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Review article

Function and repair of dental enamel – Potential role of epithelial transport processes of ameloblasts

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ABSTRACT

The hardest mammalian tissue, dental enamel is produced by ameloblasts, which are electrolyte-transporting epithelial cells. Although the end product is very different, they show many similarities to transporting epithelia of the pancreas, salivary glands and kidney.

Enamel is produced in a multi-step epithelial secretory process that features biomineralization which is an interplay of secreted ameloblast specific proteins and the time-specific transport of minerals, protons and bicarbonate. First, "secretory" ameloblasts form the entire thickness of the enamel layer, but with low mineral content. Then they differentiate into "maturation" ameloblasts, which remove organic matrix from the enamel and in turn further build up hydroxyapatite crystals. The protons generated by hydroxyapatite formation need to be buffered, otherwise enamel will not attain full mineralization. Buffering requires a tight pH regulation and secretion of bicarbonate by ameloblasts. The whole process has been the focus of many immunohistochemical and gene knock-out studies, but, perhaps surprisingly, no functional data existed for mineral ion transport by ameloblasts. However, recent studies including ours provided a better insight for molecular mechanism of mineral formation. The secretory regulation is not completely known as yet, but its significance is crucial. Impairing regulation retards or prevents completion of enamel mineralization and results in the development of hypomineralized enamel that easily erodes after dental eruption. Factors that impair this function are fluoride and disruption of pH regulators.

Revealing these factors may eventually lead to the treatment of enamel hypomineralization related to genetic or environmentally induced malformation.

Introduction

Tooth enamel is a formidable bioceramic designed to withstand enormous mechanical forces for decades while being exposed to sustained changes in temperature, pH and to microbial challenges, all that without the ability to regenerate. In accordance with this, enamel is the hardest tissue of the body: the matured enamel consisting of 96% hydroxyapatite crystals and containing only a few percent of protein and water. It is perhaps surprising that enamel is secreted by epithelial cells. However, the outcome is very different from other epithelial products like the fluid-rich secretions of salivary glands, pancreas and liver [1–5].

The process of amelogenesis

The secretion of enamel by ameloblasts is a two-stage process. The first step is building of a slightly mineralized matrix structure and the second step is remodeling of this matrix to a high mineral structure. Ameloblasts differentiate from the inner enamel epithelium, which originally derived from the oral epithelium, and take several distinct morphological forms corresponding to different functional states during their life cycle. These are the morphogenic, inductive, early secretory, late secretory, maturation ruffled-ended, maturation smooth-ended and protective forms. These ameloblast forms change according to a strict time schedule and each has a specific functional role in particular phases of amelogenesis [6] (Fig. 1A).

In the secretory stage of amelogenesis ameloblasts are tall, columnar cells rich in mitochondria, endoplasmic reticulum and Golgi apparatus according to their active transport processes.

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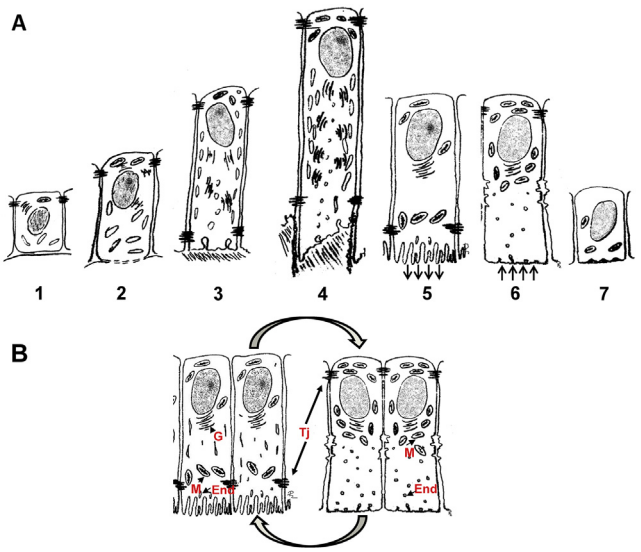

Fig. 1. Morphological and functional transitions of ameloblasts during amelogenesis. **A**: During their life cycle ameloblasts change form and structure according to their actual function following a strict schedule: 1) morphogenetic, 2) inductive, 3) early secretory, 4) secretory, 5) maturation - ruffle-ended, 6) maturation – smooth-ended, 7) protective. **B**: Maturation stage ameloblasts cycle back and forth multiple times during maturation exhibiting ruffle-ended and smooth-ended phenotypes. Cycling of the two phenotypes involves extensive remodeling of the distal cytoplasm and junctional complexes at both ends of the cells. The lysosomal apparatus and the Golgi complexes (G) are well developed in both phases. Tight junctions (Tj) shift from distal position in the ruffle-ended ameloblasts to a proximal position in the smooth-ended ameloblasts. Mitochondria (M) are located primarily in the distal cytoplasm. Endosomes (End) are present both in ruffle-ended and smooth-ended ameloblasts. During the cycling process ameloblasts create cyclical changes in pH at the apical space and absorb the digestive products of MMP-20 and kallikrein4.

Ameloblasts can be clearly distinguished from dentin producing mesenchymal odontoblasts by the ability of tight junction formation. Ameloblasts have characteristic epithelial tight junctions throughout their lifetime which close the intracellular space and separate the apical and basolateral surfaces of the cell. These tight junctions allow the maintenance of extreme concentration gradients between the apical and basolateral extracellular spaces. The Tomes process of the secretory ameloblast is a short, lance-shaped structure that provides the surface for the highly active secretory transport. Calcium and phosphate ions which are necessary for mineralization, are actively transported into the mineralization space in a basolateral to apical direction. The molecular mechanism of this mineral transport process is only partially understood at present. Recently evidence has been presented for specific ion transporters in the ameloblast membrane [7], but the extent to which these are responsible for crystal growth is not yet determined [6]. During the secretory phase the whole thickness of enamel is formed but it is only 30% mineralized. In this phase the mineral content is condensed into thin, parallel crystal ribbons while the space between the crystals is filled by matrix with high amelogenin content.

The maturation stage begins once the final tissue thickness has been laid down. During the maturation stage ameloblasts change their morphology and cyclically transform between ruffle-ended and smooth-ended forms. The importance of this cyclical modulation is that ameloblasts have a double function in this phase: they have to secrete calcium and phosphate and neutralize the protons liberated during hydroxyapatite crystal growth while they also have to reabsorb and degrade amelogenin cleaved by matrix metalloprotease-20 (MMP-20) and kallikrein-4 [8]. Degradation of amelogenin content and parallel crystal expansion in thickness continues until the whole matrix is eliminated and replaced by the tightly packed and practically impermeable crystal structure. The maturation mechanism and control is not fully understood. However, it is important to note that the papillary cells located above the ameloblasts may have an important role in supporting their transport activity and the removal of unnecessary materials [6,8] (Fig. 1B).

The result of amelogenesis is an almost completely impermeable structure. There is some density difference between the crystal rods and the matrix but the whole enamel is 96% mineralized and it is the hardest tissue in the body. After the maturation phase, the ameloblasts de-differentiate, becoming short and cuboid rather than tall and columnar, and make a protective layer until eruption [6].

Significance of intrinsically disordered proteins involved in amelogenesis

During the secretory stage of enamel formation three major structural proteins, namely amelogenin, ameloblastin and enamelin, and a number of additional minor proteins such as amelotin, follicular dendritic cell secreted protein and tuftelin are secreted. Then, during the maturation these proteins are removed in a complex process, involving two proteases, MMP-20 and kallikrein-4. The individual roles of these proteins are not fully understood but it is clear that among the enamel proteins amelogenin is present in the largest quantity [9,10]. A number of recent studies revealed that mutations of multiple proteins could cause syndrome-associated and nonsyndromic enamel defects (for review see Refs. [11], but because of the space limitation the present work can discuss only the biomineralization process in general, and the contribution to this by amelogenin, the most abundant enamel protein.

Amelogenin is coded by two genes, by AMELX and AMELY located on the X and Y chromosomes. Significantly more protein is produced from AMELX. It is synthesized as a small, globular protein, which then forms nanospheres during the self-assembly of the proteins. The nanospheres interact with each other and create grid-like sheets to control the formation and orientation of enamel crystals. During the early phase of maturation MMP-20, and later kallikrein-4, disrupt the grid structure and break down the nanospheres. The building blocks become degraded and can be taken up by the cells while the space left behind is filled with the thickening crystals. By the end of the maturation phase amelogenin has almost completely disappeared from the enamel. The unique structure of amelogenin in enamel formation which is a form of biomineralization, is absolutely important [10] (Fig. 2).

For biomineralization, commonly two types of proteins are distinguished. Framework macromolecules such as collagen, and chitin are considered organic matrix scaffolds for mineral deposition, while nucleators for crystal growth such as acidic phosphoproteins such as dentin matrix protein 1 and bone sialoprotein [12,13] are thought to act as active regulators of the process. In a recent in silico work we found that the major biomineralization proteins can be characterized by a high percentage of disordered residues, including dentin sialophosphoprotein (96%), dentin matrix protein 1 (96%), bone sialoprotein (85%), osteopontin (92%), amelogenin (67%), ameloblastin (82%), and enamelin (92%) [14]. The tooth enamel protein amelogenin is a typical intrinsically disordered biomineralization protein as it controls the growth of apatite crystals for tooth enamel formation [15,16].

Under native conditions intrinsically disordered proteins lack a well-defined three-dimensional structure [17]. These proteins are enriched in charged amino acids (such as Gln, Ser, Pro, Glu, Lys, Gly) and are depleted in hydrophobic residues (such as Tyr, Trp, Phe, Leu, Ile), which are responsible for their structure adopting multiple, rapidly changing conformational states. Bioinformatic

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