Int. J. Dev. Biol. 39: 223-230 (1995)

The mechanisms and mediators of tooth eruption — Models for developmental biologists

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ABSTRACT Tooth eruption is a localized process in the jaws which exhibits precise timing and bilateral symmetry. It involves resorption and formation of bone on opposite sides of the erupting tooth and these activities depend on the dental follicle, a thin connective tissue investment of the developing and erupting tooth. Biochemical studies have shown that during eruption cells, proteins and enzymes change in the dental follicle and several growth factors and proteins known to accelerate or retard eruption have been identified. This review discusses these aspects of tooth eruption and proposes testable hypotheses and strategies that can make studies of tooth eruption new experimental opportunities for developmental biologists.

KEY WORDS: *tooth eruption, bone resorption, bone formation, dental follicle, epithelial-mesenchymal interactions*

Tooth eruption — the differentiation and interactions of cells and tissues

Teeth develop within the jaws and their eruption is a complex process during which they move through bone to their functional positions within the oral cavity. For species with more than one set of teeth, eruption of the second set also accomplishes root resorption and exfoliation of the first (Fig. 1). Our understanding of the sequential migration, differentiation and activation of cells around erupting teeth is in its infancy. This review focuses on these events and proposes hypotheses and methods for understanding the complex regulation of this process. Because tooth eruption is a local event in each jaw which exhibits an exquisite bilateral symmetry, an exploration of tooth eruption is essentially one of dissecting the local cellular microenvironment. Because this can be approached but not duplicated *in vitro*, observations from cell and organ cultures must be pursued *in vivo* (Cielinski and Marks, 1994). Eruption of molars in rats and permanent premolars in dogs are two reproducible systems for which there is a considerable database. Because these species are readily accessible to developmental biologists, we shall concentrate on them in this review where the emphasis will be on cellular interactions in these systems as experimental models for developmental biology. Interested readers are referred to other recent reviews of tooth eruption with different emphases (Steedle and Proffit, 1985; Thesleff, 1987; Marks *et al.*, 1988; Gorski and Marks, 1992; Marks and Schroeder, 1995).

Summary of experimental analyses of tooth eruption

Studies of premolar eruption in dogs has shown that it is accomplished by localized resorption and formation of alveolar bone on opposite sides of the tooth (Cahill, 1969; Marks *et al.*, 1983; Marks and Cahill, 1986), that the cells responsible, osteoclasts and osteoblasts, respectively, are activated on bone surfaces just prior to eruption (Marks *et al.*, 1983; Wise *et al.*, 1985) and that eruption depends upon the dental follicle proper, a thin collagenous investment of the crown of each tooth (Cahill and Marks, 1980; Marks and Cahill, 1984). Just prior to eruption the coronal, resorption-associated, part of the follicle is invaded by mononuclear cells which have enzymatic and ultrastructural features of preosteoclasts (Wise *et al.*, 1985, 1988; Marks and Grolman, 1987) and the apical, formation-associated, part of the follicle binds epidermal growth factor (Marks *et al.*, 1988). Surgical manipulations of the dental follicle proper have shown that it exhibits regional control of bone resorption and formation (Marks and Cahill, 1987) and that the dental follicle and enamel epithelium, which separates the follicle from the mineralized tooth surface, are needed for eruption (Larson *et al.*, 1994). Biochemical analyses of the dog premolar follicle have shown changes in collagen and non-collagenous proteins, proteoglycans and metalloproteinases during eruption (Gorski *et al.*, 1988a,b; Gorski and Marks, 1992). The most prominent follicular protein (DF-95) is a sialoprotein of 95,000 relative molecular weight. DF-95 is fragmented at the onset of eruption and has been recently localized in the reduced enamel epithelium (Fig. 2) (Gorski

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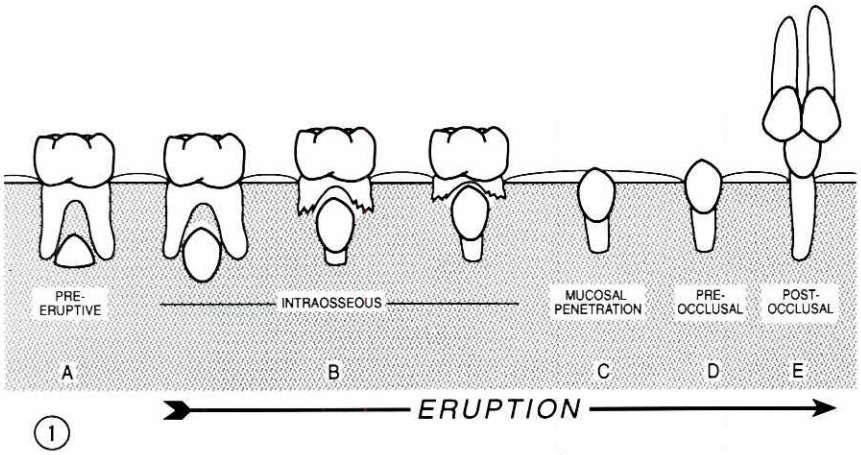


Fig. 1. Diagrammatic representation of eruption of the human first permanent mandibular premolar as seen in a radiograph. *In the preeruptive period* **(A)** *the crown of the permanent tooth forms within a bony crypt between the root tips of the first primary molar. Eruption begins after crown formation is complete and its first sign is an area of bone resorption over the cusp tip. During the intraosseous phase* **(B)** *root formation begins and the tooth moves into an eruption pathway prepared by resorption of bone and the roots of the primary tooth. After the primary tooth is lost, the erupting tooth penetrates the oral epithelium* **(C)**, *and the speed of eruption increases until occlusal contact is made with the opposing teeth* **(E)**.

et al., 1994). The enamel organ is known to contain proteases during tooth development (Nanci *et al.*, 1989), and some enamel proteins are translocated internally to odontoblasts (Nakamura *et al.*, 1994). These or different proteases may fragment DF-95 to trigger tooth eruption after the tooth crown is formed (Cahill *et al.*, 1988; Gorski and Marks, 1992; Gorski *et al.*, 1994).

Studies of tooth eruption in primates have shown that the follicle is important in eruption (Kristerson and Andreasen, 1984), that bone formation occurs eccentrically in erupting teeth to provide a mechanism for changes in the direction of eruption (Schroeder *et al.*, 1992), and that root formation and bone formation act reciprocally to move erupting teeth into the eruption pathway formed by bone resorption (Bosshardt *et al.*, 1989).

Studies of molar eruption in rodents have shown that bone resorption and formation are necessary components (Gregg and Avery, 1964; Kameyama, 1973; Marks, 1981) and that parts of the rodent dental follicle have mononuclear cells near osteoclasts on adjacent bone surfaces in the direction of eruption (Wise and Fan, 1989). Furthermore, collagen synthesis within dental follicles changes and exhibits regional differences during eruption (Shroff *et al.*, 1994). Several studies have provided evidence that epidermal growth factor (EGF), transforming growth factor-beta (TGF-ß), interleukin-1 (IL-1), colony-stimulating factor-1 (CSF-1) and two proteins isolated from the follicle and the enamel organ play some role in the regulation of eruption (Cohen, 1962; Thesleff, 1987; Topham *et al.*, 1987; Wise and Fan, 1991; Lin *et al.*, 1992a,b; Wise *et al.*, 1992a,b, 1994a,b; Lin and Wise, 1993; Cielinski *et al.*, 1994).

Taken together these studies of tooth eruption in rodents, dogs and primates show that tooth eruption is a localized, symmetrical process in the jaw which involves a polarization of alveolar bone resorption and formation which is dependent upon the dental follicle. Tooth eruption involves the interaction of local and migrating cells, may be triggered by proteolytic events in the enamel organ and involves a cascade of regulator molecules. In short, it is a process awaiting exploration by developmental biologists. We describe below current and future efforts to understand these complex interactions *in vitro* and *in vivo*.

The regulation of tooth eruption: molar eruption in rodents

Potential eruption molecules

In the past, experiments to determine the potential molecules that initiate tooth eruption have primarily involved injecting different molecules and observing their effects on eruption. In that vein, the early studies of Cohen (1962) demonstrated that injections of epidermal growth factor (EGF) resulted in premature eruption of rodent incisors. Another molecule that binds to the EGF-receptor, transforming growth factor alpha (TGF-α), also accelerates incisor eruption (Tam, 1985). However, recent "knockout" gene studies have shown that the absence of TGF-α will not delay eruption (Mann *et al.*, 1993). Of course, in such studies EGF may substitute for TGF-α and thus, one cannot fully eliminate TGF-α as a candidate molecule for eruption in normal development.

Another candidate molecule for initiating the onset of tooth eruption is colony-stimulating factor-one (CSF-1). This protein, which stimulates monocyte development, will initiate eruption in osteopetrotic rats (Iizuka *et al.*, 1992). Without CSF-1 injections, such rats are toothless presumably because a deficiency in osteoclasts in these animals prevents alveolar bone resorption and the formation of an eruption pathway. Injection of CSF-1 increases osteoclast numbers in the bony crypt and leads to eruption pathway

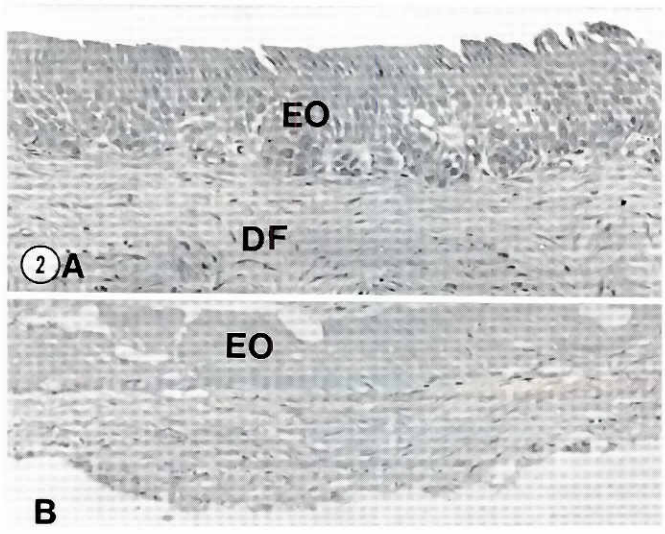


Fig. 2. Photomicrographs of the enamel organ (EO) and dental follicle (DF) from a dog premolar during eruption. *Immunohistochemical staining* **(A)** *of these tissues with an antibody to the major sialoprotein in these tissues (DF-95) shows that its distribution is limited to the enamel organ. Prior treatment of an adjacent section with preimmune serum* **(B)** *shows the specificity of the staining in* **A**. *x75.*

formation (Iizuka *et al.*, 1992). Moreover, injection of CSF-1 into normal rats will accelerate the rate of eruption, as well as increase the numbers of monocytes in the dental follicle and the number of osteoclasts in the bony crypt (Cielinski *et al.*, 1993, 1994).

It should be noted that both the molecular and cellular events that probably initiate tooth eruption occur early postnatally. At the cellular level, the influx of monocytes into the dental follicle of the rat first mandibular molar peaks at day 3 postnatally (Wise and Fan, 1989; Cielinski *et al.*, 1994), as do the number of osteoclasts. At the molecular level, EGF injections are most effective if done before day 3 postnatally (Hoath, 1986) and CSF-1 must be injected before day 1 postnatally to bring about incisor eruption in the osteopetrotic rat (Iizuka *et al.*, 1992). The receptor for EGF also is present early postnatally in the rat mandibular molars and then is absent by day 10 postnatally (Wise *et al.*, 1992c).

Another molecule that is present early in a dental tissue and then disappears is transforming growth factor beta one (TGF-ß1). The stellate reticulum (SR), an epithelial layer adjacent to the dental follicle immunostains for TGF-ß1 from day 0-2 postnatally in the first mandibular molar of the rat but there is no staining at subsequent postnatal times (Wise and Fan, 1991). The facts that TGF-ß1 is a potent chemoattractant for monocytes (Wahl *et al.*, 1987; Wiseman *et al.*, 1988) and that it is present in the SR just prior to the peak influx on monocytes into the follicle on the third postnatal day (Wise and Fan, 1989) suggest that TGF-ß1 might initiate this important cellular event of eruption.

Experiments show that *in vitro* TGF-ß1 also enhances the secretion of extracellular matrix proteins, including Type I collagen and fibronectin, by cultured dental follicle cells (Wise *et al.*, 1992b). Because the periodontal ligament is a dense connective tissue derived from the dental follicle, it is possible that TGF-ß1 is signalling the differentiation of parts of the dental follicle into the periodontal ligament.

In addition to molecules that stimulate eruption, one has been isolated that inhibits the rate of eruption. This molecule, a 167 kDa protein that is present in the DF and SR (Lin *et al.*, 1992a), delays the onset of tooth eruption when injected into rats (Lin *et al.*, 1992b). Western blots suggest that this protein may be an EGF receptor (Wise *et al.*, 1994b) and thus, it may inhibit normal EGF activity.

In vitro studies of eruption molecules

With the establishment of *in vitro* cultures of stellate reticulum cells (Wise *et al.*, 1990) and dental follicle cells (Wise *et al.*, 1992a) from rat mandibular molars, the possibility now exists to determine the direct effect of a putative eruption molecule on cells that are from tissues required for eruption. To that end, several studies now have been completed which examine a given molecule's effect upon the gene expression of the cells *in vitro*. As will be detailed, these *in vitro* results suggest that *in vivo* a cascade of molecular signalling may be required to initiate the onset of tooth eruption.

Beginning with EGF, it appears to have its major influence on the stellate reticulum. In terms of gene expression, Northern blotting demonstrates that EGF enhances the expression of TGF-ß1 mRNA in cultured SR cells (Lin and Wise, 1993). Reverse transcription polymerase chain reaction studies (RT/PCR) also show that EGF enhances the expression of interleukin-one alpha (IL-1α) mRNA in cultured SR cells in both a time-dependent and concentration-dependent fashion (Wise *et al.*, 1994b). Moreover, *in vivo* injections of EGF into rats results in an increase in immunostaining for IL-1α itself in the SR (Wise *et al.*, 1994a). TGF-ß1 also acts to stimulate expression of IL-1α mRNA in cultured SR cells (Wise *et al.*, 1994b).

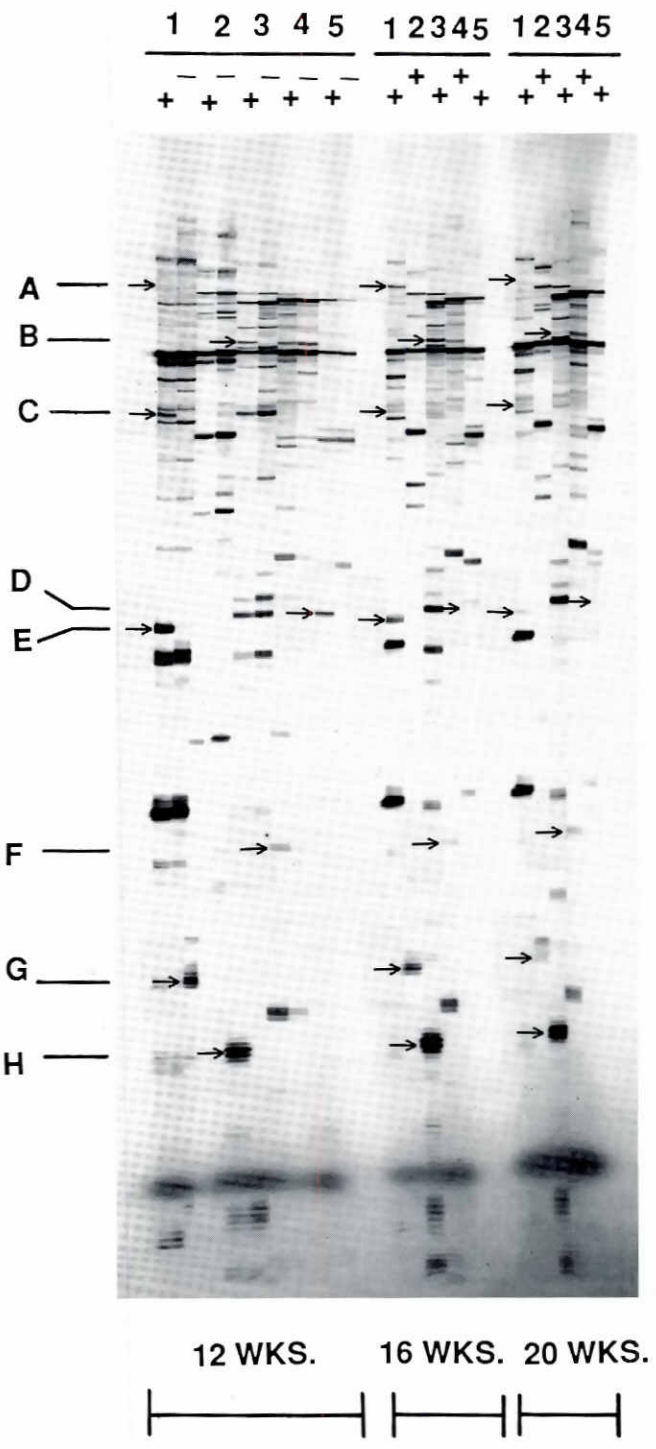


Fig. 3. Differential display of dental follicles from 12,16 and 20-week-old dogs. *Total RNA was isolated from two third and fourth premolar follicles from each age shown and subjected to differential display analysis with (^{35}S)-dATP. The resultant gel autoradiograph was produced with T_{12}MG primer for RT-PCR step and then AP 1-5 random primers (GenHunter Corp., Brookline MA, USA) added individually for subsequent PCR step (designated as 1 through 5 on the figure heading). Each follicle RNA sample was analyzed twice with each primer pair; the only difference among these assays was that incubation with reverse transcriptase was omitted from one (denoted as "-") while included in the other ("+").*

TABLE 1

STAGES AND CHARACTERISTIC FEATURES OF FOLLICULAR AND CRYPT ACTIVITIES DURING ERUPTION OF THE THIRD AND FOURTH MANDIBULAR PERMANENT PREMOLARS IN DOGS

Postnatal Age (wk)	Stage of Eruption	Characteristic Cellular Activities
12	non-eruption	near end of crown formation
14	pre-eruption	primarily pre-osteoclasts
16	early-eruption	primarily bone resorption
18	mid-eruption	mixture of bone resorption and bone formation polarized around tooth
20	late eruption	primarily bone formation
23	eruption completed	bony support consolidates

The presence of IL-1α in the SR is of interest because it enhances the expression of CSF-1 mRNA in cultured dental follicle (DF) cells (Wise and Lin, 1994). It is important to note that neither EGF nor TGF-ß1 stimulate CSF-1 mRNA expression in cultured DF cells (Wise *et al.*, 1994b). Thus, initiation of increased CSF-1 gene expression by EGF or TGF-ß1 would have to be mediated through Il-1α production in the SR.

EGF may act to prime the dental follicle to respond to the IL-1α. Recent studies have shown that injections of EGF into rats result in increased expression of the interleukin type I receptor mRNA in the dental tissues (Wise *et al.*, 1994a). *In vitro*, incubating cultured DF cells with EGF also enhances the interleukin receptor mRNA level (Wise *et al.*, 1994a).

CSF-1 itself has an autocrine effect on the CSF-1 gene in cultured DF cells (Wise and Lin, 1994). This may, in turn, result in an increase in the synthesis and secretion of CSF-1 by the dental follicle *in vivo*. Such an increase might account for the maximal influx of monocytes in the rat first molar follicle on the third postnatal day (Wise and Fan, 1989; Cielinski *et al.*, 1994). This hypothesis is supported by the findings of Cielinski *et al.* (1994) which demonstrate that injection of CSF-1 increases the number of monocytes in the follicle. The fact that this maximal number of monocytes is not maintained beyond one day may be due to the incoming monocytes endocytosing the CSF-1 similar to the manner in which hepatic macrophages clear CSF-1 from the systemic circulation (Bartocci *et al.*, 1987).

The initiation of tooth eruption — a hypothesis

Based on the aforementioned *in vitro* results, the diagram at right delineates a possible cascade of signals that might result in the onset of tooth eruption. This diagram is modified after one previously published (Wise *et al.*, 1994b).

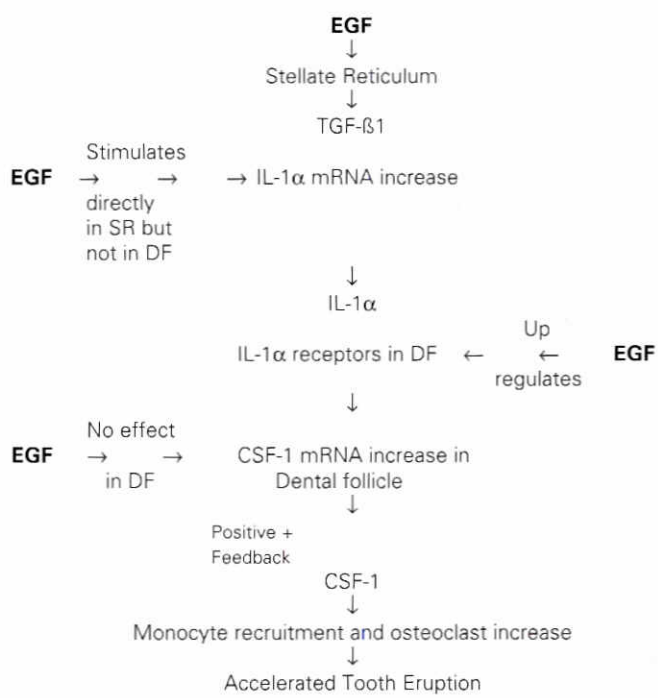
This hypothesis suggests a relationship among the molecules that previously have been shown to play a role in tooth eruption. In particular, either EGF or TGF-ß1 could initiate the cascade of molecular signals that would stimulate the onset of tooth eruption. The ultimate molecule produced, CSF-1, has the capability of stimulating the cellular events (monocyte influx and osteoclast increase) that is seen at the beginning of normal eruption (Marks *et al.*, 1983; Wise *et al.*, 1985; Wise and Fan, 1989). Whether or not this hypothesis is correct will require experiments to determine if the gene expression seen *in vitro* occurs *in vivo* in the necessary tissues and in the appropriate chronological sequence. Finally, experiments will have to be conducted to determine if a given molecule that may accelerate eruption is physiologically significant. Such studies are in progress.

Evidence from premolar eruption in dogs

Tooth eruption as a developmental cascade

We have studied eruption of canine third and fourth mandibular permanent premolars for more than a decade and delineated the morphological and biochemical landmarks of the process (see above and Table 1). Alveolar bone growth, tooth development and eruption are interdependent processes. Biochemical analyses of individual canine dental follicles during premolar eruption (Gorski *et al.*, 1988a,b) have shown that the follicle is highly hydrated throughout eruption, that its collagen and proteoglycan content increase during eruption and that the follicle contains more than 20 proteins identifiable by SDS-PAGE, the most prominent being a sialoprotein of Mr= 95,000 (DF-95). DF-95 has been located in the enamel organ, concentrated in tonofibrils between ameloblasts (Gorski *et al.*, 1994), and is partly fragmented at the onset of eruption to produce several fragments of Mr= 21-25,000. Dental follicles of erupting teeth are rich in proteases (Suga, 1970; Anderson *et al.*, 1982; Menanteau *et al.*, 1986; Overall and Limeback, 1988; Moe and Kirkeby, 1990; Smid *et al.*, 1992); interestingly, MMP-1 and MMP-3 decline as eruption proceeds (Gorski and Marks, 1992). The true dental follicle (TDF) and enamel organ can be cleanly separated by non-enzymatic treatment with EDTA (Lau and Ruch, 1983) at room temperature for 1.5-2 h. Teeth without the TDF cannot erupt while those with the TDF removed and then replaced do erupt (Larson *et al.*, 1994). These observations are of particular interest given the high incidence of disturbances of tooth eruption in children with enamel dysplasias (Sauk, 1988).

All cited work on rat and canine tooth eruption has necessarily investigated the role of defined factors on the process. This approach is limiting in that (1) it permits an examination of only one or several factors at one time, and (2) restricts mechanistic studies to characterized "candidate" molecules. Recent work with oncogenes, transcription factors and "master genes" suggests that



tooth eruption can be fruitfully viewed as a developmental cascade. Embryological development is defined by a series of inductive interactions. For example, coronal or basal dental enamel/follicle cells may send out a signal that induces differentiation of relatively featureless mesenchymal cells or precursor cells into cells with specific functions, i.e., osteoblasts or osteoclasts. Developing muscle tissue represents one of the best characterized paradigms for transcriptional control of vertebrate tissue development and differentiation (Jan and Jan, 1993; Weintraub, 1993). Muscle development depends upon the systematic expression of a series of at least four early myogenic factors, mrf-4, myogenin, myoD and myf-5, and an intermediate factor mef-2. Major effects of myogenin are seen after the 15th day of life in mice (Edmondson *et al.*, 1992) indicating control of secondary myogenic properties. The proteins, putative transcription factors, are members of the helix-loop-helix (HLH) class (Weintraub *et al.*, 1991) and contain distinct DNA binding domains (Davis *et al.*, 1987; Braun *et al.*, 1990; Miner and Wald, 1990). Myogenic transcription factors appear to function as dimers in activation of specific genes possessing "E-boxes" in their promoter (Murre *et al.*, 1989; Weintraub *et al.*, 1991); phosphorylation state of myogenic factors represents another level of control in modifying binding to the E-box (Li *et al.*, 1992). Interestingly, inactive dimer complexes can form with *id* (inhibitor of differentiation), an inhibitory HLH factor (Benezra *et al.*, 1990; Finkel *et al.*, 1993). As a result of these studies muscle development is viewed as a cascade of transcriptional activation genes for muscle growth factors, kinases, phosphatases, and ultimately leading to activation of genes for characteristic muscle structural components (Jan and Jan, 1993; Weintraub, 1993).

In *Drosophila*, segment-polarity genes direct cell development within each of 14 parasegments. Hedgehog *(hh)* and engrailed are such genes; mouse and chicken homologs play key roles in morphogenesis of limbs and the central nervous system (Echelard *et al.*, 1993; Kraus *et al.*, 1993; Riddle *et al.*, 1993). Of note, *hh* in the fruitfly induces expression of decapentaplegic *(dpp)*, a member of the transforming growth factor beta family (Heberlein *et al.*, 1993). *dpp* protein shares sequence homology with bone morphogenetic protein-2 (BMP-2) and BMP-4 in vertebrates, factors believed to play morphogenic roles in bone and tooth development (Theis *et al.*, 1992). BMP-2 and dexamethasone are potent inducers of *id* (inhibitor of differentiation) in osteoblastic cells, whereas $1,25(OH)_2D_3$ is an inhibitor of id expression in these cells (Ogata and Noda, 1991; Kawaguchi *et al.*, 1992; Ogata *et al.*, 1993). Finally, *Msx* genes are avian and mammalian analogs of *Drosophila* muscle segment homeobox *msh* gene which are expressed in neural crest cells, osteogenic tissue of mandible and maxilla, and developing teeth (Hill *et al.*, 1989; Takahashi and Le Douarin, 1990; Holland, 1991; Monaghan *et al.*, 1991; Yokouchi *et al.*, 1991; MacKenzie *et al.*, 1992). Vainio *et al.* (1993) have shown that BMP-4 induces expression of *Msx1* and *Msx2* in vertebrate mesenchyme cells, implying a role in mediating epithelial-mesenchymal tissue interactions during tooth development. Expression of MSX2 in human bone cells is regulated by $1,25(OH)_2D_3$ (Hodgkinson *et al.*, 1993). A mutation in a highly conserved position of the homeodomain region of MSX2 leads to human hereditary craniosynostosis (Jabs *et al.*, 1993), which exhibits as its primary defect premature closure of calvarial studies. These examples of "master gene" action in bone and tooth development suggest that these or new, yet to be identified, genes may control a tooth eruption cascade pathway analogous to that for muscle development.

Strategies for studying the regulation of tooth eruption

Subtractive and differential hybridization represent standard methods to identify changes in mRNA expression in comparative studies. RNA differential display is a new alternative method involving anchored oligo-dT RT-PCR of mRNAs, followed by PCR amplification of resultant cDNAs in conjunction with a decamer oligodeoxynucleotide of arbitrarily defined sequence (Liang and Pardee, 1992; Liang *et al.*, 1993). Amplified cDNA fragments of 100-500 bp derived from the 3'-termini of mRNAs are separated on sequencing gels and comparisons made among fragments from 20 different primer pairs. A major problem limiting biochemical and cell biological studies of the role of the dental follicle in tooth eruption, in both the rat and canine dentitions, has been the small quantity of tissue available. Differential display analyses can be carried out with mRNA from only two dental follicles (see below); in addition, this technique facilitates screening and comparison of the entire mRNA repertoire of dental follicle cells from distinct stages of eruption. Nishio *et al.* (1994) have used differential display to detect changes in expression as small as 50%, while other workers have successfully applied this method to analyses of gene expression of the pre-implantation mouse embryo (Zimmermann and Schultz, 1994), delayed-early action of FGF-1 in culture (Donohue *et al.*, 1994), and glucose-dependent genes in retinal pericytes (Aiello *et al.*, 1994).

We have begun differential display analyses of canine dental follicle cells from animals at 12, 16 and 20 weeks of age (Table 1). Fig. 3 depicts the gel autoradiograph comparing expression at these time points for five sets of random primers. A complete analysis will involve 20-25 different primer pairs. Total RNA was isolated from the third and fourth premolar follicles and the effect of DNA contamination on the resulting pattern of bands from differential display analyses was investigated by comparing identical RNA samples with (noted as "+" in Fig. 3) and without inclusion of a reverse transcriptase step (noted as "-" in Fig. 3). Both samples (+/-) were treated identically except for the omission of reverse transcriptase. It is assumed that bands that are present under both control conditions are due to contaminating DNA; bands that are present only after both RT-PCR and PCR steps should be derived from mRNA. Several points can be made about the banding pattern depicted (Fig. 3). First, comparison of + and - lanes for 12 week follicle RNA showed that indeed some bands are present in both lanes and generally similar in intensity, whereas other detected bands were present only in + lanes (RNA-dependent bands denoted by letters A-H on Fig. 3). For simplicity, only + lanes for 16 and 20 week samples are presented, however "-" lanes were found to be very similar to those for the 12 week sample. Second, messages A and B were found to vary over these eight weeks of development reaching maxima at 16 weeks of age. In contrast, expression of messages C,D,E and G are observed to diminish substantially over the period from 12 to 20 weeks. In contrast, messages F and H represent internal controls in the sense that their expression did not change substantially over this same period. Although further studies (Northern blotting and sequencing) are necessary to characterize these messages, our initial results validate this approach as applicable to individual canine dental follicles and demonstrate the existence of eruption-specific messages (ESMs) which vary as a function of eruption stage. We plan to use RNA differential display to identify ESMs through direct side-by-side comparisons of dental follicles from different stages with each other and with cultures of canine fibroblasts. This latter comparison will be used to select for those candidate ESMs not expressed by normal fibroblast cell

cultures. Dental follicle control of tooth eruption through regulation and coordination of regional bone resorption and bone formation is now the pre-eminent theory of tooth eruption. Our underlying hypothesis is that, in analogy with muscle and limb development, ESMs code for tooth eruption-specific regulatory proteins (i.e., transcription factors, regional growth factors) that function within pathways controlling alveolar bone resorption and bone formation.

The opportunities of tooth eruption for developmental biologists

We have summarized the current understanding of tooth eruption as a developmental process and described several candidate molecules which are known to affect it. The complexities of tooth eruption are likely to be revealed in comparative analyses of the differences in gene expression in and around the dental follicle as eruption proceeds. It is our expectation that these studies will show tooth eruption to be yet another example of the complex epithelial-mesenchymal interactions by which development is regulated. In addition, the local polarization of bone metabolism around erupting teeth represents a unique opportunity to study the local coordination (coupling) of bone formation and resorption. Finally, the discrete localization and bilateral symmetry of tooth eruption are other opportunities to study expression of genes governing temporal and spatial relationships, as illustrated by prior use of *hox* and *pax* genes to study axial development.

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