CHARACTERIZATION OF CYTOPLASMIC AND NUCLEAR GENOMES IN THE COLORLESS ALGA POLYTOMA

I. Ultrastructural Analysis of Organelles

CHI-HUNG SIU, HEWSON SWIFT, and KWEN-SHENG CHIANG

From the Whitman Laboratory and the Department of Biophysics, University of Chicago, Chicago, Illinois 60637. Dr. Siu's present address is the Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California 92037.

ABSTRACT

Electron microscope studies have been made on the fine structure of the colorless biflagellate, Polytona obtusum, with main emphasis on the structural organization of the mitochondria and the leucoplast. Both organelles have been demonstrated to contain DNA aggregates as well as ribosomal particles within their matrix material. Reconstructions from serial sections showed that (a) the mitochondria were highly convoluted and irregular in shape and size, and (b) the leucoplast was a single cup-shaped entity, with large starch grains, localized at the posterior end, and multiple sites of DNA aggregates. The starch-containing compartments appeared to be interconnected by narrow tubular or sheetlike bridges. Cytoplasmic invaginations into the plastid, often containing mitochondria, were of frequent occurrence, and membranes of mitochondria and the leucoplast appeared to be closely apposed. Membranous elements, both sheetlike and vesicular, were also present in the matrix. The Polytona leucoplast was, in certain respects, morphologically similar to the plastids of various photosynthetic mutants of Chlamydomonas, most of which show Mendelian segregation. It is suggested that Polytona arose from a Chlamydomonas-like ancestor, possibly through combined mutational processes of both chloroplast and nuclear genomes. Since Polytona leucoplasts contain both DNA and ribosomal particles, it is probable that these organelles still possess semiautonomy and limited ability for protein synthesis.
brane. Thylakoids and other elements of the photosynthetic apparatus were absent. The leucoplasts were nevertheless thought to have evolved from a typical algal chloroplast. The *Chlamydomonas* chloroplast has a unique morphology, being cup-shaped and containing many stacked thylakoids, whereas other reports (14, 17), as well as our preliminary observations (37), showed the presence of many small leucoplast profiles in *Polytoma*. That there are apparent dissimilarities in the morphology of their plastids has raised questions concerning the assertion that *Polytoma* and *Chlamydomonas* are indeed close relatives. Therefore, a detailed morphological characterization of the leucoplast seemed important for a meaningful evaluation of the evolutionary affinities of *Polytoma* and *Chlamydomonas*.

Chloroplasts and mitochondria have been shown to be semiautonomous organelles which possess an active protein synthesis system as well as a semi-conservatively replicating DNA genome (6, 10; also see 43 for review). In *Chlamydomonas*, the chloroplast contains both DNA filaments (31) and ribosomal particles (34), but Lang (17) failed to detect either filaments or particles inside the leucoplasts of *Polytoma*. Previous studies on *Euglena* showed that irreversibly bleached mutants may lose their complement of the chloroplast genome (8, 27), although whether or not small proplastid-like structures are still present in these mutants remains unclear (10, 22). On the other hand, the presence of DNA filaments and ribonucleoprotein particles has been demonstrated morphologically in the leucoplasts of the colorless cryptomonad *Chilomonas* (36). Consequently, a more careful search for DNA and ribosomes inside the *Polytoma* leucoplast seemed necessary before it could be considered a semiautonomous organelle, possessing its own genetic and protein synthesis apparatus.

In the present investigation, we shall document our findings on (a) the general morphology of *P. obtusum*, (b) the three-dimensional structure and internal morphology of the leucoplast and the mitochondria by means of serial sectioning reconstruction technique, and (c) the presence of both DNA-like filaments and ribosome-like particles (which will hereafter be referred to as DNA and ribosomes, as the presence of specific DNA and RNA components in the organelles have been demonstrated in our subsequent report [39]) inside the leucoplast and mitochondria.

**MATERIALS AND METHODS**

**Strains and Culture Conditions**

The *P. obtusum* strain 1 was kindly supplied by Dr. D. L. Provasoli, Haskins Laboratories, New Haven, Conn. *P. obtusum* was grown in Tris-acetate medium, which contained 0.5 g NH₄Cl, 0.02 g MgSO₄, 0.01 g CaCl₂·2H₂O, 0.144 g K₂HPO₄, 0.072 g K₂HPO₄, 1.33 g Trisma HCl, 0.194 g Trisma Base, 2 g sodium acetate, and 1 ml of trace element solution per liter of distilled deionized water. The trace element solution contained 25 g Na₂EDTA, 11 g ZnSO₄·7H₂O, 5.7 g H₂BO₃, 2.53 g MnCl₂·4H₂O, 2.49 g FeSO₄·7H₂O, 0.305 g CoCl₂·6H₂O, 0.785 g CuSO₄·5H₂O, and 0.55 g (NH₄)₆MoO₄·4H₂O in 500 ml of water and was adjusted to pH 6.5 with 10% KOH. Liquid cultures were grown at room temperature, aerated with filtered air (40).

**Electron Microscopy**

Cells were collected and washed with 0.05 M sodium phosphate buffer (pH 7.2), and then fixed in buffered 1% OsO₄ in the cold for 6 h or overnight. The pellet was washed repeatedly with cold buffer to get rid of excess OsO₄. Cells were dehydrated in the alcohol series and then in propylene oxide. Infiltration was carried out in a mixture of Epon and propylene oxide (1:1, vol/vol) for 2–3 h, followed by several changes of fresh Epon mixture (21). Sections were put on carbon-coated grids. Key-hole grids coated with films cast from 0.4% Formvar were used for serial sections. Sections were stained with 3% aqueous uranyl acetate for 2 h, followed by lead citrate (28), and were scanned in a Siemens Elbinskop la microscope.

**RESULTS**

**General Morphology**

The general arrangement of the cell contents can be seen in Fig. 1. The two flagella were seen to emerge from the anterior end of the cell through two sides of the papilla. Close to the flagellar basal bodies were two contractile vacuoles. The nucleus was centrally situated and was embedded in the convoluted but essentially cup-shaped leucoplast. Golgi bodies were usually seen around the nucleus. Mitochondria occurred mainly in the periphery of the cell. Small, uniformly electron-dense bodies, oval in outline and with no apparent limiting membrane, were also present. They appeared to be closely adjacent to the cell membrane. A schematic drawing of a vegetative cell of *P. obtusum* is shown in Fig. 2.

Cells of *P. obtusum* were generally elongate, with the anterior end slightly tapered toward the papilla. They were 3–5 μm in diameter and 5–10
All electron micrographs show sections of *P. obtusum* cells fixed in 1% OsO₄ in 0.05 M phosphate buffer (pH 7.2), and embedded in Epon. Thin sections were doubly stained with uranyl acetate and lead citrate.

**FIGURE 1** Longitudinal section of *P. obtusum*, showing its general morphology and internal organization. The leucoplast (*) is a single cup-shaped structure bounded by a double membrane and contains large starch granules (S) and also DNA aggregates (arrows). It surrounds a central area containing the nucleus (N), Golgi bodies (G), and the rough endoplasmic reticulum (ER). Mitochondria are largely located peripherally; just beneath the plasma membrane are some electron-dense bodies (D). In the anterior is a contractile vacuole (C); a flagellum is shown to emerge from the side of the papilla. × 32,000.
The cell was covered by a continuous cell wall made up of two electron-dense layers, each of which appeared double in some regions, and which were connected by interdigitating bridges (Fig. 3). A region of amorphous material was present between the cell wall and the plasma membrane. The plasma membrane was a single, somewhat sinuous unit membrane covering the whole cell, and it was continuous with the flagellar membrane. Beneath the basal bodies were two contractile vacuoles, probably formed by the fusion of small vesicles (Figs. 4 and 5). Narrow channels connected vesicles with the central vacuole and apparently drained the liquid into the larger reservoir. Lipid droplets were also present at the anterior half of the cell.

The cytoplasm had a relatively light matrix with numerous ribosomal particles 180–220 Å in diameter. The ribosomes mostly existed individually, but a few were clustered. There were also ribosomes attached to the endoplasmic reticulum as well as the outer membrane of the nuclear envelope. Sometimes, bound ribosomes were seen to be arranged in double rows (Fig. 23). The rough endoplasmic reticulum usually appeared to be in the form of typical flattened cisternae and was occasionally seen to be continuous with the outer membrane of the nuclear envelope (Fig. 7). There also occurred in the perinuclear cytoplasm single, gently curved and apparently rigid cisternae, which contained ribosomes on both outer surfaces (Figs. 1 and 5). Serial sections showed that this membrane system consisted of a few sheetlike structures several microns wide, apparently being a special type of rough endoplasmic reticulum.

The nucleus often appeared to be spherical or ovoid in shape and was located deep in the concavity of the leucoplast (Figs. 1, 28, and 29). The nucleoplasm had an amorphous matrix, and patches of dense fibrils representing typical areas of condensed chromatin were visible either immediately beneath the nuclear envelope or in the nucleoplasm (Figs. 10–12). The nucleus had a single centrally placed spherical nucleolus, consisting of many tightly packed dense granules and finely fibrillar material. The nuclear envelope had the usual double unit membranes transversed by typical nuclear pores (Fig. 7).

Generally, four to five Golgi bodies were located between the nucleus and the leucoplast. They were characterized by typical arrays of elongate, smooth membranes closely stacked together. The ends of the cisternae were often dilated to form vesicles of various sizes, and many similar vesicles were found at the periphery of the membrane system. The Golgi bodies were always closely associated with the rough endoplasmic reticulum. The surface of the endoplasmic reticulum that faced the Golgi apparatus was largely free of ribosomes, and small vesicles apparently blebbed out from this side (Fig. 6). These vesicles apparently fuse with the membrane system of the Golgi apparatus and thus serve as a means of transportation of material from the endoplasmic reticulum to the Golgi bodies for secretion or further transport to other regions of the cell.
Electron-dense bodies were found in the periphery of the cell, usually just beneath the plasma membrane (Figs. 1, 3, and 8). Their shape varied from spherical to ellipsoidal, 0.1-0.5 μm in diameter. They were often seen within evaginations of the cell membrane, or as membrane-bounded bodies apparently pinched off from the cell and lying between the cell membrane and the cell wall. These bodies occasionally contained ribosomes as well as dense material. It seems likely that they contain cell wall material being transported out of the cytoplasm.

**The Mitochondria**

Usually about 20 or more mitochondrial profiles could be seen in any median section of the cell. Most of them were located peripherally, forming a subcortical layer of organelles just beneath the cell membrane; very few were found in the cytoplasm between the nucleus and the leucoplast (Figs. 1, 27, and 28). The mitochondria had an average diameter of 0.3-0.4 μm. The inner mitochondrial membrane was infolded to form a moderate number of cristae, which were arranged in parallel arrays extending at different angles to the longitudinal axis (Figs. 1 and 8-12). The cristae generally had a lamellar structure, and some of them had a length of over 1 μm. The inner membrane enclosed a matrix compartment which had a homogeneous texture and occasionally also a few electron-dense particles 250-300 μm in diameter, probably representing granules of calcium phosphate. In favorable sections, a clumped filamentous component could be observed in the matrix area. Both the morphological characteristics of the filamentous component and its binding with uranyl ions were indicative of the presence of DNA (Fig. 15). These DNA aggregates were invariably present in areas of very low electron opacity within the matrix. Besides, ribosomal particles, 140-170 μm in diameter, were also present sparsely scattered in the matrix (Fig. 17).

Most of the mitochondrial profiles appeared to have a sinuous, elongate shape. Some of them were very long and filamentous, extending the full length of the cell (Fig. 8). Many showed constricted regions, some being so narrow that the matrix compartment was practically obliterated (Figs. 8 and 15). Branched mitochondria also occurred fairly frequently (Figs. 9 and 12). Three-dimensional models constructed from serial sections showed that most of the mitochondrial profiles were interconnected. Consequently, the number of independent mitochondria per cell (between 6 and 12) was much fewer than one would estimate from micrographs of random sections, and substantial variations in shape and size existed among different mitochondria. Three-dimensional models of three individual mitochondria as constructed from serial sections of one cell are presented in Fig. 13. The respective positions of these mitochondria in the cell are diagrammatically shown in Fig. 14. Some mitochondria had very complicated structures with arms extending from a sheetlike central region, which was perforated in places so that areas of cytoplasm passed through the organelle (Fig. 13 a and b), while other mitochondria were small, filamentous structures folded or bent into different shapes in the cytoplasm (Fig. 13 c).

**The Leucoplast**

Although cross sections of *P. obtusum* often revealed many small leucoplast profiles, more cri-
FIGURE 8  Section showing an elongate mitochondrion extending from the anterior end to the posterior end with characteristic narrow constrictions. The leucoplast contains starch grains, vacuole-like structures, as well as DNA clumps in the matrix. It also shows invaginations of the cytoplasm. × 19,000.

FIGURE 9  A branched mitochondrion is shown closely adjacent to the leucoplast. × 41,000.

FIGURES 10–12  Serial section micrographs showing the interconnections between mitochondrial profiles. The mitochondrion is filamentous as well as branching, and is located at the anterior half of the cell. × 31,000.
FIGURE 13  Three-dimensional models of three mitochondria (a, b and c) constructed with dental wax sheets based on serial section micrographs. Relatively thick sections (800-1,000 Å) were used for this purpose. Pictures were taken and printed at the same magnification. The contours of the organelles were traced on dental wax sheets and were then cut and glued together. The mitochondrion in (a) is filamentous but is relatively short and folded. (b) and (c) are two large and convoluted mitochondria with branches and cytoplasmic invaginations. Approx. × 31,000.

FIGURE 14  A schematic drawing of the cell from which the three mitochondria were constructed, showing their relative positions. This cell possessed several additional mitochondria.

tical reconstruction work from serial sections showed that at least in some cases there was only one leucoplast per cell. The leucoplast, like the *Chlamydomonas* chloroplast, was bounded by two unit membranes and had a relatively dense matrix. Many electron-transparent areas were seen in the matrix where clumped DNA filaments were located. Usually, the clumps were barlike with fine filaments extending out from all sides (Figs. 15, 18, and 19). Multiple sites of clumped DNA were
FIGURE 15  Section of mitochondria and leucoplast, showing prominent clumps of DNA filaments. The clumps are located in electron-transparent areas within the matrix. × 27,000. The inserts show some of the DNA aggregates at a higher magnification. × 46,000.

FIGURE 16  Ribosomal particles in the leucoplast. They are very sparse and generally located near membranous structures (arrows). × 63,000.

FIGURE 17  Ribosomal particles in both the leucoplast and the mitochondria (arrows). × 39,000.
found in each leucoplast (Figs. 1 and 15). Reconstructions from serial sections showed that there were about half a dozen separate DNA-containing sites in a single leucoplast (Table I). These DNA aggregates occurred most frequently within the lateral armlike structures of the leucoplast and were rarely seen associated with starch grains (Fig. 1). Ribosomal particles were present sparsely in the matrix. They appeared to be smaller than the cytoplasmic ribosomes, 140-170 Å in diameter as opposed to 170-220 Å. Where they were detected, the ribosomes were usually in the vicinity of some membranous structures in the organelle (Figs. 16 and 17).

Various membranous inclusions were present in the leucoplast, most being either double-membrane, sheetlike structures or vesicles of irregular shape and varying sizes (Figs. 20 and 21). Sometimes these inclusions were located against the side of small starch grains. Large starch granules were present mainly in the posterior half of the leucoplast (Fig. 1), while small ones were formed almost anywhere. Cells in the stationary phase generally had considerably more starch grains than the exponentially growing cells. Starch was apparently the major food reserve in Polytoma, although a few lipid droplets were also present, lying free in the cytoplasm.
FIGURE 20 Section of a leucoplast showing membranous structures. Some are sheetlike, made up of two membrane layers, while others are vesicular. Some of these structures are located next to a small starch grain. × 71,000.

FIGURE 21 Section of a leucoplast showing vesicular membrane structures, which are irregular in shape and size. The leucoplast also has characteristic DNA clumps and invaginations of the cytoplasm. × 47,000.

FIGURE 22 Section through daughter cells, before their emergence from the maternal cell wall. The starch grains in the leucoplasts of the daughter cells are apparently being broken down into small fragments. × 20,000.
A rapid breakdown of the starch grains was seen soon after vegetative cell division, probably to provide energy for the fast-growing daughter cells in early interphase. Large starch grains were apparently broken down from the external surfaces toward the core into small granular fragments which were probably further disintegrated before the biochemical assimilation processes (Fig. 22).

The many leucoplast profiles in the cell were interconnected by narrow tubular or sheet-like bridges of varying diameters (Figs. 23–27). The structures could be as narrow as 0.1 μm (Figs. 24 and 25). A single leucoplast often had many perforations, which were responsible for the discontinuous appearance of the organelle profiles in a given section (Figs. 26, 28, and 29). To confirm that the leucoplast was a single entity in the cell and to demonstrate that it was structurally similar to the Chlamydomonas chloroplast, reconstructions were made of the leucoplast from serial sections of three individual cells. The results indicated that the leucoplast was basically cup-shaped with large and bulky starch-containing regions at the posterior end and a slender sheetlike structure forming a cylindrical partition around the nucleus. A three-dimensional model of a leucoplast and its schematic representation are shown in Fig. 30. Although the structure was highly convoluted, particularly at the posterior half, and perforated in several places, structural continuity among the different parts of the cup-shaped leucoplast was nevertheless clearly evident.

**Mitochondria-Leucoplast Interaction**

Both random and serial sections have revealed an interesting spatial relationship between the leucoplast and the mitochondria. These two organelles were often found to be lying side by side with their contours tightly fitted together (Figs. 31 and 32). Owing to the close physical proximity, the obliquely cut double membranes of the two organelles occasionally appeared as if they were fused (Fig. 32), but outer membranes where clearly evident were always separated by a space of 100–200 Å (Fig. 31). Figs. 33–35 show how a mitochondrion may penetrate into a small concavity of the leucoplast. This intimate mitochondrial-leucoplast spatial relationship may have its functional significance in an efficient reciprocal transport system between these two organelles.

**DISCUSSION**

Our results indicate that the *Polytoma* leucoplast is a single cup-shaped entity, which possesses both a DNA genome and its own ribosomes. In their morphology, the DNA clumps are strikingly similar to the DNA fibers found in bacteria and mitochondria (23, 24, 30). Such clumps are not characteristic of the chromatin of higher organisms. However, exactly similar groups of filaments have been found in other chloroplasts (15, 31, 41) and in the nuclear regions of blue-green algae (32). About half a dozen or more discrete areas of DNA filaments were present within a single organelle. Although the possibility that they were joined by extended fine filaments cannot be excluded, the evidence nevertheless suggests that multiple copies of the genome may exist in a single plastid. This has been verified by our DNA renaturation kinetic studies which indicated the presence of about 50 copies (38). Such studies have also demonstrated that Chlamydomonas chloroplasts contain 40–50 copies of a characteristic genome in vegetative cells (3, 4, 44). The leucoplast also was shown to contain ribosomal particles, suggestive of the presence of a protein-synthesizing system. However, the number of ribosomal particles was very low. This is contrary to the Chlamydomonas case, in which about one-third of the total cellular ribosomes comes from the plastid (13), and some of the proteins of the photosynthetic apparatus are also thought to be made on the chloroplast ribosomes (1, 20). However, the Chlamydomonas ac-20 mutant was similar to *Polytoma* in that ribosomes were very scarce in the plastid when grown mixotrophically (11). Our biochemical studies (39) have also confirmed the fact that *Polytoma* leucoplasts possess rRNA characteristically different in base composition from the rRNA of the cytoplasm.

Although the leucoplast clearly has no photosynthetic capacity and no chlorophyll, some of the plastid functions appear to have been retained. The most obvious roles of the leucoplast would appear to be amylogenesis, starch deposition, and also enzymatic degradation of the starch grains associated with reutilization. Membranous structures within the plastid may represent the remnants of the chloroplast thylakoids. These membranous inclusions resemble those found in the dark-grown y-1 mutant (25) and also the U3A and U3N mutants (12, 45) of Chlamydomonas reinhardtii. Frequently, some of these membranes and ribo
somal particles were observed in the close vicinity of the starch grains, and it is a possibility that such membranes and ribosomes may be involved in the active synthesis of enzymes necessary for amylogenesis (Figs. 17 and 20).

Morphologically, the leucoplast consisted of many bulky starch-containing compartments often interconnected by narrow tubular bridges. The starch contributes a major portion of the leucoplast volume, so this may imply that the leucoplast, when depleted of its starch grains, is simply a cup-shaped structure with a thin layer of matrix material enveloped by double membranes. The membrane boundary appeared to be very irregular and thus probably quite flexible, so that invaginations of cytoplasm and mitochondria could easily occur. As starch grains grow in size, the membranes apparently simply expand, resulting in a convoluted structure with bulky bodies at the posterior end and slender arms on the lateral sides.

Most of the unicellular algae in the order Phytomonadida contain large chloroplasts, and a single cup-shaped plastid is characteristic of the families Chlamydomonadidae and Carteriidae (16). Polytoma, being a biflagellate, which also contains a single cup-shaped plastid, may therefore have evolved from a Chlamydomonas-like ancestor. There is to date no successful induction of aplastidic mutants in organisms such as Chlamydomonas. Perhaps this is related to the role of the plastid in food storage in chloromonads, and implies the presence of a certain interdependence between nuclear and plastid genomes (5). In Euglena, for instance, where paramylon is the major food reserve, the interdependence between the nucleus and the plastid may be less stringent, since paramylon formation in Euglena is outside the chloroplast, so that the enzymes for polysaccharide synthesis are presumably cytoplasmic. This may be related to the finding that bleached mutants lacking plastid DNA can readily be induced by ultraviolet light, heat or streptomycin (see 35 for review). On the other hand, irreversibly bleached mutants of Chlamydomonas such as U3A and U3N possess a degenerate chloroplast but still contain a full complement of the normal plastid DNA and ribosomes (45). Chloroplast mutants such as y, ac-20, U3A and U3N also have degenerate plastids which retain their starch storage function, and, in many respects, resemble the Polytoma leucoplast.

Arnold et al. (2) have shown that Chlamydomonas contains only about a dozen mitochondria per cell. Branchings and constrictions were common in those elongated mitochondria. Polytoma and Chlamydomonas also share the same basic structure of mitochondria. DNA aggregates and ribosomal particles were present in the mitochondria of Polytoma, and they were morphologically similar to those found in Chlamydomonas (31) and other lower eukaryotes (23, 36, 42).

Our observations also indicated that Polytoma and Chlamydomonas possess similar internal organization. Many of their organelles were of almost identical morphology. Eyespot granules, present within the Chlamydomonas chloroplast, have also been found within the leucoplast in some cultures of Polytoma (17), although they were not found in our strain of P. obtusum. Unlike Euglena with its intracellular pellicle (19), both Polytoma and Chlamydomonas have intracellular cell walls, and some species of both genera exhibit similar "crystalline" structure in the cell wall (33). Their flagella and basal bodies were also similar, with bundles of microtubules arising from the basal-body complex to run along the periphery of the cell (18, 29). The leucoplast of Polytoma was cup-shaped and its basic structure had characteristics

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**FIGURE 23** Cross section through the posterior end of the cell. The leucoplast has many starch-containing compartments, which are interconnected by narrow bridgelike tubular structures (arrow). Asterisk shows arrangement of cytoplasmic ribosomal particles in double rows. × 20,000.

**FIGURE 24** One of the connecting tubular structures in Fig. 23 (arrow) shown at a higher magnification. × 79,000.

**FIGURE 25** A higher magnification of the bridgelike connection between two leucoplast profiles (arrow) in Fig. 27. × 74,000.

**FIGURE 26** The fenestration of leucoplast profiles. × 25,000.

**FIGURE 27** Section showing leucoplast profile with interconnecting tubular structures. × 48,000.
FIGURES 28 and 29  Serial sections showing the ring of leucoplast profiles around the nucleus. Perforations of the cytoplasm appear as gaps in some of the sections. Similar regions may show continuity in other sections. × 19,500.
FIGURE 30 Three-dimensional models of the leucoplast constructed from micrographs of serial sections. Relatively thick sections (800–1,000 Å) were used and pictures were taken and printed at the same magnification. The contours of the organelle were traced on transparent acetate sheets and then sandwiched between plexiglass plates. The model was illuminated with a light box from below. The model shows the structural continuity of the leucoplast, which is cup-shaped with bulky starch-containing compartments at the posterior and a slender cylindrical structure on the lateral sides. (c) and (d) are the schematic drawings for the front (a) and the back (b) of the model, respectively.
FIGURE 31  Section showing a mitochondrion closely adjacent to a leucoplast. Their outer membranes are so close to one another that there is little cytoplasm in the space between them. × 50,000.

FIGURE 32  Section showing mitochondria closely adjacent to the leucoplast. Apparent fusion (arrow) is probably due to the oblique sectioning of their limiting membrane. × 25,000.

FIGURES 33–35  Serial section micrographs showing a mitochondrion fitting tightly into a concavity of the leucoplast. × 30,000.
that were also found in the degenerate plastids of a few Chlamydomonas chloroplast mutants. Therefore, the overall morphological similarities tend to support the close phylogenetic affinities between Polytoma and Chlamydomonas, and favor the hypothesis that Polytoma has evolved from a Chlamydomonas-like organism by a step-wise mutational mechanism, apparently initiating the loss of some, but not all, of the ability for protein synthesis in the change from autotrophic to heterotrophic nutrition. To the extent that it is still dependent on a leucoplast for its carbon metabolism (BG-19338). K. S. C. acknowledges a U.S. Public Health Service Research Career Development Award.

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