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Gene expression pattern

Dynamic *Lunatic fringe* expression is correlated with boundaries formation in developing mouse teeth

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Abstract

The formation of boundaries is a fundamental organizing principle during development. The Notch signalling pathway regulates this developmental patterning mechanism in many tissues. Recent data suggest that Notch receptors are involved in boundary determination during odontogenesis. It remains, however, uncertain if other components of the Notch pathway are also important for compartmental lineage restrictions in teeth. Here we report on the expression of the *Lunatic fringe* gene, which encodes a secreted signalling molecule regulating the Notch pathway, during the development of mouse teeth. *Lunatic fringe* is expressed in both epithelial and mesenchymal components of the developing molar. The expression pattern of *Lunatic fringe* in the epithelium is complementary to that of the Notch receptors. *Lunatic fringe* is asymmetrically expressed in the incisor epithelium during its antero–posterior rotation. This expression pattern defines the lingual comportment of the incisor epithelium whereas the labial comportment is defined by *Notch2* expression.

Keywords: Notch; Delta; Jagged; Lunatic fringe; Tooth; Dental; Incisor; Molar; Cell; Odontoblast; Ameloblast; Development; Embryonic; Asymmetry; Rotation; Pattern; Polarity; Organogenesis; Morphology; Rodent; Mouse; Signalling molecules; Epithelium; Mesenchyme; Tissue interactions; Cell fate; In situ hybridization; Boundary; Compartment

A reciprocal series of interactions between oral ectoderm and cranial neural crest-derived mesenchyme cells transform the tooth primordia into complex structures with various cell types. Our previous data suggest that dental cell specification is linked to the Notch signalling pathway (Mitsiadis et al., 1995, 1997, 1998), an evolutionarily conserved cell communication mechanism which enables neighbouring cells to adopt different fates (Artavanis-Tsakonas et al., 2000). In a more recent work we suggested that Notch is also implicated in the specification of boundaries in the developing incisor (Mucchielli and Mitsiadis, 2000). Fringe is a secreted protein which regulates the Notch pathway (Cohen et al., 1997; Micchelli and Blair, 1999) and plays a key role in defining borders in developing tissues, in both invertebrates and vertebrates (Johnston et al., 1997; Forsberg et al., 1998; Zhang and Gridley, 1998; Rauskolb et al., 1999). Three mouse *fringe* genes have been cloned and characterized: *Manic*, *Radical* and *Lunatic fringe* (Johnston et al., 1997).

1. Results and discussion

Lunatic fringe (*Lfng*) expression during mouse odontogenesis was examined by in situ hybridization with a digoxigenin-labelled probe. By E11.5, a faint *Lfng* signal is detected in a restricted part of the dental placode, a local thickening of the stomodeal epithelium (Fig. 1A). At E12.5, the dental epithelium has invaginated into the underlying mesenchyme and forms a bud around which the mesenchyme condenses. *Lfng* is weakly expressed in cells of the epithelial bud which are in close contact with the condensed mesenchyme (Fig. 1B). At the cap stage (E15), *Lfng* is strongly expressed in both epithelial and mesenchymal components of the developing molar: transcripts are detected in cells of the inner enamel epithelium and dental papilla mesenchyme (Fig. 1C). However, some territories of the inner enamel epithelium and the dental papilla are not expressing *Lfng* showing a compartmentalization of these two dental structures. Furthermore, the *Lfng*-expressing epithelial compartments abut the compartments of the dental mesenchyme where the gene is not expressed, and vice versa. Finally, a faint expression is also seen in the outer enamel epithelium. At the bell stage (E16–E18), *Lfng* is expressed in the outer and inner

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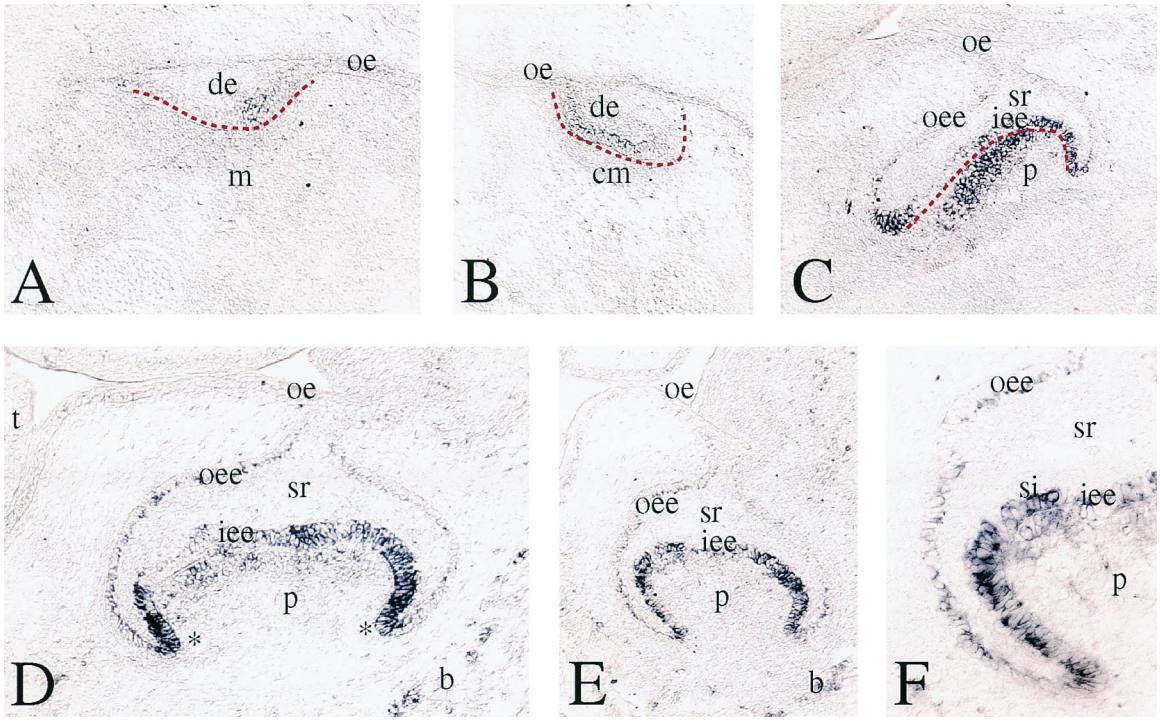

Fig. 1. Pattern of *Lunatic fringe* (*Lfng*) expression during mouse molar embryonic development. Frontal sections through the heads of E11.5–E17.5 mouse embryos. The red lines represent the borders between the dental epithelium and the underlying mesenchyme. (A) At E11.5, *Lfng* expression is restricted in some dental epithelial cells (de) adjacent to the mesenchyme (m). (B) At E12.5, transcripts are localized in dental epithelial cells adjacent to the condensed mesenchyme (cm). (C) At E15, *Lfng* is expressed in cells of the inner enamel epithelium (iee) and dental papilla mesenchyme (p). (D,E) From E16.5 to E17.5, *Lfng* transcripts are observed in cells of the inner enamel epithelium, outer enamel epithelium (oee), some cells of the dental papilla and of the alveolar bone (b). Note that expression is stronger in the inner enamel epithelium at the apical region (D, asterisks) of the molar. (F) Higher magnification of the Fig. 1E. Additional abbreviations: oe, oral epithelium; Mc, Meckel's cartilage; si, stratum intermedium; sr, stellate reticulum.

enamel epithelia of the molar (Fig. 1D–F). *Lfng* transcripts are also detected in some dental papilla cells next to the inner enamel epithelium, as well as in cells forming the alveolar bone.

The expression pattern of the Notch1 and Notch2 receptors in the molar primordia has been described previously (Mitsiadis et al., 1995,1998). Here we compared *Lfng* expression with the distribution of the Notch1 and Notch2 proteins in E16–E18 molars. At E16 (early bell stage), a particularly strong *Lfng* signal is observed in the inner enamel epithelium, whereas the Notch1 immunoreactivity is confined to the adjacent layer of cells forming the stratum intermedium (Fig. 2A). At E18 (late bell stage), *Lfng* is expressed in the inner enamel epithelium (Fig. 2B,C), whereas the Notch1 labelling is mainly detected in stratum intermedium cells at the apical region (Fig. 2B). A weaker

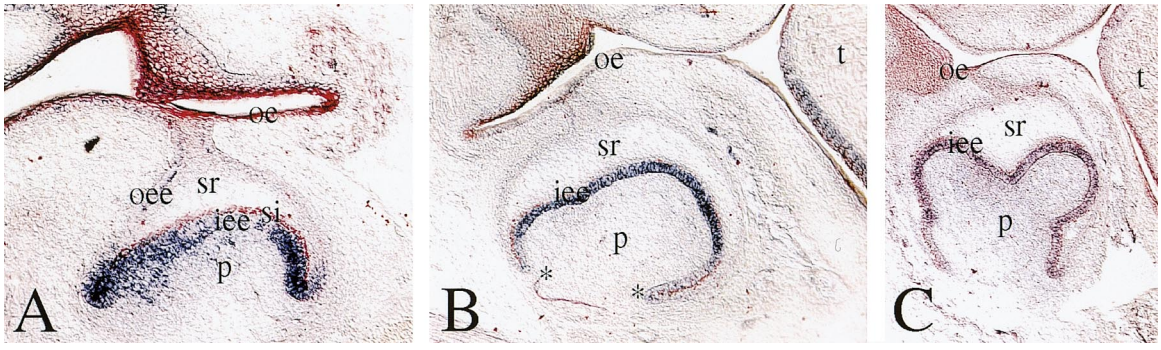

Fig. 2. Comparison between *Lfng* expression and Notch1 and 2 proteins distribution in a mouse molar at the early (A) and late bell stages (B,C). Photomicrographs of in situ hybridizations on cryosections using a digoxigenin-labelled probe (violet colour) followed by immunohistochemistry using anti-Notch antibodies (red colour) are shown. (A) At E16, *Lfng* is expressed in the inner enamel epithelium (iee), whereas the Notch1 protein is distributed in the adjacent layer of stratum intermedium cells (si). (B,C) At E18, *Lfng* transcripts are detected in the inner enamel epithelium, while Notch1 staining is observed in the stratum intermedium at the apical part (B, asterisks) and faint Notch2 reactivity is localized in stratum intermedium and in the upper part of the stellate reticulum (sr) (C). oee, outer enamel epithelium; oe, oral epithelium; p, dental papilla; si, stratum intermedium; t, tongue.

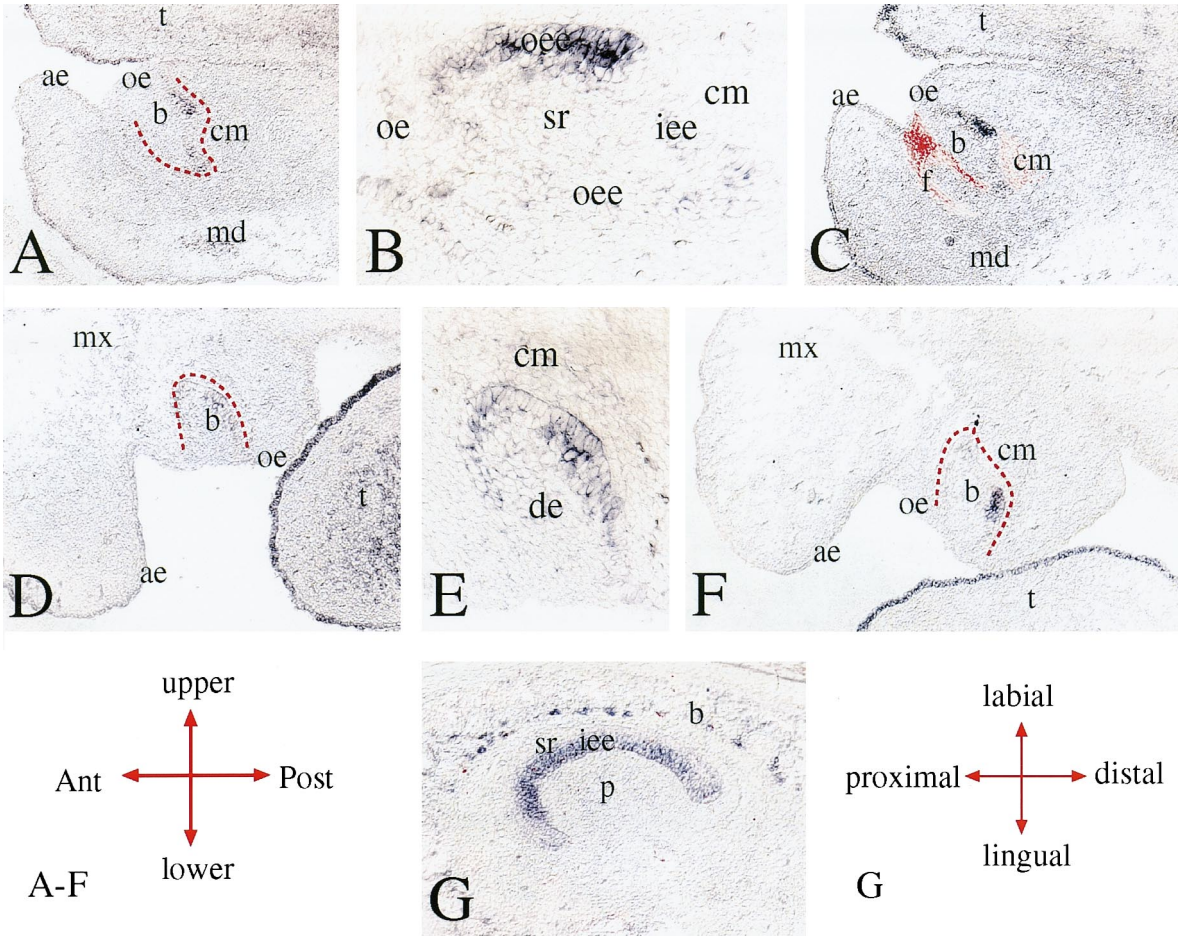

Fig. 3. Pattern of *Lfng* expression during the embryonic development of incisors. Longitudinal (A–F) and frontal (G) sections through the heads of E13.5 (A–E), E14 (F) and E18 (G) mouse embryos. Lower (A–C,G) and upper incisors (D–F). The red lines represent the borders between the epithelium and the mesenchyme of the incisors. (A) At E13.5, *Lfng* is expressed in the posterior part (Post) of the incisor epithelial bud (b). (B) Higher magnification of (A). (C) *Notch2* mRNA (in red) is seen in the anterior part (Ant) of the incisor epithelium, while *Lfng* transcripts (in violet) are found in its posterior part. (D) In the maxillary process, a faint *Lfng* signal is detected in the posterior part of the incisor epithelial bud. (E) Higher magnification of (D) showing few transcripts in the upper/anterior part. (F) *Lfng* is strongly expressed in the posterior part of an E14 incisor epithelium. (G) At E18, the *Lfng* signal is restricted to inner enamel epithelium (iee) located at the labial side of the incisor. Note also *Lfng* expression in the alveolar bone (b). ae, aboral epithelium; cm, condensed mesenchyme; f, incisor furrow; md, mandibular process; oe, oral epithelium; sr, stellate reticulum; p, dental papilla; t, tongue.

Notch2 staining is found in the upper part of the stellate reticulum and in the stratum intermedium (Fig. 2C).

Incisor development differs from that of molar. After the formation of the epithelial bud, the developing incisor rotates antero–posteriorly and becomes asymmetric at its labial–lingual axis. This asymmetry leads to the division of the incisor epithelium in two compartments, an enamel-forming compartment (labial side) and a cementum-forming compartment (lingual side). During incisor rotation, *Lfng* expression is restricted to epithelial cells located at the lingual side (Fig. 3A,B). In contrast to the expression of *Lfng*, *Notch2* expression is limited to epithelial cells at the labial side (Fig. 3C; Mucchielli and Mitsiadis, 2000). The upper incisor is developmentally retarded in comparison with the lower incisor. At this stage (E13.5), *Lfng* is strongly expressed in cells located at the posterior part of the incisor (Fig. 3D), whereas few transcripts are found at its anterior part (Fig. 3E). At E14, the signal is detected only at the posterior part of the incisor (Fig. 3F). These results show a Notch/Fringe-dependent separation of labial and lingual epithelial cells during incisor rotation. During the bell stage (E18), the incisor is not rotating anymore and the *Lfng* signal was observed at the opposite compartment of the incisor (labial side): expression was restricted in inner enamel epithelium cells (Fig. 3G) destined to become the enamel forming ameloblasts. Previous data have shown that expression of the Notch ligand Delta1 is also confined to the cells of the inner enamel epithelium, whereas the three *Notch* genes are expressed in the adjacent stratum intermedium layer (Mitsiadis et al., 1998). Taken together these findings demonstrate a segregation of two group of cells in dental epithelium, one group of cells expressing both *Delta1* and *Lfrg* and another group of cells expressing *Notch*.

2. Experimental procedures

Swiss mice were used at embryonic stages (embryonic day 11.5 to embryonic day 18; E11.5–E18). In situ hybridization on cryosections, using digoxigenin-labelled antisense riboprobes for mouse *Lunatic Fringe* (a kind gift of Dr JC Izpisúa-Belmonte) and *Notch2* (Mitsiadis et al., 1995), is performed as previously described (Mitsiadis et al., 1998). For immunohistochemistry, polyclonal antibodies against the mouse Notch1 and Notch2 proteins were used (Mitsiadis et al., 1995). In situ hybridization followed by immunohistochemistry was performed as previously described (Mitsiadis et al., 1998).

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