

## Transcript assembly and quantification identify candidate genes for foraminiferal calcification

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Foraminifera, a major group of biomineralizing eukaryotes, have two important roles in paleo and modern Earth environments. Firstly, foraminifers produce a large amount of calcium carbonate in pelagic realm due to their widespread distributions and high-biomass over the world oceans. Their calcification could largely impact on the global biogeochemical cycle of both CO<sub>2</sub> and calcium. The second point is that the foraminiferal shells have been preserved in marine sediments with an excellent fossil record, leading to reconstruction of Earth environmental changes in the past. Because foraminifers uptake the extracellular CO<sub>2</sub> from ambient sea-water during the shell-forming, these shells record the chemical condition of the ocean at that time. Therefore, many scientists have paid attention to the mechanism of foraminiferal biomineralization. The precise cell observations during the shell-forming process mentioned that the calcium ion and inorganic carbon were excreted from vesicles in the cell. Such intracellular storages of indispensable elements for calcification concern non-equilibrium state of calcium ion between sea-water and the shells. Even such studies tackled the biomineralization process, they emphasized the necessity of understanding cellular metabolism for foraminiferal biomineralization.

In order to reveal biomineralizing mechanism of foraminifers, we here present the gene expression profiles during the foraminiferal calcification for the first time. We used cultured specimens of *Ammonia beccarii*, which have hyaline calcite, and applied comparative transcriptomic survey by using high-throughput mRNA sequencing (RNA-seq). We performed *de novo* assembly using ~67 million pair-end reads of mRNAs, and then mapped back the reads to the open read frames (ORFs) for calculating the expression levels. From the results, we identified the candidate genes for the calcification process; Ca<sup>2+</sup> is transported through multiple ion channels from an extracellular region and pooled in vesicles. These Ca<sup>2+</sup> seem to play important role for mitochondrial ATP synthesis. We also found the genes coding enzymes, which are related to generate bicarbonate in a cell and to release it to extracellular. In taking account of the studies of cell observations, the present study figured out transportations of ions and molecules for calcification.

Keywords: calcification, single-cell eukaryote, gene expression