



Morphogenetic role of F-actin meshwork in chamber formation: immunolabeling results from symbiont bearing benthic foraminifera

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Foraminifera are excellent tracers of palaeoceanographic conditions recorded in their shell (test) morphology and chemical composition. Understanding foraminiferal morphology controlled by chamberwise growth can be reduced to processes of chamber formation. However, little is known about how foraminifera control the shape of the chamber wall to be biosynthesized and precipitated. Searching for fundamental morphogenetic features involved in biomineralization, we focused on foraminifers, which belong to the class Globobulimina. The most critical condition to run experiments was to have convenient access to early stages of chamber formation in any species of cultured benthic foraminifers. We have tested small foraminifers collected from the tidal flats of the North Sea. All species, including *Ammonia*, *Haynesina*, and *Elphidium*, turned out to be unsuitable due to their reproduction seasonality and/or unpredictability. The problem was solved by using symbiont bearing *Amphistegina lessonii* cultured in small aquaria. In well treated cultures, such foraminifera often reproduce on a glass wall surface, serving as a continuous source of juveniles. They tend to regularly construct chambers. Another important point is that symbiont bearing foraminifers usually do not construct opaque protective cysts from detritus that disturb observations. All these features facilitate immunolabeling experiments observed under confocal microscopy. Therefore, for the first time, we managed to label cytoskeleton proteins during the chamber formation in Foraminifera. The results show that the shape of chamber is predefined by a meshwork of F-actin, which acts as a dynamic organic scaffold most likely responsible for distribution and docking of biomineralizing molecules (glycoproteins). The F-actin meshwork interacts with microtubules and all associated proteins, which are involved in the morphogenesis of biomineralized structures. Foraminifera, like other eukaryotic cells, can form active lamellipodia, which are used for engulfing the existing test and secondary calcification. Calcium carbonate nucleation takes place within a delimited zone within the organic matrix, incl. actin meshwork, which envelopes the growing skeletal structure during its biomineralization.