

Integration of plastids with their hosts: Lessons learned from dinoflagellates

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After their endosymbiotic acquisition, plastids become intimately connected with the biology of their host. For example, genes essential for plastid function may be relocated from the genomes of plastids to the host nucleus, and pathways may evolve within the host to support the plastid. In this review, we consider the different degrees of integration observed in dinoflagellates and their associated plastids, which have been acquired through multiple different endosymbiotic events. Most dinoflagellate species possess plastids that contain the pigment peridinin and show extreme reduction and integration with the host biology. In some species, these plastids have been replaced through serial endosymbiosis with plastids derived from a different phylogenetic derivation, of which some have become intimately connected with the biology of the host whereas others have not. We discuss in particular the evolution of the fucoxanthin-containing dinoflagellates, which have adapted pathways retained from the ancestral peridinin plastid symbiosis for transcript processing in their current, serially acquired plastids. Finally, we consider why such a diversity of different degrees of integration between host and plastid is observed in different dinoflagellates and how dinoflagellates may thus inform our broader understanding of plastid evolution and function.

dinotoms | poly(U) tail | transcript editing | chloroplast genomes | minicircle

Plastids evolve through the endosymbiotic integration of two organisms: a eukaryotic host and a photosynthetic prokaryotic or eukaryotic symbiont. It is generally believed that the host initially consumes the symbiont through phagocytosis. Subsequently, over long evolutionary timescales, pathways evolve within the host to maintain the endosymbiont as a permanent, intracellular organelle (1). At least eight distinct plastid endosymbioses have been documented across the eukaryotes, giving rise to a diverse array of different photosynthetic lineages (reviewed in ref. 2). Understanding what processes underpin the integration of plastids with their hosts may provide valuable insights into the evolution and function of photosynthetic eukaryotes.

Integration in Plastid Evolution

Plastids and their hosts share intricate biological connections. For example, plastids possess transporters that enable them to export photosynthetic and photorespiratory products to the host and to import inorganic nutrients and cofactors essential for plastid metabolism (3, 4). Plastid replication and division are likewise dependent on proteins encoded within the host nucleus (5). Finally, gene expression within the plastid depends on factors expressed within the host, alongside other factors encoded within the plastid genome (6). The host factors may support the plastid, for example, by regulating plastid gene expression and, at an evolutionary level, by correcting mutations in the plastid genome that might otherwise prove deleterious (6, 7).

Each of these examples of integration depends on proteins that are encoded within the nuclear genome but are targeted to the plastid. Some of these proteins were originally of plastid

origin, with the genes encoding them having been transferred to the nucleus of the host after endosymbiosis (8, 9). In other cases, genes endogenous to the host may be recruited to support the plastid, changing its biology. It is likely that most extant plastids are supported by a mosaic of pathways, some of symbiont and some of host origin. For example, approximately half of the plant plastid proteome consists of proteins of nonplastid origin, which may thus have been acquired from the host nucleus (10).

Dinoflagellates in the Context of Plastid Integration

Some of the most extreme examples of plastid evolution are found within the dinoflagellate algae. Dinoflagellates are members of the alveolate kingdom, and their nuclei are only distantly related to those of plants (2). Dinoflagellates play important roles in aquatic ecology. Some species (e.g., *Amphidinium*, *Pyrocystis*) are principally free-living primary producers and mixotrophs whereas others (e.g., *Symbiodinium*) are symbionts of marine invertebrates, such as coral (11). Some free-living dinoflagellates (e.g., *Ceratium*, *Lingulodinium*) have important economic effects as causative agents of harmful algal blooms, which have major effects on global fisheries (11). The dinoflagellates are closely related to the coral symbiont “chromerid” algae *Chromera velia* and *Vitrella brassicaformis* and to the apicomplexans, a lineage that includes the malaria parasite *Plasmodium* (Fig. 1) (12, 13).

The cellular organization of dinoflagellates is highly unusual. For example, the dinoflagellate nuclear genome is extremely large, with many genes present in multiple copies (14). This genome is permanently condensed and uses an unusual DNA packaging protein that is evolutionarily distinct from histones (15). The dinoflagellate mitochondrial genome is likewise highly abnormal, containing only three protein-coding genes (*cob*, *coxI*, and *coxIII*), which are present in multiple, fragmented copies (16, 17).

The majority of photosynthetic dinoflagellates possess plastids that contain the accessory carotenoid light harvesting pigment peridinin (18, 19). This plastid is surrounded by three membranes, is of red algal origin, and probably originated through a secondary endosymbiotic event (2, 20). The peridinin plastid branches as a sister group to the plastids found in chromerid algae and to the vestigial, nonphotosynthetic plastids found in apicomplexans, suggesting a common endosymbiotic origin of all three plastid lineages (although chromerid and apicomplexan

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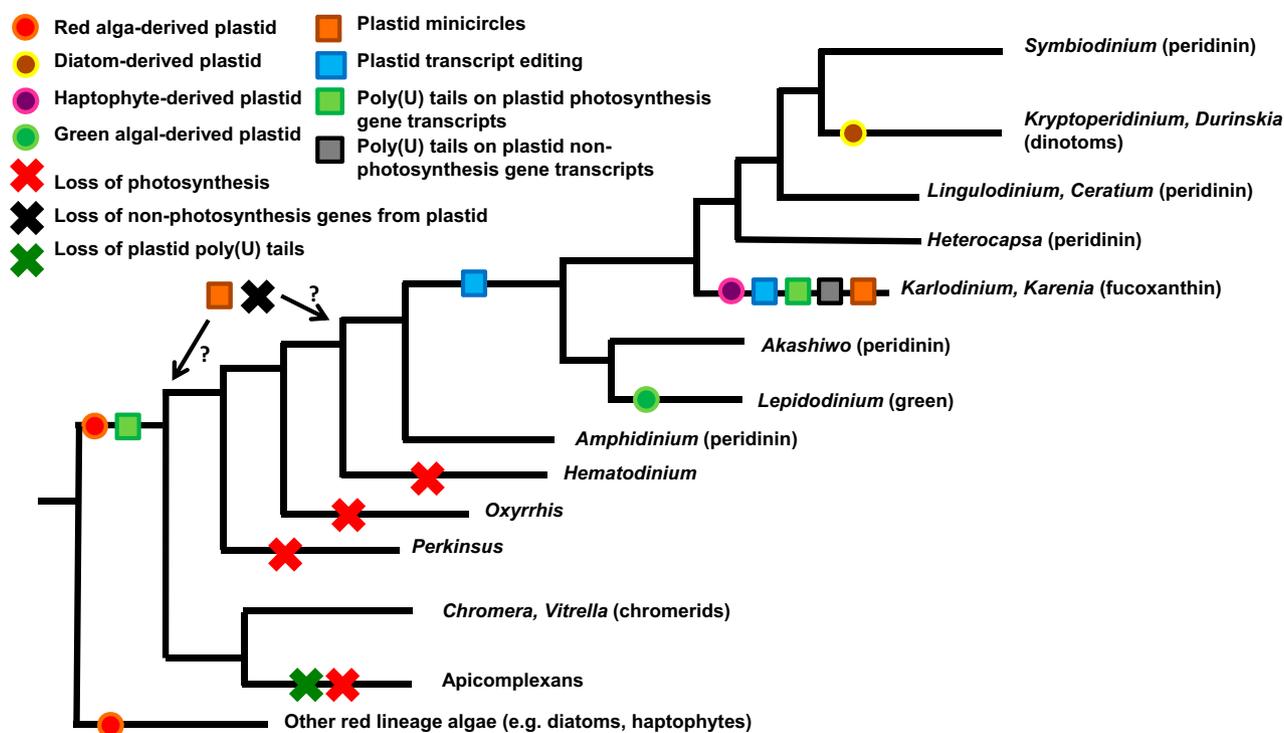
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Lineage	Plastid	Plastid Genome	Membranes	Minicircles	Editing	Poly(U) Tails
Diatoms, Haptophytes	Red algal origin	Phot + non-phot genes	4	No	No	No
Chromerids	Red algal origin	Phot + non-phot genes	4	No	No	Phot genes
Apicomplexans	Red algal origin	Non-phot genes only	4	No	No	No
<i>Oxyrrhis</i> , <i>Perkinsus</i>	Red algal origin	-	4?	-	-	-
<i>Amphidinium</i>	Red algal origin	Phot genes only	3	Yes	No	Yes
Other peridinin dinos	Red algal origin	Phot genes only	3	Yes	Yes	Yes
<i>Lepidodinium</i>	Green algal origin	Phot + non-phot genes	4	No	No	No
Dinotoms	Diatom origin	Phot + non-phot genes	5	No	No	No
Fucoxanthin dinos	Haptophyte origin	Phot + non-phot genes	3	Yes (<i>dnaK</i>)	Yes	Phot + Non-phot genes

Fig. 1. Evolution of dinoflagellates and their plastids. (Upper) An evolutionary tree of dinoflagellates and their closest relatives, adapted from ref. 41. The evolutionary relationships in this tree are taken from refs. 13, 48, and 91); for simplicity, only a representative sample of dinoflagellate species is shown. The endosymbiotic acquisition and secondary loss of each individual plastid lineage, the loss of nonphotosynthesis genes from the peridinin plastid lineage, and the origins of minicircles, poly(U) tail addition, and transcript editing in peridinin and fucoxanthin plastids are labeled on the diagram. It is not clear from current data whether the loss of nonphotosynthesis genes and the evolution of minicircle gene organization occurred in the peridinin lineage before or after the divergence of basal dinoflagellates such as *Perkinsus*, *Oxyrrhis*, and *Hematodinium* (which have since lost the capacity for photosynthesis entirely); accordingly, the earliest and latest evolutionary points at which these events can have occurred are shown on the tree, labeled with question marks. (Lower) Tabulates key features of the different plastid lineages discussed in this manuscript.

plastids are surrounded by four membranes and do not seem to contain peridinin) (Fig. 1) (12, 13, 21). The peridinin plastid is also very closely related to other plastid lineages acquired through the secondary endosymbiosis of red algae (for example, those of diatoms and haptophytes) although the current consensus is that the dinoflagellate, diatom, and haptophyte plastids have each been acquired independently by the respective host lineage, rather than all descending from one common endosymbiotic event (Fig. 1) (2, 9, 11).

Dinoflagellates present an ideal model system in which to explore the integration of host and endosymbiont biology, for several reasons. The peridinin dinoflagellate plastid is highly reduced in terms of genome content and thus is particularly dependent on proteins encoded within the host nucleus (18, 22). The peridinin dinoflagellate plastid is supported by several highly

unusual pathways that are encoded within the host nucleus: i.e., are likely to have been imposed on the plastid by the host lineage (23–25). Furthermore, some dinoflagellates possess plastids acquired through the serial endosymbiotic replacement of the ancestral peridinin lineage, and these replacement plastids show different degrees of integration with the host dinoflagellate environment (2). In this review, we discuss the integration of different dinoflagellate plastids with their hosts, with a particular focus on plastid genome organization and gene expression pathways. From this discussion, we demonstrate the insights that dinoflagellates may provide into plastid evolution across the eukaryotes.

Unusual Plastid Genome Organization in Dinoflagellates

The peridinin dinoflagellate plastid genome is very different in terms of gene content from the other plastid lineages. Typically,

the plastid genomes of plants and algae contain in the region of 60–250 genes (Fig. S1) (22, 26). These genes encode components of the photosynthesis machinery [each photosystem complex, cytochrome *b₆f* complex, ATP synthase, and ribulose bis-phosphate carboxylase (rubisco)], as well as proteins that do not directly function in photosynthesis but perform other essential plastid functions (e.g., cofactor biosynthesis, protein import, and expression of the plastid genome) (22). It has been proposed that some of these genes are retained in the plastid to allow direct regulation of their expression in response to plastid redox state (7, 27).

Studies from multiple dinoflagellate species have indicated that the peridinin plastid, in contrast to other plastids, retains fewer than 20 genes (Fig. S1) (22, 28). These genes form a subset of those found in essentially all other photosynthetic plastids, encoding subunits of the two photosystems, the cytochrome *b₆f* complex, the ATP synthase complex, rRNAs, and a small number of tRNAs (18, 28). Thus, the peridinin dinoflagellate plastid has lost all of the ancestral genes that would have encoded proteins of nonphotosynthetic function (18). There are a small number of genes that are not found in other plastid lineages and are specific to individual peridinin dinoflagellate species (29, 30). It has additionally been suggested that the plastids of the peridinin dinoflagellates *Ceratium horridum* and *Pyrocystis lunula* may contain a small number of genes acquired through lateral transfers from bacterial sources although it cannot be excluded that these genes have been misidentified from bacterial contamination in the original sequence datasets (31). Many of the genes that have been lost uniquely from the peridinin plastid genome are known to have relocated to the nucleus and have acquired targeting sequences allowing the import of the expression products into the plastid (28, 32). Thus, the peridinin plastid is particularly dependent on the expression of nuclear genes for its function.

The peridinin dinoflagellate plastid genome also has a highly unusual organization. The plastid genomes of most plant and algal species form a single chromosome, which can be represented as topologically circular (26). There are some exceptions to these features; in some species, including the chromerid alga *C. velia*, the plastid genome may adopt linear or branched forms (26, 33). However, the plastid genome of the other chromerid species, *V. brassicaformis*, has a more conventional circular structure (12). Thus, the plastid genomes of early ancestors of the peridinin plastid lineage were likely to be conventionally organized.

In contrast to more conventional plastid genomes, the peridinin dinoflagellate plastid genome is fragmented into multiple coding elements. Zhang et al. showed that a number of plastid genes were contained on plasmid-like “minicircles” in the peridinin dinoflagellate *Heterocapsa triquetra* (34). Similar organization has since been shown in other dinoflagellate species (18, 35). The minicircles contain one or a few genes and a “core” sequence, which is similar in sequence, although not identical, among the minicircles containing different genes (18). Although the location of these minicircles in the cell was debated (18, 36), recent hybridization studies have confirmed that they are situated in the plastid (37). In peridinin dinoflagellates, the copy numbers of different minicircles vary during different phases of growth and, in log-phase cultures, may reduce to fewer than 10 copies per cell (38). The low copy numbers of individual minicircles in log-phase cells might plausibly lead to minicircle loss, through unequal distribution during plastid division (38). This loss would be disadvantageous unless there were already a copy of the minicircle gene in the nuclear genome that could be expressed and rescue the plastid (8). Thus, the minicircular genome organization of the peridinin plastid may have provided a selective advantage for gene transfer from plastid to nucleus and greater integration of the plastid with its host (39).

Unusual Plastid Biochemistry in Peridinin Dinoflagellates

In addition to the highly reduced nature of the plastid genome, there is evidence for intricate functional relationships between the peridinin plastid and the host dinoflagellate nucleus. Some of the proteins that function in the peridinin plastid are clearly of nuclear or external origin and thus have been secondarily applied to the peridinin plastid by the host. For example, peridinin dinoflagellates lack a conventional form II rubisco holoenzyme, consisting of eight large and eight small subunits (as found in other plastids descended from red algae, and typically encoded in the plastid genome) and instead use a form II rubisco, consisting of two large subunits, which is encoded in the nucleus (23, 40). The form II large subunit gene is also used by chromerid algae and was acquired via lateral gene transfer from a purple sulfur bacterium into a common ancestor of the dinoflagellate and chromerid lineages (12, 40).

There are several unusual pathways associated with transcript processing in peridinin plastids. These pathways are likely to be dependent on nucleus-encoded proteins, given the absence of nonphotosynthesis genes from peridinin plastid genomes (18, 28). One such pathway is editing, which has been detected in multiple dinoflagellate species (25, 28), although plastid transcript editing does not appear to occur in *Amphidinium* (29) (Fig. 1 and Table S1). The number of sites edited varies between species and genes, with nearly 1 in 10 sites edited in some *Ceratium* transcripts (Table S1). The most common editing event in dinoflagellate plastids is A–G, followed by C–U and U–C; however, nine different events, including five different transversion substitutions, have been documented (Table S1) (28). Transcript editing is not found in the plastids of other studied algae, including those of chromerid algae (21, 41) (Fig. 1). Although editing occurs in plant plastids, it is very different from dinoflagellates, with a more restricted range (predominantly C–U) and generally lower frequency of editing events (<1 in 1,000 in angiosperms) (6). Thus, the plastid editing pathways found in peridinin dinoflagellates have evolved specifically within that lineage.

An even more remarkable processing event is the addition of a poly(U) tail to the 3' end of many transcripts. This transcript processing feature was first reported for *Lingulodinium* and *Amphidinium* (24) but has since been reported for other peridinin dinoflagellate species (42). Poly(U) tail addition has also been found in the chromerid algae *C. velia* and *V. brassicaformis*, suggesting that it is an ancestral feature of red lineage alveolate plastids although it seems not to occur in apicomplexans (12, 41) (Fig. 1). The role of the poly(U) tail remains unclear, although in chromerids it is principally added to transcripts of genes encoding photosystem subunits, suggesting that it plays a role in the expression of the photosynthesis machinery (33, 41). The role of poly(U) tail addition in dinoflagellate plastids is in contrast to poly(A) tail addition in plant plastids, which principally seems to be involved in the degradation of unwanted transcripts (43). Poly(U) tail addition has been documented in a small number of gene expression pathways in bacteria and in some eukaryotic nuclear and mitochondrial lineages, although not in those of dinoflagellates (44–46). Poly(U) tail addition is not known to occur in any plastids other than those of dinoflagellates and chromerids, indicating that it is a specific evolutionary innovation within this lineage (21).

Serial Endosymbiosis in Dinoflagellates

Not all dinoflagellates possess peridinin plastids. Many (e.g., *Perkinsus*, *Oxyrrhis*, *Hematodinium*) are nonphotosynthetic, including some species that are of ecological importance as free-living predators or as parasites of marine invertebrates (11). These species may possess vestigial plastids but have lost the capacity for photosynthesis (Fig. 1). Some of the lineages that do not possess their own plastids (e.g., *Dinophysis*) maintain transient

symbioses with other photosynthetic organisms. These short-term endosymbioses have been reviewed elsewhere and will not be discussed in further detail here (47). However, other dinoflagellates are photosynthetic and possess permanent plastids that are not of the peridinin type. Because the peridinin plastid was present in the last common dinoflagellate ancestor, these plastid types must have arisen through subsequent serial endosymbioses. Thus far, three major serially acquired plastid lineages have been documented. They are monophyletic, and each arose through independent serial endosymbiosis events (48, 49). As shown in Fig. 1, they are the *Karenia/Karlodinium*, *Kryptoperidinium/Durinskia*, and *Lepidodinium* lineages.

Dinoflagellate species that possess the accessory light-harvesting carotenoid pigment fucoxanthin (e.g., *Karenia*, *Karlodinium*) contain plastids that are derived from haptophyte algae (Fig. 1) (50). Many of the fucoxanthin-containing dinoflagellates are implicated in harmful algal blooms (49, 51). Although some early phylogenetic studies of the fucoxanthin plastid indicated that it might be closely related to the peridinin plastid (52), more recent phylogenies have confirmed that the fucoxanthin plastid arose through a subsequent serial endosymbiosis (53–55). The fucoxanthin plastid is surrounded by three membranes, similarly to the peridinin plastid, and there is no evidence for the retention of a nucleus, or mitochondria, from the haptophyte (20).

The “dinotom” algae, typified by *Kryptoperidinium* and *Durinskia*, possess complex endosymbionts derived from pennate diatoms (56) (Fig. 1). In contrast to the fucoxanthin dinoflagellates, the dinotom endosymbiont not only consists of a plastid but also contains a nucleus and mitochondria, which retain their own genomes (20, 57). The dinotom plastid is surrounded by four membranes, similarly to the plastids of free-living diatoms, and a final, fifth membrane surrounds the entire endosymbiont (20, 58). Two dinotom lineages—*Peridinium quinquecornu*, and *Peridiniopsis sp.*—have been proposed to possess endosymbionts derived from centric, rather than pennate, diatom sources (59, 60). Because relatively little is known about the molecular biology of the centric diatom endosymbionts in these species, the term “dinotom” will be used here to refer to the pennate diatom endosymbiont.

Finally dinoflagellates of the genus *Lepidodinium* possess plastids derived from green algae (Fig. 1) (61, 62). The *Lepidodinium* plastid is surrounded by four membranes, of which the innermost two correspond to the plastid membranes of the original endosymbiont lineage, and the third may correspond to the plasma membrane of the endosymbiont (20, 63). Although mitochondria or nuclei have not been documented within the *Lepidodinium* endosymbiont, membrane-bound bodies and free ribosomes have been observed between the second and third membranes, which may correspond to a highly reduced endosymbiont nucleus (20, 63).

Reductive Evolution of Serially Acquired Dinoflagellate Plastids

The extraordinary diversity of dinoflagellate plastids provides exceptional opportunities for studying the events that occur after plastid acquisition. After their acquisition, the biology of the fucoxanthin, *Lepidodinium*, and dinotom plastids must have been altered to facilitate productive associations with the host. In each lineage, for example, starch is principally detectable in the host cytoplasm (20). Thus, carbohydrates generated through photosynthesis in the plastid are exported across each of the endosymbiont-derived membranes into the host, including ones derived from the outermost membranes of the endosymbiont, which may not have been involved in carbohydrate transport before the endosymbiotic event.

Thus far, plastid genomes have been sequenced for the fucoxanthin dinoflagellate *Karlodinium veneficum* (55) and for the dinotoms *Kryptoperidinium foliaceum* and *Durinskia baltica* (64). The genomes of the endosymbiont mitochondria of both dinotoms have also been sequenced (17). The dinotom plastid and endosymbiont mitochondrial genomes are similar to those of free-living

diatoms, with almost no examples of gene loss (Fig. S1) (17, 64). The genome of the dinotom endosymbiont nucleus has not been fully sequenced, but it retains genes for complex metabolic pathways and for structural proteins (e.g., actin, tubulin) that have been lost from other vestigial nuclei found in association with plastids (e.g., the “nucleomorphs” of chlorarachniophyte and cryptomonad algae) (65–67). In contrast to the dinotoms, the *K. veneficum* plastid genome has lost over 40 genes that are present in the plastids of free-living haptophytes (Fig. S1) (55, 68). In addition, many of the individual genes contain premature termination codons and may constitute pseudogenes (55, 69).

The different reduction of each serially acquired plastid lineage is reflected by differences in the degree of gene transfer to the host nucleus. EST studies of fucoxanthin dinoflagellates have identified many gene transfers from the plastid to its host (70–73). For example, in a recent study of the fucoxanthin dinoflagellates *K. veneficum* and *Karenia brevis*, Burki et al. identified 90 ESTs of predicted haptophyte origin, including 34 that were predicted to encode a plastid targeting sequence, out of a total of 493 ESTs of definable phylogenetic affinity (74). Thus, ~7% of the fucoxanthin dinoflagellate nuclear genome may encode proteins of haptophyte plastid origin, a figure approaching that found in other plastid lineages derived through secondary or tertiary endosymbiosis (9). In the same study, the authors screened EST libraries of the dinotom algae *K. foliaceum* and *D. baltica*. Only 14 ESTs out of a total of 237 of definable phylogenetic origin resolved with diatoms, and none was predicted to encode a plastid-targeting sequence (74). The most recent study of gene transfer in *Lepidodinium* identified six ESTs, of probable green algal origin, that were predicted to contain a plastid-targeting sequence, from a total dataset of 4,746 sequences of both definable and uncertain phylogenetic origin (75). Whether gene transfer events have occurred from the serially acquired plastids in *Lepidodinium* to the same extent as in fucoxanthin dinoflagellates awaits further characterization.

Integration of Ancestral and Serially Acquired Endosymbionts

Given that genes have been relocated from serially acquired plastids to the dinoflagellate host nucleus, has there been a more intricate integration of the host and serially acquired plastid genomes? For example, serially acquired dinoflagellate plastids may have benefited from pathways that are endogenous to the host. Any dinoflagellate that undergoes serial endosymbiosis may retain pathways that had been associated with the original peridinin plastid. If these pathways were applied to the incoming replacement plastid, they might facilitate its integration into the host or even change its biology (Fig. 2).

This hypothesis is consistent with the “shopping bag” model for plastid evolution proposed by Larkum et al. (76). This model argued that the endosymbiotic origin of a plastid is unlikely to have been due to a single event at a particular time and place but instead followed multiple unsuccessful “attempts” at endosymbiosis (1). Although these previous attempts did not lead to extant symbioses, they may have contributed genes that help support present-day plastids. It has been proposed that several major photosynthetic eukaryote lineages possess genes that correspond to the “footprints” of such cryptic endosymbioses. For example, diatoms (which possess red algal plastids) may possess genes retained from an ancestral green algal symbiont and plants, and red and green algae (which possess cyanobacterial plastids) may possess genes from an ancestral chlamydiobacterial symbiont (9, 77–79). These hypotheses remain controversial because of the absence of identifiable extant descendants of the cryptic endosymbiont lineages. Serially acquired dinoflagellate plastids, in contrast, provide a well-defined opportunity to assess the impact of a historical endosymbiont on its successors.

Genes have been identified in the nuclei of fucoxanthin dinoflagellates (70, 72, 80, 81) and of *Lepidodinium* (62, 75) that

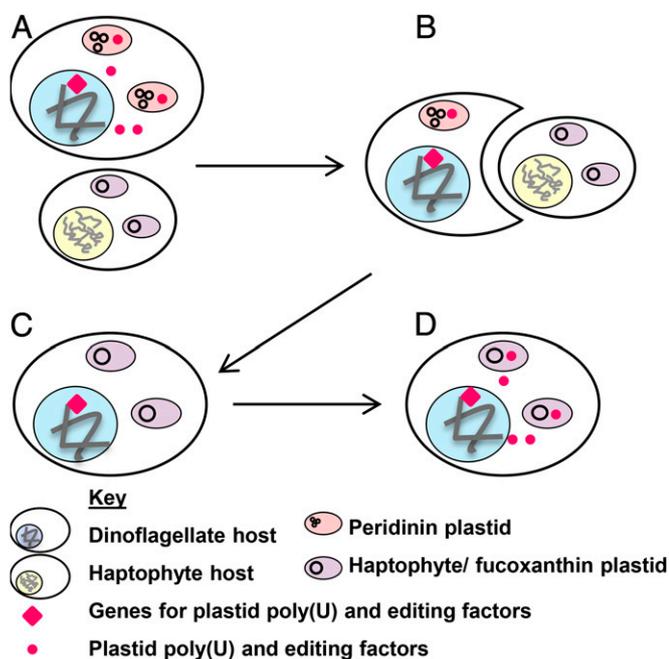


Fig. 2. Application of ancestral plastid pathways to serially acquired dinoflagellate plastids. This diagram shows how pathways associated with the peridinin plastid may have come to function in serially acquired dinoflagellate plastid lineages. For clarity, only the first membrane around each plastid is shown. Early dinoflagellates possessed a peridinin plastid, which was maintained by pathways [such as poly(U) tail addition and editing] encoded within the nucleus (A). In some lineages, this plastid was replaced by others (such as the fucoxanthin plastid) through serial endosymbiosis (B). Although the ancestral peridinin plastid was lost in these lineages, some of the nucleus-encoded genes associated with its function were retained (C) and, after the serial endosymbiosis event, were applied to the replacement plastid, changing its biology (D).

encode proteins predicted to be targeted to the plastid and are related to genes from peridinin dinoflagellates, rather than the free-living relatives of the respective serially acquired plastids (Table S2). Thus, the fucoxanthin and *Lepidodinium* plastids may be supported by pathways retained from the peridinin symbiosis. The dinotom host nucleus has likewise been shown to retain genes for components of several metabolic pathways that were likely to have functioned in the original peridinin plastid (65, 66). However, in each case, components for a second copy of the pathway, of diatom origin, seem to be encoded in the endosymbiont nucleus, and the host-derived copies do not possess targeting sequences appropriate for protein import into diatom plastids (65, 66). Thus, the dinotom plastid is supported by the diatom-derived pathways encoded in the endosymbiont nucleus, rather than the pathways from the peridinin symbiosis.

Transcript Processing in Serially Acquired Plastids

Perhaps the most compelling evidence for pathways retained from the peridinin symbiosis in serially acquired plastids comes from studies of plastid transcript processing. As previously discussed, the peridinin plastid uses two highly unusual transcript-processing pathways—poly(U) tail addition and extensive RNA editing. These pathways are not found in the progenitors of the serial endosymbionts, such as the haptophyte relatives of fucoxanthin dinoflagellates (12, 21, 33). Recently, however, we have demonstrated that plastid transcripts in the fucoxanthin dinoflagellates *Karenia mikimotoi* and *K. veneficum* receive poly(U) tails (Fig. 1) (21, 69). Furthermore, we and others have shown that fucoxanthin plastid transcripts undergo high levels of editing, involving both transition and transversion

substitutions, as occurs in the peridinin plastid (Fig. 1 and Table S1) (21, 82).

Because neither poly(U) tail addition nor transcript editing is native to free-living haptophytes, the most parsimonious explanation for their occurrence in the fucoxanthin plastid is that they are remnants of the ancestral peridinin plastid symbiosis and were applied to the incoming fucoxanthin plastid after serial endosymbiosis (Fig. 2) (21, 82). Notably, whereas editing and poly(U) addition are found in both the peridinin and fucoxanthin dinoflagellate plastids, they do not occur in dinotom or in *Lepidodinium* plastids (Fig. 1) (69). Thus, the pathways required for this unusual degree of endosymbiotic integration have been retained through some, but not all, serial endosymbioses.

Functional Consequences of Poly(U) Addition and Editing

Both poly(U) tail addition and editing are widespread features in fucoxanthin dinoflagellate plastid transcript processing. Recently, we profiled the occurrence of each pathway across the plastid genome of the fucoxanthin dinoflagellate *K. veneficum* (69). We found evidence of poly(U) and editing sites on almost every transcript (69), including those with nonphotosynthesis functions, which are not plastid-encoded in peridinin dinoflagellates (18, 28) and which generally are not polyuridylylated in chromerid algae (41).

Many of the major hypotheses for the origins of transcript processing pathways in other plastid lineages propose they are neutral overall, either compensating for changes in the underlying genomic sequence (6, 83) or having silent effects on plastid transcripts (84). Although the acquisition of foreign RNA processing pathways by the fucoxanthin plastid may have had neutral consequences for the host initially, for transcript editing and poly(U) tail addition to have become such major components of transcript processing in fucoxanthin plastids, it is likely that they conferred some advantageous effects and thus had an adaptive role in fucoxanthin plastid evolution. Poly(U) tail addition and RNA editing may have enabled the fucoxanthin plastid to tolerate the highly divergent sequence evolution of the underlying genome (55). Editing of transcript sequences may enable the compensatory removal of mutations in the genome sequence. For example, premature in-frame termination codons are removed from mRNA sequences by editing in both *K. mikimotoi* and *K. veneficum* (21, 69, 82). As detailed above, fucoxanthin plastid genomes are highly divergent from those of free-living haptophytes (55). Transcript editing, by enabling fucoxanthin dinoflagellates to recover regions of sequence that are important for the function of the protein encoded, might allow the plastid to tolerate mutations that would otherwise prove deleterious. Thus, the presence of transcript editing might enable the fucoxanthin plastid to function in a host environment subjected to elevated rates of sequence substitution.

The poly(U) machinery of fucoxanthin dinoflagellates might similarly play a role, alongside editing, in compensating for divergent evolution in the underlying genome sequence. For example, several genes in the *K. veneficum* plastid are present in multiple copies, one of which is translationally functional whereas others are pseudogenes (55, 68). Remarkably, in these cases where transcripts of the functional gene copy receive poly(U) tails and are highly edited as expected, transcripts of the pseudogene copies do not receive poly(U) tails and undergo only very limited editing (69). A similar discrimination between functional and pseudogene transcript copies by the poly(U) machinery has also been documented in the chromerid alga *C. velia* (33, 41). Thus, a preferential application of the poly(U) tail might enable the fucoxanthin dinoflagellate plastid to discriminate functional gene copies from pseudogenes in its genome.

Convergence of Peridinin and Fucoxanthin Plastid Genomes

It remains to be determined which other features of serially acquired dinoflagellate plastids, beyond transcript-processing pathways, are

derived from the ancestral peridinin plastid. There is a dramatic example of convergence between peridinin and serially acquired dinoflagellate plastids in terms of organization of the plastid genome. As discussed above, the plastid genome of peridinin dinoflagellates is fragmented into small elements termed “minicircles.” Recently, the *K. veneficum* *dnaK* gene (encoding the stromal 70-kDa chaperone) has been shown to be located on a minicircle (68, 69). This minicircle gives rise to a complete, polyuridylylated and edited *dnaK* transcript, confirming that it is located in the plastid (69). This minicircle also contains a secondary structure-rich motif that may constitute an equivalent of the peridinin dinoflagellate minicircle core (68, 69). Similar minicircles have not been reported in *Lepidodinium* or in dinotoms.

The reason why minicircles are present in fucoxanthin plastids remains to be determined. It is possible that whatever factors caused fragmentation of the peridinin plastid genome have been applied to the fucoxanthin plastid after its endosymbiotic acquisition, leading to the convergent evolution of minicircles from each plastid. The gene order in the *K. veneficum* plastid genome is highly divergent, with disruptions to gene clusters that are well conserved in other plastids (55). Thus, other rearrangement events may have accompanied the formation of minicircles in fucoxanthin dinoflagellates. The selective consequences of this fragmentation for the fucoxanthin plastid are unclear. As discussed above, the relocation of certain genes to minicircles might have adverse effects on the ability to maintain those genes in the plastid (38). If a similar situation were true in fucoxanthin dinoflagellates, a partial fragmentation of the fucoxanthin plastid genome might have driven the relocation of genes located on plastid minicircles to the nucleus of the host (55, 68).

Why Integration in Some Lineages, and Not Others?

It is apparent, from both the reduced state of the plastid genome (55) and the acquisition of host-derived pathways such as poly(U) addition and transcript editing (21, 82), that the fucoxanthin dinoflagellate plastid has become intricately integrated with that of the host. This integration is likely to have had beneficial consequences. For example, poly(U) tail addition and editing may mitigate against the divergent evolution of the plastid genome (69, 82). Although the *Lepidodinium* plastid does not use the poly(U) tail addition or editing pathways (69), it is likely that it has become similarly integrated into the host, given the evidence for endosymbiotic gene transfer and the presence of plastid-targeted proteins that are retained from the peridinin symbiosis (62, 63, 75).

In contrast to the situation for the fucoxanthin and *Lepidodinium* plastids, there is only very limited evidence for integration of the dinotom endosymbiont with its host. Not only is the endosymbiont largely unreduced in terms of genome content (17, 64), there is no significant evidence for the presence of genes in the host nucleus—of any phylogenetic derivation—that are likely to support the plastid (65, 66, 74). It seems instead that the endosymbiont nucleus plays a more significant role in supporting the plastid (65, 66). Why might the dinotom plastid be much less integrated with its host than the plastids of fucoxanthin dinoflagellates and *Lepidodinium*?

One possible reason for different degree of integration of the dinotom and fucoxanthin plastids with their respective hosts is the relative age of each lineage. The dinotom endosymbiont has been inferred to have been acquired not substantially greater than 50 million years ago whereas the fucoxanthin dinoflagellate plastid may represent a much more ancient acquisition, potentially of the order of 200 million years ago or greater (56, 85). The dinotom endosymbiont may thus simply not have had time to have reached as intimate a degree of connection with its host environment. However, plastids of an equivalent age to the dinotom endosymbiont can undergo reduction and integration with the host. For example, gene loss and functional gene

transfers have been documented in the independently acquired primary plastids of the photosynthetic amoeba *Paulinella chromatophora*, which are believed to have originated no more than 60 million years ago (86). Gene loss has even been documented in the cyanobacterial endosymbionts of the diatom *Rhopalodia gibba*, which are believed to have been acquired by their host as little as 12 million years ago (87). Furthermore, dinotoms do show evidence of postendosymbiotic divergence from one another. For example, the *K. foliaceum* endosymbiont has acquired a small number of novel coding sequences (encoding DNA recombinases and RNA maturases) in its plastid and endosymbiont mitochondrial genomes that are found neither in free-living diatoms nor in the dinotom *D. baltica* (17, 64). Thus, the biology of the dinotom endosymbiont may have changed since its initial endosymbiotic uptake; however, it has not become significantly integrated into the biology of its host.

An alternative hypothesis for the lack of integration in some lineages concerns the stages of serial endosymbiosis associated with each plastid lineage. In theory, serial endosymbiosis could occur either via the initial loss of a plastid, then the gain of a replacement, or via the initial gain of a plastid, followed by the loss of the original plastid lineage. In the latter scenario, the two plastids coexist for a certain period, allowing the recruitment of maintenance pathways from one lineage to support the other. Thus, the extreme degree of integration of the fucoxanthin plastid with its host might suggest that, for a period, the fucoxanthin and peridinin plastids coexisted in the host. In contrast, if the dinotom endosymbiont was acquired only a substantial period after loss of the peridinin plastid, plastid-associated pathways that were associated with the peridinin plastid lineage might have been lost before the acquisition of the replacement. However, as detailed above, the host nucleus of dinotom algae still possesses genes for biosynthetic pathways inferred to have functioned in the ancestral peridinin plastid (although subcellular localization predictions suggest that the expression products of these genes function elsewhere from the replacement plastid) (65, 66). Thus, the different degree of integration of fucoxanthin and dinotom plastids with their hosts is not due to a difference in the peridinin-derived genes present in the host lineage at the point of serial endosymbiosis but is due to how these gene complements have been applied to support each serial plastid lineage.

A final possible explanation for the lack of integration in dinotoms concerns the biology of its plastid. There may be specific physiological reasons why the dinotom plastid has not integrated with its host and is instead supported by the mitochondria and nucleus of the endosymbiont. There may be a selective requirement to retain plastid-targeted genes in the endosymbiont nucleus, which might prevent the transfer of these genes to the host, or the cooption of genes within the host nucleus to support the endosymbiont. In dinotoms, the outermost membrane surrounding the plastid is frequently contiguous with the endosymbiont nuclear envelope (58, 88, 89). It will be interesting to determine whether there are particularly intricate cellular connections between the two organelles: for example, in terms of the import of proteins into the plastid or the coordination of gene expression in the plastid and the endosymbiont nucleus.

Similarly, intricate mitochondria–plastid interactions have been characterized in free-living diatoms, including the use of a mitochondrial urea cycle to modulate plastid nitrogen metabolism, and potentially even pathways that redistribute electron potential between mitochondria and plastids to minimize photoinhibition (3, 90). If these mitochondria–plastid interactions also function in the dinotom endosymbiont, they might also provide a selective barrier to elimination of the endosymbiont mitochondria and greater integration of the plastid with the host.

Many questions remain to be answered in the context of serial plastid evolution in dinoflagellates. For example, the exact extent

of plastid gene transfer or the number of genes retained from the ancestral peridinin symbiosis to support each serially acquired plastid remain to be determined. In addition, it remains to be determined what the consequences of editing and poly(U) tail addition have been for fucoxanthin plastid evolution. At a broader level, the extreme diversity of integration observed between different plastids with the dinoflagellate host—ranging from the intricate cellular and evolutionary connections between

the peridinin and fucoxanthin plastids and the host nucleus to the largely autonomous function of the dinotom endosymbiont—provides insights into the diversity of evolutionary pathways that plastids and other endosymbiotic organelles may undertake. Further exploration of why different dinoflagellate plastids are so differently integrated with their hosts may provide valuable insights into the fundamental processes associated with post-endosymbiotic plastid evolution across the eukaryotes.

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