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Cell-matrix interactions in tooth development

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ABSTRACT. A chain of reciprocal interactions between the epithelial and mesenchymal tissues regulates both morphogenesis and cell differentiation in the developing tooth. The very early interactions lead to budding of the oral epithelium and to the characteristic condensation of the neural crest-derived mesenchymal cells around the epithelial bud. During the bell stage of morphogenesis, the mesenchymal cells which are in contact with the dental epithelium differentiate into odontoblasts. In this reveiw article we summarize the results of our descriptive and experimental studies, which indicate that differentiation of the dental mesenchymal cells into odontoblasts, as well the condensation of dental mesenchymal cells at the bud stage, are regulated by interactions between the cell surface and the extracellular matrix.

Transfilter studies where the dental epithelium and mesenchyme were cultured on opposite sides of Nuclepore filters, led to the hypothesis that the differentiation of dental mesenchymal cells into odontoblasts is triggered by interactions between the cell surface and the epithelial basement membrane matrix. Immunohistochemical localization of various matrix molecules showed that the matrix glycoproteins fibronectin and tenascin are accumulated in the dental basement membrane at the time of odontoblast differentiation. Fibronectin and tenascin are known to interact with each other, with other matrix molecules as well as with the cell surface, and also to influence cell shape. We suggest that fibronectin and tenascin are involved in the cell-matrix interaction which leads to the polarization and differentiation of odontoblasts.

Immunohistochemical localization of the matrix glycoprotein, tenascin, and the cell surface proteoglycan, syndecan, has indicated that these molecules, unlike other molecules studied, are accumulated in the condensing dental mesenchyme in bud-staged teeth. By using *in vitro* recombination experiments we have been able to demonstrate that the presumptive dental epithelium induces the expression of tenascin and syndecan in dental mesenchyme. Because syndecan acts as a matrix receptor in epithelial cells, it can be speculated that tenascin and syndecan are involved in the mediation of cell-matrix interactions during the condensation of dental mesenchymal cells.

KEY WORDS: *extracellular matrix, epithelial-mesenchymal interactions, morphogenesis, fibronectin, tenascin, cell surface proteoglycan.*

Introduction

Changes in the extracellular matrix (ECM) accompany different developmental events such as cell migration, cell differentiation and morphogenesis (Thesleff *et al.*, 1979; Bernfield and Barnejee, 1982; Hay, 1983, Ekblom *et al.*, 1986; Watt, 1986). Although the ECM appears to play important roles in development, the question as to how the matrix affects cellular behavior has remained unanswered for most embryonic systems. Also, it has been difficult to interpret experiments where matrix components have been introduced to differentiating cells *in vitro*. The possibility exists that the ECM molecules may be involved in the maintenance of cell shape and polarization, and so provide only a permissive environment for the action of other factors affecting gene regulation more directly.

The matrix molecules do not cross the cell membrane, and therefore they must exert their effects at the cell surface. Hence, what is needed are cell surface receptors which are able to transmit the signal from the ECM to the cell interior. Such receptors, which bind with high specificity to various matrix molecules and which interact with intracellular molecules, have been described. The best characterized are the integrin family of receptors, which bind fibronectin and other molecules containing a RGDS sequence (Hynes, 1986; Ruoslahti and Piersbacher, 1987), and certain cell surface proteoglycans, which bind several matrix molecules (Rapraeger *et al.*, 1986). In this paper, we summarize our studies on the interactions between the ECM and the cell surface, which appear to regulate the condensation of mesenchymal cells and the differentiation of odontoblasts in the developing tooth.

Tooth development starts as a thickening of oral epithelium, which subsequently buds into the underlying mesenchyme (Fig. 1). At this time mesenchymal cells condense around the epithelial bud. This condensation is apparently induced by the epithelium, which, as recently shown by Mina and Kollar (1987) and Lumsden (1988), possesses odontogenic potential. Via epithelial-mesenchymal interactions the capacity for tooth formation is shifted from the epithelium to the mesenchyme, since at the cap and bell stages (Fig. 1) the dental papilla mesenchyme expresses the odontogenic potential (Kollar and Baird, 1970, Ruch and Karcher-Djuricic, 1975; Thesleff, 1977). During the bell stage, the dental mesenchymal cells which dir-

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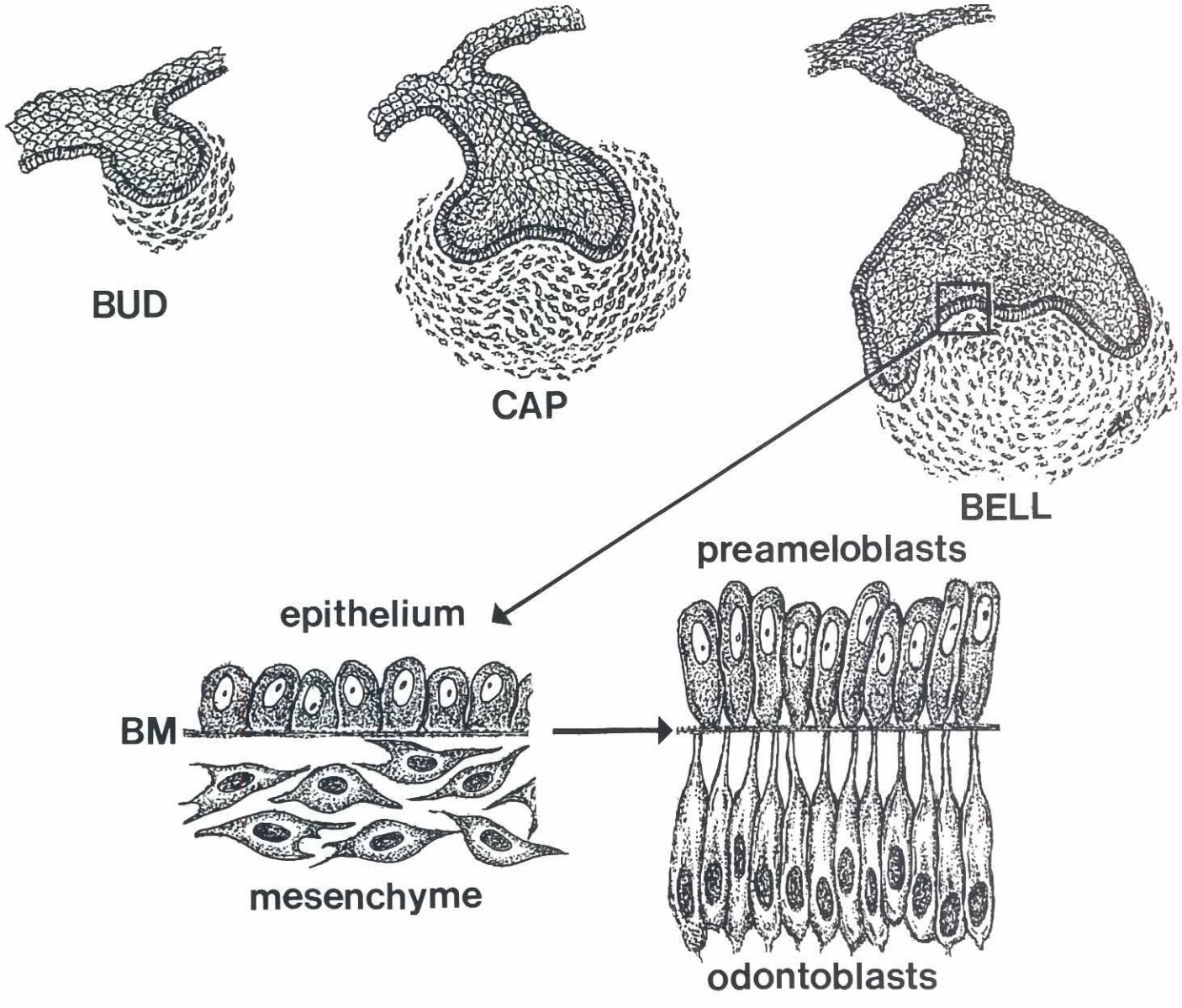

Fig. 1. *Schematic illustration of different stages in tooth morphogenesis. At bud stage neural crest derived mesenchymal cells condense around the epithelial bud. At cap stage the epithelium invaginates at its undersurface and encompasses the dental papilla mesenchyme. At the bell stage the form of the tooth crown is established, and odontoblasts and ameloblasts differentiate and start to secrete dentin and enamel matrices, respectively. The higher magnifications of the epithelial-mesenchymal interface at the bell stage illustrate the differentiation of dental papilla mesenchymal cells into odontoblasts. This results from an interaction between mesenchymal cells and the basement membrane extracellular matrix (BM, basement membrane).*

ectly underlie the epithelium differentiate terminally into odontoblasts (Figs. 1, 2A). This differentiation results also from epithelial-mesenchymal interaction.

The studies that are summarized in this review were started in 1974 in Lauri Saxén's laboratory at the Department of Pathology, University of Helsinki. At that time transfilter methodology was being successfully applied to studies on epithelial-mesenchymal interactions during early kidney development (Wartiovaara *et al.*, 1974; Lehtonen, 1976; Saxén, *et al.*, 1976) and we decided to extend this method to the analysis of tooth development. The importance of epithelial-mesenchymal interactions in tooth morphogenesis had been established years earlier (Sellman, 1946; Gaunt and Miles, 1967), and their nature was still being actively studied in several laboratories (Kollar and Baird, 1970; Slavkin, 1974; Ruch and Karcher-Djuricic, 1975). Thus, the developing tooth appeared to be an ideal model for studies on the mechanisms of tissue interactions. The mechanism of the interaction between the dental epithelium and mesenchyme, which triggers odontoblast differentiation (Fig.1), was regarded as a suitable problem for transfilter analysis.

The transfilter studies led to the hypothesis that cell-matrix interactions trigger odontoblast differentiation. The composition of the epithelial-mesenchymal interface was subsequently analysed by immunohistochemical localization of various matrix

and cell surface molecules. Further observations of the accumulation of the matrix glycoprotein, tenascin (Chiquet-Ehrismann *et al.*, 1986) and the cell surface proteoglycan, syndecan (Saunders *et al.*, 1989) in the condensing dental mesenchyme (Thesleff *et al.*, 1987, 1988) led us to study tissue interactions during earlier stages of tooth development. Our recent tissue recombination experiments, which are also reviewed below, have focused on the early epithelio-mesenchymal interactions, which lead to the condensation of tooth-specific mesenchymal cells around the epithelial bud. We have shown that at this time the presumptive dental epithelium induces the expression of tenascin and syndecan in the condensing dental mesenchyme (Vainio *et al.*, 1989).

Results and Discussion

Odontoblast differentiation is triggered by cell-matrix interaction

That an interaction with epithelium is a prerequisite for the differentiation of dental papilla mesenchymal cells into odontoblasts, has been well established (cf. Thesleff and Hurmerinta, 1981). The mechanism of this epithelial-mesenchymal interaction was examined with the transfilter technique using Nuclepore filters, which allow exact localization of cell processes in electron microscopy (Wartiovaara *et al.*, 1974). The filters were placed between the epithelial and mesenchymal tissues, which were enzymatically separated from bell-staged mouse embryonic molar teeth. The differentiation of odontoblasts was examined after various times in culture and correlated with electron microscopic observations of the interphase between the interacting tissues. The observations indicated that odontoblast differentiation did not take place through small pore size filters, which prevented the penetration of cell processes into the filter pores. When the pore size was 0.2 microns or more, odontoblasts differentiated (Fig. 2b, Thesleff *et al.*, 1977). Electron microscopical observations indicated that the differentiating mesenchymal cells had sent cellular processes through these filters. The processes were frequently seen in close contact with the basement membrane material which had been restored between the epithelial cells and the filter (Thesleff *et al.*, 1978). Since similar contacts were also observed *in vivo* at the time of odontoblast differentiation (Hurmerinta and Thesleff, 1981), it was suggested that the differentiation of odontoblasts is triggered by an interaction between the mesenchymal cell surface and the basement membrane matrix (Thesleff and Hurmerinta, 1981).

Our subsequent studies were devoted to studies of the molecular mechanism of odontoblast differentiation. Experimental *in vitro* studies where various metabolic inhibitors were applied, suggested that proteoglycans as well as glycoproteins were involved (Hurmerinta *et al.*, 1979; Thesleff and Pratt, 1980). Immunohistological analysis of the dental basement membrane indicated that basal lamina components such as type IV collagen (Fig. 3A), laminin and the BM-1 proteoglycan were evenly distributed in the dental and other basement membranes. Interestingly, fibronectin was accumulated in the dental basement membrane at the time of odontoblast differentiation (Fig. 3B, Thesleff *et al.* , 1979, 1981) and it also was shown to be of mesenchymal origin (Hurmerinta *et al.*, 1986). Our recent observations indicate that another interstitial matrix glycoprotein, tenascin (also known as cytotactin) is also enriched in the dental basement membrane at the time of odontoblast differentiation (Fig. 3C, Thesleff *et al.*, 1987). Tenascin has been shown to interact with fibronectin, and it has been suggested that a certain balance of tenascin and fibronectin is critical for their physiological action (Chiquet-Ehrismann *et al.*, 1988). It can be speculated that both tenascin and fibronectin are involved in the interaction between the basement membrane and the mesenchymal cell surface which leads to the polarization and differentiation of odontoblasts.

The molecular mechanism behind the polarization and differentiation of odontoblasts has been extensively studied by Ruch and his associates (Ruch, 1987). Their observations on changes in the odontoblast cytoskeleton and in the extracellular matrix have given additional support for the involvement of a

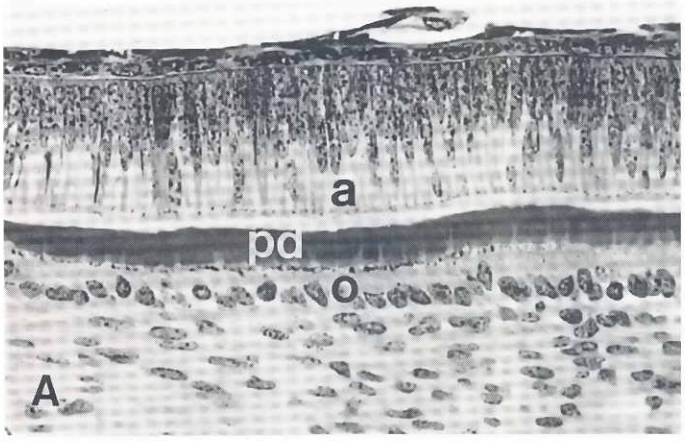

Fig. 2A. *Photomicrograph of the epithelial-mesenchymal interface of a molar tooth of a newborn mouse. The differentiated odontoblasts have secreted predentin matrix and the epithelial cells are polarizing into ameloblasts.*

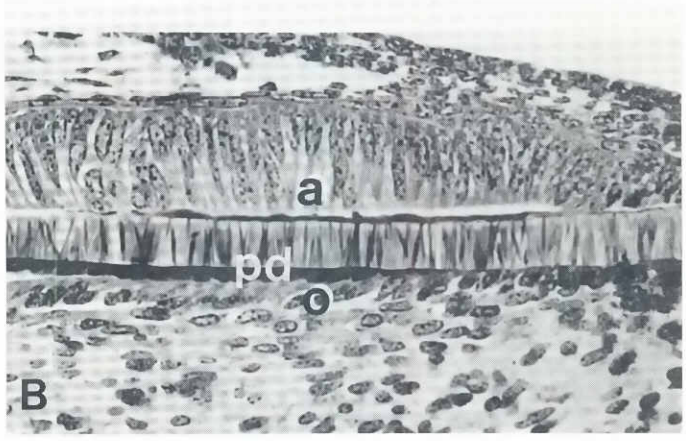

Fig. 2B. *Differentiation of the same cells as in 2A in a transfilter explant. The undifferentiated dental papilla and enamel organ were separated at early bell stage and cultured with an interposed Nuclepore filter (0.6μm pore diameter) for 7 days. o, odontoblasts, a, ameloblasts, pd, predentin.*

cell-matrix type of interaction (Kubler *et al.*, 1988). They have also recently produced a monoclonal antibody which recognizes a cell membrane protein transducing a signal from the extra- to the intracellular compartment, and which inhibits odontoblast differentiation *in vitro* (Lesot *et al.*, 1988).

The expression of the matrix glycoprotein, tenascin, and the cell surface proteoglycan, syndecan, is developmentally regulated during tooth morphogenesis

Based on *in vitro* inhibition studies, important roles in tooth morphogenesis have been suggested for members of all main categories of matrix molecules including collagens, proteoglycans and glycoproteins (Galbraith and Kollar, 1974; Hetem *et al.*, 1975; Hurmerinta *et al.*, 1979). With immunohistochemical staining it became possible to map out developmental changes in some well characterized matrix molecules, such as different types of collagens and fibronectin (Thesleff *et al.*, 1979; Lesot *et al.*, 1981; Kubler *et al.*, 1988). The observation by Chiquet-Ehrismann *et al.* (1986) that the matrix glycoprotein tenascin is accumulated in the early organ-specific mesenchyme in anlagen of teeth, hair follicles and mammary glands suggested that this particular matrix component is involved in morphogenetic tissue interactions. Other matrix components such as fibronectin and collagens did not show similar accumulation (Fig.4).

We have recently observed that the cell surface proteoglycan, syndecan, which acts as a matrix receptor on epithelial cell (Koda and Bernfield, 1984; Rapraeger and Bernfield, 1985; Rapraeger *et al.*, 1986; Jalkanen, 1987; Saunders and Bernfield, 1988; Saunders *et al.*, 1989) shows similar accumulation in the condensing dental mesenchyme as does tenascin (Fig. 4, Thesleff *et al.*, 1988). Whereas tenascin expression persists in the dental mesenchyme throughout development, syndecan expression is transient and disappears by the late cap stage of development (see Fig. 1). These observations, together with the earlier findings that this particular proteoglycan is not found in adult connective tissues (Hayashi *et al.*, 1987), suggest a morphogenetic role for syndecan. The antibody that was used (Jalkanen *et al.*, 1985) recognizes the core protein of the ectodomain of the proteoglycan, and we do not yet know whether syndecan is located on the cell surface also in the mesenchyme as has been shown for the epithelium (Rapraeger and Bernfield, 1985). Our recent molecular analysis of the syndecan synthesized by the dental mesenchyme has shown that the main GAG bound to this proteoglycan is heparan sulfate and that it has the same molecular size of 200-250 kD by SDS-PAGE as the dental epithelial proteoglycan (unpublished). This suggests that the ectodomain of the mesenchymal proteoglycan has similar properties to those of epithelial tissue and that it may function as a matrix receptor.

Tenascin is known to interact with other matrix molecules and with cells (Chiquet-Ehrismann *et al.* 1986, 1988), but its cell surface receptor has not yet been characterized. Our recent observations indicate that tenascin binds syndecan from dental tissues (unpublished). Hence, it can be speculated that syndecan and tenascin represent a couple formed by a cell membrane receptor and its matrix ligand, and that their interaction forms a basis for mesenchymal cell condensation during early tooth organogenesis.

The presumptive dental epithelium induces the expression of tenascin and syndecan in the mesenchyme

Syndecan and tenascin were present in the differentiating dental mesenchyme adjacent to the budding epithelium, and the period of their expression coincided with morphogenetic tissue interactions between the epithelium and mesenchyme (Thesleff *et al.*, 1987, 1988). This suggested to us that the two

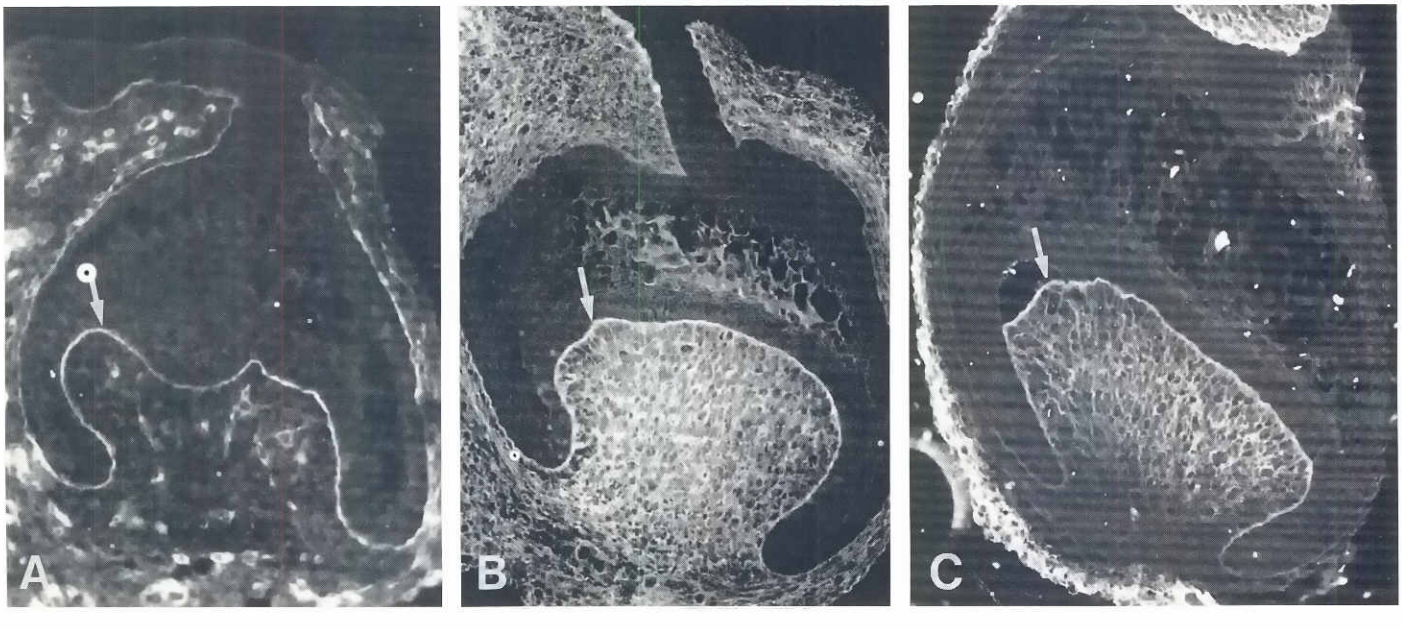

Fig. 3. *Immunofluorescent localization of type IV collagen (A), fibronectin (B) and tenascin (C) in bell-staged mouse embryonic molars. Type IV collagen is evenly distributed in all basement membranes. Fibronectin and tenascin are expressed in the mesenchymal tissue and appear accumulated in the basement membrane which underlines the enamel epithelium and which triggers odontoblast differentiation (arrows).*

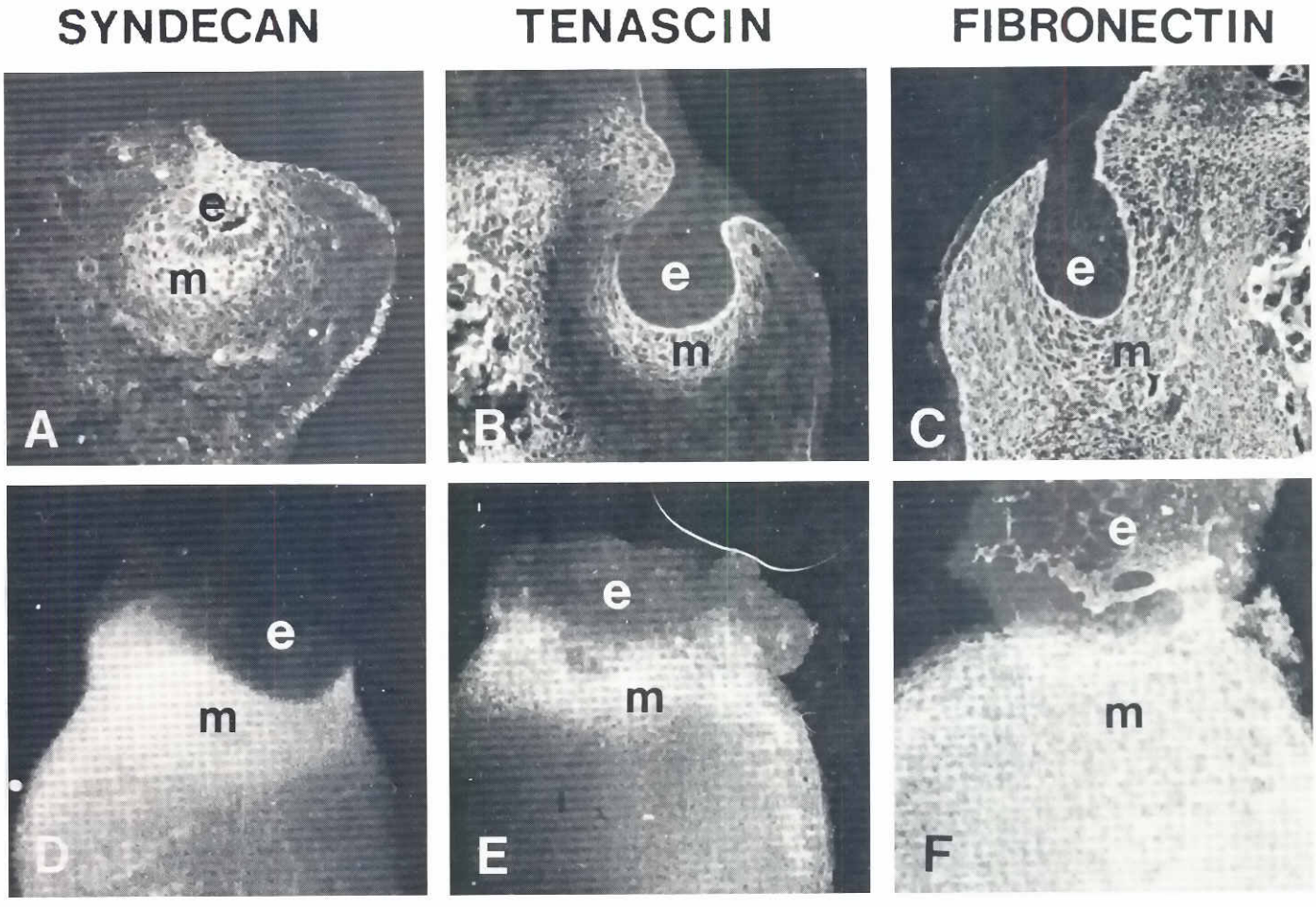

Fig. 4. *Immunofluorescent localization of cell surface proteoglycan (A, D), tenascin (B, E) and fibronectin (C, F) in bud-staged tooth germs* in vivo *(A-C) and in cultured recombinants of presumptive dental epithelium and mesenchyme (D-F). Accumulation of cell surface proteoglycan and tenascin is apparent* in vivo *in the condensed dental mesenchyme, and in the experimental recombinants in the mesenchymal tissue which is in contact with the epithelium. Fibronectin is expressed throughout the mesenchymal tissue and shows no accumulation in the condensed dental mesenchyme. e, epithelium, m, mesenchyme.*

molecules are involved in epithelial-mesenchymal interactions, and we decided to study this question in experimental tissue recombination cultures.

We designed a model system where presumptive dental epithelium and mesenchyme were separated from the lower jaw of 11 day-old mouse embryos and thereafter recombined for culture on Nuclepore filters. The appearance of tenascin and syndecan was monitored with immunofluorescent staining of the whole mounts. We were able to demonstrate that when the epithelium was cultured in contact with the mesenchyme both tenascin and syndecan appeared in the contact area. The positive area in the mesenchyme increased as culture time advanced (Fig. 4D, E; Vainio *et al.*, 1989). Other matrix molecules such as fibronectin, type III collagen and laminin did not show similar accumulation in the epithelial contact area (Fig. 4F). The expression of the syndecan by mesenchymal tissue was also verified by metabolic labeling. Furthermore, the possibility that some of syndecan would have originated from the epithelium by proteolytic cleavage of the ectodomain (Jalkanen *et al.*, 1987) was excluded by interspecies recombinations between rat and mouse tissues. The antibody used does not recognize the rat syndecan, and in combinations of mouse epithelium with rat mesenchyme no positive reaction was seen in the mesenchyme.

Our results suggest that the presumptive dental epithelium induces the neural crest-derived jaw mesenchymal cells to express the matrix glycoprotein, tenascin and the cell surface proteoglycan, syndecan. Because the condensation of mesenchymal cells is induced at the same time, it is tempting to speculate that syndecan and tenascin are involved in the mediation of cell-matrix interactions during condensation and differentiation of the dental mesenchymal cells.

Conclusions and speculations

The conclusion that the polarization and differentiation of odontoblasts results from an an interaction between the cell surface and the basement membrane extracellular matrix, is based on our transfilter studies and subsequent descriptive and

experimental studies, and supported by the work of Ruch and his collaborators (Ruch, 1987). Furthermore, the co-distribution of the matrix glycoprotein tenascin and the cell surface proteoglycan, syndecan, and their induction in the condensing dental mesenchyme suggest that cell-matrix interactions are involved also in early tooth morphogenesis. Therefore, it appears justified to include odontoblast differentiation and the condensation of dental mesenchymal cells in the list of the developmental events which are regulated by cell-matrix interactions.

Although much has been learned about the molecular basis of cell-substratum interactions in cell culture systems, we still lack definitive proof of the developmental functions of individual molecules such as tenascin or cell surface proteoglycan. The interpretation of many experimental inhibition studies is difficult because of the complexity of the embryonic systems. New methodologies may give further insights into the molecular nature and regulation of cell-matrix interactions in *in vivo* situations. Functional studies can be designed where organ development or cell differentiation is interfered with by specific antibodies, purified molecules, their fragments or synthetic peptides. Such studies have already indicated roles for fibronectin and the integrin receptors in cell-matrix interactions during neural crest migration and myoblast fusion (Bronner-Fraser, 1986; Menko and Boettiger, 1987). With the advances in molecular biology, biological functions of individual matrix and cell surface molecules, as well as the control of their expression, can now be examined by introducing genes or regulatory nucleotide sequences into developing organs or transgenic mice. With the aid of this new technology it will perhaps be possible to answer the questions of how the information in the extracellular matrix is translated into cellular behavior and, especially, whether the extracellular matrix molecules act as direct signals influencing gene expression in the developing organism.

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