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Review

Altered Ca^{2+} signaling in enamelopathies☆

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ABSTRACT

Biomineralization requires the controlled movement of ions across cell barriers to reach the sites of crystal growth. Mineral precipitation occurs in aqueous phases as fluids become supersaturated with specific ionic compositions. In the biological world, biomineralization is dominated by the presence of calcium (Ca^{2+}) in crystal lattices. Ca^{2+} channels are intrinsic modulators of this process, facilitating the availability of Ca^{2+} within cells in a tightly regulated manner in time and space. Unequivocally, the most mineralized tissue produced by vertebrates, past and present, is dental enamel. With some of the longest carbonated hydroxyapatite (Hap) crystals known, dental enamel formation is fully coordinated by specialized epithelial cells of ectodermal origin known as ameloblasts. These cells form enamel in two main developmental stages: *a)* secretory; and *b)* maturation. The secretory stage is marked by volumetric growth of the tissue with limited mineralization, and the opposite is found in the maturation stage, as enamel crystals expand in width concomitant with increased ion transport. Disruptions in the formation and/or mineralization stages result, in most cases, in permanent alterations in the crystal assembly. This introduces weaknesses in the material properties affecting enamel's hardness and durability, thus limiting its efficacy as a biting, chewing tool and increasing the possibility of pathology. Here, we briefly review enamel development and discuss key properties of ameloblasts and their Ca^{2+}-handling machinery, and how alterations in this toolkit result in enamelopathies.

1. Introduction

The evolutionary history of most mammals and their ancestors can be garnered from the analysis of their fossilized dental remains. Tooth size, shape, and enamel microstructure tracks evolution in detail because dental enamel, the outer covering of teeth, is the hardest and most mineralized tissue of vertebrates, and can withstand the trials of time reasonably unchanged. This is not to suggest that enamel formation is a passive event, but the contrary. Dental enamel formation is a remarkable example of cell-tissue interactions encompassing a number of transformations in cytoskeletal elements concomitant with functional differences [1,2]. Ameloblasts are ectoderm-derived epithelial cells responsible for orchestrating the formation and mineralization of dental enamel. Their organized assembly of enamel matrix proteins and interaction with the crystal phase occur extracellularly with limited control by the enamel cells [3]. The geometry of the crystals is guided by the assembly of proteins, many of which are unique to the ameloblasts' protein synthesis machinery [4]. The mineral phase that constitutes the fully mineralized tooth enamel is composed of calcium Hydroxyapatite (Hap) organized into some of the largest known Hap crystallites in the biological world [3] (Fig. 1).

The biogenesis of enamel (amelogenesis) is, for simplicity, commonly reduced to two main developmental stages: *a)* secretory; and *b)* maturation. Secretory ameloblasts develop a distal secretory process and initiate enamel matrix protein secretion (see below) moving in predetermined outward paths toward the outer enamel [5]. Developmentally, the earliest formed regions of the enamel are the cusps, i.e. the pointy surfaces of a chewing (molar) tooth, and the last developmental phase of enamel occurs at the base. Thus, enamel forms outward and downward. Secretory ameloblasts will transition into a maturation stage cell and, rather than dedicate themselves to synthesizing and secreting enamel matrix proteins, they engage primarily in ion transport. Enamel crystal growth magnifies at this time [6]. It is during the maturation stage that many Ca^{2+} handling proteins and Ca^{2+}-associated functions are upregulated [2] in line with the increased mineral uptake and crystal growth [7]. Ca^{2+} is a key and necessary component of enamel. Therefore, disruptions in Ca^{2+} transport may cause the enamel to mineralize poorly, or improperly, resulting in enamelopathies. Here, we review the effects of altered calcium homeostasis on enamel disease. To better understand these effects, we first provide an

Abbreviations: Hap, hydroxyapatite; SOCE, store-operated Ca^{2+} entry; RA, ruffled-ended ameloblast; SA, smooth-ended ameloblast; AI, amelogenesis imperfecta

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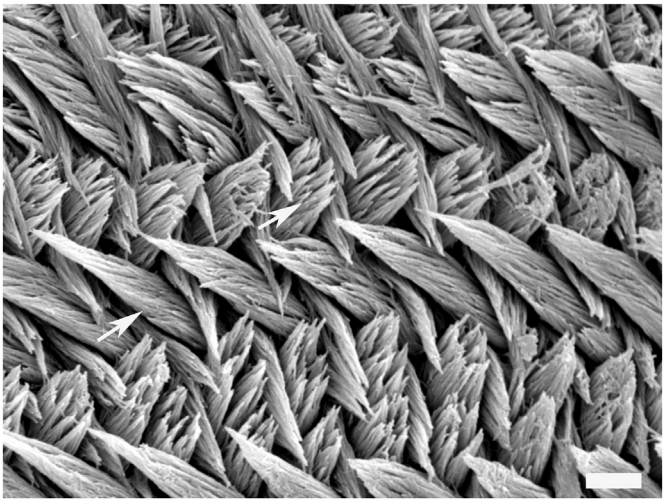

Fig. 1. Enamel pattern in mice: Rodent enamel is commonly used as a model to study mammalian enamel development. This SEM micrograph shows enamel prisms (arrowed) oriented in different directions, which is characteristic of rodent enamel but less so in human enamel. Within the prisms, single crystals can be identified. Scale bar = 5 µm.

overview of the ultrastructure of enamel cells, emphasizing the characteristics of Ca^{2+} handling organelles in these cells.

2. Enamel organ and matrix proteins

Secretory ameloblasts are post-mitotic, tall columnar, and heavily polarized cells. The cell skeleton may extend ~70 µm in height (5 µm diameter), with the nucleus found at the basal pole (away from the enamel). Mitochondria densely occupy the narrow infranuclear cytoplasm (Fig. 2A & 2B), whereas extensive endoplasmic reticulum (ER) networks populate the large infranuclear space. Abundant Golgi saccules are also present [8]. One of the most conspicuous features of secretory ameloblasts is the presence of an apical (facing the enamel) cellular extension known as the Tomes' process. Many cellular bodies fuse to the Tomes' process to exocytose and endocytose proteins (Fig. 2B). Cell-cell connections (desmosomes, tight junctions) are found both apically and distally, maintaining narrow intercellular spaces [8]. This limits the movement of molecules between cells in any direction [6]. The protein synthesis apparatus is committed to the production of a key enamel-specific protein known as amelogenin, which constitutes about 90% of all secreted products [9]. Amelogenin is required for the ordering, or alignment, of enamel crystallites in the extracellular space [3]. Other “non-amelogenin” products including enamelin and ameloblastin, are also involved in the normal development of enamel [10]. Mutations in these enamel-specific genes result in a broad range of clinical pathologies known as *amelogenesis imperfecta* (AI), defined by abnormal mineralization or deficient growth of the enamel layer [11].

Many secretory cells will transition into a maturation stage ameloblast, showing reduced height but maintaining their polarity, and losing the secretory Tomes' process. They develop deep membrane infoldings or ruffled distal border, which plays an important role in ion transport [6] (Fig. 2C & 2D). These ruffled ameloblasts have less conspicuous ER tubules and contain mitochondria both at the infra- and supranuclear regions with large clusters near the ruffled end [12] (Fig. 2C, Fig. 3). Distal cell-cell tight junctions (TJ) constrain the movement of molecules and ions between cells toward the enamel [6]. Unusually in mammalian cell biology, these ruffled cells will lose and reform these distal infoldings, periodically adopting a smooth distal pole [13] (Fig. 3). These smooth-ended cells, which are a minority of all maturation stage cells, show increased intercellular spaces and distally placed mitochondria. There is only limited knowledge of their role in amelogenesis. It has been suggested that the smooth ameloblast phase facilitates the

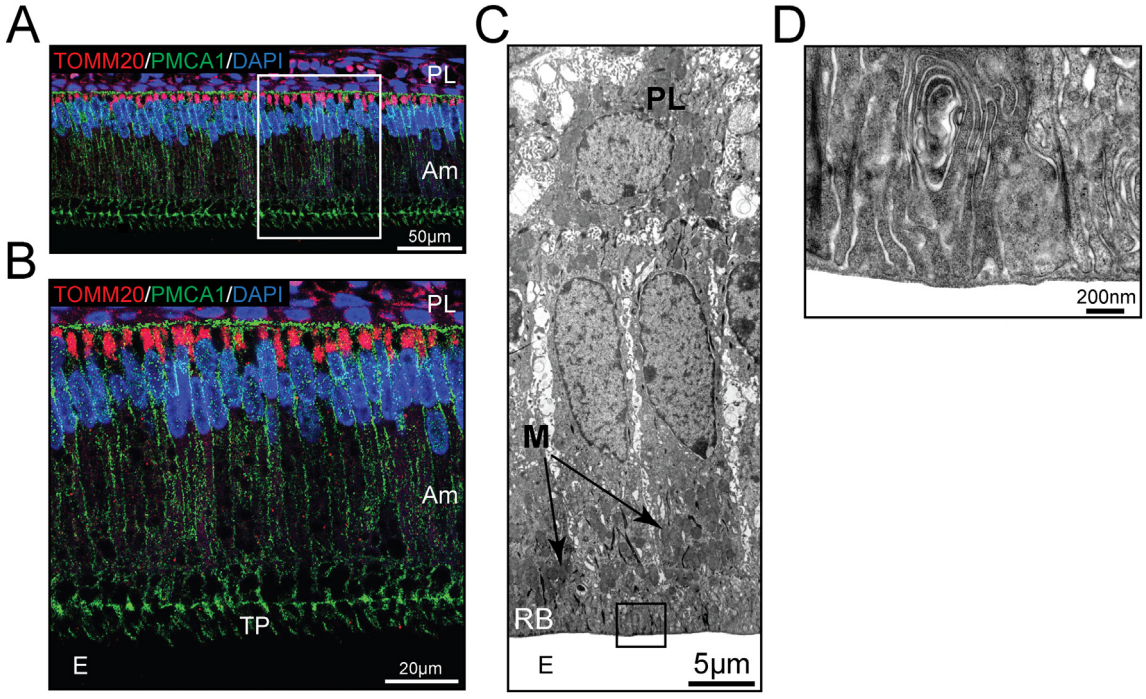

Fig. 2. Mitochondria localization in secretory and maturation stage ameloblasts. **(A)** The mitochondrial outer membrane marker TOMM20 (red) was used to visualize the dominant infranuclear localization of mitochondria in secretory stage ameloblasts. The plasma membrane of ameloblast cells was highlighted by immunofluorescence staining of PMCA (green). Cell nuclei are stained with DAPI (blue). A close-up is shown in **(B)**, which also highlights the Tomes' process (TP), a cell extension at the apical (distal) end near the enamel (E). **(C)** TEM image of maturation stage ameloblasts with mitochondria (M) clustering in areas just above the ruffled-border (RB) and below the nucleus. **(D)** A close-up of the ruffled-border showing the deep infoldings characteristic of this apical membrane structure. Am = ameloblasts. PL = papillary cell layer.