

# Morphogenesis of the lower incisor in the mouse from the bud to early bell stage

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**ABSTRACT** The development of the lower incisor in the mouse was investigated from histological sections using computer-aided 3D reconstructions. At ED 13.0, the incisor was still at the bud stage. At ED 13.5, the initial cap was delimited by a short cervical loop, the development of which proceeded on the labial side, but was largely retarded on the medial side. This difference was maintained up to ED 15.0. From ED 16.0, the bell stage was achieved. Metaphases had a ubiquitous distribution both in the enamel organ and in the dental papilla from the bud to early bell stage. Apoptosis gradually increased in the mesenchyme posteriorly to the labial cervical loop from ED 13.5 to 14.0 and then disappeared; this apoptosis was not related to the posterior growth of the incisor. From ED 13.5, a high apoptotic activity was observed in the stalk. A focal area of apoptosis was observed at ED 13.5 in the enamel organ, approaching the epithelio-mesenchymal junction at the future tip of the incisor. There, the inner dental epithelium formed a bulbous protrusion towards dental papilla, reminiscent of the secondary enamel knot of mouse molars. This epithelial protrusion was still maintained at the bell stage. The enamel knot in the incisor demonstrated specific features, different from those characterizing the enamel knot in the molar: the concentric arrangement of epithelial cells was much less prominent and the occurrence of apoptosis was very transitory in the incisor at ED 13.5. The disappearance of the enamel knot despite a low apoptotic activity and the maintenance of the protrusion suggested a histological reorganization specific for rodent incisor.

**KEY WORDS:** mouse, incisor, development, apoptosis, 3D reconstructions

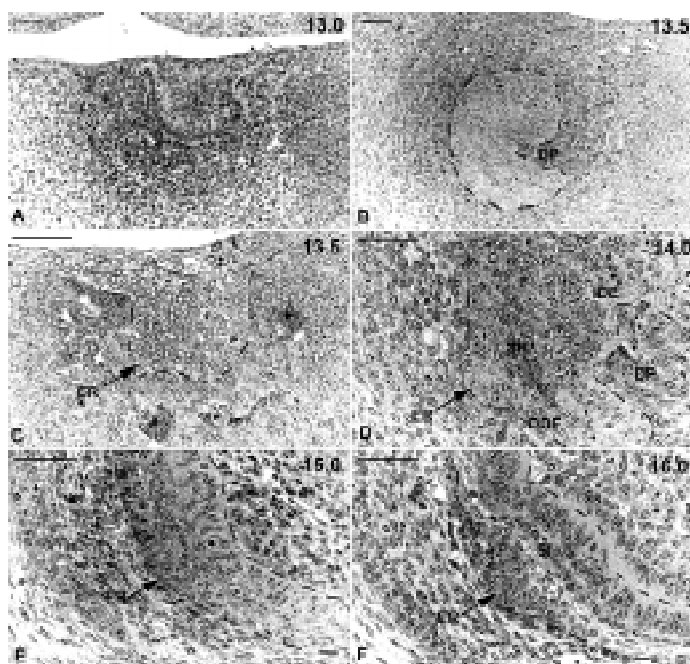
## Introduction

Dental morphogenesis is a complex multi-factorial process where differential mitotic activities, apoptosis as well as cell migration and cell adhesion may play an important role (Gaunt, 1955, 1956; Cohn, 1957; Butler and Ramadan, 1962; Ruch, 1984; Palacios *et al.*, 1995; Lesot *et al.*, 1996; Tureckova *et al.*, 1996; Vaahtokari *et al.*, 1996a; Fausser *et al.*, 1998; Peterková *et al.*, 1998). The number and the spatial distribution of the functional post-mitotic cells (odontoblasts, ameloblasts), specific for each type of tooth, determine the final shape of the crown. The functional mouse incisor shows a single cusp and enamel is present only on its labial side. The absence of enamel on its lingual surface has been suggested to result from a lack of competence of cells of the lingual inner dental epithelium (Amar *et al.*, 1987). The continuously growing rodent incisor is a widely used model to investigate

odontoblast cytodifferentiation (Hasty, 1983; Bishop and Boyde, 1986; Steinfort *et al.*, 1990) and ameloblast cytodifferentiation (DenBesten and Li, 1992; Nanci *et al.*, 1994; Matzaki *et al.*, 1995), as well as dentin and enamel matrix deposition (Cho and Garant, 1984; Yoshida *et al.*, 1998) and mineralization (Linde 1988; Robinson *et al.*, 1988, 1994; Smith *et al.*, 1989) along a simple antero-posterior gradient. Much less attention has been given to the morphogenesis of the rodent incisor. Hay (1961) has presented histological observations covering the period from embryonic day (ED) 12.0 to day 15.0 of postnatal incisor development in the mouse. Pourtois (1961) studied the development of the incisor dental bud in the mouse and Addison and Appleton (1915) the

*Abbreviations used in this paper:* ED, embryonic day; 3D, three dimensional.

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**Fig. 1.** Frontal histological sections at ED 13.0 (126-150 mg) (A); ED 13.5 (201-225 mg) (B,C); ED 14.0 (276-300 mg) (D); ED 15.0 (426-450 mg) (E) and ED 16.0 (664 mg) (F). DP, dental papilla; ER, epithelial ridge; SR, stellate reticulum; IDE, inner dental epithelium; ODE, outer dental epithelium; SI, stratum intermedium. Bar, 50 µm.

structure and growth of the rat incisor.

Systematic descriptive studies including also 3D evaluation of morphogenesis and distribution of mitosis and apoptosis, have permitted reinterpretation of several classical aspects of molar morphogenesis in the mouse (Lesot *et al.*, 1996; Peterková *et al.*, 1996; Viriot *et al.*, 1997). In the present work, the development of the lower incisor was investigated from the bud to early bell stage using serial histological sections and computer-aided 3D reconstructions. The distribution of metaphases and of apoptotic bodies were investigated to try to evaluate their role in morphogenesis.

## Results

Three aspects of the incisor morphogenesis will successively be described: the morphogenesis of the enamel organ, the morphogenesis of the anterior part of the dental papilla and the distribution of apoptosis in the mesenchyme.

### Morphogenesis of the enamel organ

#### ED 13.0

At this stage the dental epithelium was distinct from the vestibular lamina and exhibited a bud shape (Figs. 1A and 2A,B).

The dental mesenchyme was condensed at the periphery of the bud (Fig. 1A). However, moving away from the bud this condensation progressively decreased (Fig. 1A), making it difficult to distinguish the dental from the peridental mesenchyme on frontal histological sections. A clear condensation of the dental mesenchyme will not occur before ED 13.5.

Metaphases were ubiquitously distributed in the epithelium and

in the mesenchyme surrounding the bud (Fig. 2A-D). Only low apoptotic activity was observed within the oral epithelium (Fig. 2C,D).

#### ED 13.5

This chronological stage corresponded to the transition from the bud to the cap stage: two embryos at slightly different developmental stages were used to better illustrate this transition.

In the developmentally less advanced embryo, the cervical loop started to develop and extended posteriorly from the bud over 10 µm on the labial aspect of the tooth (Fig. 3A,B).

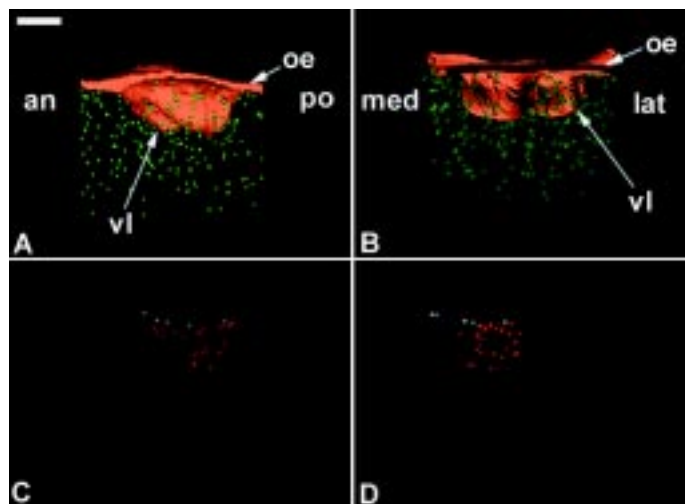
Very few metaphases were observed in the dental epithelium (Fig. 3C,D). In the mesenchyme surrounding the tooth bud, metaphases had a ubiquitous distribution (Fig. 3A,B). Apoptotic cells and bodies accumulated in the anterior part of the bud and in the adjacent oral epithelium (Fig. 3C,D).

In the developmentally more advanced specimen, the incisor had reached the cap stage and the cervical loop became obvious. The development of the cervical loop was more pronounced on the labial compared to the lingual side (Fig. 4A) and its growth was also delayed on the medial side (Fig. 4B,F).

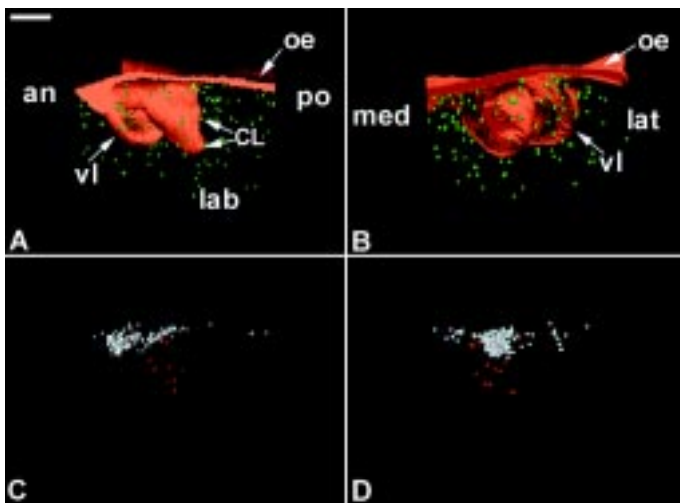
At this stage, the condensation of the dental mesenchyme increased and allowed its delimitation (compare Fig. 1B to Fig. 1A).

Initiation of histodifferentiation occurred in the anterior part of the tooth, allowing for the distinction of the prospective inner and outer dental epithelium (Fig. 5A).

Metaphases in the condensed dental mesenchyme were uniformly distributed (Fig. 4A,B). A similar situation was observed in the dental epithelium (Fig. 4C,D). Apoptotic cells and bodies were concentrated in two distinct areas in the epithelial compartment. The first was localized in the stalk which connected the anterior part of the enamel organ with the oral epithelium. In the second region, apoptosis was concentrated in the prospective inner dental epithelium close to the epithelio-mesenchymal junction. There the pro-



**Fig. 2.** 3D reconstructions of epithelium and distribution of mitoses and apoptosis of the lower incisor at ED 13.0 (126-150 mg) in medial views (A,C) and posterior views (B,D). Spatial distribution of mitoses in the mesenchyme represented as green spots (A,B), in the epithelium as red spots (C,D) and of apoptosis in the epithelium as white spots (C,D). An, anterior; po, posterior; vl, vestibular lamina; oe, oral epithelium; med, medial; lat, lateral. Bar, 100 µm.



**Fig. 3.** 3D reconstructions of epithelium and distribution of mitoses and apoptosis of the lower incisor at ED 13.5 (151-175 mg) in medial views (A,C) and posterior views (B,D). Spatial distribution of mitoses in the mesenchyme represented as green spots (A,B), in the epithelium as red spots (C,D) and of apoptosis in the epithelium as white spots (C,D). An, anterior; po, posterior; vl, vestibular lamina; oe, oral epithelium; med, medial; lat, lateral; lab, labial; CL, cervical loop. Bar, 100  $\mu$ m.

spective inner dental epithelium formed a bulbous protrusion towards dental papilla (Figs. 5A,B and 6). Frontal sections demonstrated a higher cell density in this specific region (Fig. 5A) whilst the epithelial arrangement of cells in contact with the basal membrane could only be detected on sagittal sections (Fig. 5B). The anterior and posterior limits of this structure were also observed on sagittal sections (Fig. 5B).

On the outer labial surface of the enamel organ a structure, which we termed the epithelial ridge, started to develop on the stalk (Fig. 4E). This epithelial ridge appeared in the anterior part of the tooth (Fig. 1C) and extended over 65  $\mu$ m in a posterior direction (Fig. 4E).

#### ED 14.0

The enamel organ elongated posteriorly, due to further extension of the labial and lingual parts of the cervical loop. Compared to the lingual aspect, the labial part progressed more rapidly, extending over 305  $\mu$ m posteriorly. As a result, the labio-lingual asymmetry of the cervical loop became still more pronounced (compare Fig. 7A to 4A). The medio-lateral asymmetry similarly increased during this period (compare Fig. 7F to 4F).

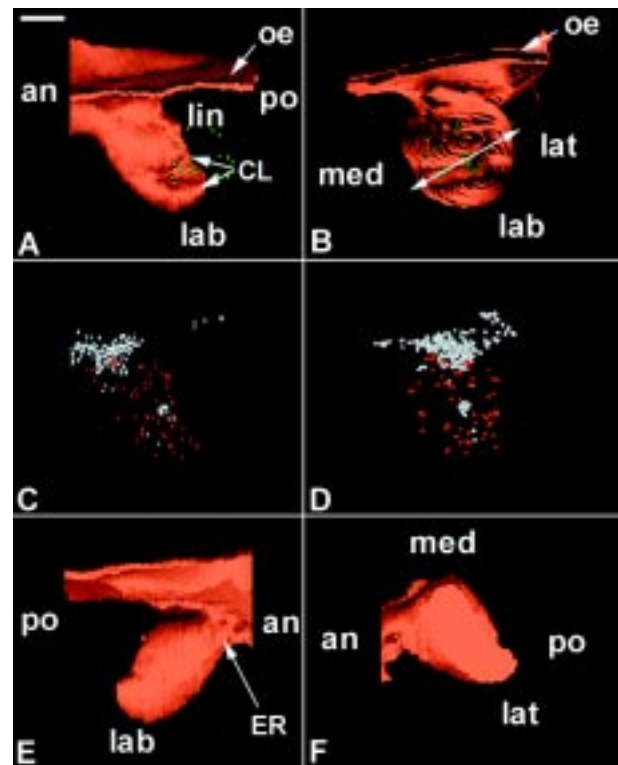
Metaphases were ubiquitously distributed in the dental mesenchyme and in the epithelium as well (Fig. 7A-D). At this stage again, apoptosis was concentrated in the stalk and in the oral epithelium (Figs. 7C,D and 10B,C). There was a sharp distinction between the dividing and the apoptotic zone, corresponding respectively to the cap itself and to the stalk (Figs. 7C,D and 10B,C). Another accumulation of apoptosis was also found in the oral epithelium above and behind the posterior end of the enamel organ (Fig. 7C,D). Almost no apoptosis was observed in the developing cervical loop (Fig. 7C). In sagittal sections, the enamel knot was characterized by condensation and concentric arrangement of epithelial cells (Fig. 5D). In frontal sections, the enamel knot protruded towards the mesenchyme (Fig. 5C) and was formed by

condensed cells (Fig. 5C). Its antero-posterior length reached 50  $\mu$ m. The distance between epithelial apoptosis located in the stalk and the anterior part of the dental papilla facing the enamel knot was about 30  $\mu$ m (Fig. 10B,C). Thus, at ED 14.0 there was no association of apoptosis with the enamel knot.

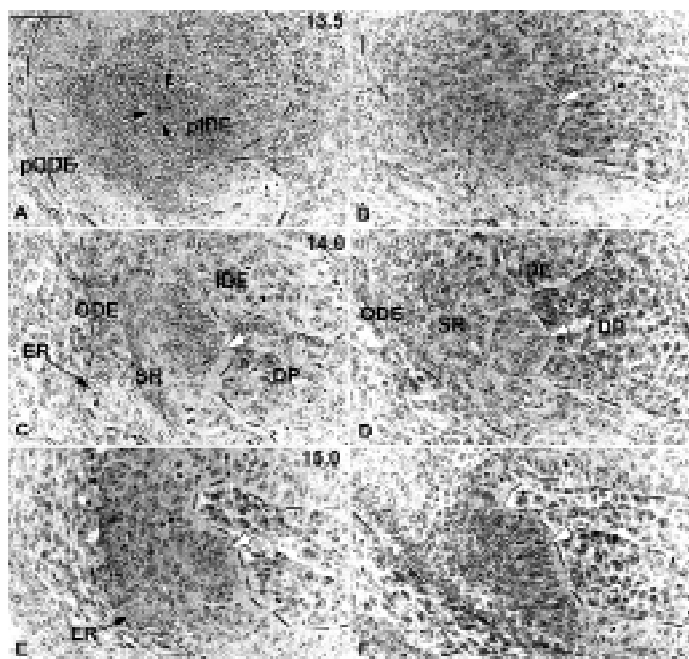
The epithelial ridge still existed and developed for 230  $\mu$ m along the tooth (Fig. 7E). In the outer labial surface of the enamel organ, the epithelial ridge formed a protrusion towards the mesenchyme (Figs. 1D and 5C). The external cells of the epithelial ridge, in contact with the basal membrane, were in continuity with the outer dental epithelial cells (Figs. 1D and 5C).

#### ED 15.0

In the well-formed cap, the labial and lingual parts of the cervical loop continued to develop in a posterior direction. The cervical loop extension was always more advanced in the labial and lateral parts (Fig. 8A,B,F). Metaphases were ubiquitously distributed in the dental mesenchyme and epithelium (Fig. 8A-D). Apoptosis was observed in the stalk and the oral epithelium (Figs. 8C,D and



**Fig. 4.** 3D reconstructions of epithelium and distribution of mitoses and apoptosis of the lower incisor at ED 13.5 (201-225 mg) in medial views (A,C) and posterior views (B,D). Spatial distribution of mitoses in the dental papilla, represented as green spots (A,B), in the epithelium as red spots (C,D) and of apoptosis in the epithelium as white spots (C,D). The lateral view (E) showed the epithelial ridge which development began at the base of the stalk. The virtual section (F) of the lingual part according to the plane represented by the arrow in Figure 5B, showed the beginning of the medio-lateral asymmetry. The vestibular lamina was voluntarily suppressed on the 3D reconstructions in order to better visualize the most anterior region of the incisor. an, anterior; po, posterior; oe, oral epithelium; med, medial; lat, lateral; lab, labial; lin, lingual; ER, epithelial ridge; CL, cervical loop. Bar, 100  $\mu$ m.



**Fig. 5.** Frontal histological sections at ED 13.5 (201–225 mg) (A); ED 14.0 (276–300 mg) (C); ED 15.0 (426–450 mg) (E) and corresponding sagittal histological sections at ED 13.5 (B); ED 14.0 (D); ED 15.0 (F). The black arrows showed the apoptosis present in the enamel organ at ED 13.5. The white arrows showed the epithelial protrusion of the EK towards the mesenchyme. DP, dental papilla; ER, epithelial ridge; pIDE, prospective inner dental epithelium; IDE, inner dental epithelium; pODE, prospective outer dental epithelium; ODE, outer dental epithelium; SR, stellate reticulum. Bar, 50  $\mu$ m.

10E,F) but the concentration of apoptotic bodies tended to decrease in the more posterior part of the oral epithelium (Fig. 8C,D). In frontal sections, the enamel knot decreased in length by about 50% at this stage and lost its histological characteristics: its cells became much less condensed (Fig. 5E,F) leading to a decrease in specific histological arrangement. At this stage the stratum intermedium did not yet exist (Fig. 5E,F).

The epithelial ridge extended over 805  $\mu$ m. It was well pronounced on the stalk and less marked in the most posterior part of the enamel organ (Fig. 8E). In the anterior part of the proper enamel organ the epithelial ridge was formed by a bulbous mass of dental epithelial cells (Fig. 5E) and in the more posterior part it was formed by three or four layers of dental epithelial cells (Fig. 1E).

#### ED 16.0

The bell stage was reached when the labial and lingual parts of the cervical loop became interconnected medially. The initial asymmetry between medial and lateral parts of the cervical loop was abolished whereas the extension of the labial part of the cervical loop was always more pronounced (Fig. 9A,B,F).

Metaphases in the epithelium and in the dental mesenchyme were uniformly distributed (Fig. 9A–D). Apoptosis was still present in the stalk and in the oral epithelium (Figs. 9C,D and 10H,I).

The epithelial ridge extended over 1000  $\mu$ m and was observed all along the tooth (Fig. 9E). The epithelial cells in the epithelial ridge were organized in a more concentric way (Fig. 1F). The

differentiation of the stratum intermedium started at this stage on the antero-labial side (Fig. 1F). At this early bell stage, odontoblast terminal differentiation had not been initiated.

Posterior view of the bell stage compared to that of the previous stages demonstrated a progressive rotation of the enamel organ around its antero-posterior axis (Fig. 9B compared to Figs. 2B,3B,4B,7B,8B).

#### Behavior of the anterior part of the dental papilla

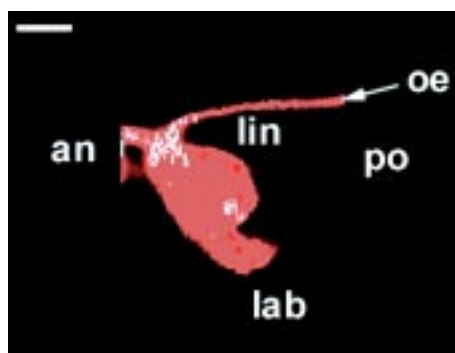
At ED 14.0, the lateral part of the anterior domain of the dental papilla formed a concavity (Fig. 10A,B). This concavity corresponded to the protrusion of the enamel knot towards the mesenchyme (Fig. 5C). At ED 15.0 this region stretched out all along the labial side of the tooth (Fig. 10D,E). At ED 16.0 the tip of the papilla was formed by a crater. Its margin was more developed on the lingual than on the labial side (Fig. 10G,H).

#### Apoptosis in mesenchyme

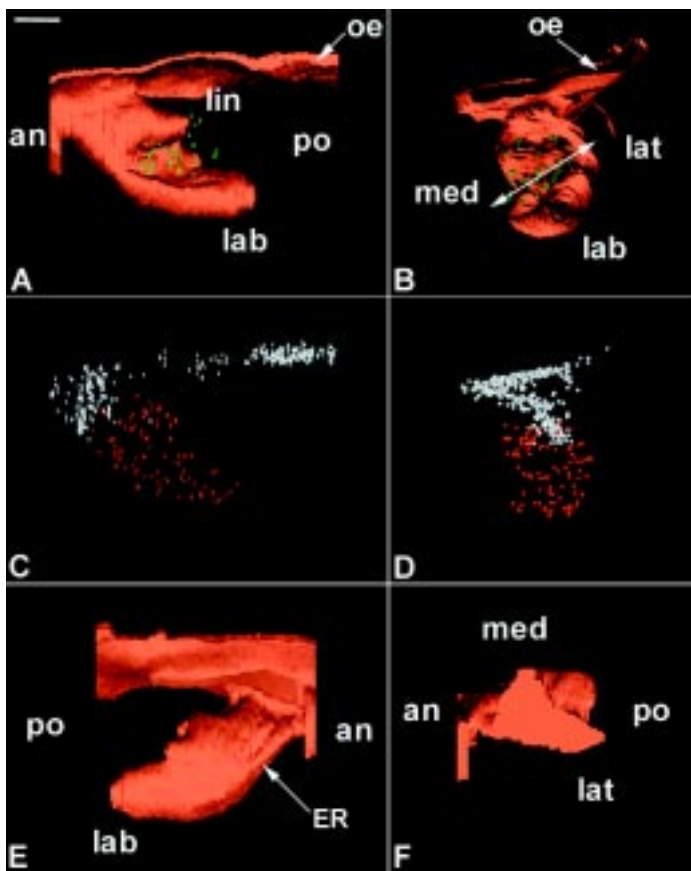
At ED 13.0, apoptosis appeared in the mesenchyme under the posterior extremity of the bud (Fig. 11A,B). At the late bud stage the number of apoptotic cells increased in a postero-lateral direction and stretched towards the non-dental mesenchyme for 135  $\mu$ m (Fig. 11C,D). From ED 13.5 to ED 14.0 the concentration of apoptosis decreased dramatically, being mainly focussed posteriorly to the labial cervical loop in the non-dental mesenchyme (Fig. 11E–H). At the late cap stage, only sparse apoptotic cells and bodies persisted in the mesenchyme (Fig. 11I,J).

#### Discussion

At ED 13.0, the incisor was still at the bud stage and the first signs of cervical loop formation appeared at ED 13.5. From ED 14.0 the tooth elongated and the labio-lingual and medio-lateral asymmetries appeared. The initial medio-lateral asymmetry ceased to be apparent at ED 16.0 (bell stage), whilst the labio-lingual asymmetry was maintained. The absence of enamel secretion on the lingual aspect of the incisor was suggested to result from a lack of competence of the inner dental epithelium to produce enamel in this region (Amar *et al* 1987; Nso *et al.*, 1992). Peterková *et al.* (1993b) showed that the upper incisor originated from five primary dental laminae (placode like structures), whose contributions appeared to differ along the antero-posterior axis of the incisor



**Fig. 6.** Virtual sagittal section, 45  $\mu$ m thick, of the 3D reconstruction at ED 13.5 (201–225 mg). Apoptosis in the epithelium were represented as white spots, metaphases in the epithelium as red spots. Lab, labial; lin, lingual; oe, oral epithelium; an, anterior; po, posterior. Bar, 100  $\mu$ m.



**Fig. 7.** 3D reconstructions of epithelium and distribution of mitoses and apoptosis of the lower incisor at ED 14.0 (276-300 mg) in medial views (A,C) and posterior views (B,D). Spatial distribution of mitoses in the dental papilla, represented as green spots (A,B), in the epithelium as red spots (C,D) and of apoptosis in the epithelium as white spots (C,D). The medio-lateral and labio-lingual asymmetries were maintained. The lateral view (E) showed the epithelial ridge. The virtual section (F) of the lingual part according to the plane represented by the arrow in Figure 7B showed the medio-lateral asymmetry. The vestibular lamina was voluntarily suppressed on the 3D reconstructions in order to better visualize the most anterior region of the incisor. an, anterior; po, posterior; oe, oral epithelium; med, medial; lat, lateral; lab, labial; lin, lingual; ER, epithelial ridge. Bar, 100  $\mu$ m.

epithelial anlage. Consequently, a different developmental origin between the labial and lingual sides of the upper incisor has been suggested (Peterková *et al.*, 1993b), which might explain the labio-lingual asymmetry. Such a situation might also exist in the lower incisor.

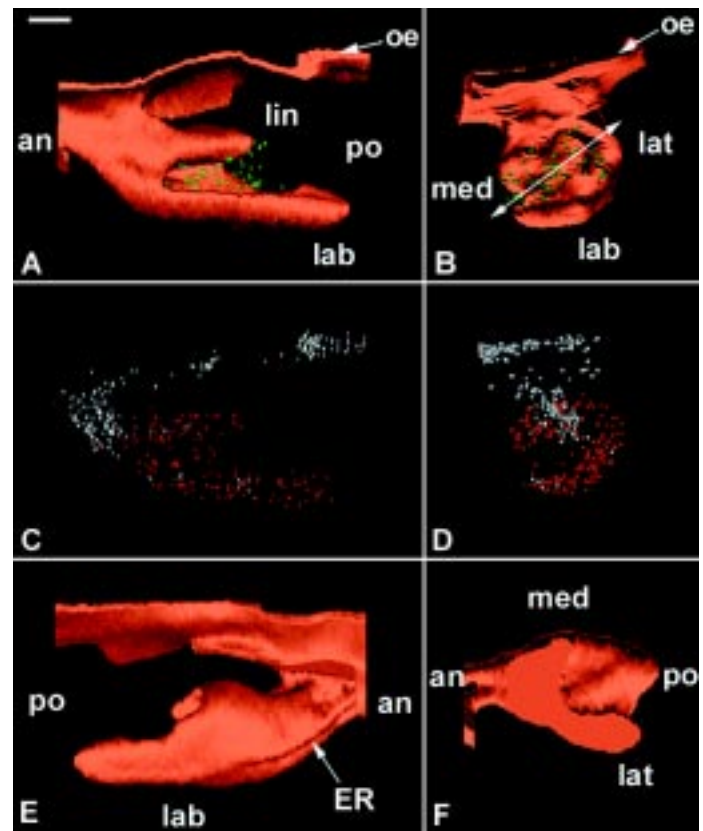
Tooth morphogenesis results from several mechanisms such as cell migration, changes in cell-cell or cell-matrix interactions (Palacios *et al.*, 1995; Salmivirta *et al.*, 1996; Fausser *et al.*, 1998; Yoshida *et al.*, 1998) as well as apoptosis and cell proliferation (Ruch, 1984; Lesot *et al.*, 1996; Tureckova *et al.*, 1996; Vaahtokari *et al.*, 1996a). In this work, we aimed to localize in 3-D reconstructed incisor the distribution of metaphases and apoptosis in the dental epithelium and mesenchyme from bud to early bell stage.

From the bud to the bell stage, metaphases in the enamel organ and in the dental papilla were ubiquitously distributed. However, these observations cannot be directly correlated to mitotic activity as with measurement of mitotic indices (Osman and Ruch, 1976).

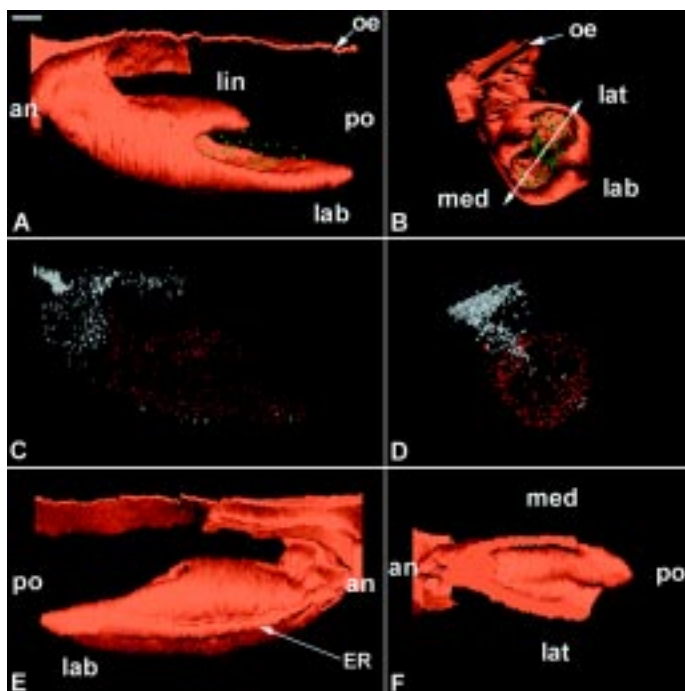
These authors demonstrated a variation of mitotic indices in the epithelium depending upon the developmental stage and the part of the tooth studied (Osman and Ruch, 1976). According to this study, from ED 14.0 the mitotic activity increases in the epithelium in apical direction. From ED 15.0 the mitotic activity is significantly higher in the inner dental epithelium (IDE) than in the outer dental epithelium (ODE). From ED 16.0, few mitoses can be observed in the anterior part of the tooth and their number increases in the posterior part (Osman *et al.*, 1976).

Apoptosis in the epithelial compartment was predominantly localized in the stalk. In this region, apoptosis was observed from the initial cap stage although this structure is maintained until tooth eruption. A similar focus of apoptosis has been observed in the stalk of upper and lower mouse molars although at the bell stage only (Lesot *et al.*, 1996; Viriot *et al.*, 1997).

At the early incisor cap stage (ED 13.5), an apoptotic zone was observed in the enamel organ at the future tip of the tooth, close to the epithelio-mesenchymal junction, before any histological arrangement for the enamel knot could be detected. The absence of



**Fig. 8.** 3D reconstructions of epithelium and distribution of mitoses and apoptosis of the lower incisor at ED 15.0 (426-450 mg) in medial views (A,C) and posterior views (B,D). Spatial distribution of mitoses in the dental papilla, represented as green spots (A,B), in the epithelium as red spots (C,D) and of apoptosis in the epithelium as white spots (C,D). The lateral view (E) showed the epithelial ridge. The virtual section (F) of the lingual part according to the plane represented by the arrow in Figure 8B showed the medio-lateral asymmetry. The vestibular lamina was voluntarily suppressed on the 3D reconstructions in order to better visualize the most anterior region of the incisor. an, anterior; po, posterior; oe, oral epithelium; med, medial; lat, lateral; lab, labial; lin, lingual; ER, epithelial ridge. Bar, 100  $\mu$ m.

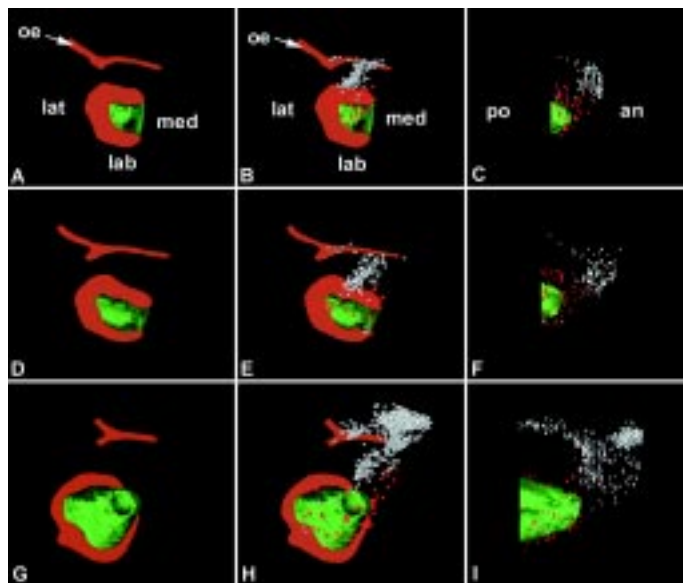


**Fig. 9.** 3D reconstructions of epithelium and distribution of mitoses and apoptosis of the lower incisor at ED 16.0 (664 mg) in medial views (A,C) and posterior views (B,D). Spatial distribution of mitoses in the dental papilla, represented as green spots (A,B), in the epithelium as red spots (C,D) and of apoptosis in the epithelium as white spots (C,D). The lateral view (E) showed the epithelial ridge. Virtual section (F) of the lingual part according to the plane represented by the arrow in Figure 9B showed the medio-lateral asymmetry abolition. The vestibular lamina was voluntarily suppressed on the 3D reconstructions in order to better visualize the most anterior region of the incisor. an, anterior; po, posterior; oe, oral epithelium; med, medial; lat, lateral; lab, labial; lin, lingual; ER, epithelial ridge. Bar, 100  $\mu$ m.

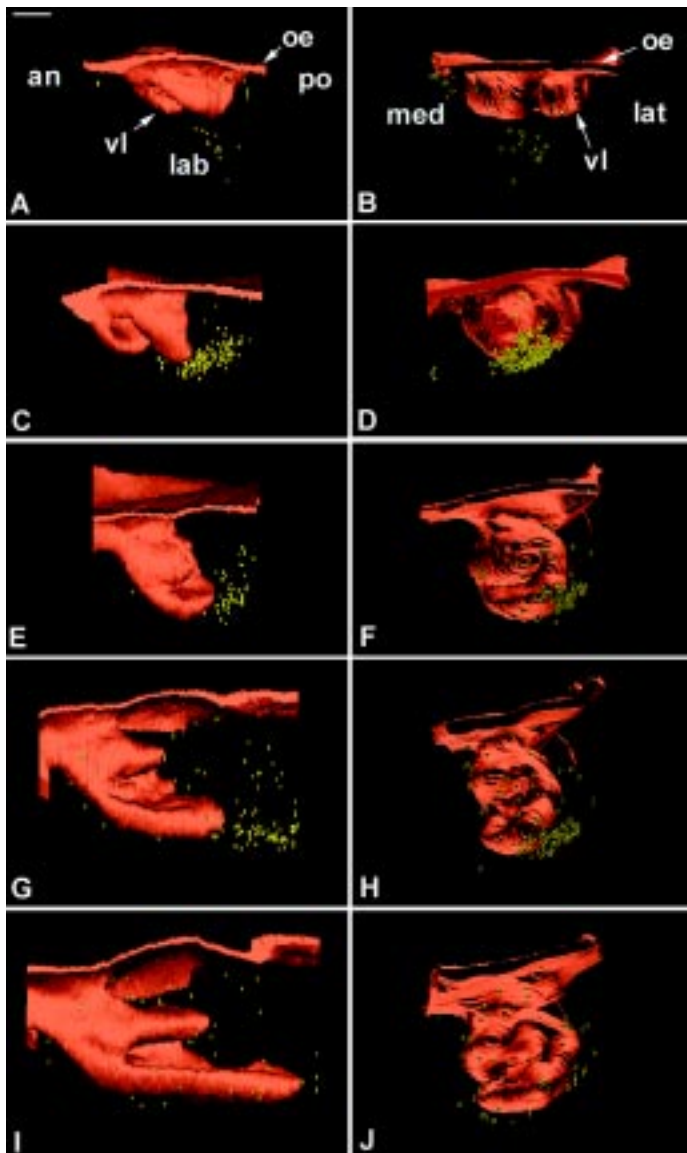
dividing cells and the presence of few apoptosis in this condensed prospective inner dental epithelial cells were reminiscent of the secondary enamel knots at the future molar cusp tip regions (Jernvall et al., 1994; Vaahokari et al., 1996a). Moreover, 12 h later when the primary enamel knot structure was formed, this apoptosis completely disappeared. The enamel knot is a structure which presents different histological appearances according to the tooth class and the species studied. In the mouse, cells of the enamel knot were found to express several signaling molecules (Shh, BMP-2, 4, 7, FGF-4) (Jernvall et al., 1994, 1998; Vaahokari et al., 1996a,b; Keranen et al., 1998) and therefore the enamel knot was suggested to act as an organizing center for tooth morphogenesis. According to these authors, these signals of the primary enamel knot induced the formation of the secondary enamel knots. Recently, Coin et al. (1999) proposed that the primary enamel knot comprising non cycling cells and intercalated cycling cells, presented a cellular continuity with the secondary enamel knots instead of inducing it. The enamel knot has been described in mouse molars as a transitory structure with a specific arrangement of the cells and accumulation of apoptotic activity (Lesot et al., 1996). In the lower molar, the primary enamel knot appeared at ED

14.0, extended at ED 15.0, and was still maintained during the cap-bell transition (Viriot et al., 1997; Lesot et al., 1999). Compared to upper and lower first molars, the life span of the enamel knot was shorter in the lower incisor. In the incisor, the enamel knot appeared at ED 14.0 and considerably decreased at ED 15.0. The concentric arrangement of enamel knot cells in the incisor was less prominent than in the molar. Depending on whether sagittal or frontal sections were analyzed, the enamel knot in the incisor did not present the same appearance. This might be correlated with the curvature of the epithelio-mesenchymal junction on the bottom of the enamel organ where the enamel knot is located. In the first lower molar, apoptosis in the enamel knot was observed from ED 14.0 to ED 15.5 (Viriot et al., 1997; Lesot et al., 1999). The fact that no or little apoptosis was found in the enamel knot in the incisor was particular to this tooth. It suggested that different types of cell populations, as far as proportion of living/quiescent/dying cells is concerned (Coin et al., 1999), exist in the enamel knot of the incisor, when compared to that of a molar (Lesot et al., 1996). The disappearance of the enamel knot in the incisor might result from reorganization of its cells at the histological level. The absence of apoptosis in the enamel knot could explain the persistence of the bulbous projection of the bottom of the enamel organ long after the enamel knot itself had disappeared. The sequence of events in the incisor enamel knot is different from that reported in the lower and upper first molars (Lesot et al., 1996, 1999). The existence of an enamel knot has already been reported in the human incisor and its position related to the incisor margin (Nozue, 1971). However, the absence of an enamel free area in the human suggests that the fate of enamel knot cells in the latter case is different to that observed in the mouse.

The mesenchymal concavity formed on the anterior part of the



**Fig. 10.** 3D reconstructions of the anterior part of the dental papilla at ED 14.0 (A-C), ED 15.0 (D-F) and ED 16.0 (G-I). The dental papilla was represented in green and the epithelium at the posterior position of the mesenchyme in brown. Spatial distribution of epithelial mitoses (red spots) and of epithelial apoptosis (white spots) were represented, in spite of the epithelium itself was not visualized here. oe, oral epithelium; lat, lateral; med, medial; po, posterior; an, anterior.



**Fig. 11.** 3D reconstructions of epithelium and distribution of mesenchymal apoptosis (green spots) in medial (A,C,E,G,I) and posterior (B,D,F,H,J) views at ED 13.0 (126-150 mg) (A,B); at ED 13.5 (151-175 mg) (C,D); at ED 13.5 (201-225 mg) (E,F); at ED 14.0 (276-300 mg) (G,H); at ED 15.0 (426-450 mg) (I,J). An accumulation of apoptosis appeared in the mesenchyme posteriorly to the labial cervical loop from ED 13.5 to ED 14.0 and then disappeared. The vestibular lamina was voluntarily suppressed on the 3D reconstructions from ED 13.5 (201-225 mg). *an*, anterior; *po*, posterior; *lab*, labial; *vl*, vestibular lamina; *oe*, oral epithelium; *med*, medial; *lat*, lateral. Bar, 100  $\mu$ m.

papilla reflected the bulbous protrusion on the bottom of the enamel organ, vaulted by the enamel knot at earlier stages. In fact, the enamel knot disappeared as a distinct histological structure at ED 15.0 before the crater was achieved. The papilla concavity moved from a labio-lateral position at ED 14.0 to the papilla tip at ED 16.0. The protrusion in the incisor was maintained at the bell stage. The crater observed at ED 16.0 corresponded to the future enamel free area of the incisor.

The combined analysis of frontal sections and 3D reconstruc-

tions allowed us to bring to the fore the existence of an epithelial ridge which could not be detected in sagittal sections. The epithelial ridge appeared at the cap stage when the histodifferentiation of the enamel organ occurred and disappeared at the late bell stage when differentiation of functional cells was achieved. The epithelial ridge was more developed at the late cap stage and at the early bell stage. At the late bell stage (ED 17.0 and ED 19.0) this epithelial ridge disappeared progressively by apoptosis and was no longer present in the posterior part of the incisor when eruption had started (unpublished data). From the cap to late bell stage, the epithelial ridge was facing the future ameloblast cells on the labial side of the enamel organ. The role of this structure remains unknown but hypothetically the epithelial ridge could be involved in the ameloblast differentiation at the cap and/or at the bell stage.

From the late bud stage (ED 13.5) to ED 14.0, apoptosis was focussed in the mesenchyme close to the posterior part of the tooth and disappeared from ED 15.0. Apoptosis was observed to be maximal at the late bud stage (ED 13.5). At ED 14.0 apoptosis tended to decrease in this area. The mesenchymal apoptosis did not seem to be a prerequisite for posterior elongation of the incisor enamel organ. In fact, they were not observed posteriorly to the lingual cervical loop and they disappeared before the maximal elongation of the cervical loop started in a posterior direction. In the maxilla, no apoptosis was found in the mesenchyme posteriorly to the incisor germs in the region of the diastema. Thus, the apoptosis present in the mandible might not be related to the disappearance of the dental mesenchyme of the diastema but they could be related to bone or nerve cells (Mina *et al.*, 1995), based on their location. Attempts will now have to be made to identify the cells involved in this apoptotic process in the mesenchyme.

## Materials and Methods

The ICR females were mated overnight, and the midnight before morning detection of the vaginal plug was taken as embryonic day (ED) 0.0. The embryos were harvested at noon and midnight from ED 13.0 to 16.0. The chronological age of embryos was specified in more detail by their wet body weight (Peterková *et al.*, 1993a). The specimens weighing up to 500 mg were distributed in 25 mg weight classes and larger specimens (up to 1000 mg) in 50 mg weight classes. The embryos were fixed in Bouin-Hollande fluid. At least one specimen from each weight class at each chronological stage was chosen and its head processed for histology.

### Histology

Series of 5  $\mu$ m frontal and sagittal sections from paraffin embedded heads were stained with alcian blue-hematoxylin-eosin.

### Mitoses and apoptoses

When representing mitoses, only metaphases were taken into account. Apoptotic cells and bodies were identified from histological sections on the basis of morphological criteria (Kerr *et al.*, 1995; Turecková *et al.*, 1996); their nature has previously been confirmed using the TUNEL method (Turecková *et al.*, 1996).

### 3D reconstructions

The contours of the mandibular dental and adjacent oral epithelium were drawn from serial frontal histological sections (5  $\mu$ m intervals) using a Leica microscope equipped with a drawing chamber at a magnification of 320x. Mitoses were recorded in the dental epithelium and mesenchyme and apoptosis in the epithelium and in the mesenchyme. The digitalization of the serial drawings and correlation of successive images (Olivo *et al.*,

1993) have been previously described (Lesot *et al.*, 1996). Software packages allowing image acquisition and treatment were developed and adapted to this work. Three-dimensional images were generated using a volume rendering program (Sun Voxel, Sun Microsystems).

### Length

The length of the tooth was determined along the antero-posterior axis by counting the number of 5 µm serial histological sections.

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