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CRAC channels in dental enamel cells

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Abstract

Enamel mineralization relies on Ca^{2+} availability provided by Ca^{2+} release activated Ca^{2+} (CRAC) channels. CRAC channels are modulated by the endoplasmic reticulum Ca^{2+} sensor STIM1 which gates the pore subunit of the channel known as ORAI1, found in the plasma membrane, to enable sustained Ca^{2+} influx. Mutations in the *STIM1* and *ORAI1* genes result in CRAC channelopathy, an ensemble of diseases including immunodeficiency, muscular hypotonia, ectodermal dysplasia with defects in sweat gland function and abnormal enamel mineralization similar to *amelogenesis imperfecta* (AI). In some reports, the chief medical complaint has been the patient's dental health, highlighting the direct and important link between CRAC channels and enamel. The reported enamel defects are apparent in both the deciduous and in permanent teeth and often require extensive dental treatment to provide the patient with a functional dentition. Among the dental phenotypes observed in the patients, discoloration, increased wear, hypoplasias (thinning of enamel) and chipping has been reported. These findings are not universal in all patients. Here we review the mutations in *STIM1* and *ORAI1* causing AI-like phenotype, and evaluate the enamel defects in CRAC channel deficient mice. We also provide a brief overview of the role of CRAC channels in other mineralizing systems such as dentine and bone.

GRAPHICAL ABSTRACT

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Keywords

Enamel; CRAC channels; mutations; amelogenesis imperfecta; hypoplasia

1. Introduction

The identification of STIM1 in 2005 [1, 2] as an ER Ca^{2+} sensor, followed by the discovery of ORAI1 a year later [3, 4] as the pore subunit of the Ca^{2+} release activated Ca^{2+} (CRAC)

channel, set the stage for a plethora of studies identifying the role of these proteins in numerous disease states (reviewed in [5]). It soon became apparent that CRAC channel deficiencies, named CRAC channelopathy, had a profound impact on many tissues and organs, including dental enamel [6–9]. These clinical reports, and subsequent functional data including the study of CRAC-deficient mouse models [10–12], firmly established CRAC channels as key elements in Ca^{2+} transport and signaling in enamel. This elegant system that monitors the refilling of intracellular Ca^{2+} stores also participating in Ca^{2+} signaling has provided clarity regarding the pathway for Ca^{2+} influx in enamel cells, which had been unresolved. Yet, it also poses new challenges as the data emerging from recent studies implicate CRAC channels in multiple functions of the enamel cells that have been thus far unrecognized [11]. Here, we review CRAC channelopathy in enamel and its impact on dental disease.

2. Enamel

Enamel is formed by specialized epithelial cells known as ameloblasts in roughly two stages: a) a developmental (secretory); and b) a mineralization (maturation) stage (Fig. 1). Growth of enamel crystals takes place in the extracellular space only, near the apical (distal) pole of the ameloblasts, which are heavily polarized cells [13]. This extracellular space is largely inaccessible to ions and molecules unless these are transiting through the ameloblasts [13, 14]. In this space, there is an aqueous solution whose composition is largely modulated by the cells, differing from the ionic composition of serum and thereby confirming the status of the enamel fluid as a specialized microenvironment [15]. The enrichment of this solution with varying species of both matrix proteins and ions is a stage-dependent process [16]. Secretory cells provide the bulk of the organic template consisting of enamel matrix proteins (EMPs). Crystals are thin and long at this stage. This early matrix-mineral interaction plays an important part in guiding the directionality of crystal growth [17]. In maturation, there is a substantial addition of mineral to the sides of the initial crystals, resulting in increased width [18] concomitant with a more active transport of ions and the removal of proteins and fluid so that the individual crystals are tightly packed with almost no space in between [19]. Mature enamel has a hardness between that of iron and carbon steel, but it is also highly elastic for a tissue that, by weight, is more than 95% mineral.

2.1 Enamel genes and the enamel matrix

Enamel contains less than 1% of organics when fully mineralized, in contrast to bone or dentine which contain 15–26% of organics, most of which is collagen [20]. By the late 1970s, the enamel matrix proteins amelogenin (AMEL) and enamelin (ENAM) were identified, with the former known to account for about 90% of the secreted proteins by ameloblasts in the secretory stage [21]. The ameloblastin (AMBN) protein joined these two proteins in the 1990s [22]. Today, all three are classically referred to as the enamel matrix proteins, or enamel structural proteins, as these have known roles in the “building” of the enamel microstructure [23, 24]. AMBN contributes ~8% to the composition of enamel organics whereas ENAM is found in very small amounts [17]. Given the dominant contribution of AMEL to the EMPs, the cloning of the amelogenin *cDNA* by Snead and colleagues [25] heightened the attention of enamel research on this protein. The *AMEL* gene

is found on the X and Y chromosomes in humans (annotated *AMELX* and *AMELY*, respectively), but only on the X chromosome in mice [26]. Once the protein is secreted, it is cleaved into a number of smaller peptides, which may have different functions [27]. Mutations in the *AMELX* gene result in abnormal enamel classified in clinical terminology as *amelogenesis imperfecta* or AI (see below), and mouse models lacking *Amelx* have abnormal enamel (reviewed in [17]). Despite reports on the expression of EMPs in other tissues, the general consensus is, based on the very limited or absence of effects elsewhere in the body, that *AMELX*, *AMBN*, and *ENAM* are considered to be products of ameloblasts and have functions directly linked to forming enamel [17]. These EMPs are enzymatically cleaved by two main proteases known as matrix metalloprotease 20 (MMP20) in secretory stage and kalikrein 4 (KLK4) in maturation [17]. Mutations in the genes coding for the EMPs or these associated proteases cause AI.

2.2 Amelogenesis imperfecta (AI)

Enamel defects can occur once the tooth has erupted into the mouth and is subjected to the oral environment stricken by challenges from chemical insults, bacterial attack, masticatory forces, or by pathologies including salivary gland dysfunction. By contrast, developmental defects are associated with multiple causes including gene mutations, metabolic disturbances, or generalized systemic disease [28], and are important because they provide direct understanding of how enamel formation, known as amelogenesis, is regulated. As defined by Witkop [29], hereditary or congenital defects that primarily affect enamel formation without causing defects in other systems are referred to as AI [28, 30]. This definition – the most widely adopted classification of AI – aims to separate disturbances caused by mutations in the enamel genes from others [29, 30] despite the growing number of systemic diseases that severely impact enamel formation [28]. Clinically, AI has been classified in four types (Types I to IV), largely based on the visual appearance and radiographic assessment of a tooth [29]. These types are categorized as follows: *type I*) hypoplastic enamel defined by enamel that has reduced thickness (volume); *type II*) hypomaturational defined by normal thickness but mottled appearance, enamel that chips away from the crown, and radiolucency of enamel similar to dentine; *type III*) hypocalcified when the thickness is normal but the enamel is poorly calcified as identified by less radiolucency of enamel relative to dentine and its appearance is orange-yellow [29]; *type IV*) a combination that includes several phenotypes such as hypomaturational-hypoplasia with taurodontism (enlarged pulp chamber). Mutations in the *STIM1* and *ORAI1* genes reported to date cause enamel defects similar to AI, primarily AI type III [5, 6, 8].

2.3 Calcium transport in enamel: a brief overview

Enamel contains the highest Ca^{2+} content of all biapatites. Compared to other tissues, enamel contains more than 9 times the Ca^{2+} content of muscle or liver [31]. Most of the Ca^{2+} (~90%) found in enamel is incorporated during maturation [19]. Ca^{2+} uptake in enamel cells was, for many years, considered to be a passive phenomenon, with Ca^{2+} following a natural concentration gradient from the high levels found in interstitial fluid to the lower concentration in the cytosol [32–34]. Once in the cytosol, monitoring of $[\text{Ca}^{2+}]_{\text{cyt}}$ to maintain normal levels was achieved by cytosolic buffers and also by the plasma membrane Ca^{2+} -ATPases localized along the cell membrane of the ameloblasts moving excess Ca^{2+} out

of the cell [34, 35]. The benchmark work of Hubbard via a series of publications critically evaluated the status quo of Ca^{2+} transport in enamel cells [32, 36, 37]. Hubbard recognized the possibility of a store-operated Ca^{2+} entry (SOCE) system and proposed that, much like in pancreatic acinar cells, Ca^{2+} entering the enamel cells would be transferred across the long body of the cell within the endoplasmic reticulum (ER), and then be exocytosed at the apical pole; a model he termed “the transcytosis hypothesis” [32]. This model received support with the finding that CRAC channel dysfunction resulted in AI [6, 9] although other aspects of this model have yet to be tested (i.e. the transit of Ca^{2+} via the ER). In recent years, we and others have suggested that the extrusion of Ca^{2+} at the apical pole of ameloblasts, is primarily mediated by NCKX4, as mutations in the coding gene (*SLC24A4*) in humans result in AI [38–40]. NCKX4 is a sodium/calcium/potassium exchanger mobilizing 4Na^+ for 1Ca^{2+} and 1K^+ . Its localization in the distal end of ameloblasts is consistent with its role as a mediator in Ca^{2+} extrusion. Combined, these findings have provided a clearer picture of Ca^{2+} influx/efflux in enamel cells.

2.4 CRAC channels in enamel organ

Ameloblasts develop and mineralize enamel surrounded by a number of other cell types at the basal end which, combined, form the enamel organ. In the secretory stage, the basal portion of the ameloblasts’ cytoskeleton is nearly juxtaposed with cells of the *stratum intermedium*, which form a discrete single cell layer (Fig. 1). Filling the additional space in the organ are the *stellate reticulum* cells. Maturation stage ameloblasts are surrounded basally by the papillary layer (PL) of cells, with the closest PL cells attaching via desmosomes to the ameloblasts. An important question to address is which of these cells have functional CRAC channels, as this is relevant to establish a model for Ca^{2+} transport in the enamel organ.

CRAC channels are formed by the endoplasmic reticulum (ER) Ca^{2+} sensor proteins STIM1 and its homolog STIM2, which upon loss of ER Ca^{2+} undergo a number of conformational changes enabling direct interaction with the pore forming ORAI proteins (ORAI1–3), the other key molecular component of the CRAC channel (reviewed in [41]). STIM-ORAI (*Stim-Orai* is used here for gene or gene products in rodents) interactions enable sustained Ca^{2+} influx in many cell types and represent the SOCE prototype [42, 43]. A study analyzing the expression pattern of ORAI proteins in mouse dental tissues found ORAI1 expression in pre-ameloblasts during the late embryonic (E17) and early postnatal stages, with relatively high expression during the secretory stage [44]. A similar pattern for ORAI2 expression was also reported in that study [44]. Unfortunately, these data are difficult to interpret because of the cytoplasmic expression pattern for ORAI1 reported. In our lab, we have seen no evidence of a cytoplasmic ORAI1 localization in ameloblasts, only in the basolateral PM with possible signals also in the distal pole, and found no evidence of expression elsewhere in the cells of the enamel organ [10, 45] (Fig. 2). The Papagerakis lab study reported that shRNA knock-down of *Orai1* in the enamel cell line HAT-7 cells resulted in decreased expression of enamel genes and decreased cell proliferation rates [44]. Our study using a different enamel cell line (LS8 cells) also showed decreased expression of enamel genes using pharmacological blockers of the CRAC channels in thapsigargin stimulated cells [46].

Murine and rat ameloblasts showed increased ORAI1 signals in the maturation stage ameloblasts [10, 45, 46]. STIM1 was found throughout the cytosol of maturation but not secretory stage ameloblasts, co-localizing with the ER chaperone calnexin [10]. These higher expression patterns of both *Stim1* and *Orai1* genes in maturation are in keeping with increased SOCE during this stage [10] (Fig. 1). It also enable us to construct a working model for Ca^{2+} uptake via the CRAC channel in ameloblasts (Fig. 3). Localization patterns of ORAI1 in the enamel organ facilitate interpreting SOCE kinetics in these cells. Considering that dissections of primary enamel organ cells might contain contaminants from cell types surrounding the ameloblasts, the localization of STIM1 and ORAI1, as they are not expressed in cells other than ameloblasts, suggest that SOCE measurements via time-lapse microscopy or Flexstation derive from ameloblasts solely.

3. Enamel defects in patients with loss-of-function mutation in STIM1 and ORAI1

Most *ORAI1* and *STIM1* mutations leading to alterations in enamel are loss-of-function mutations which result in reduced Ca^{2+} influx [5]. However, a recent report has linked enamel defects with diseases that are associated with gain-of-function mutations. In this condition, the CRAC channel remains constitutively opened leading to an array of disease states including tubular aggregate myopathy (TAM), York platelet and Stormorken syndromes [5]. A mutation in *STIM1* (D84E) resulting in TAM and Stormorken syndrome, also reported “tooth enamel hypocalcification”. Beyond this limited dental description, no other data on enamel were reported [47]. The patient showed hypocalcemia in laboratory tests which directly or indirectly affected the mineralization of the enamel as hypocalcemia in general impacts enamel [48]. Loss-of-function mutations are discussed in detail below (see also Table 1).

3.1 ORAI1 mutations

The first report on *ORAI1*-deficient patients with abnormal enamel was published in 2009 [6]. Three loss-of-function mutations (Table 1) that impaired SOCE resulted in immunodeficiency accompanied by congenital myopathy and anhidrotic ectodermal dysplasia with dental enamel defects classified as AI type III [6]. These mutations occurred in TM1, TM2, and in the first extracellular loop of ORAI1. The dental enamel phenotype of the deciduous teeth was striking, with panoramic photographs of the front teeth of patient P2 (R91W mutation) showing extreme loss of enamel at age 6 [6]. Additional photographs of the patient reported at age 10.5 show the permanent premolar and canine teeth erupting with heavily discolored enamel. Given the position in the mouth relative to other erupted teeth, it is likely that the premolar was in use for a limited time, and preserves almost intact enamel. While some differences in the enamel of deciduous vs permanent teeth may account for this difference (i.e. deciduous enamel is thinner), it is possible that loss of enamel occurs gradually, and while they retain a degree of functionality, wear occurs faster than in healthy controls. A different *ORAI1* mutation (A103E/L194P) reported in that study also caused AI type III [6].

A recent study by Lian et al [8] identified three novel autosomal recessive mutations in *ORAI1* (p.V181SfsX8, p.L194P and p.G98R). All three mutations resulted in abolished expression of ORAI1 as well as SOCE with impaired T-cell function, reduced numbers of Treg cells though invariant natural killer T (iNKT) cells [8]. Patient 1 (p.V181SfsX8 mutation) showed enamel defects at an early age with pitted enamel or hypoplasia and hypomineralization of the incisors at 8 months. The same patient had several abscesses and tooth extractions and capped crowns. Patient P3 (p.G98R mutation) was diagnosed with AI type III, supported by oral photographs taken at age 6 that showed severely cracked enamel with exposed dentine although the enamel discoloration seen in other patients is not apparent [8].

3.2 STIM1 mutations

The first report on enamel defects in patients with CRAC channel deficiency in 2009 described defective dental enamel in three siblings from one kindred presenting with *STIM1* mutations (see Table 1) [9]. Fibroblasts from two of the patients showed abrogated Ca^{2+} influx and patients presented with immune deficiency (the primary clinical manifestation), muscular hypotonia, partial iris hypoplasia and, in only one of the patients, nephrotic syndrome [9]. In that report, besides description of the dental anomalies, no additional information on the dental phenotype of these patients was presented. However, it is noteworthy that Patient V-4, a female who died after 18 months from encephalitis [9], already showed abnormal enamel at an age where all deciduous (milk teeth) are commonly erupted and available for visual examination. At this age, babies are generally breast-fed or receive soft diet so the functional constraints of the enamel of this patient were low.

Two patients with a homozygous R429C point mutation in *STIM1* were reported in 2012 as showing dental enamel defects amidst a detailed phenotypic characterization of immune cells from these patients [7]. One of the patients showed heavy dental wear particularly evident on the anterior teeth. In addition to the various channelopathies already reported in previous studies related to fungal, bacterial and viral infections, and anhydrosis, the mutation caused nail dysplasia [7]. Also of interest is that the heterozygous mother of one of the patients showed reduced (~45%) SOCE, but it is unknown whether this reduction in Ca^{2+} influx had a major impact on her teeth. We suspect that if dental records were available, we would likely observe some degree of increased wear and possibly a high number of carious lesions.

The Hu and Simmer's group reported on a novel *STIM1* mutation (p.R426C) in a patient whose chief medical complaint was in fact her dental health [40]. The patient's immunological history is unreported but presented with recurrent throat infections. Thus, it is suspected that the immune defects were mild. As this mutation is located in the CRAC activating domain of STIM1, we previously suggested that it might have interfered with SOCE activation [5]. The dental phenotype at age 6 showed significant loss of enamel and discoloration [40]. The patient also presented with nail dysplasia.

A novel homozygous missense *STIM1* mutation (c.494C>A) caused severe enamel hypoplasias and dental abscesses in two siblings; one of them reported at age 8 [49]. T-cells showed strongly reduced SOCE in both patients. The dental enamel hypoplasias shown in

the oral photographs differ from the discolored and worn enamel of other SOCE-deficient patients whom generally present with increased wear and discolored enamel. In the c. 494C>A patient, wear on the teeth was limited, although it should be pointed out that these are likely the permanent teeth (central incisors) [49].

The most recent *STIM1* mutation resulting in a dental defect was reported by Parry and colleagues in 2016 [50]. Two cousins were identified with a homozygous missense mutation (p.L74P) in the EF-hand domain of *STIM1*. Despite abnormal SOCE, these patients did not have overt clinical immunodeficiency, likely due to the relative preservation of T-cell function that compensate for the NK cell dysfunction. However, both cousins were diagnosed with hypomineralized AI supported by oral photographs showing opaque and discolored enamel. Permanent and primary teeth were affected. Additional oral radiographs of unerupted teeth showed a near-normal volume of enamel. Dental X-rays of one of the patients showed brighter radio density in enamel relative to dentine, as is the case in healthy patients. This difference is less clear in patients with hypomineralized AI. Interestingly, although both cousins reported abnormal sweating suggesting anhydrosis, they both had normal hair and nails [50].

3.3 Overall effects of *STIM1* and *ORAI1* mutations

The defects in enamel caused by loss-of-function mutations in *STIM1* and *ORAI1* are poorly known and are largely based on oral photographs of the patients. The lack of X-rays or histological studies limits our assessments on overall impact. With these limitations, the dental phenotypes observed in patients include *amelogenesis imperfecta* type III with loss of enamel either via cracking or wear, discoloration, or hypoplasias. No tooth agenesis has been reported. The published data suggest that enamel appear to develop normally but have mineralization defects in both primary and permanent teeth, although there are differences between these tooth types. The normal development of enamel suggests that the secretory stage is not under major constraints, but that the mineralization stage is disrupted. This is in keeping with the upregulation of *STIM1* and *ORAI1* during maturation [10]. The reported hypoplasias, which are thinning of enamel in some areas, are slightly difficult to interpret. These may have been caused by severe episodes of physiological insults such as repeated severe infections as these are known to affect ameloblasts. Alternatively, they may be associated with direct effects of *STIM1* and *ORAI1* mutations in enamel, as highlighted above. It may also be linked with the type of mutation and the degree to which SOCE is diminished in each.

4. Enamel defects in *Stim1*- and *Orai1*-deficient mice

Given the sparse human data on dental studies associated with loss-of-function mutations in *ORAI1* and *STIM1*, animal models are important tools for analyzing the effects of loss of SOCE in enamel. The first description of enamel defects in mice associated with loss of SOCE was included in what was primarily a description of bone phenotypes of the *Orai1*^{-/-} mice [51]. The bone study by Robinson et al. [52] showed oral photographs of the incisors of the *Orai1*^{-/-} mice as well as histological sections of the enamel organ [52]. The *Orai1*^{-/-} mice are noticeably smaller than WT littermates, which likely also explains the smaller-sized

teeth reported [52]. The decreased enamel thickness reported in that study might have also played a role in final tooth size, but the data derived from the histological sections shown are insufficient to support this. A detailed report on the enamel phenotype of *Orai1*-deficient mice is still wanting.

By contrast, the impact of *Stim1* and *Stim2* in enamel is much clearer. The *Stim1^{fl/fl}* and *Stim2^{fl/fl}* mice originally reported by Oh-Hora et al. [53] were crossed with K14-Cre mice in the Feske lab to generate *Stim1/2^{K14Cre}* mice [54] lacking both *Stim1/2* genes in keratin 14 derived tissues including ameloblasts. These mice were analyzed by our lab to study the effects of *Stim1/2*-deficiency in enamel cells and in the enamel crystals [11]. The main findings from the enamel included severe hypomineralization, diminished Ca^{2+} content in the enamel, and increased wear [11]. At the cellular level, we found nearly abolished SOCE, altered redox system, including differences in glutathione metabolism, increased ROS, and lower mitochondrial membrane potential. Maturation ameloblasts failed to form a distal ruffled-border necessary for proper ion transport [55]. Collectively, our data showed that disrupting SOCE had profound effects on cell metabolism, redox system, and impaired enamel mineralization, functions that are understudied in enamel biology [55]. A later study by Furukawa et al. reported on *Stim1/2*-deficient mice using a similar approach to analyze the dental phenotype [12]. Although the data presented in this study was more limited than in the report by Eckstein et al. [11], their findings overall agree with our study supporting the critical role of *Stim1/2* in enamel mineralization. The Furukawa et al. study, however, also presented murine data following the conditional deletion of *Stim1* and *Stim2* genes independently [12]. Deletion of *Stim1* but not *Stim2* resulted in decreased enamel mineralization, suggesting that *Stim1* is the key SOCE player in enamel cells [12].

Interestingly, both studies showed that altered SOCE in these mouse models did not have a major impact on the expression of enamel genes [12, 55]. This is in contrast to *in vitro* data using two different enamel cell lines (LS8 and HAT-7) [44, 46], which demonstrated a decrease in transcript levels of, among others, *Amelx* and *Ambn*. These data suggest differences between the two systems, perhaps because both cell lines represent early developmental stages of amelogenesis, whereas examination of gene profiles from conditional knockout (KO) mouse enamel organ cells is done by pooling both secretory and maturation stage cells. It is also possible that *in vivo* systems utilize compensatory mechanisms involved in the regulation of enamel genes rescuing possible deficiencies given the critical role of these genes in enamel formation.

5. CRAC channels and ectodermal dysplasia

Patients with mutations in *STIM1* and *Orai1* have been described as presenting with ectodermal dysplasia (ED), given that they show phenotypes that are similar to those in ED patients [6–8, 56, 57]. ED are a group of heterogeneous, heritable conditions associated with the abnormal development of ectoderm-derived structures and associated appendages [58]. In humans, these structures include hair, teeth, nails, and eccrine glands (sweat, salivary, mammary, and lacrimal glands) [59]. Organogenesis of these structures is initiated in the embryo by cross-talk between adjacent layers of epithelium and mesenchyme, leading to a thickening of the placode [59]. Morphogenesis continues postnatally by epithelial

differentiation [60]. Common definitions of ED include “...one typical clinical key finding of ectodermal origin and to confirm a diagnosis of ectodermal dysplasia by the documentation of a mutation in an important gene for the ectoderm” [58]. Although nearly 200 different dysplasias are known in ED, ~90% of these are linked to mutations in only four genes (*EDA1*, *EDAR*, *EDARAAD* and *WNT10A*) [61]. Presently, there is no clear evidence that *STIM1* or *ORAI1* are critical mediators in the morphogenesis of ectoderm-derived structures. In fact, from the available published data, it has been recognized that the morphogenesis of two ectodermal structures, sweat glands and teeth, is unaffected by mutations in *ORAI1* or *STIM1* in humans, or in animal models lacking either of these genes [8, 11, 54]. The impact of CRAC channel deficiency is, however, critical for the normal function of the developed tissues. Eccrine sweat glands rely on Ca^{2+} modulations via *ORAI1* to stimulate chloride channels necessary for fluid secretion [56], whereas *STIM1* (and its homolog *STIM2*) are necessary to enable sustainable levels of Ca^{2+} transport in ameloblasts to cause proper enamel mineralization [11]. Moreover, patients with mutations in *STIM1* or *ORAI1* do not always show signs of ED, as some patients showed obvious enamel defects but hairs or nails were normal (e.g. [50]). Thus, technically, *STIM1* or *ORAI1* mutations, as they are yet to be associated with ectoderm morphogenesis, given the similarity in phenotypic outcomes with ED, should be more appropriately described as ED-like for the time being to separate them from those whose etiologies are directly involved in ectoderm morphogenesis.

6. Is SOCE important in other mineralized tissues?

In the tooth, another mineralized tissue known as dentine underlies the enamel cap. Dentine is formed by mesenchyme-derived odontoblast cells and is considerably less mineralized than enamel with less inorganic content (70% in dentine vs 95% in enamel), also containing ~20% of organic matrix largely type I collagen [62]. Published data from patients with *STIM1* or *ORAI1* mutations does not suggest that the dentine component is vastly affected. Similarly, murine models have not reported dentine deficiencies. However, a recent *in vitro* study noted that *Orai1* shRNA knockdown in dental pulp stem cells suppressed the odontogenic differentiation and the mineralization potential of these cells [63]. Yet, it is unclear whether dentine mineralization is a principal target of CRAC channel-mediated Ca^{2+} influx *in vivo*.

Dentine is slightly harder than bone, a tissue that contains 67% mineral largely found as small plates associated with the abundant collagen fibrils [62]. Whether patients with *STIM1* or *ORAI1* mutations have clear evidence of abnormal bone homeostasis is unclear. Picard et al., for example, reported a number of defects in only one of the *ORAI1* patients that were potentially associated with abnormal bone development, including facial dysmorphism, abnormal closure of vertebral arches, and clubfoot [6]. The latter is one of the most common congenital limb abnormalities lacking a well-defined genetic basis [64] while the other defects might be directly linked with a broader *Orai1*^{-/-} function in the skeleton (see below). In addition, *STIM1*- and *ORAI1*-deficient patients show muscular hypotonia [5], which would likely prevent normal mechanical loading on the skeleton, potentially masking the impact of CRAC channel deficiency on bone tissue.

In *Orai1*^{-/-} mice, Putney's group reported decreased trabecular bone and lower bone mineral density than in controls, but without affecting the differentiation of bone-forming cells (osteoblasts) [65, 66] (but see [52]). Regarding bone-resorption by osteoclasts, it was reported that *Orai1*^{-/-} mice lacked multinucleated osteoclasts but showed no signs of osteopetrosis [52]. An additional feature of these mice was the retention of cartilage in cranial as well as in the axial and appendicular skeletons, which may explain the facial dysmorphology and abnormal closure of vertebrae in the patient with a mutation in *ORAI1* reported by Picard et al. To date, no skeletal deficiencies have been studied in Stim1-deficient mice, although the most effective models developed are conditional KO models. Collectively, the effects of ORAI1 deficiencies in bone homeostasis in humans are unclear given the lack of strong supporting data, but in murine models ORAI1-deficiency appears to have a direct detrimental impact.

7. Summary

CRAC channelopathies are primarily characterized by deficiency in immune cell responses, sweat gland and muscle function, and dental enamel mineralization. To date, most patients with *STIM1* or *ORAI1* mutations present with abnormal enamel, commonly described as being similar to AI type III. The observed abnormalities are generally demanding, and require painstaking dental restorations in order to preserve the functionality of the dental arcade. Some differences in the impact of CRAC channel deficiency in primary and permanent teeth suggest that primary teeth, which are developed *in utero*, are more greatly affected. Murine models with disrupted Stim1/2 function have shown to be a good model to study the effects of lack of SOCE in enamel cells and its impact on mineralization. Recent studies linked SOCE alterations with abnormal redox and mitochondria, which are likely playing an important but unknown role in enamel [11]. Data from *Orai1*-deficient mice is still wanting. The effects of SOCE deficiency in other mineralizing systems such as dentine and bone is unclear. Human dentine from patients with *STIM1* or *ORAI1* mutations does not appear to be grossly impacted, and the limited skeletal deficiencies reported are either uncommon or have not been properly studied. However, *Orai1*^{-/-} mice showed altered bone homeostasis.

Some gaps in knowledge remain, in particular, the role of Orai proteins in enamel are poorly understood. It would also of interest to gain a better understanding of the CRAC channel activator *in vivo*. While emerging data suggest that a number of physiological agonists such as ATP, cholecystokinin or acetylcholine, can induce a rise in intracellular Ca²⁺ via SOCE in enamel cells *in vitro* [67], the "real" physiological agonist/s remains unknown. Moreover, the role of Ca²⁺ fluxes via SOCE in signaling remain understudied. For example, how do these fluxes modulate key cellular responses such as gene expression? And, are these fluxes responsible for modulating the expression pattern of the enamel genes? CRAC channels will continue to inform us about the role of Ca²⁺ in enamel in future research.

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Abbreviations:

AI	amelogenesis imperfecta
CRAC	Ca ²⁺ release activated Ca ²⁺ channels
ED	ectodermal dysplasia
TAM	tubular aggregate myopathy
SOCE	Store-operated Ca ²⁺ entry.

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HIGHLIGHTS

- Transcellular pathways dominate ion transport in enamel cells, known as ameloblasts
- Enamel mineralization depends on Ca²⁺ availability
- Ca²⁺ uptake is mediated by CRAC channels
- Mutations in *STIM1* or *ORAI1* result in dental disease

Figure 1: Enamel formation in the rodent incisor.

The continuously growing incisors of rodents is a structure commonly used to study amelogenesis. Differentiated cells from the stem cell population at the cervical loop (CL) become secretory ameloblasts which, after a short transition period (TA), become maturation stage ameloblasts. In the latter, two sub-populations are readily identified, namely the ruffled-ended ameloblasts (RA) and the smooth-ended ameloblasts (SA). The former is directly involved in transepithelial ion transport and are the dominant cell type. In secretory stage, STIM1 and Orai1 proteins are very weakly expressed suggesting low SOCE. This is in keeping with the limited mineralization of enamel crystals at this stage. By contrast, STIM1 and Orai1 are upregulated in maturation, a stage that is also known for increased ion transport. Thus, SOCE is important in the maturation or mineralization of the enamel crystals. SI: stratum intermedium. SR: stellate reticulum. PL: papillary layer of cells. BV: blood vessels. See also text for details.

Figure 2: Localization and function of ORAI1 in ameloblasts:

Shown here is the localization of ORAI1 (green) in murine ameloblasts at the maturation stage. Dapi, for nuclear localization, shown here in blue. **A)** Overview of the enamel organ at the maturation stage with ORAI1 signals found only in the ameloblasts. **B)** Close up of maturation stage ameloblasts showing strong Orail signals emanating from the baso-lateral PM. Ca^{2+} diffuses from blood vessels, a few tens of microns from the basal pole, into the interstitial space (see Fig 1 for orientation). In this scenario, it would be intuitive to suggest that Ca^{2+} influx via ORAI1 takes place along the interstitial space given the expression pattern of ORAI1. We had previously shown that STIM1 colocalizes with the ER chaperone calnexin, and both are found throughout the cytosol [10].

Figure 3: Model for CRAC channel function in ameloblast cells:

Maturation stage ameloblasts show a predominant baso-lateral localization of ORAI1. STIM1 is found throughout the cytosol of these cells making it possible to interact with ORAI1 along the baso-lateral membrane. The interstitial fluid allows the Ca^{2+} availability along the baso-lateral membrane, suggesting the possibility of multiple points in the cell for Ca^{2+} uptake by ORAI1. In this scenario, we have also suggested that IP_3Rs are the main release channels and that SERCA2 is the main ER refilling pump. Zonulae occludens (tight junctions -TJ) are found apically limiting the passage of components of the interstitial fluid toward the enamel. Instead, at the apical pole, Ca^{2+} is extruded via the sodium/calcium/potassium exchanger NCKX4 mobilizing 4Na^+ for 1Ca^{2+} and 1K^+ . Presently, there is no clear evidence of an apical ORAI1 localization.

Table 1:Human mutations in *ORAI1* and *STIM1* affecting enamel.

Gene	Mutation	SOCE	Enamel phenotype	ED	Reference
<i>ORAI1</i>	p.R91W	–	AI Type III	Anhidrosis	[6]
	p.V181SfsX8	–	Dental enamel hypoplasia	Anhidrosis, mild hypotrichosis	[8]
	p.G98R	–	AI Type III	Anhidrosis, dry and exfoliate skin, sparse/thin/brittle hair	[8]
	p.G98R	n.t.	Dental enamel hypoplasia	Anhidrosis, dry and exfoliate skin, sparse/thin/brittle hair	[8]
<i>STIM1</i>	p.D84E*	n.t.	Dental enamel hypocalcification	–	[44]
	p.E128RfsX9	n.t.	Abnormal dental enamel	–	[9]
	n.t.	n.t.	Abnormal dental enamel	–	[9]
	p.E128RfsX9	n.t.	Abnormal dental enamel	–	[9]
	p.R429C	–	Enamel defect	Anhidrosis	[7]
	p.R429C	n.t.	Enamel defect	Anhidrosis	[7]
	p.R426C	n.t.	?	nail dysplasia	[40]
	p.P165Q	–	Enamel hypoplasia	Anhidrosis	[46]
	p.P165Q	–	Enamel hypoplasia	brittle nails	[46]
	p.L74P	–	Hypomineralized AI	Hypohidrosis	[47]
	p.L74P	–	Hypomineralized AI	Hypohidrosis	[47]

* this is a gain-of-function mutation; all others are loss-of-function mutations

ED = ectodermal dysplasia n.t = not tested