Pulp Development

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1.1 Introduction

The induction and the human dentition development takes place during embryonic, fetal, neonatal, and postnatal childhood stages of development.

Human tooth development begins with the induction of the primary dentition during the fifth week of gestation (embryogenesis). Biomineralization starts during the fourteenth week of gestation, and the permanent dentition is completed at the end of adolescence.

The tooth is composed of different tissues. The enamel, dentin, and cementum are mineralized dental tissues, whereas dental pulp is the only non-mineralized dental tissue. The dental pulp is a specialized loose connective tissue localized in the central part of the tooth.

Anatomically and functionally, the dentin (synthesized by odontoblasts) and dental pulp are considered a single entity. Both tissues are often associated as the "dentin-pulp complex."

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But, biologically, this anatomical entity has no consistency.

Understanding odontogenesis is a prerequisite to be able to understand the processes involved in dentin repair. Many studies underline that genes and signaling pathways involved in the early stages of odontogenesis also play a role in the dental pulp repair process in adults [1, 2].

1.2 Tooth Development: The Initial Steps

The odontogenesis is associated with the initial stages of craniofacial development and is regulated by epithelial-mesenchymal interactions. The epithelium may be ectodermal or endodermal. The mesenchyme in the first branchial arch is termed ectomesenchyme because neural crest cells have migrated in it [3–5].

In mammals, the ectoderm is at the origin of the oral epithelium which gives rise to ameloblasts, responsible for dental enamel formation. Odontoblasts, cells secreting dentin, derive from the ectomesenchyme.

The neural crest cells (NCC) of the rostral hindbrain (rhombomeres 1 and 2) and caudal midbrain migrate and colonize the first branchial arch, forming the presumptive territories of the teeth, mandible, and maxilla. Combinatory expression of homeobox genes (Hox) assigns an identity to the branchial arches after NCC migration. Prior to tooth bud formation, these cells

already express the LIM-homeobox-containing genes, Lhx 6 and Lhx 7, which are hallmarks of the odontogenic lineage [6].

In the mouse embryo, Hoxa2 appears to be the only homeobox gene expressed in rhombomere 2, while HOXa2 is absent in the NCC of rhombomere 1. Furthermore, the "knockout" (KO) of HOXa2 induces transformation of the skeletal elements of the second arc in those of the first arc [7]. It was noted as the absence of expression of homeotic genes in the first branchial arch [8]. This absence of expression suggests that cell fate is not "determined" at this stage, which, in term, would promote morphogenesis/differentiation of the elements of the jaw during the later stages of development.

NCCs of the first branchial arch are at the origin of the odontogenic ectomesenchyme that will interact with the oral epithelium to form presumptive territories of the incisors, canines, and molars (in humans) in each quadrant of the two jaws. The early expression of FGF8 and BMP4 in the oral epithelium allows the induction of the homeobox gene expression (Barx1, Dlx1/2, Msx1, Msx2, Alx3) in the cells of the underlying ectomesenchyme and establishes a Hox gene expression pattern specifying separate territories [9, 10].

This combinatory Hox gene expression creates a "dental homeocode" that will control the morphogenesis/differentiation. This "homeocode" assigns an identity to these "pools" of progeny cells which will form the tooth germs specific to different types of teeth and thus plays a crucial role in the spatiotemporal regulation of odontogenesis.

Tooth morphogenesis is similar to other organ's morphogenesis formed by the cells deriving from the neural crest (tooth, hair, feathers, salivary glands, mammary glands) [11]. During the initiation of these organs, the ectoderm thickens and forms the epithelial placode that buds in the underlying mesenchyme. The interaction between the ectoderm and underlying mesenchyme provokes the condensation of mesenchyme around the epithelial bud. During morphogenesis, the mesenchyme directs the folding and the ramification of the epithelium, a crucial step for the morphogenesis of the organ.

The teeth have been used as a model extensively to illustrate the importance of ectomesenchymal interactions and particularly the role of these interactions during the morphogenesis of different types of teeth.

The molecular signals mediating these interactions belong to several conserved signalization families. Many growth factors such as FGFs (fibroblast growth factors), Wnt(s), BMPs (bone morphogenetic proteins), the Hh(s) (Hedgehog), Notch, and EDA (Ectodysplasin-A) are involved in the dental development [8, 12–30], but their exact roles are not yet clear.

Specific spatial and temporal expression of a number of homeotic genes, such as Pitx2, Pax9, Msx1/2, Lhx6, Lhx7, Dlx1/2, and Barx1, marks the induction of odontogenesis and can be used as markers of tooth development [23, 31–44]. Recently, it was suggested that Sox2 regulates the progenitor state of dental epithelial cells and that the expression patterns of Sox2 support the hypothesis that dormant capacity for continuous tooth renewal exists in mammals [45].

MicroRNAs (miRNAs) are emerging as important regulators of the various aspects of embryonic development, including the odontogenesis. The small noncoding RNA function is a transcriptional and posttranscriptional regulation of gene expression. It was admitted that miRNAs have different roles in the epithelium and mesenchyme during odontogenesis. Furthermore, "microarray" and hybridization in situ analysis have identified several miRNAs having a differential expression between the incisors and molars [46–48].

Finally, although the spatiotemporal gene expression pattern was determined in the mouse embryo, the precise role of each of these actors in the development program of the tooth is far from being fully elucidated.

Next, we describe briefly the different stages during odontogenesis, without details on molecular level. There are many extensive reviews related to this topic [30, 49, 50].

1.3 Stages of Tooth Development

It is well established that the basic steps of tooth morphogenesis are similar in all vertebrates. After 5 weeks of development, continuous bands of thickened epithelium, horseshoe shaped, are formed around the mouth in the presumptive upper and lower jaws. These epithelium bands, named primary epithelial bands, will give rise to the dental lamina. The establishment of the dental lamina, the area that forms the teeth, precedes the initiation of individual teeth.

The key event for the initiation of tooth development is the formation of localized thickenings or *dental placodes* (sixth week) within the primary epithelial bands, at the site of the future dental arches in the embryonic mandible and maxilla. The *basement membrane* (BM) separates, even at this early stage, the epithelium from the underlying ectomesenchyme. The BM controls the epithelial-mesenchymal interactions and exchanges. The interactions between the surface epithelium and underlying ectomesenchyme are crucial both for the formation of dental placodes and during various stages of odontogenesis (Fig. 1.1).

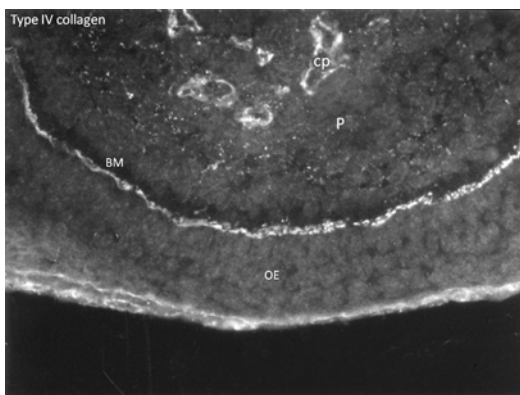The first evidence of the future teeth appears when the epithelial cells near the basement membrane begin to multiply (four to five cell layers) and invaginate into the underlying ectomesenchyme, giving rise to the *dental lamina*. The ectomesenchyme starts to change composition in response and becomes more condensed. Thus, this initial epithelial invagination clearly marks the apparition of the tooth crown area and will develop through several distinct stages (bud, cap, bell stage). Tooth development is a continuous process, so clear distinction between the transition stages is not possible.

Each dental lamina is at the origin of a tooth *bud* (Fig. 1.2). Tooth buds of the deciduous canines and incisors are apparent in the 8-week-old human embryo, and buds of the deciduous molars are formed during the ninth week. The *bud stage* is characterized by the progression of ectodermal invagination in the underlying ecto-mesenchyme, in which cells are packed closely around the epithelial bud. This will be followed by the changes in the shape of the dental bud and formation of the *dental cap* (Figs. 1.2 and 1.3). The *cap stage* is characterized by a concavity of the epithelium that partially envelops the underlying mesenchyme. During the cap stage, the epithelial outgrowth is referred widely as the *enamel organ* and is related to the differentiation of the outer dental epithelium, inner dental epithelium, and the *appearance of the enamel knot*. Also, there is a condensation of the ectomesenchyme in
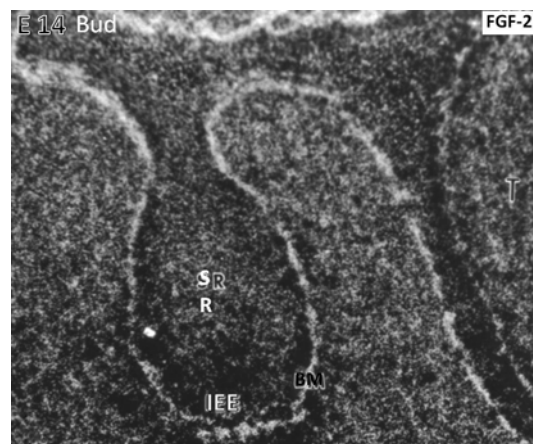

Fig. 1.1 At the initial stage of tooth germ formation, a basement membrane (*BM*) separates the outer epithelium (epithelial placode) (*OE*) from the subjacent mesenchyme. The future pulp (*P*) (condensation zone) displays type IV collagen immunostaining limiting capillaries (*cp*)

Fig. 1.2 At the embryonic day, E14 binding of the radioactive FGF-2 occurs along the basement membrane (*BM*) limiting the inner epithelial epithelium (*IEE*). The central part of the tooth bud contains the stratum reticulum (*SR*)

Fig. 1.4 At a more advanced cap stage (day 14), dense FGF-2 binding occurs along the basement membrane (*BM*). The binding is weaker in the enamel organ (*EO*), limited to the cell surface. The binding is stronger in the pulp (*p*), capillaries, and the early stages of the trabecular bone
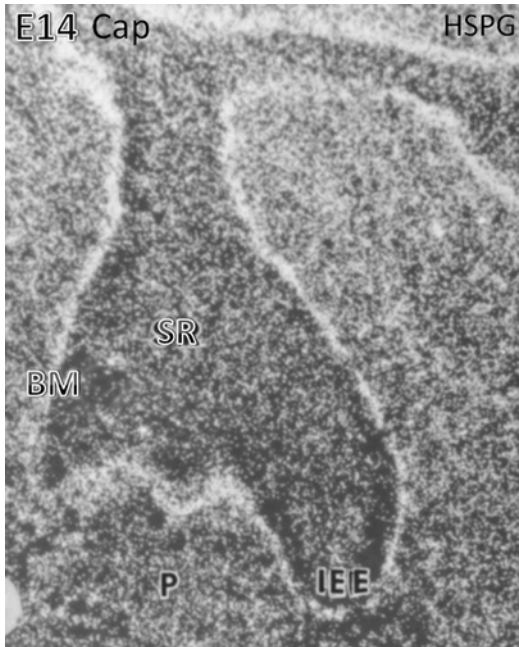
Fig. 1.3 Heparan sulfate proteoglycan (*HSPG*) binding is mostly situated along the basement membrane (*BM*) located at the early cap stage along the inner enamel epithelium (*IEE*) above the stratum reticulum (*SR*). In the embryonic pulp (*P*), numerous cells are immunostained

the concavity of the enamel organ forming the *dental papilla*, at the origin of the odontoblasts and the dental pulp [51, 52] (Figs. 1.4 and 1.5).

Starting from the late cap stage and through the transition from cap to bell stage of tooth development, many developmental changes are observed. All the elements of the *enamel organ* are well distinguished (*histodifferentiation*):

- The outer enamel epithelium located at the periphery of the cap in contact with the peridental mesenchyme.
- The inner enamel epithelium formed by cells which are precursors of ameloblasts and which are separated from the future dental pulp by a basement membrane.
- The stellate reticulum and the stratum intermedium, two intermediate layers of the enamel organ involved in the transcellular and intercellular transfer of precursors of enamel proteins, and in the provision of energy for these transfers (synthesis and degradation of glycogen). For some authors, the stratum
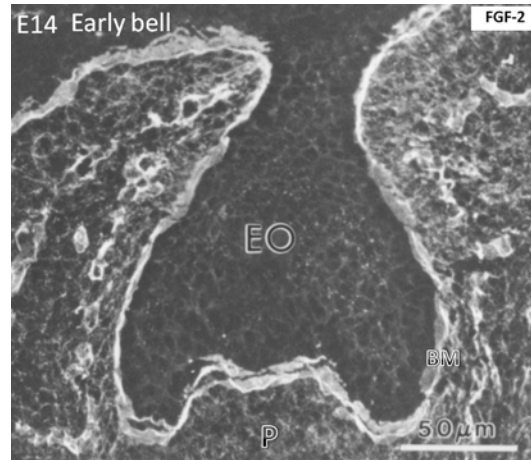
intermedium differentiates during the bell stage.

- The primary enamel knot, a transient structure situated in the center of the enamel organ that will control the morphogenesis of dental cusps and determine the final shape of the tooth [53]. Subsequently, secondary enamel knots will be formed, contributing to the formation of molar cusps. The enamel knots within epithelium are described as organizing centers composed by "clusters" of cells that secrete many morphogen signals like Shh, Wnts, FGFs, and BMPs, whose roles are not yet fully defined.

Besides the role of the enamel knots to regulate the size and shape of the teeth, the signals from the mesenchyme are also necessary for the formation and maintenance of epithelial compartments.

The cap stage is followed by the *bell stage*, during which the dental crown acquires its final shape (*morphodifferentiation*) and the formation of the cusp pattern is observed (Figs. 1.6 and 1.7).

The outer and inner enamel epithelia are continuous, and they meet at the rim of the enamel organ known as the zone of reflection or cervical loop. Extended in Hertwig's epithelial root
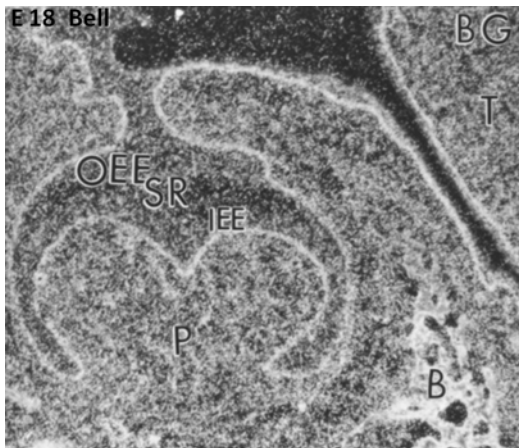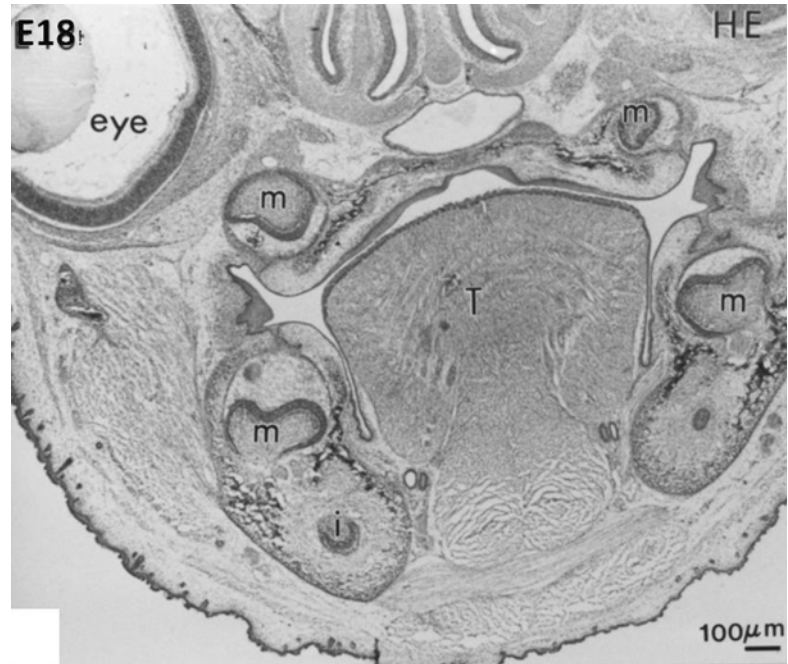
Fig. 1.6 E18. At the bell stage, the binding of FGF-2 (*BG*) concentrate along the basement membrane in the outer enamel epithelium (*OEE*), stellate reticulum (*SR*), and inner enamel epithelium (*IEE*). The pulp (*P*) is heavily labeled and the forming bone (*B*) as well. *T* tongue

area. In the pluricuspid teeth, the secondary enamel knots appear at the top of each cusp.

In the late bell stage, tooth morphogenesis is followed by a phase of cell differentiation of the inner enamel epithelium and of the ecto-mesenchymal cells at the epithelial-mesenchymal interface with the basement membrane (*histodifferentiation*). These cells will differentiate in pre-ameloblasts and pre-odontoblasts in order to become polarized and secreting ameloblasts and odontoblasts to form the enamel and dentin, respectively. The first layers of the enamel and dentine are visible at the end of the coronary morphogenesis. Thus, during embryogenesis, morphogenesis and differentiation are coupled. Cells acquire the competence to differentiate according to their position. Differentiation of pre-ameloblasts and odontoblasts is pre-coupled spatiotemporally to these morphogenetic movements that provide the pattern formation of the crown and the beginning of the root formation.

The condensed ectomesenchyme situated at the periphery of the enamel organ and dental papilla is referred as the *dental follicle or dental sac* and will give rise to the supporting dental

sheath, this cervical loop will control the formation of the root, including the root odontoblast differentiation (Figs. 1.8 and 1.9). The cervical loop progresses in apical direction, and many cell divisions sustain this preeruptive crown growth, thus delimiting increasingly the dental papilla

Fig. 1.7 E18. Bell stage. Anti-heparan sulfate proteoglycan (*AHSPG*) immunolabeling. No labeling is detectable in the enamel organ (*EO*). The basement membrane (*BM*) is densely immunostained. In the pulp (*P*), the capillaries (*CP*) are well stained. At the stage of crown formation, endothelial cells of capillaries proliferate, elongate, and form a dense vascular network
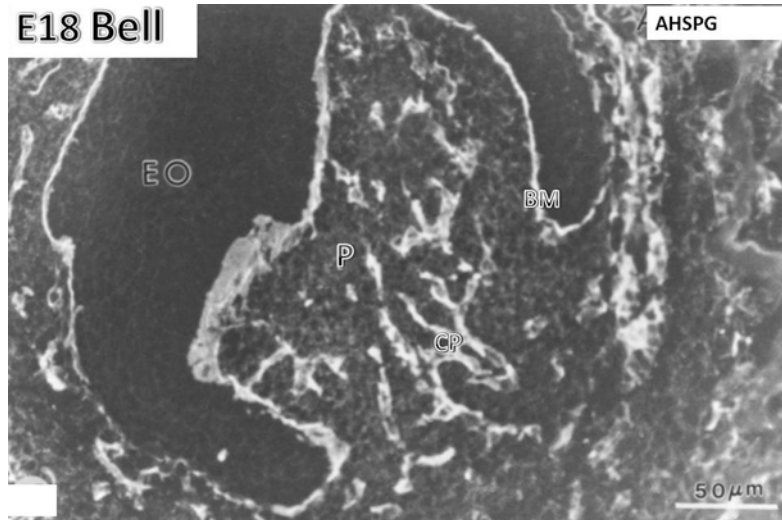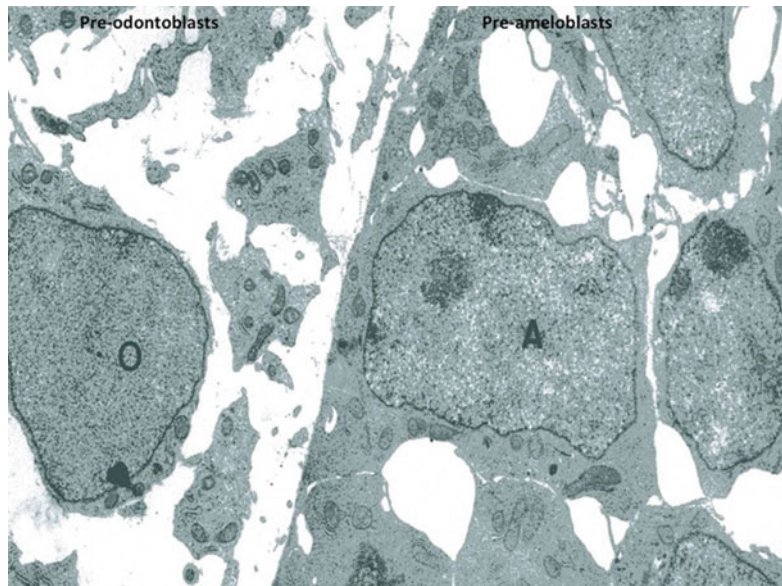

Fig. 1.8 At a later stage, pre-odontoblasts are facing pre-ameloblasts. Postmitotic polarizing ameloblasts form a cell network, with large intercellular spaces. Presecretory pre-polarized odontoblasts (*O*) are located in an electron-lucent dental pulp

tissues as the tooth cementum, periodontal ligament, and alveolar bone. Thus, the dental follicle is involved in the formation of the root and tooth eruption (Figs. 1.5 and 1.10).

The enamel organ, the dental papilla, and the dental follicle form the *dental organ or tooth germ*.

In humans, there are two dentitions, deciduous and permanent (primary, temporary). The development of the deciduous dentition begins around the sixth gestational week. Then quickly, there is coexistence of deciduous and permanent dental germs, and odontogenesis ends around the age of 18–25 years by formation of the dental root and the eruption of the third permanent molars.

The development of the permanent teeth begins during the odontogenesis of deciduous teeth. The deciduous teeth are formed from the primary dental lamina. In humans, the permanent teeth develop in two ways: (1) sequentially at the lingual region of the enamel organ of each temporary tooth (successional teeth) or (2) permanent molars grow from an extension of the

Fig. 1.9 The ultimate asymmetric division allows pre-odontoblasts to become postmitotic odontoblasts implicated in dentin formation
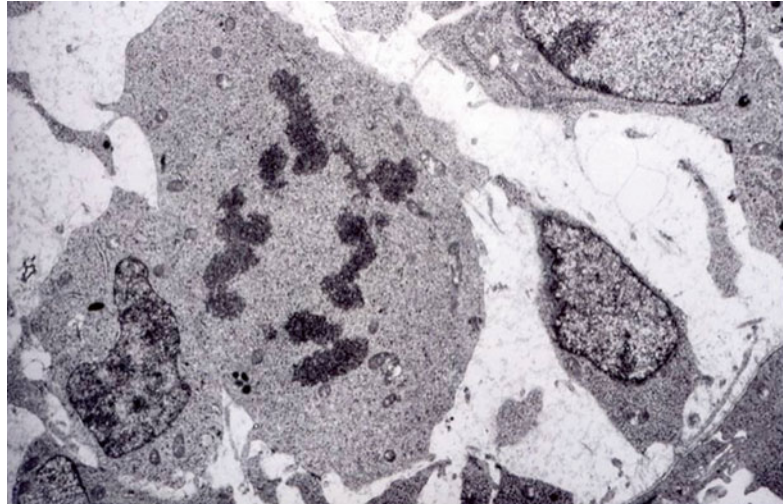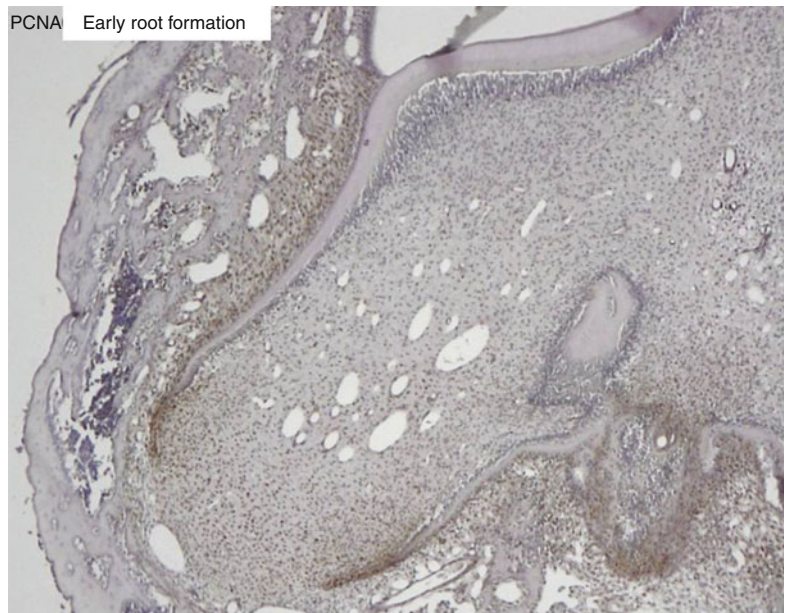

Fig. 1.10 Proliferating cell nuclear antigen (*PCNA*) labeling of the apical cells of the forming root. The epithelial Hertwig's root sheath divides and elongates, contributing to root elongation and to the tooth eruption

initial dental lamina. These are non-successional teeth.

The spatiotemporal control of dental development is orchestrated by epithelial-mesenchymal interactions. At the molecular level, these interactions provide reciprocal and sequential exchanges of signals through the basement membrane. These signals/morphogens are associated with the "determination" (commitment) of the cells in different territories during the morphogenesis.

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