The relationship between the cusp pattern and plural stem cell compartments in guinea pig cheek teeth by chasing BrdU-labeling*

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Summary. Continuously growing rodent incisors have a special epithelial structure for maintaining adult stem cells that shows a bulbous epithelial protrusion at the apical end and is referred to as an "apical bud". Guinea pig cheek teeth (premolars and molars), also continuously growing teeth, have a complex crown shape consisting of plural cusps. The present study clarifies the existence of apical buds in guinea pig premolars/molars as it examines the relationship between the crown shape and the orientation of the apical buds by micro-computed tomography (μ-CT) and immunohistochemistry for 5-bromo-2'-deoxyuridine (BrdU). One premolar and three molar teeth in each side of the maxillae and mandibles assumed characteristic features: each horizontally-sectioned tooth showing a complex zigzag shape was composed of a core of dentin covered by a layer of enamel on all axial surfaces except the buccal of the uppers and the lingual of the lowers. Furthermore, four bulbous epithelial protrusions— including the stellate reticulum— were recognized in the apical end of each tooth, where slow-cycling long-term label-retaining cells resided 20 days after a peritoneal injection of BrdU. These data indicate that guinea pig premolars/molars have four apical buds where the epithelial adult stem cells reside. In contrast, rodent incisors, which show a single cone appearance, are covered by enamel on the labial side and possess only one apical bud. The results of this study suggest that plural apical buds, being arranged bucco-lingually and mesio-distally, produce the crown mold in a zigzag fashion.

Introduction

Rodent incisors are continuously growing teeth, for which all stages of odontogenesis—including amelogenesis and dentinogenesis—can be surveyed if the sections of the tooth are prepared from the apical end to the incisal edge (Smith and Warshawsky 1975, 1976; Ohshima and Yoshida 1992). This phenomenon is

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maintained by both the cell-proliferation at the apical end and the attrition of the incisal edge. Recent molecular biological studies have demonstrated the existence of a niche for self-renewing adult stem cells in these rodent incisors as well as the molecular mechanism regulating the maintenance and cell fate decision of adult stem cells by epithelial-mesenchymal interaction through fibroblast growth factor (FGF) signaling (Harada et al., 1999, 2002a; Yokohama-Tamaki et al., 2006). The term "cervical loop" has been used to indicate the epithelial tissue situated at the proliferative end of the rodent incisor (Harada et al., 1999, 2002a; Kieffer-Combeau et al., 2001; Tummers and Thesleff, 2003; Wang et al., 2007). However, the "cervical loop" refers to the junctional zone where the inner enamel epithelium meets the external enamel epithelium at the rim of the enamel organ (Nanci, 2008). We compared the apical end of the rodent incisor to the bud-, cap-, and bell-stage-tooth germs in teeth of limited growth, and renamed the cervical loop as the "apical bud" (Ohshima et al., 2003, 2005; Harada and Ohshima, 2004). These structures were composed of the cells of basal epithelium and stellate reticulum. Furthermore, the three-dimensional features of the apical epithelial components obtained by scanning electron microscopy (SEM) clearly revealed that the bud stage-tooth germ appears as a human head-like structure in the apical end. The findings indicate that the tooth bud corresponding to the bud stage in the developing tooth germ is continually maintained at the apical end of the dental epithelium. Thus, the "apical bud" is defined as a special epithelial structure at the apical end of continuously growing teeth. It is composed of the cells of the basal epithelium and stellate reticulum, and houses adult stem cells. Nowadays, the term "apical bud" has gradually come to be used for indicating the bulbous part in the apical end of rodent incisors (Nishikawa, 2005; Merzel and Novaes, 2006; Yu et al., 2006, 2007, 2008; Sawa et al., 2008; Wu et al., 2008) although the concept of the "apical bud" is still controversial.

The stem cells divide slowly to give rise to one daughter cell, which remains in the apical region, and another cell, which enters the zone of rapidly dividing inner enamel epithelial cells (transit amplifying cell population) to differentiate into ameloblasts and deposit the enamel matrix. Previous cell proliferation assays in rodent incisors confirmed the presence of the stem cells as the source of cells for continuous growth of this type of tooth (Smith and Warshawsky, 1975, 1976, 1977; Smith, 1980; Nakasone et al., 2006). Harada et al. (1999) demonstrated the existence of self-renewing adult stem cells in the apical region by molecular biological techniques. Furthermore, our recent cell kinetic studies by a double staining of 5-bromo-2'-deoxyuridine (BrdU) and Ki67 as the markers of dividing cells clearly suggested the presence of adult stem cells in the apical end of rodent incisors (Harada and Ohshima, 2004).

The continuously growing teeth are represented not only by rodent incisors but also by cheek teeth (premolars and molars) in certain other species, including rabbits, guinea pigs, and voles. In these animals, the dental epithelium has structural similarities (Starkey, 1963; Harada et al., 2002b; Tummers and Thesleff, 2003; Huysseune and Thesleff, 2004; Ohshima et al., 2005). These specific structures are composed of a large amount of stellate reticulum and basal epithelium. One can therefore postulate that apical buds exist in the apical ends of the teeth in these animals. For example, guinea pigs possess this type of special epithelium in the apical end of premolars/molars (Hunt, 1959; Hunt and Paynter, 1963; Nataatmadja et al., 1991; Harada et al., 2002b; Jayawardena and Takano, 2004), but how the adult stem cells differentiate to become functional cells is unclear (Mataatmadja et al., 1991). Furthermore, there is no available data on the relation between the number of apical buds and the morphology of the continuously growing teeth.

Thus the present study clarifies the existence of apical buds in guinea pig premolars/molars as it examines the relationship between the crown shape and the orientation of apical buds by micro-computed tomography (μ-CT) and immunohistochemistry for BrdU.

Materials and Methods

Tissue preparation

All experiments were reviewed by the Committee on the Guidelines for Animal Experimentation of Niigata University and performed according to the recommendations or under the conditions proposed by the Review Committee. One-day- to 4-week-old guinea pigs were given an intraperitoneal injection of BrdU (150 mg/kg) 2 h, and 5, 10, 20 days before the fixation (4 groups of 2 animals each) and transcardially perfused with physiological saline followed by 4% paraformaldehyde in a 0.1M phosphate buffer (pH 7.4) under deep anesthesia by an intraperitoneal injection of chloral hydrate (350 mg/kg) (Table 1). The maxillae and mandibles including premolar and molar teeth were dissected and immersed in the same fixative for an additional 6 hours. Following decalcification in a 10% ethylenediaminetetraacetic acid (EDTA) solution for 4 weeks at 4°C, the specimens
Table 1. Ages of animals when sacrificed 2 hours, and 5, 10, 20 days after an intraperitoneal injection of BrdU (n: number of samples examined).

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Fig. 1. A diagram showing three-dimensional and transverse views at different positions (a–e) of a guinea pig mandibular premolar or molar where part of the lingual side has been sagittally removed to show the cut surfaces of dentin (D), dental pulp (DP), enamel (E), enamel organ (EO) and cartilage-like cementum (*). Four apical buds, i.e., mesial, distal, intercuspal, and lingual apical buds, exist in the apical end of a guinea pig premolar/molar, although all apical buds are never observed at the same time on the same section. See Figure 9 for the detailed observation of the apical view.
were dehydrated through a graded series of ethanol and embedded in paraffin. Serial horizontal, sagittal, and frontal sections of the premolars and molars, 5 μm thick, were stained with hematoxylin and eosin (H-E).

**Analyses by micro-computed tomography (μ-CT) and soft X-ray**

Micro-CT analysis (Elescan; Nittetsu Elex, Tokyo) and Soft X-ray (Sofron type SRO-405; Softex, Kanagawa) were performed to examine the morphological features of premolar/molar teeth before the decalcification of samples. The CT settings were as follows: pixel matrix, $256 \times 256 \times 256$; slice thickness, 31 μm; projection number, 600 × 32; magnification, $\times 2.68$; voltage, 80 kV; electrical current, 66 μA. The mandibles were reconstructed using a software program (NDTView, Sony, Tokyo; 3D bone for WinNT, Ratoc System Engineering, Tokyo) to evaluate the three-dimensionally (3D)-reconstructed and sagittal- and horizontal-views of the teeth with their surrounding bone (3D-virtual sections).
Stem cell compartments in guinea pig cheek teeth

Results

Morphological features of continuously growing teeth

Guinea pig premolars/molars assume unique features with a longitudinal, deeply folded groove on each buccal and lingual side (Fig. 1). All surfaces of the tooth are covered by enamel except the buccal of the uppers and the lingual of the lowers (Hunt, 1959; Moriyama et al., 2006). Thus, the morphology of the upper premolars/molars shows an opposite relation in comparison with that of the lowers; both Hertwig’s epithelial root sheath (HERS) and an acellular cementum layer exist in the buccal side of the uppers, whereas they exist in the lingual side of the lowers (Fig. 1, 9).

Immunohistochemical analysis

For the immuno-peroxidase procedure, sections were processed for BrdU cell proliferation assay using Calbiochem BrdU Immunohistochemistry System (EMD Biosciences, Darmstadt, Germany). The immunostained sections were counter-stained with 0.05% methylene blue. The brown color in BrdU-labeled cells in Figure 7 was altered to red and graphically emphasized by using graphic software (Adobe Photoshop CS for Windows; Adobe Systems Incorporated, San Jose, CA, USA). Immunohistochemical controls were performed by: 1) replacing the primary antibody with non-immune serum or PBS; and 2) omitting the streptavidin-peroxidase. These immunostained sections contained no specific immunoreaction product.

Fig. 3. Sagittally-sliced views at different positions from the buccal (a) to lingual (l) direction of a 5-day-old guinea pig mandible reconstructed by μ-CT (P: premolar, M1: first molar, M2: second molar, M3: third molar). Two small X-ray-translucent areas (arrows) are recognized in the buccal side of each tooth at the base of a mandible without virtual cutting (a), and each area corresponds to each buccal ridge—where each apical bud exists at the apical end—of each tooth. As the distal side of mandible is artificially destroyed during the dissection, the number of small X-ray-translucent areas is not consistent with the number of teeth. When viewing the sliced mandible forward the lingual surface, the bone at the buccal of the mandible is perforated, resulting in exposure of the X-ray-translucent spaces (arrowheads) in the apical end of each tooth (b–j). The incisor tooth of the mandible is located in the lingual side of the premolar tooth and possesses the X-ray-translucent space (arrowheads) in the apical end (i–l).
Fig. 4. H-E stained horizontally-sliced paraffin sections of the tooth germ of a maxillary premolar of 5-day- (l) and 4-week-old (a–k) guinea pigs at different positions (AB: alveolar bone, AM: ameloblasts, D: dentin, DP: dental pulp, E: enamel, EO: enamel organ, HERS: Hertwig's epithelial root sheath, OB: odontoblasts, *: cartilage-like cementum, arrows: fibers attachment to cementum pearls, arrowheads: acellular cementum). Figures e and k are higher magnifications of the boxed areas in d and j, respectively. Two bud stage-tooth germs (mesial and distal apical buds) appear at the base of the maxilla (a, b). A bell stage-tooth germ is observed at a more occlusal position where one can recognize both of the two ridges (cusp-like structures) composed of enamel and dentin and the enamel organ including ameloblasts or inner enamel epithelial cells, cells of stratum intermedium, and a large amount of stellate reticulum between these two cusp-like structures (c–e, l). Viewing the sliced jaw forward of the occlusal surface, an additional bud stage-tooth germ appears in the buccal side of the maxilla (f). At further occlusal positions, the formation of HERS and cementum occur in the buccal side of the maxilla, and amelogenesis and dentinogenesis progress, resulting in a complex zigzag shape of a tooth that is composed of a core of dentin covered by a layer of enamel on all axial surfaces except the buccal of the upper jaws (g–k). The white areas surrounding the dentin are the enamel spaces deleted by demineralization. Bars = 500 μm (c, d, f–j, l), 250 μm (a, b), 100 μm (e), 50 μm (k)
Fig. 5. Soft X-ray (a), and sagittally-sliced views reconstructed by μ-CT (b), and H&E stained sagittally-sliced paraffin sections (c–g) of 5-day- (a–c, e–g) and 3-week-old (d) guinea pig mandibles (AB: ameloblasts, D: dentin, DP: dental pulp, E: enamel, EO: enamel organ, HERS: Hertwig's epithelial root sheath, P: premolar, M1: first molar, M2: second molar, M3: third molar, OB: odontoblasts, SI: stratum intermedium, *: cartilage-like cementum). Figures e, f and g are higher magnifications views of the boxed areas in c, e, and f, respectively. X-ray-translucent spaces are observed at the apical ends of one premolar and three molar teeth covered by the X-ray-opaque alveolar bone, referred to as the "lamina dura" (arrows) (a, b). The center of the apical end in each tooth possesses two large epithelial swellings including a large amount of stellate reticulum (SR), and all stages of amelogenesis and dentinogenesis can be surveyed (c–g). The white areas surrounding the dentin are the enamel spaces deleted by demineralization. Bars = 2 mm (c), 1 mm (d, e), 500 μm (f), 100 μm (g)
Fig. 6. In vivo cell kinetic analyses by BrdU-labeling after 2 hours (a–f), and 5 (g–i) and 20 days (j–l) using horizontally-sliced sections of the tooth germ of maxillary premolar (a–f), and mandibular second (g–i) and third (j–l) molars of a 3- (j–l) or 4-week-old (a–i) guinea pig (AB: alveolar bone, AM: ameloblasts, D: dentin, DP: dental pulp, E: enamel, EO: enamel organ, HERS: Hertwig's epithelial root sheath, OB: odontoblasts, SR: stellate reticulum). Figures d, f, and i are the higher magnified views of the boxed or arrow-indicated areas in c, e, and h, respectively. Two bud stage-tooth germs (mesial and distal apical buds) appear at the base of the maxilla and mandible, and labeled cells are localized in these epithelial masses surrounded by red dots 2 days after BrdU-injection (a, b). An additional bud stage-tooth germ (buccal apical buds) appears in the buccal side of the maxilla and contains numerous BrdU-labeled cells (c, d). The inner enamel epithelium, dental papilla, dental follicle, and HERS are also labeled by BrdU, although labeled cells are not recognized in the differentiated ameloblasts or odontoblasts (c, e, f). (Continued on the next page)
Analyses by μ-CT and soft X-ray

In horizontal views (Fig. 2), two small X-ray-translucent areas were recognizable in the buccal side of each tooth at the base of the mandibles, although these are actually covered by the alveolar bone at the level of histological sections (Fig. 7a–d). When viewing the horizontally-sliced mandible toward the occlusal surface, the bone at the base of the mandibles was perforated, resulting in exposure of the X-ray-translucent space in the apical end of each tooth; the X-ray-opaque zigzag structures appeared to be increased in density and width (Fig. 2).

The incisor tooth of the mandible was located in the lingual side of the premolar tooth and possessed an X-ray-translucent space in the apical end, and the X-ray-opaque structure was increased in width when viewing the tooth toward the incisal edge (Fig. 2). In the sagittal views of the mandible reconstructed by μ-CT, the relation between the two small X-ray-translucent areas and the buccal ridges of each tooth was clearly demonstrated: each area corresponded to each buccal ridge (cusp-like structure) of each tooth (Fig. 3). Each apical bud existed at the apical end of each buccal ridge. The X-ray-translucent space in the apical end of each tooth was confirmed in the sagitally-sliced views of mandibles (Fig. 3).

Soft X-ray analysis of mandibles confirmed the X-ray-translucent spaces at the apical ends of one premolar and three molar teeth covered by the X-ray-opaque alveolar bone, which is referred to as the "lamina dura" (Fig. 5a).

Histological analysis

Morphological features of continuously growing premolar/molar teeth were consistent irrespective of the ages of postnatal animals (Fig. 4c, l, 5d, e) or tooth types such as premolar, or first, second and third molars, because permanent teeth have already erupted and are functional at birth and all premolars/molars show the same developmental stage. Serial horizontal paraffin sections from the apical end to the occlusal surface of premolars/molars showed the existence of plural bud stage-tooth germs and a bell stage-tooth germ (Fig. 4).

Two bud stage-tooth germs appeared at the base of the maxilla and mandible (Fig. 4a, b), and, subsequently, a bell stage-tooth germ was observed at a more occlusal position where we could recognize both of the two ridges (cusp-like structures) composed of enamel and dentin and the large epithelial swelling including ameloblasts or inner enamel epithelial cells, cells of stratum intermedium, and a large amount of stellate reticulum between these two cusp-like structures (Fig. 4c–e). Here, we referred to the two bud stage-tooth germs as mesial and distal apical buds (Fig. 4a, b, 7e, f, i, k), and to the apical end of this large epithelial swelling between two cusp-like structures as an intercuspal apical bud (Fig. 7g).

When viewing the horizontally-sliced jaws toward the occlusal surface, an additional bud stage-tooth germ appeared in the buccal side of the maxilla and the lingual side of the mandible. Here, this type of apical bud was referred to as a buccal apical bud in the upper jaw or a lingual apical bud in the lower (Fig. 4f, 7j).

Further toward the occlusal positions, the formation of HERS and cementum occurred in the buccal side of the upper and the lingual side of the lower, and amelogenesis and dentinogenesis progressed, resulting in a complex zigzag shape for a horizontally-sectioned tooth composed of a core of dentin covered by a layer of enamel on all axial surfaces except the buccal of the upper and the lingual of the lower (Fig. 4g–k). The serial sagittal paraffin sections of the premolars/molars revealed that each tooth possessed two large epithelial swellings, including a large amount of stellate reticulum in the center of the apical end (Fig. 5c–g, 8), which corresponded to intercuspal and buccal (or lingual) apical buds. Viewing the sagitally-sliced mandible toward the occlusal surface, all stages of amelogenesis and dentinogenesis were surveyed (Fig. 5c–g).

BrdU-labeling analysis

When BrdU-labeled cells were examined 2 h after BrdU-injection, they were found localized in the apical end of each tooth toward the incisal edge (Fig. 2). In the sagittal views reconstructed by μ-CT, the relation between the two small X-ray-translucent areas and the buccal ridges of each tooth was clearly demonstrated: each area corresponded to each buccal ridge (cusp-like structure) of each tooth (Fig. 3). Each apical bud existed at the apical end of each buccal ridge. The X-ray-translucent space in the apical end of each tooth was confirmed in the sagitally-sliced views of mandibles (Fig. 3).

Five days after BrdU-injection, the labeled cells reside in the bud stage-tooth germs (mesial apical bud) surrounded by red dots, which are composed of the stellate reticulum and basal epithelium, HERS, parts of the differentiated ameloblast and odontoblast layers, and the core of the dental papilla (g–i). BrdU-retaining cells remain in the bud stage-tooth germs (intercuspal, lingual, and distal apical buds) surrounded by red dots even 20 days after BrdU-injection (j, l). The white areas surrounding the dentin are the enamel spaces deleted by demineralization. Bars = 500 μm (c, e, h), 200 μm (b), 100 μm (a, f, g, i), 50 μm (d, j–l)
Fig. 7. Legend on the opposite page.
of the enamel organ of premolar/molar teeth and the surrounding tissue (Fig. 6a–f). Four types of apical buds, i.e., mesial, distal, intercuspal, and buccal (or lingual) ones, contained numerous BrdU-labeled cells (Fig. 6a–d). The inner enamel epithelium, dental papilla, dental follicle, and HERS were also labeled by BrdU, although labeled cells were not recognized in the differentiated ameloblasts or odontoblasts (Fig. 6c, e, f). Five days after BrdU-injection, the labeled cells resided in the apical buds, which were composed of the stellate reticulum and basal epithelium, HERS, parts of the differentiated ameloblast and odontoblast layers, and the core of the dental papilla (Fig. 6g–i). From 10–20 days after BrdU-injection, labeled differentiated cells were shifted toward the occlusal direction (data not shown). Even 20 days after BrdU-injection, labeled cells remained in the four types of apical buds, HERS, and the core of dental papilla in the apical ends of the premolar/molar teeth (Fig. 6j–l, 7e–l). Figure 8 represents the putative movement of epithelial adult stem cells in the apical buds.

**Discussion**

The present cell kinetic study, using BrdU-labeling as the markers of dividing cells, clearly demonstrated a relation between the epithelial compartments of adult stem cells and the crown shape of guinea pig premolars/molars (Fig. 8). Adult stem cells are present in many vertebrate regenerative tissues including bone marrow, neural tissue, skin, retina, and tooth (Harada et al., 1999; Fuchs and Segre, 2000; Bianco et al., 2001; Blau et al., 2001; Gronthos et al., 2002). Adult stem cells have been shown to undergo asymmetric cell division, resulting in one daughter cell remaining in the stem cell compartment and another undergoing further cell divisions to give rise to differentiated cells (Morrison et al., 1997). The dental epithelium of the guinea-pig premolar/molar may have a special structure for maintaining stem cells at the apical end, which is referred to as an "apical bud" (Ohshima et al., 2003, 2005; Harada and Ohshima, 2004). The gene expression has been examined in the mouse incisor (Harada et al., 1999) and vole molar (Tummers and Thesleff, 2003), suggesting that the local gene expression is important for the regulation of the epithelial stem cell niche.

It is reasonable to suppose that a variety of continuously growing teeth possess the dental adult stem cells (Harada et al., 2002b; Tummers and Thesleff, 2003; Harada and Ohshima, 2004; Ohshima et al., 2005). However, little is known about which cell type functions as the origin of odontogenic stem cells and how these cells differentiate to become functional. A previous autoradiographic study has demonstrated that the outer enamel epithelium (OEE) serves as a source of all odontogenic epithelium (Nataatmadja et al., 1991). The present study suggests that slow-cycling long-term label-retaining cells remain in the apical buds, dental papilla, dental follicle, and HERS even 20 days after BrdU-injection. These cells are considered to be adult stem cells in the dental epithelial and mesenchymal tissues, and the epithelial stem cells may reside mainly in the boundary between the stellate reticulum and basal epithelium (Fig. 6g, j–l, 7e–g, i–k). Thus, the epithelial stem cells are localized in the apical buds including the stellate reticulum, and the progeny of the stem cells may migrate to the IEE via the basal epithelium and form a population of transit amplifying cells to differentiate into ameloblasts. The progeny in the mesial and distal sides of the apical end of the epithelium in the sagittal view may be derived from the mesial and distal apical buds, respectively (Fig. 8).

The morphological features of the apical epithelial compartments from rodent incisors were considered to be equivalent to bud, cap, and bell stage-tooth germs at the prenatal stage in our previous studies (Ohshima et al., 2003, 2005; Harada and Ohshima, 2004). These structures were composed of the cells of the basal epithelium and

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**Fig. 7.** H-E stained frontal paraffin sections (a–d) and in vivo cell kinetic analysis by BrdU-labeling after 20 days (e–l) of a 3-week-old guinea pig mandible (AB: alveolar bone, D: dentin, DP: dental pulp, E: enamel, EO: enamel organ, HERS: Hertwig's epithelial root sheath, OB: odontoblasts, SR: stellate reticulum). Figures e–l show higher magnifications of the neighboring section, which correspond to those of the boxed areas in a–d. Frontal sections of a molar tooth display four features: the combination of a mesial apical bud and HERS at about 600 μm (a), that of mesial and intercuspal apical buds and HERS at about 1200 μm (b), that of distal and lingual apical buds at about 1900 μm (c), and that of a distal apical bud and HERS at about 2500 μm from the mesial side of the tooth (d). BrdU-labeled cells (arrowheads) remain in the four types of apical buds, HERS, and the core of the dental papilla in the apical end of the molar tooth (e–l). Bars = 500 μm (a–d), 50 μm (e–l).
stellate reticulum. These morphological features were also observed in guinea-pig premolars/molars, where plural specific proliferative regions exist at the apical end, while the serial transverse sections of the apical end reflect the development of the tooth germ with limited growth. These results suggest that such a dental stem cell niche in the rodent incisor (Harada et al., 1999, 2002a; Tummers and Thesleff, 2003; Harada and Ohshima, 2004; Yokohama-Tamaki et al., 2006; Wang et al., 2007; Klein et al., 2008) may also obtain in the tooth germ of guinea-pig premolars/molars. In the latter cases, there are four stem cell compartments: mesial, distal, intercuspal, and buccal (or lingual) apical buds. These specialized structures were confirmed by the analyses of μ-CT in the present study.

Interestingly, the frontally-sectioned views of the guinea pig premolars/molars at the mesial and distal positions show almost the same features as the sagittal view of rodent incisor teeth: the combination of an apical bud and HERS (Fig. 7a, d, 8, 9). These data indicate that guinea pig premolars/molars have four apical buds where the epithelial adult stem cells reside, resulting in a complex zigzag shape for a horizontally-sectioned tooth: this tooth is composed of a core of dentin covered by a layer of enamel on all axial surfaces except the buccal of the uppers and the lingual of the lowers. In contrast, rodent incisors, which show a single cone appearance,

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**Fig. 8.** Schematic representation of the epithelial stem cell compartments (apical buds) in the sagittal (right panel) and frontal (left panel: corresponding to the line X-Y of sagittal view) sections of a guinea pig mandibular premolar or molar (D: dentin, DP: dental pulp, E: enamel, HERS: Hertwig’s epithelial root sheath, *: cartilage-like cementum). Three apical buds, i.e., mesial, intercuspal, and lingual, are represented in these sections. The epithelial stem cells are localized in the apical buds including the stellate reticulum (SR), and the progeny of the stem cells migrate to the inner enamel epithelium and form a population of transit amplifying cells to differentiate into ameloblasts. The progeny in the mesial and distal sides of the apical end of epithelium in the sagittal view are derived from the mesial and distal apical buds which are localized in the buccal side of mandible, respectively.
are covered by enamel on the labial side and possess only one apical bud (Fig. 10). The results suggest that plural apical buds, being arranged bucco-lingually and mesiodistally, produce the crown mold in a zigzag. This concept concerning the relation between the orientation of apical buds and the crown shape of continuously growing teeth may be applied to the vole molar teeth represented by Tummers and Thesleff (2003) (Fig. 10) although they did not use the term "apical bud", but "cervical loop". Another possibility is that the zigzag shape of tooth is determined prenatally during the folding of enamel organ and that this folding also determines the position of prospective apical buds, although information on the apical bud in the vole molars is missing at present. Analysis of the prenatal developmental process of guinea pig premolars/molars will be necessary to clarify the exact mechanisms of the crown mold.

Previous studies have reported that the molecular signals regulating the maintenance and cell fate decision of adult stem cells, such as activin, bone morphogenetic protein (BMP), Notch-1, Lunatic fringe, fibroblast growth factor (FGF), and Follistatin are expressed in the epithelial structure and the surrounding mesenchyme (Harada et al., 1999, 2002; Harada and Ohshima, 2004; Harada et al., 2003) (Fig. 9).

Fig. 9. A diagram showing three-dimensional (a), frontal (b–e), and transverse views (f–h) at different positions of a guinea pig mandibular premolar/molar. Figures b–e and f–h correspond to Figures 7a–d and 4a–c, respectively. Four apical buds exist in the apical end of a guinea pig premolar/molar.
Fig. 10. A diagram showing the relation between the number of apical buds and the morphology of various continuously growing teeth. The guinea pig premolar or molar has four apical buds resulting in a complex zigzag shape for a horizontally-sectioned tooth. This tooth is composed of a core of dentin covered by a layer of enamel on all axial surfaces except the buccal of the uppers and the lingual of the lowers. In contrast, the rodent incisor, which shows a single cone appearance, is covered by enamel on the labial side and possesses only one apical bud. Judging from the findings represented by Tummers and Thesleff (2003), the vole molar may possess 17 apical buds in the apical end of a tooth germ.
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Tummers and Thesleff, 2003; Yokohama-Tamaki et al., 2006; Wang et al., 2007; Klein et al., 2008). In the case of a tooth with limited growth, the mRNA of Fgf-10 is transiently expressed during the cap stage when the tooth grows rapidly and the epithelium undergoes folding morphogenesis; their expressions cease gradually according to the progress of the tooth development (Kettunen et al., 2000). The tooth bud corresponding to the developing tooth germ at the prenatal stage is eternally maintained at the apical end of guinea pig premolars/molars as demonstrated by the present study. All continuously growing teeth may use the same molecular pathways—FGF, Notch and BMP—for the regulation of the epithelial stem cell niche (Tummers and Thesleff, 2003; Wang et al., 2007). Elucidation of the molecular mechanisms for maintaining and differentiating apical bud cells would be useful in the regenerative treatment of teeth. Further studies are needed to clarify the molecular mechanisms for adult stem cells, the stem cell niche, determination of cell fates, and the proliferation and differentiation of cells.

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References


