ULTRASTRUCTURE OF MITOSIS IN THE CHLOROMONADOPHYCEAN ALGA VACUOLARIA VIRESCENS

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SUMMARY
During preprophase in the chloromonadophycean alga *Vacuolaria virescens* microtubules are present around the flagellar basal bodies and extend over the anterior surface of the nucleus. These microtubules assist in the separation of the flagella and later enter the nucleus through polar gaps. During prophase the nucleoli begin to disperse and the chromosomes become condensed. At metaphase the nucleus assumes an elliptical shape and an equatorial plate of chromosomes becomes aligned across the long axis of the nucleus; kinetochores are recognizable on some of the chromosomes. The nuclear envelope remains intact over most of the surface and in places it forms folds. During anaphase chromosomes are less distinct and vesicles are present in the elongating nucleus. Most of the new nuclear envelope around the progeny nuclei is formed by coalescence of these membrane vesicles during late anaphase and telophase, although some of the original nuclear envelope may also become incorporated. During telophase disintegration of the original nuclear envelope becomes pronounced and portions of this structure are recognizable in the cytoplasm after completion of mitosis.

It is suggested that this unusual type of nuclear envelope behaviour may be important in ensuring the segregation of the Golgi apparatus and contractile vacuole to progeny cells. Interphase cells contain a single extensive Golgi apparatus which is located between the anterior surface of the nucleus and the contractile vacuole. The Golgi apparatus and contractile vacuole act as an osmoregulatory system and their presence is presumably essential to the existence of the organism. Formation of a new contractile vacuole and division of the Golgi apparatus occur early in mitosis and thereafter a Golgi apparatus and contractile vacuole become associated with each of the poles of the nucleus. They retain this location throughout mitosis and during cytokinesis, with the result that an osmoregulatory system is present in each of the daughter cells. In a similar manner, microbody-like organelles are associated with the nuclear envelope during mitosis but not at interphase. Growth of the nuclear envelope during mitosis may serve as the means of partitioning these organelles to the progeny cells. Thus mitosis in *Vacuolaria virescens* is responsible not only for the equal segregation of the genetic material but also for the correct distribution of some of the cytoplasmic components.

INTRODUCTION
During the last decade there have been many electron-microscope studies of mitosis in the algae. The wealth of ultrastructural information obtained has been reviewed on a number of occasions, for example, by Dodge (1973), Heath (1974), Kubai (1975) and Pickett-Heaps (1975). There now exist detailed descriptions of mitotic behaviour in at least one representative of almost all classes of algae. A conspicuous exception is the class Chloromonadophyceae (sometimes known by its
alternative name, the Raphidophyceae). Although the structure of the kinetochore has been described (Heywood & Godward, 1972), there are no other published electron micrographs of mitosis in these organisms. In this paper I describe the ultrastructure of mitosis in one member of this class, *Vacuolaria virescens* Cienkowsky. An abstract describing preliminary results of these observations has been published elsewhere (Heywood, 1973a) and there exist several light-microscope accounts of this process (Dangeard, 1939; Heywood & Godward, 1972, 1973, 1974; Poisson & Hollande, 1943; Mingot, 1967; Spencer, 1971).

**MATERIALS AND METHODS**

Cultures of *Vacuolaria virescens* Cienkowsky (Cambridge Culture Collection LB 1195/1) were grown at 23 ± 1 °C on defined medium (Heywood, 1973b) and were aerated with a gas mixture of 4 % CO₂ in air. Cells were illuminated with a cycle of 8 h dark alternating with 16 h light of intensity 180 ft.-c. (1-94 x 10⁴ lux) supplied by Ecko daylight fluorescent tubes. This growth regime resulted in partial synchronization of the culture which reached a peak of approximately 7 % of the cells in mitosis at 1-3 h after the onset of the dark period (Heywood, 1968). Cells harvested at this time were fixed for 1 h at room temperature in 3 % glutaraldehyde buffered at pH 7-0 with sodium cacodylate buffer. After being thoroughly washed in buffer, cells were postfixed for 2 h in 1 % osmium tetroxide, washed in buffer, dehydrated in an ethanol series (including staining with uranyl acetate at the absolute ethanol stage), and embedded in Epon. This procedure gave excellent fixation of most cell components, but the flagellar root system and the subunit structure of the contractile vacuole membrane were not adequately preserved. The cell in Fig. 16 (p. 48) was fixed for 6 h at 0-2 °C in 1 % chrome-osmium fixative (Dalton, 1955), dehydrated in ethanol, and embedded in Epon; this procedure gave a more intense staining of most cell components but failed to preserve some structures, for example, spindle microtubules and the subunit structure of the contractile vacuole membrane. Silver sections of Epon-embedded material were cut with a diamond knife and mounted on copper grids coated with Formvar and carbon. Sections were stained with aqueous uranyl acetate and lead citrate, and examined using a Philips 201S electron microscope.

For light microscopy sections of thickness 1-4 μm were cut with glass knives, mounted on glass slides, and examined with Nomarski differential interference microscopy using a Zeiss photomicroscope. Cells were photographed on Kodak Panatomic X film at × 330 magnification; negatives were printed at × 1320 magnification (Figs. 1-4).

**OBSERVATIONS**

The following brief description will summarize the main features of cell structure in *Vacuolaria virescens* (for additional details see accounts by Heywood, 1972, 1977a; Mingot, 1967; Poisson & Hollande, 1943; Spencer, 1971). *V. virescens* is a naked unicell of length 30-80 μm and breadth 23-55 μm. Motile organisms are longer than broad, whereas palmelloid individuals (a non-motile condition in which the cell is ensheathed by a layer of mucilage) are approximately spherical (Fig. 1). Mitosis can occur in both motile (Fig. 3) and palmelloid cells (Fig. 2). Two flagella are present: one extends anteriorly and possesses tubular flagellar hairs while the second flagellum lacks these appendages and trails posteriorly. The flagella arise subapically in a flagellar groove which is situated close to the point at which the contractile vacuole empties. Between the contractile vacuole and the anterior surface of the nucleus there occurs an extensive Golgi apparatus. Vesicles containing water are produced by the Golgi apparatus and are emptied into subsidiary vacuoles and then into the contractile
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vacuole which, in turn, discharges water from the cell (Heywood, 1978; Schnepf & Koch, 1966). Surrounding the nucleus is a region of cytoplasm containing endoplasmic reticulum and large numbers of mitochondria. Between this region and the plasmalemma occur many disk-shaped chloroplasts (Figs. 1-4).

Shortly before the onset of mitosis microtubules are observed radiating outwards from the flagellar basal bodies over the anterior surface of the nucleus (Fig. 5). These microtubules assist in the separation of the flagella but do not enter the nucleus.

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Figs. 1-4. Light micrographs of Epon sections of *Vacuolaria virescens* photographed using Nomarski differential interference microscopy. Layers of mucilage surround the palmelloid cells in Figs. 1, 2, 4; this material is absent in motile cells, for example, Fig. 3. Large numbers of disk-shaped chloroplasts are present between the nucleus and the cell membrane. Contractile vacuoles (c) are visible in Figs. 1, 3, 4. The Golgi apparatus (arrowhead) appears as a dark region on the anterior surface of the nucleus. At interphase (Fig. 1) there is a single Golgi apparatus; this has duplicated at prophase (Fig. 2) and is present on opposite sides of the nucleus at late anaphase (Fig. 3). A Golgi apparatus occurs at the anterior surface of each nucleus during early cytokinesis (Fig. 4). x 1320.
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until later. The nucleolus, nuclear envelope, and chromosomes retain their interphase morphology and hence it is appropriate to term this stage prophase.

In prophase the outline of the nucleus becomes irregular, chromosomes condense (Fig. 2), and the nucleoli begin to disperse (Fig. 8), although fragments of these structures are often still recognizable in later stages of mitosis (for example, Fig. 10). At the poles spindle microtubules enter the nucleoplasm through gaps in the nuclear envelope (Figs. 8, 9). Initially microtubules remain close to the nuclear envelope (Figs. 6, 7), but as prophase progresses they extend across the centre of the nucleus towards the chromosomes. During late interphase or early prophase 2 contractile vacuoles become recognizable. The Golgi apparatus underlying these structures becomes more extensive and eventually divides into two (Fig. 2). Throughout the remainder of mitosis and during cytokinesis a Golgi apparatus with its associated contractile vacuole occurs at or close to each of the poles of the nucleus (Figs. 3, 4, 8, 10, 16–18).

At metaphase the nucleus assumes an elliptical shape, and the metaphase plate of chromosomes becomes aligned across the long axis of this structure (Fig. 10; see also fig. 5 in Heywood & Godward, 1972). During metaphase, folds in the nuclear envelope are especially common near the poles and at the equator (Figs. 10, 11). Two types of spindle microtubules are recognizable: polar microtubules which lack connexions with the chromosomes, and chromosomal microtubules which terminate in a kinetochore of diameter approximately 0.3 μm (Heywood & Godward, 1972). In some preparations the kinetochore is less distinct and appears as an amorphous region on the surface of the chromatin (Fig. 12). During mitosis structures tentatively identified as microbodies (Heywood, 1974) occur close to the surface of the nucleus (Fig. 13) and frequently are connected by membrane to the nuclear envelope (Fig. 12); this type of association is not present at interphase.

During anaphase the chromosomes appear less distinct (Figs. 14, 15) than at other times in mitosis. The nuclear envelope is less irregular than at prophase and metaphase but folds in the nuclear envelope may persist, especially at the poles (Fig. 15). The most noticeable feature of this stage is the presence of vesicles within the nucleus between the poles and the poleward surfaces of the chromosomes (Figs. 14, 15). These structures have been observed at metaphase but are present in much greater numbers during anaphase and early telophase. The vesicles are often irregular in

Fig. 5. Preprophase. A flagellum bearing tubular flagellar hairs lies in a flagellar groove (f) close to the nucleus. Microtubules radiate outwards from the flagellar basal body (h) but do not enter the nucleus at this stage. Also present are a subsidiary vacuole (s), part of the Golgi apparatus (g), and one edge of the contractile vacuole (c). × 19,000.

Fig. 6. Pole of a prophase nucleus. Microtubules which have entered the nucleus through a polar gap (not included in this plane of section) are present in both transverse and longitudinal section. Two osmoregulatory systems occur on opposite sides of the pole: each of the contractile vacuoles (c) is associated with an underlying Golgi apparatus (g) and in one instance a collapsed subsidiary vacuole (s) is present. × 21,200.

Fig. 7. Higher-magnification view of the pole of the nucleus in Fig. 5. × 31,000.
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Outline and range from spherical structures, 90 nm in diameter, to flattened membrane profiles 600 nm or more in length. The location and morphology of these structures suggest that they are involved in the formation of new nuclear envelope around the progeny nuclei. For example, the membrane profile indicated by an arrow in Fig. 15 is similar to the other vesicles in this polar region but its flattened nature and location on the surface of the chromosome indicate that this is probably the first stage in formation of new nuclear envelope. Presumably other vesicles will attach to the edges of this structure giving rise to the situation illustrated in Fig. 18, where the new nuclear envelope has formed over most of the surface and in the remaining region vesicles are fusing together to complete this structure.

As anaphase progresses the chromosomes pull apart and the nucleus becomes more elongated. This process continues in telophase resulting in 2 progeny chromosome groups contained within the increasingly attenuated nuclear envelope of the parent nucleus (Fig. 16). At this stage the original nuclear envelope begins to disintegrate at the poles and in the interzonal region. Frequently folds of nuclear envelope are formed in the interzonal region (Fig. 16). These become detached and persist for some time in the cytoplasm as doubled nuclear envelope (Fig. 19). Some re-utilization of the original nuclear envelope occurs since that portion of it which is present along the lateral surfaces of the reforming progeny nuclei frequently becomes incorporated into their surfaces (Fig. 16). However, most of the nuclear envelope at the poleward and equatorial surfaces of the progeny chromosome groups is formed de novo. This can best be appreciated in Figs. 17 and 18 where most of the new nuclear envelope has been formed but the original nuclear envelope remains virtually intact. Eventually the latter will break down and release membrane or membrane components to the cytoplasm. Figs. 17 and 18 also provide an insight into the mechanism of nuclear envelope formation: vesicles similar to those present at anaphase (Fig. 15) are fusing together at the surface of the chromatin to form the new nuclear envelope.

As telophase progresses chromosomes decondense, nucleoli reappear, and formation of the nuclear envelope is completed. At this stage the cell contains 2 interphase nuclei each of which has a Golgi apparatus and contractile vacuole located at its anterior surface, that is, on opposite sides of the cell (Fig. 4). During cytokinesis a division furrow forms between the 2 nuclei and separates the progeny cells. This event may occur up to 4 h after completion of mitosis (Heywood, 1968) and hence it is appropriate to describe cytokinesis separately (Heywood, in preparation). However, one aspect of cytokinesis will be emphasized because it pertains to the events of mitosis. When the division furrow forms it separates 2 progeny cells, each of which

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Fig. 8. The outline of this prophase nucleus is irregular and at the polar gap (pg) the nuclear envelope is disrupted to allow entry of spindle microtubules. A Golgi apparatus (g) and its associated contractile vacuole (c) occur close to each pole of the nucleus. A fragment of nucleolus (nu) is present close to the chromosomes (ch). ×9850.

Fig. 9. Higher-magnification view of the polar gap (pg), nuclear envelope, and spindle microtubules in Fig. 8. ×28,400.
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possesses a Golgi apparatus and a contractile vacuole. This is not a fortuitous segregation: the association of the Golgi apparatus and the intact nuclear envelope that occurs at or close to each pole of the dividing nucleus ensures that the Golgi apparatus and contractile vacuole are equally apportioned to the progeny cells.

DISCUSSION

This discussion will concentrate on the most distinctive aspect of mitosis in *Vacuolaria virescens*, namely, the behaviour of the nuclear envelope. It is clear from Dangeard's observations on living cells that the nuclear envelope remains intact during mitosis (Dangeard, 1939). Light-microscope observations on fixed and stained cells indicated that the nuclear envelope is still present at metaphase in the closely related species *V. viridis* (Fott, 1935) and that the nuclear envelope remains intact throughout mitosis in *Gonyostomum semen* (Hovasse, 1945). In contrast, Mignot (1967) noted that the nuclear envelopes of *Gonyostomum semen* and *V. virescens* disappear early in mitosis. Unfortunately there are no micrographs of mitotic stages in his descriptions. However, his observation that spindle microtubules are extra-nuclear in origin is in agreement with the present account.

The fact that the nuclear envelope remains largely intact until telophase is unusual but not unique: during telophase of the second meiotic division in the sarcomastigophoran protozoan *Barbulanympha* a new nuclear envelope is formed within the disintegrating original nuclear envelope (Cleveland, 1954). And during intranuclear mitosis in the micronucleus of the ciliate *Blepharisma* a new nuclear envelope is formed within the original nuclear envelope which subsequently breaks down at telophase (Jenkins, 1973).

The presence of a Golgi apparatus and contractile vacuole at or close to each pole of the dividing nucleus is in agreement with earlier light-microscope observations (Poisson & Hollande, 1943; Spencer, 1971). This location, the close association between the Golgi apparatus and the nuclear envelope, and the persistence of the original nuclear envelope into telophase suggest that mitosis in *V. virescens* is instrumental in segregating the Golgi apparatus and contractile vacuole to the progeny cells. This viewpoint is strengthened by considering the alternative: in the absence of any...
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association between the Golgi apparatus and nucleus, the 2 osmoregulatory systems in mitotic cells might be so located that formation of a division furrow would result in both being present in one of the progeny cells. It is not known whether the other cell could form a new osmoregulatory system *de novo* but, even if this were possible, it would probably take a considerable time to accomplish and during this period the cell would presumably be damaged due to its inability to rid itself of water that had entered by osmosis. The postulated involvement of the mitotic nucleus in segregating cytoplasmic organelles has precedents; for example, it is now believed that the location of centrioles at the poles of the division spindle ensures their segregation to the progeny cells (Friedländer & Wahrman, 1970; Pickett-Heaps, 1969, 1971).

Similarly, mitosis is probably responsible for segregation of microbodies to the progeny. Cells of *V. virescens* contain few microbodies (Heywood, 1974) and consequently their association with the nuclear envelope during mitosis will ensure that this organelle is immediately available to each of the progeny cells. A special segregation mechanism for chloroplasts and mitochondria is unnecessary, since these organelles are present in large numbers (Heywood, 1977a, b).

An association between the Golgi apparatus and the poles of dividing nuclei has been demonstrated in other organisms. For example, in the phaeophycean alga *Pylaiella littoralis* Golgi bodies occur close to the nuclear envelope during interphase. Early in mitosis they migrate to the poles ahead of the centrioles and remain there throughout mitosis and cytokinesis (Markey & Wilce, 1975). It has been suggested that the Golgi bodies may be involved in formation of microtubules and in production of cell wall material (Markey & Wilce, 1975). It is therefore important that the Golgi bodies should be partitioned to the progeny cells and this is ensured by their association with the nucleus. Other instances where the Golgi apparatus is known to occur at or close to the poles of the dividing nucleus include the water mould *Harpocytrium hedini* (Whisler & Travland, 1973), the algae *Mantoniella squamata* (Barlow, 1977) and *Ochromonas danica* (Bouck & Brown, 1973; Slankis & Gibbs, 1972), and the labyrinthulid-like protists *Sorodiplophrys stercorea* (Dykstra, 1976) and *Thraustochytrium* sp. (Kazama, 1974).

During intranuclear mitosis there is often a large increase in surface area of the nucleus. For example, the surface area of the micronucleus at anaphase in *Paramedum aurelia* is approximately 5 times that of the interphase nucleus (Stevenson & Lloyd, 1971). At late anaphase and early telophase in *Vacuolaria virescens* the nucleus has a cylindrical shape and the surface is relatively smooth. Presumably the irregularities in

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Fig. 14. Chromosomes (ch) are less distinct at anaphase than at other stages in mitosis. The nuclear envelope remains intact over most of its surface and a Golgi apparatus (g) is present near one of the poles. × 12,500.

Fig. 15. Higher-magnification view of the pole of the nucleus in Fig. 14. A Golgi apparatus (g) is present on the cytoplasmic side of the nuclear envelope while spindle microtubules extend inwards through the nucleoplasm. Vesicles (v) are present close to the poleward surface of the chromosomes. A flattened membrane profile (arrow) situated on the surface of a chromosome (ch) is thought to represent the first stage in formation of new nuclear envelope. × 22,000.
the surface of the prophase nucleus and the folds in the nuclear envelope at metaphase represent new nuclear envelope, the addition of which to the existing envelope is responsible for an increase in surface area and hence permits elongation at anaphase and telophase. A similar explanation has been advanced (Heywood & Magee, 1976) to explain the irregular outline of the nucleus at prophase I of meiosis in *Ulva mutabilis* (Bråten & Nordby, 1973).

At the conclusion of mitosis portions of the original nuclear envelope are either incorporated into the nuclear envelopes of the progeny nuclei, or disperse completely, or persist for some time as membranous structures in the cytoplasm. This latter category consists of membrane profiles whose morphology resembles either nuclear envelope or doubled nuclear envelope. Although nuclear pores are absent from these membrane structures, their location and time of appearance suggest that they are derived from the nuclear envelope. Thus, they occur during late telophase and cytokinesis, and are usually located between the progeny nuclei, i.e. in the region formerly occupied by the interzonal region of the nucleus. The fold in the original nuclear envelope at telophase (curved arrow in Fig. 16) indicates a probable origin of doubled nuclear envelope from nuclear envelope in the interzonal region.

Although some of the original nuclear envelope is apparently retained by the progeny nuclei, formation of new nuclear envelope is also necessary. This is achieved by fusion of membrane vesicles which first appear at metaphase but are more numerous at anaphase and early telophase. These vesicles are spherical or irregular in outline until they become attached to the poleward surface of the chromosome groups, at which time they become flattened. Fusion of vesicles with each other and with the reforming nuclear envelope has been frequently observed. In some of these instances electron-dense material is associated with the vesicles but its significance is not known. The origin of the membrane vesicles is unclear but it is not likely that they are formed by disintegration of the original nuclear envelope since this is largely intact at the time of their formation. The membrane vesicles could be formed in the cytoplasm.
and transported into the nucleus, but this is difficult to demonstrate since vesicles are always present in the cytoplasm (especially in the region of the Golgi). It has not been possible to determine whether any of these are destined for the nucleus. In Chinese hamster cells membrane vesicles are formed near the chromosomes at anaphase and telophase and fuse to produce the new nuclear envelope (Chai, Weinfeld & Sandberg, 1974). Membrane vesicles have also been observed in mitotic nuclei of the flagellate Cyanophora paradoxa (Pickett-Heaps, 1972), and the prasinophycean alga Platymonas subcordiformis (Stewart, Mattox & Chandler, 1974). In the myxomycete Arcyria cinerea membrane vesicles apparently contribute to formation of the new nuclear envelope (Mims, 1972). Membrane vesicles are especially abundant at metaphase and anaphase in the fungus Sorosphaera veronicae (Braselton, Miller & Pechak, 1975). Some of these vesicles are associated with the poleward surface of the chromosomes while others are connected to the nuclear envelope. It has been suggested that these vesicles can form nuclear envelope (Braselton et al. 1975).

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REFERENCES


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