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Gene expression pattern

Correlation of asymmetric *Notch2* expression and mouse incisor rotation

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Abstract

Notch signaling defines an evolutionarily conserved cell communication mechanism, which enables neighboring cells to adopt different fates. Furthermore, Notch signaling may create boundaries that direct both the growth and patterning of the developing organs. Here we report on the expression of Notch receptors during the development of rodent incisors. Before the aquisition of their characteristic shape, incisors rotate antero–posteriorly and become asymmetric at their labial–lingual axis. *Notch2* is expressed only in the anterior part of the developing incisors, well before their rotation, while *Notch2* expression was symmetrical in the developing molars. This is the first demonstration of an asymmetric gene expression pattern during the rotation of the rodent incisors.

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

Teeth are organs that develop as a result of sequential and reciprocal interactions between the oral ectoderm and neural crest-derived mesenchyme. These interactions transform the tooth primordia into complex structures with various cell types. Initiation of mouse incisor and molar teeth starts at E11 as local thickenings (dental placodes) of the stomodeal epithelium which invaginate into the underlying mesenchyme and form the tooth buds by E13 (Fig. 1). At E14, the dental epithelium acquires the cap configuration and gives rise to the enamel organ.

While the initial stages of incisor development resemble those of the molars, soon after the formation of the epithelial bud, the developing incisors rotate antero–posteriorly and become parallel to the long axis of the jaws (Fig. 1). This 90° rotation is accompanied by a distinct morphological organization along the labial–lingual axis of the incisors: only the labial epithelium will give rise to the enamel-forming epithelium. Thus, the developing epithelium of the incisor, similarly to many developing epithelia, becomes not simply subdivided, but polarized. Many genes are involved in different stages of mouse teeth development (reviewed by Thesleff and Sharpe, 1997). The establishment of this planar polarity may be the result of coordinated gene action that directs the specification of a signaling center and controls the choice between different cell fates.

It is well established that the Notch signaling pathway enables neighboring cells to adopt different fates (Artavanis-Tsakonas et al., 1999). Furthermore, recent findings assign to the Notch signaling more instructive roles during development: Notch is involved in the formation of boundaries that direct organ growth and patterning (Cho and Choi, 1998; Dominguez and de Celis, 1998; Fanto and Mlodzik, 1999; Micchelli and Blair, 1999; Rauskolb et al., 1999). These studies have demonstrated that Notch has a role in the establishment of the *Drosophila* eye and wing polarity. Our previous data suggest that the Notch pathway is involved in dental cell specification (Mitsiadis et al., 1995, 1997, 1998). However, the same signaling pathway can be used to achieve another developmental goal such as the creation of a signaling center during the rotation of the incisors. Signals exchanged between large fields of cells through the Notch receptors can amplify and consolidate molecular differences (Artavanis-Tsakonas et al., 1999), influencing thus the rotation and the morphology of the incisor.

1. Results and discussion

Notch1 and *Notch2* expression during odontogenesis was examined by in situ hybridization with digoxigenin-labeled probes on cryosections. At the initiation stage (E11), *Notch1*

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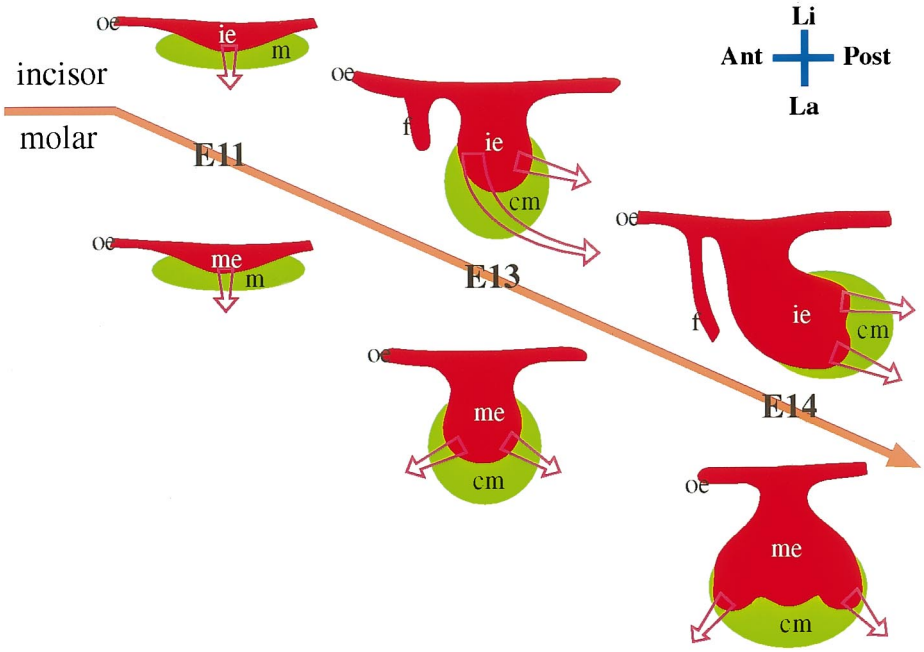

Fig. 1. Schematic representation of the mouse incisor and molar development during the initiation (E11), bud (E13) and cap (E14) stages. Dental epithelium in red and dental mesenchyme in green. The arrows represent the directed growth of the dental epithelium in incisors and molars. cm, condensed mesenchyme; f, dental follicle; ie, incisor epithelium; me, molar epithelium; m, mesenchyme; oe, oral epithelium; Li, lingual side; La, labial side; Ant, anterior side; Post, posterior side.

and *Notch2* transcripts are observed in epithelial cells of the developing incisor (Fig. 2). While *Notch1* expression is symmetric (Fig. 2A,C), *Notch2* transcripts are detected only in the anterior part of the invaginating epithelium (Fig. 2B,D). *Notch1* and *Notch2* are not expressed in epithelial cells contacting the mesenchyme.

During the rotation of the incisor (late bud stage/early cap stage; E13.5), *Notch1* is expressed in epithelial cells which

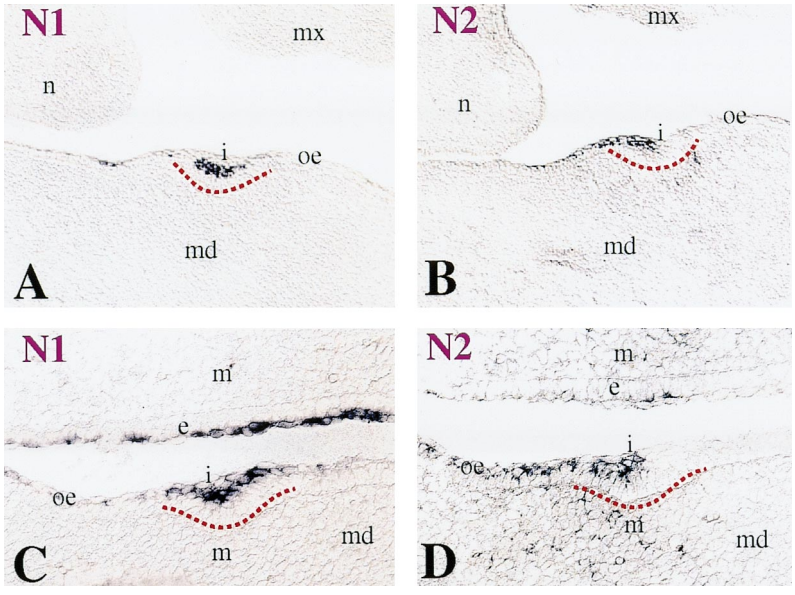

Fig. 2. Patterns of *Notch1* (*N1*) and *Notch2* (*N2*) expression during the initiation of the lower incisor development (E11). The red lines represent the borders between the epithelium of the incisors and the underlying mesenchymal tissues. (A) Symmetric *Notch1* expression in the middle of the incisor epithelium, and asymmetric *Notch2* expression in the anterior part of the epithelium (B). (C,D) Higher magnifications showing the symmetric *Notch1* expression (C) and the asymmetric *Notch2* expression (D) in the E11 incisors. Abbreviations: cm, condensed mesenchyme; e, epithelium; i, incisor epithelium; md, mandible; m, mesenchyme; mx, maxillary process; n, nose; oe, oral epithelium.

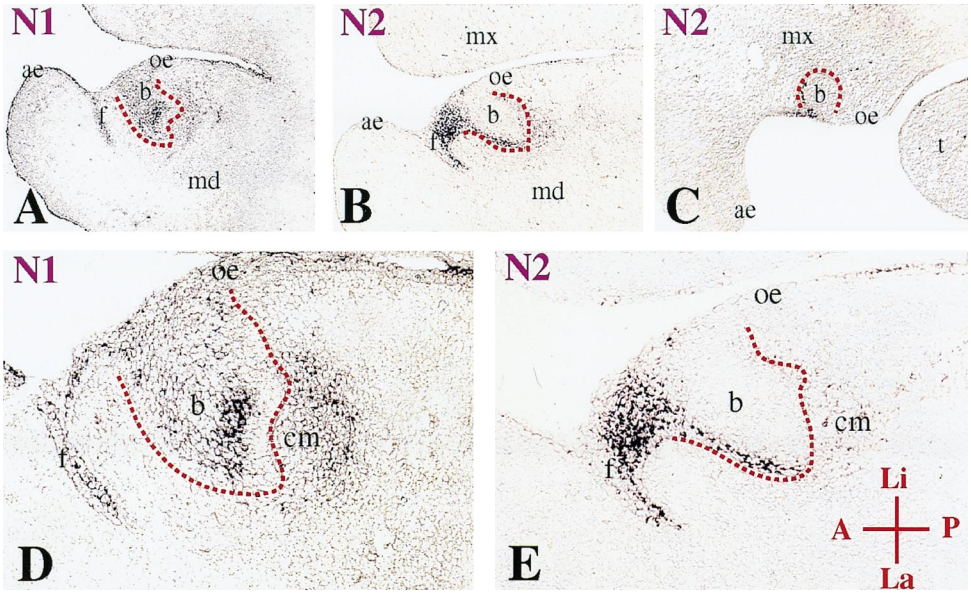

Fig. 3. Patterns of *Notch1* (*N1*) and *Notch2* (*N2*) expression during the bud and cap stages of incisor development. Longitudinal sections through the heads of E13.5 mouse embryos. Upper (C) and lower (A,B,D,E) incisors. The red lines represent the borders between the epithelium and the mesenchyme of the incisors. (A) *Notch1* is expressed in the epithelium of the incisor bud (b) during its rotation, as well as in the incisor furrow (f). (B) *Notch2* is expressed in the labial part of the incisor epithelium and in the lingual part of the incisor furrow. (C) *Notch2* mRNA is seen in the anterior part of the upper incisor epithelium. (D,E) Higher magnifications of the (A) and (B). Note that *Notch1* transcripts are absent from cells adjacent to the condensed mesenchyme (cm). ae, aboral epithelium; md, mandibular process; mx, maxillary process; oe, oral epithelium; t, tongue; Li, lingual side; La, labial side; Ant, anterior side; Post, posterior side.

are not in contact with the surrounding mesenchyme (Fig. 3A,D). The hybridization signal is also observed in epithelial cells of the dental furrow and in the condensing dental mesenchyme. By contrast to the expression of *Notch1*, *Notch2* expression is limited to cells located at the labial part of the incisor (Fig. 3B,E). It is worth noting that the *Notch2* expression in the incisor is the mirror image of the *Notch2* expression pattern in the dental furrow (i.e. lingual side). Few *Notch2* transcripts are also detected in the condensing mesenchyme. The upper incisor is developmentally retarded in comparison with the lower incisor and, at this stage, *Notch2* is expressed only in cells located at the anterior part of the incisor (Fig. 3C).

This asymmetric *Notch2* expression pattern is observed only in the primordia of the incisors. In the developing molars, expression of both *Notch1* and *Notch2* is symmetric (Fig. 4). At the initiation stage, *Notch1* and *Notch2* mRNA is detected in the middle of the epithelium (Fig. 4A,B). At the early cap stage, *Notch1* is expressed in cells of the forming stratum intermedium (Fig. 4C), while transcripts for *Notch2* are found in cells of the stellate reticulum (Fig. 4D).

In conclusion, these findings show that *Notch2* is asymmetrically expressed in the early incisor primordia prior to and during their rotation period.

2. Experimental procedures

Swiss mice were used at embryonic stages (embryonic day 11 to embryonic day 14; E11–E14). In situ hybridization on cryosections, using digoxigenin-labeled antisense

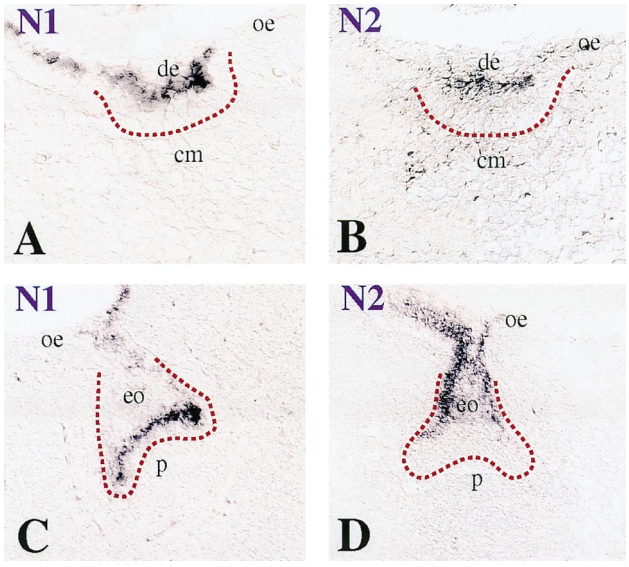

Fig. 4. Patterns of *Notch1* (*N1*) and *Notch2* (*N2*) expression during the initiation and cap stages of molar development. The red lines represent the borders between the epithelium and the mesenchyme of the molars. (A,B) At E11, symmetric *Notch1* (A) and *Notch2* (B) expression is detected in the middle of the dental epithelium (de). (C,D) At E14, *Notch1* expression in the enamel organ (eo) is restricted to cells of the forming stratum intermedium (C), while the *Notch2* transcripts are detected in cells of the forming stellate reticulum (D). Additional abbreviations: cm, condensed mesenchyme; oe, oral epithelium; p, dental papilla.

riboprobes for mouse *Notch1* and *Notch2* (Mitsiadis et al., 1995), is performed as previously described (Mitsiadis et al., 1997, 1998).

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