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Associations between transforming growth factor β1 RNA expression and epithelial–mesenchymal interactions during tooth morphogenesis

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Summary

We have studied the expression of transforming growth factor beta-1 (TGF-β1) RNA during mouse tooth development, using *in situ* hybridization and experimental tissue recombinations. Analysis of the serial sections revealed the appearance of local expression of TGF-β1 RNA in the dental epithelium at bud-staged teeth (13-day embryos). Just before transition to the cap stage, TGF-β1 RNA expression rapidly increased in the epithelial bud, and it also extended to the condensed dental mesenchyme. At cap stage (14- and 15-day embryos), there was an intense expression of TGF-β1 RNA in the morphologically active cervical loops of the dental epithelium.

During early bell stage (16- and 17-day embryos), TGF-β1 RNA expression was detected in the inner enamel epithelium where it subsequently almost disappeared (18-day embryos). After birth TGF-β1 transcripts transiently appeared in these cells when they were differentiating into ameloblasts (1-day mice). The transcripts were lost from the ameloblasts when they became secretory (4-day mice), but the expression continued in ameloblasts in enamel-free areas. Transient expression of TGF-β1 RNA was also detected in epithelial stratum intermedium cells at the time of ameloblast differentiation. In the mesenchyme, TGF-β1 RNA was not detected during bell stage until it appeared in differentiated odontoblasts (18-day embryos). The secretory odontoblasts continued to express TGF-β1 RNA at all stages studied including the odontoblasts of incisor roots.

Analysis of the distribution of bromodeoxyuridine (BrdU) incorporation indicated apparent correlations between TGF-β1 RNA expression and cell proliferation at the bud and cap stages but not at later stages of tooth development. Tissue recombination experiments of bud-staged (13-day embryos) dental and non-dental tissues showed that tooth epithelium, when cultured together with tooth mesenchyme, expressed TGF-β1 RNA. When the tooth epithelium was combined with non-dental jaw mesenchyme, TGF-β1 transcripts were not expressed. However, TGF-β1 RNA expression was seen in oral epithelium cultured with dental mesenchyme, while no expression of TGF-β1 transcripts was seen in the oral epithelium during normal development. Thus, TGF-β1 RNA expression seems to be regulated by epithelial–mesenchymal interactions.

Key words: TGF-β1, tooth morphogenesis, epithelial–mesenchymal interactions, *in situ* hybridization, BrdU.

Introduction

The tooth is an organ whose development is controlled by a chain of reciprocal interactions between its epithelial and neural crest-derived mesenchymal components (Kollar and Braid, 1970; Slavkin, 1974; Thesleff and Hurmerinta, 1981; Ruch *et al.* 1983). Tooth morphogenesis begins with the thickening of the oral epithelium. When the jaw mesenchyme condenses around the epithelial bud, the tooth germ has reached the bud stage. Tissue recombination experiments have shown that until this stage the epithelium contains all the information required for the formation of a mature tooth, but at this stage the instructions for subsequent odontogenesis shift from epithelium to mesenchyme (Mina and Kollar, 1987; Kollar and Braid, 1970). During the following cap and bell stages, the morphology of the tooth crown is established and at late bell stage the dentine-producing odontoblasts and enamel-producing ameloblasts differentiate.

Although the exact molecular mechanisms involved in the tissue interactions that regulate tooth development are largely unknown, there is evidence from our and other laboratories that ECM molecules and growth factors as well as their receptors may play central roles. These include the ECM glycoprotein tenascin and the cell surface matrix receptor syndecan which are intensely expressed in the condensing dental mesen-

chyme (Thesleff *et al.* 1987, 1988). Of the growth factors, epidermal growth factor (EGF) affects tooth morphogenesis (Partanen *et al.* 1985) and EGF receptors appear to be regulated by epithelial–mesenchymal interactions during early tooth development (Partanen and Thesleff, 1987).

TGF-β1 is a multifunctional peptide growth factor which is known to be both a positive and negative regulator of cellular growth (Roberts *et al.* 1981; Moses *et al.* 1985) and differentiation (Ignotz and Massagué, 1985; Masui *et al.* 1986; Massagué *et al.* 1986; Seyedin *et al.* 1987), and to enhance the deposition of ECM (Heino and Massagué, 1989). TGF-β1 may also modulate cell adhesion and phenotype e.g. by regulating the synthesis of integrins (Ignotz and Massagué, 1987). Growth factors belonging to the TGF-β superfamily have been shown to be involved in the induction of mesoderm formation in *Xenopus* embryos (Rosa *et al.* 1988) and in the mediation of interactions between mesoderm and endoderm in *Drosophila* embryos (Panganiban *et al.* 1990). TGF-β has also been shown to mediate epithelial–mesenchymal cell transformations during chicken heart development (Potts and Runyan, 1989; Potts *et al.* 1991).

TGF-β1, TGF-β2 and TGF-β3 RNAs have been detected in the tooth germ (Lehnert and Akhurst, 1988; Pelton *et al.* 1990), and TGF-β1 protein has been localized in developing teeth by immunohistology (Heine *et al.* 1987; Cam *et al.* 1990; D'Souza *et al.* 1990). Although TGF-β1 RNA has been localized in developing teeth, the consecutive changes in the distribution pattern of TGF-β1 transcripts during tooth development have not been studied earlier. Also, there has been no experimental evidence concerning involvement of TGF-β1 in secondary inductions during tooth development.

Materials and methods

Preparation of tissues

The regions of first (and occasionally second) molar tooth germs were removed from 11-day mouse embryos to 4-day postnatal mice (CBA×C57BL). Embryonic age was determined according to the vaginal plug (day 0). In addition, the non-mineralized apical regions of the roots of incisors were removed from 14-day mice. All tissues were staged by using morphological criteria. For *in situ* hybridization, tissues were fixed in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) and embedded in paraffin. Serial sections of 7 μm were cut and transferred to siliconized slides.

Preparation of probes

[^{35}S]UTP-labelled single-stranded RNA probes were synthesized by *in vitro* transcription from a 974 bp *Sma*I–*Sma*I fragment of murine TGF-β1 cDNA (nucleotides 421–1395 in the cDNA) (Derynck *et al.* 1986; Wilkinson and Green, 1990). Most of the sequence resides in the region encoding the TGF-β1 precursor peptide. The same region was earlier used by Lyons *et al.* (1990) and Pelton *et al.* (1990) to generate RNA probes for *in situ* hybridization. They could not detect any cross-hybridization with the other TGF-β transcripts under conditions similar to ours. Also, we have found that using the procedure described here, the distribution pattern of TGF-β3 RNA differs from that detected for TGF-β1 RNA (A. Vaahtokari, unpublished results).

pBluescript II SK(−) (Stratagene) into which TGF-β1 DNA fragment had been subcloned was linearized using *Eco*RI restriction enzyme (Boehringer Mannheim) and transcribed with T3 RNA polymerase (Boehringer Mannheim) to generate the antisense TGF-β1 RNA probe. The control sense probe was obtained using *Bam*HI-linearized plasmid and T7 RNA polymerase (Boehringer Mannheim). The probes were shortened to an average length of 100 bases by a limited alkaline hydrolysis (Cox *et al.* 1984) and separated from unincorporated [^{35}S]UTP (Amersham), using Sephadex G-50 (Pharmacia) gel chromatography. After ethanol precipitation, the probes were redissolved in the hybridization buffer and diluted to about 5×10^4 cts min^{-1} μl^{-1}. Before hybridization the probes were denaturated by heating at 80°C.

In situ *hybridization*

In situ hybridization was performed as described by Wilkinson *et al.* (1987, 1990). Briefly, tissue sections were pretreated with proteinase K (Sigma) and acetylated. Hybridization was done overnight in a moist chamber in 60% formamide at 50°C. Also, 50% formamide or 52°C were used in some experiments. The results were identical with both concentrations and temperatures. After hybridization, the tissue sections were washed using high-stringency washes as described by Wilkinson and Green (1990). Dried slides were dipped in diluted (1:1) autoradiographic emulsion (Kodak), dried and exposed for 10 days at 4°C in the presence of silica gel. Following development, the emulsion was fixed, and the sections were stained with Delafield's hematoxylin and mounted with DePeX (BDH). Photomicrography was performed using Leitz Orthoplan microscope and Ilford PanF film.

Cell proliferation assay

Dissected tooth rudiments were preincubated in a Trowell-type culture for 3 h in Minimum Essential Medium (MEM) supplemented with 10% fetal calf serum (FCS, GIBCO) at 37°C. Subsequently the tissues were incubated for 60 min in the same medium containing 5-bromo-2′-deoxyuridine (BrdU) and 5-fluoro-2′-deoxyuridine (both included in Amersham's labelling reagent, diluted in 1:1000), which increases BrdU incorporation by lowering competition by endogenous thymidine. The tissues were washed in PBS, pH 7.3, for 3×5 min at room temperature, fixed in cold ethanol and processed for immunohistology. BrdU incorporated into DNA was located in the tissue sections by using a specific mouse monoclonal antibody (Amersham). The bound antibody was detected using biotinylated secondary antibody to mouse immunoglobulins and the Vectastain–ABC kit (Vector).

Tissue recombination experiments

Dissected molar tooth germs with some surrounding tissue of 13-day embryos were incubated for 2 min in 2.25% trypsin/0.75% pancreatin on ice, and the epithelia and mesenchymes were microsurgically separated in MEM supplemented with 10% FCS. The epithelium of the tooth bud was separated from the oral epithelium and the dental mesenchyme from the non-dental jaw mesenchyme. 3–5 isolated epithelia were placed in intimate contact with 3–5 isolated mesenchymes on a polycarbonate membrane (Nuclepore Corp.). The recombinations were cultured for three days in Trowell-type cultures in MEM supplemented with 10% FCS. The explants were

fixed in PFA and processed for *in situ* hybridization as described above.

Results

Tooth development until bud stage (11- to 13-day embryos)

The examination of the serial sections indicated that during tooth development the pattern of TGF-β1 RNA expression changed along both mesiodistal and buccolingual axes corresponding with the advancing morphogenesis and cell differentiation.

During the initiation of molar tooth development (11-day embryos), no specific hybridization signal was detected either in the jaw epithelium or in the jaw mesenchyme in the region of the presumptive molar tooth germ (data not shown). Analysis of serial sections of the lower jaw of 13-day embryos indicated that rapid changes of TGF-β1 expression take place during the bud stage: no TGF-β1 transcripts were seen either in epithelium or mesenchyme at the early bud stage (data not shown), but after a short period low levels of TGF-β1 RNA expression were located at the tip of the invaginating dental epithelium (Fig. 1B). This expression was evident only in a restricted region along the mesiodistal axis of the tooth germ. At this stage, no TGF-β1 expression was detected in the dental mesenchyme. Just before transition to the cap stage, TGF-β1 RNA expression increased in the epithelium, and it also appeared in the condensed dental mesenchyme surrounding the bud (Fig. 1E), again in a localized region along the mesiodistal axis. TGF-β1 transcripts were not detected in Meckel's cartilage or in the surrounding jaw mesenchyme, which is in contrast to an earlier report according to which TGF-β1 transcripts were located in the mesenchyme between the epithelium of the tooth bud and the lip furrow in the 13-day mouse embryo (Lehnert and Akhurst, 1988). The developing mandibular bone expressed high levels of TGF-β1 RNA (Fig. 1E). In sections hybridized with the sense probe, no specific hybridization was detected at this or later stages (Fig. 3E).

Cap-staged tooth (14- and 15-day embryos)

In the 14-day embryo, first molar development has reached the cap stage. An intense expression of TGF-β1 RNA appeared in the developing cervical loops of the dental epithelium, and relatively high levels of TGF-β1 transcripts were also seen in the inner enamel epithelium between the developing cervical loops and in the mesenchyme (Fig. 1H). However, analysis of the serial sections indicated regional differences in the expression pattern: in the mesial and distal ends of the tooth, no TGF-β1 transcripts were detected in the epithelium (data not shown). In 15-day embryo, the first molar tooth germ is still at the cap stage, and the cervical loops of the dental epithelium and the inner enamel epithelium continued to express TGF-β1 RNA in the central areas of the tooth. In the mesial and distal ends, TGF-β1 transcripts were only localized in the mesenchyme (data not shown).

Bell-staged tooth (16- to 18-day embryos) and completion of crown morphogenesis (1- to 4-day mice)

During the early bell stage (16- and 17-day embryos), expression of TGF-β1 transcripts diminished in the dental epithelium so that, in comparison with the cap stage, lower levels of TGF-β1 RNA expression were detected in restricted areas in the inner enamel epithelium (Fig. 2B). In the dental mesenchyme, no TGF-β1 RNA was detected at this stage (Fig. 2B). One day later, expression of TGF-β1 transcripts almost disappeared in the dental epithelium whereas in the dental mesenchyme TGF-β1 RNA expression was detected in the polarized odontoblasts (Fig. 2D).

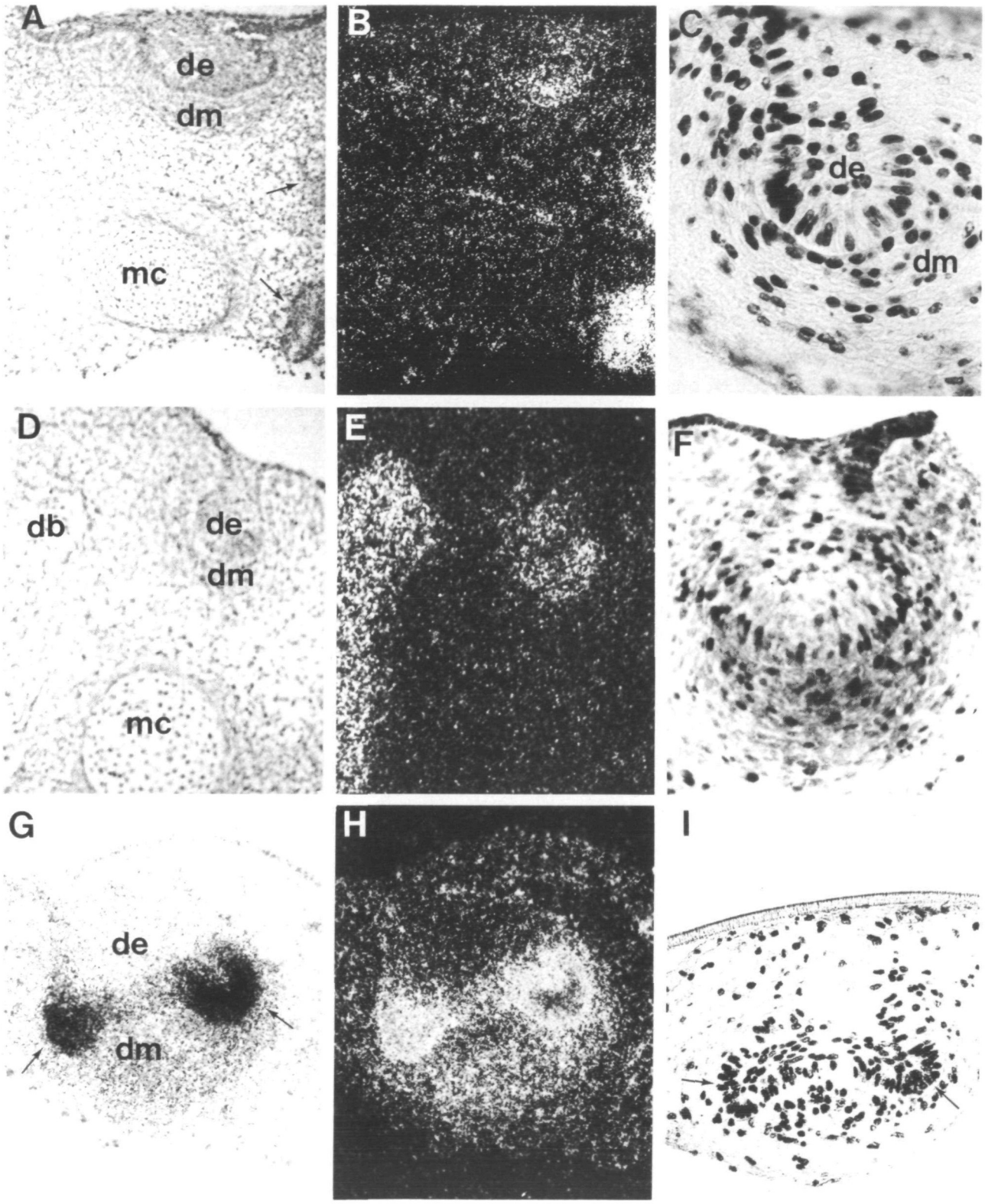
In 19-day embryos, the epithelial stratum intermedium cells transiently expressed TGF-β1 RNA (Fig. 2F). After birth, preameloblasts also expressed TGF-β1 transcripts transiently at the onset of their differentiation (Fig. 3B). Interestingly, even after crown morphogenesis was completed (4-day postnatal mice), the non-secreting ameloblasts at the tips of the cusps continued to express TGF-β1 RNA (Fig. 3D). In other areas secretory ameloblasts as well as other epithelial cells lacked TGF-β1 transcripts.

TGF-β1 RNA expression persisted in terminally differentiated odontoblasts (Fig. 3D). Secretory odontoblasts expressed TGF-β1 at all locations examined, including the roots of the incisors of 14-day mice (Fig. 3F). Examination of the second molars, which develop two days later than the first molars, revealed identical distribution of TGF-β1 transcripts as detected in the first molars (data not shown). No TGF-β1 RNA was detected in the mesenchymal and epithelial tissues surrounding the developing tooth germ except in the developing mandibular bone.

Changes in cell proliferation

Proliferating cells were localized by analyzing the incorporation of the thymidine analogue, BrdU, into replicating DNA. Incorporated BrdU was detected by a specific monoclonal antibody and immunoperoxidase staining. During active morphogenesis, both epithelial and mesenchymal cells incorporated BrdU intensely. However, regional differences were observed in the distribution of dividing cells. An apparent correlation between cell proliferation and TGF-β1 RNA expression was seen during the bud and cap stages. During the early bud stage, BrdU incorporation was evident in the dental epithelial cells and mesenchymal cells next to the basement membrane separating the epithelial and mesenchymal components of the tooth (Fig. 1C). At the late bud stage more cells in the dental mesenchyme were BrdU-positive (Fig. 1F).

At the cap stage the developing cervical loops which intensely expressed TGF-β1 transcripts also incorporated BrdU (Fig. 1I). At the same stage, dental mesenchyme which expressed TGF-β1 RNA was also BrdU-positive. However, the correlation between BrdU incorporation and TGF-β1 RNA expression was not consistent. The stellate reticulum cells incorporated BrdU but did not express TGF-β1 RNA. At later stages



of tooth development, no co-distribution of TGF-β1 transcripts and cell proliferation was detected. Thus, TGF-β1 RNA expression pattern could be correlated with cell proliferation only during the bud and cap stages.

Tissue recombination experiments

The possible role of tissue interactions in the regulation of TGF-β1 RNA expression was studied by experimental tissue recombination experiments. Epithelial and mesenchymal components of bud-staged molar tooth

Fig. 1. Localization of TGF-β1 RNA and proliferating cells in bud- and cap-staged teeth. (A) Bright-field photography of an early bud-staged tooth (13-day embryo). The developing mandibular bone is also present in the section (arrow). (B) Dark-field photography of the section shown in A. A faint hybridization signal is visible in cells at the tip of the dental epithelium. In the developing mandibular bone, strong TGF-β1 RNA expression is detected. (C) Immunoperoxidase localization of BrdU incorporation in a bud-staged tooth (corresponding to A and B). BrdU-positive cells are accumulated in the dental mesenchyme and the dental epithelium next to the basement membrane separating the epithelial and mesenchymal components of the tooth. (D) Bright-field photography of a late bud-staged tooth (13-day embryo). (E) Dark-field photography of the section shown in D, showing hybridization in both the dental epithelium and the dental mesenchyme. The developing mandibular bone is also strongly labelled, but Meckel's cartilage shows no expression of TGF-β1 transcripts. (F) Immunoperoxidase localization of BrdU incorporation in a bud-staged tooth (corresponding to C and D). BrdU-positive cells are accumulated in the dental mesenchyme and in the dental epithelium adjacent to the mesenchyme. (G) Bright-field photography of a cap-staged tooth (14-day embryo). An intense hybridization signal is visible in the developing cervical loops of the dental epithelium (arrows). The dental mesenchyme shows a weaker signal. (H) Dark-field photography of the section shown in G. (I) BrdU incorporation in a cap-staged tooth (15-day embryo). The cells of the developing cervical loops (arrows), the inner enamel epithelium and the dental mesenchyme show strong positivity. db, developing mandibular bone; de, dental epithelium; dm, dental mesenchyme; mc, Meckel's cartilage.

germs (13-day embryo) were separated and cultured *in vitro* in different combinations. After three days in recombination culture, restricted regions of the dental epithelium and dental mesenchyme formed a structure that resembled a cap-staged tooth germ. In this morphologically distinct region, local TGF-β1 RNA expression was detected in the epithelial cells adjacent to the mesenchymal component (Fig. 4B). In other parts of the epithelium and in the mesenchyme no TGF-β1 transcripts were seen. When a similar experiment was made by combining dental epithelium with non-dental jaw mesenchyme, no expression of TGF-β1 was detected either in the epithelium or in the mesenchyme (Fig. 4D). However, in the reciprocal recombinant TGF-β1 RNA expression was seen in the oral epithelium cultured with the dental mesenchyme (Fig. 4F). This expression was restricted to epithelial projections resembling the developing cervical loops of a tooth germ.

Discussion

The role of TGF-β1 in epithelial–mesenchymal interactions

Our *in situ* hybridization analysis of serial sections of carefully staged tooth germs from initiation to completion of morphogenesis indicates that marked changes take place in the expression pattern of TGF-β1. Although our studies confirm most earlier findings of TGF-β1 RNA expression in the developing tooth, interesting patterns were revealed that have gone unnoticed in previous studies.

The appearance of TGF-β1 RNA first at the tip of the epithelial bud and subsequently in the condensed mesenchyme suggests that epithelially derived TGF-β1 induces its own expression in the dental mesenchymal cells, i.e. TGF-β1 may act as an autoinducer (Van Obberghen-Schilling *et al.* 1988). Our results are in agreement with the finding that intracellular TGF-β1 protein has been found both in epithelial and mesenchymal tissues (Thompson *et al.* 1989; Flanders *et al.* 1989). The extracellular TGF-β1 protein product has only been detected in mesenchyme of developing organs including the tooth (Heine *et al.* 1987; Thompson *et al.* 1989). Although there is evidence that TGF-β1 regulates growth in some epithelial tissues, the current data can be interpreted so that the primary responding tissue for the TGF-β1 synthesized by the dental epithelium is the underlying mesenchyme (Shipley *et al.* 1986; Kurokowa *et al.* 1987).

That epithelial–mesenchymal interactions are involved in the regulation of epithelial expression of TGF-β1 RNA is suggested by our experimental tissue recombination experiments. Bud-staged dental epithelium expressed TGF-β1 RNA when cultured together with the dental mesenchyme, whereas no expression of TGF-β1 transcripts was detected when the dental epithelium was cultured with the jaw mesenchyme surrounding the tooth germ. In the reciprocal recombination, the dental mesenchyme induced the expression of TGF-β1 RNA in the oral epithelium, which does not express TGF-β1 transcripts during normal development. Thus, the bud-staged dental mesenchyme appears to regulate the expression of epithelial TGF-β1 RNA. The epithelial tissues were not cultured alone, because without any mesenchymal tissue the epithelium grows poorly and loses its normal morphology. Because the environment for development is sub-optimal in *in vitro* cultures, the levels of TGF-β1 expression may be lower than *in vivo* and possibly for that reason the less intense dental mesenchymal expression of TGF-β1 was not seen in recombinations.

One candidate molecule which may be involved in the regulation of epithelial TGF-β1 expression is homeobox gene Hox 7.1 which has been found to be expressed in the dental mesenchyme earlier than we have detected TGF-β1 RNA in tooth germs (Mackenzie *et al.* 1991). It has recently been shown that, in *Drosophila*, homeotic genes regulate the expression of *decapentaplegic* (*dpp*), which is a member of the TGF-β superfamily (Reuter *et al.* 1990). Thus, it is possible that also in tooth development the homeobox genes regulate the expression of TGF-β1.

The possible functions of TGF-β1 in the dental mesenchyme

Our BrdU incorporation analysis showed that at the

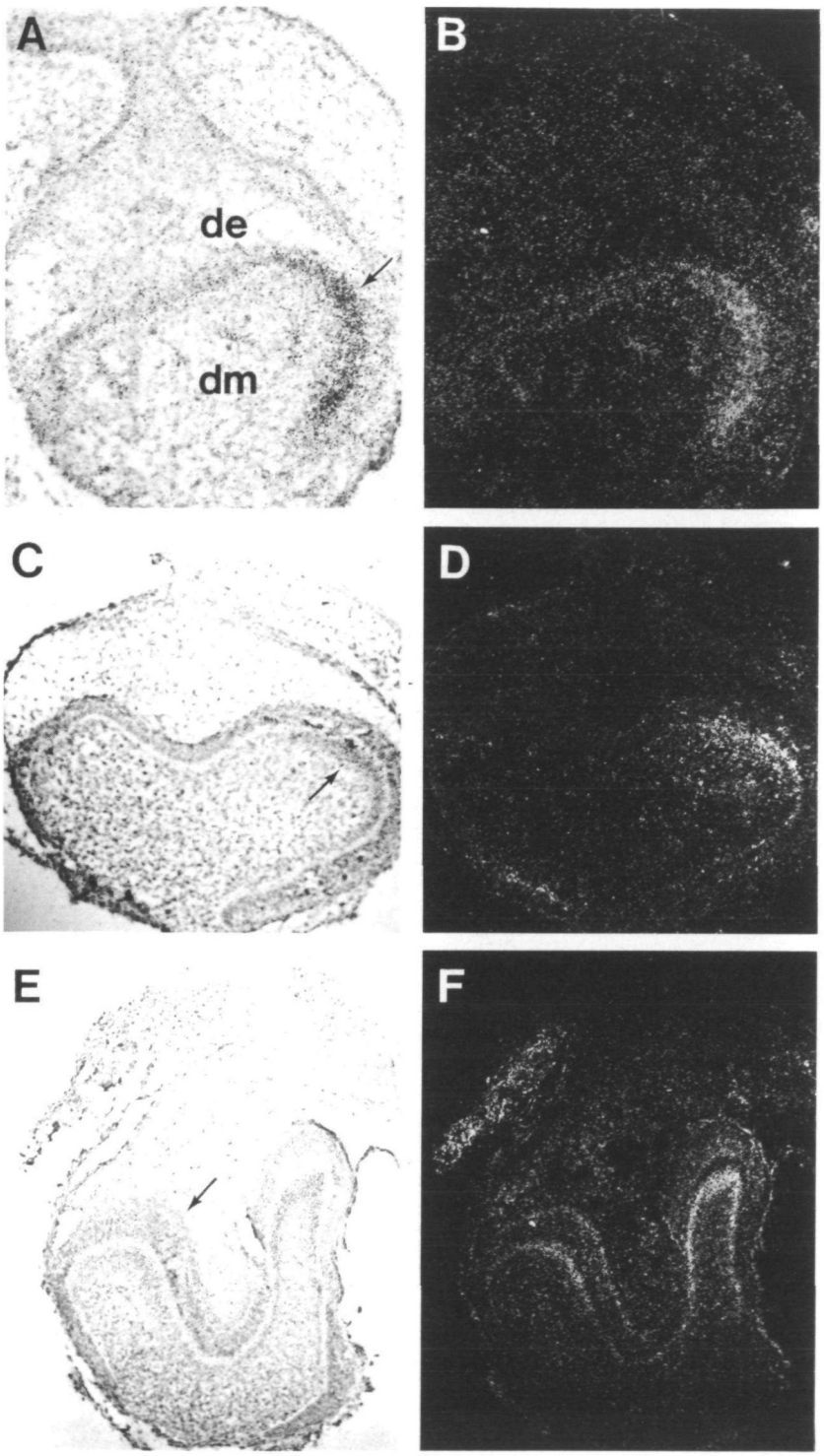


Fig. 2. Expression of TGF-β1 transcripts in 17- to 19-day embryos. (A) Bright-field photography of a section of an early bell-staged tooth (17-day embryo). (B) Dark-field photography of the section shown in A. Only the inner enamel epithelium shows TGF-β1 expression (arrow in A). (C) Bright-field photography of a section of a late bell-staged tooth (18-day embryo). (D) Dark-field photography of the section shown in C. TGF-β1 transcripts are visible in the dental mesenchyme in the first polarized odontoblasts (arrow in C). (E) Bright-field photography of a section of a tooth from 19-day embryo. (F) Dark-field photography of the section shown in E. TGF-β1 RNA expression is detected in stratum intermedium (arrow in E) and odontoblasts. The differences between the buccal and lingual cusps are due to slightly tilted plane of sectioning. de, dental epithelium; dm, dental mesenchyme.

bud and cap stages the dental mesenchyme is rapidly proliferating while the dental epithelium intensely expresses TGF-β1 RNA, and the mesenchyme itself also expresses TGF-β1 although at lower levels. It is known that TGF-β1 can function as an indirect mitogen by inducing the expression of platelet-derived growth factor A and B chains in some cells (Leof *et al.* 1986; Mäkelä *et al.* 1987). Thus, the local expression of TGF-β1 in the dental epithelium may regulate cell proliferation in the underlying dental mesenchyme and contribute to the determination of tooth morphology.

Another possible function of TGF-β1 during tooth morphogenesis is regulation of matrix deposition, because TGF-β1 is known to promote the synthesis of ECM, to modify cell surface matrix receptors and to prevent degradation of ECM (Rasmussen and Rapraeger, 1988; Rizzino, 1988). The ECM glycoprotein tenascin and the cell surface proteoglycan

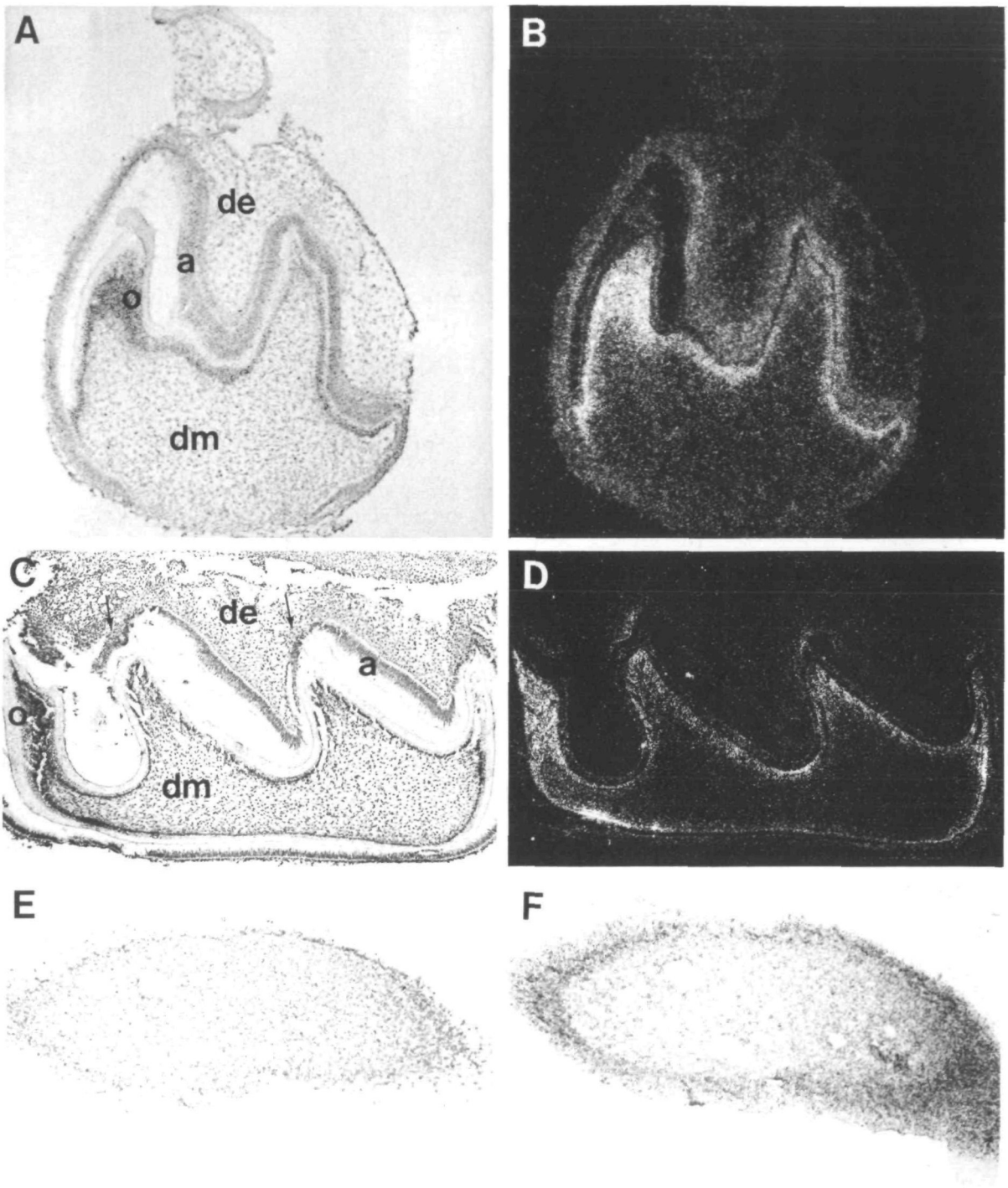


Fig. 3. Expression of TGF-β1 transcripts during completion of cuspal morphogenesis. (A) Bright-field photography of a transverse section of a molar tooth (1-day postnatal mouse). (B) Dark-field photography of the section shown in A. The mesenchyme-derived secretory odontoblasts as well as the differentiating epithelial ameloblasts show hybridization signal. (C) Bright-field photography of a sagittal section of a tooth (4-day postnatal mouse). (D) Dark-field photography of the section shown in C. TGF-β1 RNA is expressed in all secretory odontoblasts. The secretory ameloblasts do not express TGF-β1 transcripts, but the ameloblasts in enamel-free areas at the tips of the cusps (arrows in C and D) continue to express TGF-β1 RNA. (E) Bright-field photography of a horizontal section of a dental root (14-day mouse) hybridized with TGF-β1 sense probe showing non-specific hybridization. The epithelium and dentin have been lost during preparation of sections. (F) Bright-field photography of a similar section but hybridized with an antisense probe. The odontoblasts show a hybridization signal. a, ameloblastic layer; de, dental epithelium; dm, dental mesenchyme; o, odontoblastic layer.

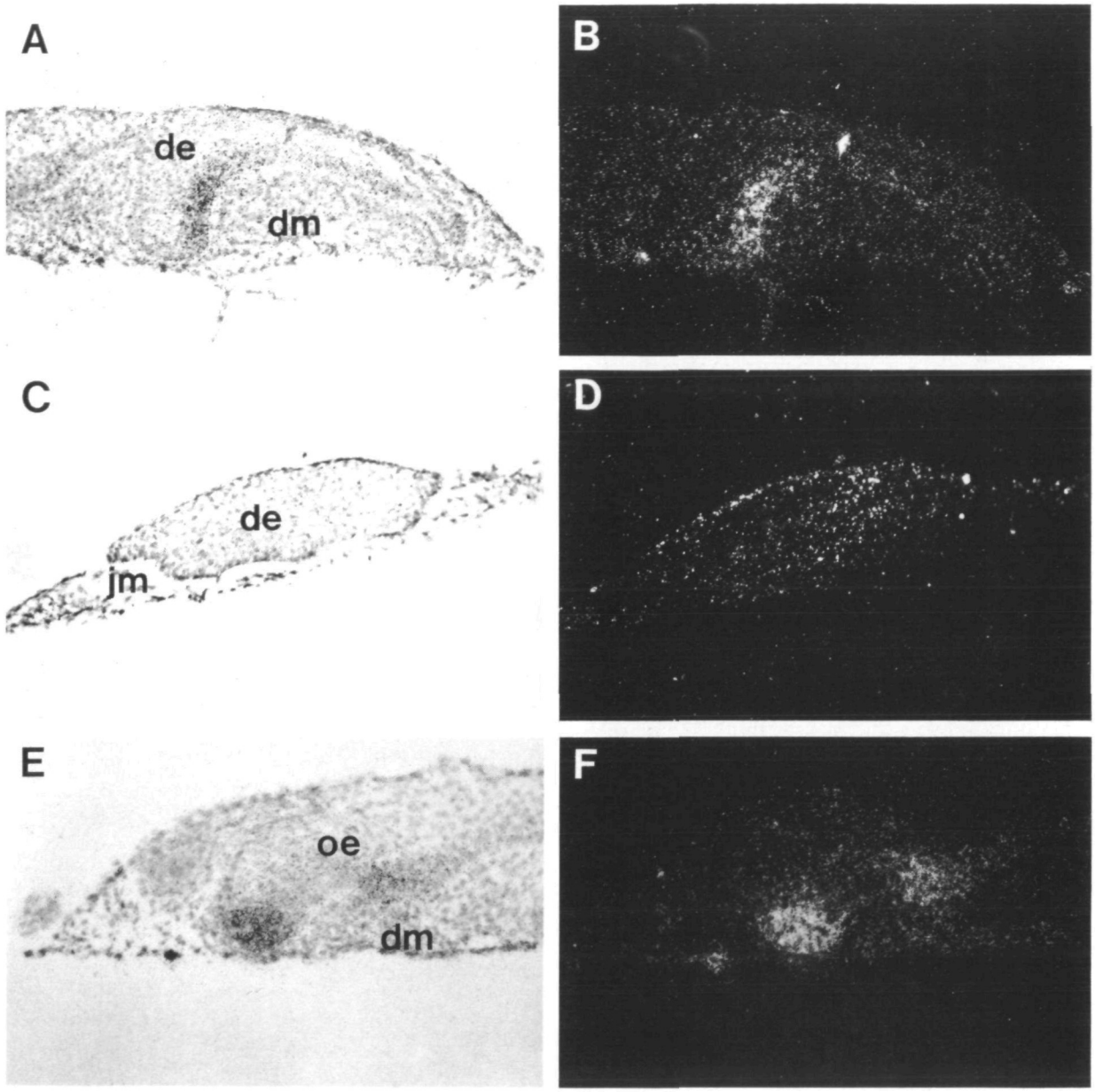


Fig. 4. Expression of TGF-β1 RNA in tissue recombination explants (from 13-day embryos) cultured for three days. (A) Bright-field image of a section of an explant of recombined dental mesenchyme and dental epithelium. (B) Dark-field image of the section shown in A. TGF-β1 RNA expression is visible in the dental epithelium adjacent to the dental mesenchyme. (C) Bright-field image of a section of an explant of recombined non-dental jaw mesenchyme and dental epithelium. (D) Dark-field image of the section shown in C which shows no specific hybridization signal. (E) Bright-field image of a section of an explant of recombined oral epithelium and dental mesenchyme. (F) Dark-field image of the section shown in E. TGF-β1 transcripts are detected in the oral epithelium adjacent to the dental mesenchyme. de, dental epithelium; dm, dental mesenchyme; oe, oral epithelium; jm, jaw mesenchyme.

syndecan are expressed in the condensed mesenchyme around the epithelial dental bud (Thesleff *et al.* 1987, 1988), and their expression is regulated by the dental epithelium (Vainio *et al.* 1989). TGF-β1 is also known to modulate both syndecan and tenascin expression (Pearson *et al.* 1988; Rapraeger, 1989). Syndecan expression in the dental mesenchyme increases significantly from the bud to cap stage and decreases rapidly during the bell stage (Thesleff *et al.* 1988), just as TGF-β1 RNA expression in the dental epithelium. Thus, it is possible that TGF-β1 is involved in regulation of syndecan and/or tenascin expression in developing tooth.

Possible autocrine functions of TGF-β1 in ameloblasts and odontoblasts

At later stages of odontogenesis, TGF-β1 RNA was detected transiently in stratum intermedium cells before the differentiation of ameloblasts. TGF-β1

synthesized by stratum intermedium may regulate the initiation of ameloblast differentiation in a paracrine way.

The expression of TGF-β1 in ameloblasts was restricted to a short period during their polarization; when ameloblasts became secretory, TGF-β1 transcripts disappeared. Only the ameloblasts at the tips of the cusps that do not differentiate terminally and which remain non-secretory continued to express TGF-β1 at least 4 days after birth (Sutcliffe and Owens, 1980). TGF-β1 RNA has been detected by northern blot analysis in the ameloblastic layer isolated from embryonic bovine teeth (Robey *et al.* 1987). However, TGF-β1 RNA has not earlier been reported in ameloblasts by using *in situ* hybridization. This may be due to the developmental stages studied because the expression of TGF-β1 RNA in most ameloblasts is transient and may therefore go unnoticed. Our data suggest that TGF-β1 may regulate the terminal differentiation and/or the secretory functions of ameloblasts.

In the dental mesenchyme, no TGF-β1 transcripts were detected in the preodontoblasts when they polarized, whereas high levels of expression of TGF-β1 transcripts were detected in secretory odontoblasts. Recent comparison of the distribution of TGF-β1, TGF-β2 and TGF-β3 by Pelton *et al.* (1990) suggested that odontoblasts do not express TGF-β1. However, TGF-β1 transcripts were detected in the subodontoblastic mesenchymal cell layer. The most probable explanation for this discrepancy in comparison to our results is a fixation artefact: in the report by Pelton *et al.* in the section that represented TGF-β1 RNA distribution the odontoblasts appeared poorly preserved and thus the expression noted in subjacent tissue may actually have represented TGF-β1 RNA in the odontoblasts.

We suggest that in odontoblasts TGF-β1 acts as an autocrine factor which regulates both its own synthesis and the formation of dentin matrix. Since TGF-β1 has been reported to induce the expression of type 1 collagen gene (Roberts *et al.* 1986) and type I collagen is the main component of dentine, the regulation of type 1 collagen is a possible function of TGF-β1 in odontoblasts. TGF-β also enhances the expression of osteonectin/SPARC (Noda and Rodan, 1987) which is a matrix molecule expressed by odontoblasts (Holland *et al.* 1987). Another possible fate for TGF-β1 protein secreted by odontoblasts is to accumulate in dentine (Harada *et al.* 1990) from which it may become released during pathological resorption associated e.g. with infections and stimulate reparative processes by regulating differentiation and/or matrix production of mesenchymal cells.

In conclusion, the stage-specific changes detected in the distribution of TGF-β1 RNA in the developing tooth suggest that TGF-β1 has multiple roles in odontogenesis. Our results support the proposal that TGF-β1 acts as a paracrine and autoinducing factor as well as participates in the epithelial–mesenchymal interactions.

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