Changes in the distribution of tenascin during tooth development

IRMA THESLEFF[1], ELEANOR MACKIE[2], SEPPO VAINIO[1] and RUTH CHIQUET-EHRISMANN[2]

[1]Department of Pedodontics and Orthodontics, Institute of Dentistry, University of Helsinki, SF-00280 Helsinki, Finland
[2]Friedrich Miescher Institute, Basel, Switzerland

Summary

Tenascin is an extracellular matrix molecule that was earlier shown to be enriched in embryonic mesenchyme surrounding the budding epithelium in various organs including the tooth. In the present study tenascin was localized by immunohistology throughout the course of tooth development in the mouse and rat using polyclonal antibodies against chick tenascin. The results indicate that tenascin is expressed by the lineage of dental mesenchymal cells throughout tooth ontogeny. The intensity of staining with tenascin antibodies in the dental papilla mesenchyme was temporarily reduced at cap stage when the tooth grows rapidly and undergoes extensive morphogenetic changes. During the bell stage of morphogenesis, the staining intensity increased and tenascin was accumulated in the dental pulp even after completion of crown development and eruption. Tenascin was present in the dental basement membrane at the time of odontoblast differentiation. The dental papilla cells ceased to express tenascin upon differentiation into odontoblasts and tenascin was completely absent from dentin. It can be speculated that the remarkable expression of tenascin in the dental mesenchymal cells as compared to other connective tissues is associated with their capacity to differentiate into hard-tissue-forming cells.

Key words: tenascin, extracellular matrix, morphogenesis, odontogenesis, tissue interactions.

Introduction

The extracellular matrix plays an important role in embryonic morphogenesis and cell differentiation. For example, branching morphogenesis in the salivary gland is accompanied by remarkable changes in the tissue distribution of collagens and proteoglycans (Grobstein & Cohen, 1965; Bernfield & Banerjee, 1982). Interactions between many defined matrix molecules and the cell surface have been associated with cell differentiation (Saxen, Ekblom & Thesleff, 1982; Hay, 1982; Orkin, Knudson & Toole, 1985; Thiery, 1985; Zanetti & Solursh, 1986). The regulative role of the extracellular matrix in tooth development has been emphasized in numerous descriptive as well as experimental studies (Kollar, 1978; Thesleff, 1980; Thesleff & Hurmerinta, 1981; Ruch, 1985). We have shown earlier that the distribution of collagens and fibronectin change during tooth morphogenesis (Thesleff, Stenman, Vaheri & Timpl, 1979) and we have suggested that the differentiation of odontoblasts and ameloblasts in the developing tooth is mediated by interactions between the cells and the extracellular matrix (Thesleff & Hurmerinta, 1981).

Tenascin is a matrix protein that was previously described as chick myotendinous antigen (Chiquet & Fambrough, 1984a,b). It is identical to the protein described as hexabrachion (Erickson & Inglesias, 1984; Vaughan, Huber, Chiquet & Winterhalter, 1987) and probably also to the proteins described as cytotactin (Grumet, Hoffman, Crossin & Edelman, 1985), J1 (Kruse et al. 1985) and GMEM (Bourdon, Matthews, Pizzo & Bigner, 1985). In embryonic teeth, as well as mammary glands and hair follicles, tenascin is present in the dense, organ-specific mesenchyme surrounding the invaginating epithelial bud, but not in the more distant mesenchyme (Chiquet-Ehrismann, Mackie, Pearson & Sakakura, 1986). It has been proposed that tenascin plays a role in the tissue interactions that govern the early development of these embryonic organs. The aim of the present study was to analyse whether changes in the distribution of tenascin can be correlated with certain stages of tooth morphogenesis and cell differentiation. For this purpose, tenascin was localized in mouse and rat teeth from the stage of epithelial bud formation until tooth eruption.

Materials and methods

Preparation of tissues for immunohistochemistry

The lower jaw of hybrid mouse embryos or newborn mice (CBA×C57B1) was removed in phosphate-buffered saline, pH 7·3 (PBS). The molar tooth rudiments with some surrounding tissue of 14- to 19-day-old embryos and of newborn mice were carefully dissected under a stereo microscope. The whole jaw of day-13 embryos was processed for histology. The tissues were either frozen in Tissue-Tek (Miles Laboratories Inc., Naperville, IL) and sectioned at 8 μm or fixed in 94 % ethanol at +4°C as described previously (Thesleff *et al.* 1979). After dehydration, the fixed tissues were embedded in Histowax (Histo-Lab LTD, Gothenburg, Sweden) at 60°C and serially sectioned at 7 μm. Frozen sections through the

whole head of 20-day-old rats were a kind gift from Dr Tuomo Kantomaa (University of Oulu). The 20 μm thick sections were taken on a plastic tape and fixed in 96 % methanol. All sections were stored in tight boxes at −20°C until used.

Anti-tenascin antiserum

Antiserum was prepared in rabbits to tenascin purified from conditioned medium from chick embryo fibroblast cultures, as previously described (Chiquet-Ehrismann *et al.* 1986).

Immunohistochemical staining

For immunofluorescent staining, the deparaffinized or frozen sections were incubated at room temperature for 30 min with tenascin antiserum (1:100 dilution). After three washes with PBS (15 min each) the sections were treated for 30 min with fluorescein isothiocyanate-conjugated swine
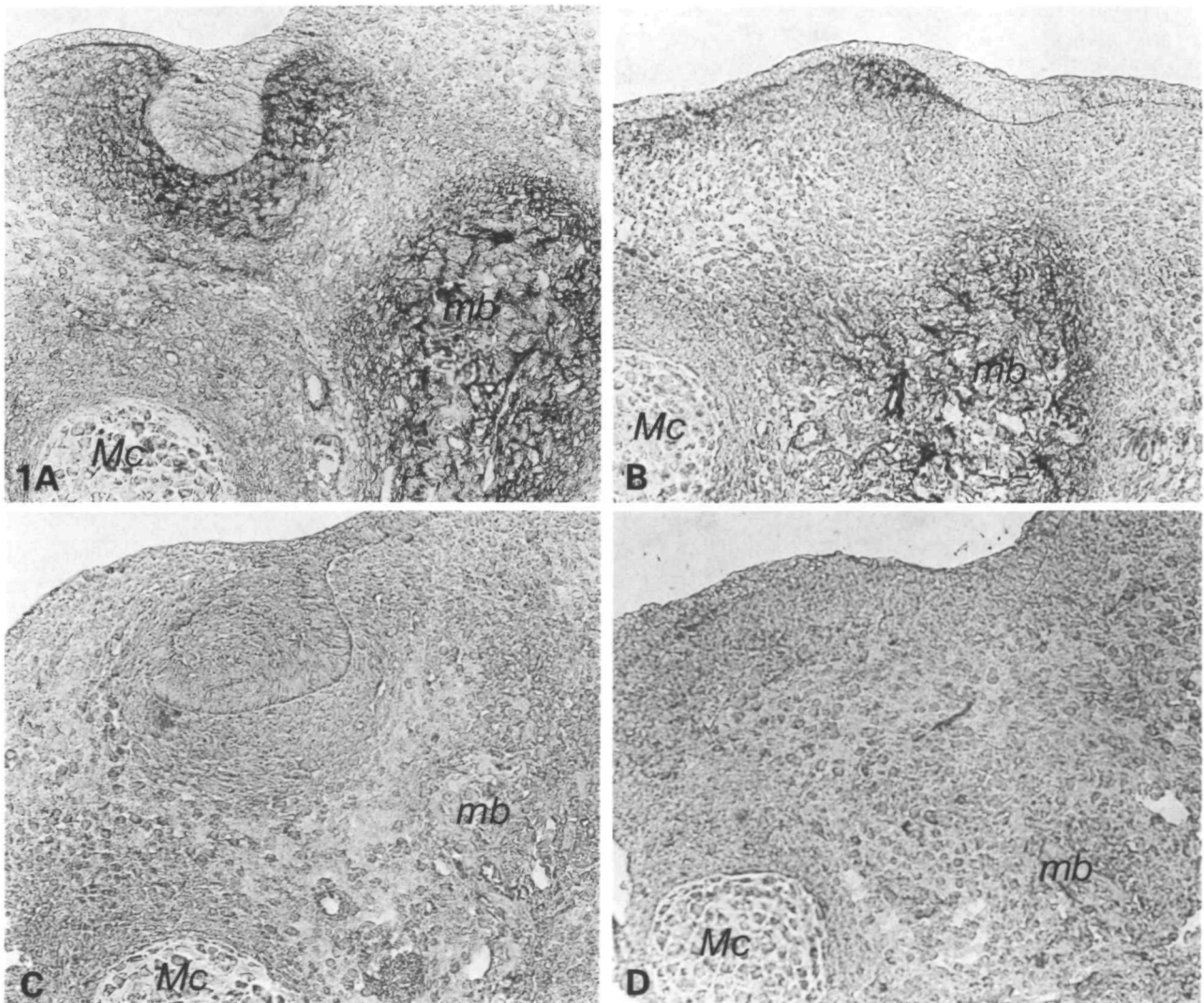
Fig. 1. Localization of tenascin by immunoperoxidase staining in the mandible of a 13-day-old mouse embryo. The transverse sections are from the level of the first molar tooth germ (A) and from the anterior aspect of the tooth bud (B). The mesenchyme surrounding the epithelial bud is intensely stained whereas the loose mesenchyme of the jaw is negative. The osteogenic mesenchyme surrounding the forming mandibular bone also shows accumulation of tenascin, whereas Meckel's cartilage is negative. The control sections (C,D), incubated with normal rabbit serum are negative. *Mc*, Meckels cartilage; *mb*, mandibular bone. ×220.

antibodies against rabbit immunoglobulins (Dakopatts, Glostrup, Denmark), washed again and mounted under a coverslip with veronal-buffered glycerol.

For immunoperoxidase staining, the Vectastain ABC kit was used (Vector Laboratories, Burlingame, CA). The sections were incubated for 1 h at 37°C with the tenascin antiserum (1:200 dilution). After staining, the sections were mounted with Aquamount (Gurr, BDH Chemicals Ltd, Poole, England). Control sections were incubated with PBS or with normal rabbit serum. In some experiments, the sections were digested prior to application of the tenascin

antiserum at 37°C with collagenase (0·05 % in 0·05 % CaCl$_2$, 20 min) or with hyaluronidase (2 mg ml^{-1}, 1 h). Both enzymes were from Boehringer Mannheim GmbH (West Germany).

Results

The mandible of 13-day-old mouse embryos was sectioned in the transverse plane at the region of the
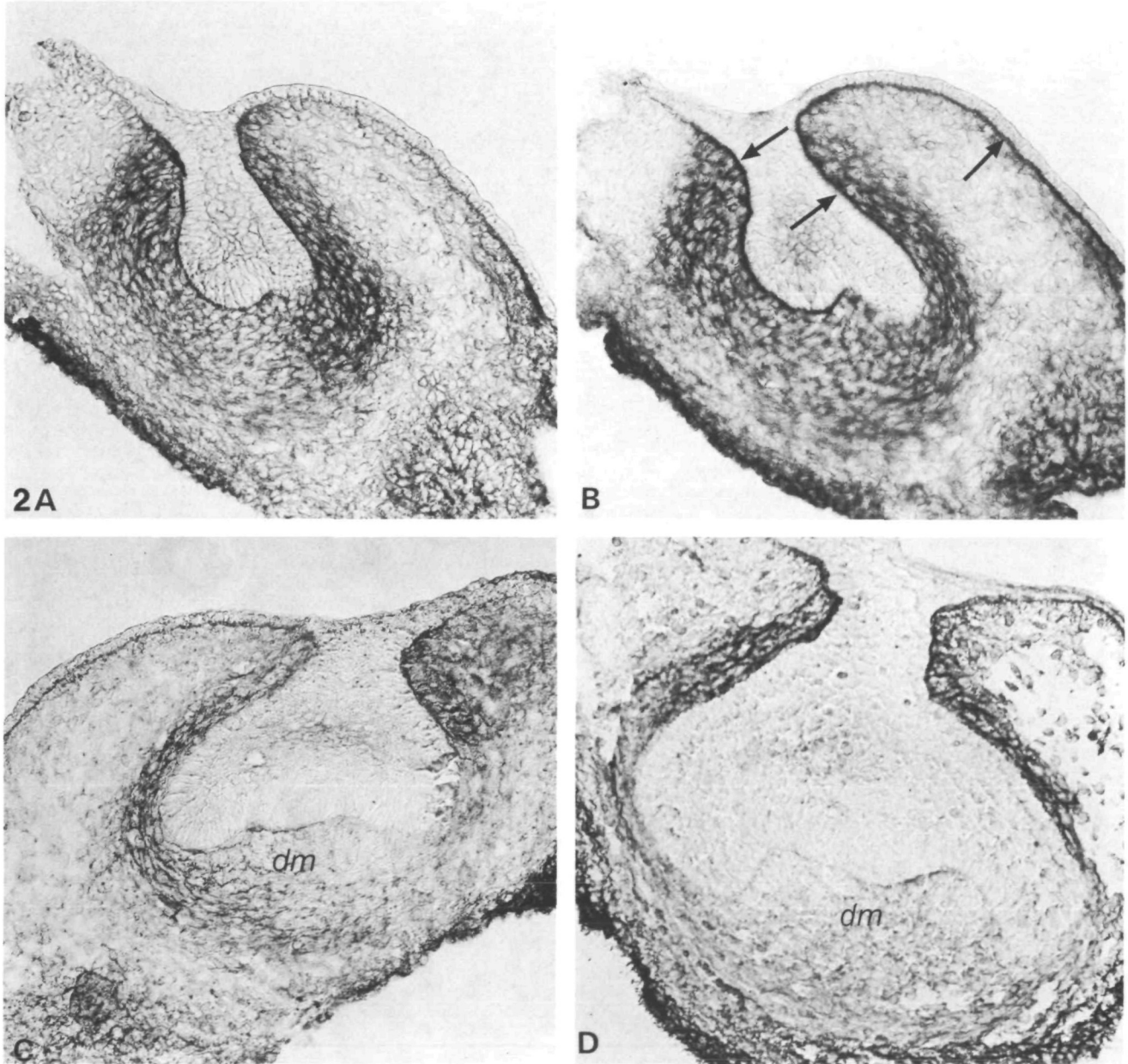
Fig. 2. Distribution of tenascin in cap-staged tooth germs of 14- and 15-day-old mouse embryos. (A,B) At the early cap stage intense staining has remained in the condensing mesenchyme around the epithelial bud. Accumulation of tenascin in the basement membranes (arrows) is evident in the section (B) which was digested with hyaluronidase prior to application of the tenascin antibodies. (C,D) With advancement of morphogenesis in the cap stage the staining in the dental papilla mesenchyme has decreased as compared to the mesenchyme surrounding the tooth germ. The section in D was digested with collagenase. *dm*, dental papilla mesenchyme. ×220.

first molar tooth bud. The staining with tenascin antiserum was very intense in the mesenchyme surrounding the epithelial tooth bud as described previously in rat embryos (Chiquet-Ehrismann *et al.*

1986; Fig. 1A). This marked mesenchymal staining was also present under the anterior aspect of the tooth bud where the onset of epithelial thickening was evident (Fig. 1B). The basement membrane
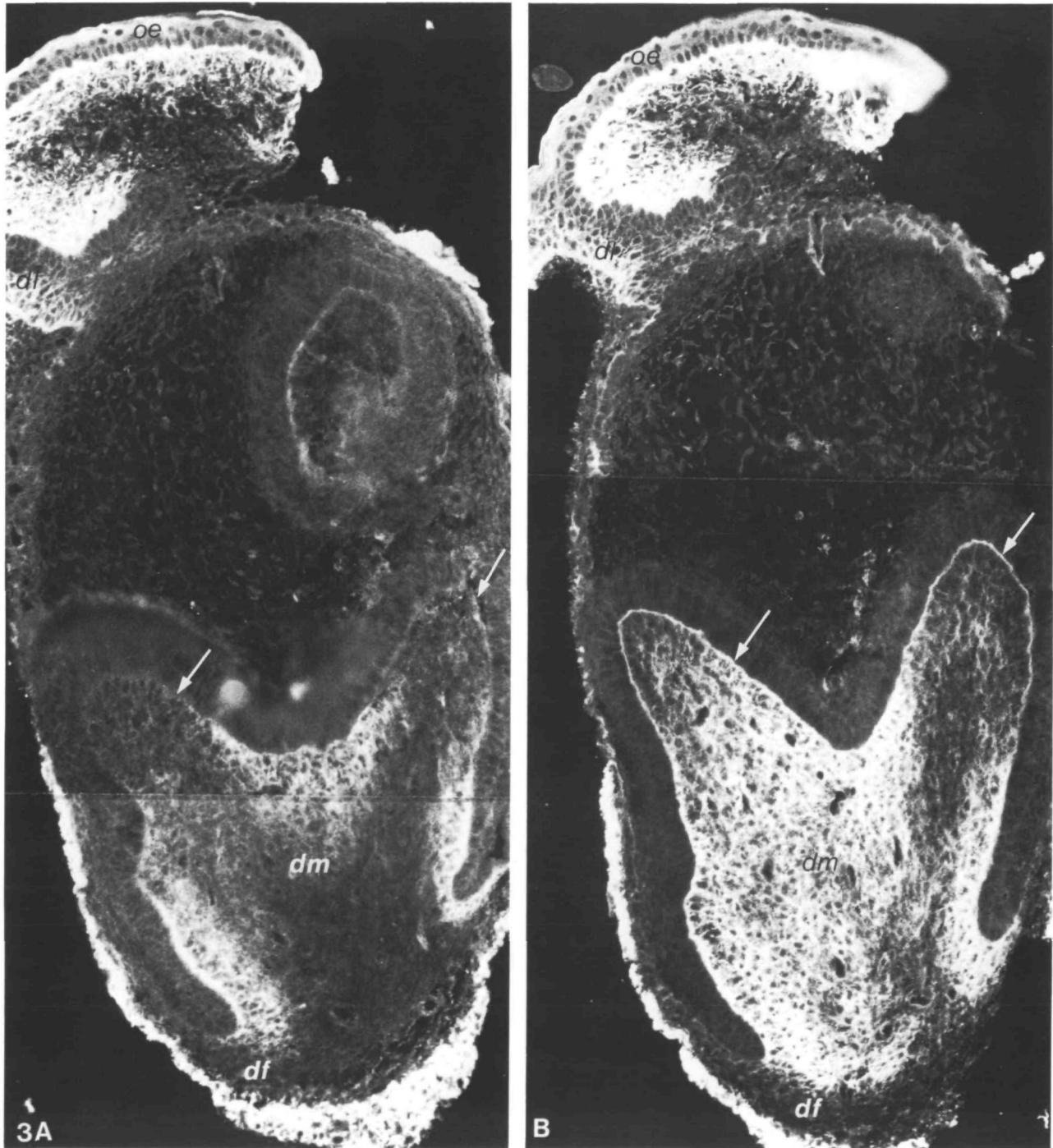
Fig. 3. Immunofluorescent localization of tenascin in a bell-staged tooth germ of a 17-day-old mouse embryo. The section in B was digested with collagenase prior to incubation with the tenascin antibodies. The mesenchyme underlying the oral epithelium and the dental lamina are intensely stained. Tenascin accumulation is also seen in the dental papilla mesenchyme and in the osteogenic mesenchyme surrounding the tooth bud. The dental follicle, a fibrocellular layer around the tooth germ, is negative for tenascin. Tenascin in the dental papilla and particularly in the dental basement membrane (arrows) is partly masked, as revealed in the collagenase-treated section (B). *dm*, dental papilla mesenchyme; *df*, dental follicle; *dl*, dental lamina; *oe*, oral epithelium. ×220.

under the oral epithelium was tenascin-positive particularly at the lingual side of the tooth bud. Intense staining was also observed in the osteogenic mesenchyme surrounding the first spicules of developing mandibular bone (Fig. 1A,B). The rest of the mesenchymal tissue as well as Meckel's cartilage were negative.

In the 14-day-old embryo the tooth germ has reached the cap stage of development where the epithelial bud has invaginated at its under surface and mesenchymal tissue, now called the dental papilla, invades the epithelial cap. The whole tooth germ is surrounded by the dental sac which consists of condensed mesenchymal cells. Intense staining with tenascin antibodies persisted in the mesenchyme surrounding the epithelial cap, particularly in the region of the dental lamina connecting the dental to oral epithelium. The intensity in the dental papilla mesenchyme was clearly less than in the surrounding mesenchyme (Fig. 2). The treatment of the sections with hyaluronidase or collagenase increased the intensity of the staining especially in the basement membranes (Fig. 2B).

During the 15th and 16th days of gestation the tooth germ grows rapidly and undergoes marked morphogenetic changes. At day 17, the tooth is in the bell stage, where terminal cell differentiation begins. Very intense staining for tenascin was seen in the mesenchyme around the dental lamina, i.e. the connection of the tooth germ to the oral epithelium (Fig. 3). Interestingly, the dental sac mesenchyme surrounding the whole tooth germ was negative for tenascin but the nondental mesenchyme surrounding the dental sac expressed tenascin intensely (Fig. 3). This outermost mesenchyme of the dissected tooth

germs represents osteogenic tissue associated with the ossifying mandibular bone. The abundance of tenascin in the dental papilla mesenchyme was clearly more prominent than in the cap stage. However, without prior enzyme digestion only the intercuspal and cervical regions of the dental papilla were stained (Fig. 3). After digestion with either collagenase or hyaluronidase, positive staining for tenascin became evident throughout the dental papilla mesenchyme and particularly in the basement membrane under the enamel epithelium (Fig. 3B).

In the tooth germs of newborn mice, remarkably intense staining for tenascin was seen throughout the dental papilla mesenchyme even without enzyme digestions (Fig. 4). The differentiated, polarized odontoblasts had become devoid of tenascin and no tenascin was seen in the predentin or dentin matrix. The marked accumulation of tenascin in the dental papilla (pulp) mesenchyme persisted after eruption of the teeth as seen in the molars and incisors of 20-day-old rats (Fig. 5). As compared to other connective tissues, the staining in the dental pulp was very intense. The odontoblast layer in the pulp as well as predentin and dentin were negative (Fig. 5C). The enamel matrix bound the peroxidase-conjugated secondary antibodies also in control sections (Fig. 5F).

Discussion

The results of the present study indicate that dental mesenchymal cells express tenascin throughout odontogenesis. We confirmed the earlier observation that tenascin is accumulated already in the condensed mesenchyme surrounding the tooth bud epithelium
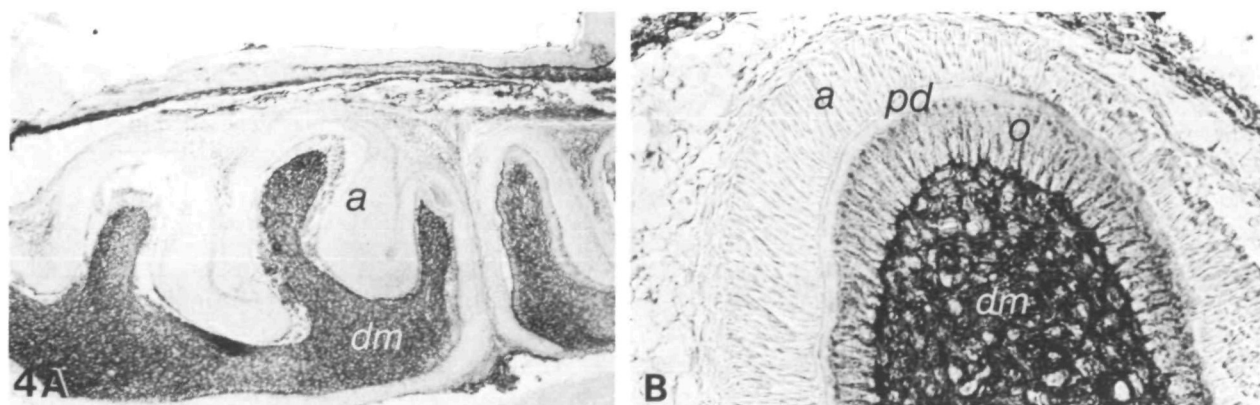
Fig. 4. Localization of tenascin in molars of a newborn mouse. (A) In the longitudinal section through the first and second molars accumulation of tenascin is evident in the dental mesenchyme. The connective tissue overlying the tooth germs is also positive. (B) The higher magnification of the dental epitheliomesenchymal interface shows that the differentiated odontoblasts are negative for tenascin. Some positive staining is seen in the intercellular spaces between the odontoblasts. Predentin is totally devoid of tenascin and the ameloblasts are negative, whereas very intense accumulation is evident in the dental mesenchyme. *dm*, dental papilla mesenchyme; *o*, odontoblasts; *a*, ameloblasts; *pd*, predentin. (A) ×90, (B) ×350.
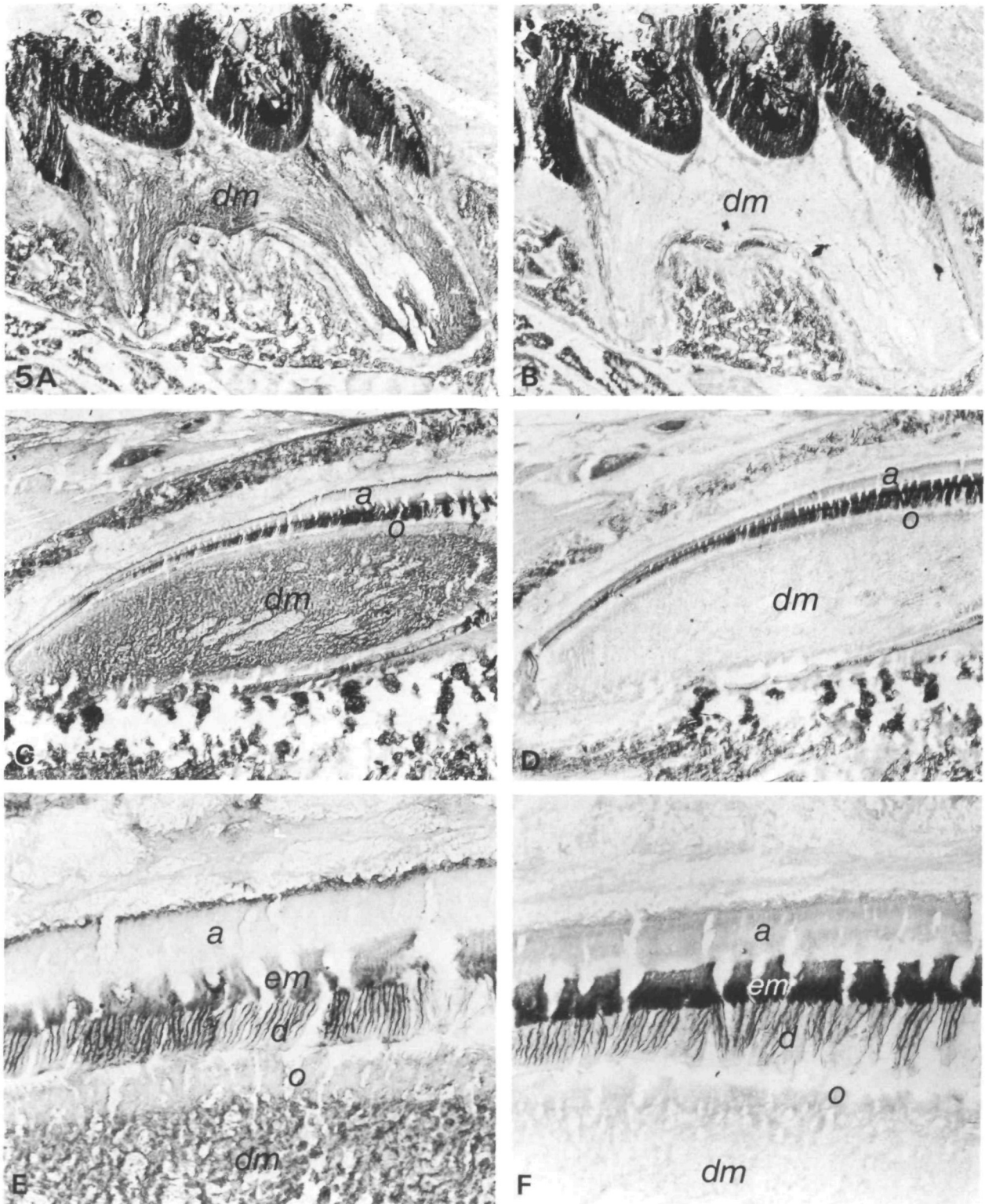
Fig. 5. Distribution of tenascin in teeth of a 20-day-old rat. Accumulation of tenascin in the dental pulp mesenchyme is evident in the erupted first molar (A) and in the incisor (C and E). The odontoblast layer as well as dentin is negative as seen in the higher magnification in E. In control sections treated with normal rabbit serum (B,D,F), dental mesenchyme is negative. The enamel matrix binds the antibodies nonspecifically. *dm*, dental mesenchyme; *o*, odontoblasts; *d*, dentin; *a*, ameloblasts; *em*, enamel matrix. (A and B) ×90, (C–F) ×350.

(Chiquet-Ehrismann *et al.* 1986). Other matrix molecules such as fibronectin and interstitial collagens are distributed evenly throughout the loose jaw mesenchyme and do not show such a limited distribution as tenascin (Thesleff *et al.* 1979; Chiquet-Ehrismann *et al.* 1986). A transient decrease was observed in staining intensity of the dental papilla for tenascin during the cap stage. This is associated with the period of remarkable morphogenetic changes and rapid cell proliferation when the cells may not be active in matrix deposition. Also interstitial collagens are less abundant in the dental papilla mesenchyme at this time (Thesleff *et al.* 1979).

At the bell stage, staining for tenascin became gradually more intense in the dental mesenchyme. Accumulation of tenascin was marked in the dental mesenchyme, or pulp of newborn mouse molars and still present in the erupted molars of 20-day-old rats. The extracellular matrix of the dental pulp is rich in collagens and fibronectin as well as other macromolecules (Linde, 1985), but these molecules are distributed widely in other connective tissues as well. Tenascin, on the other hand, shows a very limited distribution and it appears to be absent from most mature connective tissues (Chiquet & Fambrough, 1984a; Chiquet-Ehrismann *et al.* 1986). As shown in this study, dental mesenchymal cells also ceased to express tenascin upon differentiation into odontoblasts and tenascin was completely absent from dentin.

The dental follicle is a mesenchymal structure that surrounds the enamel organ and the dental papilla as a fibrocellular layer where the cells are oriented in a radial pattern. This structure gives rise to the supporting tissues of the tooth. Interestingly, the dental follicle appeared as a tenascin-free zone between the dental papilla and the osteogenic mesenchyme. The dental follicle stains positively with fibronectin and type I and III procollagen antibodies (Thesleff *et al.* 1979) and we have also demonstrated that these cells express high numbers of epidermal growth factor receptors (Partanen & Thesleff, 1987). Whether these observations are significant in terms of the differentiation of the tooth-supporting tissues remains to be seen.

Tenascin was present in the basement membrane between the enamel epithelium and dental papilla mesenchyme at the time of odontoblast differentiation. It was partly masked by other matrix molecules since digestion of the sections with collagenase or hyaluronidase resulted in enhanced intensity of staining. The dental basement membrane is believed to be significant for the alignment and differentiation of the dental papilla cells into odontoblasts (Thesleff & Hurmerinta, 1981; Ruch, 1985). We have suggested earlier that fibronectin, which is also abundant at

this location and known to mediate interactions between the cell surface and the extracellular matrix, is involved in odontoblast differentiation (Thesleff *et al.* 1979). The possibility that tenascin would play a role in interactions between matrix molecules and cells is supported by its biochemical properties. It consists of six disulphide-linked subunits of 190–240×10³ M_r and can bind proteoglycans (Chiquet & Fambrough, 1984b) and fibronectin (Chiquet-Ehrismann *et al.* 1986). Tenascin was abundant also in the basement membrane of the oral epithelium and in the mesenchyme supporting the epithelial dental lamina which connects the tooth germ to oral epithelium. It can be speculated that tenascin at these locations is involved in stimulation of epithelial cell proliferation. *In vitro* studies have shown that tenascin is mitogenic but that it does not promote cell attachment (Chiquet-Ehrismann *et al.* 1986).

The remarkable expression of tenascin by the dental pulp cells appears to be shared by other cell lineages which differentiate into hard-tissue-forming cells. The condensed mesenchymal cells surrounding the forming mandibular bone of 13-day-mouse embryos were intensely stained. At later developmental stages, the osteogenic mesenchymal tissue around the dissected tooth germs was rich in tenascin. These results are in line with observations of tenascin distribution in other bones and cartilages (Mackie, Thesleff & Chiquet-Ehrismann, 1987). Hence, it appears that tenascin expression is common to determined but relatively undifferentiated cells capable of forming the matrices of hard tissues. Since the tooth pulp is thought to provide a source of new odontoblasts e.g. after tooth injury, it can be speculated that the abundance of tenascin in this tissue is associated with the maintenance of the capacity to differentiate into hard-tissue-forming cells.

References

BERNFIELD, M. & BANERJEE, S. D. (1982). The turnover of basal lamina glycosaminoglycan correlates with epithelial morphogenesis. *Devl Biol.* **90**, 291–305.

BOURDON, M. A., MATTHEWS, T. J., PIZZO, S. V. & BIGNER, D. D. (1985). Immunochemical and biochemical characterization of a glioma-associated extracellular matrix glycoprotein. *J. cell. Biochem.* **28**, 183–195.

CHIQUET, M. & FAMBROUGH, D. M. (1984a). Chick myotendinous antigen. I. A monoclonal antibody as a marker for tendon and muscle morphogenesis. *J. Cell Biol.* **98**, 1926–1936.

CHIQUET, M. & FAMBROUGH, D. M. (1984b). Chick myotendinous antigen. II. A novel extracellular glycoprotein complex consisting of large disulfide-linked subunits. *J. Cell Biol.* **98**, 1937–1946.

CHIQUET-EHRISMANN, R., MACKIE, E. J., PEARSON, C. A. & SAKAKURA, T. (1986). Tenascin: An extracellular matrix protein involved in tissue interactions during fetal development and oncogenesis. *Cell* **47**, 131–139.

ERICSON, H. P. & INGLESIAS, J. L. (1984). A six-armed oligomer isolated from cell surface fibronectin preparations. *Nature, Lond.* **311**, 267–269.

GROBSTEIN, C. & COHEN, J. (1965). Collagenase: Effect on the morphogenesis of embryonic salivary epithelium in vitro. *Science* **150**, 626.

GRUMET, M., HOFFMAN, S., CROSSIN, K. L. & EDELMAN, G. M. (1985). Cytotactin, an extracellular matrix protein of neural and non-neural tissues that mediates glia-neuron interaction. *Proc. natn. Acad. Sci. U.S.A.* **82**, 8075–8079.

HAY, E. D. (1982). Collagen and embryonic development. In *Cell Biology of Extracellular Matrix* (ed. E. D. Hay), pp. 379–409. New York: Plenum Press.

KOLLAR, E. J. (1978). The role of collagen during tooth morphogenesis: some genetic implications. In *Development, Function and Evolution of Teeth* (ed. P. M. Butler & K. A. Joysen), pp. 1–12. London: Academic Press.

KRUSE, J., KEILHAUER, G., FAISSNER, A., TIMPL, R. & SCHACHNER, M. (1985). The J1 glycoprotein – a novel nervous system cell adhesion molecule of the L2/HNK-1 family. *Nature, Lond.* **277**, 146–148.

LINDE, A. (1985). The extracellular matrix of the dental pulp and dentin. *J. dent. Res.* **64**, 523–529.

MACKIE, E. J., THESLEFF, I. & CHIQUET-EHRISMANN, R. (1987). Tenascin is associated with chondrogenic and osteogenic differentiation *in vivo* and promotes chondrogenesis *in vitro*. *J. Cell Biol.* (in press).

ORKIN, R. W., KNUDSON, W. & TOOLE, B. P. (1985). Loss of hyaluronate-dependent coat during myoblast fusion. *Devl Biol.* **107**, 527–530.

PARTANEN, A.-M. & 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. *Devl Biol.* **120**, 186–197.

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

SAXEN, L., EKBLOM, P. & THESLEFF, I. (1982). Cell-matrix interaction in organogenesis. In *New Trends in Basement Membrane Research* (ed. K. Kuehn, H. Schoene & R. Timpl), pp. 257–264. New York: Raven Press.

THESLEFF, I. (1980). Extracellular matrix and tooth morphogenesis. In *Current Research Trends in Prenatal Craniofacial Development* (ed. R. M. Pratt & R. Christiansen), pp. 329–338. New York: Elsevier North-Holland.

THESLEFF, I. & HURMERINTA, K. (1981). Tissue interactions in tooth development. *Differentiation* **18**, 75–88.

THESLEFF, I., STENMAN, S., VAHERI, A. & TIMPL, R. (1979). Changes in the matrix proteins, fibronectin and collagen during differentiation of mouse tooth germ. *Devl Biol.* **70**, 116–126.

THIERY, J.-P. (1985). Roles of fibronectin in embryogenesis. In *Fibronectin* (ed. D. Mosher). New York: Academic Press.

VAUGHAN, L., HUBER, S., CHIQUET, M. & WINTERHALTER, K. H. (1987). A major, six-armed glycoprotein from embryonic cartilage. *EMBO J.* **6**, 349–353.

ZANETTI, N. C. & SOLURSH, M. (1986). Epithelial effects on limb chondrogenesis involve extracellular matrix and cell shape. *Devl Biol.* **113**, 110–118.