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Facts and Hypotheses Concerning the Control of Odontoblast Differentiation

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Numerous studies using amphibians have demonstrated that preodontoblasts emerging from the dental papilla are derived from cranial neural crest cells [4, 12, 46, 64]. However this has not been established for mammals. The history of odontogenesis begins during the early stages of cranial-facial development when the maxillary and mandibular processes develop. Continuous epithelio-mesenchymal interactions condition the histogenesis and morphogenesis of the teeth [24–26, 43, 44, 49, 51, 58] as well as the terminal differentiation of odontoblasts and ameloblasts [23, 47, 52, 54, 59, 61, 67].

During recent years a considerable amount of experimental data relating to differentiation of odontoblasts has been published. We summarize these data and attempt to integrate them in deductive hypotheses concerning the control of odontoblast differentiation.

Histological, Cytological, and Functional Characterization of Odontoblast Terminal Differentiation

The terminal differentiation of odontoblasts (one of the cell types present in the dental papilla) occurs for each tooth in a specific temporo-spatial pattern. For example in the first mandibular mouse molar (Laboratory-raised Swiss mice, vaginal plug = day 0) the first overtly differentiated odontoblasts are found at 18 days at the top of the principal cusps. Progressively more odontoblasts appear towards the basal parts of the cusps. The terminal differentiation is characterized by the following steps: odontoblasts become post-mitotic, assume an epithelial arrangement, and polarize. Polarization implies important cytological modifications. The cell becomes larger, the nucleus takes up an eccentric position, cisternae of granular cytoplasmic reticulum multiply, flatten, and become parallel with the long axis of the cell. The apical part of the cell forms the odontoblastic cell process which is devoid of major cytoplasmic organelles but contains coated vesicles. These cells secrete predentin [58]. These aspects of terminal differentiation last a few hours (for detailed cytological description, see [5, 8, 71]).The functional aspects of odontoblast terminal differentiation are not completely known.The preodontoblasts synthesize collagen type I, type I trimer, type III [20, 29, 30, 36], fibronectin [32, 68], and glycosaminoglycans [1, 33]. Functional differentiation of odontoblasts implies amplification of collagen type I and type I trimer synthesis and suppression of collagen type III synthesis [29, 30, 32]. Fully differentiated odontoblasts also synthesize a phosphoprotein [3, 70]. It is not yet clear if preodontoblasts and odontoblasts synthesize different glycosaminoglycans [11, 18].

Terminal Differentiation of Odontoblasts Requires Structural Integrity of Cytoskeleton

Polarized odontoblasts possess a well organized cytoskeleton [7]. Colchicine well as cytochalasin B inhibited the polarization of odontoblasts [53]. Colchicine also inhibited the secretion of predentin.

Aspects of Cell Kinetics During Differentiation of Odontoblasts

Functional odontoblasts are post-mitotic cells. It is generally believed that these cells are diploid. Nevertheless Starkey [62] using radioautographic data stressed the possibility that these cells could be tetraploid. Preliminary cytophotometric DNA quantification (Olive, unpublished data) is in good agreement with this possibility. During the last division of preodontoblasts, the spindle is always oriented perpendicularly to the basal lamina underlying the inner dental epithelium. The two daughter cells are superimposed and only the cell close to the basement membrane gives rise to a functional odontoblast [38]. Osman and Ruch [39] have established the duration of cell cycle phases during mouse odontogenesis. Cell cycle duration (Tc) increased from 8.9–13.9 h between days 11 and 17 of gestation. The lengthening of cell cycle results from the progressive lengthening of the duration of G_1 (T_{G1}). From the initiation of odontogenesis (days 11) to the terminal differentiation of odontoblasts of first mandibular molars, a maximum of 14–15 cell generations may exist. Experimental data (summarized below) suggest that odontoblast terminal differentiation can be achieved only after a minimum number of cell cycles. Supplementary cell cycles can be added [54]. Replicating preodontoblasts held in the G_1-S interface by fluorodeoxyuridine (FudR) did not polarize. This effect was reversible. FudR had no marked effect on post-mitotic odontoblasts [48, 54].

The Inner Dental Epithelium (Preameloblasts) Intervenes During Terminal Differentiation of Odontoblasts

Preodontoblasts of dental papillae, isolated by treatment with proteolytic enzymes, cultured in vitro [23, 24, 47] or in vivo [14], never became functional. Already polarized odontoblasts

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existing in teeth at the moment of papilla isolation depolarized and never became functional (even after 6–7 days in culture [54]). Predentin or dentin stabilizes the functional state of odontoblasts [50]. Iso or heterochronal associations between dental papillae and inner dental epithelia cultured in vitro [47, 54]. demonstrated that:

1. Reassociation between polarized odontoblasts and isochronal inner dental epithelium allowed maintainance and functional differentiation of odontoblasts.
2. In association between polarized odontoblasts and younger inner dental epithelium the odontoblasts depolarized and were able to incorporate ^{3}H-thymidine and to divide. Later, daughter cells became post-mitotic again, polarized, and secreted predentin.
3. Association between preodontoblasts and older inner dental epithelium (previously in contact with post-mitotic odontoblasts) did not allow anticipated terminal differentiation of odontoblasts.

Heterotopic associations between dental papillae and non-dental epithelia [25–27, 51] allowed functional differentiation of odontoblasts. In such associations the dental papilla first conditioned the transformation of the non-dental epithelium into inner dental epithelium. Later this inner dental epithelium was then able to initiate the differentiation of odontoblasts. Bromodeoxyuridine (BrdU) inhibits odontoblast terminal differentiation [31, 55, 57]; only post-mitotic but non-polarized odontoblasts were found. We have shown that this effect resulted from the incorporation of BrdU in preameloblasts which were no longer able to interact normally with preodontoblasts [55].

Differentiation of Odontoblasts May be Triggered by Contacts Between Preodontoblasts and Basement Membrane

The extracellular matrix interposed between the inner dental epithelium and the preodontoblasts (and odontoblasts before secretion of predentin) is formed by a basal lamina and associated microfibrils. We designate by basement membrane this extracellular matrix. Separation of dental papilla from dental epithelium by trypsin results in removal of the basement membrane. Culture of dental papillae and epithelia with interposed filters resulted in basement membrane redeposition if the filter pores (0.6 μm) allowed penetration of cytoplasmic processes. Differentiation of odontoblasts occurred only in explants in which a new basement membrane was formed [67]. Basement membrane reconstitution and terminal differentiation of odontoblasts have also been analyzed in isochronal and heterochronal direct associations of dental papillae and inner dental epithelia [19]. Post-mitotic odontoblasts were associated with isochronal or younger inner dental epithelium. In both cases a typical basement membrane (basal lamina and associated fibrillar and granular material) was restored within 6–18 h. In isochronal associations polarized odontoblasts always existed and these cells were functional after 24 h. In heterochronal associations polarized odontoblasts were observed only 2–3 days after the reconstitution of the basement membrane. If the basement membrane is secreted by the epithelial cells and plays a role during odontoblast terminal differentiation, then one has to assume that the membranes deposited in iso- and heterochronal associations do not have the same composition or structure, even if the cytological aspects are similar. So far attempts to induce odontoblast differentiation with gels of collagen type I, type II, and type III, as well as with collagen gels substituted with proteoglycans have not been successfull [67].

The Dental Basement Membrane is at Least Partially Epithelially Derived

Trypsin-isolated dental epithelia cultured on the chick chorio-allantoic membrane or in vitro [40, 60] restored a basal lamina with associated fibrillar and granular material. ^{3}H-proline and ^{3}H-glucosamine allowed radioautographic labelling of this basement membrane [6, 41].

Behaviour of Basement Membrane and Odontoblasts of EDTA-Isolated Dental Papillae

EDTA-dissociation of embryonic mouse mandibular molars removed the basement membrane from the basal surface of epithelium but left it in association with dental papilla [42]. In short-term culture, EDTA-isolated dental papillae remained covered by basement membrane for 15–18 h. During this time, the post-mitotic odontoblasts present at the onset of culture, but not the preodontoblasts, polarized and secreted predentin (such a result has never been observed after trypsin dissociation). Nevertheless when EDTA-dissociated dental papillae were cultured, the odontoblasts did not demonstrate the normal temporal and spatial patterns of terminal differentiation occurring in intact teeth. This was attributed to the absence of renewal of the basement membrane in EDTA-isolated dental papillae. These observations led to the hypothesis that the physiological pattern of odontoblasts terminal differentiation was correlated with a specific temporal, spatial, compositional, and structural pattern of the dental basement membrane.

Temporal Correlation Between Modifications of the Basement Membrane and Terminal Differentiation of Odontoblasts

Embryonic basement membranes contain glycosaminoglycans (GAG), collagens and glycoproteins [34]. Using indirect immunofluorescence, collagen types I, III, IV, laminin, fibronectin, and proteoglycan were localized in the basement membrane [32, 68, 69]. The main phenomenon observed by indirect immunofluorescence during the terminal differentiation of odontoblasts was the disappearance of collagen type III at the epithelial-mesenchymal junction [32] and a redistribution of fibronectin [32, 68]. The latter completely surrounded the preodontoblasts but was confined to the epithelia-mesenchymal junction when odontoblasts polarized.

^{3}H-glucosamine radioautography was performed to analyze the labelling of mouse embryonic dental basement membrane (basal lamina and associated material) before and during odontoblast terminal differentiation. Intact molars and trypsin-isolated dental epithelia were used [41]. After 6 h (or more) labelling, the epithelial mesenchymal junction was heavily labelled and the isolated epithelia accumulated substantial amounts of surface label. Before odontoblast terminal differentiation, the label always had a uniform distribution and decreased during chase without any redistribution. During terminal differentiation, the label accumulated in a unique pattern: much more label was seen at the epithelial surface corresponding to the top of the cusps than in the inferior parts. During the chase, and only in intact tooth germs, epithelial surfaces which were poorly labelled during the pulse became

labelled but those heavily labelled during the pulse lost label. This pattern existed only in the presence of mesenchyme: the mesenchyme seems to control the synthesis and/or the degradation of epithelially derived ^{3}H-glucosamine labelled material.

Ruthenium red (RR) staining and tannic acid fixation were used to analyze the fine structure of embryonic mouse dental basement membrane in intact teeth or in EDTA-isolated dental papillae [35]. The inner leaflet of the preameloblast plasmalemma (inner dental epithelium) exhibited irregularly distributed RR-positive material which was connected to intracellular tonofilaments. RR-positive granules assumed a highly ordered symmetrical array in the internal and external lamina rara. This distribution pattern was particularly clear after EDTA-dissociation. The frequency of RR-positive granules observed in the basal lamina was higher in the regions of dividing preodontoblasts than in the region of polarized post-mitotic odontoblasts. Tannic acid fixation allowed the detection within the lamina lucida of filaments connecting the lamina densa with the plasma membrane of preameloblasts. It is possible that these filaments and RR-positive granules are structurally related.

Possible Correlation Between High Adenylate-Cyclase Activity and Terminal Differentiation of Odontoblasts

The cytochemical methods of Howell and Whitfield [13] and Kempen et al. [22] have been used to investigate the localization of adenylate-cyclase activity in tooth germs [45]. Increased adenylate-cyclase activity was observed in preameloblasts and odontoblasts localized in the area where pre-

- Initiation of odontogenesis by the preodontoblasts (PO)
- Progressive addition of collagens, glycoproteins, and proteoglycans to the BL gives rise to stage specific basement membranes (BM)
- BMx might control the orientation of the spindle of PO. The daughter cells become potential mature preodontoblasts (MPO). Only the MPO in relation with the BM will overtly differentiate
- MPO controls the turn-over of proteoglycans synthesized by the preameloblasts (PA): BMx becomes BMy
- GAG modifications might increase the adenylate cyclase activity of MPO which become post-mitotic (tetraploid?) odontoblasts (PMO). These cells no longer synthesize collagen type III; fibronectin is redistributed. BMy becomes BMz
- BMz modifies the activity of the cytoskeleton: PMO become polarized odontoblasts (PoO). PoO present amplification of collagen type I and type I trimer syntheses
- The predentin (PD) secreted by functional odontoblasts (FO) maintain their functional state. The BL disappears, PA become postmitotic ameloblasts (PMA)

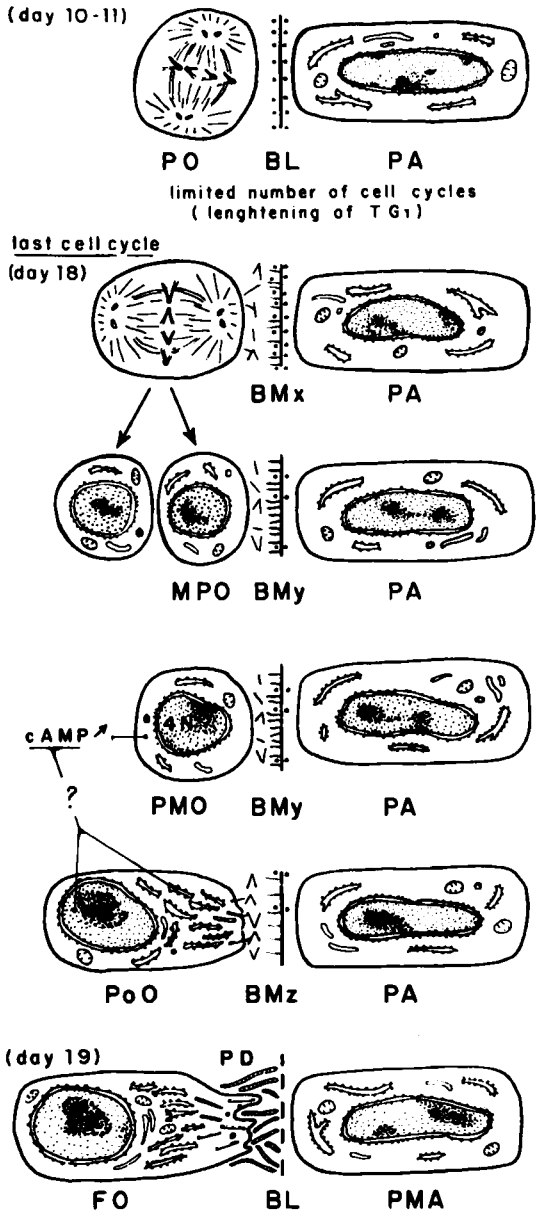

Fig. 1. Hypotheses concerning the control of odontoblast differentiation (stages corresponding to the first mandibular molar of laboratory-raised Swiss mouse embryos)

odontoblasts became post-mitotic. It is worth noting that Kelly et al. [21] have shown that glycosaminoglycan in the external cell surface of fibroblasts interact with receptor components of the adenylate-cyclase system. The reduction of glycosaminoglycan at the periphery of the cell can increase the relative activity of adenylate-cyclase [21].

Effects of Experimental Modifications of Dental Basement Membrane

Hurmerinta et al. [16] have shown that diazo-norleucine (DON) which decreased the syntheses of GAG and glycoprotein, inhibited the differentiation of odontoblasts. Nevertheless we have shown (Olive and Ruch, manuscript in preparation) that DON decreased the mitotic activity by 60–70%. This effect per se may explain the inhibition of odontoblast differentiation. Hurmerinta et al. [17] demonstrated that an excess of vitamin A prevented odontoblast differentiation. Vitamin A also inhibited the reformation of basement membrane between dental epithelium and dental papilla recombined after trypsin-pancreatin separation. Tunicamycin which inhibits glycosylation prevented the differentiation of odontoblasts [65, 66]. After tunicampycin treatment the dental basement membrane was discontinuous and fibronectin was drastically reduced.

Hypotheses Concerning the Control of Odontoblast Terminal Differentiation

Epigenetic controls are required for the progressive realization of the genetically determined phenotype of odontoblast. The terminal differentiation of odontoblasts is characterized by: (a) withdrawal from the cell cycle, (b) cytological and functional polarization, (c) transcriptional and (or) post-transcriptional modifications. The two last phenomena lead to predentin secretion.

Our hypotheses concerning the control of odontoblast differentiation are summarized in Fig. 1. The preodontoblasts (PO) probably undergo progressive maturation. Their terminal differentiation cannot be anticipated but can be delayed. The progressive lengthening of T_{G1} allows transcriptional modifications. After a minimum number of cell cycles PO become potential mature preodontoblasts (MPO). During the last cell division of preodontoblasts, the spindle is perpendicular to the basement membrane. There is actually no available evidence for a role of extracellular matrix in orientation of the spindle. The overt differentiation of the MPO occurs only in contact with stage specific basement membrane. The basement membrane components have different origins: laminin, collagen type IV, and at least some GAG (covalently bound to protein to form proteoglycan) are epithelially derived. Collagen types I and III, as well as fibronectin are produced by the dental papilla. The basement membrane presents compositional and structural modifications during the terminal differentiation: mesenchyme-dependent modifications of glycosaminoglycans (GAG) turnover, disappearance of collagen type III, redistribution of fibronectin. The modifications of GAG might increase the adenylate-cyclase activity of MPO. The increased intracellular level of cAMP could transform the MPO in post-mitotic odontoblasts (PMO).

Ruch and Karcher-Djuricic [49] postulated that the polarization of PMO was controlled by cell membrane bound 'receptors' wich could modulate the activity of the cytoskeleton. The activity or topographical distribution of these 'receptors' could be controlled by the extracellular matrix. It was shown that the PMO no longer synthesize collagen type III. Furthermore fibronectin which surrounds preodontoblasts becomes confined to the apical part of odontoblasts. This leads to modifications of the interactions between matrix components. It is known furthermore that some of these components (at least fribronectin) could interact with the cytoskeleton [2, 10, 72]. cAMP could also play a role in polarization and functional differentiation of odontoblasts: cAMP promotes polymerization of platelet tubulin [63], and in heart muscle cells, a relationship between dibutyryl cAMP and microtubules organization has been documented [37]. Furthermore cAMP might induce an increase in RNA polymerase II activity in tumour cells [28].

Odontoblast polarization is very rapidly followed by secretion of predentin. Polarized nonfunctional odontoblasts were never observed. The relationship between cytological and functional differentiation is not clear. We do not know if specific activities of the cytoskeleton per se can control transcriptional and (or) post-transcriptional modifications. Functional odontoblasts (FO) secrete predentin. This predentin, by a feed back mechanism, stabilizes the FO [50].

The stated hypotheses which still remain over simple can provide a framework for further studies. Indeed the cell surface macromolecules (glycoconjugates), which are supposed to interact with extracellular matrix components and cytoskeleton constituents, will have to be identified. Otherwise, interaction between contractile and cytoskeletal elements and other cell structures of odontoblasts will have to be studied: techniques used in other systems [9, 15] may be applied to the dental cells for this purpose. These major events controlling odontoblasts differentiation are involved in cell differentiation of most epithelial-mesenchymal interacting systems. Nevertheless unifying concepts should not actually be formulated [56].

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