

## Structural Analysis for Marine Endosymbiosis in Corals

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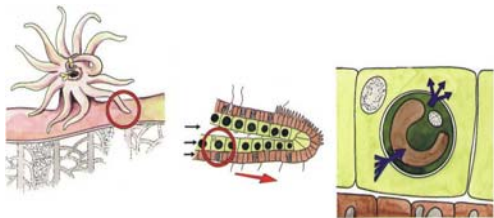
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Scleractinian corals are precious marine resources for ecosystem welfare, academic investigation, environmental regulation and economy. The endosymbiosis in cnidaria-dinoflagellate symbiodinium spp. (or zooxanthellae in generic name) association is obligatory and mutualistic for both host and symbiont. Therefore, this association preserves the vitality and regulates the productivity of corals. Environmental changes, such as irradiation, temperature increasing or pollution of sea water, devastate this nature situation and result in expulsion of symbionts from the host, eventually cause death of corals. An understanding of the mechanism of endosymbiosis becomes essential for protecting the corals.

Our investigations on cnidarian-dinoflagellate endosymbiosis (Fig. 1) with morphological and proteomic examination at molecular and cellular levels have provided the techniques for the identification of symbiotic endoderm cells[1] and isolation of the tissue layers in corals[2]. Furthermore, we demonstrated the translocation of endosymbiotic dinoflagellates in different tissue layers of coral larvae[3]. Nevertheless, the mechanistic investigation at tissue level is inhibited by the complexity of coral tissues and presence of coral skeleton. Endosymbiosis regulates photosynthesis of symbionts, which may then modulate the skeletogenesis of corals. The ability to detect endosymbiotic activity at the whole tissue level is significant in order to fully understand how this peculiar cellular activity may determine total reef formation.

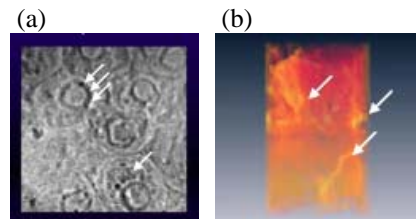


**Figure 1.** Diploblastic body pattern of a coral. The middle diagram illustrates distribution of endoderm and ectoderm cell layers. (Adapted from [http://www.Uni-koeln.de/math-nat-fak/zoologie/oekologie/marine-oekophysiologie/lb2\\_3Zooxanthellae.html](http://www.Uni-koeln.de/math-nat-fak/zoologie/oekologie/marine-oekophysiologie/lb2_3Zooxanthellae.html))

We utilize a nano-transmission X-ray microscope (TXM) with 50 nm spatial resolution and phase contrast capability, which is installed at BL01B1 end-station[4,5] at the NSRRC for structural analysis of the distribution of

Rubisco (Ribulose biphosphate carboxylase). Rubisco is the critical enzyme responsible for carbon fixation in the dark reaction of photosynthesis. Photosynthetic efficiency (Fv/Fm) of symbiont zooxanthellae correlates with endosymbiotic status. Better endosymbiotic association between coral host cells and zooxanthellae is usually accompanied by optimal photosynthetic efficiency of zooxanthellae. Preliminary gel-electrophoresis data indicate that Rubisco expression correlates with photosynthetic efficiency in free-living zooxanthellae. The expression of Rubisco will be used as an indicator for endosymbiotic activity.

We prepare the *Euphullia glabrescens* tissue with immuno-nano-gold labeling to Rubisco in zooxanthellae. The preliminary TXM images (Fig. 2) show that the Rubisco (see arrows) mostly distribute in pyrenoid and chloroplast of endosymbiotic zooxanthellae. Due to the rarity of the Rubisco and the difficulty of the immuno-gold labeling through the cell wall of zooxanthellae, we will continue to identify the treatment condition of the gold labeling more deliberately and construct the three-dimensional distribution of the Rubisco in zooxanthellae.



**Figure 2.** TXM images of the Rubisco distribution in zooxanthellae within the coral tissue. (a) Two-dimensional transmission image ( $15 \times 15 \mu\text{m}^2$ ) (b) Tomography ( $8 \times 8 \times 15 \mu\text{m}^3$ ). The arrows indicate the Rubisco.

### References

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