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Morphological Transition in Kleptochloroplasts after Ingestion in the Dinoflagellates *Amphidinium poecilochroum* and *Gymnodinium aeruginosum* (Dinophyceae)

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The unarmoured marine dinoflagellate *Amphidinium poecilochroum* and the unarmoured freshwater dinoflagellate *Gymnodinium aeruginosum* both belonging to the same clade, are known to possess cryptomonad-derived kleptochloroplasts. Previous studies revealed that *G. aeruginosum* can synchronise the division of the chloroplast with its own cell division while no simultaneous division takes place in *A. poecilochroum*, which is interpreted to mean that state of kleptochloroplastidy in *G. aeruginosum* is closer to that of the initial acquisition of the 'true chloroplast' within the lineage. Although the general ultrastructure of these two species has been reported, the changes in the kleptochloroplast with time have never been followed. We observed morphological changes in kleptochloroplasts of *A. poecilochroum* and *G. aeruginosum* following the ingestion of cryptomonad cells, using light and transmission electron microscopes. In *A. poecilochroum*, the cryptomonad ejectosomes, mitochondria and cytoplasm were all actively transferred into digestive vacuoles within 1 h of ingestion. The chloroplasts were deformed and the cryptomonad nucleus was digested after 3 h. By contrast, in *G. aeruginosum*, the cryptomonad cytoplasm and nucleus were retained for 24 h following ingestion, and the chloroplast was substantially enlarged. These differences imply that the retention of the cryptomonad nucleus is important for the maintenance of the chloroplast.

Key words: *Amphidinium poecilochroum*; cryptomonad organelle; *Gymnodinium aeruginosum*; kleptochloroplast; morphological change; ultrastructure.

Introduction

Dinoflagellates are considered to have followed highly diverse evolutionary strategies, especially with regard to their chloroplast origins. The ancestor of all dinoflagellates is considered to be

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photosynthetic and to have acquired a chloroplast derived from a red alga via secondary endosymbiosis, although the timing of acquisition is unclear (Horiguchi 2006; Keeling 2010). Typical photosynthetic dinoflagellates resulting from such an endosymbiotic event possess the peridinin chloroplasts. However, a number of dinoflagellates have lost their chloroplasts and have become heterotrophic (Saldarriaga et al. 2001). Moreover, some dinoflagellates are considered to have replaced their original peridinin chloroplasts with those of diatom or haptophyte origin via tertiary endosymbiotic events, or with that of chlorophyte origin via serial secondary endosymbiosis (Hackett et al. 2004; Horiguchi 2006; Saldarriaga et al. 2001; Stoebe and Maier 2002). Thus, dinoflagellates have a very complex history with regard to the evolution of their chloroplasts.

In addition to these permanent chloroplasts mentioned above, some dinoflagellates possess a unique form of 'chloroplast'. These dinoflagellates, which have lost the original peridinin chloroplast ingest chloroplasts (often together with other organelles) of other photosynthetic algae, and utilize them for photosynthesis. The ingested chloroplasts are temporarily retained in the dinoflagellate cell, but are eventually lost during cell division or digestion and the dinoflagellates need to feed on other photosynthetic algal cells to regain its temporary 'chloroplast'. This type of chloroplast is called a "stolen chloroplast" or "kleptochloroplast" (Schnepf and Elbrächter 1992).

The kleptochloroplast phenomenon is widely spread in dinoflagellates, from the armoured species of *Dinophysis* (Schnepf and Elbrächter 1988) and *Amylax* spp. (Koike and Takishita 2008) to unarmoured species, i.e. *Amphidinium latum* (Horiguchi and Pienaar 1992), *A. poecilochroum* (Larsen 1988), *Gymnodinium aeruginosum* (Schnepf et al. 1989), *G. acidotum* (Wilcox and Wedemayer 1984), *G. myriopyrenoides* (Yamaguchi et al. 2011), *G. eucyaneum* (Xia et al. 2013), *G.* '*gracilentum*' (Skovgaard 1998), a novel dinoflagellate (RS24 and W5-1 strains) (Gast et al. 2007). *Dinophysis* spp. acquire kleptochloroplasts not by engulfing cryptomonad cells directly, but by ingesting the ciliate *Mesodinium rubrum*, the chloroplasts of which are acquired from the cryptophyte *Teleaulax* (Nagai et al. 2008; Park et al. 2006). *Dinophysis* spp. keep only the chloroplasts in the dinoflagellate cytoplasm, and the chloroplasts are surrounded by two membranes (Schnepf and Elbrächter 1988). *Amylax* spp. possess cryptomonad chloroplasts derived from *Teleaulax* sp. (Koike and Takishita 2008). Recent phylogenetic studies indicated that the unarmoured kleptochloroplastidic dinoflagellates *A. poecilochroum, G. acidotum*, *G. eucyaneum* and *G. myriopyrenoides* are monophyletic (Xia et al. 2013; Yamaguchi et al. 2011). Moreover, it was reported that *A. latum*, *G. acidotum* and *G. aeruginosum* also form a monophyletic group (Takano and Horiguchi 2007). Taking these results into consideration, all the unarmoured kleptochloroplastidic dinoflagellates are monophyletic and a single origin of kleptochloroplastidy within this linage is highly likely. Interestingly, among the unarmoured kleptochloroplastidic dinoflagellate clade, several differences between marine and freshwater representatives have been noted with regard to the specificity of cryptomonads ingested and the dynamics of the kleptochloroplast within the host cell as mentioned below.

The marine dinoflagellate *Amphidinium poecilochroum* is sand-dwelling and usually possesses 4-8 blue-green or yellow-green cryptomonad chloroplasts in a single cell, the colour depending on the species of cryptophyte engulfed (Larsen 1988). Another marine species, *A. latum*, is also sand-dwelling and can have several cryptomonad cells, often of different colour or structure (Horiguchi and Pienaar 1992). Therefore, these marine kleptochloroplastidic species are capable of ingesting more than one species belonging to the class Cryptophyceae. These kleptochloroplasts are surrounded by four membranes; two chloroplast membranes, two chloroplast endoplasmic reticulum (ER) membranes, which is the same membrane composition as that of free-living cryptomonads. In addition to the chloroplast, the dinoflagellate engulfs cryptomonad cytoplasm and a single membrane (referred as 'phagotrophic' vacuole in Larsen (1988)) separates the cryptomonad cytoplasm from the dinoflagellate cytoplasm. The cryptomonad cytoplasm contains the cryptomonad nucleus, mitochondria and the periplastidal compartment (PPC) inside of which is the nucleomorph (Horiguchi and Pienaar 1992; Larsen 1988). When the dinoflagellates divide, the cryptomonad kleptochloroplasts are randomly distributed between the daughter cells, and no synchronization of divisions of the kleptochloroplasts and host cell has been observed.

By contrast, the freshwater dinoflagellates *Gymnodinium acidotum* and *G. aeruginosum* possess only blue-green kleptochloroplasts (Schnepf et al. 1989; Wilcox and Wedemayer 1984). In fact, the cryptomonads that *G. acidotum* can ingest are members of the genus *Chroomonas* (which are blue-green) only, and no other