Calcium ions pathways in biomineralisation of Foraminifera: live fluorescent staining experiments

Foraminifera are a group of organisms that have colonized almost all marine environments. Many species of Foraminifera form skeletons, which, depending on the species, are made of organic matter, agglutinated from grains of sand, or calcium carbonate secreted by the Foraminifera themselves. In each species, these shells take on a unique structure: they are often very complex and composed of many gradually (one by one) added chambers. These shells remain on the seafloor after the reproduction or death of the foraminifer and are very easily fossilized, i.e. transformed into fossils. To produce the calcite that builds the shell wall, the foraminiferal cell needs calcium ions, among other things. The isotopic composition of foraminiferal shells and their content of admixtures of other elements depends on the environmental conditions (temperature, salinity, pH, etc.) at the time the shells were formed. As a result, many indicators or proxies used in geology to reconstruct environmental changes in the past are based on the composition of fossil foraminiferal shells. However, this record is modified to some extent by the physiological processes of the foraminiferal cell, what constitute so-called vital effects. It is impossible to correctly interpret geological data without being able to separate the influence of these effects from the environmental signal. Unfortunately, the mechanisms by which the borer cell obtains calcium ions from seawater and the pathways by which they are transported to biomineralisation sites are still not adequately elucidated. Scientists have proposed two different models to explain this process for foraminifers belonging to the class Globothalamea: (1) a trans-membrane transport model, and (2) a seawater vacuolization model. Despite many studies, it is still unclear which of these models properly explains the phenomenon in question. Both models assume that the ultimate source of these ions for foraminifers is seawater, but they differ in their transport pathways. The trans-membrane transport model assumes that all or the vast majority of calcium ions are transported across the cell membrane by specialized proteins (ion channels or pumps) to the cytosol (the fluid filling the cell's interior), and only from there they are transported to sites of calcification. In contrast, according to the vacuolization model, calcium ions do not pass directly through the cytosol, but are transported within vacuoles containing seawater separated from the cytoplasm by a lipid membrane. The models differ in the location of calcium ion transport pathways and the physiological processes involved in this transport, so they have different implications for vital effects. Consequently, understanding the processes involved in calcium ion transport in borers is crucial for interpreting paleo-environmental indicators. An important but controversial issue is whether seawater vacuolization occurs with sufficient intensity to accumulate the amount of calcium ions needed for biomineralisation. Proponents of the trans-membrane transport model emphasize that the rate of seawater vacuolization is too low for the cell to acquire the amount of calcium ions necessary for skeleton formation in this way, and it cannot, according to them, provide the main explanation for the process in question. However, my preliminary research suggests that previous estimates of the intensity of vacuolization are significantly underestimated due to some limitations of the methods used to date to observe this process. As part of the project, a series of experiments will be performed to precisely measure the volume of vacuoles and, most importantly, the rate of seawater water exchange in vacuoles. The main method in the planned research will be fluorescence microscopy. Necessary to achieve this goal is to carry out sequential and short-lived staining of the pinholes during the addition of the new chamber with several fluorescent dyes showing seawater vacuoles, and to proceed with observations immediately after staining. In addition, imaging will be performed using transmission electron microscopy and correlative light-electron microscopy to show the detailed ultrastructure of the areas of the cell involved in seawater vacuolization.