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Special Review

Odontoblast differentiation

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Introduction

Histological and cytological organization as well as functional aspects confer specificity to the odontoblats. 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 (1990) recently discussed the evolutionary origin of vertebrate skeletogenic and odontogenic tissues and strongly supported the phylogenic emergence of distinct osteogenic and dentinogenic lineages. They postulated that the dentin of the earliest agnathian 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 primodia 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 corresponding neural crest cells which contribute to the formation of the second arch. HOXa-2 gene knockout leads to a homeotic transformation of the second arch skeletal elements into first arch components (Rijli *et al.*, 1993), however, supplementary teeth did not develop.

As far as mouse odontogenesis is concerned, the most relevant experiments have been performed by Lumsden (1984, 1988). Cranial neural crest (free margins of the neural plate at post-mesencephalic levels) recombined with oral mandibular arch epithelium and grafted to the anterior chamber of homologous adult male mice gave rise exclusively to molariform teeth. No tooth-like structures developed when the neural crest was combined with limb epithelium and neural crest grafted alone developed some neural tissue and cartilage. Neural crest cell migration (allowing specific interactions) was not necessary for their phenotypic commitment, whereas an interaction with the oral ectoderm appears to be indispensable. Furthermore, Lumsden (1988), discussing the results of experiments in which the mandibular arch was bisected into rostral and caudal halves, which were either immedialety grafted or separated into epithelial and mesenchymal components and subsequently reciprocally combined concluded: "a prepatterned

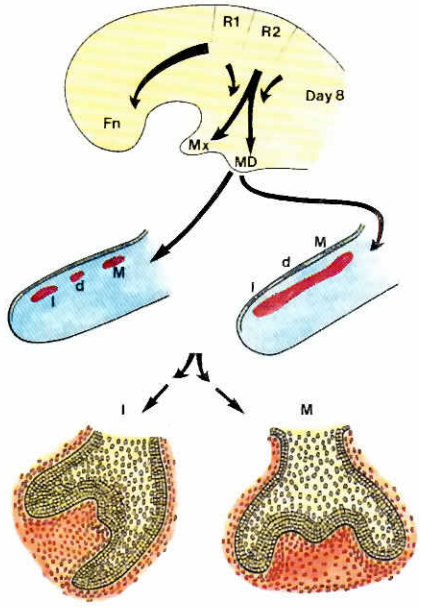


Fig. 1. In the mouse embryo, neural crest cells derived from rhombomeres 1 and 2 (R1, R2) invade the first pharyngeal arch (Mx, maxilla; MD, mandible; Fn, fronto-nasal processes). *According to Lumsden (1988) uncommitted neural crest cells might interact with a prepatterned oral ectoderm expressing incisor (I), diastemal (d) and molar (M) potencies. According to Ruch (1995 and references therein) neural crest derived "dental clones" I, d, and M, could be endowed by tooth class specific morphogenetic programming which would be revealed after interaction with unpatterned oral ectoderm. In both cases epithelial-mesenchymal signalling will trigger tooth class specific histo-morphogenesis (the diastema dilemma is discussed by Peterková et al. in this issue).*

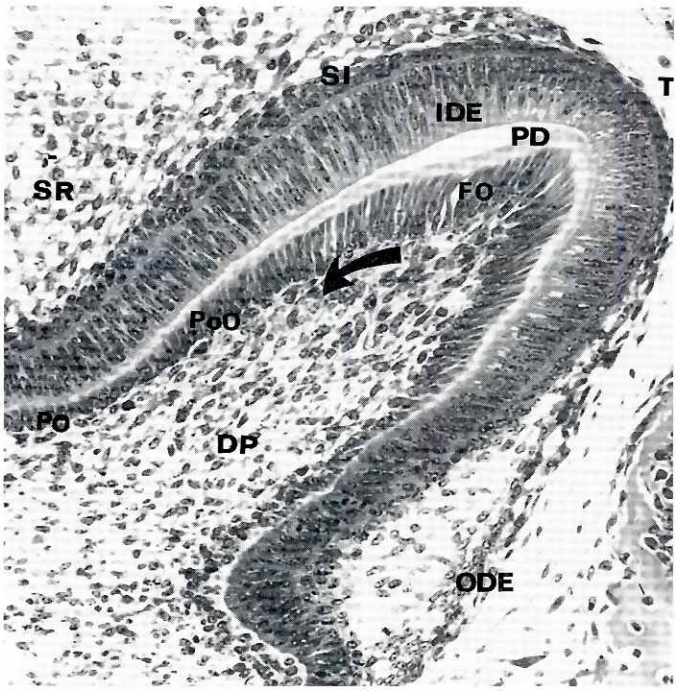


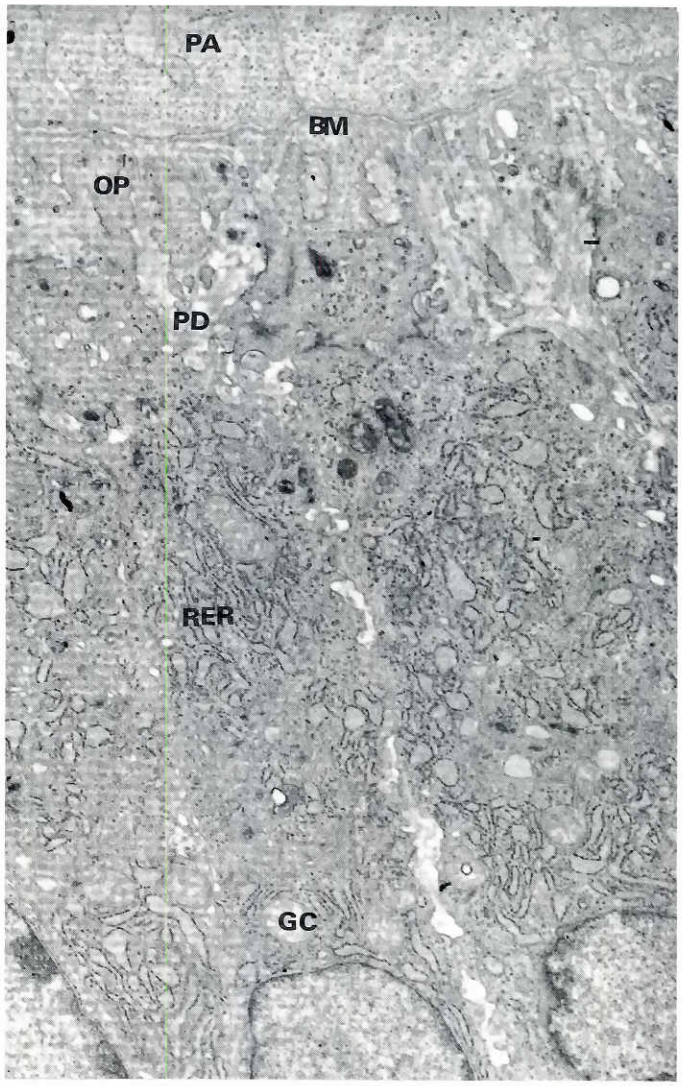
Fig. 2. Frontal section of a day 18.5 (p.c.) first lower mouse molar, illustrating the gradient of odontoblast terminal differentiation. *The first (oldest) functional odontoblasts (FO) secreting predentin (PD) are located at the tip of the cusp (T) and the progressive transition in apical direction from FO to polarizing odontoblasts (PoO) and preodontoblasts (PO) is obvious. IDE, inner dental epithelium; SI, stratum intermedium; SR, stellate reticulum; ODE, outer dental epithelium; DP, dental papilla.*

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.



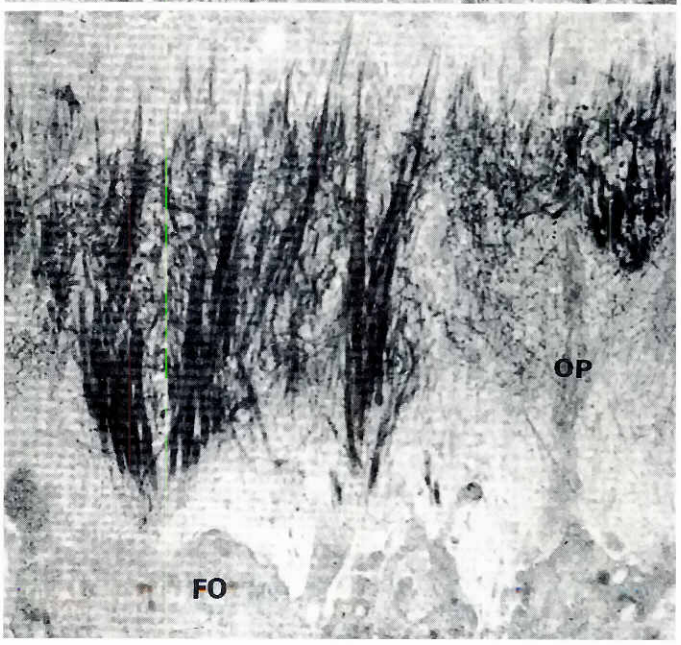


Fig. 3. Ultrastructural aspects of young functional odontoblasts. (A) *These cells are characterized by well organized rough endoplasmic reticulum (RER) and Golgi complexes (GC). The polarized cytoplasmic organization is obvious. Odontoblastic processes (OP) are embedded in the predentin (PD), extending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

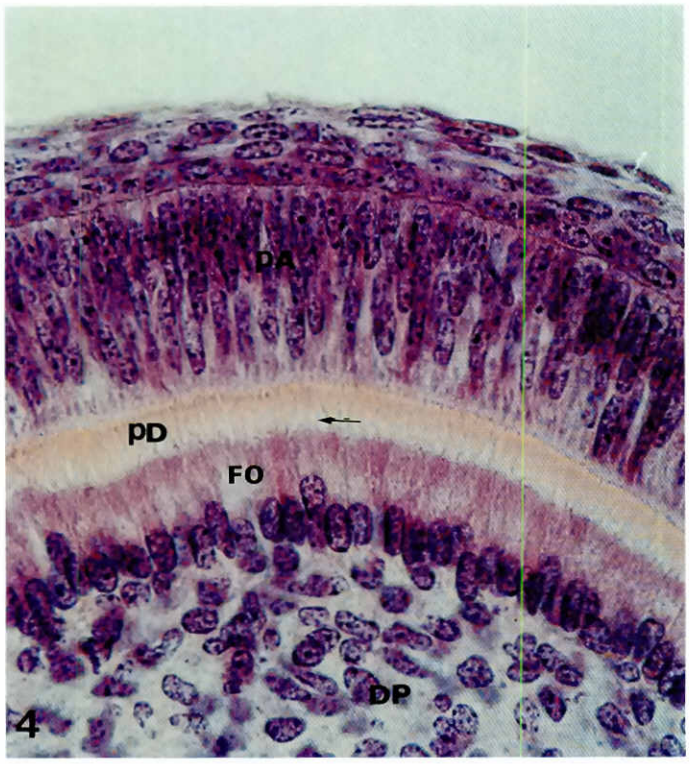


Fig. 4. Histological aspect of functional odontoblasts (FO). *The arrow points to odontoblastic cell processes. PA, preameloblast; PD, predentin; DP, dental papilla.*

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; Morriss-Kay and Tucket, 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 Tziafas 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 ~10 h to ~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 odontoblasts, 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).

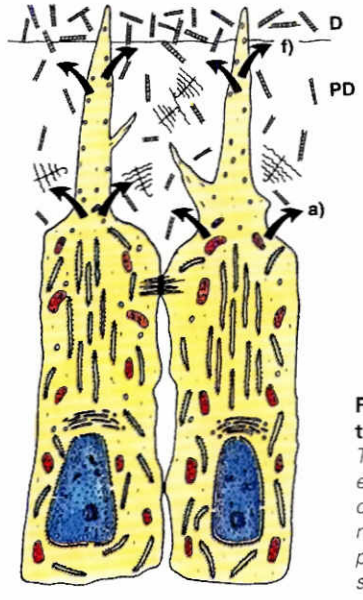


Fig. 5. Schematic representation of functional odontoblasts. *The arrows indicate the two levels of secretion: a) base of odontoblastic cell processes; f, mineralization front; PD, predentin; D, dentin (for details see Linde in this issue).*

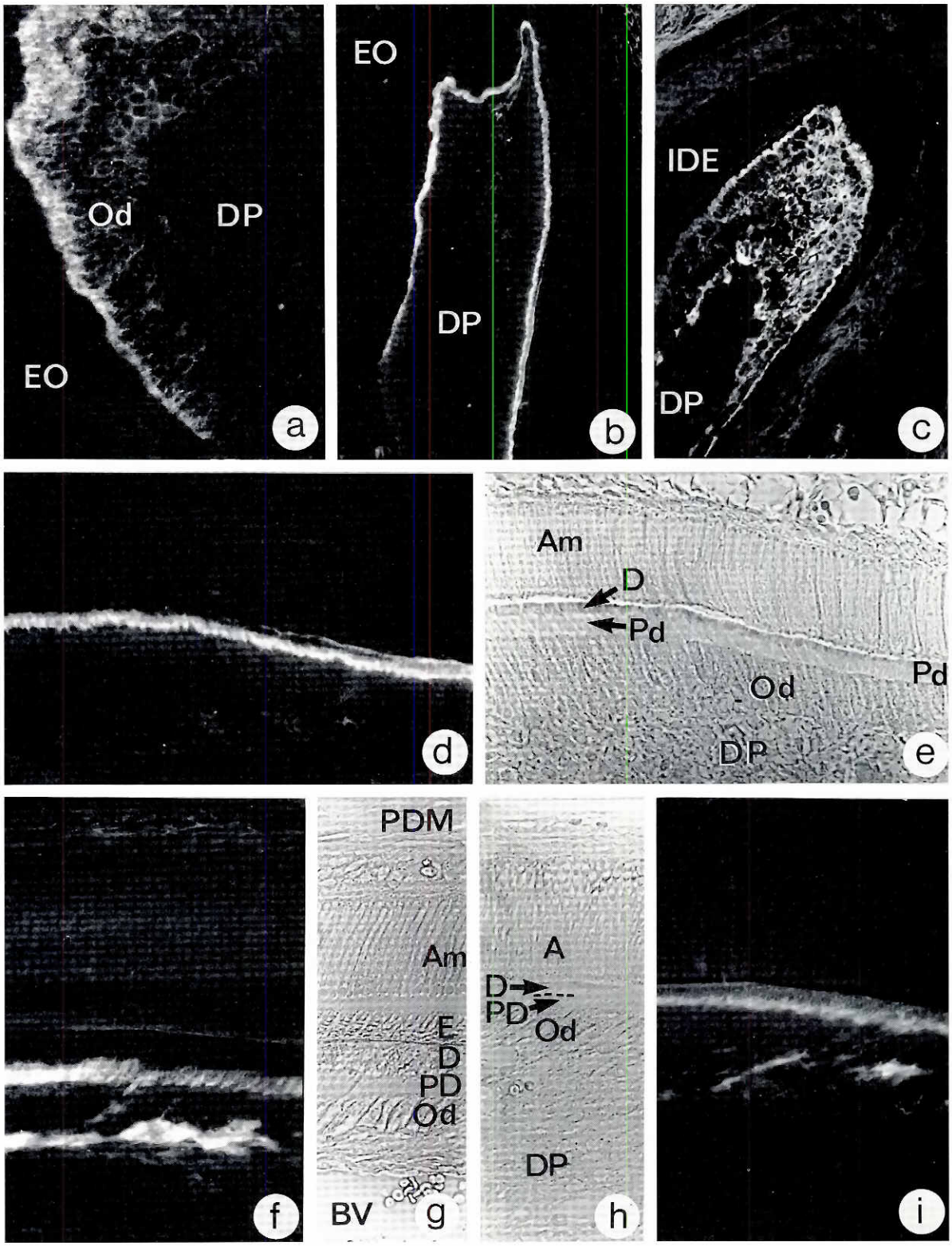


Fig. 6. Immunolocalization of decorin in mouse developing molar (a,b) and incisor (c,d,f,i). (e,g,h) *Phase contrasts corresponding respectively to d, f and i. Decorin is expressed by polarizing* **(a)** *and functional odontoblasts* **(a-i)**. *This proteoglycan surrounds the polarizing odontoblasts* **(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, peridental mesenchyme; E, enamel (the specific antibody, LF87, was kindly provided by Dr. L.W. Fisher, NIH, Bethesda, USA).*

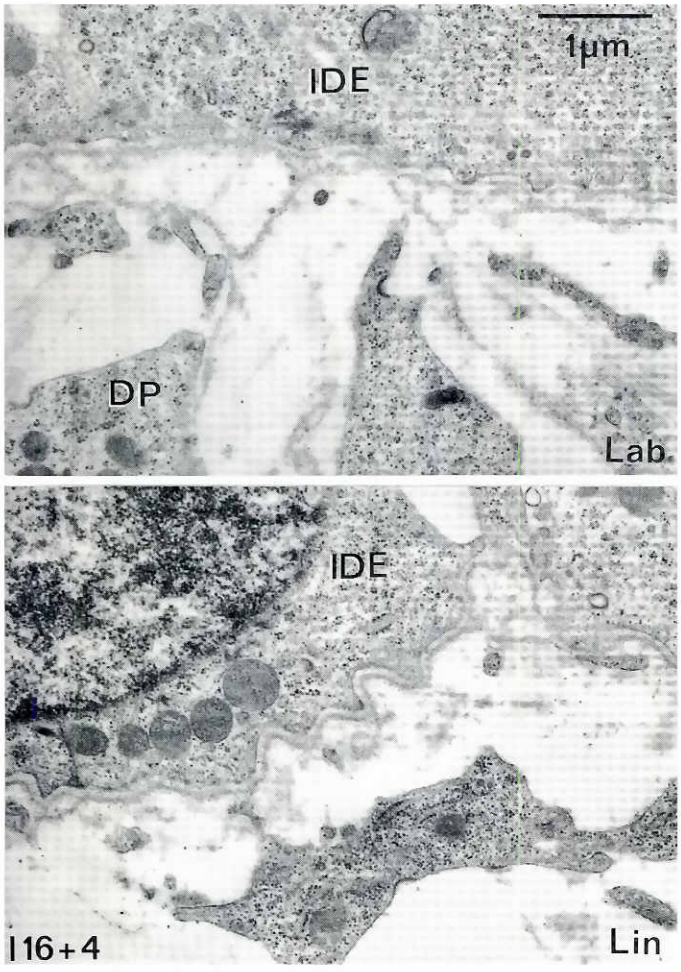


Fig. 7. Electron micrographs of day-16 mouse incisor cultured for 4 days. *Multiple superposed layers of basal laminae exist on the labial (Lab) side although the basal lamina remains unique on the lingual (Lin) side. IDE, inner dental epithelium; DP, dental papilla.*

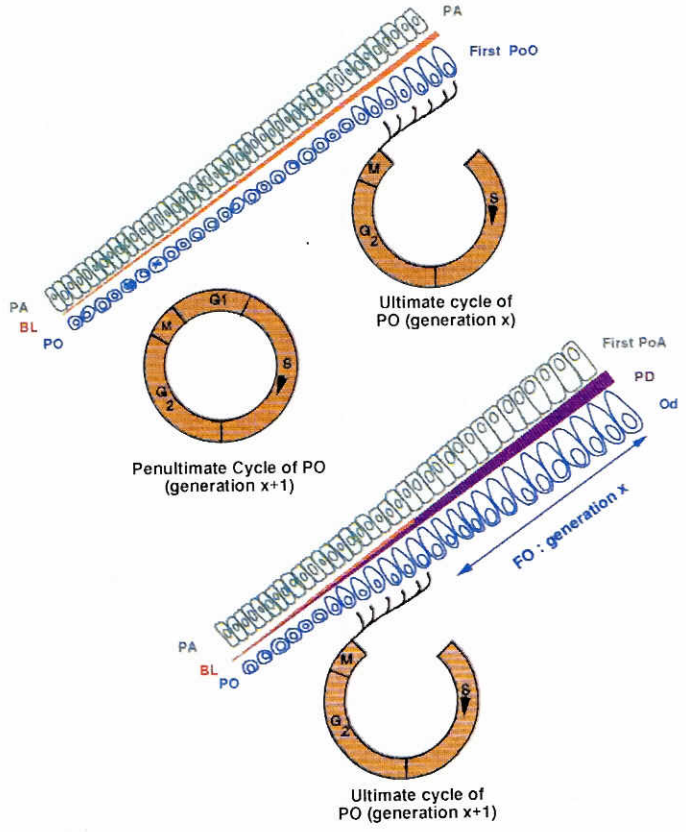


Fig. 8. The preameloblasts (PA) instruct the neighbouring preodontoblasts (PO) to differentiate along a particular pathway and in a coordinated sequential order giving rise to the gradient of terminal differentiation. *The known facts suggest both the existence of an intrinsic timing mechanism (leading to the expression of specific competence by preodontoblasts) and that division counting might be involved. PO of successive, juxtaposed generations (x, x+1...) might sequentially withdraw from the cycle and overtly differentiate. The counting of mitoses could be initiated early during odontogenesis in such a way that all PO might be able to express specific competence after the same number of cell cycles (Ruch, 1990; see also Ffrench-Constant, 1994). PoO, post-mitotic, polarizing odontoblasts; BL, basement membrane; PD, predentin; PoA, post-mitotic, polaring ameloblasts; FO, functional odontoblasts.*

The terminal differentiation of odontoblasts is characterized by the following steps: 1) preodontoblasts 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, 1976; 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.*,

1990) and microfilaments (Lesot *et al.*, 1982; Nishikawa and Kitamura, 1986; Ruch *et al.*, 1987; Kubler *et al.*, 1988) are reorganized during odontoblast elongation and polarization.

Cell membrane modifications, comprising increased adenylate cyclase activity (Osman *et al.*, 1981), and redistribution of concanavalin binding sites (Meyer *et al.*, 1981b) as well as of a 165 KDa fibronectin binding membrane protein (Lesot *et al.*, 1988) have been correlated with odontoblast terminal differentiation.

Predentin is traversed by odontoblast cell processes and in mature odontoblasts two levels of secretion probably exist (Fig. 5): a proximal one adjacent to odontoblast cell bodies where predentin components are exocytosed and a distal one at the mineralization front (see Linde in this issue).

Functional odontoblasts demonstrate intercellular junctions (see Baume, 1980 for review) and the problem of the existence of a permeability barrier in the odontoblast layer has been recently discussed (Bishop, 1985; Bishop and Yoshiba, 1992). The classical problem of the von Korff fibres, i.e. the presence of collagen fibres in between functional odontoblasts, is not yet definitively resolved (see Ten Cate, 1978, 1989; Meyer *et al.*, 1988; Bishop *et al.*, 1991, Bishop and Yoshiba, 1992; Yoshiba *et al.*, 1994). All odontoblasts are not strictly equivalent. As a matter of fact, significant cytological and functional differences exist between mouse labial and lingual incisor odontoblasts (Beertsen and Niehof, 1986). Odontoblasts also undergo age changes (Couve, 1986).

Epigenesis of odontoblast terminal differentiation

Preodontoblasts never differentiate when dental papillae, isolated by treatment with proteolytic enzymes, are either cultured *in vitro* or grafted *in vivo* (Kollar and Baird, 1970; Ruch and Karcher-Djuricic, 1971b; Kollar, 1972; Ruch *et al.*, 1973). Specific epigenetic signals trigger odontoblast terminal differentiation.

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 transfilter 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, epithelia. 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

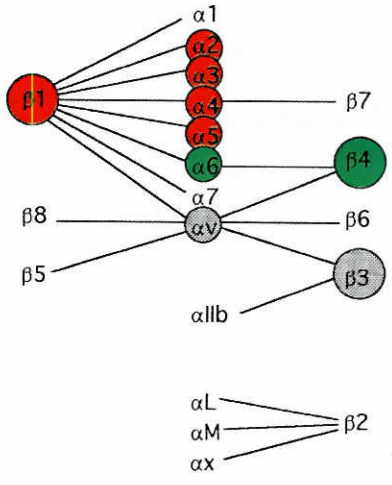


Fig. 9. α and ß subunits of integrins. *Immunostaining for ß1 chain was negative with all different antibodies directed against this antigen. This result was also confirmed when staining for α2, α3, α4 and ß5 chains: no signal was detected. Staining for αv and ß3 was very weak and ubiquitous. Only antibodies directed against α6 and ß4 chains gave strong positive signals and stained the enamel organ (Lesot et al., 1993; Meyer et al., 1995). Immunolocalization of integrins was performed on developing tooth germs sections using the following antibodies: rabbit antisera to α2 Ctal peptide (Takada and Hemler, 1989) and anti-α4 Ctal peptide (Chan et al., 1991) were obtained from Dr. M.E. Hemler, anti α3 peptide P17 (ab85) and anti-α5 (ab33 rabbit anti FNR-pept P2) were provided by Dr. McDonald (Roman et al., 1991). Rabbit antibodies to ß1 chain of integrin were provided by Dr. S. Akiyama (Akiyama and Yamada, 1987) and Dr. R.O. Hynes (Marcantonio and Hynes, 1988). Rabbit antisera directed against synthetic peptides from ßv, ß1, and ß3 were kind gifts from Dr. L.F. Reichardt (Bossy and Reichardt, 1990; de Curtis et al., 1991). Rat monoclonal antibodies directed against α6 (135-113C) and ß4 (346-11A) chains were provided by Dr. S. Kennel (Kennel et al., 1989).*

(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 dental basement membrane plays a role in odontoblast differentiation

Dissociation of tooth components by means of proteolytic enzymes such as trypsin, resulted in the degradation of the basement membrane. Recombination of such tissues allowed the in *vitro* restoration of a continuous basement membrane within 15-18 h (Meyer *et al.*, 1978). This redeposition has been shown to be a prerequisite for odontoblast differentiation (Karcher-Djuricic *et al.*, 1979; Thesleff *et al.*, 1978) and the inhibitory effects of diazo-norleucine, vitamin A and tunicamicyn on odontoblast differentiation seem to be the outcome of basement membrane alterations (Hurmerinta *et al.*, 1979, 1980; Thesleff and Pratt, 1980).

The traditional point of view implying an epithelial origin for the main basal lamina components such as collagen type IV, laminin, nidogen and basal lamina proteoglycan has to be revised. It is now clear that embryonic basal laminae are assembled from compo-

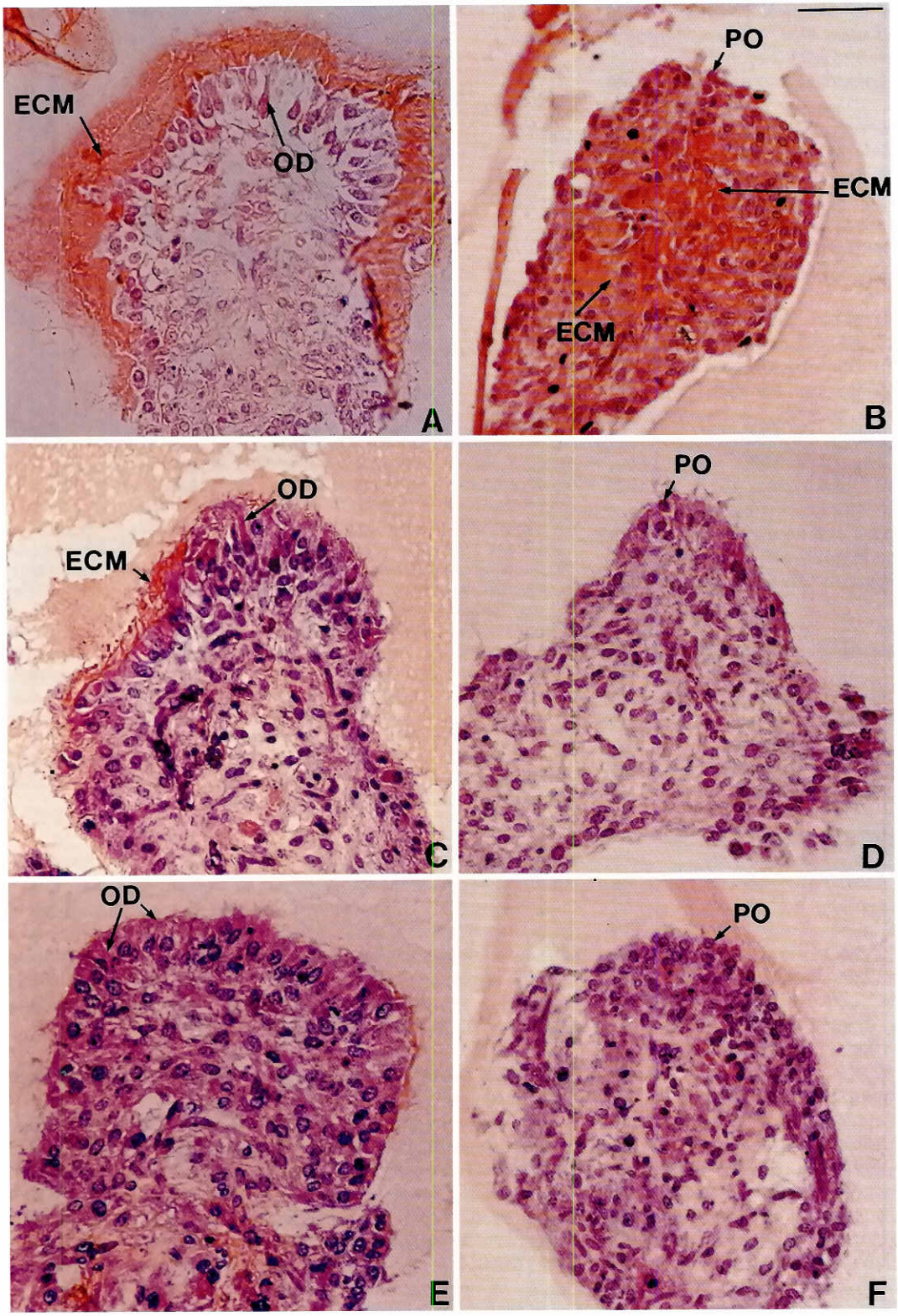


Fig. 10. Sections of dental papillae isolated by trypsin treatment from day-17 (p.c.) mouse mandibular molars and cultured for 6 days in semi-solid medium containingTGFß1, BMP2 or IGF1 either combined with heparin (HN) or alone. (A) *TGFß1-HN: extended differentiation of functional odontoblast-like cells (OD), with apical accumulation of extracellular matrix (ECM).* **(B)** *TGFß1 alone: intrapulpal accumulation of ECM. PO: preodontoblasts.* **(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.*

nents derived from epithelial and mesenchymal cells (Marinkovich *et al.*, 1993; Thomas and Dziadek, 1993). 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.

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

(Lesot *et al.*, 1981; Thesleff *et al.*, 1981; Kubler *et al.*, 1988). On the other hand, ruthenium red staining of basement membrane components revealed a changing pattern of highly ordered, symmetric arrays of anionic polysaccharides on each side of the lamina densa (Meyer *et al.*, 1981a). Changes concerning components of the lamina diffusa precede and/or accompany terminal differentiation of odontoblasts: radioautographic data concerning ^{3}H-glucosamine (Osman and Ruch, 1981b) and ^{35}Sulfate (Lau and Ruch, 1983; Lau *et al.*, 1983) incorporation in dental basement membrane as well as immunolocalization using specific monoclonal antibodies identifying different epitopes of chondroitin-4 and -6 sulfates (Mark *et al.*, 1990; see also Kogaya and Furuhashi, 1987 and Kogaya *et al.*, 1990) demonstrated stage-space specific partially mesenchyme-dependent, distribution patterns. Furthermore, during odontoblast terminal differentiation collagen type III disappeared from the epithelial-mesenchymal junction and fibronectin, which surrounded preodontoblasts, accumulated at the apical pole of polarizing cells (Lesot *et al.*, 1981; Thesleff *et al.*, 1981; Meyer *et al.*, 1989; Yoshiba *et al.*, 1994). Decorin, also present at the epithelio-mesenchymal junction, was found to change its distribution during odontoblast polarization similarly to fibronectin (Fig. 6).

Basement membrane associated tenascin (Thesleff *et al.*, 1987) increased during terminal differentiation and epithelially derived amelogenin(s) were also shown to be associated with the basement membrane during terminal differentiation of odontoblasts (Slavkin *et al.*, 1988; Inai *et al.*, 1991; Uchida *et al.*, 1991; Nakamura *et al.*, 1994).

A stage- and space-specific basement membrane is involved in odontoblast differentiation

Possible correlation between basement membrane reconstitution and odontoblast cytodifferentiation was analyzed in isochronal and heterochronal recombinations of trypsin isolated mouse molar enamel organs and dental papillae (Karcher-Djuricic *et al.*, 1979):

day-18 first lower mouse molars containing the first post-mitotic odontoblasts at the tip of the main cusps and day-16 molars containing dividing preodontoblasts were used: in day-18 dental papilla-day-18 enamel organ recombinations, a continuous basement membrane was restored and functional odontoblasts were observed after 24 h. When day-18 dental papillae were combined with day-16 enamel organs, a continuous basement membrane was also observed after 24 h, however, funtional odontoblasts were seen only after 4 days. This chronology was similar to that observed when culturing intact day-16 molars. Day-18 (and day-16 after 4 days *in vitro*) inner dental epitheliae seem to be involved in specific basement membrane modifications allowing initiation of odontoblast differentiation.

Day-18 first left and right molars were also treated respectively with either EDTA or trypsin. Under both conditions, the enamel organ could be dissociated from dental papilla (Osman and Ruch, 1981a). However EDTA treatment removed the basement membrane from the basal surface of the inner dental epithelium, and left it associated with the dental papillae, while trypsin treatment completely hydrolyzed the basal lamina. These dental papillae were cultured *in vitro* for up to 24 h. No odontoblasts differentiated in the trypsin-isolated dental papillae. The EDTA-isolated dental papillae remained covered by the basement membrane for at least 15-18 h. During this period, functional odontoblasts emerged only at the top of the main cusps and the gradient of differentiation was abolished. In intact day-18 molars, the presumed operational basement membrane is located strictly at the top of the main cusps and epithelially controlled modifications proceed in an apical direction. In EDTA-isolated dental papillae, the specific 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); FGF R_1 (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) ;
- TGFß superfamily: TGFß 1, 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 Vaahtokari, 1992; Heikinheimo *et al.*, 1993a); and $BMP_{2,4,6}$ (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 (Partanen 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 ^{3}H-thymidine radioautography of *in vitro* cultured heterochronal enamel organ - dental papilla recombinations (Ruch and Karcher-Djuricic, 1971b; Ruch *et al.*, 1976) led to the following observations:

- Day-16 preodontoblasts recombined with day-18 inner dental epithelium did not give 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 preodontoblasts held in the G_1.S interface by fluorodeoxyuridine (Ruch and Karcher-Djuricic, 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 preodontoblasts 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.

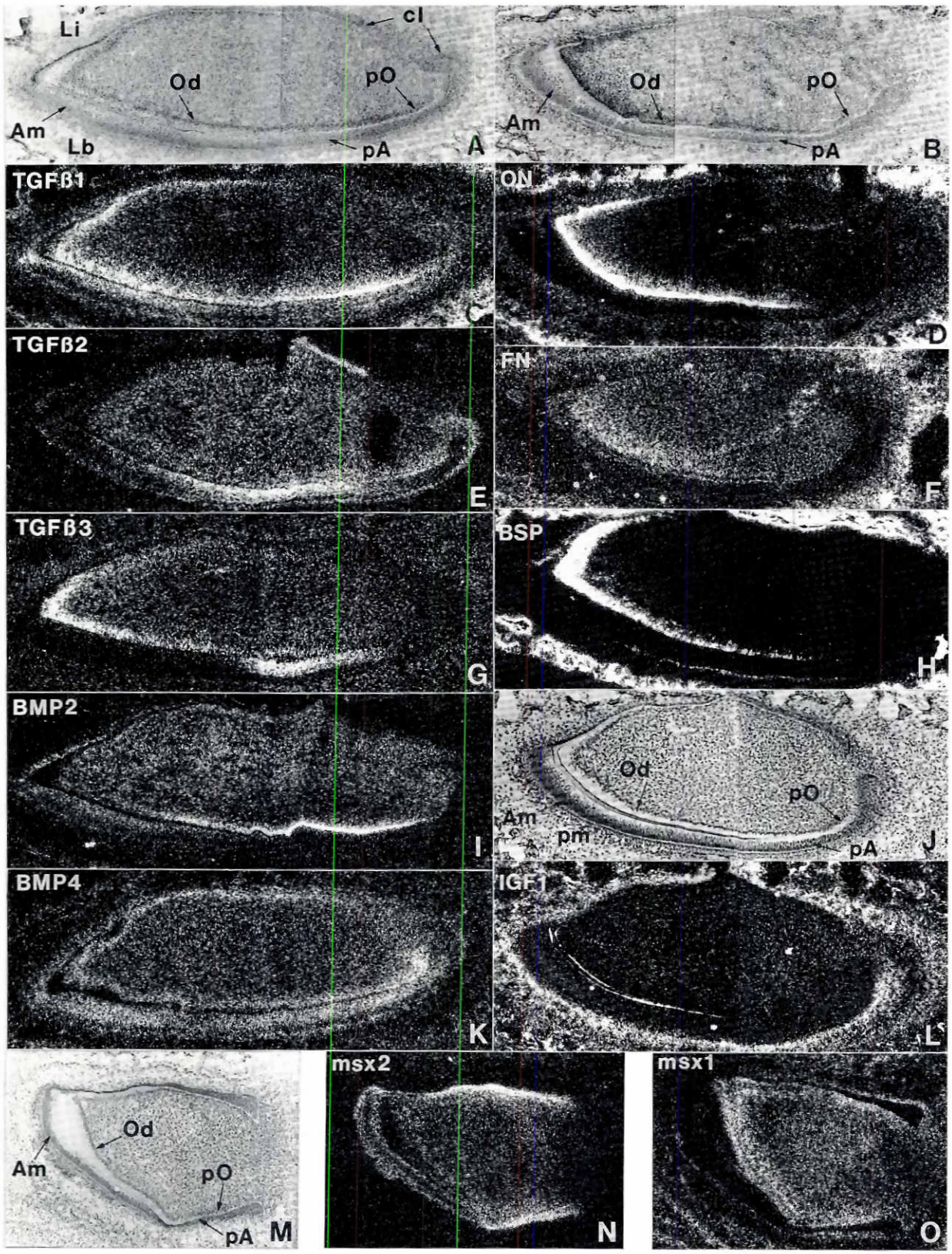


Fig. 11. TGFß1, 2, 3; BMP2, 4; osteonectin (ON); fibronectin (FN; bone sialoprotein (BSP); IGF1; and msx1 and msx2 transcripts visualized by *in situ* hybridization on sets (A,C,E,G,I,K; B,D,F,H; J,L; M,N,O) of consecutive sections of first lower incisors of new bone mice. *For details see Bègue-Kirn et al. (1994). Am, ameloblasts; PA, preameloblasts; PO, preodontoblasts; Od, odontoblasts; Li, lingual; Lb, labial; cl, cervical loop.*

TABLE 1

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

	in vivo	*in vitro*			
probes	tooth germs	EDTAsol.Fr	TGFß1-HN	BMP2-HN	IGF1-HN
TGFß1	++	++	+	+	-
TGFß2	~	-	-	-	-
TGFß3	++	++	+	~	~
BMP2	++	+	+	+	+
BMP4	+	++	++	++	++
IGF1	-	~	-	-	-
msx1	++	~	~	-	~
msx2	~	++	+	+	+
FN	-	-	-	-	-
ON	++	++	++	+	+
BSP	++	++	++	++	+

++, strong positive signal; +, positive signal; ~, weak "ubiquitous" signal; -, no signal. FN, fibronectin; ON, osteonectin; BSP, bone sialoprotein; EDTAsol.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 modulations 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.

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 ß1 subunit of integrins could interact with the microfilament system by means of either talin (Horwitz *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.*, 1988). 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, zonulae adherens and gap-junctions increases (Iguchi et al., 1984; Bishop, 1985; Callé, 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 (Fausser *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 ß3αIIb. 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 ß1α5, ß1αv, ß3αIIb, ß3αv and ß6αv integrins which are all RGD-dependent (Hynes, 1992). The 165 kDa protein was found to interact with 62 kDa proteolytic 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 ß4 and α6 chains of integrins while no reaction was

TABLE 2

EXPRESSION OF SEVERAL TRANSCRIPTS BY PREODONTOBLASTS *IN VIVO* VERSUS POTENTIAL ODONTOBLASTS CULTURED *IN VITRO* IN THE PRESENCE OF POTENTIAL INDUCERS

	in vivo	*in vitro*			
probes	tooth germs	EDTAsol.Fr	TGFß1-HN	BMP2-HN	IGF1-HN
TGFß1	+	++	+	+	+
TGFß2	~/+	+	~	~	~
TGFß3	-	~	~	~	~
BMP2	+	+	+	~	++
msx1	~	+	+	+	+
msx2	++	+	~	+	++

++, strong positive signal; +, positive signal; ~, weak "ubiquitous" signal; -, no signal; EDTAsol.Fr, EDTA soluble fraction.

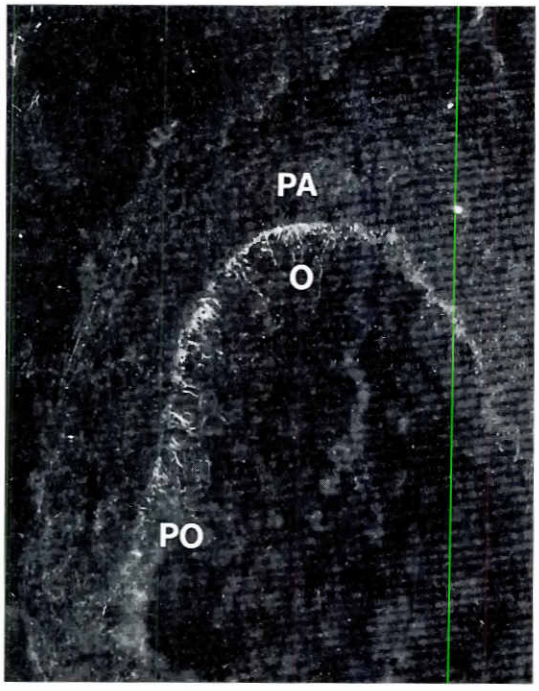


Fig. 12. Immunofluorescence localization of latent TGFß binding protein (LTBP) on frontal section of day-18 mandibular mouse molar. *LTBP is associated with the basement membrane facing preodontoblasts (PO) as well as odontoblasts (O). PA, preameloblasts (the specific antibody was generously provided by Dr. K. Miyazono, Ludwig Institute for Cancer Research, Upsala, Sweden).*

observed when using anti-ß1 antibodies (Lesot *et al.*, 1993; Meyer *et al.*, 1995). Although the specificity of α6ß4 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 (Tziafas *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 maintened *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 fibronectin 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 TGFßs, BMPs and IGFs (Finkelman *et al.*, 1990) and indeed the addition of a blocking TGFß 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 TGFß1, BMP2 and IGF1 on odontoblast differentiation

In the presence of TGFß1, BMP2 or IGF1, added alone to the agar solidified culture medium, the preodontoblasts of isolated dental papillae never overtly differentiated. When combined with heparin (or fibronectin) these growth factors had positive, albeit differential, effects (Bègue-Kirn *et al.*, 1994): TGFß1-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 TGFßs, BMPs, IGF, *msxs*, 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 TGFß1-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 TGFßs 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 TGFß 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 continous, reciprocal epithelial-mesenchymal interactions, is characterized by several steps, implying withdrawal from the cell cycle, elonga-

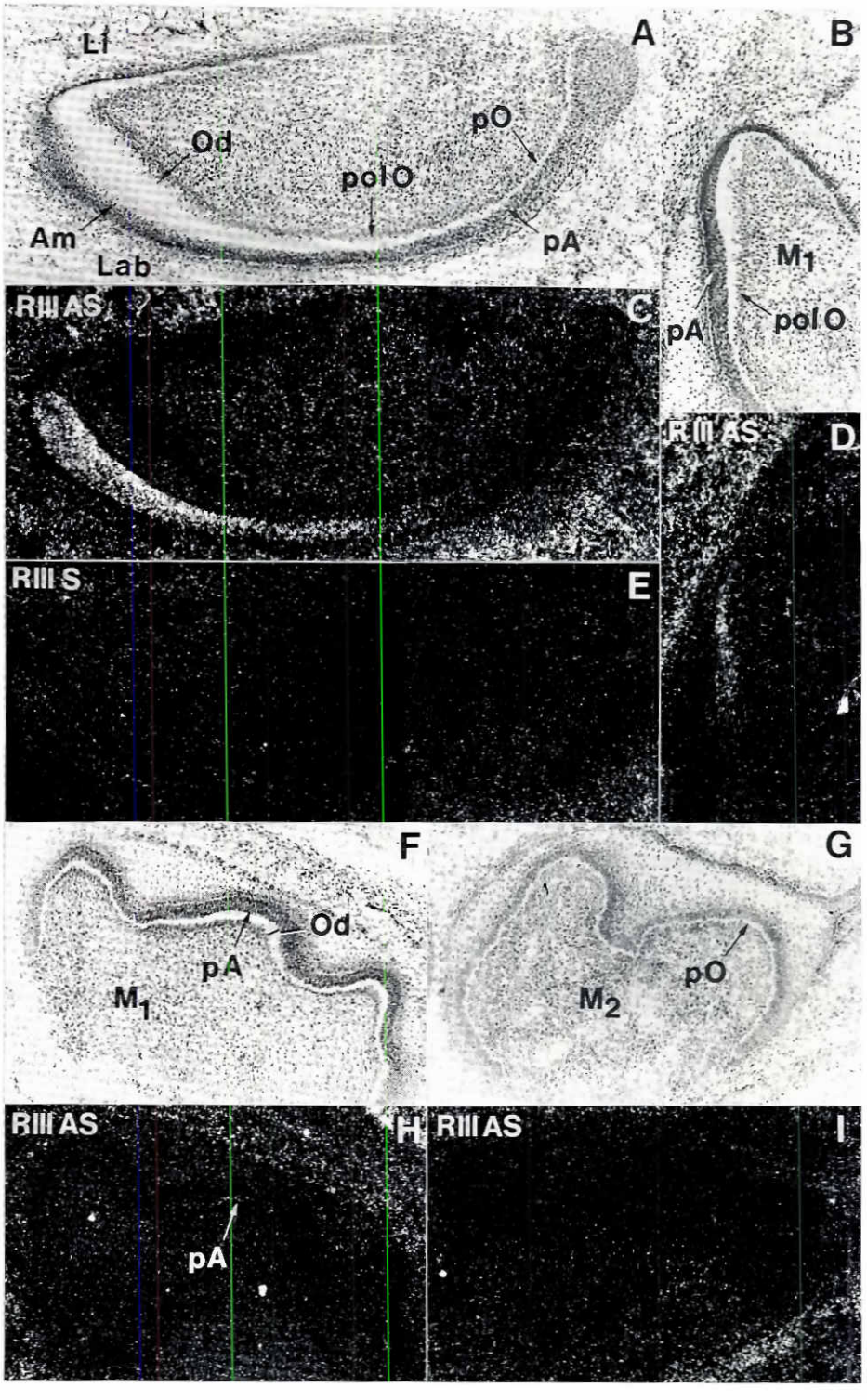


Fig. 13. RNA expression of TGFß type III receptor (RIII) in mandibular incisor (A,C) first (M1) (B,D) and second (M2) molars (G,I) of newborn rat and in first lower molar of newborn mouse (F,H). (A,B,F,G) *Bright-field images of* **(C,D,H,I)** *respectively. RIII mRNAs are expressed by preameloblasts (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 pA 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 (RIIIS)* **(E)**. *The rat RIII probe was generously provided by Dr. Weinberg of the Whithehead Institute, Cambridge, USA and hybridization steps were performed according to Bègue-Kirn et al. (1994). Od, odontoblasts; Li, lingual; Lab, labial; RIIIAS, TGFß 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 supports the hypothesis that a functional network consisting of matrix molecules including fibronectin and growth factors, including members of the TGFß 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 (Bègue-Kirn *et al.*, 1994) strongly suggests that immobilization of active growth factors (TGFß1, 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

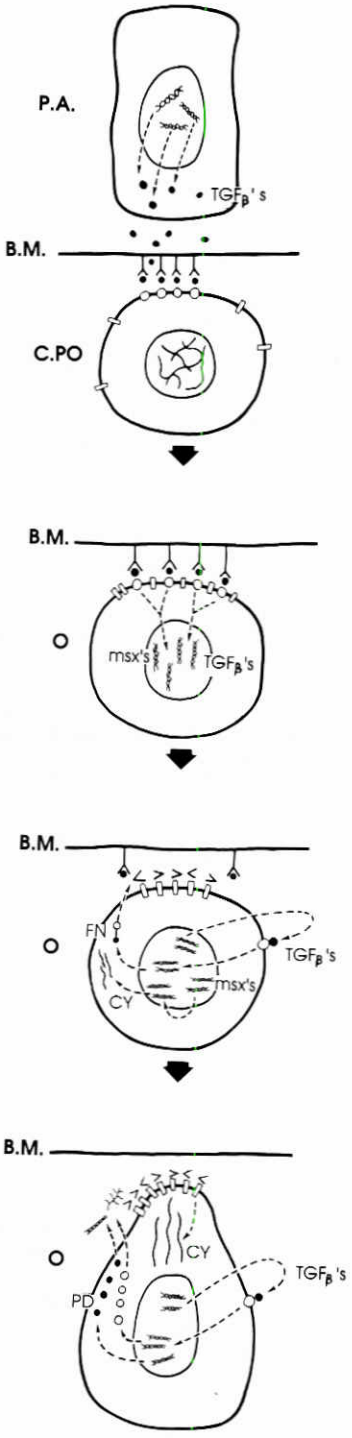


Fig. 14. Working hypothesis. *Members of the TGFß family (●) secreted by preameloblasts (PA) are trapped and activated by basement membrane (BM) associated components (λ) having an epithelial or mesenchymal origin. TGFßs interact with membrane receptors (○) expressed at the surface of competent preodontoblasts (PO).*

Transduction of signals occurs which leads to an up-regulation of TGFßs and msxs genes and capping of the 165 KDa fibronectin (FN) interacting membrane protein (▯).

Up-regulation of TGFßs leads to synthesis of FN (<) interacting with the 165 KDa protein (▯). Msxs homeoproteins might regulate transcription of cytoskeleton related genes.

The FN-165 KDa complex transduces signals leading to reorganization of the cytoskeleton (cy) allowing polarization. The endogenous TGFßs regulate transcription-translation of predentin (PD) components which are secreted at the apical pole.

accumulation. Immobilization of TGFß 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 TGFß-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 TGFß 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 (Bègue-Kirn *et al.*, 1994 and references therein) demonstrate that transcripts of genes of the TGFß 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.*, 1993). TGFß-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 TGFß1 by proteolysis is an important regulator of activity for the TGFß subclass of molecules (Kingsley, 1994).

Furthermore, latent TGFß-binding protein (LTBP) may be involved in targeting (Kanzaki *et al.*, 1990) or activation (Flaumenhaft *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 TGFß binding proteins (decorin, ß-glycan, bi-glycan) and TGFß activating/inhibiting molecules are of crucial importance. Most interestingly the transcripts for the ß-glycan (receptor TGFß type III) were localized in preameloblasts facing preodontoblasts able to differentiate (Bègue-Kirn *et al.*, unpublished data) (Fig. 13). The expression and distribution of TGFß 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) epithelially derived member(s) of the TGFß 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 TGFß 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 TGFß 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

withdrawl 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 epithelially-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 epithelially derived paracrine factors, putatively involved in odontoblast terminal differentiation and the application of adequate 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 temporo-spatial patterns and secrete predentin-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 significative 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 TGFß family. Histological aspects and the transcriptional phenotypes (transcripts of TGFßs, BMPs, msxs, IGF1, fibronectin, osteonectin, bone sialoprotein genes) are very similar *in vivo* and *in vitro*. *In vivo* members of the TGFß super family secreted by preameloblasts, trapped and activated by basement membrane associated components, might initiate odontoblast terminal differentiation.

KEY WORDS: *odontoblast, differentiation, TGFßs, BMPs, msxs*

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References

AHMAD, N. and RUCH, J.V. (1987). Comparison of growth and cell proliferation kinetics during mouse molar odontogenesis *in vivo* and *in vitro*. *Cell Tissue Kinet. 20:* 319-329.

AKIYAMA, S.K. and YAMADA, K.M. (1987). Biosynthesis and acquisition of biological activity of the fibronectin receptor. *J. Biol. Chem. 262:* 17536-17542.

BAUME, L.J. (1980). *The Biology of Pulp and Dentine Monographs in Oral Science* (Ed. H.M. Myers). S. Karger, Basel, *8:* 1-246.

BÈGUE-KIRN, C., SMITH, A., LORIOT, M., KUPFERLE, C., RUCH, J.V. and LESOT, H. (1994). Comparative analysis of TGFßs, BMPs, IGF, msxs, fibronectin, osteonectin and bone sialoprotein gene expressions during normal and *in vitro* induced odontoblast differentiation. *Int. J. Dev. Biol.* 38: 405-420.

BÈGUE-KIRN, C., SMITH, A.J., RUCH, J.V., WOZNEY, J.M., PURCHIO, A., HARTMAN, D. and LESOT, H. (1992). Effect of dentin proteins, transforming growth factor $ß_1$ ($TGFß_1$) and bone morphogenetic protein 2 (BMP_2) on the differentiation of odontoblasts *in vitro*. *Int. J. Dev. Biol. 36:* 491-503.

BEERTSEN, W. and NIEHOF, A. (1986). Root-analogue versus crown-analogue dentin: a radioautographic and ultrastructural investigation of the mouse incisor. *Anat. Rec. 215:* 106-118.

BISHOP, M.A. (1985). Evidence for tight junctions between odontoblasts in the rat incisor. *Cell Tissue Res. 239:* 137-140.

BISHOP, M.A. and YOSHIBA, S. (1992). A permeability barrier to lanthanum and the presence of collagen between odontoblasts in pig molars. *J. Anat. 181:* 29-39.

BISHOP, M.A., MALHOTRA, M. and YOSHIBA, S. (1991). Interodontoblastic collagen (von Korff fibres) and circumpulpal dentin formation: an ultrathin serial section study in the cat. *Am. J. Anat. 191:* 67-73.

BLOCH-ZUPAN, A., DECIMO, D., LORIOT, M., MARK, M.P. and RUCH, J.V. (1994a). Expression of nuclear retinoic acid receptors during mouse odontogenesis. *Differentiation 57: 195-203.*

BLOCH-ZUPAN, A., MARK, M.P., WEBER, B. and RUCH, J.V. (1994b). *In vitro* effects of retinoic acid on mouse incisor development. *Arch. Oral Biol. 39:* 891-900.

BOSSY, B. and REICHARDT, L.F. (1990). Chick integrin αv subunit molecular analysis reveals high conservation of structural domains and association with multiple ß subunits in embryo fibroblast. *Biochemistry 29:* 10191-10198.

BRONCKERS, A.L.J.J., GAY, S., FINKELMAN, R.D. and BUTLER, W.T. (1987). Immunolocalization of Gla Proteins (osteocalcin) in rat tooth germs: comparison between indirect immuno-fluorescence, peroxidase-antiperoxidase, avidin-biotin-peroxidase complex and avidin-biotin-gold complex with silver enhancement. *J. Histochem. Cytochem. 35:* 825-830.

BRONNER-FRASER, M., STERN, C.D. and FRASER, S. (1991). Analysis of neural crest cell lineage and migration. *J. Craniofac. Genet. Dev. Biol. 11:* 214-222.

BUTLER, W.T. (1989). The nature and significance of osteopontin. *Connect. Tissue Res. 23:* 123-136.

BYERS, M.R., SCHATTEMAN, G.C. and BOTHWELL, M. (1990). Multiple functions for NGF receptor in developing, aging and injured teeth are suggested by epithelial, mesenchymal and neural immunoreactivity. *Development 109:* 464-471.

CALLÉ, A. (1985). Intercellular junctions between human odontoblasts. A freeze-fracture study after demineralization. *Acta Anat. 122:* 138-144.

CAM, Y., MEYER, J.M., STAUBLI, A. and RUCH, J.V. (1986). Epithelial-mesenchymal interactions: effects of a dental biomatrix on odontoblasts. *Arch. Anat. Microsc. Morphol. Exp. 75:* 75-89.

CAM, Y., NEUMANN, M.R. and RUCH, J.V. (1990). Immunolocalization of TGFß1 and EGF-receptor epitopes in mouse incisors and molars. Demonstration of in vitro production of transforming activity. *Arch. Oral Biol. 35:* 813-822.

CAM, Y., NEUMANN, M.R., OLIVER, L., ROULAIS, D., JANET, T. and RUCH, J.V. (1992). Immunolocalization of acidic and basic fibroblast growth factors during mouse odontogenesis. *Int. J. Dev. Biol. 36:* 381-389.

CHAN, B., WONG, J., RAO, A. and HEMLER, M.E. (1991). T cell receptor-dependent, antigen-specific stimulation of a murine T cell clone induces a transient, VLA protein-mediated binding to extracellular matrix. *J. Immunol. 147:* 398-404.

CHEN, J., SHAPIRO, H.S., WRANA, J.L., REIMERS, S., HEERSCHE, J.N.M. and SODEK, J. (1991). Localization of bone sialoprotein (BSP) expression to sites of mineralized tissue formation in fetal rat tissue by *in situ* hybridization. *Matrix 11:* 133-143.

COULY, G. and LEDOUARIN, N.M. (1988). The fate map of the cephalic neural primordium at the presomitic to the 3-somite stage in the avian embryo. *Development 103:* 101-113.

COUVE, E. (1986). Ultrastructural changes during the life cycle of human odontoblasts. *Arch. Oral Biol. 31:* 643-651.

D'SOUZA, R.N., BRONCKERS, A.L.J.J., HAPPONEN, R.P., DOGA, D.A., FARACH-CARSON, M.C. and BUTLER, W.T. (1992). Developmental expression of a 53 kDa dentin sialoprotein in rat tooth organs. *J. Histochem. Cytochem. 40:* 359-366.

D'SOUZA, R.N., HAPPONEN, R.P., RITTER, N.M. and BUTLER, W.T. (1990). Temporal and spatial patterns of transforming growth factor ß1 expression in developing rat molars. *Arch. Oral Biol. 35:* 957-965.

DASCH, J.R., PACE, D.R., WEAGELL, W., INENAGA, D. and ELLINGWORTH, L. (1989). Monoclonal antibodies recognizing transforming growth factor ß. *J. Immunol. 142:* 1536-1541.

DE CURTIS, I., QUARANTA, V., TAMURA, R.N. and REICHARDT, L.F. (1991). Laminin receptors in the retina: sequence analysis of the integrin α6 subunit. *J. Cell Biol. 113:* 405-416.

FAUSSER, J.L., LESOT, H., ZIDAN, G. and RUCH, J.V. (1990). Characterization of a mouse monoclonal antibody to vimentin by indirect immunofluorescence microscopy and immunoblotting. *J. Biol. Buccale 18:* 29-33.

FAUSSER, J.L., STAUB, A., UNGEWICKELL, E., RUCH, J.V. and LESOT, H. (1993a). Absence of interaction between the 165 kDa fibronectin-binding protein involved in mouse odontoblast differentiation and vinculin. *Arch. Oral Biol. 38:* 537-540.

FAUSSER, J.L., UNGEWICKELL, E., RUCH, J.V. and LESOT, H. (1993b). Interaction of vinculin with the clathrin heavy chain. *J. Biochem. 114:* 498-503.

FFRENCH-CONSTANT, C. (1994). How do embryonic cells measure time? *Curr. Biol. 4:* 415-419.

FINKELMAN, R.D., MOHAN, S., JENNINGS, J.C., TAYLOR, A.K., JEPSEN, S. and BAYLINK, D.J. (1990). Quantitation of growth factors IGF-I, SGF/IGF-II and TGF-ß in human dentin. *J. Bone Miner. Res. 5:* 717-723.

FISHER, L.W., WHITSON, S.W., AVIOLI, L.V. and TERMINE, J.D. (1983). Matrix sialoprotein of developing bone. *J. Biol. Chem. 258:* 12723-12727.

FLAUMENHAFT, R., ABE M., SATO, Y., MIYAZONO, K., HARPEL, J., HELDIN, C. and RIFKIN, D.B. (1993). Role of latent TGF-ß binding protein in the activation of latent TGFß by co-cultures of endothelial and smooth muscle cells. *J. Cell Biol. 120:* 995-1002.

FUJISAWA, R. and KUBOKI, Y. (1988). Increase of dentin phosphophoryn with dentin formation. *Connect. Tissue Res. 17:* 231-238.

FUJISAWA, R. and KUBOKI, Y. (1989). Changes in the level of osteonectin in bovine dentin during tooth development. *Arch. Oral Biol. 34:* 89-92.

GONZALES, A.M., BUSCAGLIA, M., ONG, M. and BAIRD, D. (1990). Distribution of basic fibroblast growth factor in the 18-day rat fetus: localization in the basement membranes of diverse tissues. *J. Cell Biol. 110:* 753-765.

HEIKINHEIMO, K. (1994). Stage specific expression of decapentaplegic-Vg-related genes 2, 4 and 6 (bone morphogenetic proteins 2, 4 and 6) during human tooth morphogenesis. *J. Dent. Res. 73:* 530-597.

HEIKINHEIMO, K., HAPPONEN, R.P., MIETTINEN, P.J. and RITVOS, O. (1993a). Transforming growth factor ß2 in epithelial differentiation of developing teeth and odontogenic tumors. *J. Clin. Invest. 91:* 1019-1027.

HEIKINHEIMO, K., VOUTILAINEN, R., HAPPONEN, R.P. and MIETTINEN, P.J. (1993b). EGF receptor and its ligands, EGF and TGFα in developing and neoplastic odontogenic tissues. *Int. J. Dev. Biol. 37:* 387-396.

HOLTZER, H., BIEHL, B.A. and HOLTZER, S. (1985). Induction dependent and lineage dependent models for cell diversification are mutually exclusive. In *Advances in Neuroblastoma Research* (Ed. J. Reinert). A.R. Liss, New York, pp. 2-11.

HORWITZ, A., DUGGAN, K., BUCK, C., BECKERLE, M.C. and BURRIDGE, K. (1986). Interaction of plasma membrane fibronectin receptor with talin: a transmembrane linkage. *Nature 320:* 531-533.

HU, C.C., SAKAKURA, Y., SASANO, Y., SHUM, L., BRINGAS, P., WERB, Z. and SLAVKIN, H.C. (1992). Endogenous epidermal growth factor regulates the timing and pattern of embryonic mouse molar tooth morphogenesis. *Int. J. Dev. Biol. 36:* 505-516.

HUGGINS, C.R., McCARROL, M.D. and DAHLBERG, A.A. (1934). Transplantation of tooth germ elements and the experimental heterotopic formation of dentin and enamel. *J. Exp. Med. 60:* 199-210.

HURMERINTA, K., THESLEFF, I. and SAXÉN, L. (1979). Inhibition of tooth germ differentiation *in vitro* by diazo-oxo-norleucine. *J. Embryol. Exp. Morphol. 50:* 99-106.

HURMERINTA, K., THESLEFF, I. and SAXÉN, L. (1980). *in vitro* inhibition of odontoblast differentiation by vitamin A. *Arch. Oral Biol. 25:* 385-391.

HYNES, R.O. (1992). Integrins: versatility, modulation, and signalling in cell adhesion. *Cell 69:* 11-25.

IGUCHI, Y., YAMAMURA, T., ICHIKAWA, T., HACHIMOTO, S., HORIUCHI, T. and SHIMONO, M. (1984). Intercellular junctions in odontoblasts of the rat incisor studied with freeze-fracture. *Arch. Oral Biol. 29:* 487-497.

INAI, T., KUKITA, T., OHSAKI, Y., NAGATA, K., KUKITA, A. and KURISU, K. (1991). Immunohistochemical demonstration of amelogenin penetration toward the dental pulp in the early stages of ameloblast development in rat molar tooth germs. *Anat. Rec. 229:* 259-270.

JOSEPH, B.K., SAVAGE, N.W., YOUNG, W.G., GUPTA, G.S., BREIER, B.H. and WATERS, M.J. (1993). Expression and regulation of insulin-like growth factor-1 in the rat incisor. *Growth Factors 8:* 267-275.

KANZAKI, T., OLOFSSON, A., MOREN, A., WERNSTEDT, C., HELLMAN, U., MIYAZONO, K., CLAESSON-WELSH, L. and HELDIN, C.H. (1990). TGFß1 binding-protein: a component of the large latent complex of TGFß1 with multiple repeat sequences. *Cell 61:* 1051-1061.

KARCHER-DJURICIC, V., OSMAN, M., MEYER, J.M., STAUBLI, A. and RUCH, J.V. (1979). Basement membrane reconstitution and cytodifferentiation of odontoblast in isochronal and heterochronal reassociations of enamel organs and pulps. *J. Biol. Buccale 6:* 257-265.

KARCHER-DJURICIC, V., STAUBLI, A., MEYER, J.M. and RUCH, J.V. (1985). Acellular dental mactrices promote functional differentiation of ameloblast. *Differentiation 29:* 169-175.

KENNEL, S., FOOTE, L.J., FALCIONI, R., SONNENBERG, A., STRINGER, C.D., CROUSE, C. and HEMLER, M. (1989). Analysis of the tumor-associated antigen TSP-180. Identity with α6-ß4 in the integrin superfamily. *J. Biol. Chem. 264:* 15515-15521.

KINGSLEY, D.M. (1994). The TGF-ß superfamily: new members, new receptors, and new genetic tests of function in different organisms. *Genes Dev. 8:* 133-146.

KOCH, W.E. (1967). *In vitro* differentiation of tooth rudiments of embryonic mice: I. Transfilter interaction of embryonic incisor tissues. *J. Exp. Zool. 165:* 155-170.

KOGAYA, Y. and FURUHASHI, K. (1987). Ultrastructural distribution of sulfated glycosaminoglycans in epithelial-mesenchymal interface of developing rat tooth germs. *J. Histochem. Cytochem. 35:* 585-593.

KOGAYA, Y., KIM, S. and AKISAKA, T. (1990). Changes in ultrastructural distribution of dental basement membrane heparan sulfate during early mouse tooth development. *J. Biol. Buccale 18:* 109-115.

KOLLAR, E.J. (1972). The development of the integument: spatial, temporal and phylogenetic events. *Am. Zool. 12:* 125-135.

KOLLAR, E.J. and BAIRD, G. (1970). Tissue interactions in embryonic mouse tooth germs. II. The induction role of the dental papilla. *J. Embryol. Exp. Morphol. 24:* 173-184.

KRONMILLER, J.E., UPHOLT, W.B. and KOLLAR, E.J. (1991). EGF antisense oligodeoxynucleotides block murine odontogenesis *in vitro*. *Dev. Biol. 147:* 485-488.

KUBLER, M.D., LESOT, H. and RUCH, J.V. (1988). Temporo-spatial distribution of matrix and microfilament components during odontoblast and ameloblast differentiation. *Roux Arch. Dev. Biol. 197:* 212-220.

LAU, E.C. and RUCH, J.V. (1983). Glycosaminoglycans in embryonic mouse teeth and the dissociated dental constituents. *Differentiation 23:* 234-242.

LAU, E.C., BOUKARI, A., ARECHAGA, J., OSMAN, M. and RUCH, J.V. (1983). 35S-Autoradiographic study of sulfated gag accumulation and turnover in embryonic mouse tooth germ. *J. Craniofac. Genet. Dev. Biol. 3:* 117-131.

LEE, E.C., LOTZ, M.M., STEELE, G.D. and MERCURIO, A.M. (1992). The integrin a6ß4 is a laminin receptor. *J. Cell Biol. 117:* 671-678.

LEHNERT, S.A. and AKHURST, R.J. (1988). Embryonic expression pattern of TGFß type 1 RNA suggests both paracrine and autocrine mechanisms of action. *Development 104:* 263-273.

LESOT, H., BÈGUE-KIRN, C., KUBLER, M.D., MEYER, J.M., SMITH, A.J., CASSIDY, N. and RUCH, J.V. (1993). Experimental induction of odontoblast differentiation and stimulation during reparative processes. *Cell. Mater. 3: 201-217.*

LESOT, H., FAUSSER, J.L., AKIYAMA, S.K., STAUB, A., BLACK, D., KUBLER, M.D. and RUCH, J.V. (1992). The carboxy-terminal extension of the collagen binding domain of fibronectin mediates interaction with a 165 kDa membrane protein involved in odontoblast differentiation. *Differentiation 49:* 109-118.

LESOT, H., KARCHER-DJURICIC, V., KUBLER, M.D. and RUCH, J.V. (1988). Membrane-cytoskeleton interactions: inhibition of odontoblast differentiation by a monoclonal antibody directed against a membrane protein. *Differentiation 37:* 62-72.

LESOT, H., KARCHER-DJURICIC, V., MEYER, J.M. and RUCH, J.V. (1985a). Dental cell interaction with extracellular matrix constituents: type I collagen and fibronectin. *Differentiation 29:* 171-181.

LESOT, H., KUBLER, M.D., FAUSSER, J.L. and RUCH, J.V. (1990). A 165 kDa membrane antigen mediating fibronectin-vinculin interaction is involved in the murine odontoblast differentiation. *Differentiation 44:* 25-35.

LESOT, H., MEYER, J.M., KARCHER-DJURICIC, V., AHMAD, N., STAUBLI, A., IDRISS, H., MARK, M., BOUKARI, A. and RUCH, J.V. (1985b). Influences of matrix molecules or dental matrices on dental cell kinetics and cytodifferentiation. In *Tooth Morphogenesis and Differentiation* (Eds. A. Belcourt and J.V. Ruch). Editions INSERM, Paris, pp. 89-108.

LESOT, H., MEYER, J.M., RUCH, J.V., WEBER, K. and OSBORN, M. (1982). Immunofluorescent localization of vimentin, prekeratin and actin during odontoblast differentiation. *Differentiation 21:* 133-137.

LESOT, H., OSMAN, M. and RUCH, J.V. (1981). Immunofluorescent localization of collagens, fibronectin and laminin during terminal differentiation of odontoblasts. *Dev. Biol. 82:* 371-381.

LESOT, H., SMITH, A.J., MEYER, J.M., STAUBLI, A. and RUCH, J.V. (1986). Cell-matrix interactions: influences of non-collagenous proteins from dentin on cultured dental cells. *J. Embryol. Exp. Morphol. 96:* 195-209.

LESOT, H., SMITH, A.J., TZIAFAS, D., BÈGUE-KIRN, C., CASSIDY, N. and RUCH, J.V. (1994). Biologically active molecule and dental tissue repair: a comparative review of reactionary and reparative dentinogenesis with induction of odontoblast differentiation *in vitro*. *Cells Mater.* 4: 199-218.

LOTZ, M.M., KORZELIUS, C.A. and MERCURIO, A.M. (1990). Human colon carcinoma cells use multiple receptors to adhere to laminin: involvement of a6ß4 and a2ß1 integrins. *Cell Regul. 1:* 249-257.

LUMSDEN, A.G.S. (1988). Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. *Development 103:* 155-169.

LUMSDEN, A.G.S. (1984) Tooth morphogenesis: contributions of the cranial neural crest in mammals. In *Tooth Morphogenesis and Differentiation* (Eds. A.B. Belcourt and J.V. Ruch) Colloque INSERM, Vol. 125. INSERM, Paris, pp. 29-40.

MARCANTONIO, E.E. and HYNES, R.O. (1988). Antibodies to the conserved cytoplasmic domain of the integrin ß1 subunit react with proteins in vertebrates, invertebrates and fungi. *J. Cell Biol. 106:* 1765-1772.

MARINKOVICH, M.P., KEENE, D.R., RIMBERG, C.S. and BURGESON, R.E. (1993). Cellular origin of the dermal-epidermal basement membrane. *Dev. Dynamics 197:* 255-267.

MARK, M.P., BAKER, J.R., MORRISON, K. and RUCH, J.V. (1990). Chondroitin sulfates in developing mouse tooth germs: an immunohistochemical study with monoclonal antibodies against chondroitin-4 and chondroitin-6 sulfates. *Differentiation 43:* 37-50.

MARK, M.P., BLOCH-ZUPAN, A. and RUCH, J.V. (1992). Effects of retinoids on tooth morphogenesis and cytodifferentiation, *in vitro*. *Int. J. Dev. Biol. 36:* 517-526.

MARK, M.P., BLOCH-ZUPAN, A., WOLF, C., RUBERTE, E. and RUCH, J.V. (1991). Involvement of cellular retinoic acid-binding proteins I and II (CRABPI and CRABPII) and of the cellular retinol-binding protein I (CRBPI) in odontogenesis in the mouse. *Differentiation 48:* 89-98.

MEYER, J.M., FABRE, M., STAUBLI, A. and RUCH, J.V. (1977). Relations cellulaires au cours de l'odontogenèse. *J. Biol. Buccale 5:* 107-119.

MEYER, J.M., KARCHER-DJURICIC, V., OSMAN, M. and RUCH, J.V. (1978). Aspects ultrastructuraux de la reconstitution de la membrane basale dans des associations de constituants dentaires cultivés *in vitro*. *C.R. Acad. Sci. 287:* 329-332.

MEYER, J.M., LESOT, H., STAUBLI, A. and RUCH, J.V. (1989). Immunoperoxidase localization of fibronectin during odontoblast differentiation. An ultrastructural study. *Biol. Struct. Morph. 2:* 19-24.

MEYER, J.M., RUCH, J.V., KUBLER, M.D., KUPFERLE, C. and LESOT, H. (1995). Cultured incisors display major modifications in basal lamina deposition without further effect on odontoblast differentiation. *Cell Tissue Res. 279:* 135-147.

MEYER, J.M., STAUBLI, A. and RUCH, J.V. (1981a). Ruthenium red staining and tannic acid fixation of dental basement membrane. *Cell Tissue Res. 220:* 589-597.

MEYER, J.M., STAUBLI, A. and RUCH, J.V. (1981b). Ultrastructural localization of concanavalin A binding sites on the surface of differentiating odontoblasts. *Biol. Cell 42:* 193-196.

MEYER, J.M., STAUBLI, A. and RUCH, J.V. (1988). Ultrastructural duality of extracellular fibrillar components of the odontoblast layer in the mouse molar. *Arch. Oral Biol. 33:* 25-31.

MILLAN, F.A., DENHEZ, F., KONDAIAN, P. and AKHURST, R.J. (1991). Embryonic gene expression patterns of TGFß1, ß2 and ß3 suggest different developmental functions *in vivo*. *Development 111:* 131-144.

MITSIADIS, T.A., COUBLE, P., DICOU, E., RUDKIN, B.B. and MAGLOIRE, H. (1993). Patterns of nerve growth factor (NGF), pro NGF, and p75NGF receptor expression in the rat incisor: comparison with expression in the molar. *Differentiation 54:* 161-175.

MITSIADIS, T.A., DICOU, E., JOFFRE, A. and MAGLOIRE H. (1992). Immunohistochemical localization of nerve growth factor (NGF) and NGF receptor (NGF-R) in the developing first molar tooth of the rat. *Differentiation 49:* 47-61.

MORRISS-KAY, G. and TUCKET, F. (1991). Early events in mammalian craniofacial morphogenesis. *J. Craniofac. Genet. Dev. Biol. 11:* 181-191.

NAKAMURA, M., BRINGAS, P., NANCI, A., ZEICHNER-DAVID, M., ASHDOWN, B. and SLAVKIN, H.C. (1994). Translocation of enamel proteins from inner enamel epithelia to odontoblasts during mouse tooth development. *Anat Rec. 238:* 383-396.

NISHIKAWA, S. and KITAMURA, H. (1986). Localization of actin during differentiation of the ameloblast, its related epithelial cells and odontoblasts in the rat incisor using NBD-phallacidin. *Differentiation 30:* 237-243.

NISHIKAWA, S. and KITAMURA, H. (1987). Microtubules, intermediate filaments, and actin filaments in the odontoblast of rat incisor. *Anat. Rec. 219:* 144-151.

NISWANDER, L. and MARTIN, G.R. (1992). FGF-4 expression during gastrulation, myogenesis, limb and tooth development in the mouse. *Development 114:* 755-768.

NODEN, D.M. (1983). The role of the neural crest in patterning of avian cranial skeletal, connective and muscle tissues. *Dev. Biol. 96:* 144-165.

NODEN, D.M. (1988). Interactions and fates of avian craniofacial mesenchyme. *Development 103:* 121-140.

NODEN, D.M. (1991). Cell movements and control of patterned tissue assembly during craniofacial development. *J. Craniofac. Genet. Dev. Biol. 11:* 192-213.

OSMAN, A. and RUCH, J.V. (1975). Répartition topographique des mitoses dans les territoires odontogènes de la mâchoire inférieure de l'embryon de souris. *J. Biol. Buccale 3:* 117-132.

OSMAN, A. and RUCH, J.V. (1976).Répartition topographique des mitoses dans l'incisive et la 1ère molaire inférieure de l'embryon de souris. *J. Biol. Buccale 4:* 331-348.

OSMAN, A. and RUCH, J.V. (1978). Contribution à l'étude des paramètres du cycle cellulaire au cours de l'odontogenèse chez la souris. *J. Biol. Buccale 6:* 43-54.

OSMAN, M. and RUCH, J.V. (1981a). Behavior of odontoblasts and basal lamina of trypsin or EDTA-isolated mouse dental papillae in short term culture. *J. Dent. Res. 60:* 1015-1027.

OSMAN, M. and RUCH, J.V. (1981b). ^{3}H-glucosamine and ^{3}H-proline radioautography of embryonic mouse dental basement membrane. *J. Craniofac. Genet. Dev. Biol. 1:* 95-108.

OSMAN, M., KARCHER-DJURICIC, V. and RUCH, J.V. (1979). Rôle de la pulpe dentaire dans l'histogenèse de l'organe adamantin. *C.R. Soc. Biol. 173:* 730-735.

OSMAN, M., MEYER, J.M., STAUBLI, A. and RUCH, J.V. (1981). Cytochemical localization of adenylate cyclase in embryonic mouse molars. *Acta Histochem. 68:* 91-102.

OTEY, C.A., PAVALKO, F.M. and BURRIDGE, K. (1990). An interaction between α-actinin and the ß1 integrin subunit *in vitro*. *J. Cell Biol. 111:* 721-729.

PARTANEN, A.M. and THESLEFF, I. (1987). Localization and quantitation of ^{125}I-epidermal growth factor binding in mouse embryonic tooth and other embryonic tissues at different developmental stages. *Dev. Biol. 120:* 186-197.

PELTON, R.W., DICKINSON, M.E., MOSES, H.L. and HOGAN, B.L.M. (1990). *In situ* hybridization analysis of TGFß3 RNA expression during mouse development: comparative studies with TGFß1 and ß2. *Development 110:* 609-620.

PELTON, R.W., SAXÉN, B., JONES, M., MOSES H.L. and GOLD, J.I. (1991). Immunohistochemical localization of TGFß1, TGFß2 and TGFß3 in the mouse embryo: expression patterns suggest multiple roles during embryonic development. *J. Cell Biol. 115:* 1091-1105.

REICHERT, T., STÖRKEL, S., BECKER, K. and FISCHER, L.W. (1992). The role of osteonectin in human tooth development: an immunological study. *Calcif. Tissue Int. 50:* 468-472.

RIJLI, P.M., MARK, M., LAKKARAJUS, S., DIERICH, A., DOLLE, P. and CHAMBON, P. (1993). A homeotic transformation is generated in the rostral branchial region of the head by disruption of Hoxa-2, which acts as a selector gene. *Cell 75:* 1333-1349.

ROJKIND, M., GATMAITANT, Z., MACKENSEN, S., GIAMBRONE, M.A., PONCE, P. and REID, L.M. (1980). Connective tissue biomatrix: its isolation and utilization for long term cultures of normal rat hepatocytes. *J. Cell Biol. 87:* 255-263.

ROMAN, J., LITTLE, C.W. and McDONALD, J.A. (1991). Sequential role of RGD-binding integrins in mammalian lung branching morphogenesis. *Development 112:* 551-558.

RUCH, J.V. (1984). Tooth morphogenesis and differentiation. In *Dentin and Dentinogenesis* (Ed. A. Linde). CRC Press, Boca Raton, pp. 47-79.

RUCH, J.V. (1985). Odontoblast differentiation and the formation of the odontoblast layer. *J. Dent. Res. 64:* 489-498.

RUCH, J.V. (1987). *Determinisms of Odontogenesis.* Cell Biology Reviews Vol. 14. UBC Press, Leica.

RUCH, J.V. (1990). Patterned distribution of differentiating dental cells: facts and hypotheses. *J. Biol. Buccale 18:* 91-98.

RUCH, J.V. (1995). Tooth crown morphogenesis and cytodifferentiations: candid questions and critical comments. *Connect. Tissue Res. 31:* 1-8.

RUCH, J.V. and KARCHER-DJURICIC V. (1971a). Action de la 5-fluoro-deoxyuridine sur la différenciation *in vitro* de molaires d'embryons de souris. *Arch. Biol. 82:* 115-129.

RUCH, J.V. and KARCHER-DJURICIC, V. (1971b). Mise en évidence d'un rôle spécifique de l'épithélium adamantin dans la différenciation et le maintien des odontoblastes. *Ann. Embr. Morph. 4:* 359-366.

RUCH, J.V. and KARCHER-DJURICIC, V. (1975). On odontogenic tissue interactions. In *Extracellular Matrix Influences on Gene Expression.* II Santa Catalina Island Colloquium (Eds. H.C. Slavkin and R.C. Greulich). Academic Press, New York, pp. 549-554.

RUCH, J.V., KARCHER-DJURICIC, V. and GERBER, R. (1973). Les déterminismes de la morphogenèse et des cytodifférenciations des ébauches dentaires de souris. *J. Biol. Buccale 1:* 45-56.

RUCH, J.V., KARCHER-DJURICIC, V. and THIEBOLD, J. (1976). Cell division and cytodifferentiation of odontoblasts. *Differentiation 5:* 165-169.

RUCH, J.V., KARCHER-DJURICIC, V., OSMAN, M., MEYER, J.M. and LESOT, H. (1978). Action of 5-bromodeoxyuridine on tooth germs *in vitro*. I. Effects on cytodifferentiations. *J. Biol. Buccale 6:* 267-279.

RUCH, J.V., KARCHER-DJURICIC, V., STAUBLI, A. and FABRE, M. (1975). Effets de la cytochalasine B et de la colchicine sur les différenciations dentaires *in vitro*. *Arch. Anat. Microsc. Morphol. Exp. 64:* 113-134.

RUCH, J.V., LESOT, H. and KUBLER, M.D. (1987). Matrix-cytoskeleton interactions during odontoblast and ameloblast differentiation. In *The Cytoskeleton in Cell Differentiation and Development* (Eds. R.B. Maccioni and J. Aréchaga). ICSU Press, IRL Press, Oxford, pp. 293-303.

RUCH, J.V., LESOT, H., KARCHER-DJURICIC, V. and MEYER, J.M. (1984). Extracellular matrix mediated interactions during odontogenesis. In *Matrices and Differentiation* (Eds. J.R. Hinchliffe and R.B. Kemp). A. Liss, New York, pp. 103-114.

RUCH, J.V., LESOT, H., KARCHER-DJURICIC, V., MEYER, J.M. and OLIVE, M. (1982). Facts and hypotheses concerning the control of odontoblast differentiations. *Differentiation 21:* 7-12.

SCHWARTZ, S.A. and KIRSTEN, W.H. (1973). Tissue specific suppression of differentiation by 5-bromodeoxyuridine *in vitro*. *J. Dent. Res. 53:* 509-515.

SHARPE, P.T. (1995). Homeobox genes and orofacial development. *Connect. Tissue Res.* (In press).

SLAVKIN, H.C., BESSEM, C., BRINGAS, P., ZEICHNER-DAVID, M., NANCI, A. and SNEAD, M.L. (1988). Sequential expression and differential function of multiple enamel proteins during fetal, neonatal and early postnatal stages of mouse molar organogenesis. *Differentiation 37:* 26-39.

SMITH, A.J. and LEAVER, A.G. (1979). Non collagenous components of the organic matrix of rabbit incisor dentine. *Arch. Oral Biol. 24:* 449-454.

SMITH, M.M. and HALL, B.K. (1990). Development and evolutionary origins of vertebrate skeletogenic and odontogenic tissues. *Biol. Rev. 65:* 277-373.

SONNENBERG, A., CALAFAT, J., JANSSEN, H., DAAMS, H., van der RAAIJ-HELMER, L.M.H., FALCIONI, R., KENNEL, S.J., APLIN, J.D., LOISIDOU, M. and GARROD, D. (1991). Integrin α6/ß4 complex is located in hemidesmosomes, suggesting a major role in epidermal cell-basement membrane adhesion. *J. Cell Biol. 113:* 907-917.

STENT, G.S. (1987). Cell lineage in development. *FEBS Lett. 215:* 1-8.

TAKADA, Y. and HEMLER, M.E. (1989). The primary structure of the VLA2/collagen receptor α2 subunit (platelet GPIa): homology to other integrins and the presence of a possible collagen-binding domain. *J. Cell Biol. 109:* 397-407.

TEN CATE, A.R. (1978). A fine structural study of coronal and root dentinogenesis in the mouse: observations on the so-called von Korff fibres and their contribution to mantle dentin. *J. Anat. 125:* 183-197.

TEN CATE, A.R. (1989). Dentinogenesis. In *Oral Histology Development Structure and Function*, 3rd ed. (Ed. A.R. Ten Cate). C.V. Mosby, St. Louis, pp. 139-156.

THESLEFF, I. and PRATT, R.M. (1980). Tunicamycin inhibits mouse tooth morphogenesis and odontoblast differentiation *in vitro*. *J. Embryol. Exp. Morphol. 58: 195-203.*

THESLEFF, I. and VAAHTOKARI, A. (1992). The role of growth factors in determination and differentiation of the odontoblastic cell lineage. *Proc. Finn. Dent. Soc. 88:* 357-368.

THESLEFF, I., BARRACH, H.T., FOIDART, J.M., VAHERI, J.M., PRATT, R.M. and MARTIN, G.R. (1981). Changes in the distribution of type IV collagen, laminin, proteoglycan and fibronectin during mouse tooth development. *Dev. Biol. 81:* 182-192.

THESLEFF, I., LEHTONEN, E. and SAXÉN, L. (1978). Basement membrane formation in transfilter tooth culture and its relations to odontoblast differentiation. *Differentiation 10:* 71-79.

THESLEFF, I., MACKIE, E., VAINIO, S. and CHIQUET-EHRISMANN, R. (1987). Changes in the distribution of tenascin during tooth development. *Development 101:* 288-296.

THOMAS, P. and DZIADEK, M. (1993). Genes coding for basement membrane glycoproteins Laminin, Nidogen, and Collagen IV are differentially expressed in the nervous system and by epithelial, endothelial and mesenchymal cells of the mouse embryo. *Exp. Cell Res. 208:* 54-67.

TZIAFAS, D., AMAR, S., STAUBLI, A., MEYER, J.M. and RUCH, J.V. (1988). Effects of glycosaminoglycans on *in vitro* growing mouse dental cells. *Arch. Oral Biol. 33:* 735-740.

UCHIDA, T, TANABE, T., FUKAE, M., SHIMIZU, M., YAMADA, M., MIAKE, K. and KOBAYASHI, S. (1991). Immunochemical and immunohistochemical studies using antisera against 25 kDa amelogenin and 89 kDa enamelin and 13-17 kDa non amelogenins an immature enamel of the pig and rat. *Histochemistry 96:* 129-138.

VAINIO, S., KARAVANOVA, I., JOWETT, A. and THESLEFF, I. (1993). Identification of BMP-4 as a signal mediating secondary induction between epithelial and mesenchymal tissues during early tooth development. *Cell 75:* 45-58.

WILKINSON, D.G., BHATT, S. and McMAHON, A.P. (1989). Expression pattern of the FGF-related proto-oncogene int-2 suggests multiple roles in fetal development. *Development 105:* 131-136.

WISE, G.E. and FAN, W. (1991). Immunolocalization of transforming growth factor beta in rat molars. *J. Oral Pathol. Med. 20:* 74-80.

WRANA, J.L., ATTISANO, L., WIESER, R., VENTURA, F. and MASSAGUE, J. (1994). Mechanism of activation of the TGFß receptor. *Nature 370:* 341-347.

YOSHIBA, N., YOSHIBA, K., IWAKU, M., NAKAMURA, H. and OZAWA, H. (1994). A confocal laser scanning microscopy study of the immunofluorescent localization of fibronectin in the odontoblast laver of human teeth. *Arch. Oral Biol. 39:* 395-400.

ZHANG, C.Z., YOUNG, W.G. and WATERS, M.J. (1992). Immunocytochemical localization of growth hormone receptor in maxillary teeth. *Arch. Oral Biol. 37:* 77-84.