ON MITOSIS IN THE MULTICELLULAR ALGA
ULVA MUTABILIS FØYN

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SUMMARY

Mitosis has been studied in the multicellular green alga Ulva mutabilis by light microscopy and by electron microscopy. Mitosis seems to be of the classical type with regard to spindle, centrioles and continuous and chromosomal microtubules. The nuclear membrane, however, disappears only at the poles and persists as a boundary around the mitotic apparatus. In this respect the Ulva cell seems to be in an intermediate position between those species which have intranuclear mitosis and the forms where the nuclear membrane breaks down. A hypothesis which involves localized synthesis of membranes explains partly the intracellular rearrangement of nucleus, Golgi region and chloroplast after mitosis.

INTRODUCTION

Since Scherbaum & Zeuthen (1954) synchronized cell division in mass populations of the ciliate Tetrahymena pyriformis by heat shocks, a number of other unicellular organisms have been synchronized by other methods, e.g. Astasia (Blum & Padilla, 1962), Chlamydomonas (Bernstein, 1964), Chlorella (Lorentzen, 1957; Tamiya, 1964), Euglena (Cook, 1961), and yeasts (Williamson, 1964).

In Tetrahymena (Elliott, 1963), Astasia (Sommer & Blum, 1965), Euglena (Leedale, 1967), and yeasts (Williamson, 1966; Robinow & Marak, 1966), mitosis is intranuclear. In Chlamydomonas very large fenestrations appear in the nuclear membrane at the poles during mitosis, but the membrane does not break down (Johnson & Porter, 1968). In Chlamydomonas mitosis is followed by cytokinesis. In Chlorella, however, the nucleus may divide several times before cytoplasmic division is initiated (Tamiya, 1963).

It is of interest to know if a method which induces synchrony in cells which follow a particular division pattern, will also induce synchrony in cells which follow another pattern. If this is the case, it might indicate that the synchronizing treatment influences common division-preparing processes and that these processes are unrelated to those which ultimately manifest themselves in a particular pattern of division. We have synchronized the multicellular green alga Ulva mutabilis (Føyen, 1958) by exposing the thallus to a diurnal cycle of light and dark (Løvlie, 1964), in principle the same method as used for Chlamydomonas (Bernstein, 1964), Chlorella (Lorentzen, 1957), and Euglena (Cook, 1961). Judging by the drawings of Føyen (1934b) it is a possibility that the Ulva cell follows the classical mitotic pattern. This supposition is partly supported by the present investigation, carried out by light microscopy and electron microscopy.
MATERIAL AND METHODS

The observations are of cells from diploid sporophytes of the mutant 'Slender' (Foyn, 1959). The chance of finding cells in division is higher in the mutant than in the wild type (Lovlie, 1964). The cells of the mutant are, however, morphologically of the same kind as the cells in the blade of the wild type.

The algae were cultivated in a light-dark cycle of 17 h light (04.00–21.00) and 7 h dark in sea water with Erdschreiber (Foyn, 1934), at 19 °C. Under optimal conditions most of the cells divided during the dark period, with a division maximum around 22.00 h (Lovlie, 1964), when the cells were fixed.

For light microscopy the cells were fixed in Bouin-Dubosque's fluid (150 ml 0.75 % picric acid in 80 % alcohol + 24 ml formalin + 15 ml acetic acid) and stored in the fixative. Staining was in accordance with the chrom-haematoxylin method of Gomori (40 min) after a 15-min acid hydrolysis in 1 N HCl at 60 °C (Melander & Wingstrand, 1953).

For electron microscopy the cells were fixed for 6.5 h at room temperature in 2 % glutaraldehyde and 2 % formalin in 0.1 M cacodylate buffer, pH 7.5, osmolarity 900 m-osmoles. The specimens were washed in 4 changes of 0.1 M buffer with 3 % NaCl during a period of 4 h, and post-fixed for 1.3 h in 2 % OsO₄ in 0.1 M buffer with 3 % NaCl. Dehydration was by graded ethanol and propylene oxide, 10 min in 30, 50 and 70 %, 2 x 10 min in 96 %, 3 x 10 min in 100 %, and 2 x 10 min in propylene oxide. For infiltration the specimens were soaked in a 1 : 1 mixture of Araldite (Durcupan, Fluka) and propylene oxide for 12 h, followed by evaporation of the propylene oxide in a roller-tube rotator for 12 h, before polymerization (60 °C, 3 days).

Sections were cut on an LKB ultramicrotome and stained in a saturated solution of uranyl acetate in 50 % alcohol for 15 min followed by 15 min in Reynolds's (1963) solution of lead citrate. The pictures were taken on a Siemens Elmiskop IA electron microscope.

RESULTS

As described by Lovlie & Bråten (1968) mitosis in Ulva is preceded by intracellular rearrangements whereby the nucleus and Golgi complex move away from the chloroplast and become located above the inner cell wall. Figure 1A shows a diagram of a cross-section of a cell in interphase, and 1B of a cell after the intracellular rearrangement preceding mitosis has taken place. The chloroplast (cl) and the nucleus (n) with the Golgi complex (g) are then on each side of the vacuole (v). Figure 3 shows a

Fig. 1. A, Diagram of cell in interphase; B, diagram of cell in late interphase after the intracellular arrangements preceding mitosis have taken place. (cl, chloroplast; g, Golgi region; n, nucleus; v, vacuole.) The stippled line indicates the plane of section of the electron micrographs.
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layer of synchronized cells viewed from the inside. The cells were fixed 1 h after the light was switched off at the time of the division maximum when most of the nuclei had reached their new location. Figure 4 A–M, shows different stages of the mitotic cycle as seen by light microscopy. The stages may be designated as follows: late interphase, A; prophase, B–C; prometaphase, D; metaphase, E; anaphase, F–H; telophase, I–K; late telophase, L–M. In the late interphase nucleus the chromosomes are still diffuse and one prominent nucleolus is seen. In prophase the chromosomes seem to be located just under the surface of the nuclear membrane, leaving the central space of the nucleus empty as indicated by optical sections (c). The haploid number of chromosomes in *Ulva* spec. is reported by Føyn to be 13 (Føyn, 1934b), by Sarma to be 10 (see Godward, 1966, p. 13). In prometaphase (D) the chromosomes move towards the equatorial plane (stippled line). In metaphase (E) the chromosomes are close together and from then on individual chromosomes cannot be distinguished. The compact metaphase plate is often surrounded by a clear oval region, the border of which presumably represents the nuclear membrane (see electron micrographs). In anaphase (F) the metaphase plate divides and the chromosomes move towards the poles as chromosomal plates. Up to now no sign of a spindle-like structure has been observed with the light microscope, but as the distance between the chromosomal plates increases spindle fibres appear between them. There is, however, no sign of asters. At stage 1 when chromosomes do not seem to move further apart, the 2 compact bodies of chromosomes appear to be connected by a bridge of faintly stainable material. It is convenient to take this stage as the beginning of telophase. From then on, the 2 groups of chromosomes again move closer to each other (j, k, l) and become located close to the division furrow (l). Often they are a little displaced in relation to each other along the furrow (l). It is not possible to establish with the light microscope the stage at which the division furrow is first formed. It is clearly well developed in stage 1, but might already start to form at stage 1. When the chromosome plates are close to the division furrow the tight packing of the chromosomes loosens and the plates become transformed into round nuclei (l, m).

The electron micrographs (Figs. 5–19) are of sections cut perpendicular to the plane of section of Fig. 1 and more or less parallel to the surface (stippled line).

Figure 5 is of a nucleus presumably in late interphase and Fig. 6 of a nucleus in early prophase. Note that the nucleus in prophase appears oval and that the chromosomes are partly condensed. At this stage we have not observed microtubules within the nucleus.

Figure 7 shows a section through a stage which might be considered as late prometaphase. The chromosomes are condensed and arranged along the long axis of the oval nucleus. The undivided body, marked with arrow 3, appears more granular than the other aggregates and may represent the remnants of the nucleolus. The nuclear membrane does not show large fenestrations. However, openings or pores at the future poles of the mitotic spindle are indicated by arrows 1 and 2. A few microtubules (mt) with diameter 15–22.5 nm are located in the middle of the nucleus.

Figures 8 and 9 show sections of cells in metaphase. The microtubules within the nucleus are numerous (Fig. 9) and the nuclear membrane has polar fenestrations.
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(Fig. 8). In Fig. 8 a centriole-like body is located outside the membrane not far from one of the fenestrations. Similar bodies have been seen at the same place relative to the group of chromosomes in other cells. The diameters are about 160 nm, which is within the range covered by centrioles from other types of cell (Went, 1966).

Figure 10 shows a section through a cell in anaphase. The distance between the chromosomes is from 1 to 2 μm. When they are maximally separated the corresponding distance is 4–5 μm (Fig. 4). The picture might therefore represent a stage similar to G in Fig. 4. The polar fenestrations are large, but the nuclear membrane seems to persist as a boundary around the middle part of the mitotic apparatus. The microtubules appear more or less parallel and do not converge towards any centre. It should be emphasized that the numbers of microtubules between the 2 groups of chromosomes are fewer than between the chromosomes and the Golgi region on each side. These statements are supported by evidence from several other cells in the same stage. In a few cases, microtubules are seen which run from one side to the other, passing between the chromosomes. The constriction in the middle of the mitotic apparatus might be insignificant, as it is not seen in all cells in anaphase.

Figure 12 is presumably from a more advanced anaphase stage. The distance between the chromosome groups is between 2 and 3 μm. The arrows indicate that in addition to the polar fenestrations the nuclear membrane is no longer persistent in the equatorial region. Figure 11 may represent a similar stage. Note that the 2 centrioles are not located on the apparent axis of the mitotic apparatus.

Figure 13 shows successive sections through an advancing division furrow. The section of Fig. 13C is nearest the inner cell wall and indicates that the furrow at that level nearly transverses the cell. The region where the furrow will appear is indicated by either 1 or 2 dark lines (arrows). In the other 2 sections there is no clear sign of the furrow in the middle of the cell. There is no evidence that the furrow is formed by coalescence of vesicles. Figure 14 shows a stage which might correspond to that of Fig. 4 or a later stage. It is noted that at this stage there is no electron-dense material in the broad space (0.4 μm) between the cells. At a later stage (Fig. 15) the space appears narrower and the first signs of intracellular material can be demonstrated (arrows).

Figures 16–19 show sections through chloroplasts. The chloroplast is, as described by Bråten & Lovlie (1968), penetrated by invaginations of cytoplasm through which mitochondria (m) and ramifications of the endoplasmic reticulum (er) are brought into close contact with the interior. This is especially well illustrated in Fig. 16, where the section has also cut through the large prominent pyrenoid. Figures 17 and 18 show sections at different levels through the dividing cup-shaped chloroplast; the chloroplast of Fig. 18 has just divided. The characteristic structure of the pyrenoid (see fig. 15 in Bråten & Lovlie, 1968) disintegrates during division. The remnants of the organelle are often seen in only a part of the chloroplast which presumably will belong to only one of the daughter plastids. This might suggest that the pyrenoid does not divide, but is formed de novo in one of the plastids. Also, in the light microscope we have noticed that the stainability of the pyrenoid by haematoxylin is lost in the division period. In Uvta cells, which have been fixed during the division period,
groups of microtubules with a diameter of 25–30 nm are often seen within the chloroplast (Fig. 19). Whether they are more abundant in dividing chloroplasts than in chloroplasts fixed during interphase is at present not known. Microtubules in chloroplasts have been described by Bratén & Løvlie (1968) and by Pickett-Heaps (1968).

DISCUSSION

Among algae and other protists there are many groups which do not follow the classical pattern of mitotic division with regard to condensation of chromosomes, disappearance of nucleoli, breakdown of the nuclear membrane and the presence of a mitotic spindle with microtubules from centromeres to poles, and from pole to pole. In dinoflagellates (Dodge, 1966) and euglenoid flagellates (Leedale, 1967), the chromosomes are condensed through the whole cell cycle, the nuclear membrane remains intact throughout division and the central body or endosome, which might represent the nucleolus, does not disappear, but divides together with the chromosomes. In dinoflagellates microtubules are observed only outside the nuclear membrane (Leadbetter & Dodge, 1967) and in neither group is it likely that the chromosomes contain a localized region which might be referred to as a centromere. Intranuclear mitosis occurs also in amoeba (Roth, Obetz & Daniels, 1960; Roth & Daniels, 1962), foraminifera (Grell, 1964), and yeast (Williamson, 1966; Robinow & Marak, 1966); and in the ciliate Tetrahymena the nuclear membrane is intact during both the amitotic division of the macronucleus and the mitotic division of the micronucleus (Elliott, 1963).

In Ulva each interphase nucleus has one large nucleolus (Fig. 5), the diameter of which is 0.3 to 0.5 of the width of a metaphase plate (Fig. 4). Structures which are reminiscent of nucleoli have been seen in cells which have entered division (Fig. 7, arrow 3), but not in metaphase or more advanced stages of division. The nucleoli reappear in late telophase (Figs. 14, 15). Although we have not been able to follow its disappearace or formation, it is most likely that the nucleolus does not exist as a defined body during division.

With regard to the nuclear membrane, Ulva seems to be in an intermediate position between those species which have an intranuclear mitosis and the higher forms where the nuclear membrane breaks down (Porter & Machado, 1960). As in Chlamydomonas (Johnson & Porter, 1968), the membrane opens up or breaks down in metaphase in 2 regions opposite to each other (Fig. 8). These regions represent the future poles, a fact which is emphasized by the finding of a centriole-like structure in this position (Fig. 8). In anaphase (Fig. 10), the nuclear membrane persists as a boundary around the middle part of the mitotic apparatus. Whether the chromosomes move out of the region encircled by the nuclear membrane or not, and how the new nuclear membranes are formed, has so far not been established. One possibility is that the faintly stained bridge which connects the chromosome plates at stage 1, Fig. 4, represents the collapsed remnants of the spindle and the nuclear membrane after the chromosomes have evacuated that particular region. In this case the chromosomes might be surrounded by new membranes. It is, however, more likely that the membranous cuff of Fig. 10
divides in 2 and that each part participates in the formation of the new membranes, as is indicated by Figs. 11 and 12. All electron micrographs of nuclei reapproaching each other after division show the presence of defined nuclear membranes (Fig. 13).

In Ulva at metaphase, centriole-like bodies are located close to the future poles of the mitotic apparatus (Fig. 8). Figure 11, as well as other sections, indicates that these centrioles in anaphase or late telophase are not in the axis of the mitotic spindle, but presumably located somewhere between this axis and the inner cell wall. In Chlamydomonas, Johnson & Porter (1968) present no evidence of any structures similar to centrioles that might participate in the organization of the mitotic spindle. On the other hand, basal bodies seem to constitute a focal point of 2 sets of radiating microtubules running under the cell surface, microtubules not yet observed in Ulva. The vegetative cell of Ulva has no flagella, but the spores have. It should therefore be of interest from an evolutionary point of view to know the relation between the centrioles of the vegetative cell and the basal bodies of the spores, and their location relative to the mitotic apparatus during sporulation.

Microtubules begin to appear within the nucleus before the nuclear membrane opens (Fig. 7) and increase in number in metaphase (Figs. 8, 9) and perhaps in anaphase (Figs. 10, 12). The diameter of the tubules is about 20 nm, which is within the range of dimensions reported for similar structures in flagellates (20–24 nm, Manton, 1964), invertebrate embryos (12–15 nm, Harris, 1962; 24 nm, Rebhun & Sander, 1967), and higher plants (20 nm, Pickett-Heaps & Northcote, 1966). The fact that the number of tubules in the region between the 2 groups of chromosomes in some sections is fewer than in the region between each group and the pole, suggests that the chromosomes have centromeres with which the tubules are associated. This observation was not made in the flagellate Prymnesium parvum, where the tubules passed between the chromosomes (Manton, 1964).

The separation of chromosomes in anaphase can generally be related to 2 movements (Mazia, 1961, pp. 272–280). One is the elongation of the spindle, moving the chromosomes apart by expanding the region between them. The other is the movement of chromosomes towards the pole, accompanied by a shortening of the microtubules running between the poles and the chromosomes. So far we have not been able to demonstrate a shortening of the microtubules in question. In the light microscope, spindle fibres are seen only between the separating groups of chromosomes. Comparison of Fig. 4 F, G and H suggests that the distance between the cyttoplasmic caps on the polar sides of the chromosome plates increases through anaphase. In metaphase the Golgi regions are on both sides of the nucleus (Figs. 7, 8) and continue to be so in anaphase. We believe, therefore, that the 2 groups of chromosomes are separated mainly because of expansion of the region between them. Other examples of cases where elongation of the spindle seems to be the primary factor in chromosome separation are the giant amoebae, the parasitic flagellate Barbulanympha, and the secondary spermatocytes of an aphid. On the other hand, elongation might play a minor role in many plant cells (see Mazia, 1961, pp. 274).

Even in sea-urchin eggs which might have a storage of spindle proteins (Went, 1959), radioactive amino acids are incorporated into the spindle (Stafford & Iverson,
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1964: Mangan, Miki-Noumura & Gross, 1965). During one generation spindle proteins corresponding in amount to one half of a spindle must be synthesized before the cell can divide. If this synthesis occurs in the cytoplasm outside the mitotic apparatus in the Ulva cell, the macromolecules are supplied to the growing spindle either through the boundary represented by the nuclear membrane or via the Golgi region through the openings at the poles. In the latter case, to reach the region between the 2 groups of chromosomes, the molecules must be transported against the chromosomal movements. The proteins of the mitotic apparatus have a molecular weight of about 34,000, and the units from which the microtubules might be built measure 3.5 nm (Kiefer, Sakai, Solaris & Mazia, 1966).

[Diagrams A, B, C]

Fig. 2. The diagram illustrates an hypothesis which explains the formation of the division furrow and the intracellular rearrangements after division as a result of membrane synthesis. Stippled lines indicate newly synthesized membrane. A, Early telophase; B, the division furrow is partly formed and the nuclei are close together; C, the nuclei are brought closer to the chloroplast by the advancing division furrow. (cl, chloroplast; g, Golgi complex; n, nucleus; v, vacuole.)

Cytokinesis in algae, including the green algae, was generally believed to be characterized by a centripetal development of new cell wall. However, electron microscopy on the unicellular Chlorella (Bisalputra, Ashton & Weier, 1966) and the parenchymatous Fritschilla tuberosa (McBride, 1967), has given some evidence for formation of a cell plate from vesicles derived from the Golgi complex. In Chlorella the cell plate presumably grows outwards and is met by invaginations of the cell membrane. The present results and previous ones (Lovlie & Bråten, 1968) suggest that in Ulva cytokinesis occurs by a division furrow which is not directly formed by coalescence of vesicles. The furrow is presumably initiated at the inner cell wall, advances from there, and in time encircles the whole cell (Lovlie & Bråten, 1968, figs. 5, 9, 14, 15). The two daughter cells are presumably initially separated by a homogeneous intracellular substance with low electron density (Fig. 14) and in this substance the fibres of the new wall are founded (Lovlie & Bråten, 1968, fig. 8) and become orientated (Fig. 15; Lovlie & Bråten, 1968, figs. 9, 10). This seems to happen in the early interphase. We have so far no evidence that the accumulation and orientation of fibres is initiated from a particular region.

There is experimental support for many hypothetical mechanisms of cytokinesis in animal cells (Mazia, 1961, pp. 331-348), and one of these favours formation of new
cell surface as the furrow advances. If we assume that synthesis of new membrane preferentially takes place in the region of the Golgi complex, and that the nucleus with Golgi complex is temporarily associated with a particular part of the membrane, the intracellular re-arrangement following mitosis might partly be explained. In Fig. 2 the closed circle and the 3 open ones indicate the nucleus and Golgi complex respectively, the short line their association with a particular membrane region, and the stippled lines newly synthesized membrane. If membrane synthesis starts at telophase (Fig. 4, 1), a division furrow will be initiated and the 2 nuclei will approach each other as observed in Fig. 4, 1-M (Fig. 2B). Further synthesis might bring the nucleus and Golgi complex closer to the chloroplast (Fig. 2C) and contribute to the re-establishment of the situation at interphase.

It is a common observation that Golgi vesicles may open and release their contents on the cell surface and it seems therefore reasonable to suggest that the growth of the membrane may occur partly by its coalescence with Golgi vesicles. If there is growth of membranes during cell division, knowledge of the factors which regulate their synthesis and breakdown might be of great importance for an understanding of cytokinesis. The idea that the nucleus might be temporarily associated with a particular part of the membrane represents an extension of the hypothesis of Lettre & Lettré (1959) which proposes that there might be a permanent connexion between the chromosomes and the poles. It should in this connexion be mentioned that in the flagellates studied by Cleveland (1957a, b) each centriole is associated with the flagellated areas at the surface. In bacteria the chromosomes seem to be permanently associated with the cell membrane, and localized growth of the membrane might be involved in the separation of daughter chromosomes (Jacob, Brenner & Cuzin, 1963; Lark, 1966). Recent experiments by Lark (1966, 1967) and Lark, Consigli & Minocha (1966) with animal and plant cells suggest a mechanism for chromosomal segregation analogous to that found in bacteria. These observations may suggest that mechanism of cell division in bacteria and higher organisms is more similar than one would expect from morphological studies.

REFERENCES


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Fig. 3. Layer of synchronized cells viewed from the inner cell surface.

Fig. 4. Different stages during cell division. A, Late interphase; B, early prophase; C, prophase; D, prometaphase (stippled line indicates the future division plane); E, metaphase; F, G, H, anaphase; I, J, K, telophase; L, M, late telophase.
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Fig. 5. Section through a nucleus at late interphase. nu, nucleolus. × 23400.

Fig. 6. Section through a nucleus in early prophase. nu, nucleolus. × 24000.

Fig. 7. Section through a nucleus in late prometaphase. Arrows 1 and 2 indicate the future polar regions, arrow 3 a body which might represent the nucleolus; g, Golgi region; mt, microtubules. × 20350.

Fig. 8. Section through a metaphase plate. Arrows 1 and 2 indicate polar fenestrations; ct, centriole, which is further magnified in the inset; g, Golgi region; mt, microtubules. × 21600 and 60000.
Fig. 9. Section through a metaphase plate. × 35,650.

Fig. 10. Section through the mitotic apparatus at anaphase. Arrows point to microtubules which might run between the polar regions. × 21,850.

Fig. 11. Section through the mitotic apparatus at anaphase. The section has presumably passed through the periphery of the mitotic apparatus. Arrows point to 2 centrioles. × 22,500.
Fig. 12. Section through the mitotic apparatus at anaphase. Arrows indicate that the nuclear membrane is no longer persistent in the equatorial region. $\times 46200$. 

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Fig. 13. Three successive sections through an advancing division furrow; c is nearest the inner cell wall; n, nucleus. × 16800.

Fig. 14. Section through a cell at late telophase. Note there is no electron-dense material in the division furrow; g, Golgi complex. × 16500.

Fig. 15. Section through a cell at early interphase. Note that there is electron-dense material in the division furrow (arrows); g, Golgi complex. × 18000.
Fig. 16. Section through a chloroplast before division. Note the cytoplasmic pockets with mitochondria (m) and extensions of the endoplasmic reticulum (er); ρ, pyrenoid. × 17550.
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Fig. 17. Section through the main body of a chloroplast at division. The arrows point to the division furrow; p, pyrenoid. $\times 11,100$.

Fig. 18. Section through a divided chloroplast. Arrows point to the division furrow. $\times 9600$.

Fig. 19. A bundle of microtubules (mt) in the chloroplast. $\times 68,200$. 
Mitosis in Uloa