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DEVELOPMENTAL BIOLOGY

Developmental Biology 262 (2003) 195–205

www.elsevier.com/locate/ydbio

Review

Mechanisms of ectodermal organogenesis

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Received for publication 3 March 2003, revised 9 April 2003, accepted 28 May 2003

Abstract

All ectodermal organs, e.g. hair, teeth, and many exocrine glands, originate from two adjacent tissue layers: the epithelium and the mesenchyme. Similar sequential and reciprocal interactions between the epithelium and mesenchyme regulate the early steps of development in all ectodermal organs. Generally, the mesenchyme provides the first instructive signal, which is followed by the formation of the epithelial placode, an early signaling center. The placode buds into or out of the mesenchyme, and subsequent proliferation, cell movements, and differentiation of the epithelium and mesenchyme contribute to morphogenesis. The molecular signals regulating organogenesis, such as molecules in the FGF, TGFβ, Wnt, and hedgehog families, regulate the development of all ectodermal appendages repeatedly during advancing morphogenesis and differentiation. In addition, signaling by ectodysplasin, a recently identified member of the TNF family, and its receptor Edar is required for ectodermal organ development across vertebrate species. Here the current knowledge on the molecular regulation of the initiation, placode formation, and morphogenesis of ectodermal organs is discussed with emphasis on feathers, hair, and teeth.
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Keywords: Tooth; Hair; Feather; Mammary gland; Salivary gland; Lacrimal gland; Epithelium; Mesenchyme; Placode

Introduction

Hair, feathers, scales, teeth, beaks, nails, horns, and several eccrine glands (e.g., mammary, sweat, salivary, and lacrimal glands) are all derivatives of the ectoderm. These ectodermal organs diverge greatly from each other in shape and form (Fig. 1). Although most ectodermal organogenesis is initiated during the embryonic period, morphogenesis does continue postnatally. Tooth eruption generally occurs after birth, and the second dentition in humans develops during the first 20 years of life. Ectodermal organs also have an ability, limited though, for regeneration. The mammary gland goes through growth and differentiation during puberty and pregnancy, and this is repeated at each new pregnancy. Cyclical growth is seen in hair and feathers, where a new follicle develops from the older one. Some ectodermal organs grow continuously during adulthood, such as nails or the rodent incisor.

Despite the diversity in form and function, ectodermal organs share several common features in development. They originate from adjacent layers of epithelial (ectodermal) and mesenchymal (mesodermal or neural crest derived) tissues (Fig. 1). The first visible sign of development in most organs is the local thickening of the epithelial layer in order to make an ectodermal placode. A condensation of mesenchymal cells, a papilla, forms under the placode, which then buds into or out of the mesenchyme. Subsequent morphogenesis involves continued growth of the epithelial and mesenchymal components associated with folding and branching of the epithelium, and will then result in the final shape and size of the organ. The cellular mechanisms of ectodermal organ development have not been systematically investigated using comparable molecular markers, so, e.g., the relative contribution of proliferation and cellular migration to placode formation is not well understood (Balinsky, 1950; Wessells, 1965; Magerl et al., 2001). Growth of the epithelial bud into the mesenchyme is generally considered to be driven by proliferation of epithelial cells, though.

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doi:10.1016/S0012-1606(03)00325-7

Ectodermal organ development has been studied intensively during the last 50 years mainly using a small number of model systems such as feathers, hair, teeth, and mammary and salivary glands (reviews by Chuong, 1998; Millar, 2002; Thesleff and Mikkola, 2002a; Veltmaat et al., 2003). Tissue recombination studies have shown that organogenesis is directed by reciprocal and sequential interactions between the epithelium and mesenchyme. Also many endodermal organs, such as lung and pancreas, as well as mesodermal organs like kidney, share similar epithelial-mesenchymal interactions during early development (reviews by Hogan and Yingling, 1998, and Kuure et al., 2000). Signaling molecules belonging to the fibroblast growth factor (FGF), hedgehog (Hh), transforming growth factor β (TGFβ), Wnt, and tumor necrosis factor (TNF) families are involved repeatedly at different stages of ectodermal organogenesis, and the target genes they regulate are often the same in different ectodermal organs (Table 1). In this review we will concentrate on embryonic development of these organs, and describe current knowledge on the molecular regulation of the early stages of development.

Initiation of organs

The mesenchyme seems to supply the first signals directing organogenesis in most organs studied. The recombination of epithelium and mesenchyme between species or between different body regions has shown that the pattern and shape of organs appear to be regulated by the mesenchyme. For example, feather-forming dermis from chicken directs scale-forming epidermis from lizards to produce small protruding buds similar to feather follicles rather than the large closely packed elevations seen in lizard skin (Dhouailly, 1975). Recent evidence also indicates that premigratory neural crest mesenchyme from mouse can induce tooth-like morphogenesis in chick oral epithelium (Mitsiadis et al., 2003) and that early mammary gland mesenchyme is instructive for mammary gland development (Veltmaat et al., 2003).

Specification of the chicken feather mesenchyme

In chick the first sign of feather formation in the dorsal skin is the condensation of mesenchymal cells immediately underneath the epithelium throughout the dermis. This happens before ectodermal placode and dermal papilla formation. The mesenchyme is now called “dense dermis” as opposed to “loose mesenchyme” (Wessells, 1965; Chuong and Widelitz, 1998). The mesenchymal cells of the dermis are derived from the dermomyotome and are already determined to form feathers by signals from the dorsal neural tube. Aggregates of Wnt1-expressing cells can substitute for the dorsal neural tube (Olivera-Martinez et al., 2001). Wnt1 induces *Wnt11* in a subset of dermomyotome cells. Some of these cells will then migrate to form the dense dermis suggesting that specification of the feather mesenchyme depends on Wnt-mediated signals (Olivera-Martinez et al., 2002). Furthermore, specific Wnts can maintain the hair-inducing ability of mouse dermal papilla cells in culture (Kishimoto et al., 2000).

cDermo-1 is a transcription factor of the helix-loop-helix (HLH) family that is an early marker for the dense dermis in dorsal chick skin. BMP2, which is expressed in the overlying ectoderm, induces *cDermo-1* and can induce ectopic feather buds in vitro indicating that, in addition to Wnts, BMPs may also be involved in the specification of the mesenchyme (Scaal et al., 2002). This early function in promotion of organ formation is in contrast to the later inhibitory function of BMP during feather development (see below).

The nature of the first dermal message

In hair and feather development the first signal from the mesenchyme is called the “first dermal message,” which directs the epithelium to make an appendage (Hardy, 1992). A reaction-diffusion model has been put forward that proposes that the molecular signal(s) of the first dermal message are uniformly expressed throughout the mesenchyme, and activate both positive and negative regulators of placodal fate. Local competition will then restrict placode formation to sites of the future organs (Turing, 1952; Koch and Meinhardt, 1994; Barsh, 1999; Jiang et al., 1999). Jiang and colleagues (1999) have developed an in vitro assay where mesenchymal cells from placode-stage dorsal chick skin are dissociated and replated together with an intact epithelium. New feather follicles form in culture, but their number is dependent on the amount and density of mesenchymal cells in the assay. Based on their results Jiang and colleagues (1999) have suggested that when the density of the dermis reaches a certain threshold level small cell aggregates form in the dense dermis by random collisions. Local competitions (in line with the reaction-diffusion model) will then cause some microaggregates to enlarge and some to disappear. The remaining aggregates induce placodes in the overlying epithelium, and placodes will then induce the dermal papilla (Jiang et al., 1999). In other organs these microaggregates have not been reported.

Although the identity of the first dermal message is not known, it is suspected that Wnt family molecules may be involved. As already mentioned, *Wnt11* is expressed in the dense dermis of chick dorsal skin and is a target of Wnt1, which can mimic the feather-inducing abilities of the neural tube (Olivera-Martinez et al., 2001, 2002). Inhibition of Wnt signaling by Dickkopf1 inhibits hair, tooth, and mammary gland formation (Andl et al., 2002). Canonical Wnt signaling is mediated by the coactivity of the transcription factors beta-catenin and LEF1. Expression of beta-catenin is transiently found in the dense dermis prior to placode formation (Noramly et al., 1999). *Lef1* mutant mice lack vibris-

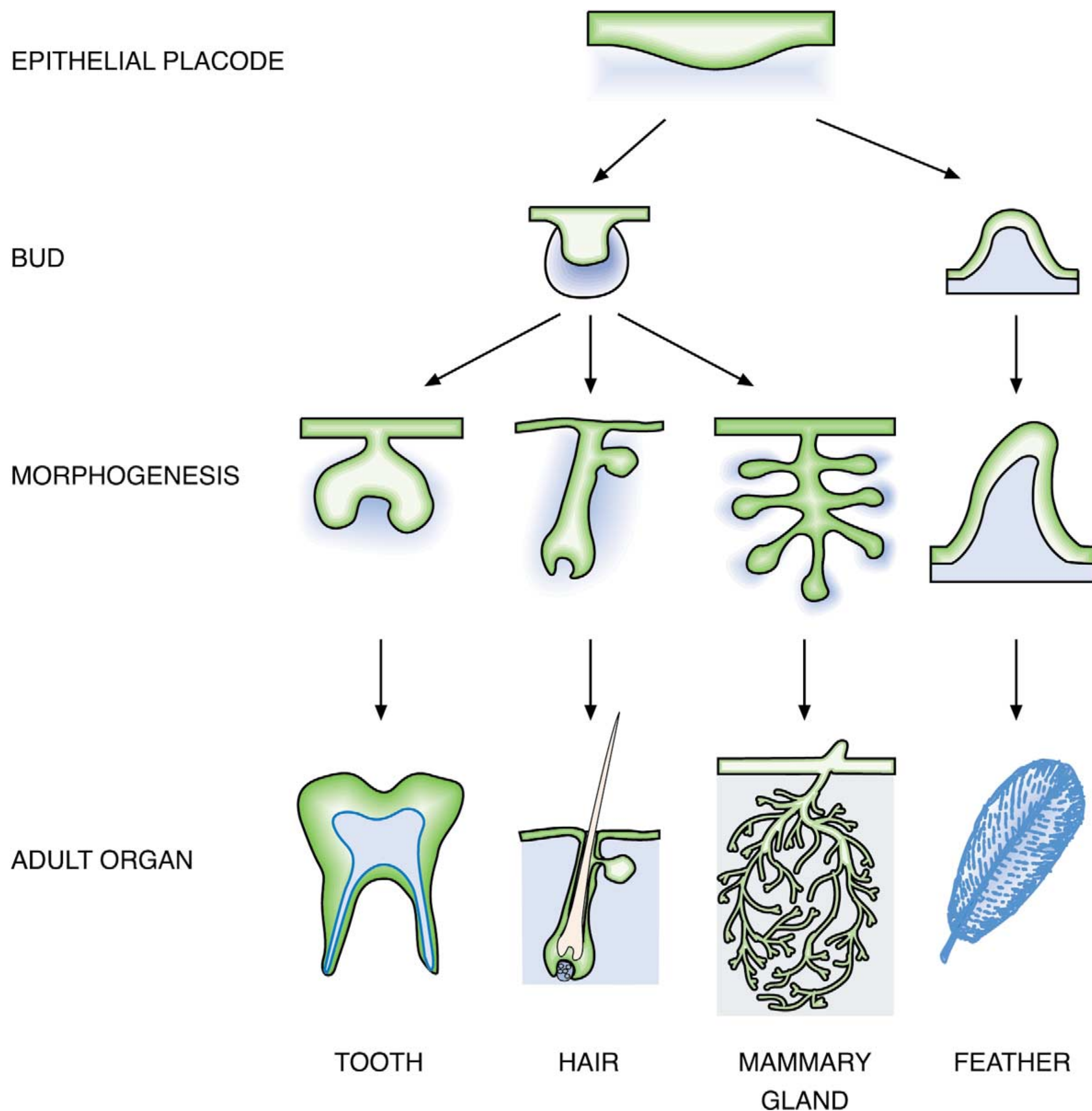

Fig. 1. Development of ectodermal organs. Morphologically very different organs, such as the tooth, hair, mammary gland, and feather, develop from two adjacent tissues: the epithelium (green) and the mesenchyme (blue). An epithelial placode is formed, which then buds either into or out of the mesenchyme. During morphogenesis the mesenchyme directs folding and branching of the epithelium, determining the shape of the organ.

sae, teeth, mammary glands, and most hairs, and LEF1 has been shown to be required specifically in the vibrissa mesenchyme prior to placode formation (van Genderen et al., 1994; Kratochwil et al., 1996). In *Lef1* mutant mice the first wave of hair follicles is nevertheless initiated. This may reflect a redundancy of LEF1 with other members of the LEF/TCF family. Wnt signaling is also required later in the formation of feather and hair placodes, and thus defining the exact roles of Wnts in organ initiation from mutational analysis is not easy.

Specifying a dental field

Mammalian teeth develop from the epithelia of the first branchial arch and the frontonasal process and neural crest-derived mesenchyme (Imai et al., 1996; Chai et al., 2000). They form from the dental lamina, a U-shaped ectodermal ridge in the mandible and maxilla. Similarly, mammary glands develop only from the bilateral presumptive milk lines on the abdomen of mammals (Veltmaat et al., 2003). Recent studies have shed some light on how the placodes are patterned in the dental field.

The patterning of the mandibular arch and consequently the definition of the dental placode locations in the lower jaws have been suggested to be determined by a balance of FGF and BMP signaling (Neubuser et al., 1997; Mandler and Neubuser, 2001). Conditional inactivation of *Fgf8* in the first branchial arch ectoderm in transgenic mice or inhibition of all FGF signaling in vitro by FGFR-specific inhibitors results in loss of tooth development (Trumpp et

Table 1
Molecules implicated in the development of at least two different ectodermal organs[a]

Molecule	Hair/feather	Tooth	Mammary gland	Lacrimal gland
FGF2/4/8	x	x		
FGF10	x	x	x	x
FGFR2b	x	x	x	x
BMP2/4	x	x		
Activin	x	x		
Wnt/Lef1	x	x	x	
Shh	x	x		
Eda/Edar/Edaradd	x	x	x	x
Msx1/2	x	x	x	
PTHrP		x	x	

[a] Only molecules that have an ectodermal organ phenotype in transgenic animals or in other functional assays are listed here. For references, see reviews by Millar, 2002 (hair), Thesleff and Mikkola, 2002a (tooth), and Veltmaat et al., 2003 (mammary gland).

al., 1999; Mandler and Neubuser, 2001). FGFs and BMPs regulate several targets in the dental mesenchyme or epithelium, some of them antagonistically (Vainio et al., 1993; Neubuser et al., 1997; Tucker et al., 1998; St. Amand et al., 2000; Mandler and Neubuser, 2001). Some of these target genes such as *Pax9, Msx1*, *Msx2*, and *Pitx2* are essential for tooth development (Satokata and Maas, 1994; Bei and Maas, 1998; Peters et al., 1998; Lin et al., 1999; Lu et al., 1999).

An early instructive signal in tooth development comes from the oral epithelium as shown by epithelial-mesenchymal recombinations (Mina and Kollar, 1987; Lumsden, 1988). A long-standing question has been whether the mesenchymal neural crest cells are prepatterned prior to the epithelial signal. The anterior-posterior body axis of most vertebrates is patterned by *Hox* genes, and it has been suggested that the Dlx homeobox transcription factors might provide similar cues for the patterning of the oral mesenchyme. *Dlx1/Dlx2* mutant mice do not develop maxillary teeth, and *Dlx5/Dlx6* mutant mandibles have maxillary identity (Thomas et al., 1997; Depew et al., 2002). Only maxillary mesenchyme is competent to express *Dlx5* upon induction by the maxillary ectoderm suggesting that maxillary mesenchymal identity differs from that of the mandibular mesenchyme. On the other hand, *Barx1*, a molar-specific marker, can be induced throughout the mandible irrespective of location (Ferguson et al., 2000). Epithelial BMP signaling negatively regulates *Barx1*, since beads of Noggin can enlarge the *Barx1* expression domain, and result in an apparent transformation of incisors into molars (Tucker et al., 1998). These findings imply that the mandibular mesenchyme may not be prepatterned into incisor and molar areas.

Placodes as signaling centers

An epithelial placode can be considered a basal unit of ectodermal organogenesis. In addition to ectodermal appendages, e.g., the olfactory, lens and otic placodes mark the early development of the corresponding sensory organs. Several signaling factors are coexpressed in the placodes,

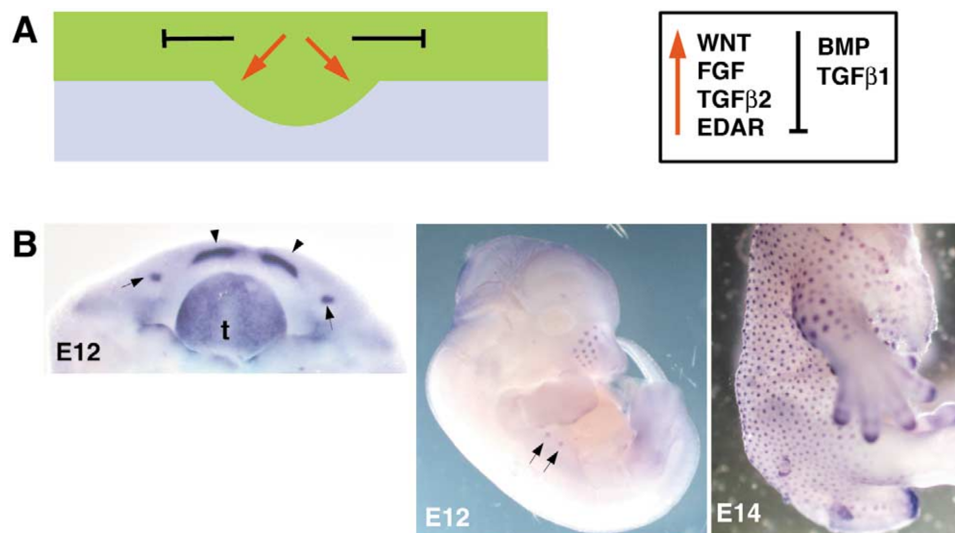

Fig. 2. Placodes as signaling centers. (A) Signaling at the hair and feather placode. Positive signaling (activators, red) promotes placode development, whereas negative signaling (inhibitors, black) represses it. The activity of the inhibitors is believed to be prevented inside the developing placode, whereas they can diffuse outside the placode to mediate lateral inhibition. (B) Placodes can be visualized with whole mount in situ hybridization detecting the restricting expression of many signaling molecules. Molar (arrows) and incisor (arrowheads) tooth placodes express *Shh* (E12 mouse mandible; t, tongue). Vibrissa and mammary gland placodes (arrows) are positive for *Edar* mRNA (E12 mouse embryo). Hair placodes express *Patched* (E14 mouse embryo, expression can also be seen at nails and joints).

and their interplay is presumably required for morphogenesis.

Lessons from feathers and hair

The reaction-diffusion model presumes that the initial mesenchymal signals regulate molecules that either promote or repress placode formation. The activators and inhibitors may affect either the initiation and/or the growth of placodes, they can act either from the mesenchyme or can be induced in the preplacode itself, and their local competition is required for placode formation. At placodal sites the relative contribution of the activators is higher and at interplacodal sites the amount of negative regulators is higher, thus inhibiting placode initiation (Fig. 2). In feathers additional complexity is created by the formation of a hexagonal pattern of placodes, which is formed in precise spatial and temporal order. The mechanisms of reaction-diffusion and lateral inhibition together with cellular proliferation, migration, and cell-cell communication must be exactly integrated to achieve the regular pattern (Jung and Chuong, 1998).

In addition to its potential role in generating the first dermal message, Wnt signaling seems also to act downstream of the first dermal message as a promoter of placode formation. The use of a reporter construct that mimics LEF1 activation and therefore possibly also Wnt activation has revealed LEF1 signaling both in the vibrissa placode itself and in the underlying mesenchyme (DasGupta and Fuchs, 1999). The relative intensities between the epithelial and mesenchymal expressions vary suggesting that they are activated sequentially. The lack of beta-catenin in the developing mouse epidermis results in lack of hair follicle formation, and conversely epithelially expressed constitutively active beta-catenin can induce ectopic feathers and hair (Gat et al., 1998; Noramly et al., 1999; Widelitz et al., 2000; Huelsken et al., 2001). These experiments cannot of course differentiate between the requirement for Wnt as a mesenchymal inducer and it role later as a placode promoter. Wnts may act as activators also in mammary placodes, as the expression of *Lef1* in the mammary epithelium is one of the earliest markers for placode development (van Genderen et al., 1994; Mailleux et al., 2002). FGFs can also promote placode growth. For example, FGF2 can induce feathers both in wild-type skin as well as in the chick mutant *scaleless*, which lacks feather follicles despite having at least a partially formed dense dermis (Goetinck and Sekellick, 1970; Song et al., 1996; Widelitz et al., 1996). FGF receptor mutants, *Fgfr2b* mice, have dysgenic hair formation (Revest et al., 2001). TGFβ2 and follistatin can both increase the number of hair follicles in vitro. In line with this, *Tgfβ2* null mice have a reduced amount of hair follicles, and *follistatin* null mice show retarded hair follicle development (Foitzik et al., 1999; Nakamura et al., 2003).

BMPs are currently the best characterized candidates for placode inhibitors, and mediators of lateral inhibition. Ectopic expression of BMP2, BMP4, or constitutively active BMPR1 in chick skin disrupts feather formation (Noramly and Morgan, 1998; Ashique et al., 2002). Similarly, BMP4 releasing beads inhibit hair and feather follicle formation (Jung et al., 1998; Botchkarev et al., 1999). Although they are expressed in the placode itself, their activity within the placode is believed to be negated by BMP inhibitors, such as Noggin. Increasing or decreasing Noggin levels has the same effect on follicle formation as decreasing or increasing BMP activity, respectively (Botchkarev et al., 1999, 2002). BMPs and FGFs seem to antagonize each other's activity by, e.g., differentially regulating the same target genes (Jung et al., 1998; Noramly and Morgan, 1998). One of their targets is probably TGFβ-stimulated clone 22 (TSC-22), a leucine zipper transcription factor, which is differentially regulated by FGF and other RTK signaling, and by BMPs in chick skin. Beads of FGF2, EGF, and TGFα all stimulate *TSC-22* expression, and BMP4 beads or transfection with BMP4 suppress it (Dohrmann et al., 2002). In addition to BMPs, TGFβ1 also inhibits hair follicle development in vitro, and *Tgfβ1* null mice have slightly advanced hair follicle formation. The negative effect may be based on inhibition of proliferation (Foitzik et al., 1999).

Signaling at the tooth placode

In teeth early epithelial signals such as FGF8 and BMP4 regulate the expression of mesenchymal transcription factors. Mutations in several of these factors (or, more precisely, double mutations of *Msx1/2* or *Dlx1/2* or *Gli2/3*) arrest tooth development to the dental lamina stage and the dental placode does not form (Bei and Maas, 1998; Thomas et al., 1997; Hardcastle et al., 1998). It is plausible that these transcription factors regulate signals, e.g., activin, that stimulate formation of the placodes (Ferguson et al., 1998; Laurikkala et al., 2001).

The dental placode, which forms in the dental lamina, is a transient epithelial signaling center, and in mouse embryos it expresses at least *Bmp2, Bmp4, Fgf8, Shh, Wnt10b, Msx2, Lef1*, and *p21* (Jernvall and Thesleff, 2000; Thesleff and Mikkola, 2002a). Comparative studies between the mouse and a related rodent, the sibling vole, which has a very different molar shape, show that the molecules of the signaling center are conserved between species (Keränen et al., 1998). Epithelial BMPs upregulate mesenchymal *Bmp4* via Msx1 and Msx2, and mesenchymal BMP4 further upregulates *Lef1* in the mesenchyme (Vainio et al., 1993; Chen et al., 1996; Kratochwil et al., 1996; Dassule and McMahon, 1998). Shh has been associated with promoting proliferation but its role in the dental placode is not clear. Ectopic infection of the oral ectoderm with Wnt7b expressing virus represses placodal *Shh* (Sarkar et al., 2000). This inhibits tooth formation but whether this is due to the lack of Shh is not certain. Reduction of Shh in the teeth of transgenic mice does not result in complete inhibition of tooth formation (Dassule et al., 2000; Zhang et al., 2000).

FGF10 promotes gland development

The interplay of promoting and inhibiting signals has been proposed as a regulatory mechanism also in gland development (Hogan, 1999). FGF10 seems to act as a promoter for epithelial budding. In an endodermal organ, the lung, *Fgf10* is expressed in the lung mesenchyme at sites where epithelial buds will form, and lack of FGF10 results in no budding. In addition, FGF10 can act as chemoattractant for the lung endothelium (Bellusci et al., 1997; Min et al., 1998; Sekine et al., 1999). Current data indicate that FGF10 is also involved in the development of the mammary gland, and lacrimal and Harderian glands of the eye. First, in all three glands, *Fgf10* is expressed in the mesenchyme underneath the epithelial bud (Makarenkova et al., 2000; Mailleux et al., 2002). In the mammary epithelium, expression of the receptor for FGF10, *Fgfr2b*, is seen throughout placode and bud development (Spencer-Dene et al., 2001; Mailleux et al., 2002). Second, the lack of FGF10 or its receptor results in loss of most mammary, lacrimal, or Harderian placodes (Govindarajan et al., 2000; Makarenkova et al., 2000; Mailleux et al., 2002). Third, misexpression of FGF10 or FGF7 in the lens is sufficient to cause ectopic ocular gland formation in the overlying corneal epithelium (Lovicu et al., 1999; Govindarajan et al., 2000). Similarly, beads of FGF10 or FGF7 can induce lacrimal glands in vitro (Makarenkova et al., 2000).

It is probable that mesenchymal FGFs are required for development of most, if not all, ectodermal organs. Defects in salivary glands, mammary glands, teeth, hair follicles, and nails have been reported in mice lacking *Fgf10* or *Fgfr2b*, and in mice misexpressing a dominant negative FGFR2 (Celli et al., 1998; De Moerlooze et al., 2000; Ohuchi et al., 2000; Mailleux et al., 2002).

A new player in the ectodermal organ field: ectodysplasin, a TNF family member

Ectodermal dysplasias are developmental syndromes that specifically affect ectodermal organs. Hypohidrotic ectodermal dysplasia patients (HED) and their mouse models *Tabby, downless*, and *crinkled (Ta/dl/cr)* have defects in teeth, hair, and, e.g., salivary, lacrimal, and sweat glands (OMIM, 2001; Blake et al., 2002). The mutated genes belong to the tumor necrosis factor (TNF) signaling pathway: ectodysplasin (*Eda*), a TNF ligand, its receptor *Edar*, and the intracellular adapter protein *Edaradd* (Thesleff and Mikkola, 2002b). These molecules are expressed during hair and tooth development first in the epithelium prior to placode formation, then in the placode, and also later during morphogenesis and cell differentiation. The early expression of the ligand, ectodysplasin, and its receptor *Edar* is overlapping, but when placodes are formed the patterns separate so that ectodysplasin is expressed in the interplacodal ectoderm and *Edar* in the placode itself (Headon and Overbeek, 1999; Laurikkala et al., 2001, 2002; Tucker et al., 2000). Although activation of the transcription factor NFκB by Edar has been shown, the actual genes regulated by Edar signaling are not known (Yan et al., 2000; Koppinen et al., 2001; Kumar et al., 2001). The first hair placodes are missing in *Ta/dl/cr* skin and consequently all placode markers so far analyzed are missing (Headon and Overbeek, 1999; Andl et al., 2002; Laurikkala et al., 2002; Nishioka et al., 2002). When Edar is overexpressed in the epithelium, upregulation of BMP4 is seen in the skin mesenchyme suggesting that BMP4 is an indirect target of Edar signaling (Pispa, J., unpublished results).

Accumulating evidence suggests that ectodysplasin-Edar signaling is an early and necessary component of placode formation in all ectodermal organs. First, *Edar* is one of the earliest markers of forming placodes. When beta-catenin activity is conditionally ablated in mouse epidermis *Edar* is expressed in a punctated placodal manner even if no hair follicles develop, and several other placode markers are missing (Huelsken et al., 2001). Second, *Ta/dl/cr* mice lack the first hair placodes (Headon and Overbeek, 1999; Laurikkala et al., 2002). Third, overexpression of ectodysplasin induces ectopic teeth and mammary glands, stimulates hair and nail growth, and increases the activity of sweat glands (Mustonen et al., 2003). *Edar* is also evolutionarily conserved as *Edar* mutant medaka fish lack scales (Kondo et al., 2001). Nevertheless, since lack of ectodysplasin-Edar signaling does not inhibit organogenesis completely it is likely that this signaling pathway can either be compensated for by a different molecular pathway or is redundant with other TNF and TNFR molecules. This is supported by overlapping expression of another TNF receptor, *TNFRSF19*, at the same sites as *Edar* (Pispa et al., 2003).

Morphogenesis

After the initial mesenchymal signals epithelial signals induce the condensation of mesenchymal cells, the papilla, around the placode. Later the mesenchyme directs morphogenesis, i.e., the organ-specific folding and branching of the epithelium (Dhouailly, 1975; Mina and Kollar, 1987; Lumsden, 1988; Hardy, 1992). For example, salivary gland mesenchyme can cause the mammary gland epithelium to branch in a salivary-like dense pattern (Kratochwil, 1969). It is noteworthy, however, that ectodermal cells in such recombinations retain their mammary gland-specific differentiation program. This indicates that morphogenesis and cell differentiation are at least partially independent processes.

The enamel knot regulates tooth shape

When the epithelial tooth bud has reached its full size it invaginates and folds at its tip. A new signaling center, the enamel knot, which is a transient structure of condensed epithelial cells, forms at the tip of the folding bud. This

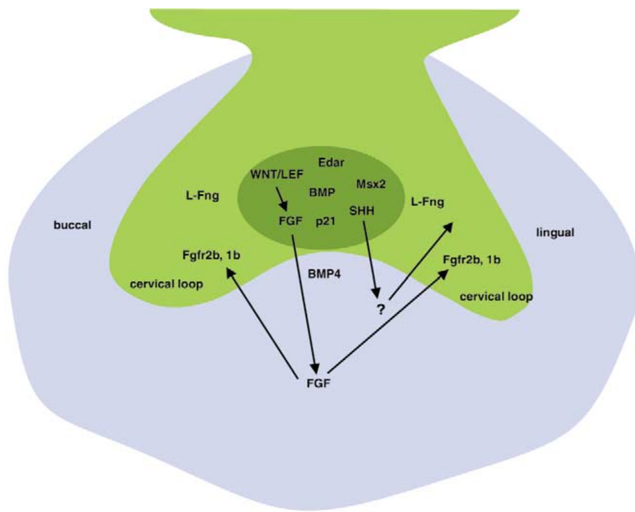

Fig. 3. Regulation of tooth morphogenesis by the signaling center, the enamel knot. More than 10 signaling molecules are locally expressed in the enamel knot (dark green), and regulate the growth and morphogenesis of tooth crown. The function of the enamel knot is regulated by at least Edar and LEF1. Wnt signaling mediated by LEF1 in the enamel knot upregulates FGF, which then induces mesenchymal FGFs, promoting proliferation in the cervical loops. SHH from the enamel knot acts via the mesenchyme to regulate epithelial growth specifically on the lingual side of the tooth germ. Lunatic fringe (L-fng) presumably contributes to the modulation of enamel knot signaling.

marks the initiation of tooth crown development. The epithelium flanking the enamel knot proliferates forming cervical loops, which grow to surround the mesenchymal dental papilla. The enamel knot cells do not proliferate, and it has been suggested that this is a mechanism for the differential growth of the tooth epithelium. The enamel knot is then removed by apoptosis (Jernvall et al., 1994; Vaahtokari et al., 1996a,b; Thesleff and Jernvall, 1997). Currently four *Fgfs*, three *Bmps*, three *Wnts, Shh, Edar, Msx2, Lef1*, and *p21* are known to be specifically expressed in the enamel knot (Fig. 3) (Thesleff and Mikkola, 2002a). Studies by Kratochwil and colleagues have shown that LEF1 is required specifically in the enamel knot during the bud-to-cap stage transition for the transduction of Wnt signals inducing *Fgf4* in the enamel knot (Kratochwil et al., 1996; Kratochwil et al., 2002). *Lef1* mutant teeth lack mesenchymal *Fgfs*, and their development can be rescued by beads releasing either epithelial or mesenchymal FGFs (Kratochwil et al., 2002). The transcription factor Runx2 is required in the mesenchyme for mediation of the same pathway (Åberg, T., Wang, X., personal communication). Mesenchymal FGFs stimulate proliferation in the epithelium, and are presumably required for the growth of the cervical loops. The FGF receptors for them, *Fgfr1b* and *Fgfr2b*, are expressed in the epithelial cervical loops, and mice carrying *Fgfr2b* mutations cease tooth development at an early stage (De Moerlooze et al., 2000). Lack of two of the molecules expressed in the enamel knot, Edar and Shh, causes defects in the patterning of cusps in molar teeth thus establishing a direct link between the enamel knot and tooth shape (Pispa et al., 1999; Dassule et al., 2000). Shh is required specifically for the growth of cervical loop at the lingual side, but this is not a direct effect, as ablation of Smo, the receptor of Shh, in epithelium does not inhibit cervical loop growth. Hence, Shh exerts its effect on epithelial growth via the mesenchyme (Fig. 3) (Gritli-Linde et al., 2002).

Mutations in some mesenchymal transcription factors such as Msx1 or Pax9 arrest tooth development at the bud stage prior to enamel knot formation (Satokata and Maas, 1994; Peters et al., 1998). It is therefore likely that mesenchymal signals regulate the formation of the enamel knot, and it has indeed been shown that exogenous BMP4 can rescue the *Msx1* phenotype and induce the expression of two enamel knot markers, *Msx2* and *p21* (Jernvall et al., 1998; Bei et al., 2000). Modulation of the enamel knot may also be achieved by signals within the dental epithelium. *Lunatic fringe (L-fng)*, a modifier of the Notch signaling pathway, is expressed in the epithelial cervical loops flanking the enamel knot. Since L-fng is important in establishing tissue boundaries in other systems it possibly assists in defining the enamel knot area. It is not indispensable for tooth development though, since the *L-fng* null mice have no tooth phenotype (Mustonen et al., 2002). Later in tooth development when the epithelial morphogenesis has progressed to the bell stage in molars, new signaling centers, the secondary enamel knots, are formed at sites of the future cusps where they will induce further folding of the epithelium (Jernvall et al., 2000).

The shape of feathers and hair

The molecular nature of the "second dermal message" that regulates epithelial downgrowth and proliferation in hair development is not known, although several molecules that are expressed in the dermal condensate exhibit a hair phenotype when mutated (Hardy, 1992; Millar, 2002). Shh is probably involved in activating this dermal message; it is expressed in the epithelium and mice lacking Shh or its transcriptional mediator Gli2 have immature hair follicles with a reduced amount of proliferation (St. Jacques et al., 1998; Mill, 2003). Despite the existence of several spontaneous and transgenic mouse models in which hair differentiation is affected, little is known about the signaling events regulating terminal differentiation of hair (Sundberg, 1994; Millar, 2002). Wnt signaling, mediated by LEF1, is likely to regulate the expression of the hair keratins, as the hair keratin promoters have LEF1 binding sites and *Lef1* is expressed in the hair matrix (Zhou et al., 1995). BMPs may be involved, as perturbations in their levels cause hair shaft differentiation defects (Blessing et al., 1993; Kulessa et al., 2000). Feathers come in diverse combinations of the rachis ("trunk") and barbs ("branches"). Recent evidence suggests that the interplay and periodicity of BMP, Noggin, and Shh is involved in the shaping of the feather filament. Suppression of BMP activity promotes rachis and barb branching,

whereas overexpression of BMPs results in repression of *Shh* and in increased rachis formation and fusion of barbs (Harris et al., 2002; Yu et al., 2002).

Future prospects

One problem in past research has been that analysis of mutant phenotypes has been hindered by the use of the same molecules reiteratively during the formation of one organ. Identifying the function of a gene in morphogenesis is difficult if it is also required in initiation of organogenesis. Combining conditional and inducible transgenic approaches to more traditional tissue recombination experiments should overcome some of these difficulties. Microarray techniques are likely to speed up the study of downstream target genes such as those that regulate cellular and structural changes such as proliferation, polarization, adhesion, and migration.

In this review we have omitted the discussion on the role of stem cells in ectodermal organs due to lack of space. Recent years have witnessed increasing interest in this line of research (Fuchs et al., 2001). The skin and its appendages are remarkable in respect that they can, to a certain degree, renew themselves. New hair follicles are initiated in the bulge region of the older degenerating follicles, where the epithelial stem cells reside, and the same stem cells appear to supply progenitors also for the sebaceous gland and the epidermis. Wnt signaling mediated by LEF1 and TCF3 is involved in their regulation (Merrill et al., 2001; Niemann et al., 2002). The rodent incisor grows continuously, which has been attributed to stem cells located in the cervical loops. Mesenchymal FGF10 and Notch pathway signals have been implicated in this process (Harada et al., 1999). The elucidation of the molecular regulation of stem cell maintenance and differentiation may obviously open up possibilities for regeneration of ectodermal organs.

If the same signals are used to make all ectodermal organs, where does the specificity come from? Global regulation by signals patterning the body axes could contribute to organ-specific development. In the control of identity of different subgroups of the same organ type, e.g., incisor and molar teeth or maxillary and mandibular molars, differential expression of transcription factors such as *BarxI* and *Dlx* genes has been implicated (Thomas et al., 1997; Tucker et al., 1998). There may also exist molecules that specify a certain type of organ. Evidence from *Caenorhabditis elegans* supports this possibility (Gaudet and Mango, 2002), but no regulatory molecules specific for individual ectodermal appendages have been discovered to date. Subtle changes in the amount and location of signaling molecules are potentially important. Manipulations in the level of beta-catenin in chick skin have shown that the identity of a feather or a scale placode may depend on the amount of beta-catenin received (Widelitz et al., 2000). The sequencing of the genomes and the development of technologies for global analysis of gene expression can be expected to reveal novel genes associated with the development of specific organs or organ types.

Variation in shape and structure is seen within ectodermal organs. For example, hair of different individuals can be curly or straight or of different color, and different species have species-specific composition of fur. The molecular basis for this variation is not well understood. Salazar-Ciudad and Jernvall (2002) have explained variation in molar tooth shape with a model based on activators and inhibitors that are expressed in the secondary enamel knots. The location of these knots will determine the location of the future cusps, and changes in the activator/inhibitor signaling will cause changes in tooth shape. With their mathematical model they have managed to predict the shape differences of the mouse and vole molars by using differential signaling parameters (Salazar-Ciudad and Jernvall, 2002). This implies that similar models may be useful in the future in determining molecular and cellular factors regulating shape variability in other ectodermal organs as well.

Acknowledgments

We thank Marja Mikkola and Tim Mitsiadis for critical reading of the manuscript, and Tuija Mustonen and Aapo Kangas for help with figures. Financial support was received from the Academy of Finland and the Sigrid Juselius Foundation.

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