The cellular mechanism for transporting calcium in the formation of dental enamel cells

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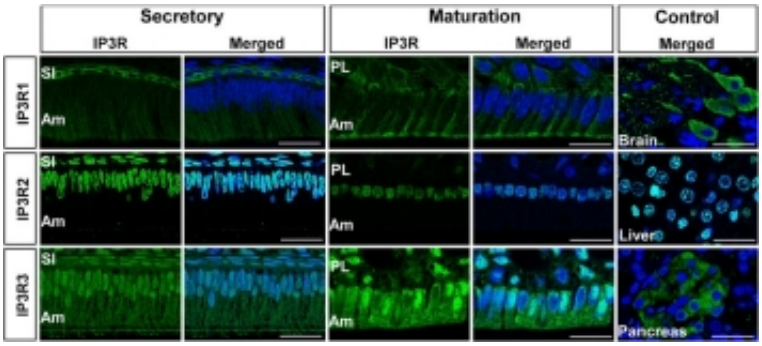


Upper panel shows IP3R1 (green) immunolocalization and nuclear DAPI (blue) staining in rat secretory and maturation ameloblasts. Brain (rat) was used as a positive control. IP3R1 is localized intracellularly as also seen in the cells of the stratum intermedium adjacent to secretory ameloblasts. In maturation stage, the papillary layer replaces the stratum intermedium. Middle panel shows IP3R2 (green) immunolocalization and DAPI (blue) in rat secretory and maturation stage ameloblasts. IP3R2 is localized to the cell nuclei. Rat liver was used as a positive control. Lower panel shows IP3R3 (green) immunolocalization and DAPI (blue) in rat secretory and maturation ameloblasts showing intracellular localization with stronger signal in maturation stage ameloblasts. Pancreas was used as a positive control. Am = ameloblasts; PL = papillary layer; SI = stratum intermedium cells. Scale bars in all images = 20 μm.

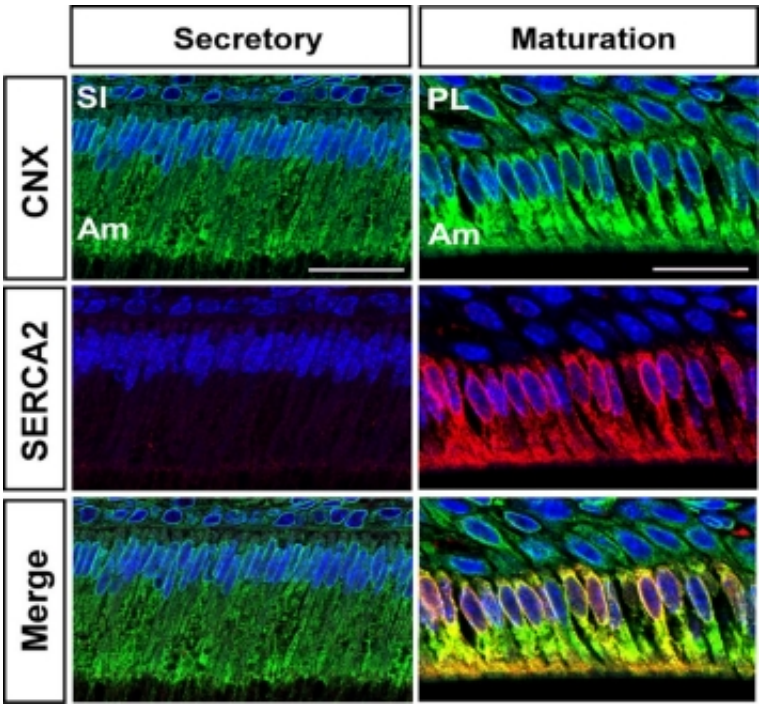
A team of researchers led by Rodrigo Lacruz, MSc, PhD, assistant professor in the Department of Basic Science and Craniofacial Biology

at NYU College of Dentistry, has published a paper in *Scientific Reports* (5:15803) titled "Dental enamel cells express functional SOCE channels," which reports the results of a study showing for the first time the mechanism of calcium transport essential in the formation of dental enamel.

The team found that the main calcium influx pathway involved in the mineralization of enamel [called the CRAC (Ca2+ release-activated Ca2+) channel—the main type of SOCE (Store-operated Ca2+ entry) channel—is critical for controlling calcium uptake, which is necessary for the development of tooth enamel. Despite calcium's central role in the development of enamel, it was not previously understood how it was transported from the bloodstream to the zone where enamel crystals grow.

The finding has important implications for people who suffer from abnormal tooth enamel due to mutations in the genes that control the activity of these channels.

"One of the main characteristics of enamel is its durability, which it owes to the particularly high amount of calcium it contains as well as other minerals," says Dr. Lacruz. "But calcium has to reach the area where crystals are forming. If this action is impeded, which happens when there are mutations in the genes that form the core of the CRAC channel, enamel is severely affected."



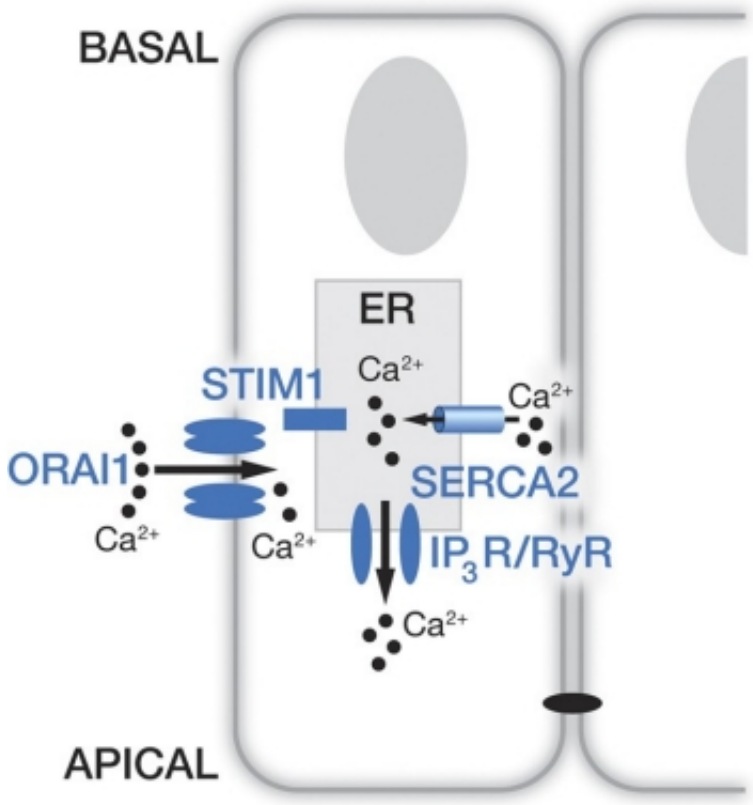
SERCA2 was almost absent in secretory stage ameloblasts but maturation ameloblasts showed strong signals localized to the cytoplasm. SERCA2 expression closely coincides with the localization of the ER as shown by the expression of the ER marker calnexin (CNX). SERCA2 are pumps involved in the transport of Ca2+ from the cytosol into the ER lumen. Am = ameloblasts; PL = papillary layer; SI = stratum intermedium cells. Scale bars in all images = 20 μm.

In this study, the team, which included Dr. Meerim Nurbaeva and Miriam Eckstein in Dr. Lacruz's lab, for the first time used freshly dissected enamel cells (ameloblasts) from rodent teeth to modulate physiological processes in order to understand the contribution of CRAC channels in enamel calcium signaling. The study builds on previous genome research that identified the genes involved in the maturation stage of enamel and other studies which showed that mutations in the genes ORAI1 and STIM1, the main components of the CRAC channel, can affect enamel development. The new study demonstrates a

physiological mechanism for calcium influx in enamel cells and shows how it can be modulated.

"By turning off calcium pumps in the endoplasmic reticulum we were able to see the contribution of CRAC channels to calcium uptake and signaling, and more specifically to how they modulate enamel gene function," says Dr. Lacruz.

Gene mutations involved in enamel mineralization through the CRAC channels were first seen in people with a type of severe combined immunodeficiency, a disorder in which—among other symptoms—tooth enamel may fall off the teeth within five or six years after birth, and the dentine becomes exposed. The number of cases of these severe genetic disorders is less than 20 worldwide, but depending on where the mutation occurs, individuals may experience tooth enamel defects combined with either minor or severe immune deficiencies in the body. Moreover, teeth might become a biomarker to identify defects in the immune system.



Working model for Ca2+ uptake by enamel cells showing maturation stage ameloblasts forming a cell barrier joined by tight junctions at the apical pole. In the endoplasmic reticulum (ER) we find that enamel cells express the sarco/endoplasmic reticulum SERCA2 as the main Ca2+ refilling pump. Inositol 1,4,5-trisphosphate receptors (IP3R) and ryanodine receptors (RyR) are also identified as release channels with the former likely being the active release system. STIM1 has a wide distribution throughout the ER and ORAI1 is found in the plasma membrane of enamel cells. As Ca2+ pools are depleted in the ER, STIM1 clusters enable Ca2+ entry via the ORAI1 channel.

Coauthor Dr. Stefan Feske, of the New York University School of Medicine Department of Pathology, in 2006 discovered ORAI channels, which open and close to allow calcium into cells. This discovery has been critical to solve an important mystery regarding the role of calcium influx in enamel mineralization in which Dr. Feske has also played an

important role.

Calcium is stored in the endoplasmic reticulum (ER) of cells until it is needed. In many cells, the protein STIM1 acts as a sensor in the ER, ensuring the right balance of calcium inside the ER and raising an alarm when calcium levels are low. STIM1 interacts with the ORAI channel in the membrane of the cell, allowing calcium from the blood into the cell to restore balance. Enamel defects can be caused by genetic mutations affecting either the STIM1 calcium sensor in the endoplasm reticulum or in the ORAI channel in the cell membrane.

According to Dr. Lacruz, this new frontier in enamel biology brings closer to reality the possibility of regenerating enamel which in the long run will benefit people who suffer from enamel-formation disorders. "You need to know the ingredients required and the mechanisms involved in the transport of calcium. In all likelihood, this research will contribute to the field of tissue regeneration. You have to know how the tissue works in order to mimic its properties."

More information: Meerim K. Nurbaeva et al. Dental enamel cells express functional SOCE channels, *Scientific Reports* (2015). DOI: 10.1038/srep15803

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