MIS27-01 Room:101B Time:May 19 14:15-14:30

Morphological changes in kleptochloroplasts after ingestion in the unarmored dinoflagellates

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Dinoflagellates are ubiquitous unicellular protists and the evolutionary scenario of their chloroplast evolution is quite complex one. About half of the dinoflagellates are photosynthetic, while rests are heterotrophic species, and the latters are thought to have evolved from the photosynthetic ancestors by losing their chloroplasts. Additionally, some dinoflagellates have replaced their original chloroplasts with those of haptophyte, diatom or green alga. In addition, some dinoflagellates possess 'kleptochloroplast', which is the temporary chloroplast 'stolen' from other photosynthetic algae.

The unarmoured dinoflagellate *Amphidinium poecilochroum* (marine) and *Gymnodinium aeruginosum* (freshwater) are closely-related to each other, and both possess kleptochloroplasts derived from cryptomonads. These dinoflagellates ingest cryptomonad cell and retain its chloroplast temporarily, but eventually lose the chloroplast due to cell division or digestion. Previous studies revealed that several differences exist between marine and freshwater representatives with regard to the cryptomonad-dinoflagellate specificity and the dynamics of kleptochloroplast processing. *A. poecilochroum* is capable of ingesting any species of cryptomonads, and synchronised division of kleptochloroplast with the host cell has never been observed. By contrast, *G. aeruginosum* can accept only members of the genus *Chroomonas* as prey and the kleptochloroplast is simultaneously divided with the host cell and being inherited by each daughter cell. Thus, the kleptochloroplastidy in *G. aeruginosum* seems to represent much more advanced stage toward acquisition of 'true chloroplast' within the linage. Therefore, unraveling the differences between the two species in detail might give us clue to understand evolutionary significance and contribution of kleptochloroplast during the quest for true chloroplasts. Although the general ultrastructure of these dinoflagellates has been studied, the morphological changes from ingestion of cryptomonad to disappearance of kleptochloroplast have never been focused and remain unclear. In this study, we observed the morphological changes of kleptochloroplast in *A. poecilochroum* and *G. aeruginosum* using light and transmission electron microscopes, and compared the differences between the two species.

The both species ingested cryptomonad chloroplast, nucleus, nucleomorph, mitochondria and ejectosomes with surrounding cytoplasm directly into the dinoflagellate cytoplasm. In *A. poecilochroum*, cryptomonad mitochondria and ejectosomes were removed together with cytoplasm, by transferring them into the food vacuole within 1 h after ingestion. The kleptochloroplast was enlarged gradually, and the cryptomonad nucleus was digested after 3 h. In *G. aeruginosum*, the cryptomonad cytoplasm, containing cryptomonad nucleus and mitochondria, was retained around the chloroplast. The chloroplast was enlarged drastically after 6 h, and eventually occupied most of the host cytoplasm by the 3rd day, forming a cup shape with several pyrenoids. The cryptomonad nucleus was positioned inside the cup-shaped chloroplast. By the day 5, the nucleomorph has undergone multiplication at the vicinity of the cryptomonad nucleus. This study revealed that *G. aeruginosum* can expand its kleptochloroplast more extensively and is capable of retaining the cryptomonad nucleus for a longer period than *A. poecilochroum*. Previous study on the kleptochloroplastidic ciliate *Mesodinium rubrum* showed that the endosymbiont nucleus plays an important role to maintain its kleptochloroplast. The diatom-harboring dinoflagellates possess diatom nucleus, and can divide the latter nucleus simultaneously with the host cell division. The differences between *G. aeruginosum* and *A. poecilochroum* indicated in this study also support that retention of endosymbiont nucleus is advantageous to maintain its chloroplast stably.

Keywords: dinoflagellate, kleptochloroplast, ultrastructure

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MIS27-02 Room:101B Time:May 19 14:30-14:45

Algivore or Phototroph? *Plakobranchus ocellatus* (Gastropoda) Continuously Acquires Kleptoplasts and Nutrition

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The sea slug *Plakobranchus ocellatus* (Sacoglossa, Gastropoda) retains photosynthetically active chloroplasts from ingested algae (functional kleptoplasts) in the epithelial cells of its digestive gland for up to 10 months. While its feeding behavior has not been observed in natural habitats, two hypotheses have been proposed: 1) adult *P. ocellatus* uses kleptoplasts to obtain photosynthates and nutritionally behaves as a photoautotroph without replenishing the kleptoplasts; or 2) it behaves as a mixotroph (photoautotroph and herbivorous consumer) and replenishes kleptoplasts continually or periodically. To address the question of which hypothesis is more likely, we examined the source algae for kleptoplasts and temporal changes in kleptoplast composition and nutritional contribution. By characterizing the temporal diversity of *P. ocellatus* kleptoplasts using *rbcL* sequences, we found that *P. ocellatus* harvests kleptoplasts from at least 8 different siphonous green algal species, that kleptoplasts from more than one species are present in each individual sea slug, and that the kleptoplast composition differs temporally. These results suggest that wild *P. ocellatus* often feed on multiple species of siphonous algae from which they continually obtain fresh chloroplasts. By estimating the trophic position of wild and starved *P. ocellatus* using the stable nitrogen isotopic composition of amino acids, we showed that despite the abundance of kleptoplasts, their photosynthates do not contribute greatly to the nutrition of wild *P. ocellatus*, but that kleptoplast photosynthates form a significant source of nutrition for starved sea slugs. The herbivorous nature of wild *P. ocellatus* is consistent with insights from molecular analyses indicating that kleptoplasts are frequently replenished from ingested algae, leading to the conclusion that natural populations of *P. ocellatus* do not rely on photosynthesis but mainly on the digestion of ingested algae.

Keywords: kleptoplasty, sacoglossan, ulvophyceae, symbiosis

MIS27-03 Room:101B Time:May 19 14:45-15:00

Evolution of symchlosomes driven by endosymbiosis of zoochlorellae in freshwater protozoa and metazoa

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Freshwater micro-predetors bearing zoochlorellae (intracellular symbiotic green algae) have been reported in various protozoan and metazoan groups. In order to extract common features among endosymbiosis of zoochlorellae in various host organisms, four 'green' species, *Mayorella viridis* (amoeboid protozoan), *Paramecium bursaria* (ciliated protozoan), *Stentor polymorphus* (ciliated protozoan), and *Hydra viridissima* (Cnidaria) were observed with a transmission electron microscope by freeze-substitution technique. Their endosymbiotic zoochlorellae formed very regulative membrane-bound photosynthetic organelles, which we named **symchlorosomes**. Symchlorosomes can be found in many freshwater micro-predatory species with a very wide genetic variety, which are ecologically important as they can provide a new niche for such mixotrophic organisms in freshwater micro-environment. We are going to introduce a possibility of ecological and evolutional researches on symchlorosomes through our resent ultrastructural study.

Keywords: protozoa, algae, endosymbiosis, zoochlorella, symchlorosome

MIS27-04 Room:101B Time:May 19 15:00-15:15

Fine-structure and molecular analyses of symbiotic algae in Radiolaria

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Marine, holoplanktonic protist radiolarians retain the algal symbionts within the cytoplasmic bodies. The majority of modern symbionts-bearing radiolarians appear to depend on their symbionts to provide photosynthetically fixed carbon and to maintain the radiolarians in low nutrient environments (e.g., Anderson 1978). Therefore, acquisitions of the photo-symbionts have may have had their survival under low nutrient condition in the geologic time. During symbiotic state, algal symbiont within radiolarians generally appear as yellow-brown minute spheres, several micrometers in diameter. Cyanobacteria, dinoflagellates, prasinophytes, and haptophytes have all been identified as symbionts of radiolarians (e.g. Anderson 1983; Foster et al. 2006; Yuasa et al. 2012). However, the accurate taxonomic affiliation of these symbionts has not been clarified by the lack of diagnostic morphological features, such as theca or flagella, during the symbiotic state. Among them, I was able to establish cultures of the symbiotic dinoflagellate and compared the motile cell morphology and the molecular phylogeny of the SSU rDNA sequences with those of related species. The features of the thecal plate pattern and the molecular phylogenetic analysis indicate that the symbiotic dinoflagellate belongs to the peridinioid genus and species. In addition, based on the ultrastructural features by scanning electron and transmission electron microscopy and the molecular phylogenetic analyses of non-motile cells of other symbiotic algae, I found that radiolarian species contained some other partners; *Synechococcus* sp. (Cyanobacteria), *Chrysochromulina* sp. (Haptophyte) and Chlorophyta gen. sp. This symbiont diversity is in contrast to many corals, which host only dinoflagellates (*Symbiodinium* spp. and others). On the other hand, the symbionts have never co-occurred in a single host radiolarians, so the notion of only one kind of symbiotic algae per individual host has been maintained. A hypothesis would be that radiolarian symbionts originated from some free-living algae. This hypothesis is in agreement with the concept that radiolarians can easily acquire cyanobacteria symbionts *Synechococcus* sp. and *Prochlorococcus* sp. from environmental pools (Foster et al. 2006; Yuasa et al. 2012). Very little is known, however, about the distribution of free-living dinoflagellate, and, as far as we know, there is no evidence for the presence of radiolarian specific dinoflagellate symbionts in the natural environment.

Keywords: Radiolaria, Symbiosis, algae, ultrastructure, molecular phylogeny

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Symbiotic relationship between *Braarudosphaera bigelowii* and cyanobacteria

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Braarudosphaera bigelowii (Haptophyta, Prymnesiophyceae) is a single-celled coastal coccolithophores, which is characterized by regular dodecahedoral exotheca consists of regular pentagonal calcareous scales called pentaliths. Fossil records of the Family Braarudosphaeraceae and *B. bigelowii* extend back to the early and late Cretaceous, respectively. Living and fossil *B. bigelowii* have significant variation in size of pentaliths. Molecular phylogenetic study of living *B. bigelowii* revealed that morphotypes of living *B. bigelowii*, which was classified based on the size of pentaliths, can be related to the 18S rDNA genotypes. Therefore, it is thought that living *B. bigelowii* is a species complex consists of at least four discrete species which can be differentiated from each other based on size of pentaliths and of 18S rDNA sequences (Hagino et al. 2009). A recent study revealed close phylogenetic relationships among *B. bigelowii* sensu stricto (morphotype Intermediate form B, 18S rDNA Genotype III), *Chrysochlomulina parkeae* (Prymnesiophyceae) and a prymnesiophyte cell that has symbiotic association with a nitrogen-fixing cyanobacterium UNYN-A. The prymnesiophyte host cell receives nitrogen from the cyanobacterium in exchange for transferring fixed carbon (Thompson et al., 2012). It was an unexpected relationship since *B. bigelowii* dissimilar to *C. parkeae* in general morphology, and *B. bigelowii* differs from UCYN-A in geographic distribution; living *B. bigelowii* is a notable coastal-neritic dweller, while the UCYN-A were abundantly reported from oligotrophic open ocean. In order to examine their relationships, we have conducted transmission electron microscopic and molecular phylogenetic studies of *B. bigelowii* and *C. parkeae*. In this talk, we will present an overview of geological history of the Family Braarudosphaeraceae, and morphological and genetic diversity in living *B. bigelowii*. We will also discuss about relationships among *B. bigelowii*, *C. parkeae* and the prymnesiophyte host of the UCYN-A based on the results from our morphological and molecular phylogenetic studies.

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Keywords: coccolithophores, cyanobacteria, symbiosis