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6B Mechanisms of Calcification

Polyp Skeleton Calcification for Polyp-Algae Symbiosis

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Abstract. A single coral polyp of Acropora tenuis created coral polyp skeleton, during which a profusion of unicellular algae of zooxanthellae were accumulated in the host polyp tissue. The controlled process of single polyp growth induced the skeleton formation after the metamorphosis of planula larvae. At the first main skeletogenesis stage the 12 sclerosepta made of $CaCO_3$ were generated beneath the 12 mesenterial septa on a basal disk substrate (DST) made of calcium carbonate-including secretion from a primary polyp in the culture dish. The interface of DST and aboral ectodermis of single polyp was identified as its calcifying space. At the second stage the sclerosepta arose from the DST, with their final ends fixed by generating a $CaCO_3$–made circle and their earliest ends fused to the DST. At the third stage a sheet of 6 branches were built up from the circle towards the central axis of coelenteron and continued to their vertical growth to fix a container of a live polyp, during which it formed tentacles and mesenterial septa. The artful pagoda of adult polyp skeleton was built up on the DST. A live polyp was tightly secured in a 6 spaces-separated container of ~450μm in diameter at ~250μm in height at the top of its skeleton. Since it was difficult to observe a single polyp skeleton without symbiont algae by optical microscope, the polyp-algae symbiosis may play a substantial role to enhance skeleton formation.

Key words Polyp skeleton · Calcification · Calcifying space · Symbiosis · Energetics.

Introduction

Coral colony is one of the optimization factors of marine ecology, as colony-building or reef-building seawater pH level indicated the vibration stability around pH 8.1 during 1700yr and 1950yr (Pelejero et al. 2005). The formation mechanism of a single polyp skeleton is of interest, as a profusion of polyps and their single skeletons constitute a coral colony. The species-specific skeleton is a masterpiece of cellular control over its extracellular environment, and of extracellular environmental control over the cells of polyp.

The calcification mechanism of single skeleton is not yet revealed. It has been investigated whether the specific calicoblastic (= skeletogenic) space is intracellular or extracelluar (Vandermeulen and Watabe 1973; Le Tissier 1988; Hallison and Wallace 1990; Hirose et al. 2008). The identification is essential for calcification reactions that pertain to material energetics (Ichikawa 2007) and physiology. The simultaneous observation of single polyp growth and its skeleton formation is essential and substantial to identify the sequence of skeleton formation and calcification mechanism. It is indispensable to clarify whether skeleton generation is influenced or not by photosynthetic activity of a number of zooxanthellae which reside in polyp tissue (Browniee 2009; Wooldrdge 2010).

In this work we identify the local space of skeleton generation by simultaneously observing polyp growth and polyp skeleton formation, and the settlement of planula larvae during its metamorphosis. The sequential views of polyp skeleton are observed and analyzed to make its formation process clear. It is also significant to reveal the calcification mechanism based on proton/hydroxide efflux in host polyp tissue within which a profusion of zooxanthellae reside as a beneficial partner (Stanley 2006; Yellowlees et al. 2008).

Material and Methods

The bundles of eggs and sperm taken from 4 colonies were immediately mixed in a bowl at high densities and left for 30 min to allow fertilization, and eggs were transferred to large tanks. Buoyant embryos/larvae were transferred daily to fresh seawater, and reared for 4 days. Obtained larvae were packed in plastic bottles at a density of 3,000 larvae per litter, and transported by air to the laboratory in Sapporo, Japan, by a commercial delivery service using larval transportation method (Petersen et al. 2005).

Hormone treatment

Primary polyps were obtained by inducing metamorphosis of larvae in vitro using a metamorphic peptide hormone, Hym-248 (EPLPIGLWamide, Takahashi et al. 1997; Iwao et al. 2002). Larvae were treated with Hym-248 (custom synthesis by Operon Biotechnologies, >90% purity) at 2 x 10^{-6}M for an overnight period to allow metamorphosis into polyps in 60mm culture dish. Polyps were reared in artificial seawater (Rei-Sea Marine, Iwaki, Japan). Symbiotic zooxanthellae were introduced into polyps using those expelled by the polyps, which had acquired zooxanthellae after administration of mashed tissues of the giant clam, Tridacna crocea.

Nuclear stain using Hoechst 33342

The live specimens were stained with 100 µg/ml Hoechst 33342 (Molecular Probes, Eugene, OR, USA) in phosphate-buffered saline solution (PBS, pH 7.4) for 5 min to visualize nuclear DNA, and washed three times in PBS solution. The specimens were observed by using a fluorescent microscope (BZ-8000, KEYENCE, Japan).

Results

Primary coral polyp of Acropora tenuis just after the settlement of planula larvae

The planula larvae metamorphosis was observed after the interchange of each other swimming and settling states in culture dish by optical microscope. The images taken from above, looking down on a primary polyp showed the imperfect six mesenteries, as indicated by arrowheads (Fig. 1 upper a, b, Hirose et al. 2008). The disk substrate (DST) made of the secretion of the primary polyp with calcium carbonate (Raz-Bahat 2006) was observed beneath primary polyp on culture dish (white arrowheads of Fig. 1 lower a, b). The DST and primary polyp were simultaneously observed, with a polyp diameter ~ 520µm which was smaller than the DST diameter ~ 620µm. The primary polyp was underpinned by many sticks (ellipses of Fig. 1 lower a, b) at more or less 50µm.

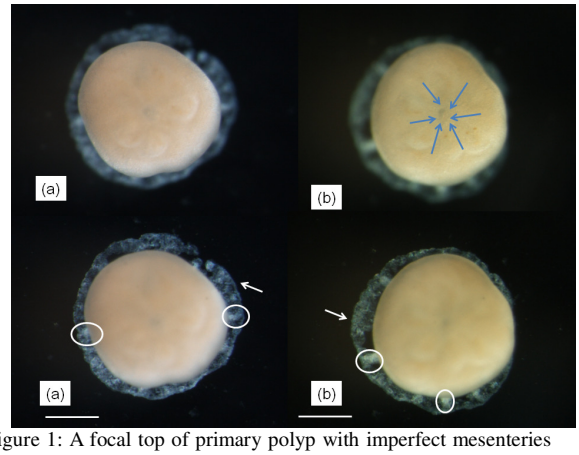

Figure 1: A focal top of primary polyp with imperfect mesenteries (upper a and b. imperfect mesenteries: blue arrows ob b). The focal DST just above culture dish (lower a and b). Scale: 200µm

Correlation between coral polyp skeleton formation and polyp growth

The immature tentacles and mouth were imaged by top-view of a coral polyp (Fig. 2a). No DST was imaged from above by looking down on a polyp because the polyp growth was from ~500µm (Fig. 1) to ~700µm in diameter (Fig. 2a). Live coral polyp images showed many constricted points at polyp margin (white arrow in Fig. 2a).

Figure 2: The polyp growth and polyp skeleton generation. The growth process of mature (white arrow) and immature (black arrow) tentacles, and mouth were imaged by top-view of a coral polyp (b, c). Scale: 200µm

At the first main skeletogenesis stage the secondary mesentery formation-initiating single polyp generated the 12 $CaCO_3$–made branches (red ellipse in Fig. 3a) of ~25µm in thickness beneath the 12 septa of aboral ectodermal layer (Vandermeulen and Watabe 1973; Le Tissier 1988; Hallison and Wallace 1990; Hirose et al. 2008; Fujiwara et al. 2009).

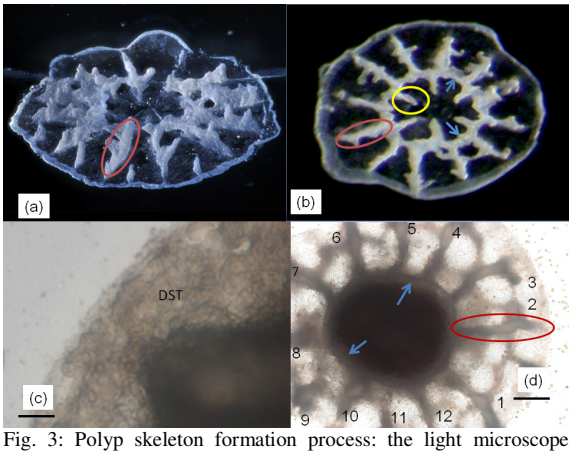

Fig. 3: Polyp skeleton formation process: the light microscope images observed at main three skeletogenesis stages of (a) 5, (b) 6 and (d) 8 days post metamorphosis. The branches surrounded by red ellipse, the circle branch pointed out by blue arrows and the stick branch were generated at 1st, 2nd and 3rd skeletogenesis stages. The images of (c) and (d) were observed by inverted microscopy. Scale: (c) 10μm and (d) 100μm

At the second stage the 12 branches arose from the DST, and their final upper ends were fixed by generating a $CaCO_3$–made circle (pointed out by blue arrows of Fig. 3b, d). Their earliest lower ends were tightly fused to the DST, as confirmed by the inverted microscope image (Fig. 3c). The circle was roughly estimated as ~450μm in diameter at ~250μm in height. At the third stage 6 branches (yellow ellipse in Fig. 3b) were generated from the circle towards the central axis of coelenteron and continued their vertical growth to fix a container of live polyp (blue arrows in Fig. 4b, and Fig. 2c), during which single polyp generated six mesenterial septa, and six mature and six immature tentacles (white and black arrows in Fig. 2b, c). At the later stages the growth of many circular and vertical $CaCO_3$–made branches continued for adult polyp skeleton (Fig. 4b), associated with the formation of secondary mesenterial septa and the growth of twelve mature tentacles, during ~15 days post metamorphosis.

The top view of artful skilled structure showed the six separated spaces by M-type wall made of $CaCO_3$ (blue ellipse in Fig. 4a, b). A live adult polyp secured in a container that consisted of six M-type spaces; two tentacles were put into the respective M-type spaces. The DST diameter was increased from ~600μm for primary polyp (Fig. 1) to ~900μm for the polyp at the later skeletogenesis stages (Fig. 2c, 4a, b). A single polyp was expanded or contracted on the way to grow up from primary polyp to the adult polyp secured in a skeleton container. Many zooxanthellae reside within the tentacles and coelenteron of a live polyp at some skeletogenesis stages (Fig. 2b, c and Fig. 4a). The live zooxanthellae were also observed at the DST by transmitted light and fluorescent microscope, as shown in two lower images of Fig. 4.

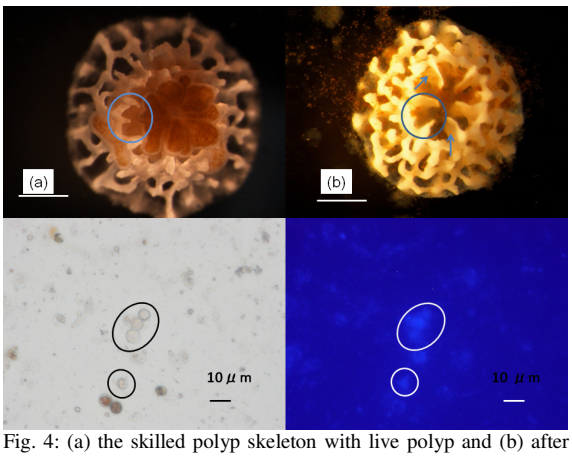

Fig. 4: (a) the skilled polyp skeleton with live polyp and (b) after polyp expulsion from it. (a, b) The top view of polyp skeletons was corrected to the same resolution for any height. The live zooxanthellae on DST were observed by transmitted light and fluorescent microscope; the two lower images confirmed the live cells of zooxanthellae from the observed white circles by using nuclear stain. Scale: (a, b) 250μm.

Discussion

The DST-aboral ectoderm interface was identified as the calcifying space for coral polyp skeleton production. Real skeletogenesis spaces were mainly located beneath the mesenterial septa at different skeletogenesis stages. The diameter of the DST made of polyp secretion with calcium carbonate was increased from ~600μm of primary polyp (Fig. 1) to ~900μm (Fig. 1, Fig. 2c, Fig. 4a). Since the reactants HCO_3^- and Ca^{2+} of calcifying reaction (Goreau 1959; Ichikawa 2007) are supplied from ectodermal cells into calcifying space, the $CaCO_3$-made twelve branches were built up on the DST (red ellipses in Fig. 3). Their lower edges were tightly fused to the DST (Fig. 3c, d). A live polyp secured in a container divided into six parts; the container of ~450μm in diameter and ~250μm in height was constructed at the later skeletogenesis stages (Fig. 2c and 4a). Since it was difficult to observe the skeleton formation for a single polyp without algae zooxanthellae by optical microscope, they may play a substantial role to enhance skeleton formation (Browniee 2009; Wooldrdge 2010). The symbiotic algae need CO_2(aq) for the production of assimilated photosynthates (Goiran et al. 1996; Furla et al. 2000) and generate it from HCO_3^- by the dehydration reaction (Ichikawa 2010) in endodermal cells of coral polyps within which zooxanthellae reside. It is mentioned that the physiological mechanisms of symbiosis and calcification are central to coral polyp health (Weis and Allemand 2009) and the perfect

formation of polyp skeleton. The direct correlation between polyp physiology and polyp calcification may be elucidated by cellular studies (Moya et al. 2008) and material energetics (Ichikawa 2007).

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