Int. J. Dev. Biol. 39: 35-50 (1995)

Special Review

Regulation of organogenesis. Common molecular mechanisms regulating the development of teeth and other organs

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0214-6282/95/$03.00

Printed in Spain

Introduction

All organs develop from epithelial and mesenchymal tissues, and during the early stages of morphogenesis many organs share common morphological features. The epithelial components usually originate as thickenings which subsequently form buds around which the underlying mesenchymal cells condense (Fig. 1). The advancing morphogenesis, i.e. the development of form, involves complex growth, such as branching and/or folding of epithelia. These morphological changes at cellular and tissue levels have been known for many decades. Also, certain general regulatory mechanisms of organ development, in particular epithelial-mesenchymal interactions, were described in detail by experimental embryologists as early as in the 1950's and 1960's. Progress in the field of developmental biology was rather slow during the following two decades until great advances in molecular biology and gene technology made it feasible to study developmental processes at the molecular level.

The molecular basis of early vertebrate development, such as the mechanisms of patterning of axial structures and mesoderm induction is already understood in great detail (McGinnis and Krumlauf, 1992; Slack, 1994). Consequently, there is increasing interest among developmental biologists in mechanisms regulating organ development. Limb development at the molecular level is the focus of interest of several groups (Niswander *et al.*, 1993; Riddle *et al.*, 1993; Francis *et al.*, 1994) and so is the analysis of development of other organs, such as kidney, heart, mammary gland, and hair (Vainio *et al.*, 1989b; Sariola *et al.*, 1991; Hardy, 1992; Runyan *et al.*, 1992; Bard *et al.*, 1994; Sympson *et al.*, 1994). In addition, the production of transgenic mice with deficient gene function has led, sometimes quite unexpectedly, to the identification of molecules that are required for the development of specific organs. As a result of various experimental approaches, regulatory functions have been ascribed to many kinds of molecules, including transcription factors, growth factors, components of the cell surface and extracellular matrix (ECM), and matrix degrading enzymes. In spite of the wide variety of molecules involved, we are now beginning to see common molecular mechanisms that appear to govern the development in different organ systems. Thus, the morphological resemblance of the early development of various organs seems to reflect similarities in the underlying molecular machinery.

Teeth are among the organs on which a reasonable amount of data on developmental regulation has accumulated recently. Molecular changes that correlate with advancing tooth morphogenesis have been mapped by many research groups, and the regulatory roles of several molecules have been elucidated by experimental studies (reviewed by Thesleff *et al.*, 1990, 1995). Here, we review some of the current literature concerning molecular regulation of organogenesis, and, specifically, we compare the mechanisms of tooth morphogenesis with those of other organs. Due to the increasing amount of data, particularly on developmental changes in the expression of various molecules in different organs, it is not possible to discuss every study in this review. The focus will rather be on those molecular mechanisms which appear to be shared in the developmental regulation of different organs.

General features of organ development

Epithelial-mesenchymal interactions

Organ development is characterized by coordinated growth and differentiation of cells in epithelial and mesenchymal cell lineages. Spemann and his colleagues demonstrated that interactions between tissues are crucial for organogenesis (Spemann, 1938). Thereafter, experimental embryologists analyzed the nature of epithelial-mesenchymal interactions in a number of different organ systems and showed that such interactions, which were also termed secondary induction, regulated both morphogenesis and cell differentiation. It was observed, for instance, that epithelial branching did not occur in the absence of mesenchymal tissue (Grobstein, 1955; Kollar and Baird, 1969; Wessells, 1970; Sakakura *et al.*, 1976). Neither did mesenchymal cells differentiate without signals from the epithelial tissue: isolated mesenchyme of the tooth did not differentiate into odontoblasts, and metanephric mesenchyme did not differentiate into epithelial kidney tubule cells unless cultured with an inducing tissue (Grobstein, 1955; Kollar and Baird, 1970; Ruch, 1987; Saxén, 1987).

It was discovered that epithelial-mesenchymal interactions are *sequential*, i.e. there is a chain of interactive events that gradually govern advancing development. The interactions were also shown to be *reciprocal* occurring in both directions between the epithelial and mesenchymal tissues (Lawson, 1974; Sakakura *et al.*, 1976; Sengel, 1986). In tissue recombination experiments where epithelia and mesenchymes from different organs were cultured together, it was observed that, depending on the organ and its developmental stage, either the epithelium or the mesenchyme possessed the information for organ-specific morphogenesis and differentiation. For instance in the kidney, several heterologous tissues induced kidney tubule formation in metanephric mesenchyme, which, on the other hand, was the only mesenchyme responding to inductive signals by differentiation into kidney tubules (Saxén, 1970). Thus, epithelial-mesenchymal interactions were divided into *permissive* and *instructive* (directive) ones (Saxén, 1977; Wessells, 1977).

In the tooth, the early dental epithelium was shown to possess the potential to induce non-dental, neural crest-derived mesenchyme to form a tooth whereas during the early bud stage odontogenic mesenchyme gained the ability to instruct non-dental epithelium to form tooth-specific structures, which synthesized enamel proteins (Fig. 2; Kollar and Baird, 1970; Mina and Kollar, 1987; Lumsden, 1988). Epithelial-mesenchymal interactions are now considered to constitute the single most important mechanism regulating organ development in vertebrates (Gurdon, 1992). Studies during the last decade have indicated numerous molecules whose expression is regulated by tissue interactions (see below). Today it is possible to analyze the molecular basis of the organ- and tissue-specificity and of the commitment of cell groups and to identify the molecules involved in the transmission of epithelial-mesenchymal signalling.

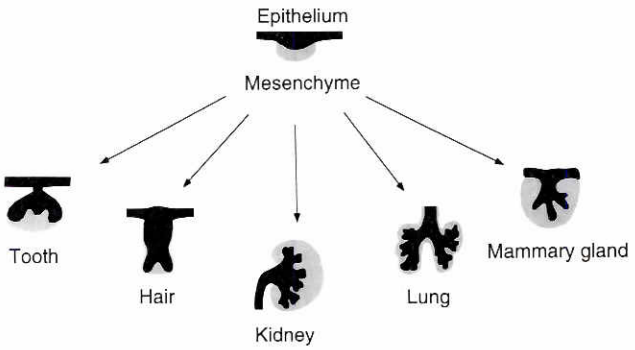


Fig. 1. Most organs are formed from epithelial and mesenchymal tissues, and during their early development they share common morphological features. *Mesenchymal cells form condensations and the epithelia undergo organotypic morphogenesis. Epithelial-mesenchymal interactions constitute a central mechanism of morphogenetic regulation.*

Condensation of organ-specific mesenchymal cells

The condensation, or aggregation, of mesenchymal cells has long been recognized as a central feature in morphogenesis. Mesenchymal cell condensates are seen during somite formation, during cartilage and bone development, and next to organ-specific epithelium during the early stages of development of practically every organ (Fig. 1). The fate of the condensed cells is different among different tissues. In chondrogenic and osteogenic condensates the cells in the central part differentiate into chondroblasts and osteoblasts, respectively. Defects in osteogenic condensations have been associated with abnormal sizes and shapes of bones (Kingsley, 1994). In many organs the mesenchymal cells remain as stromal cells which support epithelial morphogenesis and differentiation. The mesenchymal cell condensates in the developing teeth most closely resemble those in derivatives of the skin (feathers, scales, hairs, and vibrissae) in which the mesenchymal cells form papillary structures surrounded by epithelial cells (Sengel, 1986; Hardy, 1992; Panaretto, 1993).

The aggregation of cells has been proposed to result from changes in the adhesiveness of the cells, cell migration, and/or increased proliferation, but the actual mechanisms are not known, and they may involve combinations of the above features. It is not known to what extent the mechanisms of condensation are shared in the various organ systems; clearly there are similarities. The condensed cells usually acquire a shape that differs from the surrounding mesenchymal cells. Cell morphology is partly regulated by binding of cells via matrix receptors to extracellular matrix (ECM), and there are many ECM molecules and cell surface receptors, the expression of which is upregulated in the mesenchymal cells in several different organ-specific condensates (see below).

Epithelial morphogenesis

Although the exact patterns of epithelial morphogenesis are characteristic of individual organs (Fig. 1), common features are that the epithelial cells are actively proliferating and invade the surrounding mesenchyme and that interactions between the two tissues regulate epithelial behavior (Wessells, 1977; Sengel, 1986; Ruch, 1987). Tissue recombination studies in which epithelium and mesenchyme from different organs were cultured together indicated that in many organs the pattern of epithelial branching is regulated by mesenchymal tissue (Lawson, 1974; Sakakura *et al.*, 1976). A particular example of this is the hormone-dependent morphogenesis of the mammary gland and the urogenital tract epithelium. In these cases the mesenchymal tissue is the target of hormones and mediates the effects of hormones upon the epithelium (Kratochwil, 1977; Cunha, 1994). In the tooth the epithelial morphogenesis, which determines the form of the tooth, is also regulated by the dental mesenchyme (Kollar and Baird, 1970).

Rapid growth and morphogenesis of the epithelium obviously cannot take place without extensive remodelling of the ECM at the epithelial-mesenchymal interface (Saxén *et al.*, 1982; Adams and Watt, 1993). This ECM is composed of the basement membrane and underlying mesenchymal stroma, and its importance in epithelial morphogenesis was demonstrated as early as 30 years ago by *in vitro* experiments where the deposition of various matrix components was interfered with by enzymes or chemicals. This approach has shown that glycosaminoglycans and collagens are important for epithelial morphogenesis e.g. in salivary gland, pancreas, and lung (Grobstein and Cohen, 1965; Wessells and Cohen, 1968; Bernfield and Banerjee, 1982) as well as in tooth (Hetem *et al.*, 1975; Hurmerinta *et al.*, 1979; Thesleff and Pratt, 1980).

Molecular mechanisms of organ development

Transcription factors in the initiation and early morphogenesis of organs

Traditionally, organ development has been divided into phases of *initiation, morphogenesis,* and *differentiation.* Initiation begins before the organ anlage is morphologically visible. At present, little is known about the molecular basis of organ initiation, although it is conceivable that unique combinations of morphogens and transcription factors, which are involved in the establishment of the primary body plan, may also regulate the patterning in early organ development.

Transcription factors are DNA-binding proteins that control the activity of other genes. There are several groups of transcription factors containing conserved DNA sequences, such as homeoboxes, paired boxes, and zinc finger encoding motifs. The *Hox* cluster genes are involved in the regulation of anteroposterior patterning and establishment of positional information during embryonic axis forma-

Stage	tooth epithelium + other mesenchyme	other epithelium + tooth mesenchyme
	+	—
	—	+

Fig. 2. Summary of results from tissue recombination experiments using heterotypic recombinations of dental and non-dental epithelium and mesenchyme. *This illustrates the reciprocal and sequential nature of epithelial-mesenchymal interactions (Mina and Kollar, 1987; Lumsden, 1988). When tissues are dissected from early tooth germs (prior to the bud stage, E9-E10 mouse embryos), the dental epithelium instructs tooth development when cultured with non-dental neural crest-derived mesenchyme. After the bud stage (E12 onwards), dental mesenchyme governs tooth development when cultured with non-dental epithelium; i.e. the potential to direct tooth development has shifted to the mesenchyme.*

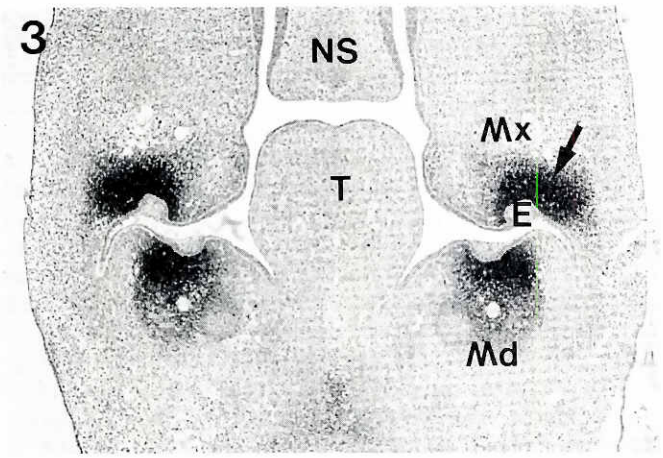


Fig. 3. *In situ* hybridization analysis of the expression of the homeobox-containing transcription factor *Msx-1* during early tooth development. *A frontal section through the molar tooth germs of an E12 mouse embryo. Intense expression (arrow) is restricted to the presumptive dental mesenchymal cells around the forming epithelial tooth buds (E). T, tongue, NS, nasal septum, Mx, maxilla, Md, mandible.*

tion (McGinnis and Krumlauf, 1992), but they may not encode individual structures (Slack *et al.*, 1993). It is probable that the next levels of patterning, including establishment of positional information for organ development and specification of tissue identities, are regulated by other homeobox-containing genes and other specific transcription factors. The elucidation of the *Hox* codes in the branchial arches (Hunt and Krumlauf, 1991; Thorogood, 1993) and the lineage studies of the migrating cranial neural crest cells (Fraser *et al.*, 1990; Prince and Lumsden, 1994) can be expected to lead to a better understanding of the initiation of tooth development as well as that of the other organs in the branchial arches.

Some transcription factors, including *Dlx-1, Dlx-2* (Dollé *et al.*, 1992; Sharpe, 1995; Thomas *et al.*, 1995), and *LEF-1* (Oosterwegel *et al.*, 1993), are expressed in the thickened presumptive dental epithelium - the first morphological sign of tooth development — suggesting that they may be downstream-target genes for the morphogens and homeobox genes determining the sites of tooth initiation. Interestingly, *LEF-1* was recently shown to be a necessary regulatory molecule for tooth development, as in the *LEF-1* knockout mouse mutant, teeth were missing (van Genderen *et al.*, 1994). In these mice hair development was also deficient, and vibrissae as well as mammary glands were missing. These organs are all initiated from the surface ectoderm, their development share similar features, and epithelial-mesenchymal interactions govern their morphogenesis. Hence, although the target genes of *LEF-1* in these organs are not known, it is conceivable that *LEF-1* has a similar developmental regulatory function in all affected organs.

The homeobox-containing genes *Msx-1* and *Msx-2* are expressed in the early tooth rudiment, and their distributions suggest patterning functions during early tooth morphogenesis (Fig. 3; MacKenzie *et al.*, 1991, 1992). The significance of the *Msx-1* gene for tooth development was recently demonstrated in transgenic mice that lack a functional *Msx-1* gene (Satokata and Maas, 1994). These knockout mice had cleft palate, and their teeth did not develop beyond the bud stage.

Although most transcription factors studied so far are expressed in several developing organs, evidence from experiments with transgenic mice suggests that in some cases their developmental regulatory functions are restricted to only one or a few organs. For

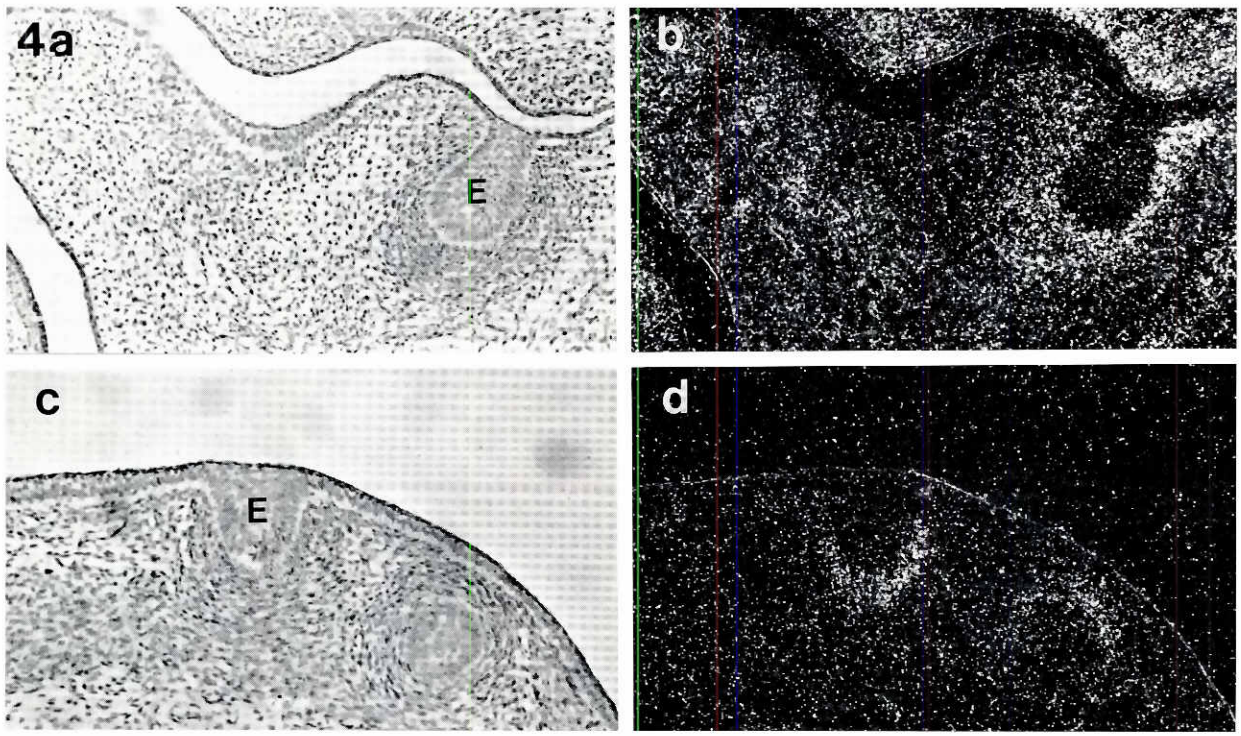


Fig. 4. *In situ* hybridization analysis of expression of the growth factor *BMP-4* in the developing tooth (a and b) and vibrissa (c and d) of a E13 mouse embryo. *In both organs, expression is intense in the mesenchymal cells surrounding the epithelial buds (E).* **b** *and* **d** *represent dark field illumination of the sections in* **a** *and* **c**.

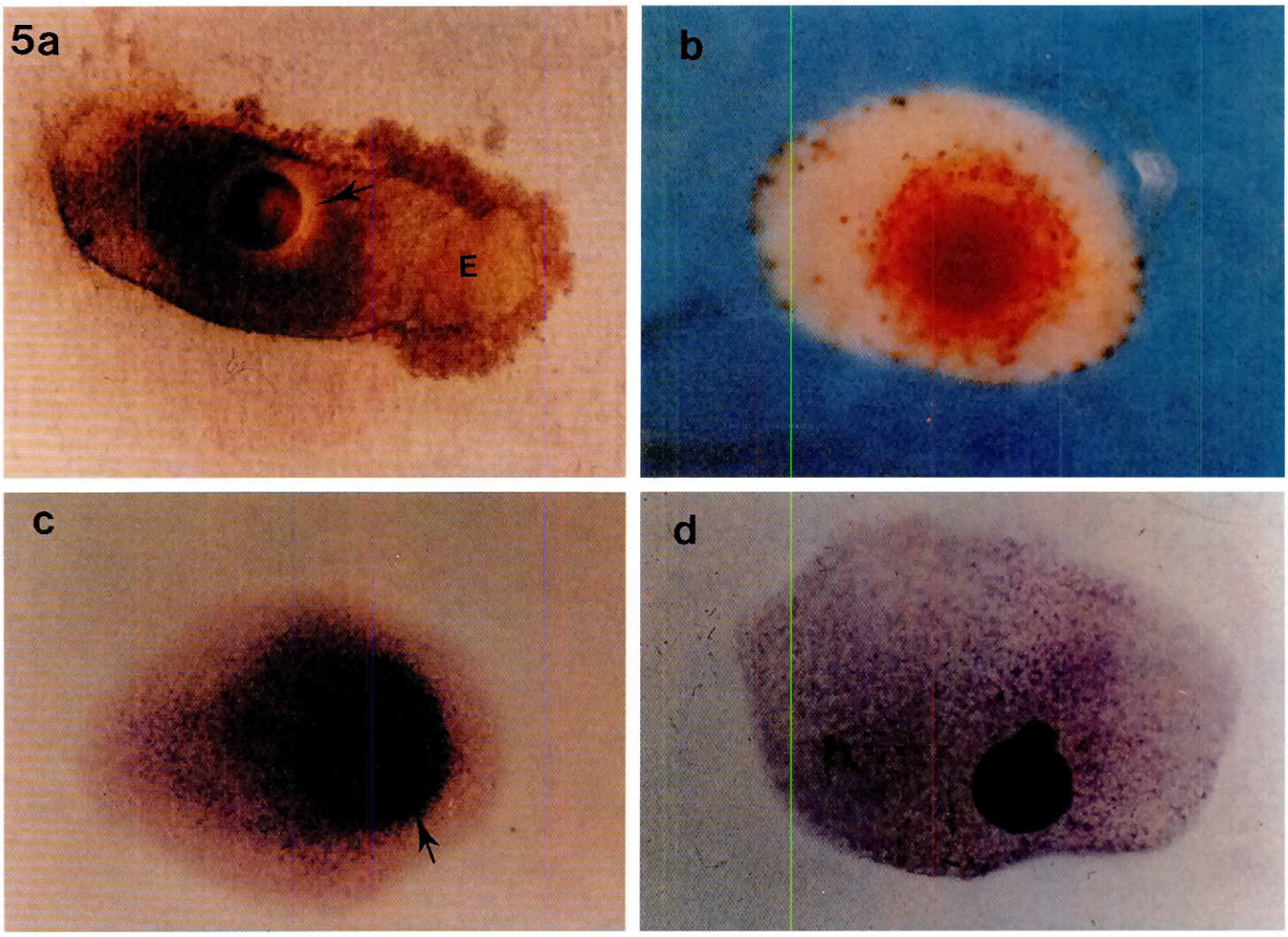


Fig. 5. Analysis on the effects of growth factors on dental mesenchymal tissue *in vitro*. *Agarose beads which were soaked in BMP-2 or FGF-4 protein and cultured in contact with dental mesenchyme for 24 h had similar effects as dental epithelium.* **(a)** *The beads (in this case FGF-4) induced a translucent zone which appears morphologically similar to that induced by the dental epithelium (arrow).* **(b)** *FGF-4 has stimulated mesenchymal cell proliferation (BrdU-incorporating cells are visualized by immunohistological staining).* **(c)** *BMP has induced the expression of the transcription factor Msx-1 (arrow, whole-mount in situ hybridization).* **(d)** *Control* **(c)** *hybridized with the sense probe. (Vainio et al., 1993; Jernvall et al., 1994). E, epithelium.*

example, the inactivation of the *Msx-1* gene caused failure of tooth and palate development, but no malformations were seen in limbs where the gene is also intensely expressed (Davidson and Hill, 1991; Satokata and Maas, 1994). Another example is the homeobox gene *Hox-11*, which is widely expressed in the embryo, including the craniofacial region (Raju *et al.*, 1993). However, in the *Hox-11* knockout transgenic mice, abnormalities were seen only in the spleen, which fails to develop (Roberts *et al.*, 1994). The significance of the expression of *Msx-1* and *Hox-11* in the organs which were unaffected in the knockout mice is not known, but there may either be superfluous expression of the proteins or RNAs, or functional redundancy with related molecules (Erickson, 1993).

Other examples of organ-specific transcription factors are *Pax-* genes, which contain a paired box. Mutations in *Pax-2* cause kidney abnormalities (Dressler *et al.*, 1993), and a mutation in *Pax-3* is the cause of Waardenburg syndrome, a condition where neural crest cells are affected (Tassabehji *et al.*, 1992). In turn, the Wilm's tumor gene *(WT1)* is a zinc finger transcription factor which regulates the development of the kidneys and gonads (Hastie, 1993).

The downstream target genes of the transcriptional regulators of development have so far been poorly characterized. However, there is evidence of regulatory loops between homeobox genes and potential morphogens such as retinoic acid and some growth factors (Tabin, 1991; Conlon and Rossant, 1992; Morriss-Kay, 1993; Thüringer and Bienz, 1993). In developing organs, it is often seen that the expression of growth factors and their receptors is not restricted to nor associated with a specific cell lineage. Rather, their expression has been associated with sites where the development of form is regulated by differential cell proliferation or cell adhesion, or with sites of epithelial branching (see below). This suggests that growth factors and their receptors may be targets of transcription factors regulating pattern and form of organs.

Growth factors as signals mediating epithelial-mesenchymal interactions

Roles of growth factors as inductive signals in vertebrate embryogenesis were first established in studies on mesoderm formation in *Xenopus* embryos. Members of the FGF (fibroblast

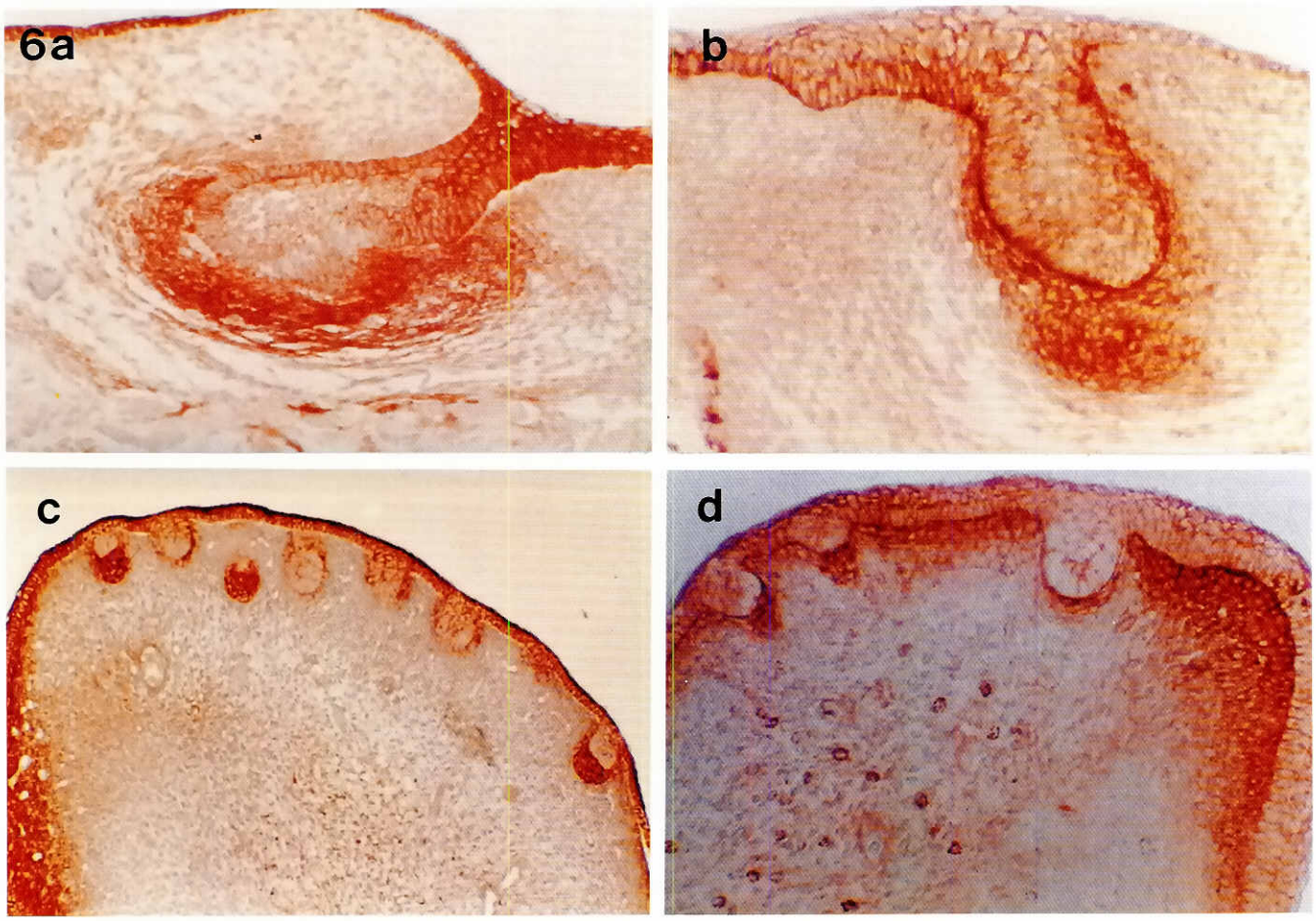


Fig. 6. Localization of the cell surface heparan sulfate proteoglycan syndecan-1 (a,c) and the heparin binding growth factor-like molecule midkine (MK, b,d) during tooth (a,b) and skin development (c,d). *Both molecules are present on the surfaces of epithelial cells and very intensely in the condensed mesenchyme surrounding tooth and vibrissa epithelium (immunohistological localization) (Mitsiadis et al., 1995b,c).*

growth factor) and TGFß (transforming growth factor ß) families were localized at the right times and places; they mimicked the effects of inductive tissues; and their roles as inductive signals were confirmed by inhibition experiments by using dominant negative mutations of growth factor receptors (Slack, 1994). During recent years, evidence has accumulated for similar roles of growth factors in organ development, and it is now believed that the same growth factors act as inductive signals in different morphogenetic tissue interactions (Jessell and Melton, 1992; Vainio *et al.*, 1993; Francis *et al.*, 1994; Thesleff *et al.*, 1995). It is noteworthy that many of these growth factors are homologous to those signalling between tissue layers in the developing *Drosophila* embryo, indicating evolutionary conservation in molecular mechanisms of morphogenetic signalling (Thüringer and Bienz, 1993).

Circumstantial evidence that growth factors may signal in epithelial-mesenchymal interactions during organ development has come from descriptive studies on expression patterns of genes and proteins. For example, the expression of TGFß-1,-2 and -3 is associated with epithelial-mesenchymal interactions in many organs (Pelton *et al.*, 1991). In the tooth germ, *TGFß-1* expression is first seen in the epithelium at the early bud stage, and then the expression extends to the condensing mesenchyme, which supports a role in mediation of epithelial-mesenchymal interactions (Vaahtokari *et al.*, 1991).

Growth factors and their respective receptors, or growth factor mRNA and protein, have in some cases been localized in adjacent interacting tissues. Such observations have suggested that growth factors in the TGFß and PDGF (platelet-derived growth factor) families act in a paracrine way between epithelium and mesenchyme in developing organs, including salivary glands, hairs and teeth (Lehnert and Akhurst, 1988; Heine *et al.*, 1989; Vaahtokari *et al.*, 1991; Orr-Urtreger and Lonai, 1992). In addition, several tyrosine kinase receptors, which are predominantly expressed in epithelial cells in many organs have been characterized recently, and their respective ligands appear to be expressed in adjacent mesenchyme, suggesting signalling across the tissue layers (Birchmeier and Birchmeier, 1993). Of these, the *c-met* receptor is expressed in tooth epithelium, and its ligand, *SF/HGF* in dental mesenchyme (Sonnenberg *et al.*, 1993)

Bone morphogenetic proteins (BMPs) consist of a family of eight differentiation factors which belong to the TGFß superfamily. Most of them initiate bone induction in vertebrate tissues, but they have several other functions in developmental regulation across the animal kingdom (Reddi, 1992; Wozney, 1992). In particular, BMPs

act as early signals during mesoderm formation (Jones *et al.*, 1992), and in many organs, including the tooth, BMP expression has been associated with epithelial-mesenchymal interactions (Fig. 4; Lyons *et al.*, 1991; Vainio *et al.*, 1993; Heikinheimo, 1994). Overexpression of BMP-4 disturbs hair morphogenesis in transgenic mice (Blessing *et al.*, 1993), and BMP-2 acts instructively in patterning of limbs (Francis *et al.*, 1994). As the functional BMP molecules are dimers, it is possible that different BMPs act in concert, perhaps by forming heterodimers.

During early tooth morphogenesis, *BMP-4* transcripts are present in the thickened presumptive dental epithelium, and they shift to the condensing dental mesenchyme. This corresponds to transfer of the potential to induce tooth formation from epithelium to mesenchyme during bud stage (see above). *BMP-2*, on the other hand, is expressed in dental epithelium from the early bud stage until the cap stage, when it shifts to mesenchyme (Fig. 4; Vainio *et al.*, 1993; Thesleff *et al.*, 1995). Results from *in vitro* studies indicate that BMP-2 and/or BMP-4 function as epithelial signals regulating gene expression in dental mesenchyme. When agarose beads releasing BMP protein were placed on dental mesenchyme *in vitro*, their effects were similar to those of dental epithelium. Like the epithelium, the beads induced the appearance of a translucent zone in the surrounding mesenchyme as well as expression of the homeobox-containing genes *Msx-1* and *Msx-2* (Fig. 5; Vainio *et al.*, 1993).

Recently, the production of transgenic mice with defective growth factor functions have implicated developmental roles for some growth factors in organogenesis. Of particular interest are the roles of FGFs in epithelial morphogenesis (see below). From observations of the phenotypes of transgenic mice, it cannot be concluded whether growth factors function in inductive epithelial-mesenchymal signalling, or whether they signal between homotypic cells or have autocrine functions. There is, however, evidence from other types of experiments which indicates signalling functions for FGFs in tissue interactions during mesoderm formation as well as during the development of some organs. It appears that the FGFs may in some cases have synergistic effects with TGFß-family growth factors (Slack, 1994). Both TGFß-1 and FGF-2 are epithelial factors regulating mesenchymal chondrogenesis in the developing ear (Frenz *et al.*, 1994). FGF-4 and BMP-2 have synergistic effects on mesenchyme in the limb bud (Niswander and Martin, 1993a). Also in the tooth germ, the expression patterns of growth factors in the TGFß, BMP and FGF-families suggest synergistic functions.

In the embryonic tooth, *FGF-3* transcripts appear in the dental mesenchyme during the late bud stage and are intensely expressed in the dental papilla until the bell stage (Wilkinson *et al.*, 1989; Vaahtokari *et al.*, in preparation). Expression of *FGF-3* RNA is restricted to mesenchymal cells and is closely associated with cell proliferation, but it is not clear whether FGF-3 functions in an autocrine fashion in the dental mesenchyme or whether it signals to epithelium (Vaahtokari *et al.*, in preparation). *FGF-4* transcripts, on the other hand, are restricted to the epithelial enamel knot of teeth, and they appear to be associated with cuspal morphogenesis (Niswander and Martin, 1992; Jernvall *et al.*, 1994; see below). FGF-4 stimulates cell proliferation in dental epithelium and mesenchyme *in vitro* (Fig. 5), but as the *in vivo* distribution of the protein has not been reported, we do not know whether the epithelium and/or mesenchyme are the target tissues of FGF-4. In general, the patterns of expression of both *FGF-3* and *FGF-4* in the embryo are very restricted and transient in several developing, apparently unrelated organs (Wilkinson *et al.*, 1989; Niswander and Martin, 1992). This may indicate that the sites of morphogenetic functions of these FGFs are determined rather by the expression of the ligands than by the expression of their receptors, which have been detected in all organs studied (Orr-Urtreger *et al.*, 1991). Although no obvious dental defects have been reported in the homozygous *FGF-3* knockout mice, a more detailed analysis might reveal minor developmental abnormalities (Mansour *et al.*, 1993).

Members of the Wnt family of signalling molecules have recently been implicated in inductive signalling. They are involved in patterning of the mesoderm and central nervous system as well as in the development of kidneys (Parr and McMahon, 1994; Stark *et al.*, 1994). At present, there is no information about the expression of Wnts in teeth. Sonic hedgehog (shh or vertebrate hh, vhh) is another signalling molecule that participates in the molecular cascade in patterning of the limb and nervous system (Echelard *et*

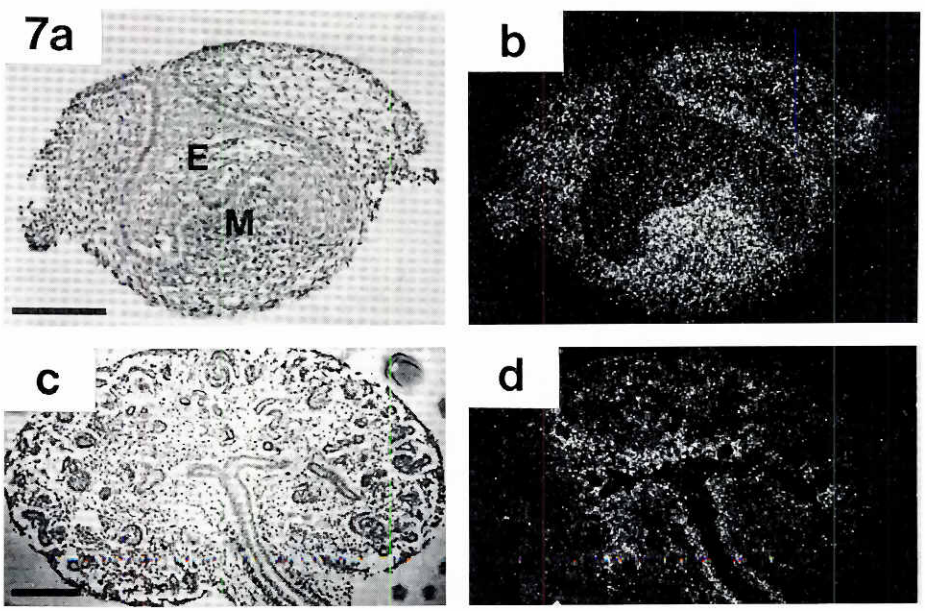
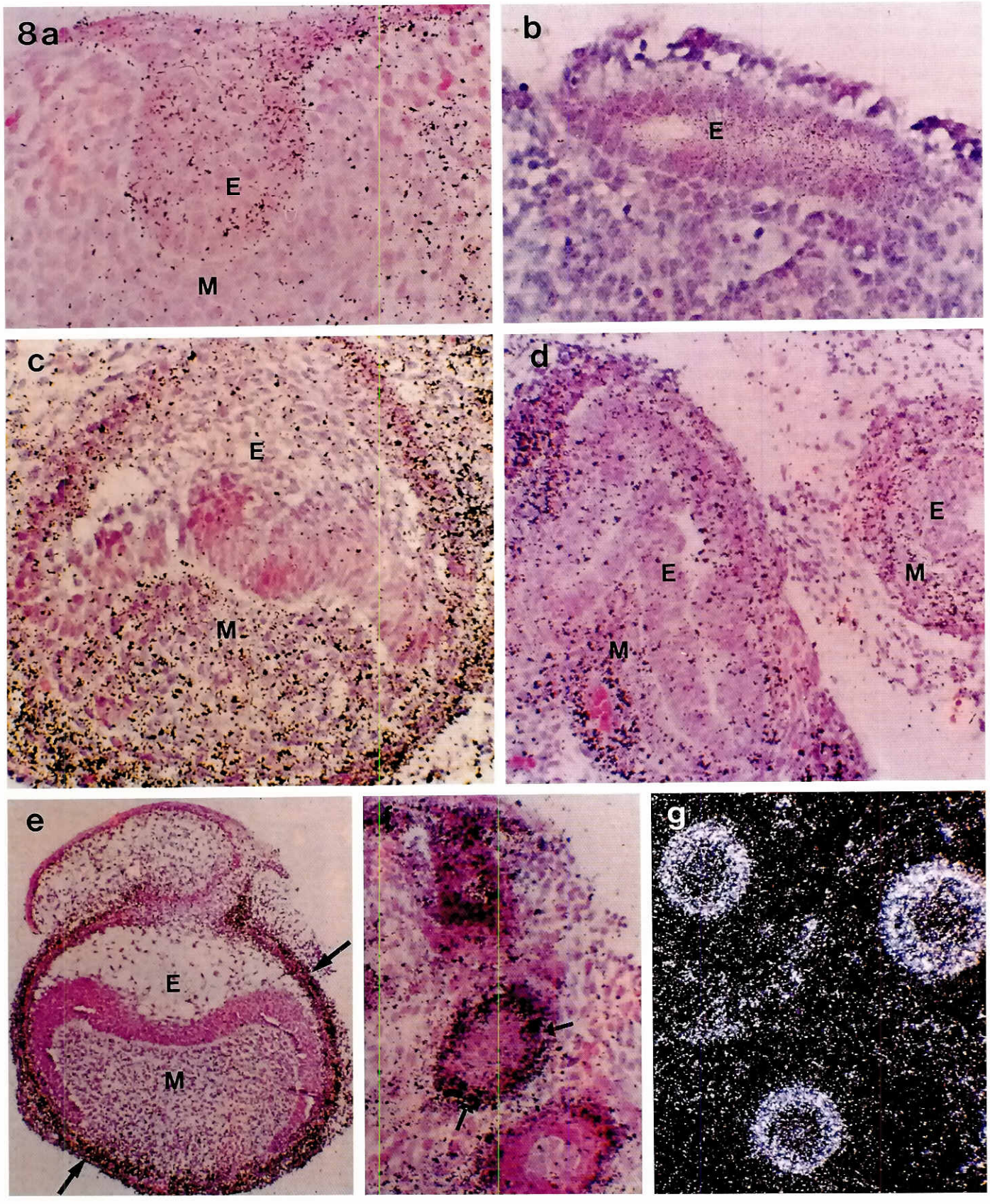


Fig. 7. *In situ* hybridization analysis of the expression of *gelatinase A*, the 72 kDa type IV collagenase in a E14 mouse embryo in a cap-staged tooth germ (a,b) and in the developing kidney (c,d). *Intense expression is seen in mesenchymal tissue whereas the epithelia are negative. No particular association is seen with epithelial morphogenesis. E, epithelium, M, mesenchyme (Reponen et al., 1992).*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al., 1993; Riddle *et al.*, 1993). Shh is associated with BMP-signalling, but its possible role in tooth development has not yet been analyzed.

Cell and tissue responses to the inductive signals transmitted between epithelium and mesenchyme differ greatly depending on organs and developmental stages. As discussed above, epithelial-mesenchymal interactions govern both cell fate and morphogenesis, which are tightly coupled. Hence, the signals conceivably regulate a variety of genes: those involved in the determination of cell fate, others that have an effect on growth, as well as genes which encode structural components of the cells and ECM and thus contribute to changes in tissue structure. During the early stages of organ development, the inductive signals may induce the expression of master regulatory genes which initiate organ-specific cascades of regulatory events.These interactions would belong to the category of instructive epithelial-mesenchymal interactions. Signals during more advanced organogenesis may, in turn, mediate permissive interactions and regulate genes affecting the functions of already committed cells (Fig. 10).

There is evidence, particularly concerning *Drosophila* development, for gene-regulatory loops between growth factors and transcription factors which play roles in positional signalling and pattern formation as well as in the determination of cell fate (Panganiban *et al.*, 1990, Thüringer and Bienz, 1993). Also in vertebrate organogenesis, growth factors regulate the expression of transcription factors. Epithelial FGF-4 induces the expression of the homeobox gene *Evx-1* in the limb mesenchyme (Niswander and Martin,1993b), and as already discussed, BMP-2 and BMP-4 regulate the expression of *Msx-1* and *Msx-2* in the dental mesenchyme *in vitro* (Fig. 5; Vainio *et al.*, 1993). During more advanced tooth development, when growth factors of the TGFß family trigger the terminal differentiation of odontoblasts (Bègue-Kirn *et al.*, 1992), they may regulate the deposition of cell-specific extracellular matrix by the already committed mesenchymal cells.

Molecular changes in the condensing mesenchyme

The first molecules that were specifically localized in several organ-specific mesenchymal condensates were tenascin and syndecan-1. Tenascin is a large glycoprotein of the ECM, and it interacts with cells and other matrix molecules (Erickson, 1993). It was initially localized in the mesenchyme of developing teeth, mammary glands, and vibrissae as well as in the stroma of some tumors (Chiquet-Ehrismann *et al.*, 1986). Later, tenascin was found in the organ-specific mesenchyme of the gut (Aufderheide and Ekblom, 1988) and developing hair follicles (Tucker, 1991; Jiang and Chuong, 1992). Experimental studies of dental tissues and other organs have shown that tenascin expression in the mesenchyme is regulated by epithelial signals (Aufderheide and Ekblom, 1988; Vainio *et al.*, 1989a).

Syndecan-1 is a cell-surface heparan sulfate proteoglycan which acts as a receptor for several matrix molecules (Bernfield *et al.*, 1992). It also binds growth factors, particularly FGF, and may be required for binding of FGF to its receptor (Rapraeger *et al.*, 1991). However, the observation that overexpression of syndecan-1 in epithelial cells renders them unresponsive to FGF has led to the speculation that syndecan-1 may regulate cell proliferation negatively (Mali *et al.*, 1993). The developing tooth was the first organ where syndecan-1 was localized in mesenchymal tissue and where it was shown to be regulated by epithelial signals (Fig. 6; Thesleff *et al.*, 1987; Vainio *et al.*, 1989a). Subsequently, syndecan-1 has been found in many organ-specific mesenchymal cells including kidney, lung, and vibrissae, and it appears that its expression in these organs is also controlled by epithelial-mesenchymal interactions (Fig. 6; Vainio *et al.*, 1989b; Trautman *et al.*, 1991; Mitsiadis *et al.*, 1995c).

Syndecan-1 isolated from dental mesenchyme binds to tenascin, suggesting that interactions between the two molecules may play a role in the condensation of the mesenchymal cells in the tooth germ (Salmivirta *et al.*, 1991) as well as in vibrissae, where the two molecules are co-expressed (Panaretto, 1993). Because similar co-expression is not seen in all organs, other explanations for cell condensation in these organs are needed. There is some controversy about the functions of tenascin in cell attachment and cell adhesion, and there is evidence that in some circumstances tenascin has antiadhesive properties (Erickson, 1993). It is also noticeable that transgenic mice lacking a functional tenascin gene do not have visible defects in the development of teeth or other organs (Saga *et al.*, 1992), indicating that either tenascin is not required for organogenesis or that other related molecules may substitute for it.

Midkine (MK) and pleiotropin (HB-GAM) are heparin-binding molecules, perhaps growth factors, the functions of which are presently unknown (Rauvala, 1989; Muramatsu, 1994). These molecules are induced by retinoic acid, and they have recently been associated with mesenchymal cell condensation in many organs including teeth and vibrissae (Fig. 6; Mitsiadis *et al.*, 1995b,c). In addition to mesenchyme, MK and HB-GAM are present in the epithelia of these organs, and their expression appears to correlate with epithelial-mesenchymal interactions. In many organs they are codistributed with syndecan-1, which may point to a possible role for syndecan-1 as a receptor for these molecules (Mitsiadis *et al.*,1995c). In fact, another member of the gene family, N-syndecan, was recently shown to act as a receptor for HB-GAM in nervous tissue (Raulo *et al.*, 1994).

In the condensing dental mesenchyme many other molecules, including *TGFß1* and *BMP-4*, are upregulated (Vaahtokari *et al.*, 1991; Vainio *et al.*, 1993). Whether the expression of these growth factors implies regulation in an autocrine way in the condensing cells or paracrine signalling to the epithelium is at present not known. There is evidence that BMPs play important roles in the mesenchymal cell condensates of developing bones (Kingsley, 1994): in the short ear mouse mutation, where many bones are absent or abnormal in shape and there is a deficiency in the early condensates, the mutation is in the *BMP-5* gene (Kingsley *et al.*, 1992). A mutation in *GDF-5* (growth and differentiation factor-5), a gene belonging to a *TGFß* subfamily closely related to *BMPs*, was shown to be the cause of brachymorphism in mice, and it was demonstrated that *GDF-5* was expressed in the osteogenic condensates of the affected long bones

Fig. 8. Localization of EGF receptors in different mouse organs by autoradiography of ^{125}I-EGF binding (a-f) and by *in situ* hybridization analysis (g). **(a)** *In the bud-staged tooth germ and* **(b)** *E11 kidney EGF binding is seen in epithelium.* **(c)** *In the cap-staged tooth EGF binding is detected also in mesenchymal cells, and in the epithelium binding is mainly localized in the outer dental epithelium.* **(d)** *In the E14 lung, intense binding is seen in mesenchymal cells facing the epithelia.* **(e)** *In bell-staged tooth, binding is intense in outer dental epithelium and mesenchymal dental sac cells surrounding the tooth germ (arrows).* **(f)** *In hair follicles (E16) outer epithelial root sheath and surrounding mesenchymal cells bind EGF (arrows).* **(g)** *EGF receptor mRNA localization in hair follicles indicates similar distribution as EGF binding* **(f)**. *E, epithelium; M, mesenchyme (Partanen and Thesleff, 1987).*

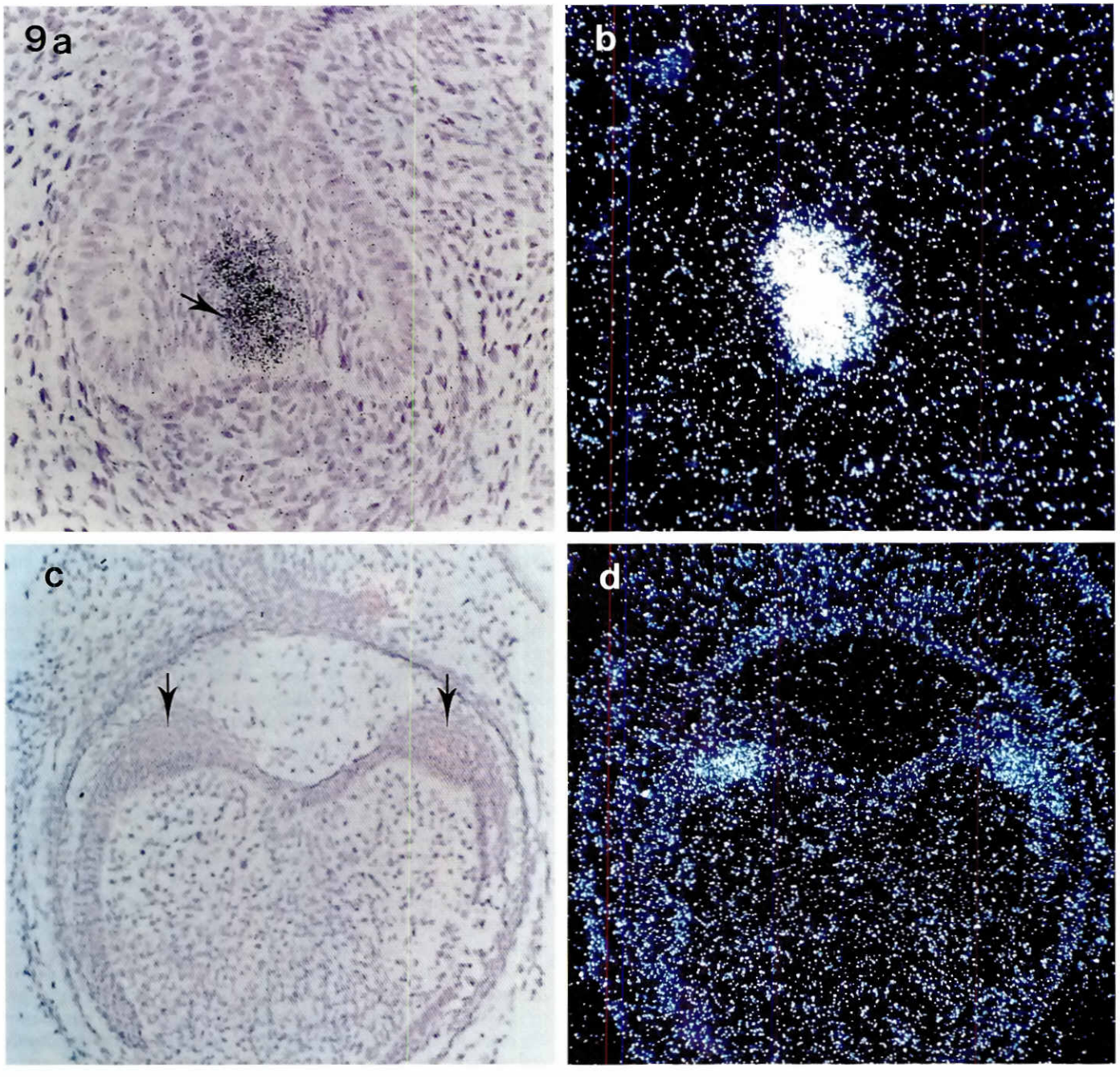

Fig. 9. *In situ* hybridization analysis of *FGF-4* expression during the cap-stage (a and b) and bell stage of tooth morphogenesis (c and d). *Intense expression is restricted to the epithelial enamel knots (arrows) which participate in the regulation of cuspal morphogenesis in teeth (Niswander and Martin, 1992; Jernvall et al., 1994).*

(Storm *et al.*, 1994). The functions of BMPs in the mesenchymal cell aggregates are not known. BMPs did not affect cell proliferation in dental mesenchyme (Vainio *et al.*, 1993), and they rather decreased than stimulated cell proliferation in the limb bud mesenchyme (Niswander and Martin, 1993a). One possibility is that BMPs affect the adhesiveness of cells by regulating cell surface-associated molecules. This may occur via homeobox-containing transcription factors, as in the early dental mesenchyme, where BMP-2 and BMP-4 stimulate the expression of *Msx-1* and *Msx-2 in vitro* (Vainio *et al.*, 1993). *Msx-1* and *Msx-2* are also intensely expressed in the condensing dental mesenchymal cells *in vivo* (Fig. 3; MacKenzie *et al.*, 1991, 1992), and they are regulated by the dental epithelium (Jowett *et al.*, 1993). As mentioned above, a functioning *Msx-1* gene is absolutely necessary for tooth morphogenesis (Satokata and Maas, 1994).

Roles of ECM molecules and growth factors in epithelial morphogenesis

The budding, folding, and branching of epithelial tissue is associated with extensive remodelling of the ECM, in particular the

basement membrane, at the epithelial-mesenchymal interface. The basement membranes in various organs have been considered to be similarly composed; they include type IV collagen, laminin, nidogen (entactin) and various proteoglycans. These molecules have also been localized by immunohistochemistry in developing teeth (Thesleff *et al.*, 1981), where the basement membrane regulates differentiation of underlying dental mesenchymal cells into odontoblasts (reviewed by Ruch, 1987). The molecular basis of interaction between the dental basement membrane and preodontoblasts has been analyzed in detail (Ruch, 1987; Lesot *et al.*, 1990).

Recently it has become apparent that there is genetic diversity between molecules in different basement membranes. Several different laminin and type IV collagen genes have been identified, and their functions are being elucidated (Rohrbach and Timpl, 1993; Yurchenco and O'Rear, 1994). For epithelial morphogenesis of kidney, binding of laminin to epithelial cells was shown to be necessary (Klein *et al.*, 1988). Furthermore, the integrity of the basement membrane is a prerequisite for tooth morphogenesis as indicated by functional *in vitro* studies (Hurmerinta *et al.*, 1979; Thesleff and Pratt, 1980; Ruch, 1987).

Although basement membranes are predominantly of epithelial origin, mesenchymal tissue also contributes to their formation (Bernfield and Banerjee, 1982). In developing kidney and lung, nidogen in the basement membrane is synthesized by the mesenchyme, and antibodies against the nidogen binding site in laminin B2 chain perturb branching of epithelium in both organs *in vitro*. Based on these functional studies it was suggested that nidogen is a mesenchymal ECM molecule which regulates early epithelial morphogenesis (Ekblom *et al.*, 1994). Whether similar molecular interactions regulate morphogenesis in the tooth is not known.

Matrix-degrading metalloproteinases have important functions in the regulation of the integrity of the ECM (Matrisian, 1992). Evidence for morphoregulatory roles of metalloproteinases was recently provided by the observation that targeted expression of the metalloproteinase stromelysin-1 in developing mammary glands dramatically altered epithelial morphogenesis (Sympson *et al.*, 1994); supernumerary branches developed in primary ducts and alveoli developed precociously. Laminin and type IV collagen were degraded in lactating glands which resulted in loss of basement membrane integrity and alteration of alveolar morphology.

Another metalloproteinase which cleaves type IV collagen, *gelatinase A* (72 kDa type IV collagenase), is widely expressed in embryonic mesenchyme but no preferential distribution was observed in association with epithelial morphogenesis in any of the organs studied, including the tooth (Fig. 7; Reponen *et al.*, 1992). Increased accumulation of transcripts was seen transiently only after the completion of cuspal morphogenesis in odontoblasts, and this corresponded to final degradation of the dental basement membrane (Sahlberg *et al.*, 1992). Because enzymatic activity of metalloproteinases is significantly regulated by their inhibitors in developing organs (Talhouk *et al.*, 1992), it is possible that gelatinase A affects basement membrane remodelling during organogenesis, although no changes in its expression were detected.

Growth factors, particularly members of the EGF- and FGF-families have been implicated in epithelial morphogenesis. The patterns of epithelial branching and folding are associated with spatiotemporal differences in the mitotic rates of epithelial cells, and, as discussed earlier, epithelial morphogenesis depends on mesenchymal tissue. Hence, the effects of growth factors on epithelial morphogenesis are presumably at least partly related to signalling between the interacting tissues. Localization of binding sites for epidermal growth factor (EGF) has indicated correlations between epithelial morphogenesis in several organs, including salivary gland, lung, kidney, and tooth (Fig. 8; Partanen and Thesleff, 1987). A direct effect of EGF on epithelium was suggested by results of organ culture studies where isolated epithelia of embryonic submandibular glands and ureter buds underwent epithelial branching morphogenesis in the presence of EGF and proper matrix molecules (Nogawa and Takahashi, 1991; Perantoni *et al.*, 1991). On the other hand, the location of EGF binding in the mesenchyme of the cap-staged tooth and branching lung suggests that the stimulatory effect of EGF may be mediated by epithelial-mesenchymal interactions. In organ cultures of cap-stage teeth, exogenous EGF stimulated proliferation of dental epithelium, but inhibited proliferation of dental mesenchyme (Partanen *et al.*, 1985). The binding pattern of EGF in bell-stage teeth is analogous to that in hair follicles (Fig. 8). Abundant binding is evident in the outer epithelium (outer enamel epithelium/outer epithelial root sheath) and in the mesenchymal cells surrounding the epithelia (dental follicle/connective tissue sheath). Taken together, these results suggest that regulation of cell proliferation via the EGF receptor pathway is a common feature of organ morphogenesis.

We have not been able to detect *EGF* expression in tooth germs by *in situ* hybridization, although *EGF* mRNA has been detected by PCR in mouse embryonic mandibles (Kronmiller *et al.*, 1991a; Shum *et al.*, 1993). However, there is a discrepancy in the PCR results, since Kronmiller *et al.* detected expression at stages E9-E10 but not at E11-E17, when Shum *et al.* detected transcripts in increasing numbers. EGF has also been suggested to be necessary for tooth initiation because antisense oligonucleotides inhibited tooth development *in vitro* (Kronmiller *et al.*, 1991b).

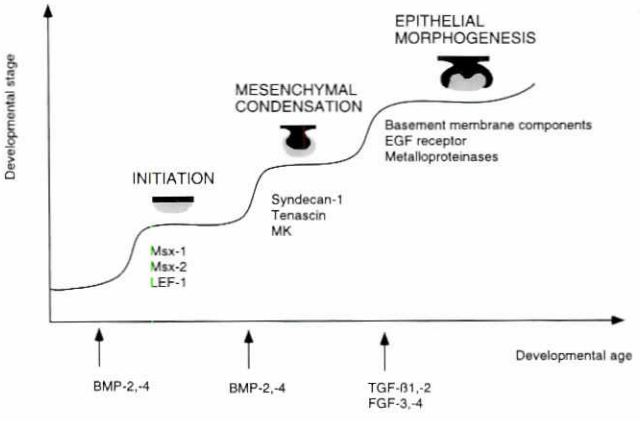

Fig. 10. Scheme of advancing tooth morphogenesis and some potential regulatory molecules. *Growth factors that may be involved in transmitting the sequential and reciprocal interactions between the epithelial and mesenchymal tissues are shown below the x-axis. Transcription factors, induced during the initiation of tooth development are thought to be master genes which regulate the expression of structural as well as regulatory genes directing subsequent morphogenesis. Condensation of mesenchymal cells involves interactions between molecules of the cell surface and the extracellular matrix. Remodelling of the ECM as well as differential epithelial growth, regulated by proteolytic enzymes and growth factors and their receptors, respectively, are central features of epithelial morphogenesis. All molecules that are indicated here are expressed in other developing organs as well.*

TGFα is closely related to EGF and it binds to the same receptor; it is expressed in tumors and, at lower levels, during embryogenesis (Derynck, 1992). TGFα has been recently identified as a regulator of hair morphogenesis, since transgenic mice with a non-functional TGFα gene had curly whiskers and wavy hair, accompanied by abnormalities in hair follicles (Luetteke *et al.*, 1993; Mann *et al.*, 1993). Interestingly, the mouse mutation waved-2, which has a similar phenotype as that of TGFα deficient mice is a mutant allele of the EGF-receptor (Luetteke *et al.*, 1994). So far, we have not been able to detect *TGFα* expression in developing teeth (unpublished observations). There are actually several other ligands for the EGF receptor which may regulate epithelial morphogenesis in the tooth, and the EGF-like domains in many ECM proteins can potentially bind to EGF receptors. Complexity to the EGF-receptor signalling is added by the fact that ligands like EGF and TGFα, as well as several other molecules in the EGF family, are synthesized as large, bioactive integral membrane proteins.

FGFs have been recently shown to regulate lung and hair morphogenesis. Targeted expression of a dominant negative FGF-receptor caused inhibition of lung morphogenesis in transgenic mice (Peters *et al.*, 1994), and mice in which the function of the FGF-5 gene was deficient, showed abnormal hair development (Hébert *et al.*, 1994). In developing tooth, FGF-4 has been associated with epithelial morphogenesis (Niswander and Martin, 1992; Jernvall *et al.*, 1994). Morphogenesis of the tooth cusps starts at the beginning of the cap stage. Appearance of the enamel knot, a cluster of non-dividing epithelial cells at the site of the future first cusp, has been associated with cuspal initiation in both incisors and molars (Butler, 1956). Careful 3-dimensional analysis showed that non-dividing enamel knot cells expressed *FGF-4* transcripts (Fig. 9). As FGF-4 protein was shown to stimulate proliferation of both epithelial and mesenchymal dental cells (Fig. 5), it was suggested that the enamel knot directs cuspal morphogenesis in the tooth by remaining non-proliferative itself and by concurrently stimulating proliferation of nearby cells (Jernvall *et al.*, 1994). In the developing limb, FGF-4 has an important growth and pattern regulatory role which may be analogous to its functions in tooth morphogenesis: epithelial FGF-4 stimulates mesenchymal cell division and regulates expression of homeobox-containing transcription factors in limb mesenchyme (Niswander and Martin, 1993b).

Concluding remarks

In almost all organs, morphology of early development has common features including condensations of mesenchymal cells and thickening, folding or branching of epithelial sheets (Fig. 1). As we have discussed, epithelial-mesenchymal interactions play a central role in the regulation of these events. Many cell adhesion molecules, ECM components and cell surface matrix receptors have been associated with morphogenesis, and they appear to play similar roles in different organs. Furthermore, there is evidence that same growth factors act as inductive signals in various tissue interactions. So far, no organ-specific molecules have been identified which would function in these cell-matrix interactions or in inductive signalling. However, it should be born in mind that there are thousands of genes which have not yet been cloned.

If most genes regulating morphogenesis are shared by organs, how is the creation of diversity between different organs possible? Although the genetic events that control the development of individual organs are not understood at present, it can be suggested that the specific patterning and morphogenesis of different organs is regulated by combinatorial gene programs, as has been shown for *Hox* genes in the development of axial skeletal structures (Condie and Capecchi, 1994). As a result, the response of individual cells to their microenvironment will depend on their specific lineage history, and it is determined largely by the arrays of transcription factors in their nuclei as well as receptor molecules in their cytoplasms and at the cell surfaces.

The capacity of a tissue to respond to inductive signals in a special way has been termed competence, and it is a central feature in all epithelial-mesenchymal interactions (Gurdon, 1992). A good example is determination of cells in the odontoblastic cell lineage: neural-crest-derived mesenchymal cells acquire increasing levels of specification during advancing morphogenesis, and, as a consequence, they are the only cells which respond to signals from the enamel epithelium by differentiating into odontoblasts (Thesleff *et al.*, 1990). On the other hand, in the epithelium of the developing tooth, the expression of enamel proteins can be detected as early as the cap stage when the cells appear morphologically quite undifferentiated (Couwenhoven and Snead, 1994). Hence, differentiation of cells is an advancing process which probably involves a series of cell fate decisions at specific developmental stages. *Egr-1 (Krox-24)* as well as members of the *Notch* gene family are transcription factors which have been associated with switches in differentiation programs in many organs, including the tooth (McMahon *et al.*, 1990; Karavanova *et al.*, 1992; Mitsiadis *et al.*, 1995c).

In Figure 10, some suggestive features of the molecular regulation of tooth morphogenesis are schematically presented. The acquisition of higher levels of development is regulated by a chain of inductive interactions between the epithelial and mesenchymal tissues. Changes in morphology of the organ is accompanied by changes in gene expression, and some molecules which have been discussed in this review are indicated. Growth factors that have been suggested to act as inductive signals are indicated below the x-axis. As has been discussed here, many aspects of this scheme can be applied to other developing organs. Early signals (in the tooth current evidence suggests that BMPs are involved) regulate expression of homeobox-containing genes and/or other transcription factors (in the tooth, *Msx-1* and *Msx-2* are regulated by BMPs). The morphogens and homeobox genes specify early patterning of the organ through regulation of molecules at the cell surfaces and in the extracellular matrix (in the tooth, syndecan-1 and tenascin may play roles). Changes in cell adhesion molecules and matrix remodelling contribute to organotypic condensations of mesenchymal cells and to epithelial morphogenesis. Since the signals are reciprocal, epithelial signals presumably control the expression of subsequent mesenchymal signals and vice versa (FGF-3 may be a mesenchymal signal in the tooth).

If same signalling and receptor molecules, transcription factors as well as cell adhesion and ECM molecules participate in the regulation of development in many different organs, an important implication is that defective functions of such molecules can be expected to lead to impaired development of several organs. Hence, they are potential candidate genes for congenital malformation syndromes in which defects are seen in several, seemingly unrelated organs. Examples of syndromes in which teeth are also affected are ectodermal dysplasia and cleidocranial dysplasia. In the former, the development is deficient in teeth as well as in other derivatives of the surface ectoderm, such as hair and sweat glands, and in the latter, supernumerary teeth and aberrant tooth morphogenesis are seen in association with abnormal bone devel-

opment. Identification of mutations causing such syndromes may lead to the discovery of new genes regulating the morphogenesis of both teeth and other affected organs.

Acknowledgments

We thank Professor Lauri Saxén for valuable suggestions during preparation of this manuscript. Our original research reviewed in this paper has been supported by the Academy of Finland.

Summary

Vertebrate organs develop from epithelial and mesenchymal tissues, and during their early development they share common morphological features. These include condensation of the mesenchymal cells and thickening, folding or branching of epithelial sheets. Sequential and reciprocal interactions between the epithelial and mesenchymal tissues play central roles in regulation of the morphogenesis of all organs. During recent years increasing amounts of molecular data have accumulated from studies describing developmental changes in expression patterns of molecules, as well as from functional *in vitro* studies and from the generation of transgenic mice. In this review article, we discuss common features in the molecular regulation that appear to be shared by the developing tooth and other organs. Several growth factors have been shown to act as inductive signals mediating epithelial-mesenchymal interactions in different organs. The early signals are proposed to regulate the expression of master regulatory genes, such as transcription factors. In early tooth germ, bone morphogenetic proteins BMP-2 and BMP-4 regulate expression of the homeobox containing genes *Msx-1* and *Msx-2*. These may specify early patterning of organs through regulation of molecules at the cell surface and the extracellular matrix, such as syndecan-1 and tenascin. Changes in cell adhesion and matrix remodelling, particularly in the organ-specific mesenchyme and in basement membrane contribute to formation of mesenchymal cell condensations and to epithelial morphogenesis. Several growth factors and their receptors, particularly in the TGFß-, FGF- and EGF- families, have been implicated in formation of mesenchymal condensates and in epithelial morphogenesis of many organs, including the tooth. It is apparent that molecules which regulate morphogenesis in different organs are potential candidate genes for congenital malformation syndromes in which several organs are affected.

KEY WORDS: *organ development, epithelial-mesenchymal interactions, mesenchymal condensation, differentiation, growth factors, odontogenesis, embryonic induction, extracellular matrix, basement membrane, homeobox genes.*

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