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Dinoflagellate with Blue-Green Chloroplasts Derived from an Endosymbiotic Eukaryote

Abstract. *The dinoflagellate,* Amphidinium wigrense, *contains triple membrane-bound bodies we have termed "blue-green chloroplasts." We believe these chloroplasts were derived from a cryptomonad endosymbiont similar to that present in another blue-green dinoflagellate,* Gymnodinium acidotum. *These dinoflagellates provide evidence that a chloroplast has evolved from an endosymbiotic eukaryote.*

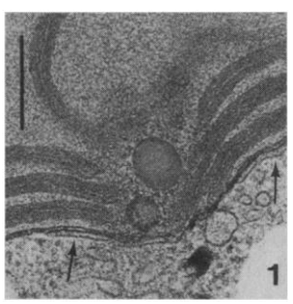
The chloroplasts of red and green algae (and higher plants) evolved from photosynthetic, prokaryotic endosymbionts (*1, 2*). These chloroplasts are surrounded by an envelope consisting of two membranes. With few exceptions (*2, 3*), however, chloroplasts of other photosynthetic eukaryotes are bound by either three membranes (euglenoids and dinoflagellates) or four membranes (chlorophyll c–containing organisms other than dinoflagellates). It has been proposed that these chloroplasts resulted from the acquisition and degeneration of endosymbiotic eukaryotes (*1, 2, 4, 5*).

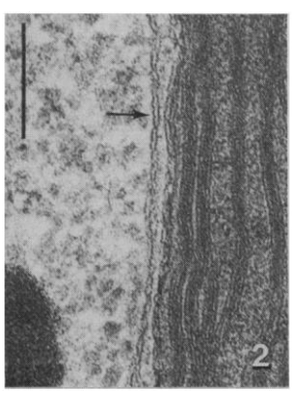
Evidence that a chloroplast arose from a eukaryotic endosymbiont has come from studies on the Cryptophyceae, a small group of flagellates whose photosynthetic members contain phycobilin accessory pigments in addition to chlorophylls a and c_2. The nucleomorph, a double membrane–bound body characteristic of Cryptophyceae, is found in the space called the "periplastidal compartment" between the two pairs of membranes surrounding the cryptomonad chloroplast. The periplastidal compartment also contains starch and eukaryotic-sized ribosomes (*6–8*). The nucleomorph may represent the degenerate nucleus of a red alga–like cell, to which the ribosomes presumably once belonged (*5, 7, 8*). This view has been strengthened by the demonstration of DNA and RNA in the nucleomorph (*9*). The red alga–like cell is thought to have taken in a prokaryote, which eventually became its chloroplast, and, in turn, to have been acquired by some ancestral cryptomonad and subsequently reduced to its present state (*5, 7, 8*).

The blue-green dinoflagellate *Gymnodinium acidotum* contains an endosymbiotic cryptomonad (*10*). Like the ciliate *Mesodinium rubrum*, which also harbors a cryptomonad (*11, 12*), and the dinoflagellates *Peridinium balticum* and *Kryptoperidinium foliaceum*, which contain chrysophycean endosymbionts (*13*), *G. acidotum* and its endosymbiont may resemble a relatively early stage in the evolution of a eukaryotically derived chloroplast.

In contrast to their free-living counterparts, the cryptomonad endosymbionts of *G. acidotum* and *M. rubrum* each lack a periplast, ejectosomes, and a flagellar apparatus (*10, 12*). Cells of one *M. rubrum* strain have numerous discrete compartments of endosymbiont cytoplasm, each containing a chloroplast, microbody, and mitochondrion. These "chloroplast-mitochondrial complexes" are apparently not connected to the portion of cytoplasm containing the endosymbiont nucleus (*11*). Moreover, a cell of *G. acidotum* was found which lacked an endosymbiont nucleus (*10*). Similar changes would be expected to occur during the transformation of a whole cell endosymbiont into a chloroplast.

The blue-green dinoflagellate, *Amphidinium wigrense* (*14*), contains bodies we have chosen to call "blue-green chloroplasts" on the basis of their consistent





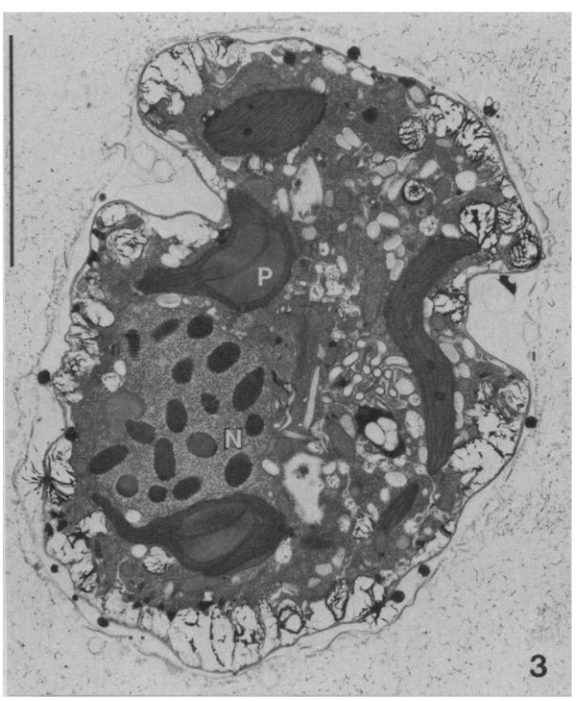


Fig. 1. The three bounding membranes of a blue-green chloroplast are indicated by arrows. The electron-opaque contents of the paired thylakoids are visible. Scale bar, 0.25 μm. Fig. 2. Chloroplast adjacent to nucleus showing the nuclear envelope (arrow) and the three membranes of the chloroplast envelope lying next to it. Scale bar, 0.25 μm. Fig. 3. Longitudinal section of an *A. wigrense* cell showing its nucleus and profiles of several of this cell's seven chloroplasts. The fibrillar material external to the cell is a mucilaginous substance released from peripheral vacuoles upon fixation. *N*, nucleus; *P*, pyrenoid; scale bar, 5 μm.

possession of only three surrounding membranes (Figs. 1 and 2) and the apparent lack of any other "non-dinoflagellate" organelles in the *A. wigrense* cell. These chloroplasts are cryptophycean in origin since their photosynthetic lamellae consisted mainly of paired thylakoids with electron-opaque lumens (Figs. 1 and 2). Such a chloroplast structure is characteristic of the cryptomonads (*5, 15*).

Pyrenoids, which were present in most of the chloroplasts, are like those of many cryptomonads (Fig. 3). No nucleomorphs or eukaryotic-sized ribosomes were associated with these blue-green chloroplasts. The number of chloroplasts in living cells was variable, as determined by light microscopic analyses. For more precise quantitation, five cells were serially thin-sectioned and contained, respectively, 7, 6, 4, 3, and either 2 or 3 chloroplasts per cell (with the uncertainty in the quantitation of the last cell occurring because of the loss of a few sections).

It seems reasonable to assume that the blue-green chloroplasts of *A. wigrense* resulted from a further reduction of an already partially degenerate endosymbiont like that in *G. acidotum*. If cryptomonads acquired their chloroplasts in this manner, then three symbiotic events must have taken place to give rise to the blue-green chloroplasts (Fig. 4, steps 1, 2, and 3). If a single endosymbiont was reduced to form the blue-green chloroplasts of an *A. wigrense* cell, then the chloroplasts have proliferated, as most free-living cryptomonad cells have only one or two chloroplasts. An even greater proliferation of chloroplasts seems to have occurred in some *M. rubrum* cells (*11*).

It is generally accepted that the inner of the two bounding membranes of green and red algal chloroplasts represents the plasma membrane of a prokaryotic endosymbiont, and the outer, a vacuolar membrane of the host (*1, 2, 16*). An alternative view suggests that both may have been present in the prokaryote (*17*). In chloroplasts with three (or four) surrounding membranes, the inner pair is thought to be equivalent to these two membranes (*1, 2, 16*). We feel that this is a reasonable assumption for the inner pair of membranes of the blue-green chloroplasts as well.

There is less agreement concerning the source of the outer membrane (or membranes) of chloroplasts with more than two bounding membranes. Whatley *et al.* (*1*) suggested that the outermost membrane of the triple membrane–bound euglenoid and dinoflagellate chloroplasts was derived from a host vacuole. They proposed that these plastids were acquired as isolated chloroplasts, which would be similar to the uptake and maintenance of chloroplasts of certain green algae by marine molluscs. Gibbs (*2*), however, proposed that euglenoid and dinoflagellate chloroplasts resulted from the degeneration of a whole-cell endosymbiont, and that in each the outer membrane is homologous with the plasma membrane of the endosymbiont.

Our work on *G. acidotum* and *A. wigrense*, along with the studies on *M. rubrum*, suggests that an entire organism was reduced to form the blue-green chloroplasts. Even if the blue-green chloroplasts were acquired as isolated entities, we believe that they were once present in a eukaryote. The discovery of the blue-green chloroplast provides indirect support for the view that other chloroplasts with more than two surrounding membranes have had a eukaryotic origin.

The endosymbiont in *G. acidotum* is separated from the host cytoplasm by a single membrane, which is probably the endosymbiont plasma membrane. The endosymbiont plasma membrane would probably be necessary for its survival, while a host vacuolar membrane would be more dispensable (*2*). In *G. acidotum*, five membranes (the putative plasma membrane and the four membranes surrounding the chloroplast) separate the chloroplast stroma from the dinoflagellate cytoplasm. If the triple membrane–bound blue-green chloroplast of *A. wigrense* developed from an endosymbiont that at one time resembled the one in *G. acidotum*, then two of the five membranes have been lost. The outer membrane of the blue-green chloroplast may represent the endosymbiont plasma membrane, or one of the two outer membranes that surround the chloroplast. The outermost membrane of the cryptomonad chloroplast characteristically has ribosomes on its cytoplasmic surface, while the outermost membrane of the blue-green chloroplast does not. Thus we believe the outer membrane of the blue-green chloroplast is homologous to either the endosymbiont plasma membrane or to the inner membrane of the

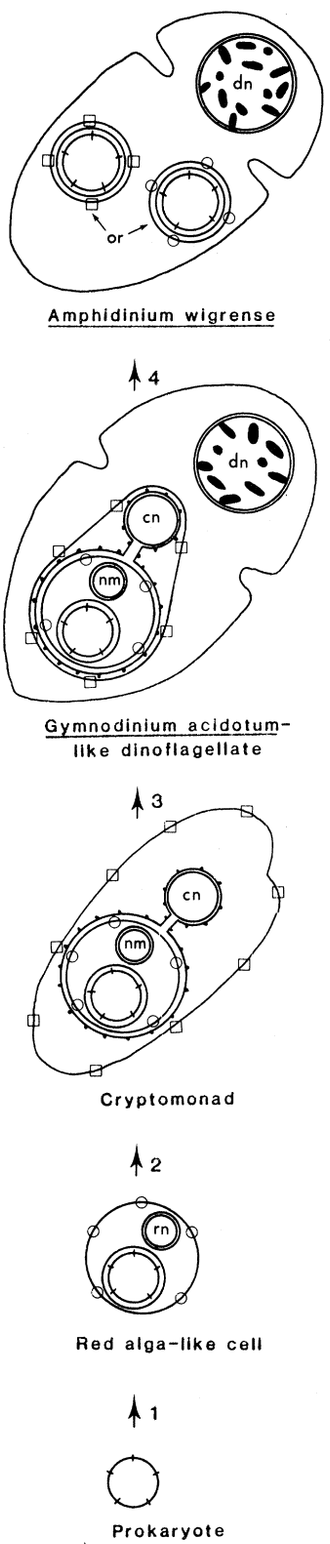


Fig. 4. Possible sequence of events giving rise to the blue-green chloroplast of *A. wigrense*. In agreement with the proposal of Whatley and Whatley (*1*), the outermost membrane of the cryptomonad chloroplast is derived from the endoplasmic reticulum of the cryptomonad, and the next-to-outermost is derived from the plasma membrane of the red alga–like cell. (Step 1) Incorporation into host vacuole. (Step 2) Incorporation into host vacuole; loss of organelles; degeneration of nucleus to nucleomorph. (Step 3) Acquisition of cryptomonad by dinoflagellate; loss of periplast, ejectosomes, sometimes nucleus of endosymbiont; loss of dinoflagellate phagocytic vacuole. (Step 4) Loss of cryptomonad organelles except chloroplast; loss of nucleomorph; proliferation of chloroplasts. Abbreviations: *dn*, dinoflagellate nucleus; *or*, organelles; *cn*, cryptomonad nucleus; *nm*, nucleomorph; *rn*, nucleus of red alga–like cell.

outer pair of membranes surrounding the cryptomonad chloroplast (Fig. 4).

Taylor (*18*) considered the possibility that the chloroplast-mitochondrial complexes of *M. rubrum* may have been able to exist separately from the endosymbiont nucleus because of the presence of a nucleomorph. This appears not to be the case with the blue-green chloroplast of *A. wigrense*, as it lacks a nucleomorph and yet exists as a distinct entity. This could indicate that the cryptomonad nucleomorph may not contain information vital to the chloroplast. Alternatively, in *A. wigrense*, such information may have been transferred, probably to the dinoflagellate nucleus. The absence of both a nucleomorph and a periplastidal compartment in the chloroplast of *A. wigrense* supports the idea that the nucleomorph is necessary for the maintenance of the periplastidal compartment (*7*).

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Evoked Mechanical Responses of Isolated Cochlear Outer Hair Cells

Abstract. *Intracellular current administration evokes rapid, graded, and bidirectional mechanical responses of isolated outer hair cells from the mammalian inner ear. The cells become shorter in response to depolarizing and longer in response to hyperpolarizing currents in the synaptic end of the cell. The cells respond with either an increase or decrease in length to transcellular alternating current stimulation. The direction of the movement with transcellular stimuli appears to be frequency dependent. Iontophoretic application of acetylcholine to the synaptic end of the cell decreases its length. The microarchitecture of the organ of Corti permits length changes of outer hair cells in a manner that could significantly influence the mechanics of the cochlear partition and thereby contribute to the exquisite sensitivity of mammalian hearing.*

Two types of mechanosensory hair cells are found in the mammalian inner ear. Outer hair cells (Fig. 1) differ from inner hair cells in their morphology, afferent and efferent innervation, and biochemistry (*1*). Outer hair cells are required for normal cochlear transduction, but their precise role has been the matter of considerable speculation. Modeling efforts argue that the exquisite tuning and sensitivity of the intact cochlear partition is possible only if a negative damping component (a source of active mechanical energy) is present in the organ of Corti (*2*). Acoustic energy of cochlear origin can be measured in the ear canal, and stimulation of efferent fibers terminating on outer hair cells modulates its intensity (*3*), both of which suggest the presence of an active mechanical process associated with outer hair cells.

We developed procedures to dissociate outer hair cells from the guinea pig organ of Corti by techniques routinely used to isolate solitary vertebrate photoreceptors (*4–6*). Intracellular studies were performed with techniques and equipment identical to those used to study photoreceptors (*5*). Transcellular stimulation was achieved by means of a pair of tungsten electrodes placed in the bathing medium (*4*). Micropipettes filled with 2*M* acetylcholine were used for extracellular iontophoretic applications.

Outer hair cells retained their unique morphological features in vitro (Fig. 1). There was almost a fourfold difference in length between the shortest and longest outer hair cells, a range most likely representing cells originating in the base and apex of the cochlea, respectively (*7*). The passive mechanical properties of outer hair cells differed from those of the other cell types in the organ of Corti. Probing the lateral walls between the nucleus to just below the cuticular plate with micropipettes revealed stiffness and resistance to electrode penetration. The unusual mechanical properties of the lateral membranes may be due to a unique complex of organelles, called the laminated cisterna, located immediately beneath the cytoplasmic membrane in this region of the cell (*8*). Penetration of the cell membrane was achieved most easily in the synaptic region, where the laminated cisterna are not found. Inner hair cell and supporting cell membranes were easily penetrated with the same type of pipette. When a pipette damaged the membrane of a healthy outer hair cell, the cytoplasmic contents were frequently ejected through the resulting hole as if under great pressure. Evidence for a similar cytoplasmic pressure was not observed for inner hair cells or supporting cells.

When current was applied intracellularly (*9*) to the synaptic end of outer hair cells, a conspicuous mechanical response resulted. A 200-msec depolarizing current step resulted in a rapid decrease in cell length and increase in its width. Hyperpolarizing currents evoked the opposite response, an increase in length and a decrease in width. The shape change was not confined to a specific location along the cell but seemed to be distributed over the entire length of the cell between the nucleus and the cuticular plate. The mechanical response was dramatic and easy to detect despite the fact that the absolute differences in cell length were under 1 μm. The magnitude of the response in either direction was graded; at the threshold for visual detection [using ×250 magnification with Hoffman optics (*5*)] the stimulating current was between 100 and 200 pA, with smaller cells requiring lower injection currents. The shape change seemed to be maintained