The stages of calcium deposition during the formation of sea urchin embryonic and larval spicules

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Abstract

Sea urchin larvae have an endoskeleton consisting of two calcitic spicules. We have reconstructed stages of the formation pathway of calcium carbonate from calcium ions in the sea water to mineral deposition and integration into the forming spicules. Monitoring the calcium uptake with the fluorescent dye calcein shows that calcium ions first penetrate the embryo and are later deposited intracellularly. Surprisingly, calcium carbonate deposits are widely distributed all over the embryo, including in the primary mesenchyme cells (PMCs) and in the endoderm cells. Using confocal microscopy we show that the intra-cellular calcium carbonate deposits are contained in vesicles of sizes 1.5-3 μm. Combined with the newly developed airSEM, the vesicle contents were confirmed to be composed of solid calcium carbonate phase, appearing as aggregates of 30-50 nm nanospheres, consistent with amorphous calcium carbonate (ACC). The aggregates are finally introduced into the spicule compartment, where they integrate with the growing spicule. The mineral-bearing vesicles observed in the ectoderm cells may be part of the spicule mineralization process or may be a designated reservoir for future post larval mineralization events.

1. Calcein uptake from sea water into the embryo

Confocal micrographs of calcein-labeled sea urchin embryos at the gastrula stage. A-C: Green calcein fluorescence; D-F: Fluorescence merged with white light. A: 10 min calcein pulse, 1h chase in seawater. Calcein appears as a cloud in the blastocoel, not in the intracellular environment. B, E: 40 min calcein pulse, 1h chase. Fluorescence is observed both in the ectoderm and in other cells. C, F: Embryo continuously developed in calcein-labeled sea water. Micrometer size calcein-labeled granules are observed all over the embryo. Scales: 20 μm.

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G: Cryo-SEM micrograph of a high-pressure-frozen and freeze-fractured sea urchin embryo at the gastrula stage. The spicule cross-sections are marked with arrows. The ectoderm (E) and endoderm (En) cells of the embryo are fractured, showing large numbers of intra-cellular vesicles of sizes 1.2-1.5 μm. Note the multi vesicular body adjacent to the PMC (arrowhead).

2. There is a correlation between calcein signal and calcium

Correlative airSEM image of a 5100 μm slice of a fixed embryo at ambient conditions showing the back scattered electrons signal (A), calcium EDS map (B) and calcein fluorescence (C). D: The correlation between the calcium EDS signal (B) and calcein fluorescence (C) is shown in the superimposition of the two pictures. Yellow: co-localized signals; green and red: fluorescence and calcium EDS signals appearing separately. Slight deformation of the surface, resulting from electron beam damage, may have decreased the actual co-localization area.

3. Solid calcium deposits inside ectoderm cells

Back scattered electrons (BSE) image (white) and calcium EDS map (red) of a fixed and sliced embryo imaged with the airSEM. A: Whole embryo, calcium EDS map and BSE superimposed, Arrow: spicule, arrowheads: membrane enveloping the embryo; B: Square-labeled area in A, arrow: spicule, arrowhead: cells; BSE. C: Region in B, with calcium EDS map superimposed. S: spicule, C: cell group. EDS quantitative analysis of the two marked areas in C is shown in the table below. The calcium content is lower than of pure CaCO₃ (40 wt%), due to the presence of Na and Cl from sea water, C and O from the mounting gel and the cell components, but is much higher than any value that could be obtained from concentrated cytoplasm or sea water.

4. Abundant intracellular nanosphere-containing vesicles

Cryo-SEM micrograph of a high-pressure-frozen and freeze-fractured sea urchin embryo at the gastrula stage. S, spicule; N, nucleus. A: The primary mesenchyme cells (PMC) and ectoderm cells (Ec) of the embryo are fractured, showing large numbers of intra-cellular vesicles. B: C: Fractured vesicle from PMCs (B) and ectoderm cell (C) containing nanospheres of sizes 20-30nm.

Conclusions

During the process of calcium carbonate deposition in sea urchin embryos at the gastrula stage, calcium is first introduced into the blastocoel in a dispersed form. The mineral then appears inside PMCs as well as in ectoderm cells as membrane bound micrometer-size granules, composed of 20-30nm nanospheres.