Cell Calcium 65 (2017) 1–7

Contents lists available at ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

Mini Review

Enamel: Molecular identity of its transepithelial ion transport system

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ARTICLE INFO

Article history:
Received 28 February 2017
Received in revised form 27 March 2017
Accepted 27 March 2017
Available online 29 March 2017

Keywords:
Enamel
Ion transport
pH
CRAC channel

ABSTRACT

Enamel is the most calcified tissue in vertebrates. It differs from bone in a number of characteristics including its origin from ectodermal epithelium, lack of remodeling capacity by the enamel forming cells, and absence of collagen. The enamel-forming cells known as ameloblasts, choreograph first the synthesis of a unique protein-rich matrix, followed by the mineralization of this matrix into a tissue that is ~95% mineral. To do this, ameloblasts arrange the coordinated movement of ions across a cell barrier while removing matrix proteins and monitoring extracellular pH using a variety of buffering systems to enable the growth of carbonated apatite crystals. Although our knowledge of these processes and the molecular identity of the proteins involved in transepithelial ion transport has increased in the last decade, it remains limited compared to other cells. Here we present an overview of the evolution and development of enamel, its differences with bone, and describe the ion transport systems associated with ameloblasts.

Contents

1. Introduction

Tooth enamel is pretty close to being just a mineral in the mouth. The inorganic content in enamel is about 95% by weight [1], much higher than in bone. The basic structure of tooth enamel is the formation of millions of nanometer wide hydroxyapatite-like (Hap) crystals. Each of these crystals run for hundreds of micrometers bundled into groups called enamel prisms or rods [2]. The Hap-like crystals contain large amounts of Ca^{2+}, being in fact the most highly calcified structure found in vertebrates containing about 60% Ca^{2+} by mass [2,3]. However, despite its complex dependency on Ca^{2+}, dental research seldom reaches the scientific attention that other topics in the Ca^{2+} signaling field receive.

This is in part associated with the fact that enamel research has largely focused on the chemical events and interactions of the ions involved in the formation of the enamel crystals, that is, the extracellular milieu, rather than the cellular events involved. As in any biological system, it is important to identify how cells orchestrate the functions that are relevant for developing a tissue. For example, recognizing that Ca^{2+} is essential for cell differentiation and survival, gene expression and other processes [4], should beckon

Abbreviations: Hap, hydroxyapatite; NCKX, sodium/calcium/potassium exchanger; SLC, solute carrier; STIM, stromal interaction molecule; SOCE, store-operated Ca^{2+} entry; CRAC, Ca^{2+} release activated Ca^{2+}.

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http://dx.doi.org/10.1016/j.ceca.2017.03.006

us to consider how the physiology of the enamel generating cells is designed to cope with handling potentially highly toxic levels of Ca^{2+} before this cation is delivered outside the cell [5]. Ameloblasts are the master makers of enamel. Their capacity to transport Ca^{2+} to mineralize the extracellular matrix can be distinguished from the potential role of Ca^{2+} as an intracellular signaling messenger. Little is known about either role. Consequently, the potential effects of changes in the intracellular concentration of Ca^{2+} in regulating enamel crystal growth are poorly understood.

Ca^{2+} and $PO_4{}^{3-}$ are the dominant ions contained in mineralized enamel crystals. For these crystals to develop properly, their often variable stoichiometric composition also requires other ions including bicarbonate, Cl^-, K^+, Na^+ and Mg^{2+} [6]. The delivery of these ions to the extracellular milieu is closely coordinated by the ameloblasts [7]. Yet knowledge of the ameloblast's decision making process to switch functions from a tissue-forming to a mineralizing cell is extraordinarily poor and their physiology is ill-defined. Although recent studies have improved our overall knowledge of the ion transport system in ameloblasts, it remains limited. Here we succinctly describe the evolutionary origins of enamel and its differences with bone, and review what is known about the process of enamel formation, or amelogenesis, and then discuss the molecular identity of ion transport proteins that have thus far been identified in ameloblasts.

1.1. Teeth and bones

The skeletonized structures of vertebrates are recognized in the fossil record dating back to the Cambrian period well in excess of 500 million years ago. Early vertebrates developed bones and teeth separately [8]. It is considered that the bony-like skeletons of early vertebrates originally derived as a reservoir for calcium and phosphate. From these, and with the availability of collagen fibers already present in invertebrate organisms, the earliest mineralization of skeletonized structures appeared [8]. The earliest vertebrates do not show evidence of internal bony elements, which likely appeared millions of years after the outer skeleton [8]. Teeth may have developed ahead of the inner skeleton but the origin of teeth is not fully understood. Teeth either evolved from dermal denticles that migrated into the rudimentary oral space, or from specialized oropharyngeal denticles which in this case provided insulation for the primitive electrosensory organ [9,10]. Besides differences in evolutionary origin, bones and teeth also differ in the combination of cell types that assemble to provide their embryonic origin. Enamel develops from ectodermal epithelium whereas bone cells derive from mesenchyme [11]. Moreover, although both tissues are formed via matrix-mediated mineralization, bone is much less mineralized than enamel and contains a large percentage of collagen which is nearly absent in enamel [12]. Bone becomes mineralized as it is formed, whereas enamel is mineralized in two stages, as described below. Enamel shows complex patters of hardness and resistance to fracture [13]. Importantly, bone is a living tissue and the cells that form and maintain this tissue (osteoblast, osteoclast, osteocytes) are active for the majority of the person's life in non-pathological conditions. However, enamel does not contain any living cells and once formed, it does not regenerate.

1.2. Life and death of a very tall cell

Ameloblast cells are complex. They develop from ectodermal epithelium at the interface with mesenchymal cells which, upon condensation, instruct the epithelium to undergo cytodifferentiation [14,15]. From here on, the post-mitotic epithelial cells will increase in height, develop a protein synthesis apparatus and a distal cell process (Tomes' process) that is important for the organization of the crystals [16]. The secretory stage defines the first stage of amelogenesis [17]. Secretory ameloblasts are tall, reaching heights around 70 μm but maintaining a narrow 4–5 μm diameter and form a semipermeable barrier [18] (see also Fig. 1). At this stage, enamel is only partially mineralized and the ameloblasts primary function is to synthesize and secrete a number of structural enamel matrix proteins to build the volume of the tissue [19]. In the next stage of amelogenesis coined the maturation stage, enamel undergoes two main processes: degradation and removal of organic matrix, and increased mineralization [7,20]. Maturation stage ameloblasts re-transform from the previous stage by changing their morphology in a number of ways. First, they decrease their height to about 40 μm or less and lose the Tomes' process [21]. Instead of the Tomes' process, the bulk of the cells in the maturation stage show a ruffled-border [1]. Ameloblasts at this stage will lose and reform this ruffled-border in alternating waves of ruffled-ended to smooth-ended morphology a number of times (Fig. 1). The mechanisms and signals triggering these waves are poorly understood but recent evidence suggests that changes in $[Ca^{2+}]_{cyt}$ or extracellular pH might be associated with the modulation from RA to SA [22,23]. At the end of the maturation stage, ameloblasts reduce their height and regress forming a layer of epithelial cells around the enamel that is lost during eruption [1]. In rodents, the most widely used model in tooth development, the secretory stage lasts about 7 days and the maturation stage twice as long [1]. Shortly after, the teeth can be seen emerging through the gingiva into the oral cavity. In the erupted teeth of all mammals, there are no living ameloblasts, so enamel cannot remodel or self-repair.

1.3. Enamel crystal formation and enamel matrix proteins

Enamel is a remarkable example of cell-directed mineralization. Enamel crystals form in the extracellular compartment by supersaturation of minerals guided or mediated by matrix proteins [24]. Crystal growth requires nucleation events as a first step, or the clustering of minerals into recognizable structures, of which the smallest unit is referred to as a unit cell [6]. For dental enamel, the chemical formula of the unit cell of these carbonated hydroxyapatite (Hap) crystals that are formed is: $Ca_{10}(PO_4)_6(OH)_2$. Hexagonally-shaped enamel crystallites are formed as ameloblasts develop their Tomes' process. These crystallites of just a few nanometers in diameter grow in length to some hundreds of micrometers and are surrounded by organic matrix [25]. These events occur in the secretory stage. The maturation of the crystals involves removal of matrix proteins and the increase in transport of Ca^{2+} and $PO_3{}^-$ which enables an increase in width and thickness of the crystals to the point that there is almost no separation between them [2,26]. The crystals are packed into units called enamel prisms or rods of ~5um in diameter [2,25]. The arrangement of these enamel rods can vary from species to species. When fully mineralized, enamel hardness has been likened to that intermediate of iron and carbon steel, while maintaining an unusual level of elasticity [6].

At least three main proteins are recognized as the main structural mediators of enamel crystal growth [19,27]. These proteins are: amelogenin (AMELX), ameloblastin (AMBN) and enamelin (ENAM). They all have in common that mutations in their coding genes affect enamel development to varying degrees. The resulting phenotypes are directly associated with *amelogenesis imperfecta* (AI) [28]. AI is a clinical term that broadly defines abnormal enamel formation. As will be discussed later, a number of mutations in many other genes that are widely expressed in the body organs besides ameloblasts which also impact enamel [29].

AMELX is by far the most abundant product synthesized by ameloblasts with as much as 90% of the total output of proteins secreted by ameloblasts being AMELX [30]. Secreted AMELX