific primordia and the control of cell diversity generated in each primordia.

Tooth patterning

According to Noden (1983, 1988) avian neural crest precursor cells acquire spatial programming while they are still part of the neural epithelium and the pattern of skeletal elements in the pharyngeal arches is determined in the crest according to their rostro-caudal position. HOX genes may contribute to the molecular identity of the rhombomeres and then of the corresponding crest cells (see Mark et al. in this issue). HOXa-2 seems to be the only HOX gene to be expressed in mouse rhombomere 2, but not in corresponding crest cells participating in the formation of the first arch. HOXa-2 is expressed in the rhombomere 4 and correspond-
distribution of inductive potency may exist in the epithelium of the early mandibular arch. However, according to Ruch (1995), further investigations are required to verify one of the two possible current hypotheses:

- either, competent, uncommitted neural crest “dental cells” require instructive interaction with a prepatterned oral ectoderm to express space specific tooth histo-morphogenesis and cytodifferentiation (Fig. 1);

- or, competent “dental” neural crest cells with differential spatial programming, follow particular migratory pathways to and in the forming jaws and expression of their specific potencies will be initiated by permissive interaction with a homogenous oral ectoderm (Fig. 1).

Sharpe (1995), analyzing by means of in situ hybridization, the expression of different homeobox genes (msx’s, dlx’s, and goosecoid), suggested that the expression domains of these genes overlap to divide the mandibular and maxillary processes into regions where the ectomesenchymal cells might have different homeoprotein combinations which could specify tooth initiation and morphogenesis.

**Fig. 3. Ultrastructural aspects of young functional odontoblasts.** (A) These cells are characterized by well organized rough endoplasmic reticulum (RER) and Golgi complexes (GC). The polarized cytoplasmic organization is obvious. Odontoblastic processes (OP) are embedded in the predentin (PD), extending to the basement membrane (BM). PA, preameloblasts. (B) High power electron micrograph illustrating bundles of collagen fibres secreted by functional odontoblasts (FO).
Generation of tooth cell diversity

Cell diversity may be lineage dependent and/or "induction" dependent (Holtzer et al., 1985; Stent, 1987).

Most of our current knowledge concerning the neural crest derives from experiments performed in avian embryos (for review see Couly and Le Douarin, 1988; Bronner-Fraser et al., 1991; Morris-Kay and Tuckett, 1991; Noden, 1991). Much of this data suggests that at least some of the neural crest cells are multipotent prior to their migration. As far as odontogenesis is concerned, a subpopulation of neural crest cells acquire, through unknown mechanisms, a "dental" competence. These multipotent "dental cells" (with or without differential morphogenetic programming) are progenitors of dental papilla cells, odontoblasts, peridental fibroblasts, cementoblasts and some bone cells.

In each tooth primordium changing, reciprocal epithelial-mesenchymal signalling will trigger tooth-class specific morphogenetic processes which, in turn, will amplify and diversify the epigenetic interactions allowing for time-space dependent emergence of tooth-specific phenotypes including odontoblasts (Ruch, 1995; Thesleff et al., in this issue). Morphogenetic dependent compartmentalization of neural crest derived cells into periodontal mesenchyme and dental papilla may account for segregation of periodontal (cementoblasts, fibroblasts, osteoblasts) and dental papilla (pulp cells, odontoblasts) potencies. As far as dental papilla cells are concerned we ignore whether distinct odontoblast and pulp cell progenitors coexist or if (more probably) all the dental papilla cells are potential odontoblasts but only some of them will, according to a particular spatial distribution of epigenetic signals, overtly differentiate (see Tziatas and Smith et al. in this issue).

Tooth morphogenesis allows for tooth specific patterning of the epithelio-mesenchymal junction (that later becomes the dentin-enamel junction). Presumptive odontoblasts are located closest to the inner dental epithelium (preameloblasts). The genotype related crown size and morphology of a specific embryonic tooth is a function mainly of a finite number of post-mitotic odontoblasts (and ameloblasts) and of their spatial distribution. Cell proliferation kinetics during in vivo mouse odontogenesis have been analyzed (Osman and Ruch, 1975, 1976, 1978; Ruch, 1984). Differential mitotic activities are related to tooth specific histo-morphogenesis and during odontogenesis a significant lengthening of the duration of the cell cycle from \( \pm 10 \) h to \( \pm 14 \) h, due to the lengthening of the duration of G1, exists in vivo. From dental lamina formation to the appearance of the first mouse molar post-mitotic odontoblast, a maximum of 14-15 cell cycles may exist. In vitro, the terminal differentiation of odontoblasts is delayed in a reproducible manner. The comparison of cell proliferation kinetics during mouse molar odontogenesis in vivo and in vitro (Ahmad and Ruch, 1987) demonstrated a significant lengthening of the cell cycle in vitro. The same number of cell cycles may exist in vitro and in vivo before the emergence of functional odontoblasts (and ameloblasts). The developing teeth express the whole set of retinoic acid receptors (Mark et al., 1991; Bloch-Zupan et al., 1994a) and retinoic acid is required for in vitro mouse incisor and molar morphogenesis, controlling tooth specific differential mitotic activities (Mark et al., 1992; Bloch-Zupan et al., 1994b).

Cytological and functional aspects of odontoblast terminal differentiation

The terminal differentiation of odontoblasts, characterized by a sequence of cytological and functional changes, occurs in each cusp according to a specific temporo-spatial pattern. For example, in the first lower Swiss mouse molar the first overtly differentiated odontoblasts are found on day 18 (of gestation) at the tip of the principal cusps and progressively, further functional odontoblasts emerge towards the basal (apical) parts (Fig. 2).
Fig. 6 Immunolocalization of decorin in mouse developing molar (a,b) and incisor (c,d,f,i). (e,g,h) Phase contrasts corresponding respectively to d, f and i. Decorin is expressed by polarizing (a) and functional odontoblasts (a-i). This proteoglycan surrounds the polarizing odontoblasts (a) and accumulates at the epithelio-mesenchymal junction (b,d,f,i). It is a constituent of predentin (a-d,f) and of osteo-dentin at the tip of developing incisor (c). Decorin as a constituent of dentin could only be visualized after demineralization (i). Od, odontoblast; EO, enamel organ; DP, dental papilla; IDE, inner dental epithelium; Am, ameloblast; D, dentin; Pd, predentin; PDM, pendental mesenchyme; E, enamel (the specific antibody, LF87, was kindly provided by Dr. L.W. Fisher, NIH, Bethesda, USA).
The terminal differentiation of odontoblasts is characterized by the following steps: 1) predontoblasts withdraw from the cell cycle. During the last division the mitotic spindle lies perpendicular to the epithelio-mesenchymal junction, the basement membrane (Osman and Ruch, 1975; Ruch, 1987). 2) The daughter cell, in contact with the basement membrane elongates and polarizes: nuclei take up an eccentric basal position and cisternae of granular endoplasmic reticulum develop, flatten and become parallel to the long axis of the cells (Fig. 3). 3) These cells start to synthesize first predentin then dentin components including collagen type I, type I trimer, types V and VI. Functional odontoblasts (Figs. 3, 4) also secrete proteoglycans such as decorin and bi-glycan (Cam et al., unpublished data) and also non collagenous proteins including bone sialoprotein (BSP) (Fisher et al., 1983; Chen et al., 1991), dentin sialoprotein (DSP) (D’Souza et al., 1992), Gla proteins such as osteocalcin (Bronckers et al., 1987), as well as phosphophoryn (Fujisawa and Kuboki, 1988), osteopontin (Butler, 1989), osteonectin (Fujisawa and Kuboki, 1989; Reichert et al., 1992). Among these dentin constituents, only DSP and phosphophoryn appear to be specific for dental tissues (see Butler et al., and Linde et al., in this issue).

The polarization of odontoblasts is cytoskeleton dependent. Colchicine as well as cytochalasin B (Ruch et al., 1975) interfere with terminal differentiation: microtubules (Nishikawa and Kitamura, 1987), intermediate filaments (Lesot et al., 1982; Fausser et al.,...
Cultured recombinations of either control or BrdU-treated dental papillae with either control or BrdU-treated enamel organs were analyzed. The primary effect of BrdU on odontoblast differentiation was a consequence of its incorporation in the preameloblasts (inner dental epithelium) which were no longer able to trigger odontoblast differentiation (Ruch et al., 1978).

During odontogenesis, from dental lamina formation to ameloblast differentiation, a continuous basement membrane (basal lamina and associated material) which connects the developing enamel organ and dental papilla has been suggested to be involved in the epithelio-mesenchymal interactions controlling odontoblast differentiation (Meyer et al., 1977).

The inner dental epithelium controls odontoblast terminal differentiation

Huggins et al. (1934) transplanted isolated tooth germ tissues in heterotopic positions and suggested that the inner dental epithelium plays a role in odontoblast differentiation. Koch (1967) analyzing cultures of transfert associations of enamel organs and dental papillae concluded that epithelio-mesenchymal interactions were necessary for the functional differentiation of odontoblasts. Ruch and Karcher-Djuricic (1971b) stressed the fact that a stage-space specific inner dental epithelium was involved. Furthermore, Kollar (1972) and Ruch et al. (1973) demonstrated that odontoblast differentiation can be "induced" by homospecific, non dental, epithelium. Interestingly, in such experimental conditions the dental papilla first promoted the transformation of the non dental epithelium into an inner dental epithelium which then was able to initiate odontoblast differentiation (see Osman et al., 1979). The data concerning hetero-specific recombinations are discussed by Lemus in this issue. The role of the inner dental epithelium in odontoblast differentiation has also been deduced from results of BrdU incorporation: in vitro, BrdU incorporation in intact teeth inhibited odontoblast and ameloblast differentiation (Schwartz and Kirsten, 1973; Ruch et al., 1978). Cultured recombinations of either control or BrdU treated dental papillae with either control or BrdU-treated enamel organs were analyzed. The primary effect of BrdU on odontoblast differentiation was a consequence of its incorporation in the preameloblasts.
Dental basement membrane changes accompany odontoblast differentiation

Immunolocalization of collagen type IV, laminin, nidogen, (entactin) and heparan sulfate proteoglycan using polyclonal antibodies, did not show obvious changes during odontogenesis.

Differential time and space regulated assembly of basement membranes by intermolecular interactions between matrix molecules allows the assembly of dynamic and tissue specific basement membranes.

Fig. 10. Sections of dental papillae isolated by trypsin treatment from day-17 (p.c.) mouse mandibular molars and cultured for 6 days in semi-solid medium containing TGFβ1, BMP2 or IGF1 either combined with heparin (HN) or alone. (A) TGFβ1-HN: extended differentiation of functional odontoblast-like cells (OD), with apical accumulation of extracellular matrix (ECM). (B) TGFβ1 alone: intrapulpal accumulation of ECM. PO, predontoblasts. (C) BMP2-HN: localized differentiation of OD, secreting ECM. (D) BMP2 alone: neither OD nor ECM accumulation were observed. (E) IGF1-HN: extended polarization of OD, without apical accumulation of ECM. (F) IGF1 alone: neither OD nor ECM accumulation were observed.
abolished. In intact day-18 molars, the presumed operation were cultured it associated with the dental papillae, while trypsin treatment or gang could be dissociated from dental papilla (Osman and Ruch, 1981a). However EDTA treatment removed the basement membrane at the tip of the main cusps promotes functional differentiation, but apical progression of the phenomenon is hampered and may be due to an absence of epithelially dependent changes.

More recently Meyer et al. (1995) have shown that cultured mouse incisors displayed major modifications in basal lamina deposition without further effect on odontoblast differentiation (Fig. 7). These data stress the fact that components of the lamina diffusa (components proper or trapped molecules, like growth factors rather than components of the basal lamina) play a role.

**Growth factors and odontoblast differentiation: descriptive data**

- Immunolocalization and/or in situ hybridizations of growth factors and receptors and/or specific transcripts, although in some cases providing somewhat conflicting data, suggest intervention of growth factors in odontoblast terminal differentiation:
  - growth hormone receptor (Zhang et al., 1992);
  - IGFs and receptors (Joseph et al., 1993); aFGF, bFGF (Gonzales et al., 1990; Cam et al., 1992); FGFR R, (Niswander and Martin, 1992); int-2 (Wilkinson et al., 1989);
  - NGF and receptors (Byers et al., 1990; Mitsiadis et al., 1992, 1993 and this issue);
  - TGFs superfamily: TGFβ1, 2 and 3 (Lehnert and Akhurst, 1988; Cam et al., 1990; D'Souza et al., 1990; Pelton et al., 1990, 1991; Millan et al., 1991; Wise and Fan, 1991; Thesleff and Vaathokari, 1992; Heikinheimo et al., 1993a); and BMP2,4,9 (Lyons et al., 1990; Heikinheimo, 1993b, 1994; Vainio et al., 1993).

Members of the EGF family may be involved during tooth initiation and morphogenesis rather than during odontoblast differentiation (Partanan and Thesleff, 1987; Cam et al., 1990; Kronmiller et al., 1991; Hu et al., 1992; Heikinheimo et al., 1993b).

**Aspects of cell proliferation kinetics: the problem of competence**

Histological investigations, combined with 3H-thymidine radioautography of in vitro cultured heterochronal enamel organ -dental papilla recombinations (Ruch and Karcher-Djuric, 1971b; Ruch et al., 1978) led to the following observations:

- Day-16 odontoblasts recombined with day-18 inner dental epithelium did not rise to the anticipated differentiation of odontoblasts.

- Day-18 post-mitotic, polarizing odontoblasts combined with day-16 inner dental epithelium demonstrated depolarization and re-entering of the cell cycle. After 3 days in culture these cells withdrew from the cell cycle again and overtly differentiated.

Furthermore, replicating odontoblasts held in the G1, S interface by fluorodeoxyuridine (Ruch and Karcher-Djuric, 1971a) did not overtly differentiate. This effect was reversible and fluorodeoxyuridine had no marked effects on post-mitotic odontoblasts.

This data suggests that the expression of competence of odontoblasts to respond to specific epigenetic signals triggering terminal differentiation requires a minimal number of cell cycles. Terminal differentiation cannot be anticipated. On the other hand, supplementary cell cycles do not hamper terminal differentiation and may allow regulative phenomena.
Fig. 11. TGFβ1, 2, 3; BMP2, 4; osteonectin (ON); fibronectin (FN); bone sialoprotein (BSP); IGF1; and msx1 and msx2 transcripts visualized by in situ hybridization on sets (A,C,E,G,I,K; B,D,F,H; J,L) of consecutive sections of first lower incisors of newborn mice. For details see Bégue-Kim et al. (1994). Am, ameloblasts; PA, preamaloblasts; PO, preodontoblasts; Od, odontoblasts; Li, lingual; Lb, labial; cl, cervical loop.
**Fibronectin plays a role in odontoblast polarization**

Several reports have shown that fibronectin could interact with cell surfaces, for example, by means of integrins (Hynes, 1992) and also that the β1 subunit of integrins could interact with the microfilament system by means of either talin (Hotypitz et al., 1986) or, more probably, α-actinin (Otey et al., 1990). When investigating the ability of fibronectin to interact with plasma membrane proteins transferred to PVDF, we observed an interaction with three high molecular weight proteins present in membranes prepared from dental mesenchyme but not from the enamel organ (Lesot et al., 1985a). An immunological approach allowed study of one of these proteins with an apparent molecular weight of 165 kDa. Two monoclonal antibodies directed against this protein were used to investigate its localization and function. The 165 kDa protein and fibronectin transitorily accumulated at the apical pole of polarizing odontoblasts (Lesot et al., 1990, 1992). A monoclonal antibody, which recognized an extracellular epitope of the 165 kDa protein, was found to specifically interfere with the organization of microfilaments, to have no effect on microtubules and to block odontoblast elongation and polarization. At later stages, the maintenance of odontoblast polarization no longer required fibronectin-165 kDa protein interaction (Lesot et al., 1998). At this stage, both fibronectin and the 165 kDa protein tended to disappear from the apical pole of odontoblasts while the formation of junctional complexes including tight junctions, zona adherens and gap-junctions increases (Iguchi et al., 1984; Bishop, 1985; Calle, 1985). The molecular mechanisms supporting the role of the 165 kDa protein in the reorganization of microfilaments during odontoblast polarization still remain unknown. No direct interactions could be detected between the membrane protein and either α-actinin, vinculin or talin (Fauser et al., 1993a,b). Either direct interactions are mediated by a ligand different from those we tested or the strength of the interaction was too weak to be maintained in the experimental conditions used. Another possibility could be that there is no direct interaction, but a transduction pathway (Lesot et al., 1994).

Several complementary approaches indicate that the 165 kDa protein is not a member of the integrin family. The 165 kDa protein is a monomer and its interaction with fibronectin is calcium independent, thus differing from integrin 83kDαβ. A large 175 kDa C-terminal fragment of fibronectin, including the RGD sequence, did not interact with the membrane protein. This observation was confirmed by the fact that GRGDS peptides did not interfere with odontoblast differentiation, although the 165 kDa protein plays a major role in this process. The protein would thus differ from 81α5, 81αV, 83kDαβ, 83kDαβ and 86kDαβ integrins which are all RGD-dependent (Hynes, 1992). The 165 kDa protein was found to interact with 62 kDa proteolysis fragments of fibronectin comprising the collagen-binding domain and the first type III repeat of fibronectin; no integrin interacts with this region of fibronectin (Lesot et al., 1988, 1992, 1993). The 165 kDa protein is a minor constituent among membrane proteins and attempts are now being made to get more information by cloning the corresponding gene.

As far as integrins are concerned, immunofluorescent staining of mouse tooth germ frozen sections with specific antibodies (Fig. 9) revealed positive staining of the inner dental epithelium corresponding to β4 and α6 chains of integrins while no reaction was detected between the membrane protein and either α-actinin, vinculin or talin (Fauser et al., 1993a,b). Either direct interactions are mediated by a ligand different from those we tested or the strength of the interaction was too weak to be maintained in the experimental conditions used. Another possibility could be that there is no direct interaction, but a transduction pathway (Lesot et al., 1994).

**TABLE 1**

**EXPRESSION OF SEVERAL TRANSCRIPTS BY ODONTOBLASTS IN VIVO VERSUS ODONTOBLAST LIKE CELLS INDUCED IN VITRO**

<table>
<thead>
<tr>
<th>probes</th>
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<tbody>
<tr>
<td>TGFβ1</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>TGFβ2</td>
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<td>BSP</td>
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+, strong positive signal; -, weak "ubiquitous" signal; EDTA5oI.Fr, EDTA soluble fraction.

The progressive emergence of competent preodontoblasts might be related to cell kinetics according to Ruch (1990, 1995); sequential withdrawal of competent preodontoblast from the cell cycle could explain the gradient of differentiation (Fig. 8). Further investigations are required to prove or to rule out a cell kinetic dependent expression of competence, i.e. to know if quantal cell cycles according to Holtzer et al. (1985), Ruch et al. (1976), Ruch (1990) exist. All together these data demonstrate that the dental basement is a dynamic, asymmetric interface demonstrating compositional and conformational modifications and strongly suggests that a stage-space specific basement membrane, acting both as a specific substrate and as a reservoir for paracrine (autocrine) factors, plays a role in odontoblast terminal differentiation. This hypothesis (Ruch and Karcher-Djuricic, 1975; Ruch et al., 1982, 1984; Ruch, 1985, 1987) implicates:

- time and space specific information encoded in the basement membrane;
- reading of this information by competent adjacent preodontoblasts and;
- transduction and interpretation by these cells.

Complementary investigations comprising study of possible interactions of matrix molecules with the odontoblast plasma membrane and the analysis of the effects of matrix molecules and growth factors on odontoblast differentiation have been performed.

**TABLE 2**

**EXPRESSION OF SEVERAL TRANSCRIPTS BY PREODONTOBlastS IN VIVO VERSUS POTENTIAL ODONTOBlastS CULTURED IN VITRO IN THE PRESENCE OF POTENTIAL INDUCERS**

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<tr>
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<tr>
<td>BMP4</td>
<td>+</td>
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<tr>
<td>IGF1</td>
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<td>++</td>
</tr>
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</table>

+, strong positive signal; -, weak "ubiquitous" signal; EDTA5oI.Fr, EDTA soluble fraction.
observed when using anti-81 antibodies (Lesot et al., 1993; Meyer et al., 1995). Although the specificity of α6β4 integrin is controversial (Lotz et al., 1990; Sonnenberg et al., 1991), it has been reported to mediate an RGD-independent interaction with laminin (Hynes, 1992; Lee et al., 1992).

Effects of matrix molecules on odontoblast differentiation in vitro

Trypsin isolated mouse molar dental papillae were cultured on Millipore filters coated with either fibronectin, laminin, collagen type I or with fibronectin and collagen type I. None of these substrates promoted odontoblast differentiation (Lesot et al., 1985b).

Dentin matrix components were also isolated from rabbit dentin after exhaustive extraction by EDTA in the presence of protease inhibitors followed by digestion of the insoluble matrix with collagenase (Smith and Leaver, 1979). Day-18 dental papillae were cultured on Millipore filters coated with these various isolated dentin fractions (Lesot et al., 1986). Some fractions allowed maintenance of polarization without de novo initiation of differentiation. Millipore filters coated with a dental biomatrix (prepared according to Rojkind et al. (1980) and containing collagen type I and IV, fibronectin and laminin) also allowed the maintenance of odontoblast polarization and functional activity (Cam et al., 1986). Hyaluronic acid or chondroitin sulfates added to the cultures medium also had such an effect (Tziasfas et al., 1988). Intact predentin was unable to promote terminal differentiation (Karcher-Djuricic et al., 1985).

Isolated dental papillae cultured on Millipore filters lost their morphological integrity. On the other hand, when isolated dental papillae were embedded in agar solidified culture medium, the cusp pattern and developmental gradients could be maintained in vitro (Bègue-Kirn et al., 1992). Trypsin-isolated day-17 mouse molar dental papillae (containing only preodontoblasts) were cultured for six days in semi-solid agar medium containing the same EDTA-soluble dentin matrix fractions separated by ion-exchange chromatography as mentioned above. The total unpurified EDTA-soluble fraction neither promoted nor maintained odontoblast differentiation. However, dentin matrix fractions retained on DEAE (diethylaminoethyl)-Cellulose (Bègue-Kirn et al., 1992) were observed to initiate the differentiation of odontoblast-like cells at the periphery of the explants (Fig. 10). Furthermore, in these conditions the normal pattern of a gradient of odontoblast differentiation could be seen in the dental papillae which was initiated at the tips of the main cusps and progressed in an apical direction. Collagen type I, decorin and biglycan but no fibronecint were found to be present in the extracellular matrix which accumulated at the secretory pole of the elongated cells (Lesot et al., 1994).

The active components present in the EDTA-soluble fraction of dentin could be retained on heparin-agarose columns and might include growth factors such as TGFβs, BMPs and IGFs (Finkelman et al., 1990) and indeed the addition of a blocking TGFβ antibody (Dasch et al., 1989) to the culture medium abolished the biological effects of the active dentin matrix fraction in odontoblast differentiation (Bègue-Kirn et al., 1992). These data prompted investigations on the effects of growth factors on odontoblast differentiation.

Effects of TGFβ1, BMP2 and IGF1 on odontoblast differentiation

In the presence of TGFβ1, BMP2 or IGF1, added alone to the agar solidified culture medium, the preodontoblasts of isolated dental papillae never differentiated. When combined with heparin (or fibronecint) these growth factors had positive, albeit differential, effects (Bègue-Kirn et al., 1994): TGFβ1-heparin (HN) (like EDTA-soluble fractions of dentin) induced gradients of cytological and functional differentiation (Fig. 10); BMP2-heparin allowed functional differentiation in restricted areas, i.e. at the tips of the cusps (Fig. 10); and IGF1-heparin induced extended cytological differentiation without apical matrix deposition (Fig. 10). By means of in situ hybridization, we compared TGFβs, BMPs, IGF, msx, fibronectin, osteonectin and bone sialoprotein gene expression during in vivo and in vitro induced odontoblast differentiation (Bègue-Kirn et al., 1994). The main data are summarized in Table 1: very similar expression patterns existed for TGFβ1-HN, BMP2-HN and EDTA-soluble dentin fraction induced odontoblasts and physiological (in vivo) odontoblasts (Fig. 11), with differences relating to the expression of msx1 and msx2 which appeared respectively over and under expressed in polarized odontoblasts in vivo. However, most interestingly, msx2 was strongly expressed in preodontoblasts in vivo just before terminal differentiation (Table 2). IGF-HN induced odontoblasts (unable to deposit apical matrix) did not express TGFβs genes and furthermore, we were unable to detect IGF1 transcripts either in the inner dental epithelium or in physiological and induced odontoblasts. These data and complementary investigations (Bègue-Kirn et al., 1994) suggested that up-regulation msx2 transcription as well as up-regulation of members of the TGFβ super-family are prerequisites for terminal differentiation of odontoblasts allowing polarization and apical accumulation of matrix respectively.

Current hypotheses and questions

Terminal differentiation of odontoblast, resulting from continuous, reciprocal epithelial-mesenchymal interactions, is characterized by several steps, implying withdrawal from the cell cycle, elonga-
Fig. 13. RNA expression of TGFβ type III receptor (RIII) in mandibular incisor (A,C) first (M1) (B,D) and second (M2) molars (G,I) of newborn rat and in first lower molar of newborn mouse (F,H). (A,B,F,G) Bright-field images of (C,D,H,I) respectively. RIII mRNAs are expressed by preameloblasts (pA), facing polarizing odontoblasts (pol O) (C,D), and the signal is increased in functional ameloblasts (Am) (C). A weak hybridization signal exists in mouse pAm which are developmentally younger than the rat pA (H). The other dental cells did not express RIII transcripts (C,D,H,I). No signal could be detected with the RIII sense probe (RIIS) (E). The rat RIII probe was generously provided by Dr. Weinberg of the Whitehead Institute, Cambridge, USA and hybridization steps were performed according to Bégué-Kim et al. (1994). Od, odontoblasts; Li, lingual; Lab, labial; RIIIAS, TGFβ type III antisense probe.

...tion and cytological polarization as well as transcriptional and translational modifications enabling the cells to deposit predentin-dentin components. These processes take place according to very specific temporo-spatial patterns. All the experimental data summarized above support the hypothesis that a functional network consisting of matrix molecules including fibronectin and growth factors, including members of the TGFβ superfamily, and their receptors/activators, might be involved in terminal differentiation of odontoblasts, at least in the control of functional polarization. Our in vitro data (Bégué-Kim et al., 1994) strongly suggests that immobilization of active growth factors (TGFβ1, BMP2 and IGF1) by means of heparin or fibronectin, immobilisation which might favor a polarized interaction with preodontoblasts, is a prerequisite for induction of cytological polarization and eventually apical matrix...
accumulation. Immobilization of TGFβ with heparin in our in vitro conditions may simulate possible in vivo immobilization by basement membrane components.

Most interestingly in our best in vitro conditions (EDTA-soluble fraction of dentin or TGFβ-HN) gradients of odontoblast differentiation are expressed: like in vivo, the differentiation is initiated at the tip of the cusps and proceeds in an apical direction. A space and time regulated expression of specific competence by preodontoblasts seems to be preserved in isolated dental papillae. It will be essential to analyze cell proliferation parameters of cultured dental papillae to test our hypothesis concerning the possible role of cell kinetics in regulating the expression of competence. The molecular aspects of this competence are completely unknown. Since members of the TGFβ family are able to increase matrix production of most of the preodontoblasts and dental papilla cells, these cells probably express the required receptors and are able to transduce specific signals. The particular competence of preodontoblasts able to give rise to functional odontoblasts might specify quantitative-qualitative differences in the metabolic answers to the signal, triggering a chain reaction leading to polarized secretion of predentin-dentin-components.

In physiological conditions, stage-specific preameloblasts trigger odontoblast terminal differentiation through matrix-mediated interactions. This mediation could require complementary levels of control such as immobilization, potentiation and special patterning of epithelially-derived paracrine signals. In situ hybridization (Begue-Kirn et al., 1994 and references therein) demonstrate that transcripts of genes of the TGFβ family are present in these cells. However, these transcriptions were not restricted to preameloblasts facing preodontoblasts able to overtly differentiate and it is not yet known if these preameloblasts release growth factors which could be trapped by matrix molecules of the lamina diffusa. However, such a pattern probably exists and release of BMPs by young dental epithelium has been documented (Vainio et al., 1983). TGFβ-related proteins are synthesized as larger precursor proteins and the actual signaling molecule is a homo- or heterodimer of a small carboxy-terminal domain. Activation of the large latent complex of TGFβ1 by proteolysis is an important regulator of activity for the TGFβ subclass of molecules (Kingsley, 1994).

Furthermore, latent TGFβ-binding protein (LTBP) may be involved in targeting (Kanzaki et al., 1990) or activation (Flaumenhaff et al., 1993) of the complex and LTBP has been localized in the dental basement membrane (Cam et al., unpublished data) (Fig. 12).

Further investigations on the cellular origin and distribution of TGFβ binding proteins (decorin, β-glycan, bi-glycan) and TGFβ activating/inhibiting molecules are of crucial importance. Most interestingly the transcripts for the β-glycan (receptor TGFβ type III) were localized in preameloblasts facing preodontoblasts able to differentiate (Begue-Kirn et al., unpublished data) (Fig. 13). The expression and distribution of TGFβ receptors I and II transmembrane serine/threonine kinases, (Wrana et al., 1994) by preodontoblasts/odontoblasts should be investigated.

As a simplified, schematic working hypothesis (Fig. 14) we might suggest the following: one (or several) epithelial-derived member(s) of the TGFβ family, trapped (and activated) by basement membrane associated components interact with the apical plasma membrane of competent preodontoblasts or post-mitotic cells and regulate the transcription of genes encoding for proteins belonging to the TGFβ family and homeoproteins such as msx2. The msx2 transcription factor might in turn modulate the expression of genes involved in cytoskeleton assembly. Endogenous members of the TGFβ family could regulate matrix production including transitory synthesis of fibronectin which interacts with the 165 kDa, non integrin, membrane receptor and thus co-regulates processes involved in cell elongation and polarization. Clearly the mechanisms involved in odontoblast differentiation are very complex and synergistic interactions with other endogenous or circulating growth factors (IGFs, FGFs) probably exist. We do not know if
withdrawn from the cell cycle, correlated with increased cAMP (Osman et al., 1981) and cytological and functional polarization are co-regulated.

Conclusion

As stated by Noden (1991): "cells of the neural crest must resolve two problems: phenotypic commitment, i.e. what to become, and spatial programming i.e. where to do it". When and how the odontogenic cells are committed is not known but odontoblasts will do it at locations defined by tooth-specific morphogenesis and thanks both to delivery of epithelioid-derived signals and time-space dependent expression of competence. We know neither to what extent nor how neural crest-derived cells control tooth-class specific morphogenesis and as yet, only circumstantial evidence suggests that the variation of competence might be either dependent on the number of mitoses or on the transition from proliferating to post-mitotic stage. The identification of epithelioid derived paracrine factors, putatively involved in odontoblast terminal differentiation and the application of advanced technologies will probably allow elucidation of the molecular network involved in the control of odontoblast terminal differentiation. Future research will also clarify, slowly but hopefully, how tissue specific and regionally specified gene expression is controlled and is involved respectively in tooth-specific histomorphogenesis allowing particular patterning of preodontoblasts and in expression of competence.

Summary

Odontoblasts are post-mitotic, neural crest-derived, cells which overtly differentiate according to tooth specific temporospatial patterns and secrete preodontin-dentin components. Neither the timing nor the molecular mechanisms of their specification are known and the problem of their patterning in the developing jaws is far from being solved. On the other hand, some significant strides were made concerning the control of their terminal differentiation. Fibronectin interacting with a 165 kDa, non integrin, membrane protein intervenes in the cytoskeletal reorganization involved in odontoblast polarization and their terminal differentiation can be triggered in vitro by immobilized members of the TGFβ family. Histological aspects and the transcriptional phenotypes (transcripts of TGFβs, BMPs, msxs, IGF1, fibronectin, osteonectin, bone sialoprotein genes) are very similar in vivo and in vitro. In vivo members of the TGFβ super family secreted by preameloblasts, trapped and activated by basement membrane associated components, might initiate odontoblast terminal differentiation.

KEY WORDS: odontoblast, differentiation, TGFβs, BMPs, msxs

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