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A Dinoflagellate with Both a Mesocaryotic and a Eucaryotic Nucleus

I. Fine Structure of the Nuclei

J. D. Dodge

Department of Botany, Birkbeck College, University of London, England

With 13 Figures

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Summary

Glenodinium foliaceum Stein (*Pyrrophyta*) has a single mesocaryotic nucleus which contains numerous typically dinoflagellate chromosomes and one or more nucleoli with a structure similar to that of nucleoli in higher organisms. In addition this organism possesses another nucleus-like organelle which is here termed the eucaryotic nucleus. This is a polymorphic body which varies in shape from ovoid to a branched filamentous form. As with the mesocaryotic nucleus it is surrounded by a perforated envelope. The organelle contains granular material and usually several nucleoli which again appear to have the typical form of nucleoli. No other dinoflagellate is known in which two nuclei of differing types are found. The function and significance of the presence of the two nuclei is discussed.

1. Introduction

For many years it has been known that dinoflagellates possess unusual nuclei, one characteristic of which is that the chromosomes can be stained and observed at all stages of the mitotic cycle. With the advent of electron microscopy further unique features became apparent (see review in: Dodge 1971) especially the fact that the chromosomes consist of only DNA fibrils which are arranged in a complex manner. This type of nucleus was named the mesocaryotic type (Dodge 1965, 1966) to distinguish it from eucaryotic and procaryotic nuclei with each of which it has some similarities and some differences. Recently Zingmark (1970) has pointed out that in some dinoflagellates such as *Noctiluca* the nucleus does pass through a phase in which the chromosomes are not "visible". Such nuclei he describes as nocticaryotic to distinguish them from the normal dinocaryotic condition which is found in most dinoflagellates.

In the course of a survey of fine structure in the *Dinophyceae* some 40 taxa have recently been examined by electron microscopy (for lists see: Dodge and Crawford 1970, 1971). In all of these organisms, except one, each cell possesses a single mesocaryotic nucleus whose size and shape varies from species to species. In the one known exception to this rule, *Glenodinium foliaceum* Stein, a single ovoid mesocaryotic nucleus is present but, in addition, there is another structure which also looks like a nucleus and which stains with nuclear stains. The problem at this point is what to call this organelle. As will become evident later in the paper its fine structure is very similar to that of the eucaryotic nucleus of a higher organism. So, in spite of its often bizarre form and its strange association with a cell which already has a mesocaryotic nucleus, this other nucleus-like organelle will be referred to as the eucaryotic nucleus for the present. The object of this paper is to describe and compare the fine structure of the two nuclei.

2. Material and Methods

The isolate of *Glenodinium foliaceum* used for this study was obtained from Dr. K. Gold (New York) who had isolated it in the vicinity of Puerto Rico. Electron microscopical methods were as already described (Dodge and Crawford 1969) and consisted of standard glutaraldehyde-osmic acid fixation and araldite embedding. For light microscopy, material was concentrated by centrifugation, fixed in methanol and stained with acetocarmine, azur B or Feulgen. Temporary preparations were examined and photographed using a Vickers M 41 photomicroscope.

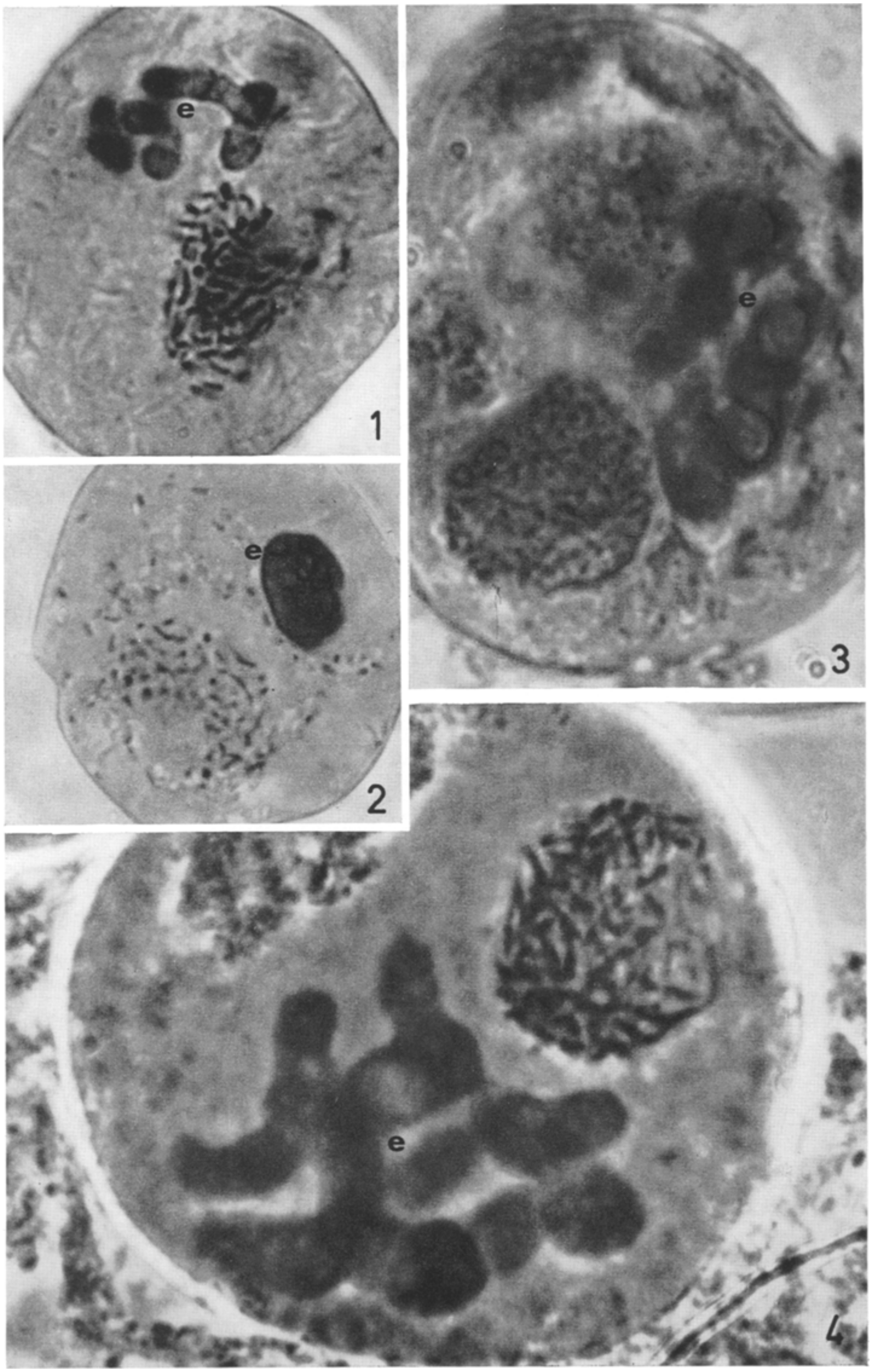
3. Observations

3.1. The General Organization of the Cell

The structure of the *Glenodinium foliaceum* cell as seen in the light microscope (Fig. 1) has been described by several authors (Biecheler 1952, Prager 1963, Silva 1962). With the electron microscope we find (Fig. 5) that the cell contains a large number of chloroplasts which are mainly arranged radially. Each chloroplast contains a simple pyrenoid. On the ventral side of the cell, adjacent to the flagellar bases, are situated the eyespot and associated lamellar body (Dodge and Crawford 1969). The mesocaryotic nucleus is situated just below the centre of the cell and a little to one side. The other nucleus is almost always found in the anterior half of the cell and often is adjacent to the mesocaryotic nucleus. The remainder of the cytoplasm (see Fig. 5) consists of Golgi bodies, mitochondria, trichocysts, pusules, flagellar-hair vesicles, ribosomes, and peripheral vesicles containing occasional crystals.

3.2. The Mesocaryotic Nucleus

This is approximately 12 μm long by 10 μm wide. It contains a large number of chromosomes which can be clearly seen in the light microscope after



Figs. 1–4. Light micrographs of *Glenodinium foliaceum* cells. Each cell contains a mesocaryotic nucleus in which chromosomes can be seen and a eucaryotic nucleus (*e*). Figs. 1 and 2, aceto carmine, ×2,000. Fig. 3, Feulgen stain, ×3,000. Fig. 4, aceto carmine, ×3,000

Fig. 5. A longitudinal section through a *G. foliaceum* cell showing the mesocaryotic (*m*) and eucaryotic (*e*) nuclei, numerous chloroplasts (*c*) with pyrenoids, a fibrous vesicle (*f*) part of the pusule (*p*), and mitochondria, golgi, ribosomes etc. ×8,400. All electron micrographs are of material fixed in glutaraldehyde + osmic acid and stained with uranyl acetate and lead citrate

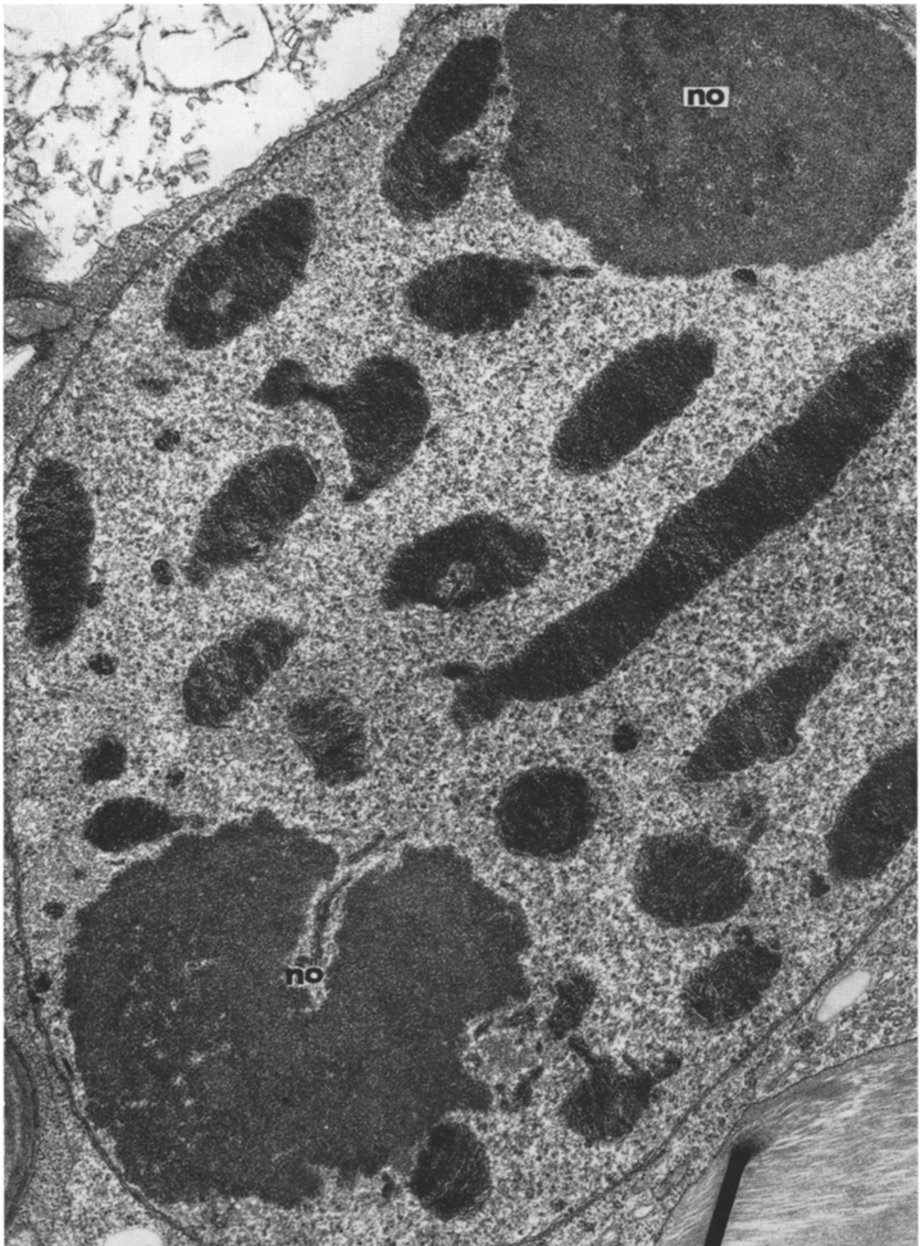


Fig. 6. A more highly magnified section of the mesocaryotic nucleus which shows two nucleoli (*no*) and chromosomes cut in various planes. ×21,000

staining with aceto-carmine or Feulgen (Figs. 1–4). With the electron microscope the chromosomes are cut at random so it is usually impossible to determine their lengths but one which was sectioned completely longitudinally was over 5.6 μm long. Chromosomes appear to have rather varied forms (Figs. 5–6) and thicknesses but the average width is abut 450 nm. As is usual in dinoflagellates the chromosomes consist of compactly arranged fibrils which are probably entirely composed of DNA. The arrangement of the fibrils gives the chromosomes a somewhat banded appearance when seen in longitudinal section. In transverse section some of the chromosomes may show a central lighter zone (Fig. 5) which appears to consist of nuclear matrix. This may be similar to the supposed chromosome axes which have been reported from some other dinoflagellates (Soyer 1967). In *Glenodinium* there is much space between the chromosomes and this is occupied (Fig. 6) by a granular nuclear matrix which clearly consists of at least two sizes of granules, large grains 20–30 nm diameter which look like clustered ribosomes and small particles of about 5 nm diameter which are particularly abundant around the nucleoli. Small chromosome spurs, or threads, of about 100 nm thickness are frequently seen (Fig. 6).

Each nucleus contains at least one nucleolus. These are dense bodies (Figs. 6–8) about 2 μm in diameter and they mainly consist of a close aggregation of 10–20 nm particles which are similar in size to the granular component of angiosperm nucleoli (Lafontaine and Chouinard 1963). Small fibrous patches can be seen in the nucleoli (Fig. 7) and occasionally chromosomes appear to be partly embedded in the nucleolar matrix (Fig. 8). These chromosomes, which are always surrounded by a fibrillar zone, appear similar to the nucleolar organizing chromosomes of higher plant nucleoli (Hyde 1967).

The nucleus is surrounded by a two membrane envelope which is perforated by numerous nuclear pores (Figs. 6 and 7). The envelope does not appear to be connected to any of the other membrane systems of the cell and in this differs from what is usually found in higher organisms.

Little information is available about nuclear division in *G. foliaceum* as yet, but such observations as have been made do suggest that the process is similar to that reported from various other dinoflagellates (Leadbeater and Dodge 1967, Kubai and Ris 1969). During mitosis the nucleus is penetrated by a number of cytoplasmic tunnels which contain microtubules (Fig. 9). There is a suggestion that for the anaphase separation some of the chromatids may be attached to the tunnels in depressions of the bounding envelope. Two such

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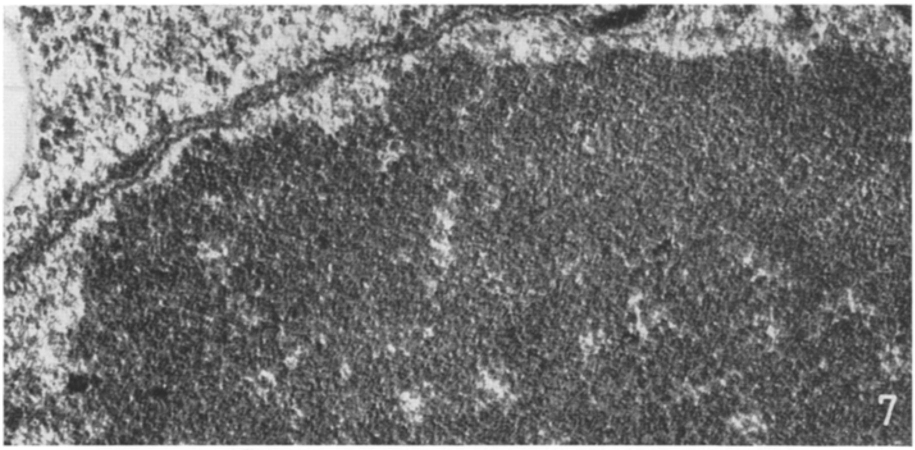
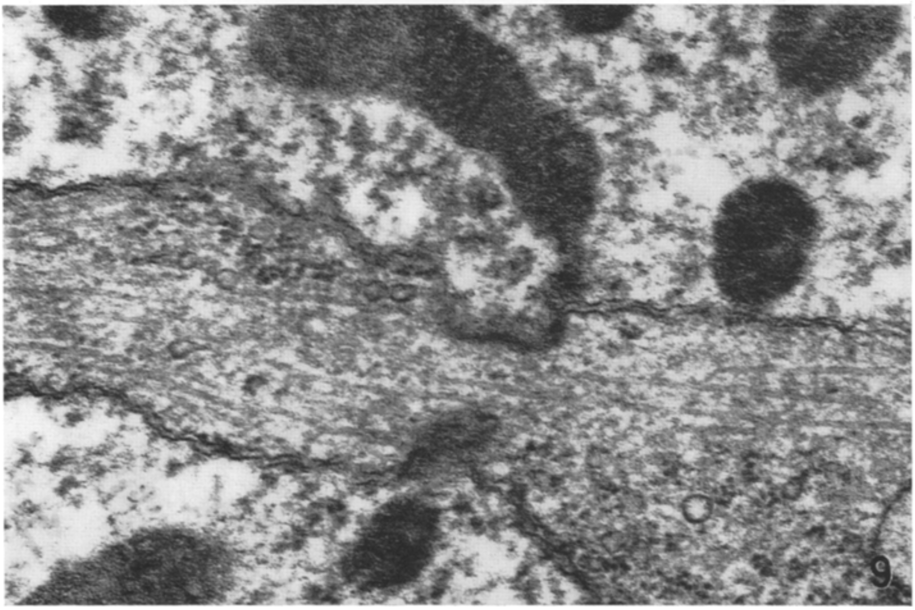
Figs. 7 and 8. Two portions of nucleolus to show the granular contents and in Fig. 8 a portion of chromosome which is embedded in the nucleolus. ×78,400

Fig. 9. This shows a small part of a dividing nucleus with a tunnel containing microtubules running across the picture. Two chromatids appear to be attached to depressions in the surface of the tunnel. ×45,800





Figs. 7–9

depressions can clearly be seen in Fig. 9. Other chromatids may be attached to the peripheral nuclear envelope.

3.3. The Eucaryotic Nucleus

Every cell of the isolate which has been studied appears to contain one nucleus-like body in addition to the mesocaryotic nucleus described above. With the light microscope this nucleus is seen to have a very varied form. Sometimes (Fig. 2), it is an entire ovoid body but more frequently it is polymorphic and may be a twisted filiform structure (Fig. 1) or it is perhaps branched (Fig. 4). It stains very densely with aceto-carmine (Figs. 1, 2, and 4) and also with Feulgen (Fig. 4) and azur B. It clearly contains much DNA.

With the electron microscope we find that this nucleus is bounded by an envelope consisting of two membranes with typical nuclear pores (Figs. 10 and 12). In glancing sections of the envelope (Fig. 13) the pores show the central dark core, light ring and dark periphery which has been described from nuclear pores in other organisms (ROBERTS and NORTHCOTE 1970). The pores appear to be partly arranged in hexagonal arrays (Fig. 13, arrow) and partly at random, although this latter effect may be due to the plane of the section. Often this nucleus is partly surrounded by a cytoplasmic band of varying thickness which contains evenly dispersed granules which look like ribosomes (Fig. 11). This suggests that ribosomes are being produced in the eucaryotic nucleus and are passing out into the cell. A single membrane can be seen separating this zone from the normal cell cytoplasm. However, no wall or distinctive sheath surrounds this eucaryotic nucleus and no connections have been observed between the nuclear envelope and the cell endoplasmic reticulum.

Internally, this nucleus consists mainly of granular material having various densities (Figs. 5, 10, 11, and 12) and looks rather similar to a conventional interphase nucleus. The dark-staining granules which tend to be aggregated into small clumps (Fig. 10) probably consist of heterochromatin. As in many micro-organisms this chromatin appears to be much more randomly dispersed than, for example, is the case in animal cells where dense aggregations are usually seen adjacent to the nuclear envelope in interphase cells. Several nucleoli are present and these are rather varied both in appearance and size. Some are entirely dense, but close examination may reveal (Fig. 12) that the central portion is composed of tightly packed small granules and the periphery is made up of less closely packed larger granules of about 15 nm diameter. Other nucleoli (Fig. 10) have very much less dense centres which contain what looks like the smaller components of the nuclear matrix. Such nucleoli have also been seen in higher plants (HYDE 1967). No obvious chromosomes have been observed either in the nuclei or associated with the

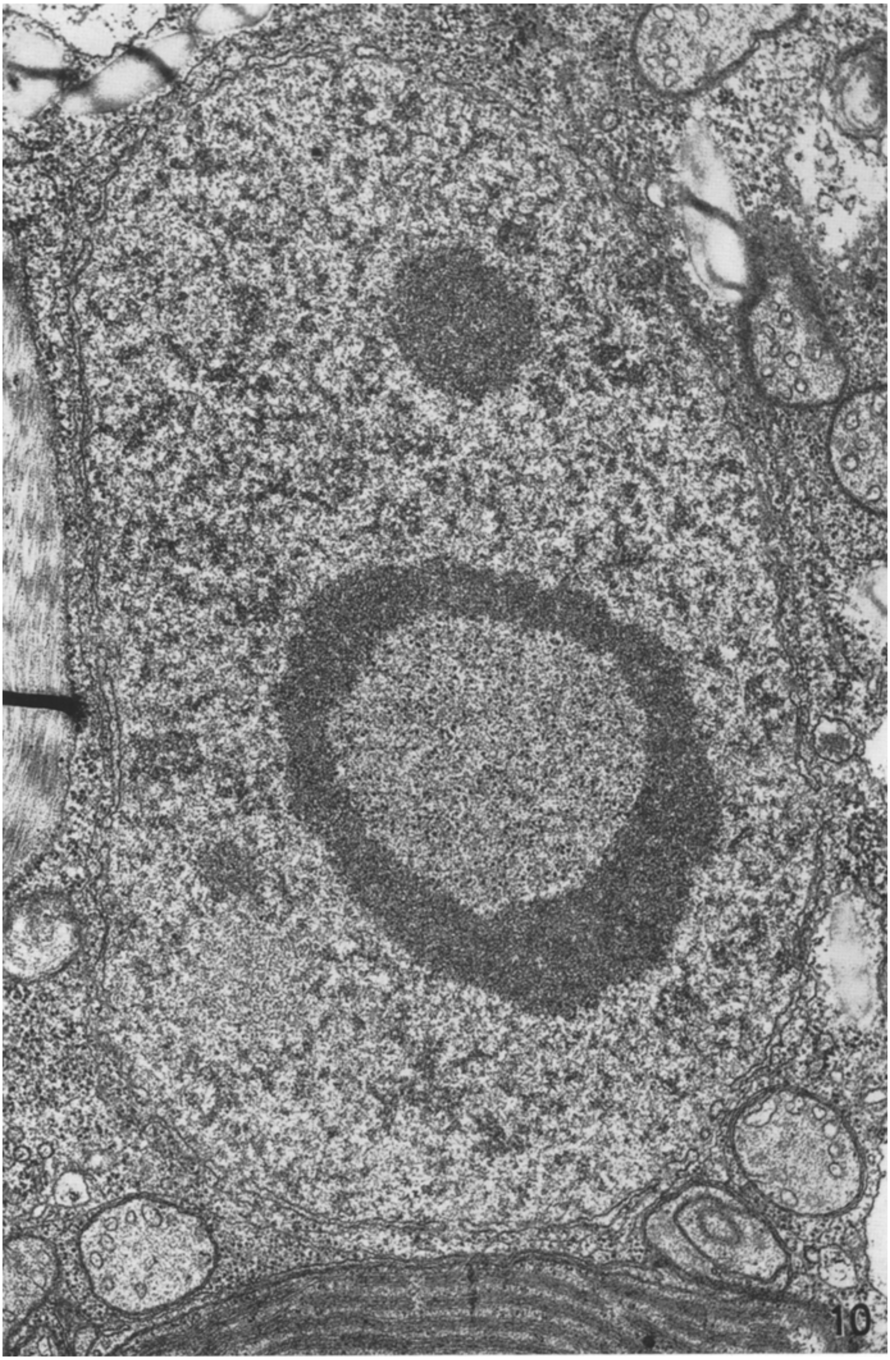


Fig. 10. A section through part of a eucaryotic nucleus of *G. foliaceum*. Note the granular contents and the profiles of two nucleoli. ×28,000

nucleoli. No observations have been made, as yet, on what happens to these structures when the mesocaryotic nucleus and the cell are in division.

4. Discussion

Glenodinium foliaceum is clearly a rather unusual dinoflagellate. In the first place it has had a chequered taxonomic history. First put in the genus *Glenodinium* by STEIN nearly 100 years ago, it has subsequently been placed by various authors in the genera *Kryptoperidinium* (LINDEMANN 1924, LEBOUR 1925), *Phyllodinium* (CONRAD 1926), and *Peridinium* (BIECHELER 1952) whilst most recent authors (SILVA 1962, PRAGER 1963, DODGE and CRAWFORD 1969) have preferred to return it to the original genus.

The second unusual feature is the eyespot and associated lamellar body, which has recently been described (DODGE and CRAWFORD 1969). Although some other dinoflagellates do have eyespots, those examined by electron microscopy have all been different in structure and less complex than that of *Glenodinium* and no other member of the group or any other alga has yet been found to also possess the lamellar body, which appears to be some sort of photoreceptor.

The third notable feature is the additional nucleus described in the present paper. Again, this appears to be a unique structure with no known parallel in any other alga. It would seem not to be confined to the particular isolate of *Glenodinium foliaceum* used for the present study as it appears to be present in some photographs of SILVA (Plate 3, Figs. 5 and 6, 1962) who worked with isolates from Portugal. It was not noted by PRAGER (1963) in a strain from New York but perhaps he did not try the effect of nuclear stains. The nuclear envelope of *G. foliaceum* was recently investigated by freeze-etching (WECKE and GIESBRECHT 1970). It is an open question which nucleus those authors actually examined but they reported that in contrast to what is found in some other dinoflagellates the nuclear pores show random distribution. As the present work shows the pores in the eucaryotic nucleus to be at least partly regularly arranged, it would seem likely that these authors were looking at the surface of the mesocaryotic nucleus.

The question now arises as to what is the status and function of the eucaryotic nucleus in *Glenodinium foliaceum*. If it were a parasite or symbiont one

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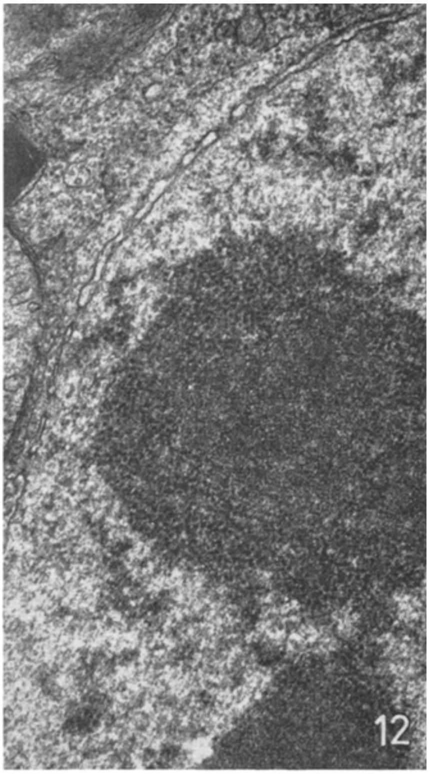
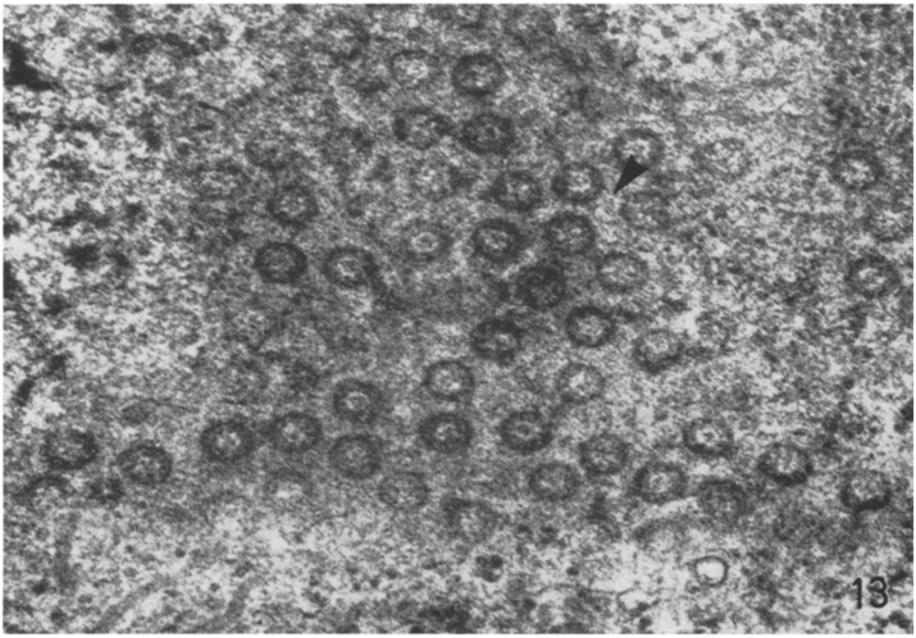
Fig. 11. Part of a eucaryotic nucleus which is partly surrounded by a granular zone (*g*). Note the single membrane which surrounds this zone. ×24,500

Fig. 12. Section of part of another eucaryotic nucleus to show at higher magnification the granular nucleolus and the perforate nuclear envelope. ×34,300

Fig. 13. A glancing section through the nuclear envelope of a eucaryotic nucleus showing the structure and arrangement of the nuclear pores. In places the arrangement is hexagonal (arrowhead). ×67,500





Figs. 11–13

would at least expect to find a sheath or entire membrane separating the extraneous organism from the host. Parasites are not normally enclosed only by a perforated envelope. Another suggestion is that it is a stage in the nuclear cycle of a special sexual nucleus. Although the shape of the eucaryotic nucleus is very different from the other one, its size is roughly comparable. The two nuclei appear to have rather similar envelopes and nucleoli. They differ only in that one has "visible" chromosomes and the other does not. However, in lacking condensed chromosomes it could be said that the eucaryotic nucleus is like the vegetative nucleus of *Noctiluca* (ZINGMARK 1970). SOYER (1970) has recently shown that when this starts to divide to give rise to gametes the chromosomes become more and more condensed with each successive division. It is possible that some such process happens here.

In one group of organisms, the *Ciliata*, each cell normally possesses a macronucleus and several micronuclei. The two types of nucleus are quite distinct and appear to perform different functions (DU PRAW 1970). The macronucleus is very large, it divides amitotically, contains very many nucleoli and is surrounded by an annulate envelope. It can regenerate from small fragments and during sexual reproduction it may break down completely only to be reformed later by the conversion of a micronucleus. By way of contrast the micronuclei are very small and they divide mitotically. They contain no nucleoli, probably synthesize no RNA and the envelopes are imperforate. Their sole function appears to be to participate in sexual reproduction. As noted above, the two *Glenodinium* nuclei are similar in several respects and there would therefore seem to be no analogy with the situation found in ciliates.

Thus, at present it is not possible to say with any degree of certainty what is the function of this organelle nor can we speculate on its origin. It remains to be discovered how *Glenodinium* functions with what appear to be two perfectly good nuclei of different types. Future work will be directed to an examination of the behaviour of the eucaryotic nucleus during division in the hope that this will shed some light on its function and affinities.

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Author's address: Dr. J. D. DODGE, Department of Botany, Birkbeck College, Malet Street, London, WC 1 E 7 HX, U.K.