NUCLEAR ENVELOPE–CHLOROPLAST RELATIONSHIPS IN ALGAE

SARAH P. GIBBS, Ph.D.

From the Biological Laboratories, Harvard University, Cambridge, Massachusetts

ABSTRACT

In *Ochromonas danica* and two related species (Chrysophyceae) and in *Rhodomonas lens* and *Cryptomonas* sp. (Cryptophyceae), the chloroplast is surrounded by an outer double-membraned envelope which lies outside the usual double-membraned chloroplast envelope. At the borders of the area where the chloroplast lies adjacent to the nucleus, this outer envelope is continuous with the outer membrane of the nuclear envelope as a double-membraned outfolding, so that the entire chloroplast in these species lies within a double-membraned sac, one wall of which is the nuclear envelope. In *Olisthodiscus* sp. (Chrysophyceae ?), each of the small peripheral chloroplasts is surrounded by a similar double-membraned outer envelope, but in this species no connections with the nuclear envelope were observed. In the Ochromonadaceae, a characteristic array of tubules is present within the sac in the narrow space which separates the chloroplast from the nucleus. In the other species studied, tubules are present at places between the chloroplast envelope and the outer envelope. In the Cryptophyceae, the starch grains lie outside the chloroplast envelope, but within the outer double-membraned sac. A double-membraned outer envelope appears to be present outside the chloroplasts of the Phaeophyta and Euglenophyta, but seems to be absent in the other groups of algae.

In the course of an investigation of the ultrastructure of the chloroplasts of a variety of algae (6), a unique relationship between the nuclear envelope and the chloroplast was observed. In several species, an outfolding of the outer membrane of the nuclear envelope surrounds the single chloroplast as a double-membraned outer envelope. This paper illustrates the structure of this outer envelope in species belonging to the Chrysophyceae and Crytophyceae and reports preliminary observations on its distribution in the other groups of algae.

MATERIALS AND METHODS

The species studied included four Chrysophytes, *Ochromonas danica* [Pringsheim], *O. malhamensis* [Pringsheim], *Poteriochromonas stipitata* [Lewin, New Haven, Connecticut], and *Olisthodiscus* sp. [Conover, Milford, Connecticut], two Cryptomonads, *Rhodomonas lens* [Lasker, Gulf Stream] and *Cryptomonas* sp. [Guillard, Milford, Connecticut]; and one brown alga, *Pylaiella littoralis*. The isolator and, when known, the place of isolation of the particular strain used are given in brackets. The three species of Ochromonadaceae were grown at 26°C in the media described by Aaronson and Baker (1) either in the dark or under fluorescent lights (*ca.* 450 ft-c). *Olisthodiscus* and the two Cryptomonads were grown at 15°C in Guillard's medium "f-1" (11) under incandescent lamps (*ca.* 350 ft-c). *Pylaiella littoralis* was collected at Sandwich, Massachusetts.

All species were fixed in a cold 1 per cent solution of osmium tetroxide buffered to pH 7.3 to 7.9 for times varying between 2 and 11½ hours. The three species of Ochromonadaceae were fixed in the osmium solution previously used for *Euglena gracilis* (5); the other species are all marine and were fixed in a sea water–osmium solution (6). After fixation, the cells were dehydrated in a graded ethanol series and embedded in 60 per cent *n*-butyl, 40 per cent ethyl methacrylate, catalyzed with 1.5 per cent Luperco CDB. Sections were cut with a glass knife on a Porter-Blum microtome, collected on collodion-coated copper screens, and stained with potassium permanganate following the method of Lawn (15). Sections were examined with an RCA EMU-2D electron microscope.

RESULTS

Ochromonas danica

This is a small unicellular alga belonging to the Chrysophyceae. Light-grown cells possess a large bilobed chloroplast, whose two major lobes are connected anteriorly by a bridge which surrounds the nucleus on three sides. Dark-grown cells have a very reduced chlorophyll content and contain a single small U-shaped proplastid which hugs the nucleus. The micrographs used here to demonstrate the outer chloroplast envelope in this species are of cells from dark-grown cultures which had been placed in the light for varying lengths of time in order to study pigment synthesis and the concomitant growth of the chloroplast (7). Fig. 1 is a section through a developing chloroplast and part of the adjacent nucleus of a cell from a culture which had been exposed to light for 18 hours. It can be seen that the nucleus is separated from the chloroplast by a narrow space (usually 400 to 500 A wide) which contains a number of circular profiles. These circular profiles represent sections of membrane-limited tubules (Figs. 2 and 3), which measure approximately 250 to 350 A in diameter and have been seen in longitudinal sections to extend to up to 0.45 μ. In some sections, tubules are not present in the space between the nucleus and the chloroplast, and here the distance between the nuclear envelope and the chloroplast envelope is less than 100 A. It can also be seen clearly in Fig. 1 that, although the single membranes of the nuclear and chloroplast envelopes are about the same thickness (50 to 60 A), the chloroplast envelope is characteristically much narrower (approximately

FIGURE 1

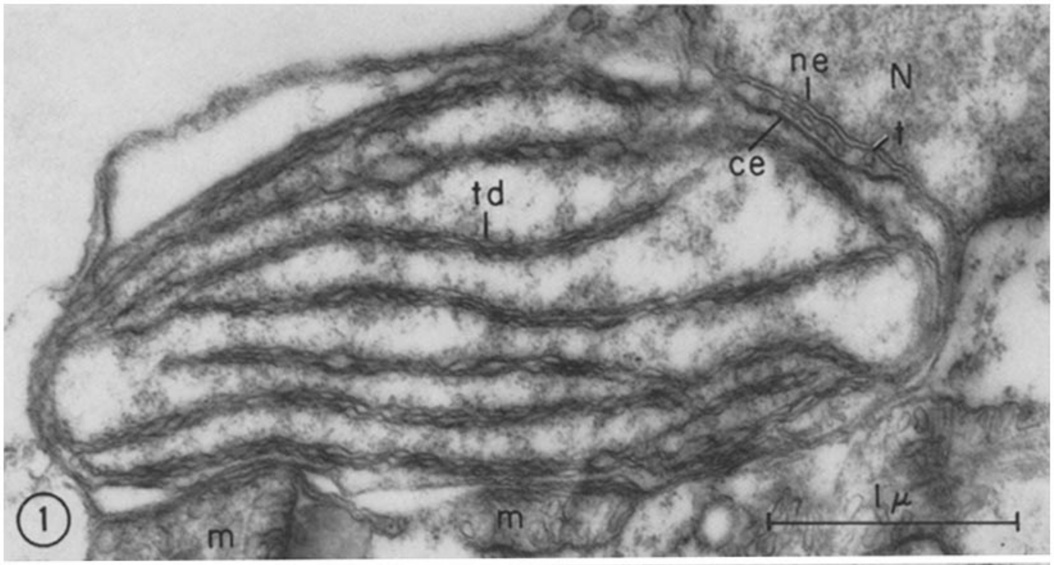
Section through the chloroplast and part of the nucleus (*N*) of *Ochromonas danica*. The tubules (*t*) which lie in the narrow space separating the nuclear envelope (*ne*) from the chloroplast envelope (*ce*) are cut in cross-section. The chloroplast contains a number of three-disc bands (*td*). A number of mitochondria (*m*) are present just outside the outer envelope of the chloroplast. This cell is from a 7-day dark-grown culture which had been exposed to light for 18 hours. Its chlorophyll *a* content was approximately 15 per cent that of a light-grown cell. × 33,000.

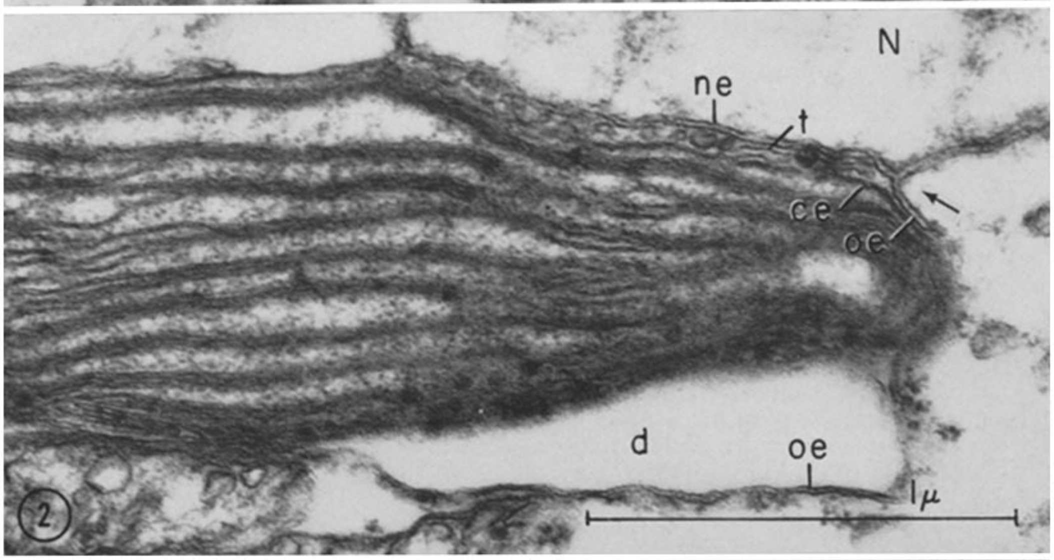
FIGURE 2

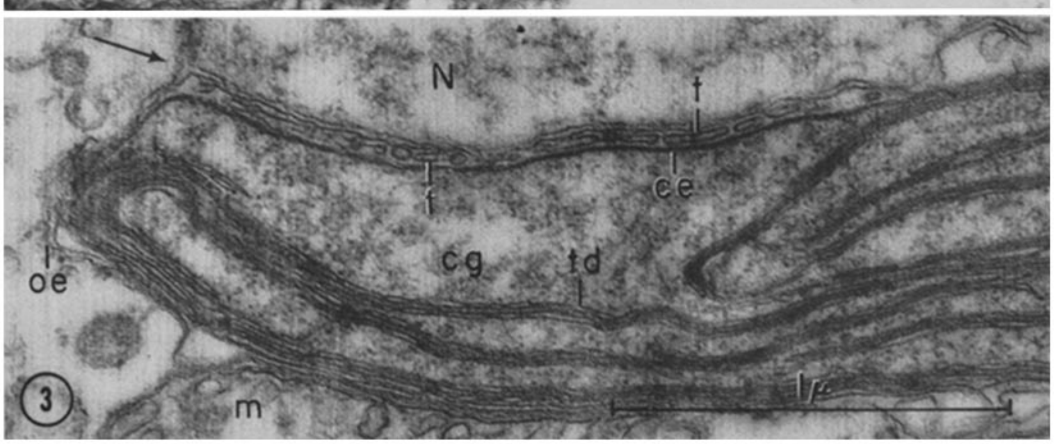
Section through part of the chloroplast and nucleus (*N*) of *Ochromonas danica*. At the arrow the outer membrane of the nuclear envelope (*ne*) outfolds to form a double-membraned outer envelope (*oe*) which extends around the chloroplast. One of the tubules (*t*) in the space between the nuclear envelope (*ne*) and the chloroplast envelope (*ce*) is sectioned longitudinally. The area of low density (*d*) lying between the outer envelope (*oe*) and the chloroplast in the lower part of the micrograph probably represents a dissolved-out leucosin granule. This cell is from an 8-day dark-grown culture which had been exposed to light for 40 hours. Its chlorophyll *a* content was approximately 40 per cent that of a light-grown cell. × 57,000.

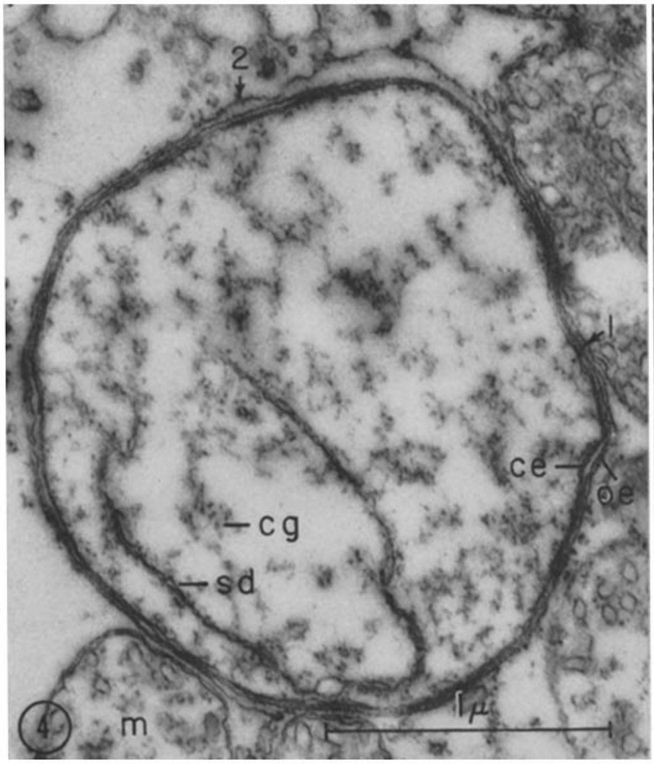
FIGURE 3

Section through part of the chloroplast and nucleus (*N*) of *Ochromonas danica*. Some of the tubules (*t*) which lie between the nuclear envelope and chloroplast envelope (*ce*) are cut longitudinally. At the arrow the outer membrane of the nuclear envelope outfolds to form the outer envelope (*oe*) of the chloroplast. Present in the chloroplast are numerous chloroplast granules (*cg*) and a number of three-disc bands (*td*). An elongated mitochondrion (*m*) lies just outside the outer envelope of the chloroplast. This cell is from a 7-day dark-grown culture which had been exposed to light for 2 days. Its chlorophyll *a* content was approximately 50 per cent that of a light-grown cell. × 53,000.


1
ne
N
t
ce
td
m
m
1 μ


2
N
ne
t
ce
oe
d
oe
1 μ


3
N
t
t
ce
oe
cg
td
m
1 μ



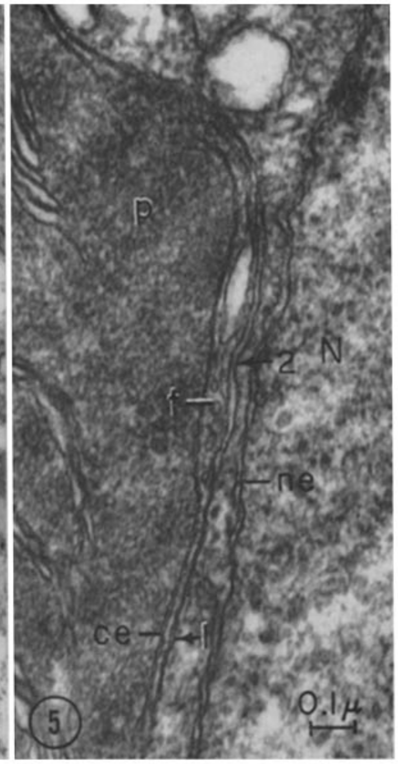


FIGURE 4

Proplastid of *Ochromonas danica*. In this section the outer double-membraned envelope (*oe*) can be observed to completely surround the proplastid. At arrow 1, the outer envelope is as narrow as the adjacent chloroplast envelope (*ce*); at arrow 2, it is considerably wider. The proplastid contains a few single discs (*sd*) and a number of small dense granules (*cg*). The double membrane of an adjacent mitochondrion (*m*) lies close to, but not appressed to, the outer envelope of the proplastid. This cell is from a 7-day dark-grown culture which had been exposed to light for 1 hour. Its chlorophyll *a* content was only 1.4 per cent that of a light-grown cell. × 38,000.

FIGURE 5

Section through part of the nucleus (*N*) and the pyrenoid (*p*) of the neighboring chloroplast of *Olisthodiscus* sp. A double-membraned outer envelope is present outside the chloroplast envelope (*ce*). At arrow 1, the outer envelope is as narrow as the adjacent chloroplast envelope (*ce*). At arrow 2, it is wider and resembles the nuclear envelope (*ne*). Where the outer envelope is wide, a row of tubules (*t*) separates it from the chloroplast envelope. × 54,000.

130 to 150 A) than the nuclear envelope (approximately 200 to 220 A). Pores are present in the region of the nuclear envelope which borders on the cytoplasm, but are absent in the region which lies adjacent to the chloroplast.

In Fig. 2 (arrow) it can be seen that at the border of the area where the nucleus and chloroplast are closely associated, the outer membrane of the nuclear envelope outfolds to form a double-membraned envelope which extends around the chloroplast. This envelope lies outside the double-membraned chloroplast envelope (Fig. 2, *ce*) and is designated the outer envelope of the chloroplast. It is not possible to trace the outer envelope all the way around the chloroplast figured in Fig. 2, but it is possible to follow the outer envelope around the entire circumference of the small developing chloroplast sectioned in Fig. 4. This is a micrograph of a cell from a dark-grown culture which had been in the light for 1 hour and still contained only a very small amount of chlorophyll. It can be seen that the chloroplast envelope is characteristically narrow throughout, but that the outer envelope at places may be as narrow as the chloroplast envelope (Fig. 4, arrow 1) and at others considerably swollen (Fig. 4, arrow 2). Because of the obliquity of a section, it is difficult to trace the outer envelope around the whole circumference of a fully developed chloroplast of a light-grown cell, but wherever the plane of section is perpendicular to the chloroplast envelope and the underlying bands of discs, the outer envelope is always observed. It appears therefore that the entire chloroplast in *Ochromonas danica* is enclosed within a double-membraned sac, one wall of which is the nuclear envelope proper. These relationships are diagrammed in Fig. 6.

Frequently the outer envelope does not lie close to the chloroplast envelope, but is separated from it by a space of low electron opacity (Fig. 2, *d*). Possibly this space in the living cell was filled with leucosin, the storage carbohydrate of the Chrysophyceae, which has been dissolved out in fixing and embedding the cells. Leucosin ultimately accumulates in this species in a large membrane-enclosed granule which fills much of the posterior half of the cell (Fig. 6, *leu*).

The two close relatives of *Ochromonas danica* which were studied, *O. malhamensis* and *Poteriochromonas stipitata*, have a similar outfolding of the outer nuclear membrane which envelops the single chloroplast. Tubules are also present in the narrow space between the chloroplast and the nucleus.

Olisthodiscus sp.

This small flagellate was described by Carter (3) who tentatively assigned it to the Xanthophyceae. However, Yenstch (personal communication) has found that this stock contains chlorophyll *c*, a pigment not found in the Xanthophyceae, but which is present in many members of the Chrysophyceae (4, 14). The cells of *Olisthodiscus* possess a large central nucleus surrounded by six to twelve small peripheral chloroplasts (see reference 8, Fig. 4). Each of these chloroplasts is completely enclosed within an outer double-membraned envelope. Around most of the chloroplast this outer envelope lies about 100 A outside the chloroplast envelope and has the same aspect as the chloroplast envelope (Fig. 5, arrow 1). Here both the chloroplast envelope and outer envelope consist of two 50 to 60 A membranes lying so close together that the total width of the envelope is only 120 to 150 A. However, over part of the surface of the pyrenoid, which in this alga forms a small projection on the surface of each chloroplast facing the nucleus, the outer envelope bulges out slightly over a row of membrane-limited tubules (Fig. 5, *t*). These tubules may be only 250 A in diameter or at places moderately swollen. Where it encloses these tubules, the outer envelope becomes 200 to 300 A wide and has the aspect of a typical nuclear envelope (Fig. 5, arrow 2). However, in this species, unlike *Ochromonas danica*, the outer envelope of the chloroplast has never been seen to be connected with the outer membrane of the nuclear envelope.

Rhodomonas lens

This is a small flagellate belonging to the Cryptophyceae. The cell in Fig. 7 has been sectioned close to the median longitudinal plane; the anterior end of the cell is uppermost, the dorsal and ventral surfaces towards the left and right, respectively. The cell contains a posterior nucleus and a large chloroplast whose dorsal and ventral lobes are connected to each other by a bridge of dense pyrenoid matrix material (Fig. 7, *p*). Large starch grains are present both surrounding the pyrenoid and lying beside the ventral lobe of the chloroplast. In each case the starch grains lie outside the chloroplast envelope, but are separated from the cytoplasm by a double membrane (best seen at arrow 2). This double-membraned envelope is an outfolding of the outer membrane of the nuclear envelope (arrow 1, Fig. 7; enlarged in Fig. 8). Since the outer envelope is always observed when the plane of section is perpendicular to it, it appears that the whole chloroplast in this species, as in *Ochromonas danica*, is enclosed within a double-membraned outfolding of the outer membrane of the nuclear envelope. It can be seen in Fig. 7 that in this species the nucleus lies adjacent to the pyrenoid region of the chloroplast and is separated from it by starch grains, rather than the tubules present in *Ochromonas*.

The individual membranes of the outer envelope are always approximately 50 to 60 A wide, but the distance between the two may vary considerably. Where the outer envelope encloses a starch grain, it is moderately wide (about 200 to 300 A) and resembles the nuclear envelope (Fig. 7, arrow 2, and Fig. 8, *oe*). Where it passes over the narrow

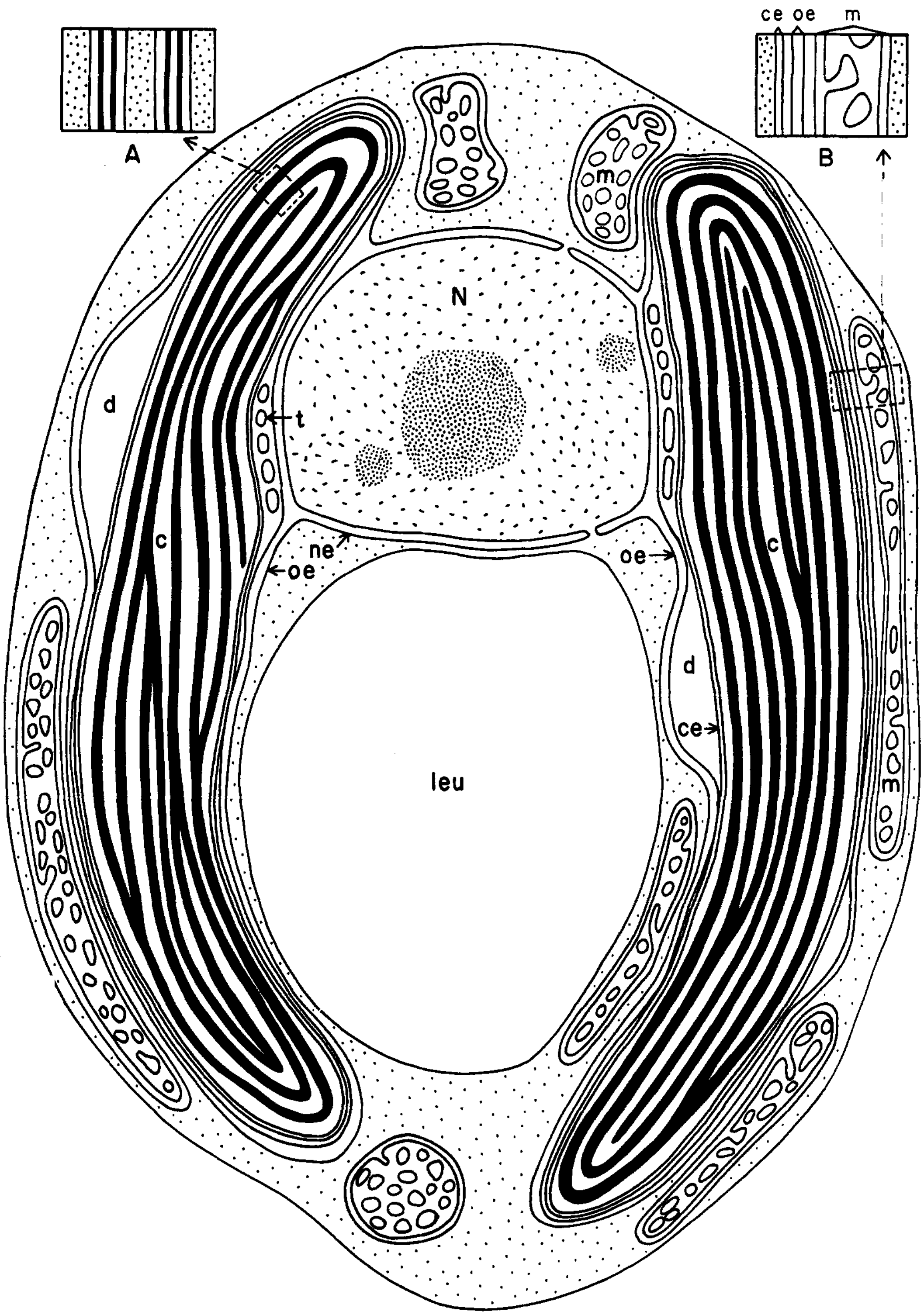


Figure 6

cytoplasmic groove which lies on the interior face of the pyrenoid (8), the outer envelope becomes as narrow (approximately 130 to 150 A) as the adjacent double membrane of the chloroplast (Fig. 9). Around the rest of the circumference of the chloroplast it may do two things. Very frequently the two membranes of the outer envelope separate and the inner one approaches the chloroplast envelope (Fig. 11, arrows). At these places the chloroplast appears to be limited by a thick membrane, which actually consists of the two membranes of the chloroplast envelope and the inner one of the outer envelope, and an outer thin membrane, which is the outer membrane of the outer envelope (see also Fig. 7, arrow 3). At other places along the circumference of the chloroplast, the two membranes of the outer envelope run together as a moderately wide envelope as they do outside the starch grains. At these places, the outer envelope may lie close to the chloroplast envelope (Fig. 10, arrow 1) or separate from it to enclose an area containing dense granules, which resemble the ribosomes of the cytoplasm, and an occasional membrane-limited tubule (Fig. 10, arrow 2).

The other cryptomonad studied, *Cryptomonas* sp., also has a large bilobed chloroplast which is enclosed in a double-membraned outfolding of the outer membrane of the nuclear envelope. Since this outer envelope appears similar to that of *Rhodomonas lens* in all respects, it is not illustrated here.

Pylaiella littoralis

This is a filamentous brown alga (Phaeophyta) whose cells contain a number of small lens-shaped chloroplasts. Each of these chloroplasts appears to be enclosed by a double-membraned outer envelope, but the preservation of this material was not adequate to determine this with certainty. However, in some sections, a distinct double membrane could be seen outside the narrow chloroplast envelope, and several times a row of circular profiles was present between the two envelopes.

DISCUSSION

An outer envelope has been previously noted by Manton and Leedale in two members of the Chrysophyceae, *Chrysochromulina ericina* (17) and *C. minor* (18). They believe, however, that in these species the cytoplasmic boundary of the perinuclear space (*i.e.*, the outer membrane of the nuclear envelope) disappears in the region where the nucleus abuts on the chloroplast or pyrenoid and that a common cytoplasmic boundary covers both the nucleus and the plastid. According to their interpretation, the outer membrane of the nuclear envelope potentially encloses the nucleus and chloroplast in a common sac. I believe that higher resolution micrographs of these species would show that the outer membrane which extends around the chloroplast is in actuality a double-membraned outfolding of the outer membrane of the nuclear envelope, as has been illustrated here for *Ochromonas danica* and *Rhodomonas lens*, and that the interior of the nucleus is always separated from the chloroplast by a double-membraned nuclear envelope.

In another species of Chrysophyceae, *Chromulina psammobia*, it can be clearly seen in a micrograph of Rouiller and Fauré-Fremiet (reference 20, Fig. 9) that both the chloroplast envelope and the double-membraned nuclear envelope are present

FIGURE 6

Diagram of a median longitudinal section of a light-grown cell of *Ochromonas danica*. The outer envelope (*oe*) of the chloroplast is shown to extend completely around the single chloroplast (*c*), as hypothesized in the text, and to be continuous with the outer membrane of the nuclear envelope (*ne*) as illustrated in Figs. 2 and 3. Other cell structures shown are the nucleus (*N*), mitochondria (*m*), the large posterior leucosin granule (*leu*), and the tubules (*t*) which lie in the narrow space between the chloroplast and the nucleus. The areas of low density (*d*) which are present between the outer envelope and the chloroplast envelope may represent dissolved-out leucosin. *A*. Detail of the chloroplast bands. Each band shown as a wide line in the main figure consists of three closely appressed discs (6). *B*. Enlargement of area where a mitochondrion lies adjacent to the chloroplast. The chloroplast envelope (*ce*), outer envelope (*oe*), and the limiting double membrane of the mitochondrion lie close to each other, but do not fuse.


tr
2
c
s
p
m
1
2
2
N
s
c
etr
3
m
7
1 μ

in the area where the nucleus and the chloroplast lie closely appressed and that, at the place where they separate, the outer membrane of the nuclear envelope outfolds to form a double-membraned envelope which can be traced around the chloroplast for a short distance. This micrograph also shows that in the region where the nucleus and the chloroplast lie against each other, the two membranes of the nuclear envelope come to lie as close together as those of the chloroplast envelope and at places appear as a single thick membrane. This is distinctly different from what happens in *Ochromonas danica* (Fig. 1), but may explain why Manton and Leedale believe that the outer nuclear membrane disappears in the region where the nucleus abuts on the chloroplast in *Chrysochromulina*.

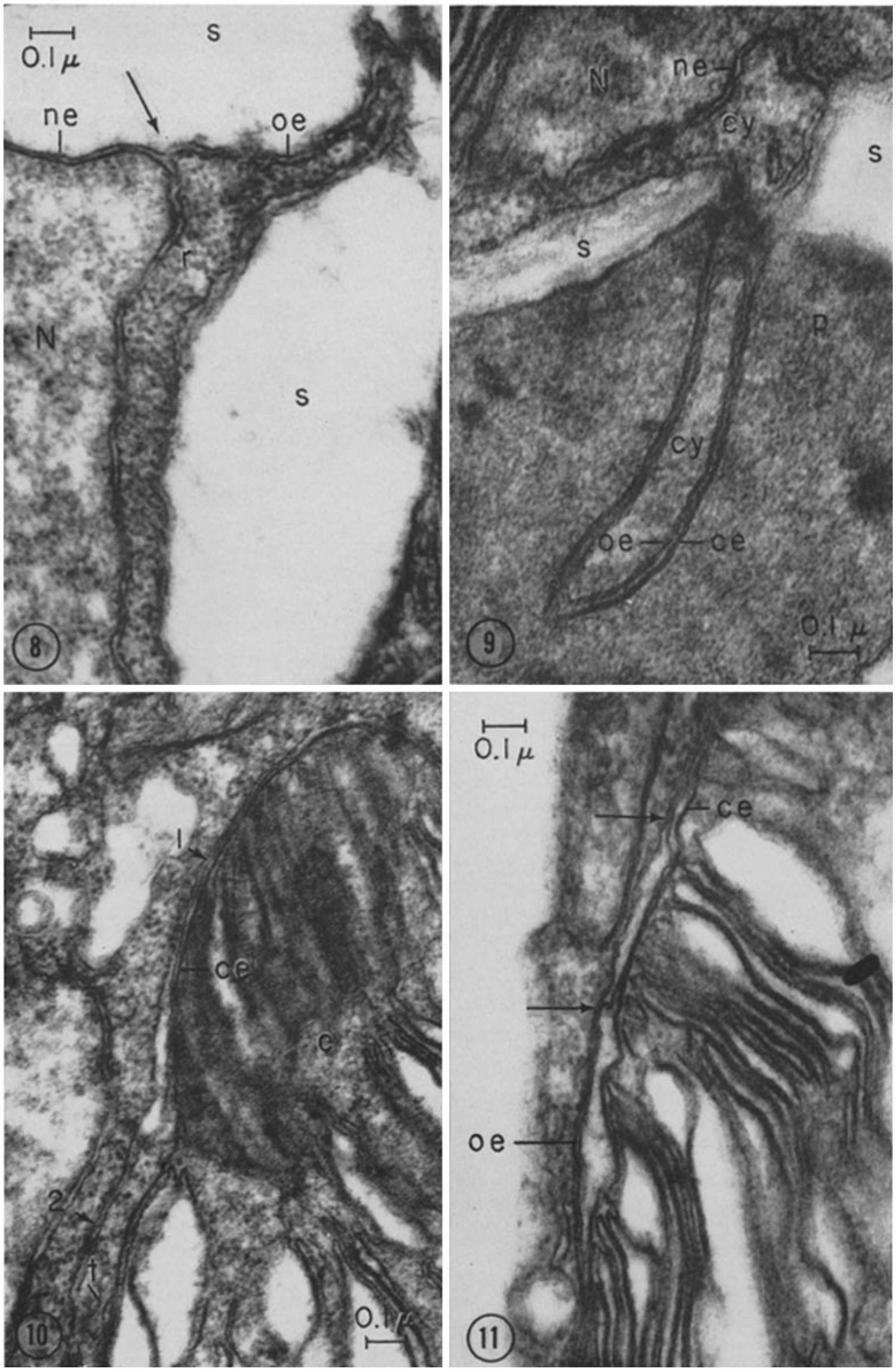
Hovasse and Joyon have observed that in another species of the Chrysophyceae, *Hydrurus foetidus*, profiles of smooth endoplasmic reticulum are present in the narrow space between the chloroplast and the nucleus, and their micrograph (reference 13, plate 9) suggests that the same nuclear envelope–chloroplast relationships may exist in this species as in *Ochromonas danica.* It appears probable, therefore, that in five genera of the Chrysophyceae, namely *Ochromonas*, *Poteriochromonas*, *Chromulina*, *Hydrurus*, and *Chrysochromulina*, the chloroplast is surrounded, in part, if not entirely, by an outer envelope which is continuous with the outer membrane of the nuclear envelope. The fact that the chloroplasts of *Olisthodiscus* are also enclosed within a double-membraned sac lends support to the evidence given by the presence of chlorophyll *c* that this flagellate should be assigned to the Chrysophyceae rather than the Xanthophyceae. The electron microscope studies of *Vaucheria* (9, 10, 12) suggest that an outer chloroplast envelope is not present in the Xanthophyceae, but more studies should be made to establish this with certainty.

A few comments can be made about the presence or absence of an outer chloroplast envelope in the other groups of algae. In Manton's (16) micrographs of the zoospore of the brown alga, *Scytosiphon lomentarius*, it can be clearly seen (Figs. 5, 6, 7, and 8) that an extension of the outer membrane of the nuclear envelope at least partly encircles the chloroplast. Whether or not this extension of the outer nuclear membrane is a double-membraned outfolding cannot be determined, but Manton's micrographs added to the present observations on *Pylaiella* indicate that the chloroplasts of the Phaeophyta possess an outer envelope, which in some species at least is continuous with the nuclear envelope. The author has previously shown (reference 5, Fig. 6) that the chloroplasts of *Euglena gracilis* (Euglenophyta) are limited by a double membrane inside of which there is a single, or sometimes double, membrane. In light of the present studies, the inner membrane, which only at places can be resolved into two membranes, should be interpreted as the chloroplast envelope and the outer distinctly double membrane as an outer envelope. In Heitz's micrograph of *Euglena* sp. (reference 12, Fig. 5), it can be seen that the chloroplast is limited by an inner denser membrane (the chloroplast envelope) and an outer double membrane. Siegesmund, Rosen, and Gawlik (21) have also noted that the chloroplasts of *Euglena gracilis* are enclosed by a triple membrane, but they see no evidence that the innermost membrane is double.

On the basis of existing studies, it can be con-

FIGURE 7

Longitudinal section of a cell of *Rhodomonas lens*. The anterior end of the cell is towards the top of the page; the dorsal and ventral surfaces towards the left and right respectively. The dorsal and ventral lobes of the chloroplast (*c*) are connected by a pyrenoid-containing bridge (*p*) which passes anterior to the nucleus (*N*). Mitochondria (*m*) and trichocysts (*tr*) are present in the peripheral cytoplasm. A number of trichocysts have apparently been discharged, for they appear empty (*etr*). Arrow 1 indicates where the outer membrane of the nuclear envelope outfolds to form a double-membraned outer envelope which completely encloses the chloroplast and its surrounding starch grains (*s*). Where the outer envelope passes over the starch grains it is moderately wide and resembles the nuclear envelope (arrow 2). At arrow 3, the inner membrane of the outer envelope lies tightly appressed to the chloroplast envelope whereas the outer membrane has lifted slightly away. × 30,000.


0.1 μ
s
ne
oe
r
N
s
8
N
ne
cy
s
s
p
cy
oe
ce
0.1 μ
9
I
ce
c
2
t
0.1 μ
10
0.1 μ
ce
oe
11

cluded that the chloroplasts of red algae (Rhodophyta) and green algae (Chlorophyta) do not possess an additional envelope outside the double-membraned chloroplast envelope. This judgment is based on Bouck's (2) excellent micrographs of the red alga, *Lomentaria baileyana*, and personal observations on a variety of species of red and green algae (6). In addition, the author (6) did not observe an outer envelope outside the chloroplasts of diatoms (Bacillariophyceae, Chrysophyta) and dinoflagellates (Dinophyceae, Pyrrophyta), but this conclusion should be regarded as provisional, awaiting higher resolution micrographs. An outer envelope has not been observed in any of the many electron microscope studies of the chloroplasts of higher plants.

The outfolding of the outer nuclear membrane which encloses the chloroplasts of a number of algae can be interpreted as a sac of endoplasmic reticulum. It is well established that the outer membrane of the nuclear envelope is often continuous with elements of the endoplasmic reticulum (19, 22, 23). However, the relationship observed here of a cell organelle's (in this case, the chloroplast) being enclosed within a sac of endoplasmic reticulum, one wall of which is the nuclear envelope, has not been observed in other cells. In view of this distinctive relationship, it seemed best to give this structure a separate name, "outer envelope of the chloroplast," to distinguish it from the general endoplasmic reticulum of the cytoplasm, which in these species consists almost entirely of small vesicles (personal observation). The function of this nuclear envelope–connected sac is unknown, but it is easy to conceive of its playing a role in nuclear-chloroplast interactions, since material may pass directly between the nucleus and chloroplast without entering the cytoplasm. It is probably also functionally significant that the starch grains in the Cryptophyceae are deposited in the space between the chloroplast envelope and the outer envelope.

I am grateful to Dr. R. R. L. Guillard, Dr. S. H. Hutner, and Dr. H. D. Isenberg for providing cultures of the organisms studied. This study was made while the author was a National Science Foundation Predoctoral Fellow.

Received for publication, March 12, 1962.

References on next page.

FIGURE 8

Enlargement of part of Fig. 7. At the arrow, the outer membrane of the nuclear envelope (*ne*) outfolds to form the outer envelope (*oe*) which encloses the chloroplast and its surrounding starch grains (*s*). *N*, nucleus; *r*, ribosomes. × 54,000.

FIGURE 9

Cross-section through the pyrenoid (*p*) of *Rhodomonas lens* where it is bisected by a groove of cytoplasm (*cy*). The outer envelope (*oe*) where it lines this groove is as narrow as the chloroplast envelope (*ce*). *N*, nucleus; *ne*, nuclear envelope; *s*, starch grain. × 77,000.

FIGURE 10

Section through part of a chloroplast (*c*) of *Rhodomonas lens*. At arrow 1, the double-membraned outer envelope lies close to the chloroplast envelope (*ce*); at arrow 2, it is separated from the chloroplast envelope by an area containing dense granules and an apparent tubule (*t*). × 52,000.

FIGURE 11

Section through part of a chloroplast of *Rhodomonas lens*. At the arrows the two membranes of the outer envelope (*oe*) separate and the inner one lies appressed to the chloroplast envelope (*ce*). × 65,000.

REFERENCES

1. Aaronson, S., and Baker, H., A comparative biochemical study of two species of *Ochromonas*, *J. Protozool.*, 1959, **6**, 282.
2. Bouck, G. B., Chromatophore development, pits, and other fine structure in the red alga, *Lomentaria baileyana* (Harv.) Farlow, *J. Cell Biol.*, 1962, **12**, 553.
3. Carter, N., New or interesting algae from brackish water, *Arch. Protistenk.*, 1937, **90**, 1.
4. Dougherty, E. C., and Allen, M. B., Is pigmentation a clue to protistan phylogeny?, *in* Comparative Biochemistry of Photoreactive Systems (M. B. Allen, editor), New York, Academic Press, Inc., 1960, 129.
5. Gibbs, S. P., The fine structure of *Euglena gracilis* with special reference to the chloroplasts and pyrenoids, *J. Ultrastruct. Research*, 1960, **4**, 127.
6. Gibbs, S. P., The ultrastructure of the chloroplasts of algae, *J. Ultrastruct. Research*, in press.
7. Gibbs, S. P., Chloroplast development in *Ochromonas danica*, *J. Cell Biol.*, in press.
8. Gibbs, S. P., The ultrastructure of the pyrenoids of algae, exclusive of the green algae, *J. Ultrastruct. Research*, in press.
9. Greenwood, A. D., Observations on the structure of the zoospores of *Vaucheria*, II, *J. Exp. Bot.*, 1959, **10**, 55.
10. Greenwood, A. D., Manton, I., and Clarke, B., Observations on the structure of the zoospores of *Vaucheria*, *J. Exp. Bot.*, 1957, **8**, 71.
11. Guillard, R. R. L., and Ryther, J. H., Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran., *Canad. J. Microbiol.*, 1962, **8**, 229.
12. Heitz, E., Das lamellare Dünn-Dick-Muster der Chloroplasten von *Chlamydomonas, Euglena, Fucus, Vaucheria*, *Z. Zellforsch. u. mikr. Anat.*, 1961, **53**, 444.
13. Hovasse, R., and Joyon, L., Contribution à l'étude de la Chrysomonadine *Hydrurus foetidus*, *Rev. Algologique*, 1960, **5**, 66.
14. Jeffrey, S. W., Paper-chromatographic separation of chlorophylls and carotenoids from marine algae, *Biochem. J.*, 1961, **80**, 336.
15. Lawn, A. M., The use of potassium permanganate as an electron-dense stain for sections of tissue embedded in epoxy resin, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 197.
16. Manton, I., Observations with the electron microscope on the internal structure of the zoospore of a brown alga, *J. Exp. Bot.*, 1957, **8**, 294.
17. Manton, I., and Leedale, G. F., Further observations on the fine structure of *Chrysochromulina ericina* Parke & Manton, *J. Marine Biol. Assoc. United Kingdom*, 1961, **41**, 145.
18. Manton, I., and Leedale, G. F., Further observations on the fine structure of *Chrysochromulina minor* and *C. kappa* with special reference to the pyrenoids, *J. Marine Biol. Assoc. United Kingdom*, 1961, **41**, 519.
19. Porter, K. R., and Machado, R. D., Studies on the endoplasmic reticulum. IV. Its form and distribution during mitosis in cells of onion root tip, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 167.
20. Rouiller, C., and Fauré-Fremiet, E., Structure fine d'un flagellé Chrysomonadien: *Chromulina psammobia*, *Exp. Cell Research*, 1958, **14**, 47.
21. Siegesmund, K. A., Rosen, W. G., and Gawlik, S. R., Effects of darkness and of streptomycin on the fine structure of *Euglena gracilis*, *Am. J. Bot.*, 1962, **49**, 137.
22. Watson, M. L., The nuclear envelope. Its structure and relation to cytoplasmic membranes, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 257.
23. Whaley, W. G., Mollenhauer, H. H., and Leech, J. H., Some observations on the nuclear envelope, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 233.