論文内容の要旨

Analysis of endosymbosis genes in coral harboring zooxanthellae

(サンゴと渦鞭毛藻の細胞内共生に関与する遺伝子の解析)

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Reef-forming scleractinian corals harbor endosymbiotic dinoflagellates in the genus *Symbiodinium* spp. (referred to as zooxanthellae) in their endodermal cells and build the structural and trophic foundation of coral reefs in the tropical and subtropical zones. The symbiotic relationship is crucial for the coral life, as extended loss of zooxanthellae (coral bleaching) due to physical stress or bacterial infection is fatal to the host. In addition to the role for survival, zooxanthellae cells also enhance the growth rate of the host coral through increased calcification rate of the host skeleton. It is apparent that zooxanthellae transfer photosynthetic products to the host tissue

to maintain endo-symbiosis. In return, zooxanthellae receive inorganic and organic nutrients from the host metabolites including sulfate, ammonium and phosphate. However, the mechanism of endo-symbiosis remains largely unknown, particularly at the molecular level, in the reef-forming scleractinian corals. Therefore, I aimed to identify coral genes that are involved in endo-symbiosis of zooxanthellae by comparing mRNA expression between symbiotic and aposymbiotic corals and to assess the function of some genes in the symbiosis.

As an initial step toward this end for this purpose, I established a culture system of the coral polyp to which cultured monoclonal *Symbiodinium* was infected. In this system, I could culture the polyp without symbionts (aposymbiotic coral juvenile) for extended days. Two *Symbiodinium* cells (PL-TS-1 and CCMP2467 strains) are shown to be effective for acceleration of polyp growth. Therefore, I compared the gene expression profiles among aposymbiotic, PL-TS-1-inhabited and CCMP2467-inhabited corals to identify the genes that respond significantly to endo-symbiosis. High coverage expression profiling (HiCEP) methods was applied to the juvenile coral inhabiting *Symbiodinium* cells for 20 days. In total, 25 genes were identified as symbiosis-related genes, of which 11 increased and 14 decreased in the symbiotic corals. The change of the expression level was confirmed by the quantitative RT-PCR. The genes were annotated by the homology

search using the Blastx program. Annotated genes could be categorized according to their function; 1) lipid metabolism, 2) ion transport, 3) cell signaling, and 4) bone morphogenesis. These results suggest that several genes are involved in endo-symbiosis by changing the metabolism and growth of the host corals.

Among the identified genes, I chose the sulfate transporter gene and performed further analysis to assess its function in endo-symbiosis. Because mucus and skeleton of corals are known to contain sulfate glycosaminoglycans, sulfate transporter may have important regulatory roles in bone morphogenesis and mucus production that are up-regulated by the endo-symbiosis. The determination of the whole cDNA sequence and subsequent molecular phylogenetic analysis of the precursor protein strongly suggested that the identified sulfate transporter is an ortholog of SLC26A11, a Na^+-independent sulfate transporter, of vertebrates and invertebrates. The antiserum was raised against the coral protein and localization of the transporter was examined in the host coral. It was shown that the immunoreactive sulfate transporter was localized in mucus cells and the tissue between the coelenteron and skeleton. These results suggest the possibility that the sulfate transporter protein is involved in the uptake of SO_4^{2-} for synthesis of sulfated macromolecules that are necessary for the formation of calcified skeleton and mucus. These expression analysis

and functional analysis provide useful information for future investigation of the molecular mechanisms involved in coral-zooxanthellae symbiosis.