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# Growth factors and tooth development

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ABSTRACT. The effects of various growth factors on tooth development were studied in organ cultures of mouse embryonic tooth germs. Transferrin was shown to be a necessary growth factor for early tooth morphogenesis. Transferrin was required for the development of bud- and early cap-staged teeth, and it was shown to be the only serum protein that was needed by early cap-staged teeth in organ culture. Promotion of tooth morphogenesis and dental cell differentiation was shown to be based on the stimulation of cell proliferation.

The roles of polypeptide growth factors in tooth development were studied by adding these factors to the transferrin-containing chemically-defined culture medium which supports early tooth morphogenesis and cell differentiation. Fibroblast growth factor or platelet-derived growth factor did not affect cell proliferation or morphogenesis of tooth germs in culture. On the contrary, epidermal growth factor (EGF) stimulated cell proliferation in tooth explants, but at the same time inhibited tooth morphogenesis and dental cell differentiation. Autoradiographic localization of proliferating cells revealed that dental tissues responded to EGF with different proliferation rates. The responsiveness to EGF was stage-dependent, early cap-staged teeth were sensitive to EGF but late cap-staged and bell-staged teeth developed normally in the presence of EGF in the culture medium.

The presence and distribution of receptors for both transferrin and EGF were studied in mouse embryonic teeth at various developmental stages by incubating freshly-separated tooth germs with $^{125}$Iodine-labeled transferrin or EGF, and then processing the tissues for autoradiography. The number of transferrin receptors in embryonic teeth correlated with the proliferation rate, and the receptors were uniformly distributed between the dental epithelium and the dental mesenchyme. The responsiveness of embryonic teeth to EGF in organ culture correlated with the expression of EGF receptors in dental tissues. However, the distribution of receptors changed markedly from bud stage to cap stage and further to bell stage of development. Furthermore, at early cap stage, which is responsive to EGF, the distribution of EGF receptors between the dental epithelium and the dental mesenchyme was not related to the stimulation of cell proliferation by EGF in these tissues. These results suggest that epithelial-mesenchymal tissue interactions may control the response of dental tissues to EGF.

KEY WORDS: *morphogenesis, transferrin, epidermal growth factor, EGF receptors, transferrin receptors*

## Introduction

Tooth morphogenesis starts with the thickening of the presumptive dental epithelium to dental lamina, and is followed by intrusion of the actively proliferating dental epithelium into the jaw mesenchyme. The neural crest-derived mesenchymal cells under the dental epithelium condense to form the dental mesenchyme. The multistep process of tooth morphogenesis and dental cell differentiation proceeds in a specific temporal and spatial pattern. It is known that from the earliest stages of morphogenesis, tooth development is tightly regulated by reciprocal epithelial-mesenchymal tissue interactions in which extracellular matrix molecules appear to play important roles (Kollar and Baird, 1970; Slavkin, 1974; Thesleff and Hurmerinta, 1981; Ruch *et al.*, 1983; Mina and Kollar, 1987; Thesleff *et al.*, 1987a, 1988; Lumsden, 1988).

Results from the active research in the field of growth factors in recent years indicate that these molecules have important physiological functions in embryonic development. The presence of growth factors and their receptors in embryos, and their differential expression at various developmental stages suggest that these growth-promoting polypeptides are involved in the regulation of morphogenetic and differentiation events (for review see Mercola and Stiles, 1988). We have examined the effects of some growth factors on tooth development and the presence of growth factor receptors in embryonic teeth. These studies, which are discussed below, indicate that besides the extracellular matrix, growth factors are involved in tooth development, particularly during the early stages of morphogenesis.

### *Transferrin is a necessary growth factor for tooth morphogenesis*

The effects of various factors on tooth development can be examined under controlled conditions in organ culture of embryonic tooth germs. The use of chemically-defined culture media makes possible exact analysis of the influence of different supplemented factors because numerous undefined serum components are not present.

Transferrin, the iron transporter protein of vertebrate serum, is an essential growth promoting component in chemically-defined culture media used in monolayer cell cultures (Barnes and Sato, 1980). The stimulation of cell proliferation by transferrin is based on the delivery of iron to cells by receptor-mediated endocytosis. The main site of transferrin synthesis in adult mammals is the liver. Besides the yolk sac, which is the

main source in embryos, several fetal tissues seem to synthesize transferrin (Adamson, 1982; Levin *et al.*, 1984; Meek and Adamson, 1985). Transferrin is apparently provided for the rapidly growing embryonal tissues both as maternal transferrin through the placenta and as intrinsic embryonic transferrin (Booth and Wilson, 1981; McArdle and Priscott, 1984). Hence, transferrin can be regarded as an embryonic growth factor. The essential role of transferrin in organogenesis has been experimentally demonstrated in organ culture studies of mouse embryonic kidney where transferrin was the only serum protein that was needed for differentiation (Ekblom *et al.*, 1981).

Previous organ culture studies of mouse embryonic teeth have established that teeth which have reached the bell stage of development undergo morphogenesis as well as differentiation of odontoblasts and ameloblasts in a basal chemically-defined culture medium containing the necessary amino acids, vitamins and minerals, but no hormones or growth factors (Thesleff, 1976; Yamada *et al.*, 1981), while serum or embryo extract supplement is needed for the development of teeth at earlier stages. We have studied the growth requirements of teeth at early developmental stages, i.e. bud and cap stages, by adding various hormones and growth factors to the culture

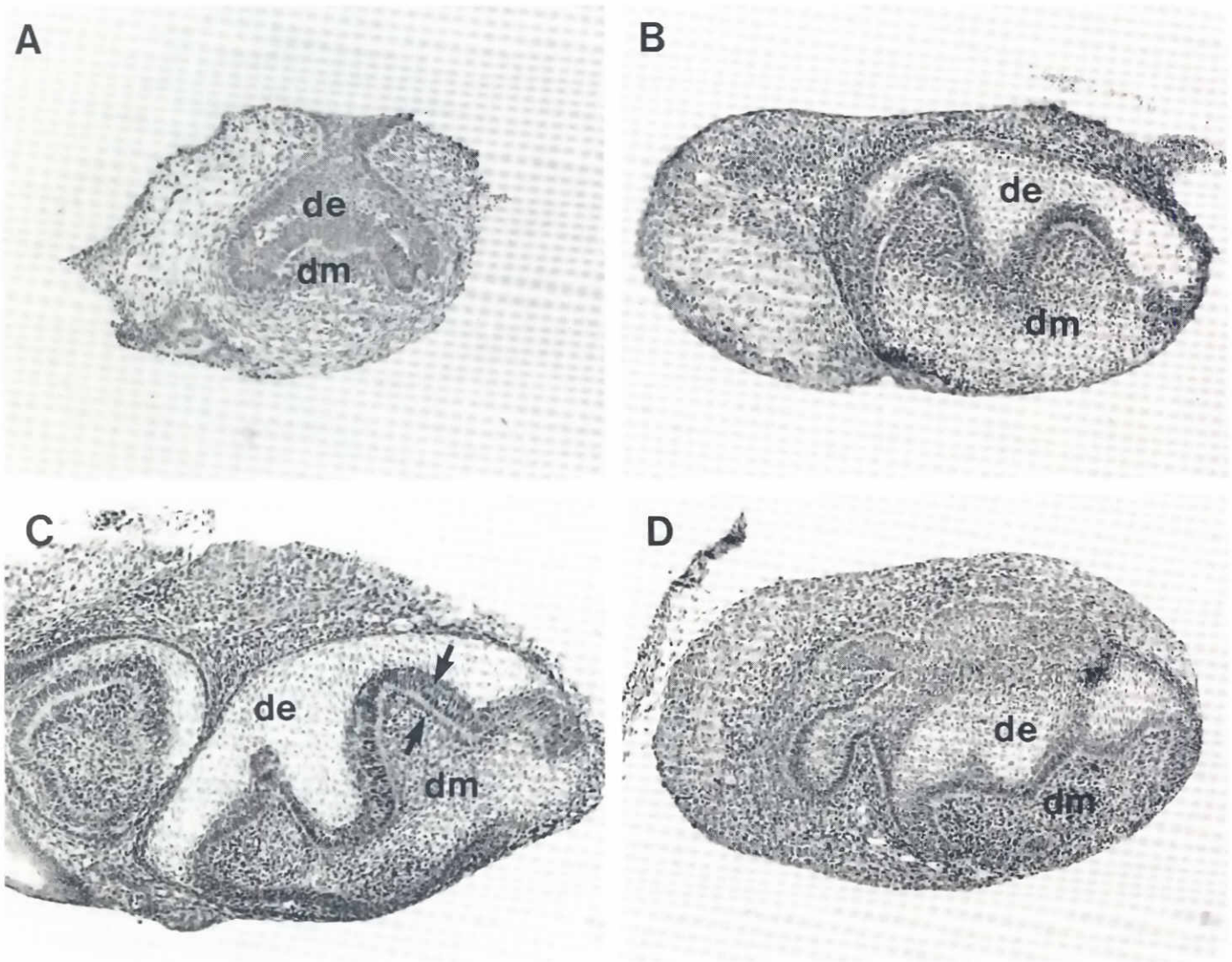



**Fig. 1.** *Light micrographs illustrating the effects of transferrin and epidermal growth factor (EGF) on the development of early cap-staged first mandibular molar teeth in organ culture.*

*A. First mandibular molar tooth from 14-day mouse embryo (day 0 = day of vaginal plug) prior to culture. Tooth germs were dissected free from surrounding tissues. Some adherent oral epithelium and surrounding jaw mesenchyme were left in the explants. At early cap stage the condensation of dental mesenchymal cells (dm) is covered by dental epithelium (de) which has begun to grow around the mesenchymal papilla.*

*B. After 6 days of culture in the chemically-defined basal culture medium, improved Eagle's minimum essential medium (I-MEM), which contains the necessary amino acids and minerals, but not any hormones or growth factors, the tooth explant has grown in size, and cuspal morphogenesis has advanced to some extent.*

*C. After 6 days of culture in I-MEM supplemented with transferrin (50 μg;ml), the tooth has undergone morphogenesis, and differentiation of dental cells, mesenchymal odontoblasts and epithelial ameloblasts is proceeding at cuspal tips (arows). The developing second molar tooth is visible on the left side.*

*D. Epidermal growth factor (20 ng;ml) in the transferrin-containing culture medium has inhibited tooth morphogenesis. The tooth germ has grown slightly, but morphogenesis has not proceeded. The surrounding nondental mesenchymal tissue is stimulated. Magnification: x 105.*

medium, and observing the effects on morphogenesis, cell proliferation and differentiation. Transferrin was shown to be necessary for the development of bud and cap-staged teeth (Partanen *et al.*, 1984). Furthermore, transferrin turned out to be the only serum protein required for the development of early cap-staged teeth in organ culture (Fig. 1). Promotion of tooth morphogenesis and dental cell differentiation by transferrin was shown to be based on the stimulation of cell proliferation in tooth germs. The DNA content of teeth cultured in the presence of transferrin was nearly two-fold as compared with the teeth cultured without transferrin supplement (Fig. 2, Partanen *et al.*,

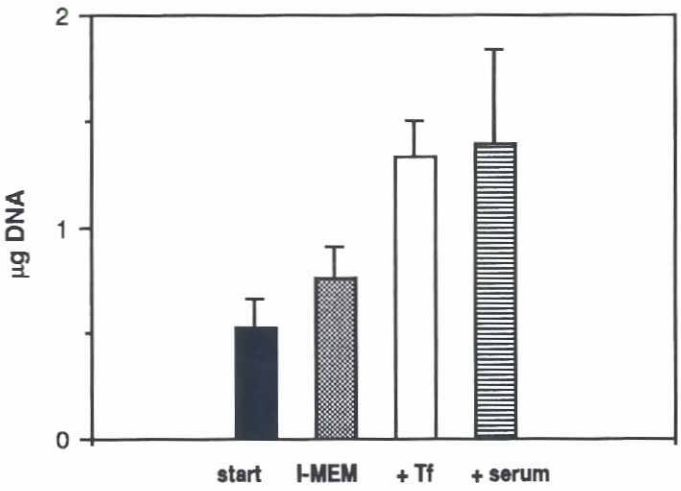



**Fig. 2.** *The effects of various culture media on the DNA content of early cap-staged day-14 tooth explants after 6 days of culture. The DNA content of tooth explants cultured in the basal chemically-defined culture medium, improved Eagle's minimum medium (I-MEM), increased about 1.5-fold. Addition of 50 µg/ml of transferrin or 10% fetal calf serum to the basal medium increased the DNA content of tooth explants 2.5-fold during culture.*

1984). Transferrin can be replaced by a synthetic lipophilic iron chelator, ferric pyridoxal isonicotinoyl hydrazone, in the culture medium (Thesleff *et al.*, 1985). This implies the crucial role of iron transport in the growth stimulation by transferrin.

The need for exogenous transferrin was shown to be stage-dependent so that late cap-staged teeth developed *in vitro* even without transferrin supplement (Partanen *et al.*, 1984). This was unexpected, because there is active cell proliferation still in the bell-staged tooth (Ruch *et al.*, 1972).

The need for transferrin for proliferation at bell stage could be satisfied either by endogenous synthesis of transferrin in dental tissues, or by storage and reuse of serum transferrin present in tooth explants transferred to organ culture. We could not detect synthesis of transferrin in embryonic teeth, but immunoblotting the electrophoretically-separated proteins from tooth explants and culture media by transferrin antibodies showed that the bell-staged teeth were able to retain transferrin considerably long in culture (Partanen and Thesleff, 1987a). This amount of transferrin appears to be sufficient for the proliferation and proceeding of morphogenesis.

### *Epidermal growth factor inhibits early tooth morphogenesis in organ culture*

Polypeptide growth factors can be grouped into several families based on their molecular structure, structural homologies and receptor-binding activity (Mercola and Stiles, 1988). Some members from all these families seem to be involved in proliferation and differentiation during embryonic development Representatives from three different groups were tested in organ culture of mouse embryonic tooth germs, and their effects on morphogenesis, cell proliferation and differentiation were examined during culture. The chemically-defined transferrin-containing culture medium which supports tooth morphogenesis (see above) was used as the basal control medium to which other growth factors were added.

Fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) are potent mitogens for various cell types of mesodermal, and in case of FGF, also of neuroectodermal origin (Gospodarowicz *et al.*, 1986; Ross *et al.*, 1986). These factors did not detectably affect the development of early cap-staged teeth. However, the addition of epidermal growth factor (EGF), which is mitogenic for both mesenchymal and epithelial cells, inhibited morphogenesis and dental cell differentiation (Fig. 1 o, Partanen *et al.*, 1985). At the same time, the same concentration (20 ng/ml of EGF) was the most stimulatory to cell proliferation, when measured as incorporation of $^{3}$H-thymidine in tooth explants. Localization of proliferating cells by autoradiography in histological tissue sections showed that the various cell lineages in a tooth germ responded to EGF with different proliferation rates. EGF appeared to stimulate proliferation of dental epithelial cells and surrounding nondental jaw mesenchyme, while proliferation in the dental mesenchymal papilla was inhibited. This inhibition apparently prevented tooth crown morphogenesis. Because of the combined inhibitory and stimulatory effects on cell proliferation, no differences could be measured at the end of culture in the DNA content of EGF-treated explants as compared to control cultures.

The responsiveness to EGF during tooth development was shown to be dependent on the developmental stage, so that only teeth at the early cap stage responded to EGF, while the late cap-staged and bell-staged teeth developed normally even in the presence of EGF in the culture medium (Partanen *et al.*, 1985). In our subsequent studies we were able to demonstrate that the loss of responsiveness correlates with the loss of EGF receptors in dental tissues (Partanen and Thesleff, 1987b).

### *Receptors for transferrin and epidermal growth factor in embryonic teeth are developmentally regulated*

Growth factors, including transferrin and epidermal growth factor, mediate their biological effects to cells by binding to specific cell surface receptors, which span the cell membrane. However, the mechanisms of mitogenesis and processing of the ligand and the receptor molecule are different with transferrin and EGF.

The mitogenic effect of transferrin is based on iron transport into cells. Iron is released intracellularly from the receptor-bound transferrin into the acidic environment of the endocytotic vesicles, and both apotransferrin and receptor molecules are returned to the cell surface and are reused (Karin and Minz, 1981; Hopkins and Trowbridge, 1983). In the case of EGF, binding of EGF to the receptor activates the intrinsic tyrosine kinase activity in the receptor molecule. Autophosphorylation in the intracellular domain of the receptor molecule apparently plays an important role in transmitting the mitogenic signal to the nucleus (Schlessinger, 1987). After internalization of the ligand-receptor complex both the EGF molecule and the receptor molecule are degraded in lysosomes.

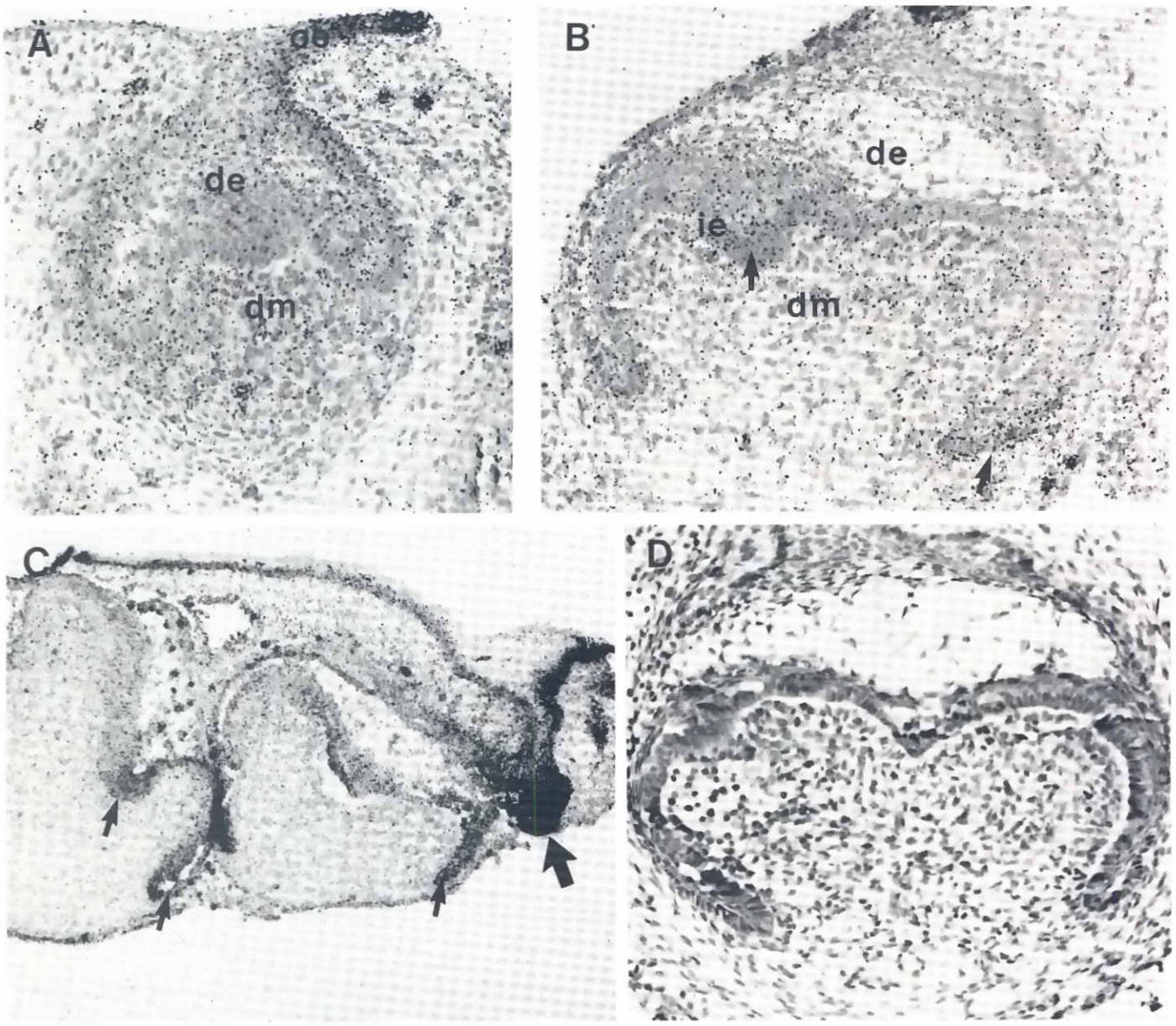



**Fig. 3.** *Autoradiographs of $^{125}$I-transferrin binding in mandibular tooth germs at different stages of development. The freshly separated tooth explants were incubated with 1 μg;ml of $^{125}$I-transferrin.*

*A. The early cap-staged first mandibular tooth of 14-day mouse embryo binds $^{125}$I-transferrin more intensely than the surrounding jaw mesenchyme. The label of bound $^{125}$I-transferrin is uniformly distributed in the dental epithelium (de) and the condensed dental mesenchyme (dm). The oral epithelium (oe) is heavily labeled.*

*B. The bell-staged first mandibular molar tooth of 16-day mouse embryo has less label than the cap-staged tooth. The dental epithelium (de) has grown downwards to surround the dental mesenchymal papilla (dm). There are more intensely labeled areas in the inner dental epithelium (ie) at cuspal folds and at the cervical loops (arrows). These areas are known to be sites of active cell proliferation in bell-staged tooth.*

*C. In the mandible of newborn mouse all three molar tooth germs are visible. The first molar tooth (left) has undergone cuspal morphogenesis, and differentiation of dental cells is proceeding. The second molar is at the bell stage, and the third molar (right) is at the bud stage of development. Both in the first and second molar teeth there is intense label of bound $^{125}$I-transferrin in cervical areas and at cuspal folds (small arrows). The epithelial bud of the third molar tooth is heavily labeled (large arrow). Also the basal cell layers of the oral epithelium are intensely labeled.*

*D. Autoradiograph of a bell-staged first mandibular molar tooth incubated with 1 μg;ml of $^{125}$I-transferrin and 1000-fold concentration (1 mg;ml) of unlabeled transferrin in the incubation medium. The label of $^{125}$I-transferrin is absent. This confirms the specificity of the labeling in tooth explants. Magnifications: A, B, D x 215; C x 85. (From: Partanen, A-M. and Thesleff, I. 1987c).*

The expression of receptors by cells is obviously essential for their responsiveness to growth factors. Hence, the presence of receptors for transferrin and EGF was studied in embryonic teeth at various developmental stages to find out how it was related to the requirement for and responsiveness to these factors. For this, tooth germs were separated from the jaw and were immediately incubated with $^{125}$iodine-labeled transferrin or EGF. Binding of the $^{125}$I-labeled ligand was localized by autoradiography in histological tissue sections.

The number of transferrin receptors in mouse embryonic

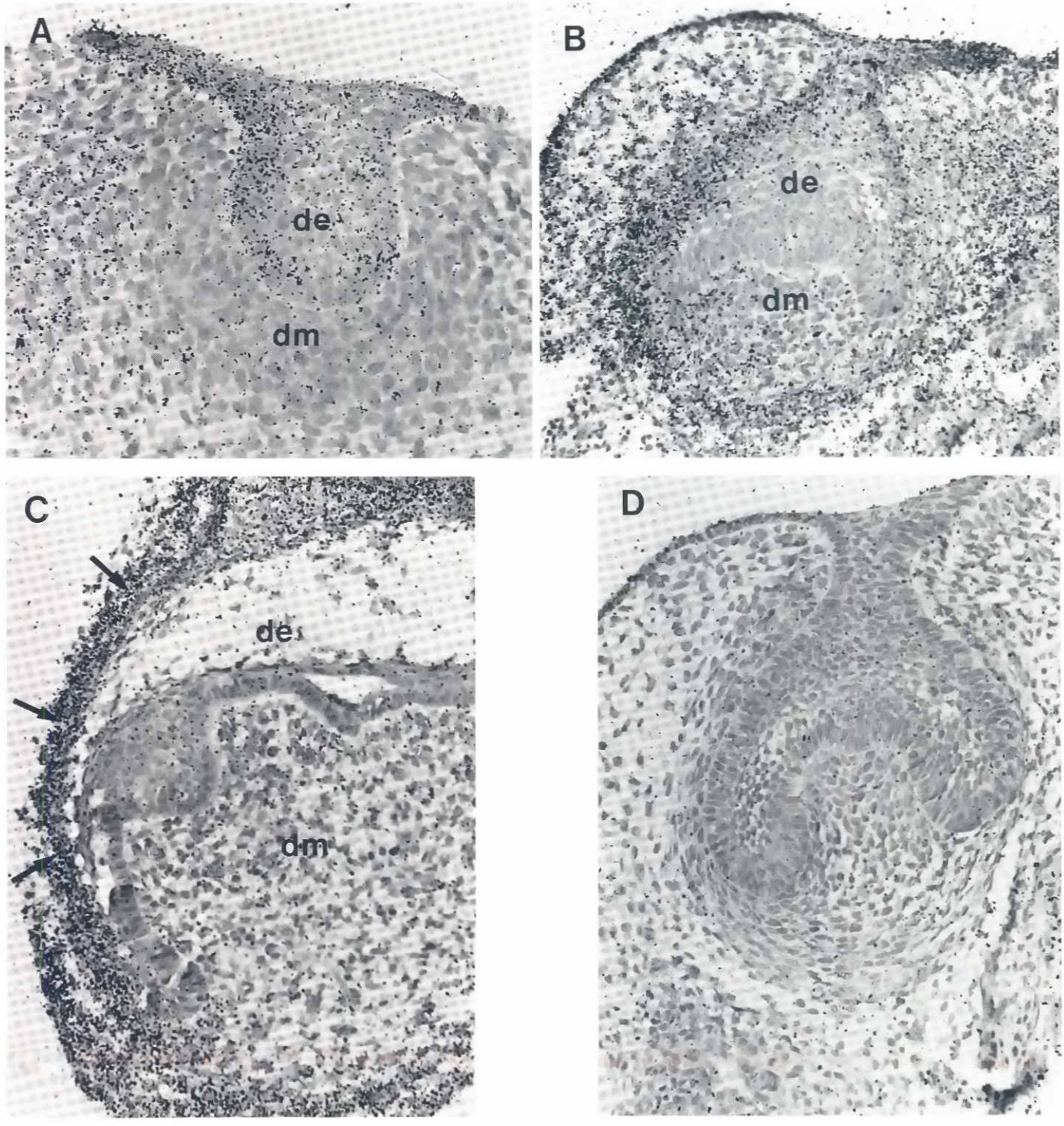
**Fig. 4.** *Autoradiographs of $^{125}$I-epidermal growth factor ($^{125}$I-EGF) binding in embryonic first mandibular molar tooth germs at various stages of development. The separate tooth germs were incubated with 5 ng;ml of $^{125}$I-EGF.*

*A. In the bud-staged first mandibular molar tooth of 13-day mouse embryo, the bud of dental epithelium (de) is heavily labeled by bound $^{125}$I-EGF, while the labeling is sparse in the condensed dental mesenchymal cells (dm) around the epithelial bud. The jaw mesenchyme surrounding the tooth germ shows more intense labeling than the dental mesenchyme.*

*B. In the early cap-staged tooth of 14-day mouse embryo there is label in the dental mesenchyme (dm), but in the dental epithelium (de) only the outer dental epithelial cells are labeled. The inner dental epithelium facing the dental mesenchyme is unlabeled. The surrounding nondental mesenchyme is intensely labeled.*

*C. In the bell-staged tooth of 16-day mouse embryo there is only very sparse labeling in the dental mesenchyme (dm) and the dental epithelium (de), whereas the dental follicle, which consists of condensed mesenchymal cells (arrows) around the tooth germ, and the jaw mesenchyme are heavily labeled.*

*D. In the cap-staged first mandibular molar tooth, which was incubated with 5 ng;ml of $^{125}$I-EGF and a 1000-fold concentration of unlabeled EGF in the incubation medium, the label of $^{125}$I-EGF is removed. Magnifications: A x 340; B-D x 215.*



teeth correlated with the proliferation rate and was decreased with overt differentiation (Fig. 3). In the early cap-staged tooth which required exogenous transferrin in organ culture, binding of transferrin was more intense in the dental tissues than in the surrounding jaw mesenchyme, and the grains were uniformly distributed in the dental epithelium and the dental mesenchyme (Fig. 3A). The amount of transferrin receptors did not, however, correlate with the loss of transferrin requirement of bell-staged teeth *in vitro*, since at bell stage there was still moderate binding of transferrin (Fig. 3B). The binding sites were aggregated at sites of active cell proliferation (Partanen and Thesleff, 1987c). It is thus evident that transferrin is still needed and used for cell proliferation in bell-staged teeth. As discussed above, the requirement for transferrin is apparently satisfied by retention of transferrin by the bell-staged teeth in culture.

The number of EGF receptors was considerably high in mouse embryonic teeth, but there was a remarkable change in the distribution of binding sites from bud stage to cap stage and further to bell stage of development (Partanen and Thesleff, 1987b). In bud-staged teeth the dental epithelium bound EGF. The condensed dental mesenchymal cells around the epithelial bud did not have many EGF receptors, while the surrounding nondental mesenchyme showed intense binding of EGF (Fig. 4A). In early cap-staged teeth the number of EGF receptors had decreased, and epithelial cells near the epithelio-mesenchymal interface lacked receptors totally. Now the dental mesenchyme had EGF receptors which were uniformly distributed in the dental papilla. In the nondental mesenchyme around the tooth, binding of EGF was still more intense than in the tooth mesenchyme (Fig. 4B). EGF binding decreased markedly from early to late cap stage, and at bell stage had nearly totally disappeared from both the dental epithelium and the dental mesenchyme. At the same time, abundant binding of EGF was observed in the cells of the dental follicle which consists of condensed mesenchymal cells around the tooth germ (Fig. 4C).

Thus, the responsiveness of embryonic teeth to EGF was related to the expression of receptors in dental tissues. The distribution of receptors did not, however, correlate with the stimulation of cell proliferation by EGF in the early cap-staged teeth. The cells of the dental epithelium were stimulated by EGF, but did not bind EGF, while the cells of the dental mesenchyme bound EGF, but their proliferation was prevented.

Hence, the response of dental tissues to EGF may be controlled by epithelial-mesenchymal tissue interactions, where the dental mesenchyme is the primary target tissue. This was suggested also by the results from cell culture studies; EGF stimulated the proliferation of disaggregated dental mesenchymal cells in monolayer cultures (Partanen *et al.*, 1985), and EGF enhanced the stimulation of proliferation in dental epithelial cells in the presence of dental mesenchymal tissue (Brownell and Rovero, 1980). Regulation of hormonal response by tissue interactions occurs in the development of other epithelial-mesenchymal organs, like mammary gland, prostate and lung, and the mesenchymal stroma seems to be the primary target tissue (Kratochwil and Schwartz, 1976; Cunha and Lung 1978; Beer *et al.*, 1984).

EGF receptors decreased in the dental epithelium and the dental mesenchyme with advancing morphogenesis and declining cell proliferation, and totally disappeared with dental cell differentiation. The binding of EGF increased, however, at the same time in the dental follicle around the tooth germ. The abundant binding of EGF by follicular cells was still evident in erupting teeth (Thesleff *et al.*, 1987b), and besides the dental sac, EGF binding was observed also in the apical mesenchymal tissue, in the cervical loop epithelium and around the blood vessels. It has been suggested that these tissues play a role in tooth eruption (Massler and Shour, 1941; Ten Cate 1969; Berkovitz, 1971), but the role of dental follicle has been emphasized (Cahill and Marks, 1980). EGF was originally discovered due to its ability to stimulate tooth eruption and eyelid opening in newborn mice (Cohen, 1962). The presence of EGF receptors in tissues which are involved in tooth eruption may be associated with the responsiveness of these tissues to EGF during the process.

## Conclusions and Hypotheses

The effects of transferrin and EGF on tooth development in organ culture, and the appearance of their receptors in embryonic tooth germs indicate that both transferrin and EGF, or EGF-like factors, play physiological roles in tooth development. The number of receptors for both transferrin and EGF in embryonic teeth is highest during the stages of most active cell proliferation, but decreases with overt differentiation of odontoblasts and ameloblast. Thus, transferrin and EGF-like growth factors are apparently involved in the regulation of cell proliferation.

Effects of EGF on embryonic tissues *in vivo* and *in vitro* (Hassel, 1975; Sundell *et al.*, 1975; Goldin and Opperman, 1980), and the presence of EGF receptors in embryonic tissues (Adamson *et al.*, 1981; Hortsch *et al.*, 1983; Partanen and Thesleff, 1987b) indicate that EGF is involved in embryonic development. However, synthesis of EGF at the level of transcription was not detected in mouse embryos, but only two weeks postnatally (Popliker *et al.*, 1987). It seems that another member of the EGF family, namely transforming growth factor -alpha (TGF-alpha), which is structurally analogous to EGF and binds to the same receptor molecule, represents the embryonic form of EGF-like activity (Nexo *et al.*, 1980). This is suggested also by the presence of TGF-alpha in mouse embryos from early gestation onwards (Twardzik, 1985).

Furthermore, the changes in the pattern of EGF binding at the early stages of tooth development suggest that the action of EGF in tooth morphogenesis is somehow associated with epithelial-mesenchymal tissue interactions. Both EGF and TGF-alpha are synthesized as larger precursor molecules which are thought to be transmembrane peptides (Rall *et al.*, 1985; Teixido *et al.*, 1987). It is possible that these precursor molecules could mediate cell-cell interactions when binding to a receptor molecule on another cell.

In future, the technique of *in situ* hybridization of TGF-alpha transcripts in tissue sections could give more information on the role of EGF-like growth factors as paracrine or autocrine regulators of embryonic development.

The biological functions of growth factors in tissue interactions may also be intimately linked with extracellular matrix. Growth factors have been shown to affect the production and turnover of matrix molecules, and may thus control cell-matrix interactions by regulating the composition of extracellular matrix (Chen *et al.*, 1977; Massague, 1987). On the other hand, the responses of cells to growth factors are influenced by extracellular matrix molecules. It is possible that growth factors can be stored in tissues by binding to matrix molecules. Hydrolysis of extracellular matrix occurs during morphogenetic events

(Bernfield *et al.*, 1984; Gospodarowicz *et al.*, 1986) whereby growth factor molecules can be released locally to stimulate cell proliferation.

## References


ADAMSON, E.D. (1982). The location and synthesis of transferrin in mouse embryos and teratocarcinoma cells. *Dev. Biol. 91:* 227-234.

ADAMSON, E.D., DELLER, M.J. and WARSHAW, J.B. (1981). Functional EGF receptors are present on mouse embryo tissues. *Nature (London) 291:* 656-659.

BARNES, D. and SATO, G. (1980). Serum free cell culture: a unifying approach. *Cell 22:* 649-655.

BEER, D.G., BUTLEY, M.S., CUNHA, G.R. and MALKINSON, A.M. (1984). Autoradiographic localization of specific ($^3$H) dexamethasone binding in fetal lung. *Dev. Biol. 105:* 351-364.

BERKOVITZ, B.K.B. (1971). The effect of root transection and partial root resection on the unimpeded eruption rate of the rat incisor. *Arch. Oral Biol. 16:* 1033-1043.

BERNFIELD, M.R., BANERJEE, S.D., KODA, J.E. and RAPRAEGER, A.C. (1984). Remodelling of the basement membrane as a mechanism of morphogenetic interaction. In *The Role of Extracellular Matrix in Development* (Ed. R.L. Trelstad). Alan R. Liss, Inc., New York, pp. 545-572.

BOOTH, A.G. and WILSON M.J. (1981). Human placental coated vesicles contain receptor-bound transferrin. *Biochem. J. 196:* 355-362.

BROWNELL, A.G. and ROVERO, L.J. (1980). DNA synthesis of enamel organ epithelium *in vitro* is enhanced by co-cultivation with nonviable mesenchymal cells. *J. Dent. Res. 59:* 1075-1080.

CAHILL, D.R. and MARKS, S.C., Jr. (1980). Tooth eruption: evidence for the central role of the dental follicle. *J. Oral Pathol. 9:* 189-200.

CHEN, L.B., GUDOR, R.C., SUN, T-T., CHEN, A.B. and MOSESSON, M.W. (1977). Control of a cell surface major glycoprotein by epidermal growth factor. *Science 197:* 776-778.

COHEN, S. (1962). Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the newborn animal. *J. Biol. Chem. 237:* 1555-1562.

CUNHA, G.R. and LUNG, B. (1978). The possible influence of temporal factors in androgenic responsiveness of urogenital tissue recombinants from wild-type and androgen-insensitive (Tfm) mice. *J. Exp. Zool. 205:* 181-194.

EKBLOM, P., THESLEFF, I., MIETTINEN, A. and SAXÉN, L. (1981). Organogenesis in a defined medium supplemented with transferrin. *Cell Differ. 10:* 281-288.

GOLDIN, G.V. and OPPERMAN, L.A., (1980). Induction of supernumerary tracheal buds and the stimulation of DNA synthesis in the embryonic chick lung and trachea by epidermal growth factor. *J. Embryol. Exp. Morphol. 60:* 235-243.

GOSPODAROWICZ, D., NEUFELD, G. and SCHWEIGERER, L. (1986). Molecular and biological characterization of fibroblast growth factor, an angiogenic factor which also controls the proliferation and differentiation of mesoderm and neuroectoderm derived cells. *Cell Differ. 19:* 1-17.

HASSEL, J.R. (1975). The development of rat palatal shelves *in vitro* and ultrastructural analysis of the inhibition of epithelial cell death and palate fusion by the epidermal growth factor. *Dev. Biol. 45:* 90-102.

HOPKINS, C.R. and TROWBRIDGE, I.S. (1983). Internalization and processing of transferrin and the transferrin receptor in human carcinoma A 431 cells. *J. Cell Biol. 97:* 508-521.

HORTSCH, M., SCHLESSINGER, J., GOOTWINE, E. and WEBB C.G. (1983). Appearance of functional EGF receptor kinase during rodent embryogenesis. *EMBO J. 2:* 1937-1941.

KARIN, M. and MINZ, B. (1981). Receptor-mediated endocytosis of transferrin in developmentally totipotent mouse teratocarcinoma cells. *J. Biol. Chem. 256:* 3245-3252.

KOLLAR, E.J. and BAIRD, G.R. (1970). Tissue interactions in embryonic mouse tooth germs. *J. Embryol. Exp. Morphol. 24:* 159-171.

KRATOCHWIL, K. and SCHWARTZ, P. (1976). Tissue interactions in androgen response of embryonic mammary rudiment of mouse: identification of target tissue for testosterone. *Proc. Natl. Acad. Sci. USA 73:* 4041-4044.

LEVIN, M.J., TUIL, D., UZAN, G., DREYFUS, J.C. and KAHN, A. (1984). High levels of transferrin mRNA in fetal muscle and brain. *Biochem. Biophys. Res. Commun. 122:* 212-217.

LUMSDEN, A.G.S, (1988). Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. In *Craniofacial Development* (Eds. P. Thorogood and C. Tickle). The Company of Biologists Ltd., pp. 155-169.

MASSAGUE, J. (1987). The TGF-beta family of growth and differentiation factors. *Cell 49:* 437-438.

MASSLER, M. and SHOUR, I. (1941). Studies in tooth development. Theories of eruption. *Am. J. Orthod. Oral Surg. 27:* 552-576.

McARDLE, H.J. and PRISCOTT, P.K. (1984). Uptake and metabolism of transferrin and albumin by rat yolk sac placenta. *Am. J. Physiol. 247:* C409-C414.

MEEK, J. and ADAMSON, E.D. (1985). Transferrin in foetal and adult mouse tissues: synthesis, storage and secretion. *J. Embryol. Exp. Morphol. 86:* 205-218.

MERCOLA, M. and STILES, C.D. (1988). Growth factor super-families and mammalian embryogenesis. *Development 102:* 451-460.

MINA, M. and KOLLAR, E.J. (1987). The induction of odontogenesis in non-dental mesenchyme combined with early murine mandibular arch epithelium. *Archs. Oral Biol. 32:* 123-127.

NEXO, E., HOLLENBERG, M.D., FIGUEROA, A., and PRATT, R.M. (1980). Detection of epidermal growth factor-urogastrone and its receptor during fetal mouse development. *Proc. Natl. Acad. Sci. USA 77:* 2782-2785.

PARTANEN, A-M., EKBLOM, P. and THESLEFF, I. (1985). Epidermal growth factor inhibits morphogenesis and cell differentiation in cultured mouse embryonic teeth. *Dev. Biol. 111:* 84-94.

PARTANEN, A-M. and THESLEFF, I. (1987a). Transferrin and tooth morphogenesis: Retention of transferrin by mouse embryonic teeth in organ culture. *Differentiation 34:* 25-31.

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

PARTANEN, A-M. and THESLEFF, I. (1987c). Levels and patterns of $^{125}$I-transferrin binding in mouse embryonic teeth and kidneys at various developmental stages. *Differentiation 34:* 18-24.

PARTANEN, A-M., THESLEFF, I. and EKBLOM, P. (1984). Transferrin is required for early tooth morphogenesis. *Differentiation 27:* 59-66.

POPLIKER, M., SHATZ, A., AVIVI, A., ULLRICH, A., SCHLESSINGER, J. and WEBB, C. (1987). Onset of endogenous synthesis of epidermal growth factor in neonatal mice. *Dev. Biol. 119:* 38-44.

RALL, L.B., SCOTT, J., BELL, G.I., CRAWFORD, R.A., PENSCHOW, J.D., NIALL, M.D. and COGHLAN, J.P. (1985). Mouse preproepidermal growth factor synthesis by the kidney and other tissues. *Nature (London) 313:* 228-231.

ROSS, R., RAINES, E.W. and BOWEN-POPE, D.F. (1986). The biology of platelet-derived growth factor. *Cell 46:* 155-169.

RUCH, J.V., LESOT, H., KARCHER-DJURICIC, V., MEYER, J.M. and MARK, M. (1983). Epithelial-mesenchymal interactions in tooth germs: mechanisms of differentiation. *J. Biol. Buccale 11:* 173-193.

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

SCHLESSINGER, J. (1987). Allosteric regulation of the epidermal growth factor receptor kinase. *J. Cell Biol. 103:* 2067-2072.

SLAVKIN, H.C. (1974). Embryonic tooth formation. A tool for developmental biology. In *Oral Sciences Reviews, Vol. 4* (Eds. Melcher, A.H. and Zarb, G.A.). Munksgaard, Copenhagen, pp. 1-136.

SUNDELL, H., SERENIUS, F., BARTHE, T., FRIEDMAN, C., KANAREK, K., ESCOBEDO, M., ORTH, D.M. and STAHLMAN, M.T.(1975). Effect of epidermal growth factor on fetal lamb lung maturation. *Pediatr. Res. 9:* 371-376.

TEIXIDO, J., GILMORE, R., LEE, D.C. and MASSAGUE, J. (1987). Integral membrane glycoprotein properties of the prohormone protransforming growth factor-α. *Nature (London) 326:* 883-885.

TEN CATE, A.R. (1969). The mechanism of tooth eruption. In *Biology of the Periodontium* (Eds. Melcher, A.H. and Bowen, W.H.) Academic Press, New York, pp. 91-103.

THESLEFF, I. (1976). Differentiation of odontogenic tissues in organ culture. *Scand. J. Dent. Res. 84:* 353-356.

THESLEFF, I. and HURMERINTA, K. (1981). Tissue interactions in tooth development. *Differentiation 18:* 75-88.

THESLEFF, I., JALKANEN, M., VAINIO, S., BERNFIELD, M. (1988). Cell surface proteoglycan expression correlates with epithelial-mesenchymal interactions during tooth morphogenesis. *Dev. Biol. 129:* 565-572.

THESLEFF, I., MACKIE, E., VAINIO, S. and CHIQUET-EHRISMANN, R. (1987a). Changes in the distribution of tenascin during tooth development. *Development 101:* 289-296.

THESLEFF, I., PARTANEN, A-M., LANDSCHULZ, W., TROWBRIDGE, I.S. and EKBLOM, P. (1985). The role of transferrin receptors and iron delivery in mouse embryonic morphogenesis. *Differentiation 30:* 152-158.

THESLEFF, I., PARTANEN, A-M. and RIHTNIEMI, L. (1987b). Localization of epidermal growth factor receptors in mouse incisors and human premolars during eruption. *Eur. J. Orthod. 9:* 24-32.

TWARDZIK, D.R. (1985). Differential expression of transforming growth factor-α during prenatal development of the mouse. *Cancer Res. 45:* 5413-5416.

YAMADA, M., BRINGAS, P., GRODIN, M. MACDOUGALL, M., CUMMINGS, E., GRIMMET, J., WELIKY, B. and SLAVKIN, H.C. (1980). Chemically-defined organ culture of embryonic mouse tooth organs: morphogenesis, dentinogenesis and amelogenesis. *J. Biol. Buccale 8:* 127-139.