Second-hand Chloroplasts:
Evolution of Cryptomonad Algae

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I. INTRODUCTION

Oxygenic photosynthesis is believed to have evolved at least 3500 million years ago, when prokaryotes were the principal life form (Schopf and Walter, 1982; Krishna Rao et al., 1985; Walsh and Lowe, 1985). Since then, the autotrophic way of life has become widespread among prokaryotes and eukaryotes and has radically altered the biosphere through the increase of oxygen concentration. The global success of photosynthetic organisms attests to the selective advantage of being able to synthesize food from inorganic nutrients and light energy. It follows that natural selection could well favour any means by which the capacity for photosynthesis could be acquired by heterotrophs.

The theory of endosymbiosis proposes that green algae, and their descendants the land plants, acquired photosynthetic capacity by engulfing a photosynthetic prokaryote and retaining it within (Margulis, 1981). A wealth of morphological and biochemical evidence (reviewed in Cavalier-Smith, 1982; Gray and Doolittle, 1982; Taylor, 1987; Gray, 1988, 1989) indicates that either a cyanobacterium (Pace et al., 1986; Giovanni et al., 1988; Turner et al., 1989; Morden and Golden, 1991) or a chlorophyll b-containing prokaryote (Morden and Golden, 1989), was “captured” by a nucleated cell and retained for the purpose of carrying out photosynthesis. The captured prokaryote has subsequently been reduced to an endosymbiont, the chloroplast, by reduction of its genome through transfer (or duplication) of the majority of its genes into the host’s nucleus (Weeden, 1981; Harrington and Thornley, 1982). The inner of the two chloroplast membranes is proposed to represent the plasma membrane of the prokaryote endosymbiont, while the outer membrane is proposed to derive either from the phagocytotic vacuole of the host (Dodge, 1979; Whatley et al., 1979; Whatley and Whatley, 1981) or the outer membrane of the endosymbiont (Cavalier-Smith, 1987).

While endosymbiosis of a photosynthetic prokaryote can explain the origin of green algae and subsequently the land plants, the origin of photosynthetic capacity in other algae is much less certain. The diverse morphology and biochemistry of algal chloroplasts have prompted speculation that different algal chloroplasts arose from separate endosymbiotic events, perhaps involving as many as three (Raven, 1970; Larkum and Barrett, 1983; Morden and Golden, 1991), four (Sagan, 1967) or even six (Mereschkowski, 1910) different prokaryotic endosymbionts. Furthermore, it has been proposed that several algal groups acquired their chloroplasts “second-hand” by engulfing photosynthetic eukaryotes, as opposed to photosynthetic prokaryotes (Dodge, 1979; Whatley et al., 1979; Gibbs, 1981a; Whatley and Whatley, 1981; Whatley, 1989). These evolutionary scenarios involve two sequential endosymbiotic events, one between a nucleated cell and a prokaryote to create a chloroplast-containing eukaryote, and a second
between another phagotrophic eukaryote and the photosynthetic eukaryote (Fig. 1).

In cryptomonad algae several lines of molecular and morphological evidence combine to indicate that a heterotrophic flagellate engulfed a red alga to acquire a chloroplast. This review weighs that evidence and examines the symbiotic relationship that apparently involves at least four cells, two prokaryotes and two different eukaryotes, amalgamated into a single cell.

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**Fig. 1.** The second hand chloroplast hypothesis. All chloroplasts (C) are proposed to ultimately derive from one or more prokaryotic endosymbionts. The primary endosymbiosis results in a double membrane-bound compartment with a prokaryotic genome—the chloroplast. The chloroplasts of some algae are believed to have been acquired by a secondary endosymbiosis of a photosynthetic eukaryote. The chloroplast-containing eukaryote is hijacked by a second phagotrophic eukaryote (nucleus N'). In cryptomonads, the nucleus (N) and cytoplasm of the photosynthetic eukaryote are apparently retained.
II. OVERVIEW OF CRYPTOMONAD FEATURES

The first cryptomonad was described by Ehrenberg in 1832. Currently there are approximately 50 genera and some 200 species described in the Division Cryptophyta, but the systematics of the group is in a state of flux (see Section IX: Taxonomic Appendix). Cryptomonads are abundant in marine and freshwater habitats. Cells are typically ovoid to bean-shaped, and somewhat flattened laterally. The two flagella, which can both bear tubular hair-like appendages known as mastigonemes or "flimmers", are inserted into an anteriolateral invagination known as the gullet. Cells lack a rigid wall but are covered in an elaborate array of scales and pellicular plates. A characteristic feature is the presence of "ejectisomes": extrusive organelles of unknown function (Anderson, 1962; Wehrmeyer, 1970). The single, ramified mitochondrion has flat cristae (Santore and Greenwood, 1977). The nucleus is typically located in the posterior of the cell and mitosis involves partial breakdown of the nuclear envelope and an open, barrel-shaped spindle without centrioles (see Meyer and Pienaar, 1984, for references). Sexual reproduction is not clearly established but may occur (Hill and Wetherbee, 1986; Kugrens and Lee, 1988). Further details of cryptomonad fine structure can be found in previous reviews (Gantt, 1980; Oakley and Santore, 1982; Gillott, 1990).

III. THE NUCLEOMORPH

A. NUCLEUS-LIKE ORGANELLE

A key feature of the cryptomonads is a small organelle termed the nucleomorph. This structure, first described in 1974 (Greenwood, 1974), is proposed to be the vestigial nucleus of the eukaryotic endosymbiont (Greenwood et al., 1977; and see Fig. 1). The nucleomorph is located in the periplastidal space, a cytoplasmic compartment between the chloroplast envelope and the extension of the rough endoplasmic reticulum that envelopes the chloroplast (Fig. 2). Within the periplastidal space are particles resembling eukaryotic (80S) ribosomes and starch grains (Sepenswol, 1973; Gillott and Gibbs, 1980). All cryptomonads with a chloroplast (even non-pigmented chloroplasts—leucoplasts) contain a nucleomorph, and those species having two chloroplasts have two nucleomorphs. The heterotrophic cryptomonad genus, *Goniomonas*, has no chloroplast and no nucleomorph (Mignot, 1965; Hill, 1991a).

The nucleomorph exhibits many characteristics of a eukaryotic nucleus, albeit a very small one. The nucleomorph is round to ovoid with a diameter of about 1 μm (Figs. 3 and 4). It is delimited by a double membrane envelope with several pores reminiscent of nuclear membrane pores (Gillott
Fig. 2. Layout of cryptomonad cell. The cryptomonad apparently contains a photosynthetic eukaryotic endosymbiont, and the nucleomorph and periplastidal space are the vestiges of the endosymbiont's nucleus and cytoplasm (compare with Fig. 1). The chloroplast is enveloped by four membranes. Between the two inner and the two outer membranes is a compartment known as the periplastidal space, within which is a small nucleus known as the nucleomorph. The two inner membranes are probably homologous to the envelopes of other chloroplasts. The outer two membranes are termed the chloroplast endoplasmic reticulum (CER). The outermost membrane is continuous with the endoplasmic reticulum-nuclear envelope and bears ribosomes. The third membrane, counting out from the chloroplast, could represent the plasma membrane of the eukaryotic endosymbiont, in which case the endosymbiont is actually within the lumen of the host cell's endoplasmic reticulum.

and Gibbs, 1980; Morrall and Greenwood, 1982; Santore, 1982). The contents of the nucleomorph are distinguished into three components: a background matrix; several electron-dense, spherical to rod-shaped structures; and a relatively large granular zone of medium electron density that resembles a nucleolus (Figs. 3 and 4). Nucleomorph division is dissimilar to normal nuclear division in that no spindle is visible; the nucleomorph
Fig. 3. Longitudinal section of a cryptomonad (Komma caudata) showing the main nucleus (Nu) and the nucloemorph (Nm). The chloroplast (Chl) contains stacks of thylakoids with electron-dense material in the lumina and a pyrenoid (Py). The mitochondrion (Mi) has flat cristae. Scale bar = 1 μm.
Fig. 4. The nucleomorph of *Plagioelmis palustris*. Between the inner and outer pairs of membranes surrounding the chloroplast is the periplastidal space (asterisk) which contains eukaryotic ribosomes and starch grains (not shown). Also located within the periplastidal space is the nucleomorph, a miniature nucleus complete with a double membrane interrupted by pores (arrowheads), a nucleolus-like zone (open arrow), and unidentified electron-dense bodies (small arrows). Scale bar = 0.5 μm.

division apparently occurs by pinching in two (McKerracher and Gibbs, 1981; Morrall and Greenwood, 1982). Chromatin is not distinguishable within the nucleomorph during the division process (McKerracher and Gibbs, 1981; Morrall and Greenwood, 1982). The nucleomorph is the first structure to divide in the cell, followed by the chloroplast, then the main nucleus.

B. DNA IN THE NUCLEOMORPH

Several histochemical studies demonstrate the presence of DNA in the nucleomorph, further confirming its nucleus-like status. Staining with the DNA-binding fluorochrome 4′-6-diamino-2-phenylindole (DAPI) shows fluorescence in the cryptomonad nucleomorph (Hansmann et al., 1985; Ludwig and Gibbs, 1985). The DNA content of the nucleomorph was estimated at approximately 10^{8} or 10^{9} daltons (Ludwig and Gibbs, 1985). Immunogold techniques have localized DNA to the matrix of the
nucleomorph and to a part of the nucleolus-like zone (Hansmann et al., 1986). The anti-DNA antibody did not bind to the electron-dense globules in the nucleomorph, so the composition and role of these structures remains enigmatic.

C. EUKARYOTIC RIBOSOMES AROUND THE NUCLEOMORPH

Since the nucleomorph appeared to be a eukaryotic nucleus within a subcompartment of the cryptomonad cell, it was relevant to enquire: (1) were the ribosome-like particles present in the periplastidal space around the nucleomorph really ribosomes; (2) were they prokaryotic or eukaryotic ribosomes; and (3) were these ribosomes perhaps encoded by nucleomorph DNA? Once again, histochemical techniques were employed. Using the enzyme–gold method with a ribonuclease (Bendayan, 1981), Hansmann (1988) demonstrated the presence of RNA in the periplastidal space, supporting the idea that the 22 nm particles within it were ribosomes. The RNase–gold also indicated RNA within the nucleolus-like portion of the nucleomorph, suggesting that transcriptionally active ribosomal RNA (rRNA) genes could be present in this zone of the nucleomorph.

If the nucleomorph and periplastidal space of the cryptomonad are the vestiges of the nucleus and cytoplasm of a eukaryotic endosymbiont, then the rRNA genes in the nucleolus-like portion of the nucleomorph and the rRNAs in the periplastidal ribosomes should share sequence identity with the other eukaryotes. The author has been able to localize either eukaryotic or prokaryotic rRNAs at the ultrastructural level using techniques of high-resolution in situ hybridization (McFadden et al., 1990; McFadden, 1991; see also Fig. 5). Probing of cryptomonad cells with eukaryotic- and prokaryotic-specific rRNA clones shows that the eukaryotic rRNAs are present in the periplastidal space (Figs. 6 and 7; see also McFadden, 1990a,b). Prokaryotic rRNAs are found in the chloroplast (Fig. 8) and also in the mitochondrion (McFadden, 1991). The in situ hybridization technique also detects transcripts of rRNAs in the nucleolus (McFadden et al., 1988, 1990; McFadden, 1989, 1991). The nucleolus-like zone of the nucleomorph labels with the eukaryotic rRNA probe (Figs. 9a,b), indicating that rRNA genes are possibly being transcribed therein (McFadden, 1990a,b).

Microscopy has thus demonstrated that the nucleomorph has four characteristics of a eukaryotic nucleus: a double membrane with pores, DNA, self-replication, and a nucleolus-like zone in which eukaryotic rRNAs are apparently transcribed.

The nucleomorph is surrounded by eukaryotic ribosomes in the periplastidal space which is isolated from the rest of the cell by the chloroplast endoplasmic reticulum (CER). Are the rRNAs in the periplastidal ribosomes encoded by DNA in the nucleolus-like zone of the nucleomorph? No
Fig. 5. Diagrammatic representation of the in situ hybridization procedure used to localize either prokaryotic or eukaryotic rRNA (McFadden, 1991). A biotin-labelled probe is synthesized from a clone of either a prokaryotic or eukaryotic rRNA gene. The probe is hybridized to complementary nucleic acid sequences (rRNAs) in the ribosomes at the surface of the section. The biotin tag is then detected with an antibody to biotin developed in goats. These goat IgGs are then detected with rabbit-anti-goat IgGs conjugated to colloidal gold markers. The diagram is not to scale and is not intended to represent the way in which the antibodies recognize their determinants.

Fig. 6. Transmission electron micrograph (longitudinal section of Komma caudata) of a cryptomonad showing the nucleomorph (Nm) and the periplasidal space (arrows) within which is a starch grain (S). The chloroplast (Chl) and pyrenoid (Py) are also visible. The main nucleus (Nu) is toward the cell posterior. Scale bar = 0.5 μm.
Fig. 7. Localization of eukaryotic rRNA in a section similar to Fig. 6. The markers indicate the presence of eukaryotic rRNAs in the main cytoplasm and the nucleolus of the main nucleus. In addition, the periplastidal space (arrows) is clearly positive for eukaryotic rRNA (arrows). Scale bar = 0.5 μm.

Fig. 8. Localization of prokaryotic rRNA in a section similar to those shown in Figs. 6 and 7. The ribosomes in the chloroplast stroma are heavily labelled with the prokaryotic probe, but the periplastidal space (arrows) and the main nucleocytoplasmic area are not labelled. Scale bar = 0.5 μm.
firm proof is yet forthcoming but the answer is very likely to be in the affirmative. The only other eukaryotic rRNA genes located in cryptomonads thus far occur in the main nucleus (McFadden, 1990b). In order to reach the periplastidal space, rRNAs from the main nucleus would need to cross the two membranes of the CER. Certain short RNAs apparently cross
the mitochondrial membrane (Chang and Clayton, 1987; Maréchal-Drouard et al., 1988), but no mechanisms of membrane translocation for rRNAs is yet known.

D. ORIGIN OF THE NUCLEOMORPH

1. Autogenous or exogenous origin
Taken together, the morphological and histochemical data outlined above are congruent with the presence of a vestigial photosynthetic eukaryote within the cryptomonad, and support the idea of a secondary endosymbiosis as represented in Fig. 1. The microscopical data do not, however, discount the possibility that the nucleomorph and periplastidal space are of auto-
genous rather than exogenous origin. Autogenous origin, where the nucleo-
morph is derived from the main nucleus and partitioned off into the periplastidal space by the CER, is difficult to explain (Cavalier-Smith, 1986), but examples of genome partitioning such as the micro- and macro-
nuclei of ciliates do occur (Lynn and Small, 1990). Small blebs of dinoflagellate nuclei, reminiscent of the cryptomonad nucleomorph, are sometimes partitioned off by evaginations of the nuclear envelope (Lewis and Burton, 1988), but whether these blebs contain any DNA or have any function is undetermined. Bogorad (1975, 1982) discussed the possible autogenous origin of mitochondria and plant chloroplasts by partitioning of the nuclear genome, but he did not include the cryptomonad chloroplast and nucleomorph in his discussion.

2. Cryptomonads have two different sets of rRNA genes
The definitive test is to compare gene sequences from the nucleomorph with gene sequences from the main nucleus. If the nucleomorphy is autogenously derived, the sequences can be expected to be similar, although some divergence might have occurred if the nucleomorph “broke away” a long time ago. Conversely, if the nucleomorph is indeed the genome of a foreign cell, then the gene sequences could be expected to be radically divergent. The latter is exactly what was found (Douglas et al., 1991a). Since the nucleomorph apparently contained rRNA genes, these were the logical sequences to compare. Moreover, the expanding database of rRNA gene sequences could be cross-checked to sift through the eukaryotes for possible relatives of the putative symbionts. But how to get the sequences out of the two nuclei? A means of separating the nucleomorphy from the main nuclei was not available, so Douglas et al. (1991a) employed the polymerase chain reaction (PCR; Saiki et al., 1988). They reasoned that if the 16S-like rRNA genes of the nucleomorph and nucleus were divergent, then they might be different in size. Using primers that direct amplification of the eukaryotic 16S-like rRNA gene, Douglas et al. (1991a) amplified total cryptomonad
DNA and obtained two products. These PCR products were cloned and sequenced, and the two sequences aligned. Alignment revealed that both were obviously eukaryotic 16S-like rRNAs, but they shared only 70% positional identity, indicating highly divergent sequences. It has been previously demonstrated that two different rRNA sequences can occur in a single cell (Gunderson et al., 1987a), but the divergence is considerably less and one sequence is clearly a derivative of the other (Enea and Corredor, 1991). Secondary structure models indicate that the two cryptomonad rRNAs would have distinct structures and that one is not a derivative of the other (Douglas et al., 1991a). The shorter 16S-like gene was assigned to the nucleus on the basis that it alone could be amplified from DNA prepared from isolated cryptomonad nuclei. By a process of elimination, the longer sequence was assumed to derive from the nucleomorph.

Proof that both cryptomonad sequences are active genes was obtained by positive Northern hybridization using sequence-specific synthetic oligonucleotides (Douglas et al., 1991b). The nucleus-specific probe hybridized to an RNA of 1800 bp. Two nucleomorph-specific probes were used, and each probe hybridized to a different RNA, one of 1450 bp and one of 250 bp. The nucleomorph transcript is apparently discontinuous, and Douglas et al. (1991a) postulate that post-transcriptional excision of a 166 bp spacer from variable region V2 is the probable explanation. Discontinuities in nucleocytoplasmic 23S-like rRNAs of certain insects, the chloroplast 23S-like rRNAs of plants and green algae (Appels and Honeycutt, 1986), and the mitochondrial 16S-like rRNAs of a ciliate (Schnare et al., 1986) have been previously reported, but the nucleomorph rRNA is the first known bipartite nucleocytoplasmic 16S-like rRNA.

3. The nucleomorph is derived from a red algal nucleus

Phylogenetic trees, based on comparison of the cryptomonad nucleus and nucleomorph rRNA sequences determined by Douglas et al. (1991a) with other eukaryote sequences, reveal several interesting affiliations (Fig. 10). The nuclear sequence and nucleomorph sequence are clearly not closely related. The nuclear sequence branches with the rhizopod Acanthamoeba, and these two form a sister group to the land plants and green algae. The nucleomorph sequence affiliates with two red algal sequences (Fig. 10), supporting the theory that the eukaryotic endosymbiotic in cryptomonads was a red alga (Dodge, 1979; Whatley et al., 1979; Gillott and Gibbs, 1980; Gibbs, 1983).

4. Are cryptomonads the ancestors of chromophyte algae?

An important finding of the rRNA sequence phylogenetic analyses (Perasso et al., 1990; Douglas et al., 1991b; Eschbach et al., 1991b) is that the cryptomonad nuclear sequence does not branch together with members of the chromophyte algae (chlorophyll c-containing algae including diatoms and chrysophytes but excluding dinoflagellates; see Fig. 10). It had
previously been suggested that cryptomonads were perhaps the ancestors of the chromophytes because they also possessed chlorophyll c, four membranes surrounding the chloroplasts (chloroplast ER), and tubular mastigonemes on the flagella (Dodge, 1979; Cavalier-Smith, 1986, 1989). The chromophytes would thus derive from cryptomonads by loss of the nucleomorph, periplastidial ribosomes, and phycobilins (Coombs and Greenwood, 1976; Dodge, 1979; Gibbs, 1981b; Cavalier-Smith, 1986, 1989).

In addition, the oomycetes, hypochytrids and thraustochytrids (all of which produce heterokont zoospores with tubular mastigonemes) were held not to be true fungi but merely chromophytes that had lost their chloroplasts (Cavalier-Smith, 1986, 1989). The evolutionary progression would thus be cryptomonads → chromophytes → zoosporic fungi (Cavalier-Smith, 1986).

Fig. 10. Phylogenetic tree showing the relationship of the two cryptomonad rRNA sequences to other eukaryote sequences (redrawn from Douglas et al., 1991b, with permission). The two cryptomonad sequences are not closely related. The sequence believed to derive from the nucleomorph branches with the red algae (Gracilaria and Gracilariorpsis), while the sequence from the main nucleus is affiliated with Acutinanhoea. A cadd containing the cryptomonad algae (Ochromonas and Skeletonema) and the oomycete (Achyta) is remote to the cryptomonad nuclear sequence, refuting the suggestion that chromophytes and oomycetes are descendants of a cryptomonad. The scale bar represents 10 changes per 100 nucleotides.
PHYLOGENETIC TREES BASED ON BOTH MOLECULAR AND MORPHOLOGICAL CHARACTERS

GROUP CHROMOPHYTES AND ZOOSPORIC FUNGI TOGETHER (Gunderson, et al., 1987b; Bhattacharya and Druel, 1988; Beakes, 1989; Whalley, 1989; Aitzia et al., 1991; Bhattacharya et al., 1991; Williams, 1991), but a recent analysis indicates that zoosporic fungi diverged before the acquisition of a chloroplast produced the chromophyte lineage (Williams, 1991).

Several cryptomonad characteristics make it unlikely that chromophytes are their descendants. First, cryptomonad mitochondria have flat cristae while those of chromophyte mitochondria are tubular (Dodge, 1979). Second, the cryptomonad periplast is unique and not at all similar to the surface coverings of chromophytes. Finally, cryptomonads are anisokont while chromophytes (with the exception of Prymnesiophytes) are classically heterokont (Moestrup, 1982).

From the available morphological and molecular data it would seem that the chromophytes and zoosporic fungi are probably not descendants of cryptomonads. This means that CER and tubular mastigonomes are either shared primitive characters or have evolved convergently in cryptomonads and chromophytes. Cavalier-Smith (1986) argues that CER probably only evolved once, but host ER is associated with the endosymbiont in the ciliate Myrionecta (see Section V), suggesting that a CER-like arrangement of membranes may not be so unique. It also seems doubtful that the tubular appendages on cryptomonad flagella are homologues of those on chromophyte flagella (Moestrup, 1982). Cryptomonads have tubular appendages on both flagella, so they are not heterokont like most of the chromophytes and zoosporic fungi. Moreover, the cryptomonad appendages are bipartite whereas the chromophyte flagella appendages are tripartite (Moestrup, 1982). Similarly, chlorophyll c could have perhaps evolved twice from chlorophyll a (Jeffrey, 1989), or perhaps both cryptomonad and chromophyte chloroplasts developed from closely related primary prokaryotic endosymbionts containing chlorophyll c (Larkum, 1991; see also Section IV.D).

E. ISOLATION OF THE NUCLEOMORPH

1. SEPARATION OF NUCLEOMORPH AND NUCLEAR DNA

While the PCR analysis reveals two distinct sets of rRNA genes in cryptomonads, definitive proof that one set is from the nucleomorph and the other from the nucleus is not yet available. Such proof could come from either in situ hybridization or blot hybridizations using isolated nucleomorphs and nuclei. Attempts to separate nuclear DNA from nucleomorph DNA by isopycnic centrifugation, based on differences in buoyant density, have been without success (Hansmann et al., 1987). Two low-density bands contained circular chromosomes of approximate size 130 kb and 43 kb that are believed to represent chloroplast and mitochondrial DNA respectively (Hansmann et
Fig. 11. Longitudinal section of *Rhodomonas salina* showing the nucleomorph (arrow) within the pyrenoid (Py). The pyrenoid has an invagination, which is lined by the two innermost chloroplast membranes, within which the nucleomorph is positioned. Scale bar = 1 μm.

*al., 1987*). The major high-density band contained nuclear DNA, but no band corresponding to nucleomorph DNA could be found.

Recently, a lateral approach to isolation of the nucleomorph was developed using a cryptomonad in which the nucleomorph was encapsulated in the pyrenoid (Fig. 11). Hansmann and Eschbach (1990) lysed crypto-
Fig. 12. Electron micrograph of isolated pyrenoid/nucleomorph complexes from *Rhodomonas salina*. Each pyrenoid contains a nucleomorph. The preparation is 99.8% pure with respect to nuclear contamination. Scale bar = 1 μm.
monads and banded the lysate on Percoll step gradients to isolate the pyrenoids, still containing their nucleomorphs. Nucleomorph/pyrenoid complexes isolated from *Rhodomonas salina* using the protocol of Hansmann and Eschbach (1990) are shown in Figs. 12 and 13. DAPI fluorometry of the isolated nucleomorphs shows between 1.3 Mb and 2.8 Mb of DNA per nucleomorph (Hansmann and Eschbach, 1990). If the 2.8 Mb examples represent DNA doubling prior to nucleomorph division, and one assumes that the nucleomorph contains a diploid set of chromosomes, then the haploid size of the nucleomorph genome is 0.7 Mb—about 1/30th the size of the cyanobacterium *Anacystis nidulans* or 1/7th the size of *Escherichia coli*. If the nucleomorph is indeed the vestige of a red algal nucleus, it would seem to be drastically reduced in DNA content. The separation of nucleomorph and nuclear DNA will allow construction of a nucleomorph genomic library from which it should be possible to isolate nucleomorph genes.
2. A diploid nucleomorph with three short, linear chromosomes

An electrophoretic karyotype of the nucleomorph has been established by pulsed-field gel electrophoresis of isolated nucleomorphs and shows three linear chromosomes of 195 kb, 225 kb and 240 kb (Eschbach et al., 1991a). How the three linear chromosomes are segregated during division of the nucleomorph, in the absence of a detectable spindle, is an intriguing question. The total of 660 kb for the three chromosomes is in good agreement with the DAPI estimates and is congruent with a diploid nucleomorph. Southern blotting of the isolated chromosomes with probes for either 16S-like or 23S-like rRNA genes indicates that each chromosome carries at least one set of rRNA genes (Eschbach et al., 1991a). A dot blot of nuclear and nucleomorph DNA from Rhodomonas salina probed with rDNA of pea (Jorgensen et al., 1987) also indicates presence of rRNA genes in the nucleomorph DNA (Fig. 14). It should now be possible to probe isolated nucleomorph DNA with the oligonucleotide probes believed to be specific for either nuclear or nucleomorph DNA (Douglas et al., 1991a) to test whether the putative nucleomorph sequence represented in Fig. 10 is indeed derived from the nucleomorph.

![Slot blot of nucleomorph and total Rhodomonas salina DNA probed with rRNA genes of pea (2.6 kb BamHI fragment; Jorgensen et al., 1987). The nucleomorph DNA is positive for rRNA genes.](image)

Fig. 14. Slot blot of nucleomorph and total Rhodomonas salina DNA probed with rRNA genes of pea (2.6 kb BamHI fragment; Jorgensen et al., 1987). The nucleomorph DNA is positive for rRNA genes.
IV. THE CHLOROPLAST

A. CHLOROPLAST MEMBRANES

Most cryptomonads have a single chloroplast, but species of the genera Cryptomonas and Campyloponas have two. The chloroplast is bounded by four membranes—an inner pair of membranes comprising the plastid envelopes, and two outer membranes, known as the chloroplast endoplasmic reticulum (CER), which are an extension of the endoplasmic reticulum/nuclear envelope (Figs. 2, 3, 4). The outermost CER membrane of cryptomonads bears ribosomes, as does the outermost CER membrane in chromophytes (Gibbs, 1979, 1981b). The cryptomonad thylakoids are typically organized in pairs but loose stacks of several thylakoids occur (Figs. 3, 4, 6, and 11). The thylakoids are relatively thick and contain electron-dense material (Figs. 3, 4, 6, and 11).

B. STORAGE PRODUCT

No starch is stored in the chloroplast, but starch grains are found in the periplastidal space (Fig. 6). This location of starch is consistent with cryptomonads harbouring a red algal endosymbiont (Gillott and Gibbs, 1980), since red algae store starch in their cytoplasm. However, the cryptomonad starch contains amyllose (an α(1,4)-linked linear glucan) and amylepectin (an α(1,4,6)-branched glucan) and thus resembles starch of land plants (Antia et al., 1979; McCracken and Cain, 1981), while red algal starch (termed floridean starch) was initially reported to contain no amyllose (Percival, 1968; Craigie, 1974). Further investigations have revealed the presence of amyllose in unicellular (McCracken et al., 1980) and multicellular (McCracken and Cain, 1981) red algal starch, and the so-called floridean starch is probably restricted to certain multicellular red algae. Hence, the presumably primitive unicellular red algae—which are perhaps the ancestors of cryptomonad chloroplasts, periplastidal space and nucleomorph—probably had amyllose.

C. PHOTOSYNTHETIC PIGMENTS

A unique combination of light-harvesting pigments distinguishes the cryptomonad chloroplast from other algae. In addition to chlorophylls a and c₂, the cryptomonads have phycobiliproteins. Depending on the species, either phycoerythrin or phycocyanin is present (Hill and Rowan, 1989). Cryptomonad phycobiliproteins are apparently homologues of phycobiliproteins present in red algae and cyanobacteria (Glazer and Apell, 1977; Wehrmeyer, 1983; Guard-Friar et al., 1986; Reith and Douglas, 1990).
However, the cryptomonad phycobiliproteins are not organized into phycobilisomes like those of red algae and cyanobacteria (Glazer, 1982). Moreover, only one phycobiliprotein occurs in each cryptomonad, while two or three are present in red algae and cyanobacteria. Another essential difference is in phycobiliprotein location. The phycobilisomes of red algae and cyanobacteria are attached to the stromal side of the thylakoid membrane (Glazer, 1982), but cryptomonad phycobiliprotein occurs within the lumen of the thylakoid membranes (Gantt et al., 1971; Dwarte and Vesk, 1982; Spear-Bernstein and Miller, 1987) and is probably attached to the luminal side of the thylakoid membrane (Ludwig and Gibbs, 1989a). The unique electron-dense nature of the cryptomonad thylakoid lumina (Figs. 3, 4, 6 and 11) is attributed to the phycobiliprotein therein (Dodge, 1969; Gantt et al., 1971; Rhiel et al., 1985) and absence of phycobilisomes may allow the stacking of thylakoids in cryptomonads (Spear-Bernstein and Miller, 1985), a chloroplast feature not observed in red algae or cyanobacteria.

The cryptomonad phycobiliprotein is known to act as the major light antenna (Haxo and Fork, 1959; Gantt, 1979), but exactly how the excitation energy is transferred to photosystem II in the absence of the usual intermediary structures in the phycobilisome is unknown. Since the phycobiliprotein is on the luminal or “wrong” side of the thylakoid, it was suggested that perhaps cryptomonad thylakoids are “inside-out” and that energy transfer to photosystem II would thus be possible (Gantt et al., 1971; Gantt, 1979, 1980). However, freeze-fracture analysis indicates normal topology for cryptomonad thylakoid membrane proteins (Spear-Bernstein and Miller, 1985), and it must be assumed that energy transfer from an antenna on one side of the membrane to a reaction centre on the other side is occurring. Possibly the intraluminal arrangement of the light antenna compensates for the lack of phycobilisomes (Lichtlé et al., 1987; Ludwig and Gibbs, 1989a), or, alternately, phycobilisomes are required for energy transfer if the antenna is not within the lumen. In red algae, the presumed endosymbiotic in cryptomonads, the linker peptides of the phycobilisome are nuclear-encoded (Grossman et al., 1986) so the linker protein genes, were they present in cryptomonads, would be expected to reside in the nucleomorph. Ludwig and Gibbs (1989a) suggested that perhaps the linker peptides were lost from nucleomorph, and that a mechanism for translocation of β-phycoerythrin to the thylakoid lumen had previously evolved to compensate for the loss in ability to form phycobilisomes.

To further confound the issue, cryptomonad β-phycoerythrin, which is chloroplast genome-encoded, lacks the standard leader peptide that directs proteins from the stromal space into the thylakoid lumen (Howe and Wallace, 1990; Reith and Douglas, 1990). It is interesting in this respect that the α-phycoerythrin subunits of cryptomonads are apparently not related to the α-phycoerythrin subunits of red algae (Sidler et al., 1985, 1987), and
these may perhaps be involved in transporting the β-phycoerythrin subunit across the thylakoid membrane (Reith and Douglas, 1990).

D. CHLOROPLAST GENOME

Analysis of the plastid genome from cryptomonads could be expected to help identify the eukaryotic endosymbiont, and perhaps even the primary prokaryotic endosymbiont. Determination of the content, arrangement, coding patterns and copy number of the various genes in the chloroplast genome has helped in establishing evolutionary relationships among land plants and, to a lesser extent, among the algae (Palmer, 1985, 1990; Cattolico, 1986; Cattolico and Loiseaux-de Goër, 1989).

1. Structure of the chloroplast chromosome

Physical mapping of a cryptomonad chloroplast genome reveals a circular chromosome of 118 kb containing two small inverted repeats (Douglas, 1988) which is thus superficially similar to chloroplast chromosomes of land plants and most algae. Surprisingly though, the gross structure is not equivalent to red algal chloroplast chromosome structure. Two red algal chloroplast chromosomes have been mapped (Li and Cattolico, 1987; Shivji 1990), and they are both different from the cryptomonad. One red alga, Griffithsia pacifica, has only one set of rRNA genes and hence no inverted repeat whatsoever (Li and Cattolico, 1987). The other species, Porphyra yezoensis, has inverted repeats, but they are situated in the chromosome in the reverse orientation to cryptomonads with respect to the large single-copy region containing rbcL (operon encoding the large and small subunits of Rubisco) and tufA (protein synthesis elongation factor Tu). Since the inverted repeats in the green algae are oriented in the opposite direction to those of their descendants, the land plants (Palmer, 1985), it is difficult to determine the significance of the anomalous repeat orientation in cryptomonads and red algae. Moreover, since the loss of one repeat is known to have occurred in Pisum sativum and Vicia faba (Palmer and Thompson, 1982), it is possible that cryptomonad chloroplast chromosomes are derived from a Griffithsia-like ancestor with the same repeat orientation that has since lost one repeat. Another explanation could be that the inverted repeat has evolved independently in red algae and cryptomonads. Palmer and Thompson (1982) propose that the inverted repeat serves to stabilize the chloroplast genome from rearrangement and prevent recombinational gene loss. Such a beneficial trait could well have evolved convergently and the differences in inverted repeat content argue for parallel development.

Difficulties in rationalizing chloroplast chromosome structure are also apparent when comparing cryptomonads with chromophytes. The inverted repeats of the two brown algae Pylaiella littoralis (Loiseaux-de Goër et al.,
1988) and Dictyota dichotoma (Kuhshel and Kowallik, 1987) and the chrysophyte Ochromonas denticola (Cattolico and Loiseaux-de Goër. 1989) have the same orientation as cryptomonads with respect to rbcL but not psbA. In two diatoms (Odontella and Coscinodiscus) and the xanthophyte Vaucheria (Kowallik, 1989), the inverted repeat is oriented in the same way with respect to psbA (D₁-reaction centre polypeptide of photosystem II). psbA is actually contained within the inverted repeat of Coscinodiscus (Kowallik, 1989). The rbcLS genes of Vaucheria have not been mapped. In another chromophyte, Olisthodiscus luteus, the 23S rRNA genes are proximal to the single-copy region containing psbA (Reith and Cattolico, 1986; Delaney and Cattolico, 1989), as occurs in cryptomonads. However, rbcLS is contained within the inverted repeat Olisthodiscus luteus (Reith and Cattolico, 1986; Delaney and Cattolico, 1989). Rearrangements of chloroplast genes are common (Palmer, 1985), and even occur within members of the same genus of chromophytes (Kowallik, 1990), so comparison of the gross morphology of chloroplast chromosomes is perhaps premature given the small data set for algae. Once more detailed maps for a wider selection of algal taxa are available, it should be possible to draw more conclusive comparisons, as has been possible with land plants (Palmer, 1990).

2. Chloroplast gene sequences
Several protein, tRNA, and rRNA genes have been sequenced from the cryptomonad chloroplast chromosome (Douglas and Durnford, 1989; 1990a, b; Douglas et al., 1991a; Douglas, 1991; Douglas and Turner, 1991). Like land plants and other algae, the cryptomonad tRNA^{Ile} and tRNA^{Ala} genes are located in the spacer between 16S and 23S rRNAs (Douglas and Durnford, 1990a). The cryptomonad tRNA^{Ile} and tRNA^{Ala} genes are similar to those of other algae in that they lack introns (Douglas and Durnford, 1990a) characteristic of the land plant chloroplast lineage (Manhart and Palmer, 1990). Like all chloroplast tRNA^{Ile} and tRNA^{Ala} genes, the cryptomonad genes do not encode the CCA 3' termini (Douglas and Durnford, 1990a). These results indicate that the inverted repeats of chloroplast chromosomes in land plants and algae (including cryptomonads) are all homologous and that they could well be derived from the rRNA operon of bacterial endosymbionts, since the latter usually contain tRNA^{Ile} and tRNA^{Ala} genes (Sprinzl et al., 1989).

3. Rubisco phylogeny
Genes for the large and small subunits of ribulose-1,5-bisphosphate carboxylase/oxygenase (rbcL and rbcS) have now been sequenced from a wide variety of organisms including cryptomonads (Douglas and Durnford, 1989; Douglas et al., 1991a). Phylogenetic trees based on comparison of rbcL and rbcS sequences cluster cryptomonads with red algae (Douglas and Durnford, 1989; Valentin and Zetsche, 1990a, b; Douglas et al., 1991b;
Morden and Golden, 1991), again supporting the hypothesis that cryptomonads contain a red algal endosymbiont. The cryptomonad sequence shares more positional identity with the unicellular red algae *Porphyridium aerugineum* than with *Cyanidium caldarium*, which is an unusual thermo-acidophilic unicellular red alga (Valentin and Zetsche, 1990b).

Rubisco from chromophyte is similar to that from red algae (Newman et al., 1989), and phylogenetic trees derived from rbcL and rbcS sequences group the chromophytes with red algae and cryptomonads (Douglas et al., 1991a; Morden and Golden, 1991). The other major cluster contains all the green or chlorophyll b-containing chloroplasts (i.e. euglenoids, green algae and the land plants; Douglas et al., 1991a; Morden and Golden, 1991). To explain this dichotomy of rbcL and rbcS sequences, it has been postulated that chloroplasts have polyphyletic origins. The chlorophyll b line of chloroplasts is suggested to derive from a cyanobacterial ancestor, while the red algal, cryptomonad and chromophyte chloroplasts would seem to derive from β-purpe bacteria, as represented by the chemolithotrophic *Alcaligenes eutrophus* (Boczar et al., 1989; Douglas et al., 1991a; Morden and Golden, 1991). In *Alcaligenes eutrophus* (formerly known as *Hydrogenomonas*), carbon fixation is driven by oxidation of molecular hydrogen. *Alcaligenes eutrophus* lacks a photosystem, so postulating this bacterium as a progenitor for chloroplasts in cryptomonads, red algae and chromophytes implies that oxygenic photosynthesis has evolved twice. Also indicated would be the parallel evolution of chlorophyll a and phycobiliproteins, since these pigments are not known in β-purple bacteria. The other, seemingly unlikely, alternative would be that a common ancestor of β-purple bacteria and cyanobacteria possessed oxygenic photosynthesis, and that after establishment of an endosymbiotic relationship leading to the non-chlorophyll b line, remaining β-purple bacteria underwent secondary loss of the ability to use water as an electron donor.

4. *Chloroplast rRNA and protein gene phylogeny*
Phylogenies based on chloroplast DNA sequences other than rbcLs do not invariably support the polyphyletic origin of chloroplasts. Sequence of the quinone-binding, photosystem II-associated D1 protein (psbA) from a red alga has a high level of positional identity when compared to psbA of land plants, but also contains a seven amino acid insertion at the carboxyl terminus that is characteristic of cyanobacteria (Maid et al., 1990).

Phylogenies inferred from chloroplast 16S rRNA gene sequences do not suggest a β-purple progenitor for any chloroplasts (Douglas and Turner, 1991). Rather, all chloroplasts are monophyletic and have a cyanobacterium as a sister group (Douglas and Turner, 1991). There is, however, a deep divergence in the chloroplast lineage separating red algal, cryptomonad, *Euglena* and chromophyte chloroplasts into one clade, and chlorophyll b-containing chloroplasts (with the exception of *Euglena*) into another. The
discrepancy between the phylogeny inferred from rbcL sequences and that inferred from 16S rRNA data remains unresolved. If the 16S rRNA phylogeny is correct, the similarities between rbcL sequences of *Alcaligenes eutrophus* and non-chlorophyll *b*-containing chloroplasts could be explained in several ways:

1. Displacement of the cyanobacterial endosymbiont's rbcLS operon with an *Alcaligenes*-like operon by horizontal gene transfer before, during, or after the establishment of the endosymbiosis;
2. Existence of an unknown prokaryote with a photosystem, phycobilins, and an *A. eutrophus*-like rbcLS operon;
3. Convergent evolution of the rbcLS sequences in *A. eutrophus* and non-chlorophyll *b*-containing chloroplasts;
4. The possibility that all plastids derive from a cyanobacterium with two different rbcL genes, a different one of which was subsequently lost in the two divergent lines;
5. That rbcL in rhodophytes and chromophytes is derived from the mitochondrion (proposed to be derived from a purple bacterium) by inter-organelle transfer; and
6. Failure of the rbcLS-based trees to predict the correct phylogeny of bacteria and chloroplasts, perhaps due to biases in A + T content of the genomes examined (Lockhart *et al.*, 1992).

It should also be noted that an alternative phylogeny, inferred from structure function and dispersion of Rubisco, places the chemosynthetic bacteria as descendants of cyanobacteria and a sister group of chloroplasts (McFadden *et al*., 1986).

V. CRYPTOMONADS AS ENDOSYMBIONTS, PARASITES OF CRYPTOMONADS AND ENDOSYMBIONTS OF CRYPTOMONADS

So, naturalists observe, a flea
Hath smaller fleas that on him prey
And these have smaller fleas to bite 'em
And so proceed ad infinitum

Jonathun Swift (1733)

Cryptomonads exist as endosymbionts within the cells of ciliates and dinoflagellates. The gymnostome ciliate *Myrionecta rubra* (formerly *Mesodinium rubrum* or *Cyclotrichium meunieri*), which commonly causes non-toxic red tides, contains numerous cryptomonads (Hibberd, 1977; Oakley and Taylor, 1978). Curiously, the cryptomonad endosymbiont is sometimes subdivided into separate membrane-bound compartments in the ciliate. In one type of compartment are found several chloroplasts, each with its
peri plastidal space and nucleomorph, as well as the mitochondria and surrounding cytoplasm presumably derived from the main cryptomonad cytoplasm. The main cryptomonad nucleus along with more mitochondria is found in another type of compartment. Both types of compartment have a single smooth membrane dividing the endosymbiont cytoplasm from the host cytoplasm. In some places, a layer of host rough ER overlays the dividing membrane. Exactly how the chloroplasts and mitochondria are maintained in a compartment remote from their nucleus is a mystery. The cryptomonad appears to be a permanent endosymbiont and has lost its flagellar apparatus, ejectosomes, and periplast.

Several dinoflagellates have been shown to contain cryptomonads (Wilcox and Wedermayer, 1984, 1985; Larsen, 1988; Schnepf and Elbrächter, 1988; Schnepf et al., 1989; Farmer and Roberts, 1990). That the cryptomonads are true endosymbionts, and not merely incompletely digested prey cells, has not been unequivocally established (Larsen, 1988; Fields and Rhodes, 1991). The dinoflagellates involved feed by myzocytosis, a process in which the dinoflagellate seems to suck out the contents of the prey cell through a tube known as the peduncle (Schnepf and Elbrächter, 1988). The cryptomonad "endosymbionts" in the dinoflagellates frequently lack their nucleus and sometimes only the chloroplast is found in the dinoflagellates, and it is possible that the dinoflagellate sometimes fails to ingest all the organelles of the prey cell. Chloroplasts obtained in this manner have been dubbed "cleptochloroplasts" (Schnepf et al., 1989) and it is possible that they represent an early stage in the establishment of a true endosymbiont-derived organelle.

A kinetoplast flagellate known as Proteromonas steinii parasitizes several species of Cryptomonas (Ettl and Moestrup, 1980; Ettl, 1984). The parasite occupies up to half the cryptomonad cell and is released either as a cyst or numerous swarmers when the host cell eventually dies (Ettl and Moestrup, 1980).

Some chloroplast-containing cryptomonads can contain bacterial endosymbionts. The bacteria occur in the main cytoplasmic compartment and can be in a peribacterial membrane, a specialized vacuole, or free in the cytoplasm (Kugrens and Lee, 1990; Schnepf and Melkonian, 1990). The bacterial endosymbionts contain bacteriophage-like particles (Schnepf and Melkonian, 1990).

VI. SECOND-HAND CHLOROPLASTS IN OTHER ALGAE

Do any other algae have second-hand chloroplasts? Indirect acquisition of photosynthetic capacity has been proposed for euglenoids, dinoflagellates, chromophytes and Chlorarachniion. A variety of evolutionary schemes postulate photosynthetic eukaryotic endosymbionts as chloroplast progenitors
in these algae (see Gibbs, 1978, 1981a, 1985, 1990; Dodge, 1979; Whatley and Whatley, 1981; Sitté, 1990). The chloroplasts of these algae have three, or sometimes four, membranes surrounding them, and these extra membranes have been proposed to represent either the plasma membrane of the endosymbiont or the phagocytic membrane of the host, or both in the case of those chloroplasts having four limiting membranes (Fig. 2). While the hypotheses for chromophyte and dinoflagellate chloroplast origins are still highly speculative, the chloroplasts of Chlorarachniion and the dinoflagellate Lepidodinium viridae are very likely to derive from green algae.

As outlined in Section III.D.4, the chromophyte chloroplasts are probably not derived from cryptomonad chloroplasts by loss of the nucleomorph and periplastidal ribosomes. Another explanation could be that chromophyte chloroplasts are derived from a red algal endosymbiont that subsequently lost the phycobiliproteins, which would explain the similarities in chromophyte and red algal ribulose-1,5-bisphosphate carboxylases. However, other features, including the accessory pigments and storage products in chromophytes, are dissimilar to red algae, so the origin of the chromophyte chloroplast remains uncertain.

Dinoflagellates typically have three membranes delimiting the chloroplast and possibly acquired their chloroplasts from a chromophyte (Tomas and Cox, 1973; Gibbs, 1981a)—a case of a “hand-me-down” chloroplast.

The euglenoid chloroplast shares certain features, including chlorophyll b, with green algae (Palmer, 1985), but sequences of nuclear-encoded 16S-like rRNA ally the euglenoids with kinetoplastids (parasitic flagellates including trypanosomes, Crithidia and leishmanias; Wolters, 1991; see also Fig. 10). Euglenoids may be flagellates that engulfed either a green algal cell (Gibbs, 1978, 1985, 1990), or some other eukaryotic alga (Douglas and Turner, 1991), which is now reduced to a chloroplast with three membranes.

Chlorarachniion and the dinoflagellate Lepidodinium viridae almost certainly contain a eukaryotic endosymbiont, as they both contain a nucleus-like structure analogous to the cryptomonad nucleomorph. Chlorarachniion is a reticulopodial amoeba that contains several green chloroplasts containing chlorophylls a and b (Hatakeyama et al., 1991). Like cryptomonads, the chloroplasts of Chlorarachniion are surrounded by four membranes and between the inner and outer pairs is a periplastidal space with eukaryotic-size ribosomes and a nucleus-like structure (Hibberd and Norris, 1984) that contains DNA (Ludwig and Gibbs, 1987, 1989a). The Chlorarachniion nucleomorph apparently encodes rRNAs (McFadden, 1989), but sequences are not yet available. It seems most likely that Chlorarachniion acquired its chloroplast by engulfing a green alga (possibly a prasinophyte), and that the nucleomorph is the homologue of the green algal nucleus. It will be most interesting to determine the coding function of the Chlorarachniion nucleomorph DNA and compare the location of chloroplast protein genes in Chlorarachniion with the green algae and land plants.
The dinoflagellate *Lepidodinium viridae* is different from typical dinoflagellates in that it contains green chloroplasts with chlorophylls *a* and *b* (Watanabe et al., 1987). Like cryptomonads and *Chlorarachniion*, the *Lepidodinium viridae* chloroplasts have four membranes with a periplastidal space and a nucleus-like organelle surrounded by ribosome-like particles (Watanabe et al., 1987). The presence of DNA in the nucleus-like structure has not been demonstrated but *Lepidodinium viridae* seems likely to contain a reduced green alga.

From these preliminary observations it would seem that conversion of phagotrophic protists to an autotrophic life-style by engulfing and retaining photosynthetic eukaryotes may have occurred several times during evolution. Protists would seem to have a propensity for “kidnapping” autotrophic cells and putting them to work as slaves (Cavalier-Smith and Lee, 1985), or, conversely, algae may have a propensity for invading phagotrophs and remaining permanently inside the host cell. Either way, distantly related protists seem to have acquired chloroplasts of the same type by harbouring closely related eukaryotic endosymbionts. It is therefore important to distinguish between the original evolution of chloroplasts and their subsequent dissemination among a variety of protists by secondary endosymbioses.

**VII. ROLE OF THE NUCLEOMORPH**

What is the role of the nucleomorph and why does it persist? The nucleomorph is situated in a subcompartment of the cryptomonad cell and is surrounded by eukaryotic ribosomes. Preliminary indications are that nucleomorph DNA encodes rRNAs for the surrounding ribosomes (see Section III) and it is likely that nucleomorph DNA could encode ribosomal proteins and housekeeping genes. But what is the ultimate purpose of this second set of eukaryotic translation machinery in the periplastidal space? Presumably, the nucleomorph DNA encodes proteins essential to the chloroplast. The chloroplast chromosome has the capacity to encode roughly 100 protein genes, so the many hundreds of other chloroplast proteins must be encoded elsewhere—either in the nucleus or nucleomorph. Only one cryptomonad chloroplast protein, an α-subunit of phycoerythrin (cpeA), has thus far been demonstrated to be encoded outside the chloroplast. In red algae and cyanobacteria cpeA is usually located on the chloroplast chromosome adjacent cpeB (the β-subunit of phycoerythrin), but in cryptomonads the cpeA gene is apparently absent from the chloroplast genome (Reith and Douglas, 1990). A cpeA gene isolated from a library of total cryptomonad DNA (Jenkins et al., 1990) contains features typical of a “nuclear” gene (CAT and TATA boxes, polyadenylation site, 66% G + C content, and an N-terminal transit sequence), but it has not yet been
determined whether cpeA resides in the nucleus or in the nucleomorph of the cryptomonad, or even in both.

Available evidence suggests that the cryptomonad chloroplast originated from a red alga, so it might be expected that the nucleomorph encodes many of the same chloroplast proteins as does the red algal nucleus. In modern day red algae the nucleus presumably encodes the majority of chloroplast proteins, but thus far only the genes for phycobilisome linker polypeptides have been demonstrated to be nuclear-encoded (Egelhoff and Grossman, 1983; Grossman et al., 1985). Since cryptomonads lack phycobilisomes, they probably lack these genes altogether. Other likely candidates for nucleomorph-encoded chloroplast proteins might be ferredoxin, cytochrome and chlorophyll a/c binding protein, since these proteins, or their homologues, are usually nuclear-encoded in plants and several different algae.

Clearly the nucleomorph has been retained by all the chloroplast-containing cryptomonads. Several herbivorous molluscs and planktonic ciliates are known to sequester algal chloroplasts (Hinde, 1983; Stoecker et al., 1987). The chloroplasts carry out photosynthesis for a limited period but eventually degenerate, probably through lack of essential components normally produced by the nucleocytoplasm of the alga. The molluscs periodically renew the chloroplasts by further grazing (Hinde, 1983). The cryptomonad flagellate host would seem to have obviated the need to renew its chloroplasts by retaining the algal nucleus in the form of the nucleomorph.

In addition to providing some essential component(s) for the biogenesis or maintenance of the chloroplasts, the nucleomorph may have a pivotal role in the endosymbiotic relationship. The nucleomorph and periplastidal cytoplasm are located between the primary producer (the chloroplast) and the consumer (the host flagellate cell). Significantly, this intermediary zone is where excess photosynthetic is stored (as starch in the periplastidal space), and screening of a nucleomorph library for starch synthetases and hydrolyases may be a fruitful exercise. It is also noteworthy that the cryptomonad nucleomorph is invariably closely associated with the pyrenoid (Figs. 3 and 6), even being encased within the latter in some species (Figs. 11–13). This association even extends to the nucleomorph of Chlorarachnion reptans, which is also situated in a notch of the pyrenoid (Hibberd and Norris, 1984). The role of pyrenoids is not clear, but they are usually close to the site of starch deposition, perhaps further implicating the nucleomorph in starch metabolism.

In the unicellular red alga Rhodella, a protrusion of the nucleus invades the pyrenoid (Evans, 1977; Patrone et al., 1991), so perhaps the pyrenoid/nucleus association in cryptomonads is carried over from the eukaryotic endosymbiont. The Rhodella pyrenoid appears to be involved in mitosis, apparently encasing the dividing nucleus. The cryptomonad nucleomorph
might be sited near the pyrenoid to ensure that the daughter nucleomorphs are segregated with the two halves of the chloroplast during division.

The periplastidal compartment probably serves as a transit zone, through which there is bidirectional traffic of a variety of substances. Photosynthetic must go from the chloroplast to the host; numerous metabolites (including ATP) must be conveyed from the mitochondrial-containing host compartment to the periplastidal compartment and chloroplast. All of this traffic must also cross the two pairs of membranes that separate the three compartments. Gibbs (1979, 1981b) proposed that the ribosomes on the CER surface might translate proteins into the lumen between the CER membranes, and that vesicles might convey molecules across the periplastidal space to the chloroplast. According to this hypothesis, Nuclear-encoded chloroplast proteins in cryptomonads should bear an N-terminal signal peptide rather than a transit peptide. Nucleomorph-encoded chloroplast proteins, on the other hand, would be expected to have a transit peptide. Sequencing of nuclear-encoded chlorophyll a/c fucoxanthin binding proteins (fcp) of diatoms, which have a CER but no nucleomorph or periplastidal ribosomes, reveals the presence of a possible N-terminal signal peptide (Grassman et al., 1990). In vitro studies with the diatom fcp clones should establish whether these chloroplast proteins are first directed into the lumen of the ER en route to the chloroplast.

The nucleomorph contains only 660 kb of DNA and must be much reduced in comparison to the original red algal nucleus. Similarly, the periplastidal space is estimated to perform a mere 3% of the translation in the cell (Sitte and Baltes, 1989). Since many functions would be duplicated in a cell with two nucleocytoplasmic compartments, it is reasonable to assume that the nucleomorph has lost many redundant genes—mitochondrial proteins are an obvious case. It is also possible that nucleomorph genes have been transferred to the main nucleus.

VIII. SUMMARY

Cryptomonads are apparently chimaeras derived from a phagotrophic flagellate and a eukaryotic, chloroplast-containing cell. The cell contains the genomes of at least four different evolutionary lineages: mitochondrial DNA (probably from a purple non-sulphur eubacterium), chloroplast DNA (from a photosynthetic eubacterium?), main nucleus (phagotrophic flagellate), and nucleomorph (red algal nucleus?).

The identity of the phagotrophic flagellate that engulfed the endosymbiont is unknown. Similarities in flagellar apparatus between cryptomonads and trichomonads (parasitic flagellates) have been noted (Roberts et al., 1981), but phylogenetic trees based on 23S-like rRNA sequences do not ally cryptomonads and Trichomonas (Perasso et al., 1989).
The eukaryotic endosymbiont is most likely to have been a red alga, as first proposed several years ago (Dodge, 1979; Whatley et al., 1979). This conclusion is based on several lines of evidence: (1) the presence of phycobiliproteins in both cryptomonads and red algae; (2) the similarities of cryptomonad and red algal rbcLS sequences; (3) the similarity of cryptomonad and red algal chloroplast 16S rRNA sequences; (4) the similarity of the nucleomorph 16S-like rRNA gene sequence to the nucleocytoplasmic 16S-like rRNA of red algae; and (5) the presence of starch in the cryptomonad periplastidal space and starch in the cytoplasm or red algae.

The identity of the primary prokaryotic endosymbiont that gave rise to the chloroplast of the red algae is undetermined but seems most likely to have been a cyanobacterium. Further study of red algae and cryptomonads will hopefully reveal whether the chloroplasts of red algae, cryptomonads and chromophytes are derived from the same primary cyanobacterial endosymbiont as the chloroplasts of green algae and land plants.

The endosymbiosis between cryptomonad and red alga probably developed from a predator–prey relationship. Digestion of the prey cells by the flagellate was postponed in order to allow the engulfed cell to photosynthesize. By holding the photosynthetic cell captive, the phagotroph was able to benefit from the autotrophic ability of its prey. Eventually, a permanent endosymbiotic relationship was established. The endosymbiont's mitochondria became redundant and were lost. Its nuclear genome became much reduced, perhaps through transfer of genes to the nucleus of the host cell. The diversity and distribution of cryptomonads suggest that the endosymbiosis was not recently established, although it apparently post-dates the divergence of the red algal lineage.

IX. TAXONOMIC APPENDIX

Several of the cryptomonads used in laboratory studies reviewed here have been reclassified. The strain designated as Cryptomonas θ has been described in the new genus Guillardia as Guillardia theta D. Hill et Wetherbee (Hill and Wetherbee, 1989a). The strain designated Cryptomonas Φ remains undescribed but is considered an ally of species in the genus Teleaulax (Hill, 1991b). The species first described as Cryptomonas salina Wislouch and subsequently moved to Chroomonas salina (Wislouch) Butcher, and then to Pyrenomonas salina (Wislouch) Santore has been transferred to Rhodomonas salina (Wislouch) Hill et Wetherbee (Hill and Wetherbee, 1989b). Similarly, Cryptomonas maculata Butcher and Cryptomonas abbreviata Butcher, which were transferred to Pyrenomonas as Pyrenomonas maculata (Butcher) Santore and Pyrenomonas abbreviata (Butcher) Santore are now known as Rhodomonas maculata Butcher ex Hill et Wetherbee and Rhodomonas abbreviata Butcher ex Hill et Wetherbee
(Hill and Wetherbee, 1989b). *Chroomonas caudata* Geitler has been transferred to the new genus *Komma* to become *Komma caudata* (Geitler) Hill (Hill, 1991c). The chloroplast-lacking *Cyathomonas* is considered synonymous with *Goniomonas* (Hill, 1991a).

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NOTE ADDED IN PROOF

Definitive evidence that the red algal-like rRNA gene of cryptomonads is located in the nucleomorph was recently published. Using DNA from isolated nucleomorphs in PCR experiments, Maier et al. (1991) were able to amplify and clone the endosymbiont 16S-like rRNA gene of *Pyrenomonas salina*. Phylogenetic trees incorporating the endosymbiont gene sequence indicate that it is related to the red algae. As final proof that cryptomonads are composed of one eukaryotic cell operating inside another eukaryotic cell, it is now necessary to demonstrate that the nucleomorph gene encodes rRNAs for ribosomes in the periplastidal space, and that the nuclear gene encodes rRNAs for the host cytoplasm.

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