

Cell–matrix interactions: influence of noncollagenous proteins from dentin on cultured dental cells

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SUMMARY

Matrix-mediated epitheliomesenchymal interactions control dental cytodifferentiations. Experiments were performed in order to study the effects of noncollagenous proteins extracted from dentin on cultured enamel organs and dental papillae.

Seven noncollagenous protein fractions were prepared from rabbit incisor dentin and used as substrates to coat Millipore filters. Embryonic mouse tooth germs were dissociated and the isolated tissues were cultured for 4 days on these different substrates as well as on noncoated Millipore filters.

When compared to control cultures, only two protein fractions affected the behaviour of epithelial cells. A slight elongation of the cell body and a preferential localization of the nuclei at the basal pole of the cells in contact with the filter was observed with protein fractions 5 and 6.

When dental papillae were cultured on Millipore filters coated either with protein fraction 2 or fraction 6, the mesenchymal cells in contact with the filter elongated, polarized and demonstrated a high metabolic activity. Such modifications in the cell organization, implying changes in the cytoskeleton organization and, or, activity, never occurred spontaneously or in the presence of isolated collagens (I–V), laminin or fibronectin.

INTRODUCTION

Several experimental approaches have demonstrated the importance of the extracellular matrix in the mediation of epitheliomesenchymal interactions during embryonic development (Reddi, 1984). However, the nature and the mechanisms of the cell–matrix interactions that are involved in the control of organogenesis and cell differentiation are still under investigation (for reviews, see Yamada, 1983; von der Mark *et al.* 1984; Bernfield, Banerjee, Koda & Rapraeger, 1984). Different models have been used for these studies, including the tooth (Thesleff, Lehtonen & Saxen, 1978; Ruch *et al.* 1982; Slavkin, 1982), which is representative of such interactions.

The tooth germ consists of two interacting tissues: the enamel organ and the dental papilla. At the epitheliomesenchymal junction, the ectomesenchymal cells in contact with the basement membrane differentiate into functional odontoblasts

Key words: cell–matrix interaction, dentin, proteins, dental papilla, enamel organ, polarization, tissue culture.

secreting the predentin. Cells of the inner dental epithelium differentiate into functional ameloblasts, which secrete the enamel. However, preodontoblasts and preameloblasts do not differentiate spontaneously. In order to differentiate, preodontoblasts have to interact with the mesenchymal face of a stage-specific basement membrane (Osman & Ruch, 1981) and preameloblasts require interaction with the predentin (Ruch, Karcher-Djuricic & Gerber, 1972).

Attempts have been made to promote the terminal differentiation of these cells using artificial substrates made of collagens (Thesleff, 1978; Lesot *et al.* 1985), noncollagenous glycoproteins (Lesot *et al.* 1985) and glycosaminoglycans (Ruch, 1985). Up to now, the polarization of odontoblast-like cells could only be obtained in the presence of either hyaluronic acid or chondroitin sulphate (Ruch, 1985). Recently, the cytological and functional differentiation of ameloblasts has been observed when cells from the inner dental epithelium were cultured in association with a specific region of isolated dental matrices (Karcher-Djuricic, Staubli, Meyer & Ruch, 1985).

In the present study, we report experiments in which enamel organs and dental papillae have been cultured on Millipore filters coated with noncollagenous matrix proteins extracted from rabbit incisor dentin. The behaviour of the cultured dental cells was examined at the histological and cytological levels.

MATERIALS AND METHODS

Rabbit incisors

Dentin was prepared from incisors extracted from New Zealand White rabbits as previously described (Smith & Leaver, 1981) and powdered in a percussion mill cooled with liquid nitrogen.

Dentin demineralization

The powdered dentin was demineralized for 10 days at 4°C with 10% EDTA, pH 7.2, containing 10 mM-*N*-ethylmaleimide and 5 mM-phenylmethylsulphonyl fluoride as protease inhibitors (Smith & Smith, 1984). The demineralizing solution was changed every 48 h and the EDTA-soluble fraction in the supernatant collected after centrifugation. The combined EDTA-soluble fractions were exhaustively dialysed against distilled water, reduced in volume by vacuum dialysis and dialysed against two changes of 0.05 M-tris-HCl, pH 7.2, prior to DEAE-cellulose chromatography.

DEAE-cellulose chromatography

The total EDTA-soluble fraction from demineralization of the dentin was applied to a column (18×1.0 cm) of DEAE-cellulose equilibrated with 0.05 M-tris-HCl, pH 7.2, and after elution of the pregradient material with the same buffer, elution was continued with a salt gradient of 0–0.7 M-NaCl in 0.05 M-tris-HCl, pH 7.2. Column effluents were monitored as previously described (Smith & Leaver, 1979). The contents of the tubes were pooled into fractions 1–5, designated as in Fig. 1, and dialysed against distilled water and lyophilized.

Collagenase digestion and urea extraction

After washing with distilled water, the demineralized EDTA-insoluble dentin residue was incubated with 1000 units of collagenase (Sigma type VII from *Clostridium histolyticum*, 1600 units per mg) in 0.025 M-tris-HCl–0.3 M-calcium acetate buffer, pH 7.2, at 37°C. After two

days, a further 500 units of collagenase were added and the incubation continued for a further three days. The digested material was then centrifuged at 12 000 *g* for 20 min to separate the insoluble collagenase-released residue (ICR). The soluble collagenase-released fraction in the supernatant (CRF, 'fraction 6') was decanted off, dialysed against distilled water and lyophilized. The ICR fraction was washed with distilled water and extracted with 8 M-urea at 4°C until the absorption of the extracts at 280 nm became minimal. The extracts were dialysed against distilled water and lyophilized (ICR-U, 'fraction 7'). Protease inhibitors (10 mM-*N*-ethylmaleimide, 2 mM-phenylmethylsulphonyl fluoride, 25 mM-EDTA) were included at all stages to prevent artefactual degradation.

Amino acid analysis

After hydrolysis in 6 N-HCl, the amino acid compositions of the hydrolysates were determined using an LKB 4400 autoanalyser.

Sugar analysis

Samples of the fractions were analysed for hexose (Dubois *et al.* 1956), hexosamine (Blumenkrantz & Asboe-Hansen, 1976), uronic acid (Blumenkrantz & Asboe-Hansen, 1973) and sialic acid (Skoza & Mohos, 1976).

Polyacrylamide gel electrophoresis

Noncollagenous protein fractions from rabbit incisor dentin were separated by electrophoresis in 0.1 % sodium dodecylsulphate (SDS) on a linear 6/10 % polyacrylamide gradient gel (PAGE) using the buffer system described by Laemmli (1970). The proteins were stained by the silver procedure (Oakley, Kirsch & Morris, 1980).

Tooth germs

First lower molars of Swiss mouse embryos were used. Tooth germs were removed on day 18 (vaginal plug = day 0) of gestation. The teeth were staged according to morphological features (size, form, vascularization, etc.). These teeth contain dividing preameloblasts and preodontoblasts and the first postmitotic odontoblasts (Karcher-Djuricic *et al.* 1985).

Trypsin dissociation

Enamel organs and dental papillae were isolated from tooth germs enzymically dissociated with 1 % trypsin (Difco 1:250) in Hanks' balanced salt solution as previously described (Ruch, Karcher-Djuricic & Thiebold, 1976). Soybean trypsin inhibitor, 1 % in phosphate-buffered saline (PBS), was used to stop the activity of the enzyme.

Tissue culture

Day-18 isolated enamel organs and dental papillae were cultured for 4 days on Millipore filters (150 µm thickness and 45 µm pore size) coated or noncoated with 100–150 µg of noncollagenous proteins (applied as solution and dried) isolated as above. The culture medium was Dulbecco's Minimum Essential Medium (MEM) supplemented with 10 % foetal calf serum and was changed every two days. Cultures were performed in a humidified incubator at 37°C in an atmosphere of 5 % CO₂ in air.

Histology

The tissues were fixed with Bouin's fixative and paraffin-embedded specimens were cut in 5 µm thick serial sections and stained with Mallory Alun haematoxylin.

Transmission electron microscopy

Specimens were fixed for 60 min at 4°C in a 2 % glutaraldehyde solution buffered with 0.1 M cacodylate, pH 7.3, and postfixed for 30 min in 1 % osmium tetroxide in the same buffer. The specimens were embedded in Epon 812 and processed for electron microscopy.

RESULTS

Seven noncollagenous protein fractions were prepared from rabbit incisor dentin and used as substrates to coat Millipore filters. For each protein fraction, 60–100 dental papillae and enamel organs were cultured on these as well as uncoated control Millipore filters and observed after 4 days in culture. The protein fractions used in these experiments represented three independent isolations from rabbit dentin.

Substrates

Ion-exchange chromatography on DEAE-cellulose of the EDTA-solubilized material from dentin resulted in fractions 1–5 (Fig. 1). Examination of these fractions on SDS-PAGE followed by silver staining confirmed that the individual peaks demonstrated heterogeneity in their protein content (Fig. 2). Fractions 6 and 7 were extracted, respectively, after collagenase and after 8 M-urea treatment of the EDTA-insoluble residue and also demonstrated protein heterogeneity on electrophoresis. The amino acid and sugar compositions of those fractions (2, 5 and 6) producing morphological and cytological changes in the cultured dental cells reported below are shown in Table 1. Appreciable amounts of the anionic amino acids, aspartic acid, glutamic acid and serine are present, although more anionic fractions are present in dentin. The carbohydrate content indicates the presence of glycoproteins in the fractions and the hexosamine and uronic acid contents of fraction 5 are in accord with the presence of glycosaminoglycans in this fraction.

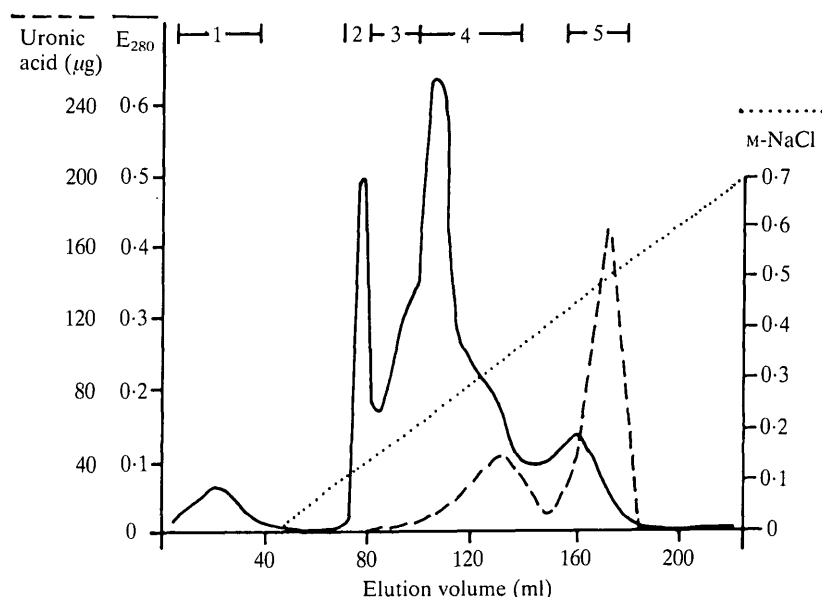


Fig. 1. DEAE-cellulose chromatography of rabbit incisor dentin total EDTA-soluble material. Tubes were pooled into fractions 1–5 as designated by the bars at the top of the figure.

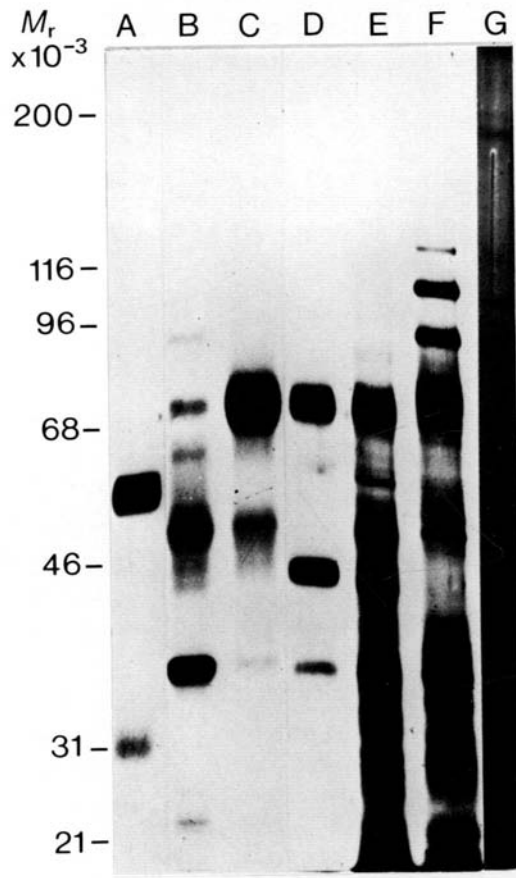


Fig. 2. SDS-polyacrylamide slab gel electrophoresis of noncollagenous proteins extracted from rabbit dentin. (A-E) Fractions 1 to 5 of the EDTA-soluble material obtained after DEAE-cellulose chromatography (Fig. 1). (F) Fraction 6 solubilized by collagenase treatment and (G) fraction 7 insoluble after collagenase treatment and extracted with 8M-urea. The dissociation and electrophoresis were performed as described in Materials and Methods. Proteins were detected by silver staining. The relative molecular mass markers were myosin (200 000), β -galactosidase (116 000), phosphorylase B (96 000), bovine serum albumin (68 000), ovalbumin (46 000), carbonic anhydrase (31 000) and soybean trypsin inhibitor (21 000).

Cultured dental papillae

After 4 days in culture, the mesenchymal cells in contact with Millipore filters coated with rabbit dentin protein fraction 2 or fraction 6 demonstrated important changes in their morphology (Fig. 3B) as compared with the same cells cultured in contact with noncoated Millipore filters (Fig. 3A). Cells in contact with Millipore filters coated with one of these two substrates had an epithelial arrangement and appeared elongated and polarized (Fig 3B). The nuclei migrated to the basal pole of the cells (Figs 4C, 5A). When compared with control cultures, these cells demonstrated the widening of the cisternae of the rough endoplasmic reticulum (Fig. 4B) and distended Golgi cisternae (Figs 4C, 5B). Coated vesicles originated

from ergastoplasmic cisternae and fused with the Golgi apparatus which produced secretory granules (Fig. 5B,C). No excretion of granules was observed and there was no deposition of an extracellular matrix (Fig. 4C). 55 % of the cultured dental papillae demonstrated this behaviour. After 4 days in culture, in control experiments, the corresponding cells neither polarized nor elongated (Fig. 4A).

Mesenchymal cells grown in contact with Millipore filters coated with protein fractions 1, 3, 4, 5 or 7 were similar at the histological level to control cultures.

Cultured enamel organs

In control cultures where cells from the inner dental epithelium were associated with the filter, an epithelial arrangement of the cells could be seen after 4 days (Fig. 3C, 6A). The nuclei did not demonstrate any preferential localization (Fig. 6A).

In 40 % of the cultured enamel organs, cells of the inner dental epithelium demonstrated morphological changes including a slight elongation and also a preferential localization of the nuclei at the basal pole of these cells (Figs 3D, 6B) when filters coated with protein fractions 5 or 6 were used as substrates. However, the elongation of these cells was much less than that of functional ameloblasts in normal teeth. Furthermore, the mitochondria did not migrate to the infranuclear compartment as they normally do during the terminal differentiation of ameloblasts. However, epithelial cells in contact with filters coated with protein fractions

Table 1. *Amino acid (residues/1000) and sugar (% by weight) compositions of rabbit dentin fractions*

	Fraction 2	Fraction 5	Fraction 6
Asp	103	118	107
Thr	52	52	47
Ser	52	92	102
Glu	143	159	133
Pro	99	78	79
Gly	83	150	139
Ala	99	80	65
1/2 Cys	—	—	—
Val	73	33	49
Met	2	—	—
Ile	30	27	39
Leu	80	60	52
Tyr	26	23	51
Phe	32	25	37
His	24	21	20
Lys	59	46	38
Arg	43	36	42
Hexose	8.4	17.4	12.0
Hexosamine	2.8	5.2	2.1
Sialic acid	0.8	2.8	0.7
Uronic acid	2.7	5.6	4.0

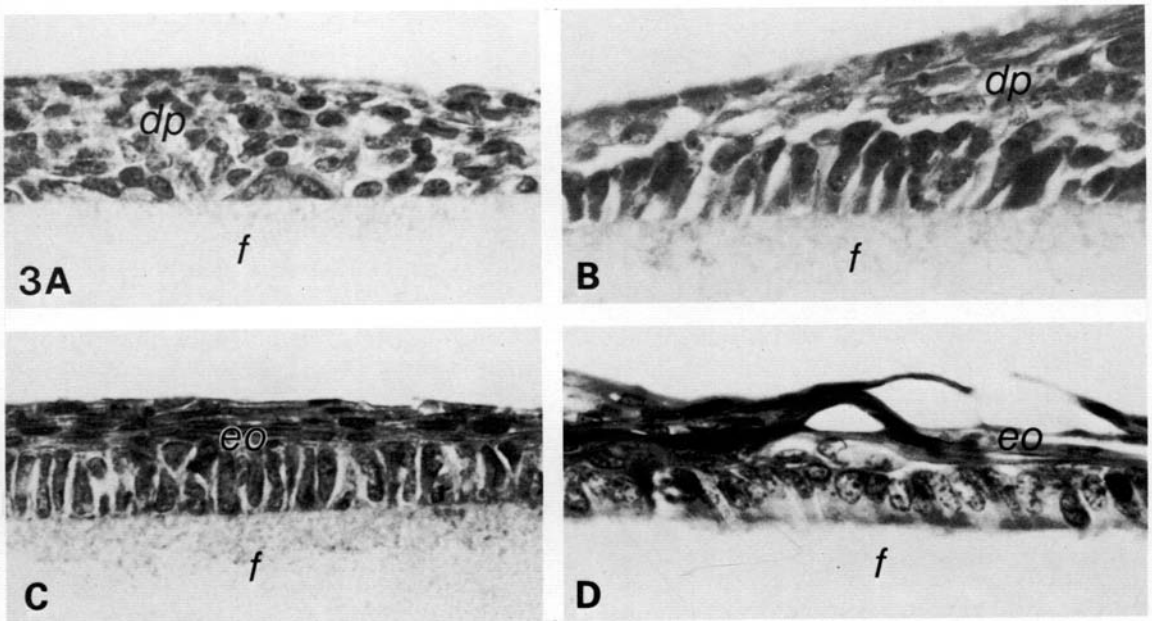


Fig. 3. Histological sections of dental papillae (*dp*) and enamel organs (*eo*) cultured for 4 days on uncoated (A,C) and coated (B,D) Millipore filters (*f*).

(A) Dental papillae cells in contact with uncoated Millipore filters demonstrated a flat shape ($\times 175$).

(B) Dental papillae cells cultured on Millipore filters coated with protein fraction 6 elongated and polarized ($\times 175$).

(C) The cells of the inner dental epithelium, cultured in contact with uncoated Millipore filters, showed an epithelial arrangement ($\times 175$).

(D) The same cells in contact with Millipore filters coated with protein fraction 5 polarized ($\times 175$).

5 or 6 displayed condensation of their chromatin and a development of the ergastoplasm which indicated an increased metabolic activity. In these conditions, as well as for control cultures, there was no evident reconstitution of a basal lamina in between epithelial cells and coated filters.

DISCUSSION

Reciprocal epitheliomesenchymal interactions control the terminal differentiation of odontoblasts and ameloblasts (Kollar & Baird, 1970; Slavkin, 1978; Thesleff & Hurmerinta, 1981; Ruch *et al.* 1982). Since these interactions are matrix mediated (Osman & Ruch, 1981; Karcher-Djuricic *et al.* 1985), attempts have been made to understand the nature and mechanism(s) of cell-matrix interactions. Several artificial substrates have been tested for their ability to replace the physiological requirements for odontoblast and ameloblast differentiation including different collagen types (Thesleff, 1978; Lesot *et al.* 1985), noncollagenous glycoproteins such as fibronectin and laminin (Lesot *et al.* 1985) and glycosaminoglycans (Ruch, 1985).

In the present study, substrates comprising noncollagenous proteins extracted from rabbit incisor dentin have been tested for their effects on dental epithelial and mesenchymal cells in culture. Previous studies have shown that noncollagenous proteins extracted from rat dentin possessed bone and cartilage morphogenetic activity (Bang & Urist, 1967; Yoemans & Urist, 1967; Huggins, Wiseman & Reddi, 1970). Structural analysis has shown that three of these proteins could be solubilized after collagenase treatment, two of which were acidic proteins and the third one was a serine-rich phosphoprotein (Butler, Mikulski & Urist, 1977).

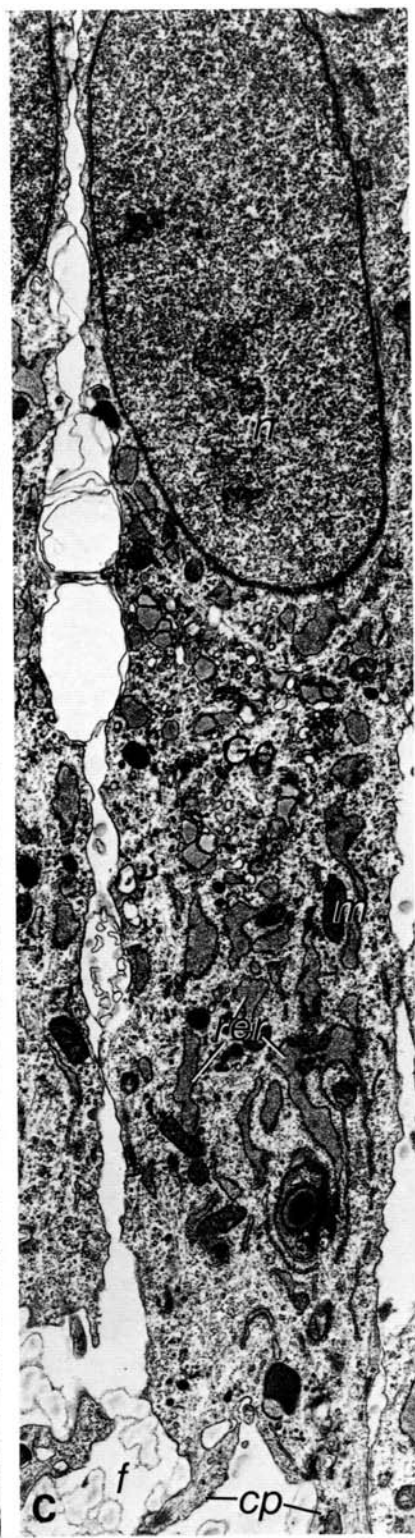
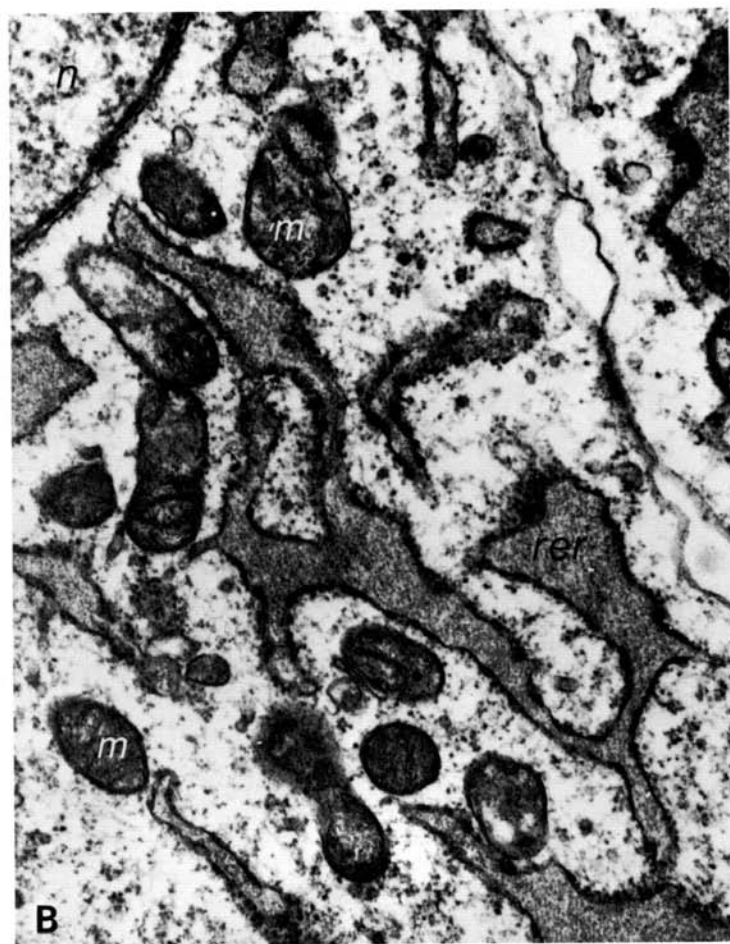
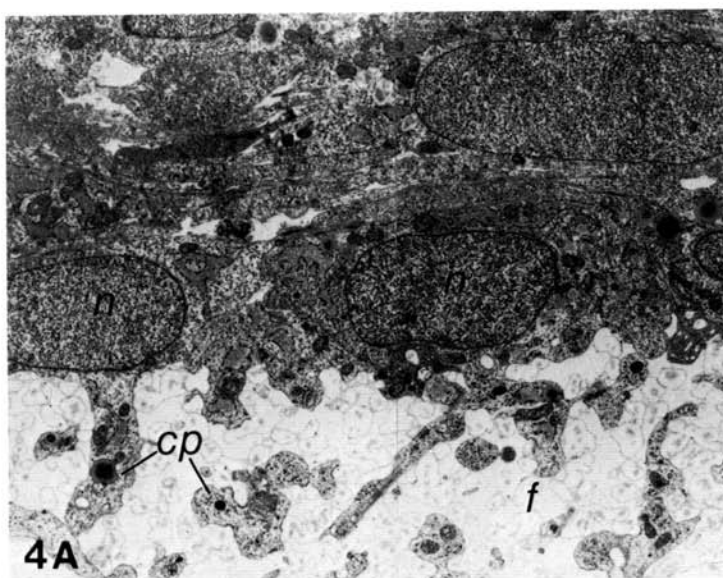
The noncollagenous proteins from rabbit incisor dentin isolated in the present study were prepared essentially as previously described (Smith & Leaver, 1979) except that protease inhibitors were included at all stages to prevent artefactual degradation. The fractions 1–5 from DEAE–cellulose chromatography of the EDTA-soluble material have been previously described as less-acidic glycoproteins (fractions 2 and 3), anionic glycoproteins (fraction 4) and a proteoglycan-containing fraction (fraction 5), whilst fraction 1 appears to include collagenous material (Smith & Leaver, 1979). Fraction 6 represents noncollagenous proteins tightly associated, possibly covalently, with the collagen of the tissue matrix and was only released after digestion of the demineralized dentin residue (Smith, Price & Leaver, 1979). The remaining insoluble residue after collagenase digestion could be partially solubilized by 8 M-urea extraction and has been shown to include proteins of the high aspartic acid and serine type similar to the anionic phosphoproteins (Richardson, Beagle, Butler & Munksgaard, 1977) and also acid structural proteins (Smith *et al.* 1979). The composition of the fractions 2, 5 and 6 reported here to show effects on the cultured dental cells are similar to those described in these earlier studies, although the hexose of fraction 5 indicates that glycoprotein material is present in addition to the proteoglycan material.

When compared to control cultures, cells from the inner dental epithelium cultured in contact with Millipore filters coated with protein fractions 5 or 6 demonstrated slight changes in the cell shape (elongation) and a preferential localization of the nuclei at the basal pole. However, it was not possible to observe the differentiation of functional ameloblasts. When enamel organs were grown on noncoated Millipore filters there was no reconstitution of a basal lamina. This observation agrees with previous data from Thesleff *et al.* (1978). However, when enamel organs were cultured on agar or plasma, basal lamina was deposited after a few hours while no polarization occurred (Osman & Ruch, 1980). The presence

Fig. 4. Electron micrographs of dental papillae cultured for 4 days.

(A) The mesenchymal cell in contact with uncoated Millipore filter neither elongated nor polarized. Cell processes (*cp*) invaded the pores of the filter (*f*) ($\times 3200$).

(B,C) Mesenchymal cells grown on Millipore filters coated with protein fraction 6. The rough endoplasmic reticulum (*rer*) accumulated electron-dense material (B) ($\times 27000$). These cells elongated and polarized (C). The nuclei (*n*) migrated toward the basal pole of the cells. Numerous Golgi vesicles (*Go*) are observed in the supranuclear region. Cell processes (*cp*) invaded the pores of the filter (*f*) ($\times 6500$). *m*, mitochondria.



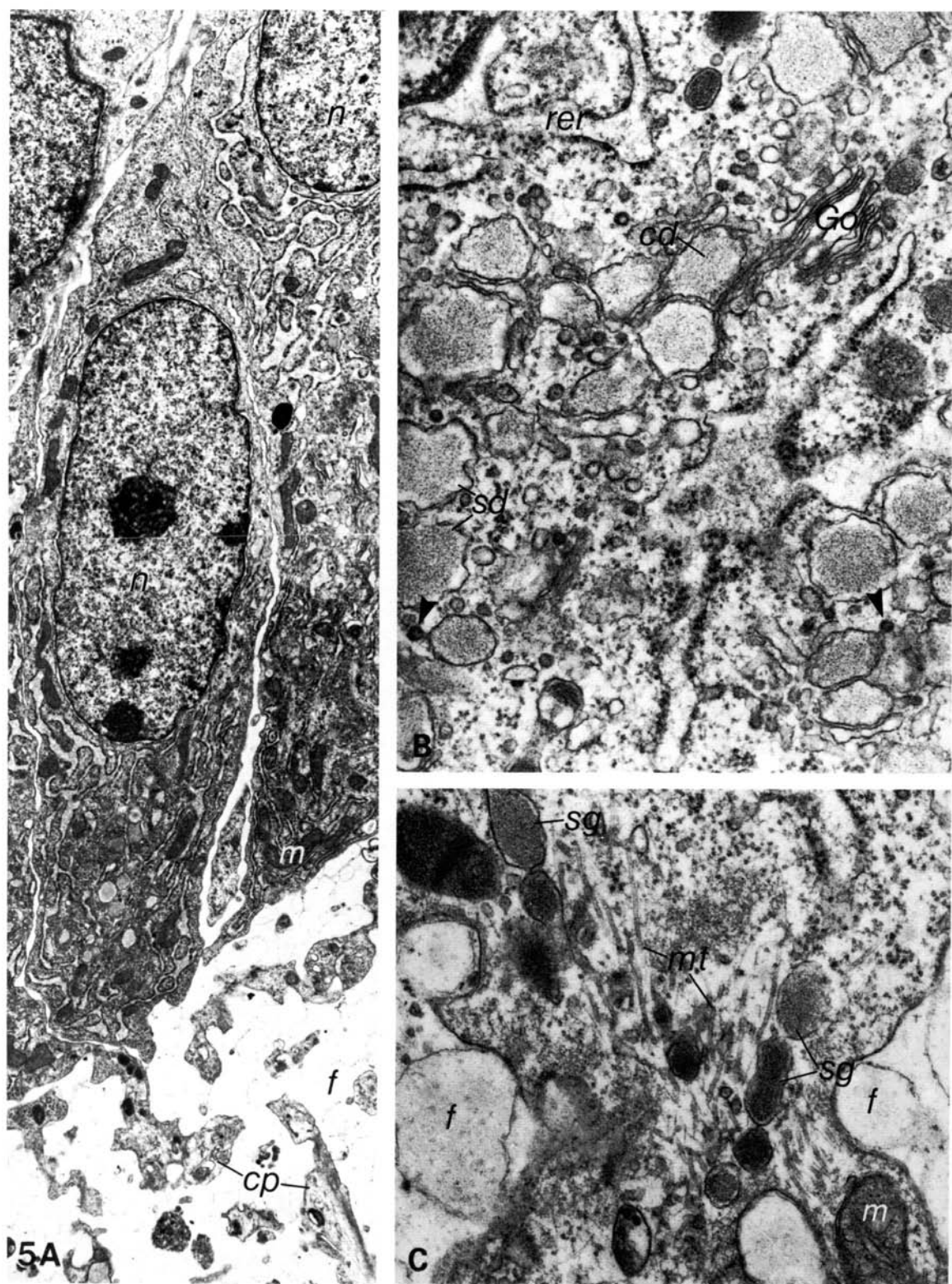


Fig. 5. For legend see p. 206

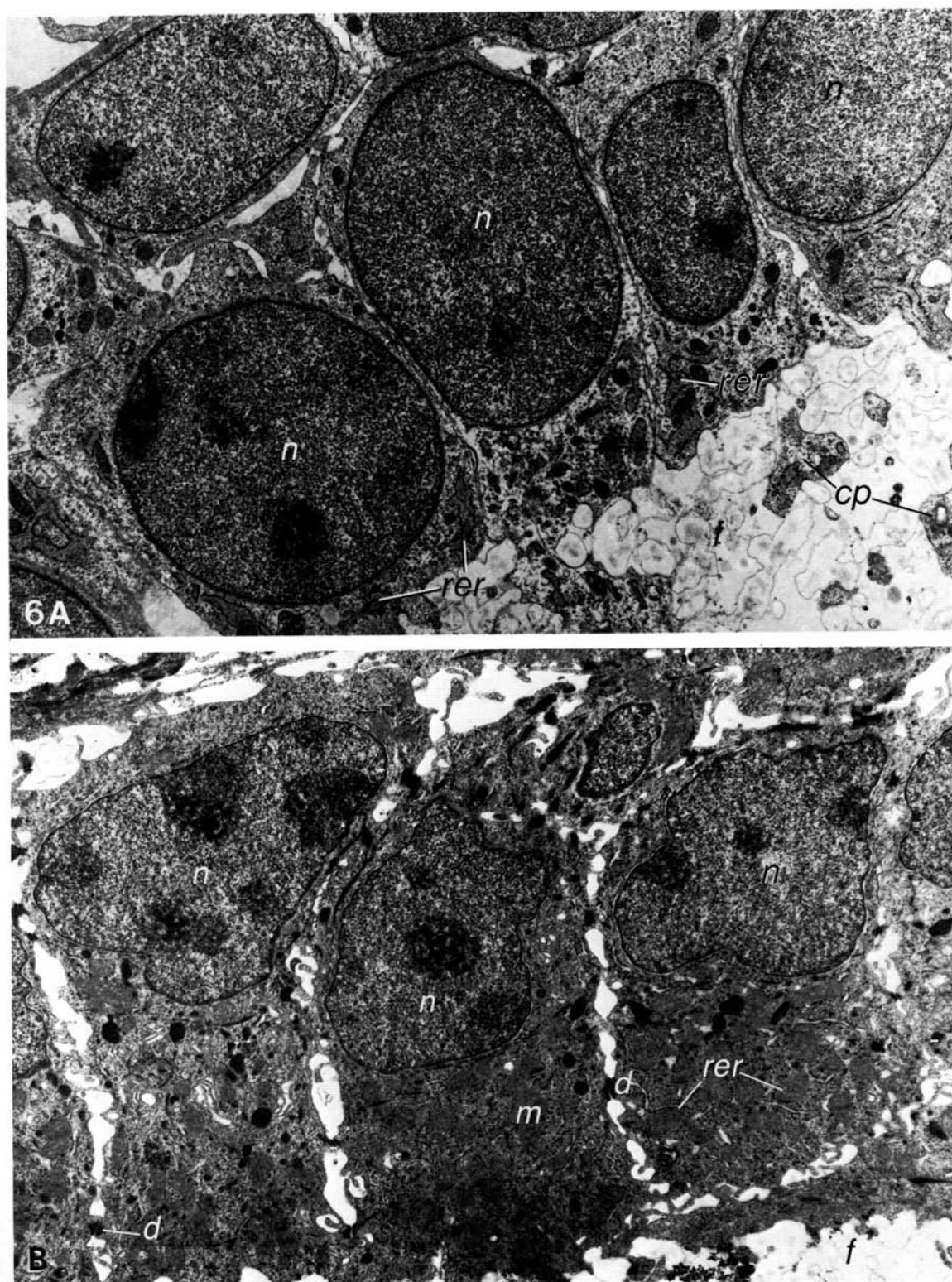


Fig. 6. For legend see p. 206

of protein fractions 5 or 6 led to modification in the cell shape but not to the deposition of a new basal lamina. The concomitant existence of polarized mammalian ameloblasts and a continuous basal lamina has been documented for tooth germs cultured in a serum-less chemically defined medium (Slavkin *et al.* 1983). In physiological conditions, the degradation of the basal lamina precedes and might be a prerequisite for the terminal differentiation of ameloblasts. Karcher-Djuricic *et al.* (1985) have shown that functional ameloblasts differentiated when enamel organs from stage 18 were cultured for 4 days in contact with the epithelial side of predentin of isolated dentin matrices. The extraction of these isolated dental matrices, either with 4 M-guanidine-EDTA or 0.5 M-acetic acid, resulted in the loss of the morphogenetic properties. Collagen type I, a major constituent of predentin-dentin, does not seem to have morphogenetic properties since isolated molecules of type I collagen are not able to promote the differentiation of ameloblasts. However, protein fraction 6 (and possibly fraction 5) is tightly associated with collagen type I and the possible physiological properties of protein fractions 5 and 6 might depend on interactions with collagen as far as their configuration and spatial distribution is concerned. Such a possibility is in agreement with previous observations showing that bone matrix possessed inductive properties which (1) were lost after 4 M-guanidine extraction and (2) could be restored after reconstitution of the matrix (Sampath & Reddi, 1981; Sampath, Nathanson & Reddi, 1984). It is possible that intimate relationships exist between several components of the matrix that are important for morphogenesis and cytodifferentiation. To investigate this, we propose to use new substrates including both type I collagens and noncollagenous protein fractions 5 or 6 in order to see whether it might improve the differentiation process of ameloblasts.

When dental papillae are cultured on noncoated Millipore filters, elongated and polarized cells are never observed. On the other hand, mesenchymal cells in

Fig. 5. Electron micrographs of dental papillae cultured for 4 days on Millipore filters coated with protein fraction 2.

(A) Mesenchymal cells in contact with the filter (*f*) elongated and polarized. Numerous cell processes (*cp*) invaded the filter ($\times 5000$).

(B) Golgi region of a polarized cell demonstrating successive compartments of the rough endoplasmic reticulum (*rer*)–Golgi (*Go*) pathway of protein synthesis. It includes spherical distensions (*sd*), cylindrical distensions (*cd*) and round buds (arrowhead) which have been described by Marchi & Leblond (1984) ($\times 37000$).

(C) Secretory pole of a polarized cell in contact with the filter (*f*) demonstrating secretory granules (*sg*) close to microtubules (*mt*) ($\times 37000$). *m*, mitochondria; *n*, nucleus.

Fig. 6. Electron micrographs of dental epithelial cells cultured for 4 days.

(A) The cuboidal cells of the inner dental epithelium cultured on noncoated Millipore filter (*f*) maintained an epithelial arrangement. There was no preferential localization of the nuclei (*n*) ($\times 6000$).

(B) Cells in contact with filters coated with protein fraction 5 elongated and the nuclei (*n*) migrated toward the basal pole. The mitochondria (*m*) had mainly a supranuclear distribution. The cytological aspect of the nuclei attested a high metabolic activity ($\times 6000$). *cp*, cell processes; *d*, desmosome; *f*, filter; *rer*, rough endoplasmic reticulum.

contact with Millipore filters coated with protein fractions 2 or 6 elongated, polarized and demonstrated widening of cisternae of the rough endoplasmic reticulum and of the Golgi, suggesting a high metabolic activity. However, these cells did not deposit an extracellular matrix. Polarized, but nonfunctional, odontoblasts have been recently reported by Sakakura, Iida, Ishizeki & Nawa (1984) when developing mouse tooth germs were treated with calcitonin *in vitro*. In physiological conditions, the terminal differentiation of odontoblast requires interaction of the differentiating cells with a specific basement membrane (Osman & Ruch, 1981). Immunochemical studies are in progress in order to determine if proteins from fractions 2 and 6 are also constituents of this extracellular matrix.

The elongation and polarization of mesenchymal cells interacting with protein fractions 2 and 6 as well as the more discrete effects of protein fractions 5 and 6 on cultured epithelial cells are related to changes in the organization and, or, the activity of the cytoskeleton. A direct effect of matrix molecules such as collagens, fibronectin or laminin on the activity of the cytoskeleton has been reported for different cell types in culture (Sugrue & Hay, 1981; Tomasek & Fugiwara, 1982) and these effects were suggested to be mediated by membrane receptors (Sugrue & Hay, 1982). Membrane proteins interacting specifically with collagens (Chiang & Kang, 1982; Kurkinen, Taylor, Garrels & Hogan, 1984; Mollenhauer & von der Mark, 1983), fibronectin (Hughes, Butters & Aplin, 1981; Pytela, Pierschbacher & Ruoslahti, 1985; Lesot *et al.* 1985) or laminin (Malinoff & Wicha, 1983; Lesot, Kuhl & von der Mark, 1983) have been identified. Similar investigations will have to be performed with the noncollagenous proteins tested here in order to know whether these interact directly or indirectly with the plasma membranes of cultured dental cells.

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REFERENCES

- BANG, G. & URIST, M. R. (1967). Bone induction in excavation chambers in matrix of decalcified dentin. *Archs Surg., Chicago* **94**, 781–789.
- BERNFELD, M., BANERJEE, S. D., KODA, J. E. & RAPRAEGER, A. C. (1984). Remodelling of the basement membrane: morphogenesis and maturation. In *Basement Membranes and Cell Movement* (ed. R. Porter & J. Whelan), pp. 179–212. London: Pitman.
- BLUMENKRANTZ, N. & ASBOE-HANSEN, G. (1973). New method for quantitative determination of uronic acids. *Analyt. Biochem.* **54**, 484–489.
- BLUMENKRANTZ, N. & ASBOE-HANSEN, G. (1976). An assay for total hexosamine and a differential assay for glycosamine and galactosamine. *Clin. Biochem.* **9**, 269–274.
- BUTLER, W. T., MIKULSKI, A. & URIST, M. R. (1977). Noncollagenous proteins of a rat dentin matrix possessing bone morphogenetic activity. *J. dent. Res.* **56**, 228–232.
- CHIANG, T. M. & KANG, A. H. (1982). Isolation and purification of (I) receptor from human platelet membrane. *J. biol. Chem.* **257**, 7581–7586.

- DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A. & SMITH, F. (1956). Colorimetric method for the determination of sugars and related substances. *Analyt. Chem.* **28**, 350–356.
- HUGGINS, C., WISEMAN, S. & REDDI, A. H. (1970). Transformation of fibroblasts by allogenic and xenogenic transplants of demineralized tooth and bone. *J. exp. Med.* **132**, 1250–1258.
- HUGHES, R. C., BUTTERS, T. D. & APLIN, J. D. (1981). Cell surface molecules involved in fibronectin-mediated adhesion. A study using specific antisera. *Eur. J. Cell Biol.* **26**, 198–207.
- KARCHER-DJURICIC, V., STAUBLI, A., MEYER, J. M. & RUCH, J. V. (1985). Acellular dental matrices promote functional differentiation of ameloblasts. *Differentiation* **11**, 169–175.
- KOLLAR, E. J. & BAIRD, G. R. (1970). Tissue interaction in embryonic mouse tooth germs. II. The inductive role of the dental papilla. *J. Embryol. exp. Morph.* **24**, 173–186.
- KURKINEN, M., TAYLOR, A., GARRELS, J. I. & HOGAN, B. L. M. (1984). Cell surface associated proteins which bind native type IV collagen or gelatin. *J. biol. Chem.* **259**, 5915–5922.
- LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.
- LESOT, H., KARCHER-DJURICIC, V., MARK, M., MEYER, J. M. & RUCH, J. V. (1985). Dental cell interaction with extracellular matrix constituents: type I collagen and fibronectin. *Differentiation* **11**, 176–181.
- LESOT, H., KUHLE, U. & VON DER MARK, K. (1983). Isolation of a laminin-binding protein from muscle cell membranes. *EMBO J.* **2**, 861–865.
- LESOT, H., MEYER, J. M., KARCHER-DJURICIC, V., AHMAD, N., STAUBLI, A., IDRIS, H., MARK, M., BOUKARI, A. & RUCH, J. V. (1985). Influences of matrix molecules or dental matrices on dental cell kinetics and cytodifferentiation. In *Tooth Morphogenesis and Differentiation* (ed. A. B. Belcourt & J. V. Ruch), pp. 89–108. Paris: INSERM.
- MALINOFF, H. L. & WICHA, M. S. (1983). Isolation of a cell surface receptor protein for laminin from murine fibrosarcoma cells. *J. Cell Biol.* **96**, 1475–1479.
- MARCHI, F. & LEBLOND, C. P. (1984). Radioautographic characterization of successive compartments along the rough endoplasmic reticulum–Golgi pathway of collagen precursors in foot pad fibroblasts of ³H proline-injected rats. *J. Cell Biol.* **98**, 1705–1709.
- MOLLENHAUER, J. & VON DER MARK, K. (1983). Isolation and characterization of a collagen-binding glycoprotein from chondrocyte membranes. *EMBO J.* **2**, 45–50.
- OAKLEY, B. R., KIRSCH, D. R. & MORRIS, N. R. (1980). A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. *Analyt. Biochem.* **105**, 361–363.
- OSMAN, M. & RUCH, J. V. (1980). Secretion of basal lamina by trypsin-isolated embryonic mouse molar epithelial cultured *in vitro*. *Devl Biol.* **75**, 467–470.
- OSMAN, M. & RUCH, J. V. (1981). Behaviour of odontoblasts and basal lamina of trypsin- or EDTA-isolated mouse dental papillae in short-term culture. *J. dent. Res.* **60**, 1015–1027.
- PYTELA, R., PIERSCHBACHER, M. D. & RUOSLAHTI, E. (1985). Identification of a 140 Kd cell surface glycoprotein with properties expected of a fibronectin receptor. *Cell* **40**, 191–198.
- REDDI, A. H. (1984). Extracellular Matrix and Development. In *Extracellular Matrix Biochemistry* (ed. K. A. Piez & A. H. Reddi) pp. 375–412. New York: Elsevier.
- RICHARDSON, W. S., BEAGLE, W. F., BUTLER, W. T. & MUNKSGAARD, E. G. (1977). The phosphoprotein of rabbit incisors. *J. dent. Res.* **56**, 233–236.
- RUCH, J. V. (1985). Odontoblast differentiation and the formation of the odontoblast layer. *J. dent. Res.* **64**, 489–498.
- RUCH, J. V., KARCHER-DJURICIC, V. & GERBER, R. (1972). Quelques aspects du rôle de la pré-dentine dans la différenciation des adamantoblastes. *Archs Anat. microsc. Morph. exp.* **61**, 127–138.
- RUCH, J. V., KARCHER-DJURICIC, V. & THIEBOLD, J. (1976). Cell division and cyto-differentiation of odontoblasts. *Differentiation* **5**, 165–169.
- RUCH, J. V., LESOT, H., KARCHER-DJURICIC, V., MEYER, J. M. & OLIVE, M. (1982). Facts and hypotheses concerning the control of odontoblast differentiation. *Differentiation* **21**, 7–12.
- SAKAKURA, Y., IIDA, S., ISHIZEKI, K. & NAWA, T. (1984). Ultrastructure of the effects of calcitonin on the development of mouse tooth germs *in vitro*. *Archs oral Biol.* **29**, 507–512.
- SAMPATH, T. K., NATHANSON, M. A. & REDDI, A. H. (1984). *In vitro* transformation of mesenchymal cells derived from embryonic muscle into cartilage in response to extracellular matrix components of bone. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3419–3423.

- SAMPATH, T. K. & REDDI, A. H. (1981). Dissociative extraction and reconstitution of extracellular matrix components involved in local bone differentiation. *Proc. natn. Acad. Sci. U.S.A.* **78**, 7599–7603.
- SKOZA, L. & MOHOS, S. (1976). Stable thiobarbituric acid chromophore with dimethyl sulphoxide – application to sialic acid assay in analytical de-O-acetylation. *Biochem. J.* **159**, 457–462.
- SLAVKIN, H. C. (1978). The nature or nurture of epithelial–mesenchymal interactions during tooth morphogenesis. *J. biol. Buccale* **6**, 189–203.
- SLAVKIN, H. C. (1982). Combinatorial process for extracellular matrix influences on gene expression: a hypothesis. *J. craniofacial Genet. devl Biol.* **2**, 179–189.
- SLAVKIN, H. C., BROWNWELL, A. G., BRINGAS, P., MACDOUGALL, M. K. & BESFEM, C. (1983). Basal lamina persistence during epithelial–mesenchymal interactions in murine tooth development *in vitro*. *J. craniofacial Genet. devl Biol.* **3**, 387–409.
- SMITH, A. J. & LEAVER, A. G. (1979). Non-collagenous components of the organic matrix of rabbit incisor dentine. *Archs oral Biol.* **24**, 449–454.
- SMITH, A. J. & LEAVER, A. G. (1981). Distribution of the EDTA-soluble non-collagenous organic matrix components of rabbit incisor dentine. *Archs oral Biol.* **26**, 643–649.
- SMITH, A. J., PRICE, R. & LEAVER, A. G. (1979). Components of the organic matrices of rabbit incisor and molar dentine isolated after digestion of the demineralized tissues with collagenase. *Archs oral Biol.* **24**, 955–963.
- SMITH, A. J. & SMITH, G. (1984). Proteolytic activity of rabbit incisor dentine. *Archs oral Biol.* **29**, 1049–1050.
- SUGRUE, S. P. & HAY, E. D. (1981). Response of basal epithelial cell surface and cytoskeleton to solubilized extracellular matrix molecules. *J. Cell Biol.* **91**, 45–54.
- THESLEFF, I. (1978). Role of the basement membrane in odontoblast differentiation. *J. biol. Buccale* **6**, 241–249.
- THESLEFF, I. & HURMERINTA, K. (1981). Tissue interactions in tooth development. *Differentiation* **18**, 75–88.
- THESLEFF, I., LEHTONEN, E. & SAXEN, L. (1978). Basement membrane formation in transfilter tooth culture and its relation to odontoblast differentiation. *Differentiation* **10**, 71–79.
- TOMASEK, J. J., HAY, E. D. & FUJIWARA, K. (1982). Collagen modulates cell shape and cytoskeleton of embryonic corneal and fibroma fibroblasts: Distribution of actin, α -actinin and myosin. *Devl Biol.* **92**, 107–122.
- VON DER MARK, K., MOLLENHAUER, J., KUHLE, U., BEE, J. & LESOT, H. (1984). Anchorins: a new class of membrane proteins involved in cell–matrix interactions. In *The Role of Extracellular Matrix in Development* (ed. R. L. Trelstad), pp. 67–87. New York: Alan R. Liss.
- YAMADA, K. M. (1983). Cell surface interactions with extracellular materials. *A. Rev. Biochem.* **52**, 761–799.
- YOEMANS, J. D. & URIST, M. R. (1967). Bone induction by decalcified dentin implanted into oral and muscle tissues. *Archs oral Biol.* **12**, 999–1008.

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