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Mitosis and Cytokinesis in *Polytomella agilis*

A Thesis
Presented to the School of Graduate Studies
of
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in partial fulfillment of requirements for a
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in Biology
by
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I dedicate this thesis to my wife Cathy, without whose support this project would never have materialized.

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ABSTRACT

Polytomella agilis is a colorless flagellate of problematical affinities. It has been classified as a close relative of the photosynthetic flagellate (phytomonad), Chlamydomonas reinhardi, based on the presence of starch-filled plastids, anterior isokont flagella and basal body associated fibrous structures. It has also been classified as a Prasinophyceae due to the lack of a cell wall. Recently, the reclassification of several flagellates has been proposed based on the characteristics of cell division. The phytomonads can be grouped into two major divisions: the Chlorophyceae and the Charophyceae.

Cultures of P. agilis, synchronized by cold shocks, have been utilized to study cell division. Mitosis in P. agilis begins with the appearance of dispersed chromosomes in a darkened karyoplasm. As the nucleus gradually elongates into a spindle-shaped structure, short microtubule segments first appear next to the poles of the nuclear envelope. The microtubules gradually become longer and more numerous within the karyoplasm. The spindle is perpendicular to the cell's longitudinal axis and the nuclear envelope surrounding it, remains intact throughout mitosis (closed spindle). The poles of the spindle eventually come to rest adjacent to the plasma membrane on each side of the cell. No specialized structures (i.e. centrioles) are visible in the vicinity of the spindle poles (acentric spindle). The basal bodies remain attached

to the flagella at the anterior end of the cell, quite removed from the dividing nucleus. A metaphase plate has not been observed. Two masses of chromosomes are separated to opposite poles during anaphase. At telophase, the mid portion of the cylindrical nucleus constricts and the spindle poles move away from the plasma membrane as the nucleus acquires a dumbbell shape. Finally, adjacent daughter nuclei in which the nucloli have reappeared, occupy the center of the cell.

Cytokinesis occurs only after mitosis has been completed. The formation of new basal bodies involves the sequential assembly of microtubules to a nine singlet stage adjacent and perpendicular to the old basal bodies. The two newly formed sets of flagellar apparatuses migrate to opposite ends of the cell. The daughter cells are then produced by furrowing at the cell's mid portion.

The mitotic characteristics support the classification of Polytomella within the Chlorophyceae, while the differences in mitosis and cytokinesis between Polytomella agilis and the other Chlorophyceae indicate that this organism diverged from a chlamydomonad-like ancestor early in its history.

RESUME

Polytomella agilis est un quadriflagellé incolore d'affinité problématique. Il a été classifié comme étant relié de près au biflagellé photosynthétique (phytomonade), Chlamydomonas reinhardi, en raison de la présence de plastes remplis d'amidon, de flagelles à longueurs égales et de structures fibreuses associées aux grains basaux. Il a aussi été classifié parmi les Prasinophyceae grâce à l'absence d'une paroi cellulaire. Récemment, la reclassification de certains flagellés a été proposée en raison de caractéristiques de la division cellulaire. Les phytomonades se regroupent en deux divisions majeures: les Chlorophyceae et les Charophyceae.

Des cultures de P. agilis synchronizées par chocs à température froide ont été utilizées pour étudier la division cellulaire. La mitose dans P. agilis commence avec la disparition du nucléole et l'apparition des chromosomes dispersés dans un karyoplasme devenu foncé. Pendant que le noyau s'allonge graduellement en une structure fusiforme, de courtes microtubules apparaissent en premier lieu près des pôles de l'enveloppe nucléaire. Par la suite, les microtubules deviennent plus longues et plus nombreuses dans le karyoplasme. Le fuseau est perpendiculaire à l'axe longitudinal de la cellule et l'enveloppe nucléaire entourant le fuseau demeure intacte durant la mitose (fuseau fermé). Les pôles du fuseau

aboutissent éventuellement près de la membrane cytoplasmique de chaque côté de la cellule. Aucune structure spéciale (ex. centriole) n'est visible dans la proximité des pôles du fuseau (fuseau acentrique). Les grains basaux demeurent attachés aux flagelles à la partie antérieure de la cellule, très éloignés du noyau en division. Une plaque métaphasique n'a pas été observée. Deux masses de chromosomes sont séparées aux pôles opposés durant l'anaphase. A la télophase, la portion centrale du noyau cylindrique se rétrécit et les pôles du fuseau s'éloignent de la membrane cytoplasmique pendant que le noyau acquiert la forme d'une haltère. Finalement, les noyaux filles adjacents, chacun démontrant, un nucléole, occupent le centre de la cellule.

La cytodiérèse ne se poursuit qu'après la complétion de la mitose. La formation de nouveaux grains basaux comprend l'assemblée en séquence de microtubules à un stage de neuf "singlets", adjacente et perpendiculaire aux vieux grains basaux. Les deux nouveaux appareils flagellés émigrent aux bouts opposés de la cellule. Les cellules filles sont alors produites par sillonnement de la partie centrale de la cellule.

Les caractéristiques de la mitose appuient la classification de <u>Polytomella</u> parmi les Chlorophyceae, tandis que les différences de la mitose et de la cytodiérèse, entre <u>Polytomella</u> <u>agilis</u> et les autres Chlorophyceae, indiquent que cet organisme a divergé d'un ancêtre à caractéristiques de chlamydomonad tôt dans son histoire.

INTRODUCTION

The problem of establishing evolutionary relationships (phylogeny) within the protists, and in this case more specifically within the unicellular green algae, has been indicated by several authors on several occasions. For example, the external morphology of the organism, utilized as a phylogenetic criterion for more advanced plants and animals, is practically useless for many unicellular algae which essentially all look the same. Most recently, the characteristics of the flagellar apparatus and those of cell division have been widely utilized as phylogenetic indicators for the green algae. Since the characteristics of the flagellar apparatus of Polytomella agilis has already been studied and published in our laboratory (Patenaude, 1974; Brown, Massalski and Patenaude, 1976c), the goal of this thesis is to study the characteristics of cellular division in Polytomella agilis and to assess the phylogeny of this colorless flagellate.

I. Evidence for Two Lines of Evolution within the Green Algae.

I.1. Symmetry of Motile Cells.

The green algae, which share common pigments (chlorophylls a and b) with the higher land plants, are generally considered to be on a direct line of evolution leading to these higher plants. In a search for an ancestor to this line of evolution, the unicellular biflagellate, Chlamydomonas, has been proposed as a potential candidate on several occasions (ie. Scagel et al., 1965; Klein and Cronquist, 1967). In contrast to the views expressed by these authors, Manton (1965) has proposed that the ancestor of land plants would likely be an asymmetric green alga, since all motile cells (ie. sperm) of land plants exhibit a fundamental asymmetry. This view would favor a member from the Prasinophyceae[1] sensu Round (1971), a class of asymmetrical, scaly unicells, over the symmetrical Chamydomonas. In the same article, Manton has drawn attention to the potential phylogenetic value of the flagellar apparatus which combines both a conservative feature (the 9 + 2 structure) and variable acessory structures (ie. the flagellar scales, roots and bases) in eukaryotic cells.

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[1] The taxa utilized in this thesis agree with the five kingdom classification proposed by Whittaker (1969) and modified by Margulis (1974, 1976) which attempts to eliminate problems such as the classification of Polytomella both as a plant by botanists (ie. Smith, 1950) and as an animal by protozoologists (ie. Kudo, 1971).

I.2. Flagellar Root Structures

The taxonomic value of flagellar roots was first applied in the green algae by Moestrup (1972). Of the eleven "normal" green algae listed (Table 1, p. 181), ten possess a cruciate arrangement of four roots in a X-2-X-2 pattern (X varies from 3 to 8 where the digits represent the number of microtubules per root) while Chara and Nitella, more complex genera, have roots lacking this cruciate pattern. The presence of scales covering the cells and flagella of the Charales, the Chaetosphaeridium (from the Coleochaete group), the Prasinophyceae and the sperm of the Bryophytes and Fern suggests an affinity between these groups (Moestrup, 1974).

TABLE I. Flagellar root types in the Chlorophyceae *sensu* Christensen (1966)

Name	Root type	Striated fibrils	No. of flagella	Author(s)
Stigeoclonium	Cruciate: 5-2-5-2	2 present	4	Manton, 1964
Draparnaldia	Cruciate: 5-2-5-2	?	4	Manton, Clarke & Greenwood, 1955
Chaetomorpha	Cruciate: 3-2-3-2	?	2	Manton *et al.*, 1955
Ulothrix	Cruciate: 5-2-5-2	?	4	Manton, 1965
Chlamydomonas	Cruciate: 4-4-4-4	Absent	2	Ringo, 1967
Schizochlamys	Cruciate	?	4	Lembi & Walne, 1969
Tetraspora	Cruciate	?	2	Lembi & Walne, 1969
Golenkinia	Cruciate: 3-1-3-1	Absent	2	Present paper
Enteromorpha	Cruciate	Present	4	Evans & Christie, 1970
Volvox	Cruciate: 4-4-4-4	Present	2	Olson & Kochert, 1970
Bryopsis	Possibly missing*	?	2	Burr & West, 1970
Oedogonium	Many roots: 3-3-3—	Many present	Many	Hoffman & Manton, 1962, 1963
Nitella	Not cruciate, one row with many microt.	Absent	2	Turner, 1968
Chara	Not cruciate	Absent	2	Pickett-Heaps, 1968 Moestrup, 1970b
Pedinomonas (Loxophyceae *sensu* Christensen)	Cruciate: 3-2-3-2	Present, but different	1	Ettl & Manton, 1964

Based on the pattern of the flagellar roots, two categories of motile green algae have been established by Birbeck, Stewart and Mattox (1974):

a) the chlorophycean type with anteriorly inserted flagella and four cruciately arranged microtubular roots and

b) the charophycean type with a broad, flat band of microtubules associated with the flagella, very similar to that of motile cells of land plants.

Based on this and on other characteristics (cell division and biochemical features, to be discussed below), Birbeck et al. (1974) have proposed that the charophycean-type cells, but not the chlorophycean-type, are near the line of evolution leading towards the land plants. The asymmetry of these charophycean-type cells also supports Manton's (1965) earlier view. The presence of body scales on the zoospores of both charophycean and chlorophycean algae (Mattox and Stewart, 1973) also indicates the likelihood that both lines evolved from asymmetrical, scaly phytoflagellates.

The euglenoids are possibly located on a third evolutionary line (Moestrup, 1978), since the presence of chlorophyll a and b seems to be the only characteristic shared with the green algae (Round, 1971).

The structure of the microtubular root systems appears to be conservative within the Chlorophyceae and Prasinophyceae (Moestrup, 1978). Melkonian (1980) has suggested that basal body associated fibrous structures, which apparently display greater variation, could be more useful in elucidating the phylogeny of these groups.

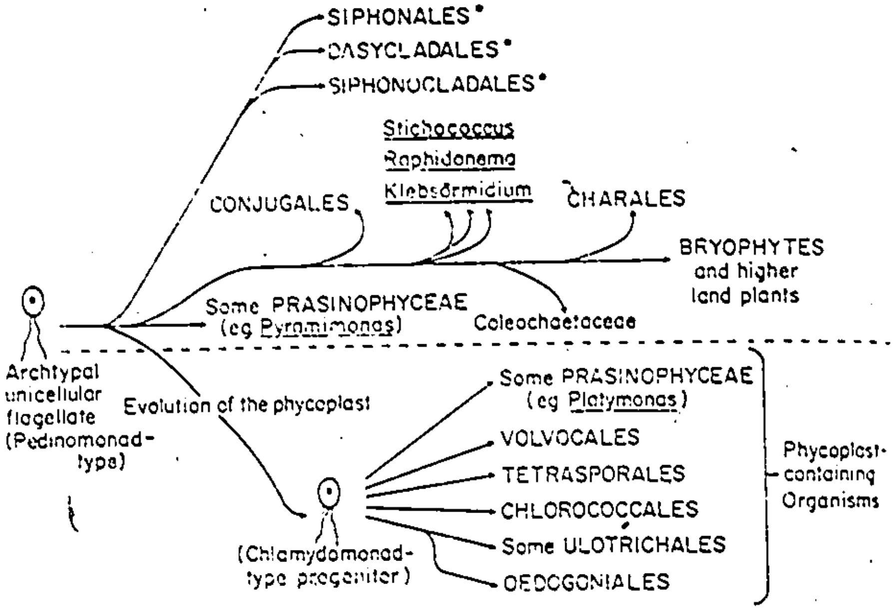
I.3. Mitosis and Cytokinesis

Pickett-Heaps (1975) originally proposed that mitosis and cytokinesis, being conservative processes, should be valuable phylogenetic indicators. Studies of mitosis and cytokinesis have also supported two lines of evolution within the green algae. The following is a brief review of such studies as previously surveyed by Pickett-Heaps (1975) and Stewart and Mattox (1975).

Pickett-Heaps (1967) has shown that cell division (mitosis and cytokinesis) in Chara is similar to that of vascular plants: the mitotic spindle is open (the nuclear envelope is absent) and cytokinesis involves a phragmoplast/cell plate which develops within a persistent interzonal spindle at telophase. On the other hand, the Oedogoniales were shown to have a closed mitotic spindle (completely surrounded by a nuclear envelope throughout mitosis) as reported by Pickett-Heaps and Fowke (1969 and 1970 a) while the Chlorococcales have a partially closed spindle in which the nuclear envelope has polar interruptions (polar fenestrae) at metaphase (Pickett-Heaps, 1972 a). In the latter two organisms, the interzonal spindle disperses at telophase and a system of microtubules appears between both nuclei. These microtubules are parallel to the plane of division, that is, perpendicular to the elongated mitotic nucleus. This system of microtubules has been termed the "phycoplast" by Pickett-Heaps (1972 b). The phycoplast apparently facilitates the formation of the new septum (Pickett-Heaps, 1972 b) which can develop as a cell plate (ie. *Oedogonium*, Pickett-Heaps and Fowke, 1969 and 1970 a) or as a furrow (ie. *Tetraedron*, Pickett-Heaps, 1972 a). The daughter nuclei are close together in the cells with a phycoplast, unlike those with a phragmoplast. In the Zygnematales (Fowke and Pickett-Heaps, 1969 a and 1969 b; Pickett-Heaps, 1972 a; Pickett-Heaps and Fowke, 1970 b), the spindle tends to be open and cytokinesis usually involves furrowing without a system of microtubules. *Spirogyra* (Fowke and Pickett-Heaps, 1969 b) combines both furrowing and cell plate formation in its division (furrowing occurs until it meets with a persistent interzonal spindle and a phragmoplast - like septum develops at that point). This has led these authors to speculate that the phragmoplast of higher plants evolved in green algae whose cell division resembled that of *Spirogyra*.

Similar comparative studies of cell division have been performed on the ulotrichalean green algae, relatively unspecialized filamentous organisms. *Klebsormidium* has been shown to differ considerably in its mode of division from *Ulothrix*, two algae originally considered to be closely related (Floyd, Stewart and Mattox, 1972 a and

1972 b). Cell division in *Ulothrix* is characterized by a closed spindle and the development of a phycoplast/cell plate between adjacent daughter nuclei, whereas, *Klebsormidium* has a fully open spindle and a furrow which, during cytokinesis, cleaves between widely separated daughter nuclei without a phycoplast (Floyd et al, 1972 a and 1972 b). Due to the persistent telophase spindle between the daughter nuclei, *Klebsormidium* has been suggested to belong near the line of evolution leading to land plants (Pickett-Heaps, 1972 b). Pickett-Heaps and Marchant (1972) have proposed a new phylogeny for the green algae, where two main lines of evolution arise from primitive, flagellated ancestors which are characterized by a persistent telophase spindle, and cytokinesis is achieved by furrowing. One of the lines, leading to the bryophytes and higher land plants, is characterized by a phragmoplast and includes the Conjugales (also known as Zygnematales), *Klebsormidium*, Coleochaetaceae, and Charales. The other line, leading away from the higher land plants, is characterized by a phycoplast and includes the Volvocales, Tetrasporales, Chlorococcales, some Ulotrichales and the Oedogoniales. Further information on the above groups can be found in Smith (1950) and Pickett-Heaps (1975). An updated version of this phylogeny (from Pickett-Heaps and Ott, 1974), which also includes some members of the Prasinophyceae, is shown here:



In their review of comparative cytology in the green algae, Stewart and Mattox (1975) have proposed a preliminary partial classification (until more information could be obtained) of the green algae reflecting both lines of evolution. Briefly, one class, the Charophyceae, is characterized by a persistent interzonal spindle during cytokinesis, glycolate oxidase, (see section I.4 on biochemical evidence) and motile cells (if produced) in which laterally attached flagella are associated with a single broad band of closely adjacent microtubules. The other class, the Chlorophyceae, is characterized by a collapsing interzonal spindle during cytokinesis, glycolate dehydrogenase, and motile cells (if produced) in which anteriorly attached flagella are associated with four (or more) cruciately arranged, relatively narrow microtubular roots. This classification includes mostly filamentous green algae because the authors felt that too few of the unicellular green algae had been studied to include them.

I.4. Biochemical Evidence

Differences exhibited by the glycolate pathway appear to have phylogenetic significance in the green algae. Higher plants utilize a different enzyme (glycolate oxidase) than the algae (glycolate dehydrogenase) to oxidize glycolate to glyoxylate as part of the photorespiratory carbon metabolism within their peroxisomes (Frederick, Gruber and Tolbert, 1973). Selecting representative species of green algae from both evolutionary lines, Frederick et al. (1973) have shown a correlation between those algae with a phragmoplast and the presence of glycolate oxidase and between those with a phycoplast (collapsing telophase spindle) and the presence of glycolate dehydrogenase. This confirms the phylogeny (Pickett-Heaps, 1975) and classification (Stewart and Mattox, 1975) proposed above for the green algae.

II. Studies of Cell Division In Unicellular Green Algae.

II.I The Chlorophyceae Sensu Round (1971)

The first detailed ultrastructural study of cell division in a unicellular green alga has been that of Chlamydomonas reinhardi (Johnson and Porter, 1968). In brief, the following features are characteristic of the organism:

1. a closed spindle with polar fenestrae at metaphase;
2. a collapsing telophase spindle (not mentioned in the article but visible in the photomicrographs) with "microtubules comprising the cleavage apparatus" (the phyoplast of Pickett-Heaps, 1972 b);
3. a centric mucleus, that is one in which centrioles[2] are located near the poles. This feature was reported by Coss (1976), using thick sections and high voltage electron microscopy. In the 1968 paper, Johnson and Porter reported that the basal bodies did not appear at the poles;
4. a loss or resorption of the flagella with consequent loss of motility during division (for a review of flagellar resorption, see Bloodgood, 1974);
5. basal body formation during early mitosis.

A later study of cell division in Chlamydomonas moewusi (Triemer and Brown, 1974) has revealed many similarities (ie. features 2 to 5) and the following differences: kinetochores on the chromosomes, a crescent-shaped metaphase spindle without polar fenestrae and a ribosome-free area surrounding the nucleus.

[2] The term centriole in this thesis refers to a basal body which has detached from the flagellum and which takes a position near the pole of the mitotic nucleus.

Triemer and Brown (1974) have postulated that since polar fenestrae have been observed in C. reinhardi (Johnson and Porter, 1968) and in Volvox (Deason and Darden, 1971), members at opposite ends of the volvocine line of evolution (Pickett-Heaps, 1975), the formation of these structures may be a brief event which could have been missed in their organism.

A closely related green biflagellate, the naked Dunaliella bioculata (Marano, 1976) was shown to have similar features of cell division (ie. features 1, 2 and 5). Although the flagella remain attached to the basal bodies in Dunaliella, the basal bodies separate along the plasma membrane, next to the mitotic nucleus. A characteristic cytoplasmic feature of this flagellate is the presence, in depressions of the nuclear envelope, of two electron dense bodies which appear to initiate the assembly of the mitotic spindle. Marano has referred to these as microtubule-organizing-centres (MTOC, terminology of Pickett-Heaps, 1969).

Cell division in Tetraspora (Pickett-Heaps, 1973), an organism closely related to Chlamydomonas and which commonly forms colonies of non-motile unicells held by a fragile matrix, shows many similarities to that of C. reinhardi, as described above. The spindle may differ by possibly being unicentric (only one pole is adjacent to the basal bodies) and new basal bodies appear to form only in the later stages of mitosis.

Basal body formation in all of the unicellular green algae appears to be linked to the process of mitosis. The timing of basal body formation has been proposed as occuring early in mitosis (i.e. C. reinhardi, Johnson and Porter, 1968) or near telophase (i.e. Tetraspora, Pickett-Heaps, 1973).

The role of the basal bodies (centrioles) in mitosis remains to be elucidated. Some authors (i.e. Stubblefield and Brinkley, 1967) have proposed that the centrioles are required to initiate and control the assembly of the mitotic spindle whereas others (i.e. Pickett-Heaps, 1969) have proposed that the position of the centrioles at the poles only ensures their distribution to both daughter cells.

The stages of development in the formation of the basal bodies has been debated on several occasions. Gould (1975) has proposed that basal bodies simply elongate from a probasal body annulus connected to the parent organelle. Dippel (1968) and Cavalier-Smith (1974) on the other hand, have reported that the formation of this organelle involves the sequential addition of tubules to an initial stage of nine singlets.

II.2 The Prasinophyceae sensu Round (1971).

Cell division has also been studied in several species of the Prasinophyceae, commonly known as scaly green monads because of the scales present on their flagella and/or cell surface. Since these organisms are both scaly and asymmetric, they are considered to be among the most primitive of the green algae (Stewart, Mattox and Chandler, 1974). Cell division is very heterogeneous in the Prasinophyceae and this has led certain authors to question the validity of this class (Stewart and Mattox, 1975).

Platymonas subcordiformis has a striated fibrous root, the rhizoplast, which contracts and is linked to flagellar activity (Salisbury and Floyd, 1978). It also appears to contribute to the formation of the spindle microtubules (Stewart et al, 1974). The nuclear envelope is vesiculate, allowing microtubules to extend from the rhizoplast in the cytoplasm to the nucleoplasm. The flagella are detached from the basal bodies which remain at their interphase position during mitosis (acentric nucleus). Newly formed daughter nuclei approach each other and appear to attach to the basal bodies via rhizoplasts. Cytokinesis occurs after the telophase spindle has collapsed and involves a cleavage furrow with a phycoplast between adjacent daughter nuclei. Stewart et al (1974) have indicated that due to their similarities of cell division, Platymonas and Chlamydomonas are potentially more closely related than previously suggested and that ancestors of Chlamydomonas probably had many characteristics of Platymonas.

Asteromonas gracilis, a green, naked biflagellate, has been classified in the family Polyblepharidaceae (order Volvocales, class Chlorophyceae) because of the absence of a cell wall (Fritsch, 1935, Smith, 1950). Peterfi and

Manton (1968) have suggested a close relationship to the Prasinophyceae because of the lack of both a cell wall and sexual reproduction, although anterior attachment of the two isokont flagella and symmetrical cell shape suggests an affinity with the chlorophycean algae. Cell division in *Asteromonas* (Floyd, 1978) is very similar to *Dunaliella* (Marano, 1976) and *Chlamydomonas moewusi* (Triemer and Brown, 1974). In all three, new basal bodies are formed by late prophase and the crescent-shaped nucleus approaches the anterior end of the cell such that each basal body is positioned lateral to the poles, between the crescent area and the plasma membrane. In these three organisms, each set of basal bodies separate from each other, in parallel with the separation of the chromosomes, both in time and space; also, the telophase spindle collapses and cytokinesis occurs by furrowing along a phycoplast. Kinetochores, similar to those found in *C. moewusi* (Triemer and Brown, 1974) are present in *Asteromonas* (Floyd, 1978). Floyd (1978) concludes that the above three organisms are closely related. He also postulates a close relationship between *Asteromonas* and *Platymonas* (Stewart et al., 1974) because both have mitotic similarities (i.e. a collapsing telophase spindle and phycoplast) and similar pyrenoid structures.

The other three prasinophytes in which cell division has been studied, show a diverse array of features. *Pedinomonas*, a naked uniflagellate, is said to have a persistent telophase spindle between the widely separated daughter nuclei, thus allowing a cleavage furrow to bissect the cell without a phycoplast (Pickett-Heaps and Ott, 1974). It also features a totally closed spindle, basal bodies which remain attached to the flagellum near each pole and a rhizoplast which does not appear to participate in cell division.

Pyramimonas parkae, a marine quadriflagellate, appears to have an open spindle, cleavage without a phycoplast between adjacent daughter nuclei and basal bodies which, while attached to the flagella, separate along with the chromosomes (Pearson and Morris, 1975).

Heteromastix angulata, a scaly biflagellate, has a vesiculate spindle with wide open poles at metaphase (Mattox and Stewart, 1977). The microtubules of the spindle appear to originate from the rhizoplasts. Rhizoplasts and flagellar apparatus both separate along with the chromosomes. Cleavage proceeds without a phycoplast across a persistent telophase spindle. According to Pickett-Heaps and Ott (1974), the Prasinophyceae represent a collection of different but related taxa.

To facilitate the comparison of cell division in the unicellular green algae discussed above, their characteristics have been listed in Table I. Heath's (1980) review of mitosis in the protists and fungi provides a similar but more extensive list of characteristics.

TABLE 1

Organism	a Spindle type	b Basal body separation	c Rhizoplast	d Telophase behavior	e Cytokinesis
CHLOROPHYCEAE					
Chlamydomonas reinhardi	closed(f)	+	–	collapsing	phycoplast
Chlamydomonas moewusi	closed	+	+/–	collapsing	phycoplast
Dunaliella bioculata	closed(f)	+	–	collapsing	phycoplast
Tetraspora sp.	closed(f)	?	–	collapsing	phycoplast
Asteromonas gracilis	closed	+	–	collapsing	phycoplast
Platymonas subcordiformis	closed(v)	?	+	collapsing	phycoplast
PRASINOPHYCEAE					
Pedinomonas minor	closed	+	+/–	persistent	furrow
Heteromastix angulata	closed(v)	+	+	persistent	furrow
Pyramimonas parkae	open?	+?	+/–	persistent	furrow

NOTE: See the legend on the following page.

LEGEND

a) the mitotic spindle can be closed, closed with polar fenestrae (f), vesiculate (v) or open.

b) + : the basal bodies migrate with the chromosomes
? : the characteristic is not treated in the paper

c) + : the rhizoplast appears to be involved in spindle formation
- : no rhizoplasts have been observed
+/- : the rhizoplast does not appear to be involved in spindle formation

d) the collapsing telophase spindle allows the daughter nuclei to become positioned adjacent to each other in the center of the cell;
the persistent telophase spindle maintains the daughter nuclei separated and removed from the plane of division

e) cytokinesis proceeds by furrowing with or without a phycoplast.

NOTE: *Asteromonas* and *Platymonas* have been included with the Chlorophyceae as suggested by Floyd (1978).

III. A Capsule Review of the Genus Polytomella

Polytomella agilis was the first member of the genus to be described (Aragao, 1910). Fritsch (1935) has classified Polytomella in the family Polyblepharidaceae (algae that have lost their cell wall), order Volvocales (unicellular or colonial organization) and class Chlorophyceae (algae with isokont flagella and pigments similar to those of the higher plants...), whereas Parke and Dixon (1968) list the Polyblepharidaceae under the Prasinophyceae. According to Fritsch (1935), the colorless algae, such as Polytomella and Polytoma, are descendants of the pigmented forms (i.e. Chlamydomonas) which have lost their photosynthetic pigments but not their starch storing ability within plastids.

Specimens of Polytomella have been found in a variety of places such as earth holes of rotting hemp in Capua, Italy to garden ponds and tree sap in England (Pringsheim, 1955).

Most species of Polytomella have been grown in either a complex culture medium (0.1% sodium acetate, 0.1% yeast extract and 0.2% tryptone) or in a simple defined medium (sodium acetate, ammonium chloride and thiamine, Pringsheim, 1955). As in the case of other flagellates, P. agilis can utilize simple organic acids and alcohols but not sugars as a source of carbon and energy (Little, Oleson and Williams, 1951; Wise, 1959). These simple components can be converted to polysaccharides and stored in starch granules within plastids (Bebbington, Bourne and Wilkinson, 1952; Sheeler et al., 1968b). Metabolic adaptation of acetate-grown cells to propionate and butyrate as carbon sources, has been shown by Cantor and James (1965).

Changes in protein, carbohydrate, nucleic acid and cell volume in P. agilis have been utilized as parameters of growth. Increases in protein levels, a major structural component of the cell, did not correlate with an increase in cell size as expected (Sheeler, Cantor and Moore, 1968a). To overcome this problem, Cantor and Klotz (1970) decided to synchronize the growth of the culture, utilizing a repetitive 22 hour-cold block every 24 hours. In this method, cellular division ceases and biosynthetic activities continue during the cold temperature. Cantor and Klotz (1971) have shown an increase in protein, DNA and RNA levels during the cold block. When the optimal growth temperature (25 C) has been restored, the population undergoes a doubling within one half of the mean generation time (4 hours) and the protein, DNA and RNA levels decrease.

The swimming motion (Gittleson and Noble, 1973), the ultrastructure of both the vegetative cell (Moore et al., 1970) and of the flagellar apparatus of P. agilis (Brown et al., 1976c) have been described. One pair of the four basal bodies, organized into two "nearly opposite" pairs, has a set of proximal and distal connecting fibers similar to that of Chlamydomonas (Brown et al., 1976c). Basal body-rootlet complexes have been isolated (Stearns, Connolly and Brown, 1976) and shown to serve as microtubule-organizing centers (MTOC).

The vegetative cells may transform into round cysts which can survive dessication (Pringsheim, 1955). Although the primary stimulus for cyst formation in Polytomella remains unknown (Kater and Burroughs, 1926; Sheeler, Cantor and Moore, 1970), the sequence of structural events in encystment and excystment has been reported (Brown, Leppard and Massalski, 1976a; Brown, Massalski and Leppard, 1976b).

Sexual reproduction in Polytomella has been a subject of controversy over the years. Conjugation, reported by Aragao (1910), has been questioned by several authors (i.e. Pringsheim, 1955). Recently, Lewis et al. (1974) and Moore and Cushing (1979) have described sexual reproduction in P. caeca and P. agilis respectively.

The most "recent" reports of asexual division for Polytomella, based on light microscopy (Pringsheim, 1955; Lewis, 1974) indicate that cytokinesis begins from the posterior end and proceeds toward the flagellar apparatus at the anterior end. This method of division, as described, would differ from that of all other unicellular green algae described so far.

In this thesis, mitosis and cytokinesis will be studied in Polytomella agilis, using both light and electron microscopy. The phylogeny of this unicellular alga will also be discussed.

METHODS AND MATERIALS

CELL CULTURES

Cultures of Polytomella agilis were grown in 15ml, 300ml and 1000ml lots utilizing erlenmeyer flasks (50ml, 1 liter and 4 liter respectively). These were grown in complex medium containing 0.1% tryptone, 0.2% yeast extract and 0.2% sodium acetate at 25°C in the dark (Sheeler et al. 1968a). Cultures were inoculated from log phase cultures to give concentrations of approximately $5X10^4$ cells/ml. The mean generation time is 4 hours. Cells in 1 liter lots or larger volumes were harvested by centrifugation in a Sorvall RC2-B centrifuge with a GSA head in 290ml polycarbonate bottles at 4,000 rpm (or 2,600 g) for 5 minutes.

CELL COUNTS

Aliquots of 0.5 or 1.0ml were fixed in 2% gluteraldehyde, 0.1M phosphate solution buffered at 7.4 and counted either by hemacytometer or Coulter Counter.

SYCHRONIZATION

Sychronization, modified from that of Cantor and Klotz (1971), involved inoculating a culture with 0.5 to $1X10^5$ cells and letting it grow at 25°C for 6 hours, transferring the culture to an ice bath to bring down the temperature to 12°C and then incubating for 22 hours at 9°C. The temperature was then raised by placing in a 37°C water bath to bring the temperature to 25°C.

Synchronization was monitored by taking cell counts for at least 2 hours following the cold shock. During this period, cells were fixed for electron microscopy.

PROTEIN, DNA AND RNA DETERMINATIONS

Proteins and nucleic acids were precipitated by adding 6ml of cold 20% trichloroacetic acid (TCA) to 18ml culture samples. The organic components were pelleted (1500g at 4yoC for 5 minutes) and washed twice with 5ml of cold 5% TCA. The precipitate is resuspended in 1ml of 0.5M perhloric acid and incubated for 20 min. at 70°C while shaking, to solubilize the nucleic acids. The solution is cooled, then centrifuged at 4°C for 7½ min. at 1500g.

The precipitate was dissolved in 10ml in 1N NaOH. Aliquots (0.1ml and 0.2ml) were made up to 1ml with water. These were utilized in the Hartree (1972) method of protein determination, with bovine serum albumin as the standard.

The supernatant was divided into 0.4ml aliquots for DNA determinations (Burton, 1956) and 0.05ml aliquots for RNA determinations (Schneider, 1957). The DNA aliquots were mixed with two volumes of diphenylamine reagent, incubated at 30°C for 20 hours and read at 600nm. The RNA aliquots were mixed with 1.2ml of 0.5M perchloric acid and 1.2ml orcinol reagent (Lin, 1969). This solution was incubated for 20 min. at 100°C, cooled and read at 660 nm.

ISOLATION OF BASAL BODY ROOTLET COMPLEXES

This procedure has been described by Stearns et al.

(1976). Basal body-rootlet complexes were isolated from both log-phase cultures and synchronized cultures. In each case, three liters of cells at a density of 1 to $5X10^6$ cells/ml were collected by centrifugation and deflagellated. The cell bodies were separated from the flagella by centrifugation and resuspended in GDMP. The mixture was made 0.1% (vol/vol) Triton X-100 and the cells were broken by gentle vortex agitation in a fluted tube. The released basal body-rootlet complexes could be observed by phase microscopy. The complexes were purified from denser cell debris by successive centrifugations and resuspended in 0.2ml of 5% sucrose solution.

MICROSCOPY

a) Light Microscopy

Living cells were photographed with a Zeiss microscope equipped with Nomarski optics and a Zeiss 60 W.S. electronic flash.

Thick-sections (approx 1 μm) were cut with a diamond knife on a Porter-Blum MT2-B ultramicrotome. Sections were floated on a glass slide and air dried. These were stained with 0.1% aqueous Azure II and heated until evaporation began. They were then washed with water and air dried. Thick sections were photographed utilizing brightfield microscopy.

b) Electron Microscopy

Cell samples (30ml.) were collected and centrifuged for 2 min. on an IEC clinical centrifuge set at 7. The supernatant was discarded and the pellet fixed in 2% buffered gluteraldehyde. The pellet was transferred to a smaller glass tube and allowed to stand at least 1½ hours at room temperature. The pellet was then washed for 15 min. (four times) and the last wash was done on ice. The pellet was then fixed on ice for 1½ hours in 1.0% osmium tetroxide - 0.05 M phosphate solution buffered at 7.4. The pellet was then dehydrated on ice for 15 min. in each of a 10, 30, 50, 70, 90, 100, 100, 100% acetone series. The last 100% acetone wash was done at room temperature. The pellet was embedded in Spurr's plastic (Spurr, 1969) and left overnight. The pellet was placed in fresh embedding medium for 5½ hours and placed in castings and then filled with embedding medium. These were incubated at 60°C for 8 hours.

Pellets of isolated basal body-rootlet complexes were also treated in this fashion.

Thin sections (60-100 nm) were cut with a diamond knife on a Porter-Blum MT2-B ultramicrotome. Sections were stained with a 2% uranyl acetate (in 5% ethanol) and 1% lead citrate (Reynolds, 1967) to be examined in an AEI-EM 6B or Philips 201C electron microscope.

Some of the basal body-rootlet complexes were observed after negative staining with 2% phosphotungstic acid solution on 200 mesh, formvar-carbon coated grids, rinsed before and after with 0.4% photo-flo.

Over one hundred cells have been observed in one stage or another of mitosis or cytokinesis during the course of this project.

RESULTS

In order to facilitate the study of cell division in Polytomella agilis, the first objective was to synchronize cell division based on a modified method of Cantor and Klotz (1971). Figure I shows the result of a temperature shock applied to a log phase culture. The number of cell divisions per unit time is drastically reduced by the cold temperature while synthesis of macromolecules does not appear to be inhibited, as shown by increases in RNA, DNA and protein levels (Figure 2). Once the optimal temperature is restored, the generation time is reduced by roughly one half that of the control culture and the concentration of RNA, DNA and protein decreases within the cells (figure 2). Although the degree of synchronization achieved by this method was relatively low (84% maximum cell increase over two hours) and inconsistent (less than 1 successful trial out of every 10), several of the experiments facilitated the task of finding cells in stages of mitosis and cytokinesis.

Interphase

The external morphology and cytoplasmic organization of Polytomella agilis, as described before (Moore, Cantor, Sheeler and Kahn, 1970; Brown, Massalski and Patenaude, 1976), is briefly reviewed.

The external morphology is best seen in the scanning electron micrographs (Figure 3a and 3c). The cell shape is basically ovoid with four flagella emerging from depressions below an anterior, cruciform papilla (Figures 3a and

Figure 1 Growth curve of a control culture at 25°C and a synchronized culture of *P. agilis*. The arrows indicate the time period (22 hours) spent at 9°C by the synchronized culture. Open dots: control culture, closed dots: synchronized culture.

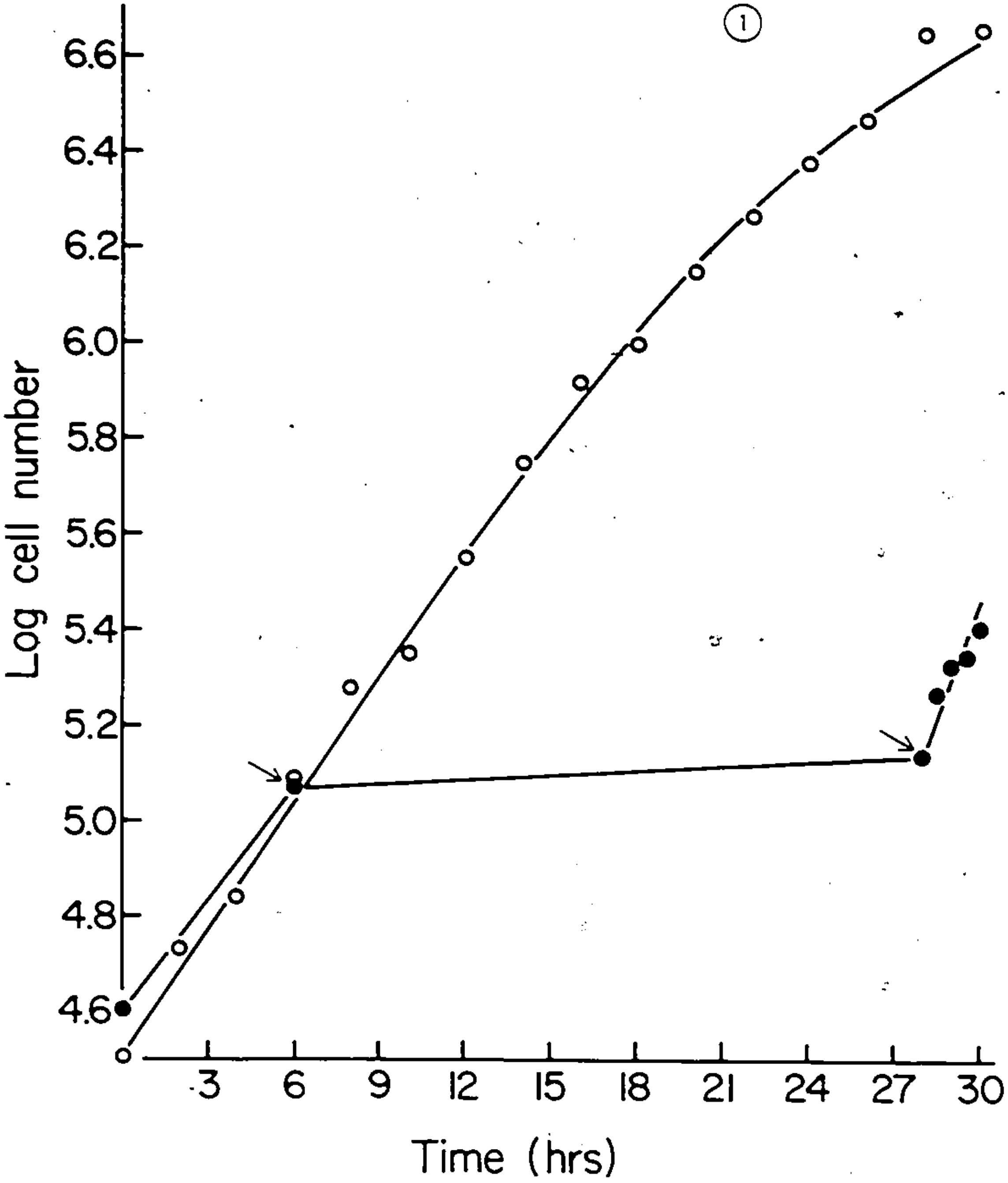
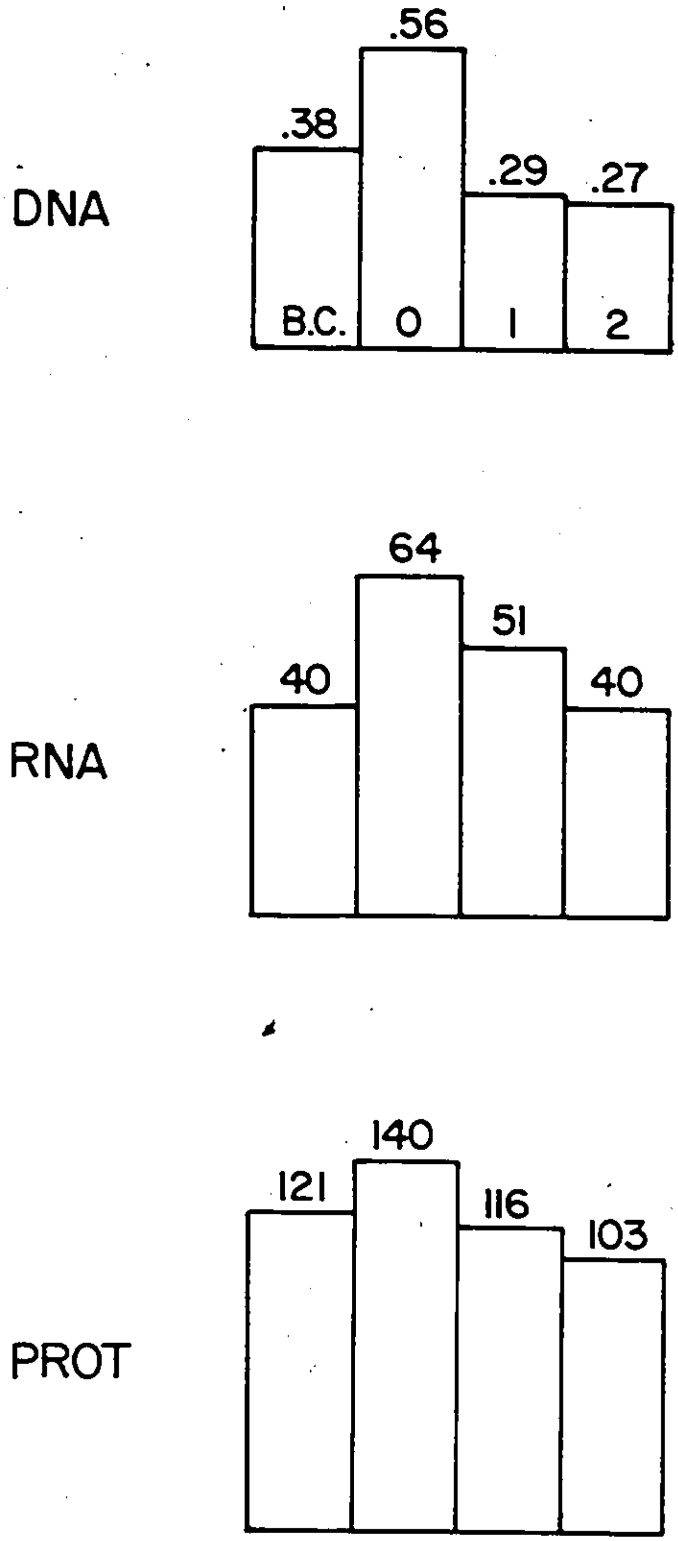

①
Log cell number
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
3
6
9
12
15
18
21
24
27
30
Time (hrs)

Figure 2 These bar charts indicate that the concentration of DNA, RNA and protein increases within the cells of _P. agilis_ when cell division is arrested by the cold temperature (from B.C. to 0). Once the optimal temperature is restored, the cells can divide and the concentration of these substances decreases within the cell (from 0 to 2). BC: determination made on a sample of log phase culture before the cold shock; 0,1 and 2 refer to the number of hours after the termination of the cold shock. Concentrations are expressed in $\mu g/10^6$ cells. These results were obtained from a single experiment.



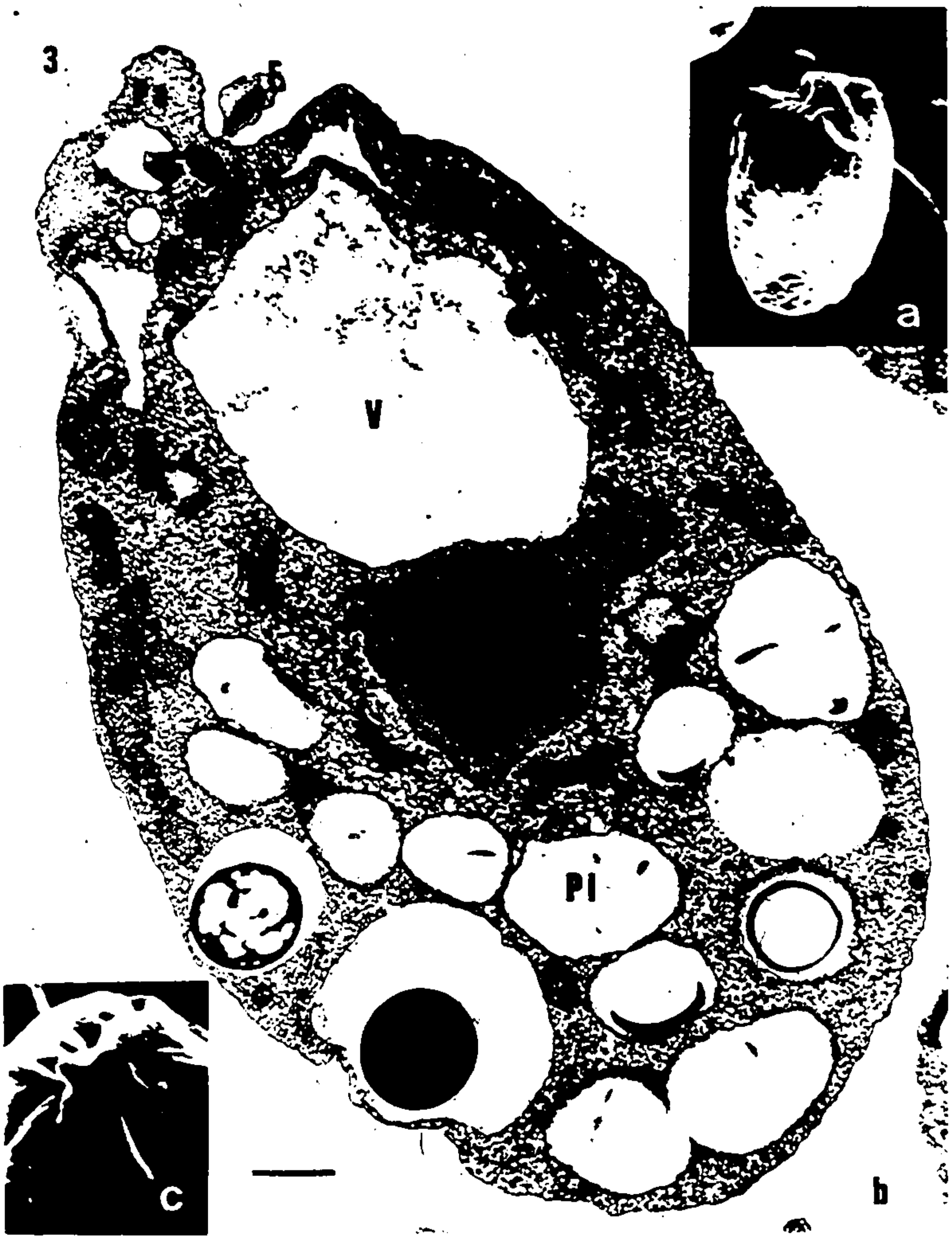
②

c

Figure 3 External and internal morphology of *Polytomella agilis*. a and c, Scanning electron microphaphs of the whole cell showing the four flagella emerging from depressions below the cruciform papilla at the anterior end of the cell.
a) X 2,300; c) X 5,500
b, Transmission electron microphaph (TEM) of a longitudinal section through the mid portion of the cell. F, Flagellum; G, Golgi apparatus; M, Mitochondrion; N, Nucleus; Pa, Papilla; Pl, Starch-filled plastid. X 11,500
Bar: 1.0 µm.

Figure borrowed from Brown et al., 1976c.

3.



and 3c). The cytoplasmic organization is shown in a longitudinal section through the cell (Figure 3b). In the center of the cell, a nucleus with its large nucleolus is surrounded by several Golgi bodies. Contractile vacuoles are usually seen anterior to the nucleus. Mitochondrial profiles are observed at the periphery of the cell. Starch containing plastids occupy roughly the posterior half of the cell. A small amount of endoplasmic reticulum and numerous ribosomes are dispersed throughout the finely granular cytoplasmic matrix except in the anterior papilla where the cytoplasm, free of organelles, is characterized by a filamentous appearance (Figure 3b).

Mitosis

The characteristic stages of mitosis and the orientation of the mitotic spindle are shown in a series of light micrographs in Figure 4. The nucleolus (Figure 4a) disappears as the spherical nucleus elongates into a spindle-shaped structure at prophase (Figure 4b). At metaphase, the spindle extends across the entire width of the cell (Figure 4c). The cylindrical nucleus of anaphase (Figure 4d) constricts at telophase (Figure 4e) to yield daughter nuclei (Figure 4f).

Prophase

In early prophase, the nucleolus disappears and the chromatin condenses into scattered chromosomes within a densely stained karyoplasm (Figures 5a and 5b). The distribution of cytoplasmic organelles in the early stage

Figure 4 Light microphaphs (LM) of stained thick sections showing the structural characteristics of the of the-nucleus and its orientation with the cytoplasm at different stages of mitosis. a, Interphase; b, Prophase; c, Metaphase; d, Anaphase; e, Early telophase; f, Late telophase. The relative position of the other visible organelles within the cell appear to remain unchanged. X 3,000
Bar: 3.0 µm

4

Figure 5 TEM showing the transition from a) an interphase nucleus with a distinct nucleolus to b) an early prophase nucleus with dispersed chromosomes and a densely stained karyoplasm. Microtubules are not yet visible in the karyoplasm.
a) X 26,000; b) X 21,000 Bar: 0.5 μm


5
b

of prophase (Figure 6) is similar to that of the interphase cell (Figure 3b). A basal body attached to its flagellum can be seen at the anterior end of the cell. Microtubules first appear as short segments which predominate next to the nuclear envelope at the poles of the spindle (Figure 7). The chromosomes[3] are dispersed throughout most of the nucleoplasm during early prophase (Figures 6 and 7). The roughly spherical nucleus gradually elongates into a spindle-shaped nucleus (Figures 5, 7 and 9). The distribution of the chromosomes in the prophase spindle is shown in Figure 9. A magnified view of a late prophase spindle pole (Figure 8) shows long microtubules projecting into the karyoplasm from the pole and the odd short segment next to the chromosomes. Chromosomes are still present near a pole which is approaching the plasma membrane (Figure 8).

Metaphase.

The spindle-shaped nucleus is perpendicular to the cell's long axis (Figure 4c) and the poles of the nucleus approach the plasma membrane (Figures 4c and 10). No specialized structures can be observed at the poles between the plasma membrane and the nucleus (Figure 10). The distance between the chromosomes and the pole (Figure 10) appears to have increased since late prophase

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[3] The word chromosome is utilized loosely in this thesis and refers to the darker staining bodies within the mitotic nucleus.

Figure 6 Longitudinal section through the mid portion of a cell in early prophase showing that the cytoplasmic organelles, and more specifically, the basal bodies remain in their interphase position. X 13,000 Bar 1.0 µm.

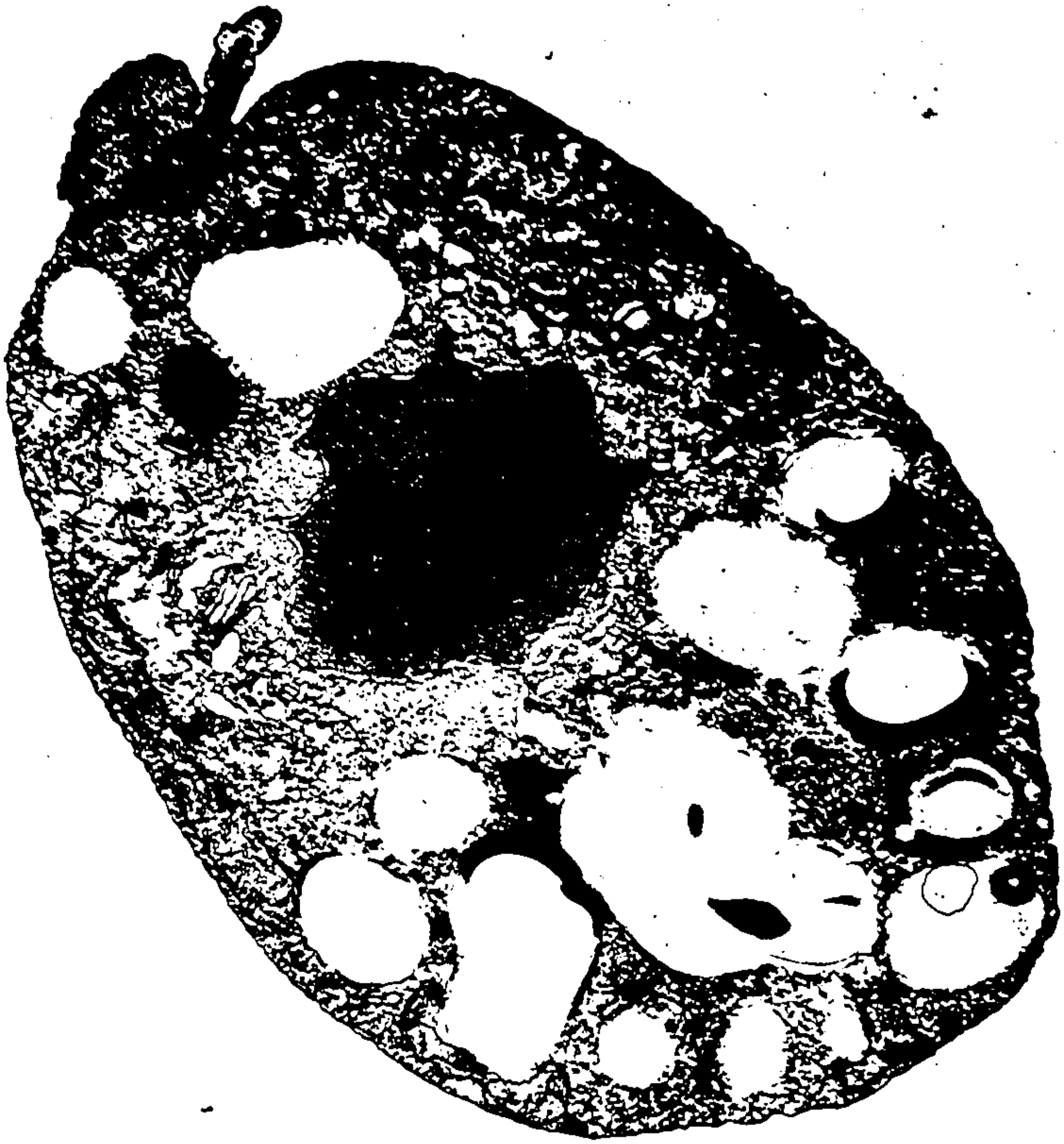

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Figure 7 Section through a prophase nucleus. Chromosomes are visible throughout the karyoplasm. Short microtubules are now visible at the poles (arrows) and near the chromosomes (arrowhead).
X 38,000 Bar: 0.2 µm

7

Figure 8 One pole of a late prophase spindle. Arrow shows longer microtubules projecting away from the pole while short segments are sometimes observed next to the chromosomes (large arrowheads). The chromosomes are still near the pole as it approaches the plasma membrane. The nuclear envelope remains intact. X 39,000 Bar: 0.3 μm


82

Figure 9 View of the entire spindle showing the distribution of the chromosomes during prophase.
X 20,000 Bar: 0.5 μm

Figure 10 The proximity of the spindle pole to the plasma membrane indicates a stage of metaphase. No specialized structures are observed between the pole and the plasma membrane.
X 30,000 Bar: 0.3 μm

9

10

Figure 11 Metaphase as indicated by the large number of microtubules near the chromosomes in this cross-section of the mid portion of the spindle. X 48,000 Bar: 0.2 µm


11

(Figure 8). The nuclear envelope remains intact, even at the poles (Figure 10). Golgi bodies are sometimes observed adjacent to a pole of the nucleus (Figure 10). The spindle is acentric, that is, no centrioles are present at the poles. A cross-section of the mid portion of the spindle (Figure 11) shows numerous microtubules and several chromosomes dispersed throughout the karyoplasm. Kinetochores and alignment of the chromosomes at the equator of the spindle to form a metaphase plate have not been observed in this organism.

Anaphase

Two distinct chromosome masses are now visible within the intact nuclear envelope (Figures 4d and 12). The shape of the nucleus no longer resembles a spindle. The poles, still adjacent to the plasma membrane, are less tapered, more blunt and the central portion is more cylindrical (Figures 4d and 12). Segments of microtubules appear over most of the length of the anaphase nucleus (Figure 12).

The basal bodies are still located at the anterior end of the cell (Figure 12). In the separation of chromosomes at anaphase, the chromosome-to-pole distance appears to decrease (compare Figure 12 to Figure 10).

Telophase

In early telophase, when the poles of the nucleus are still within proximity of the plasma membrane, the central

Figure 12 Two distinct masses of chromosomes are shown during anaphase. A basal body (large arrowhead) can be observed at the anterior end of the cell. Segments of microtubules (arrows) are visible throughout the cylindrical nucleus. X 19,000 Bar: 0.5 µm.


12

portion of the nucleus constricts between the two chromosome masses (Figures 4e and 13). A bundle of microtubules appears within the constricted karyoplasm (Figure 13b). The nucleus becomes more dumbbell-shaped as the nuclear envelope is withdrawn from the plasma membrane, and the constriction in the mid portion of the nucleus becomes more pronounced in mid telophase (Figure 14). Fewer microtubules are visible within the densely stained karyoplasm (Figure 14). In late telophase, the newly formed daughter nuclei, adjacent to each other, occupy the center of the cell (Figures 4f and 15). The karyoplasm at this stage (Figure 15) resembles that of an early prophase nucleus (Figure 6): the karyoplasm is densely stained, the chromosomes are dispersed and spindle microtubules are no longer visible (collapsed telophase spindle). Figure 16 shows a later stage of telophase, where a nucleolus reappears in each of the adjacent nuclei. Microtubules have not been observed in the cytoplasm between the daughter nuclei following mitosis. During the stages of mitosis described above, the outer morphology of the cell and the location of the cytoplasmic organelles do not appear to differ from that of the interphase cell (Figures 3, 4 and 6). Mitosis is thus fully completed before the formation of new flagella and the initiation of cytokinesis.

Cytokinesis

The motion of the cell during the separation of the

Figure 13 a) Early telophase as indicated by the proximity of the nuclear envelope to the plasma membrane and the constriction in the mid portion of the nucleus.
X 20,000 Bar: 0.5 μm
b) At higher magnification, a bundle of microtubules is visible in the constricted region. X 50,000 Bar: 0.2 μm

13

Figure 14 At mid telophase, the nuclear envelope is no longer near the plasma membrane and the constriction in the mid portion of the nucleus has become more pronounced. Only a few short microtubules (arrows) can be seen in the karyoplasm of the dumbbell-shaped nucleus. X 33,000 Bar: 0.3 μm


14

Figure 15 Adjacent daughter nuclei in late telophase. The densely stained karyoplasm is similar to that observed during early prophase before the nucleoli reappear. A portion of the papilla is visible at the anterior end of the cell. X 25,000 Bar: 0.4 μm

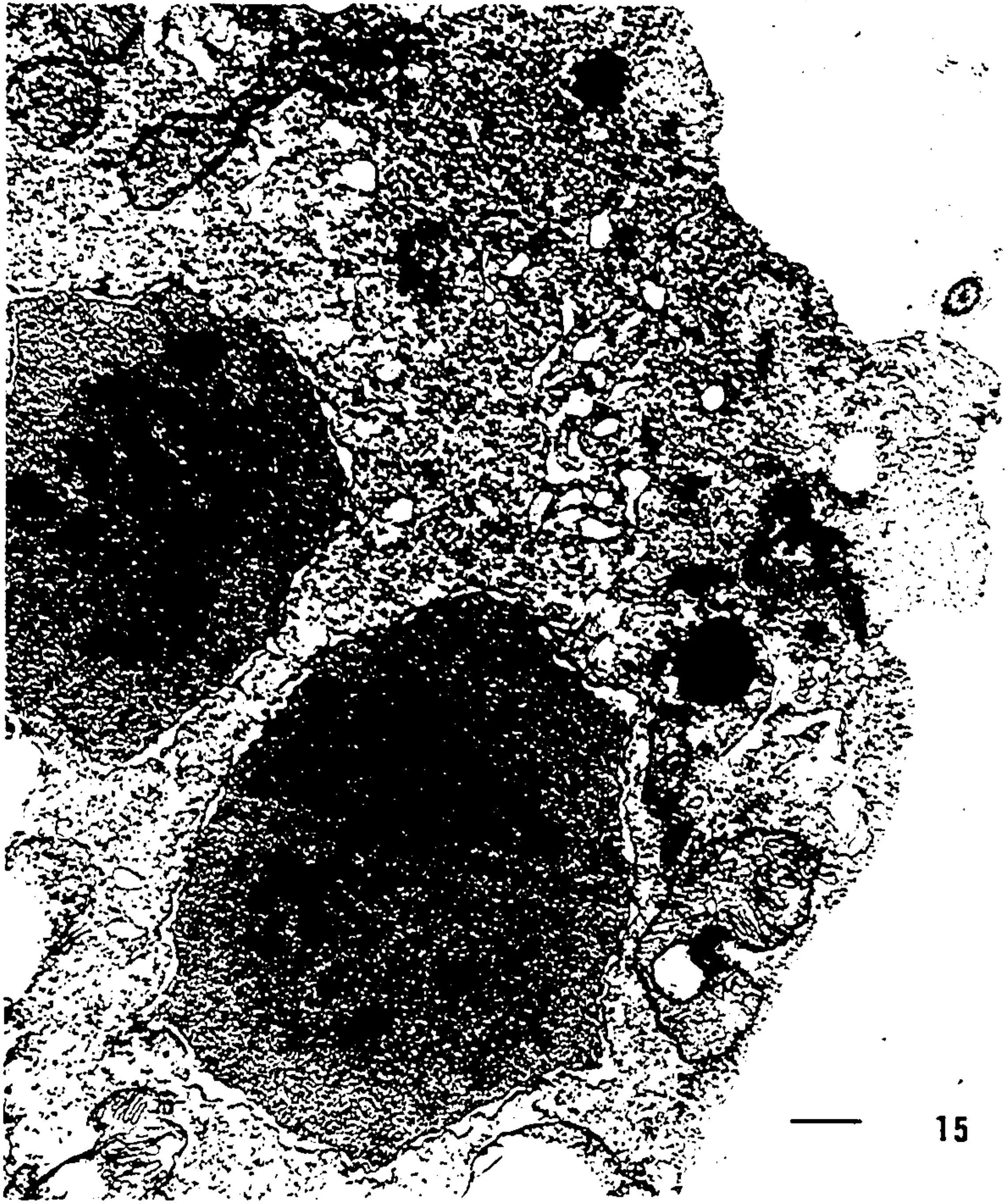

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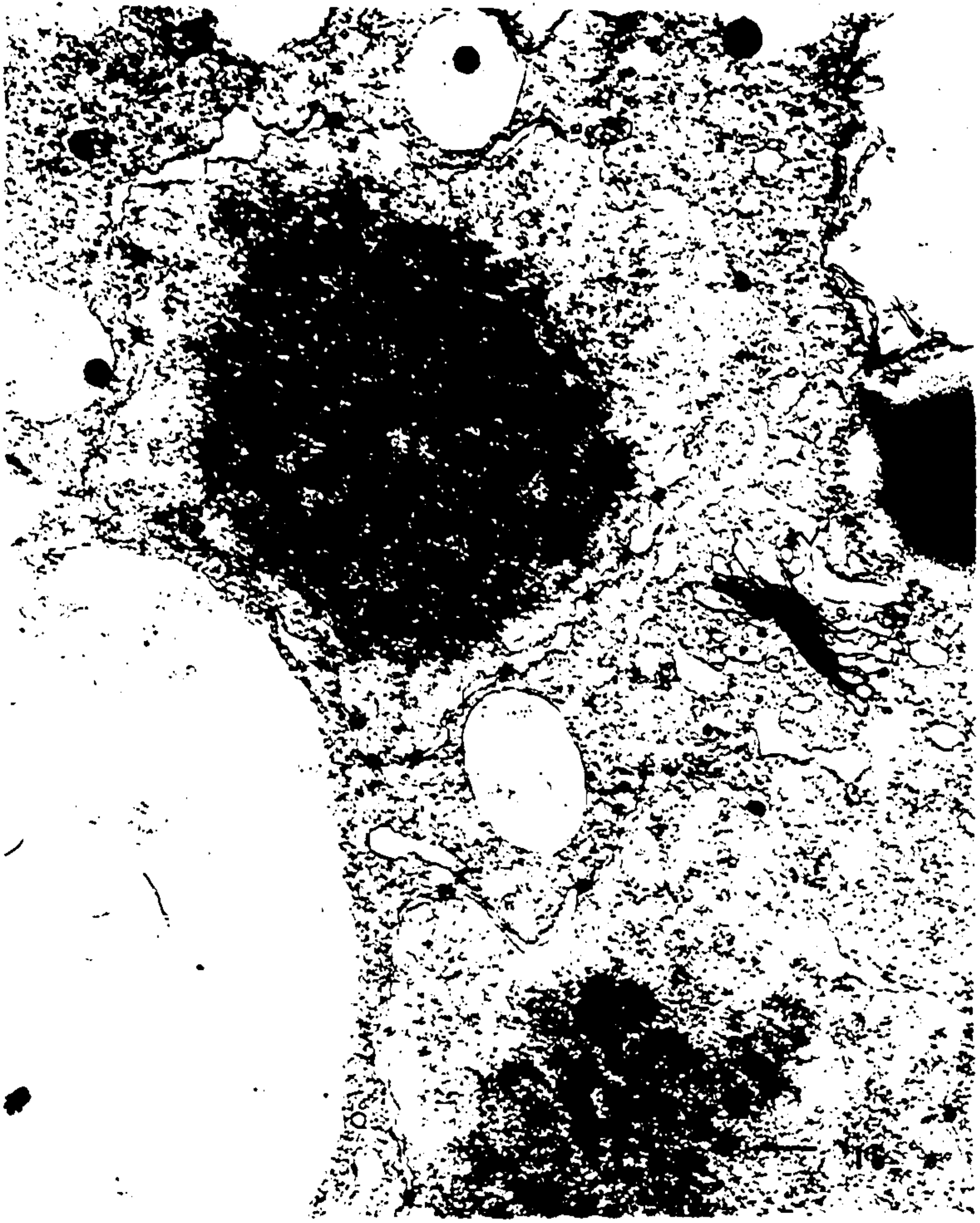
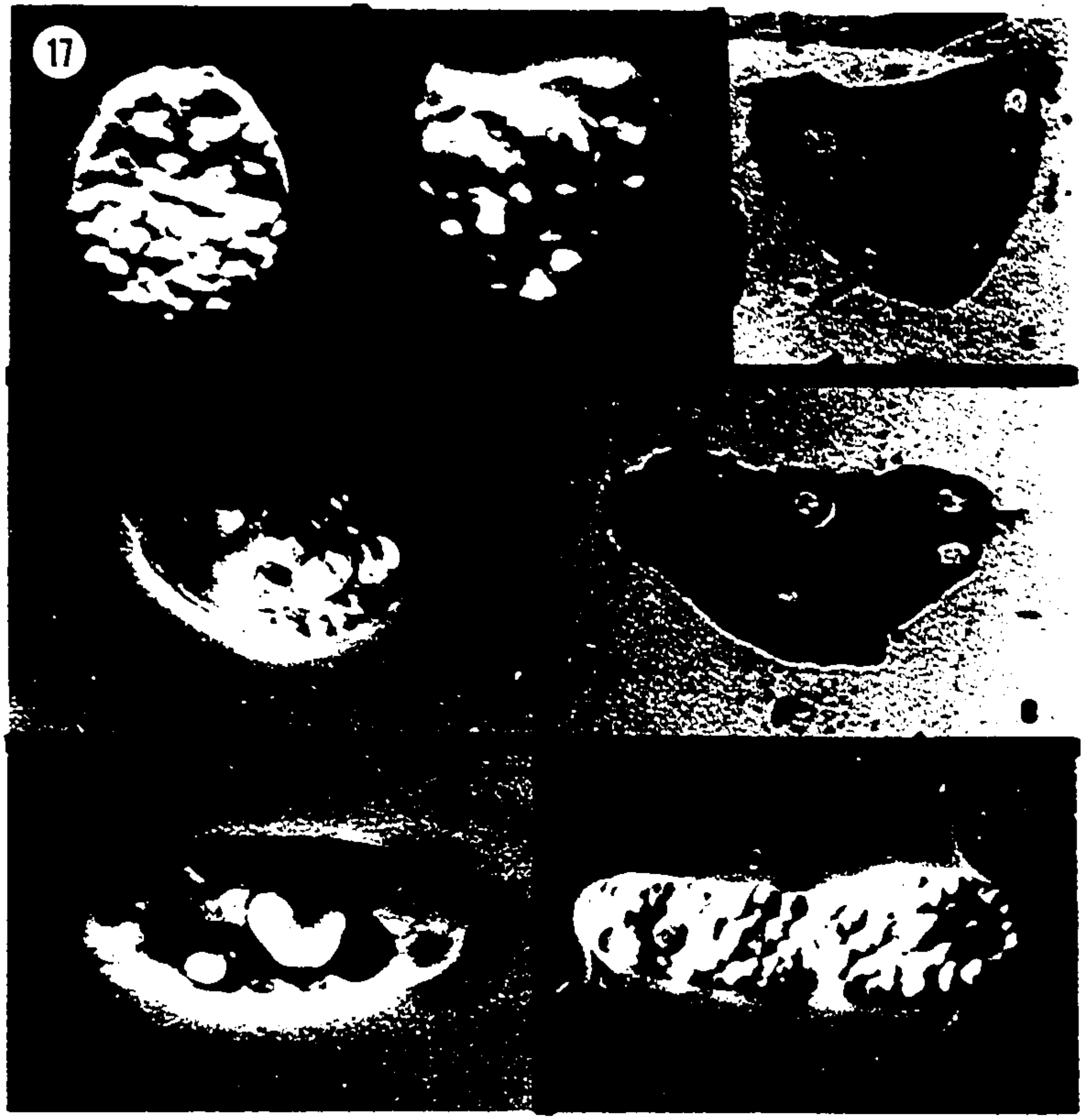
Figure 16 A nucleolus has reformed in each daughter nucleus. Following mitosis, microtubules have not been observed in the cytoplasm between the adjacent daughter nuclei.
X 25,000 Bar: 0.4 µm



flagella in early cytokinesis differs from that of the interphase cell (Brown et al, 1976). Instead of darting across the field of view in a straight path, a cell with an extra set of flagella tends to move slower and continously changes direction as it revolves along its long axis. This type of motion allows one to single out cells in early stages of cytokinesis from the rest of the population.

The separation of the flagellar apparatuses is shown in Figure 17. Cytokinesis begins with the formation of a second flagellar apparatus at the anterior end of the cell (Figure 17a). The separation of the two flagellar apparatuses is shown by the increasing distance between the papillae (Figures 17a to 17e). An indentation (furrow?) is usually present in the plasma membrane on the short side between the papillae (Figures 17b to 17e). The nuclei appear to remain close to each other in the central area of the cell during these events (Figures 17c and 17e). The separation of the papillae, which lasts roughly twenty minutes, can usually be followed until the papillae are at opposite poles of the cell. The cells usually remain in this stage until they begin to deteriorate due to the drying effect generated by the heat of the microscope (Figure 17f). On one occasion, the appearance of a furrow at the mid point between the two papillae has been witnessed and photographed (Figure 17g). The nuclei appear to have moved away from the site of furrowing at this stage (Figure 17g).

Figure 17 Light micrographs a,b,d,f and g show cytokinesis in swimming cells. LM c and e of stained thick sections confirm the presence of daughter nuclei during early cytokinesis.
a) Early stage of cytokinesis in which two adjacent papillae and at least six flagella are visible. Daughter nuclei can also be observed.
b) The papillae are further apart. A slight indentation is visible at the anterior end, not the posterior end.
c) View of adjacent daughter nuclei in the central area of the cell.
d) The papillae have migrated away from each other to the point where they are nearly at opposite poles. An indentation (furrow?) is still visible on the short side between the papillae.
e) A view showing that the daughter nuclei can still be found adjacent to each other in the central area of the cytoplasm when the papillae are nearly at opposite ends of the cell.
f) A later stage at which the papillae are at opposite ends of the cell. This cell shows signs of deterioration as the wet mount begins to dry.
g) Late stage showing signs of furrowing all around the plasma membrane at the mid point between both papillae. Note that the nuclei now appear to have moved away from the site of furrowing. X 2,500 Bar: 4.0 µm.


⑰

Formation of Basal Bodies

New basal bodies are formed adjacent and perpendicular to the pre-existing pairs (Figures 18a and 18b). New basal bodies first appear as nine singlets (Figure 18c) at the base of which exists a cartwheel structure (Figure 18d). Figure 19 shows a forming basal body in a cell with two nuclei. Serial sections of the same cell (Figure 20) show all four of the forming basal bodies.

The full complement of extra basal bodies has also been observed in basal body-rootlet complexes isolated by the method of Stearns et al. (1976). Figures 21a and 21b show isolated complexes with and without basal bodies. Thin sections of complexes, isolated at different time intervals after the termination of a cold shock, show a forming basal body adjacent to a mature basal body (Figures 21 c and 21 d).

Figure 18 Formation of basal bodies.

a, Longitudinal and tangential sections through forming basal bodies (arrows) adjacent to mature ones (arrowheads).
X 50,000 Bar: 0.2 µm

b, Tangential sections through forming basal bodies (arrows) adjacent and perpendicular to mature ones (arrowheads). X 52,000 Bar: 0.2 µm

c, Cross section through a singlet stage.
X 68,000 Bar: 0.1 µm

d, Cartwheel structure within nine singlets.
X 62,000 Bar: 0.1 µm

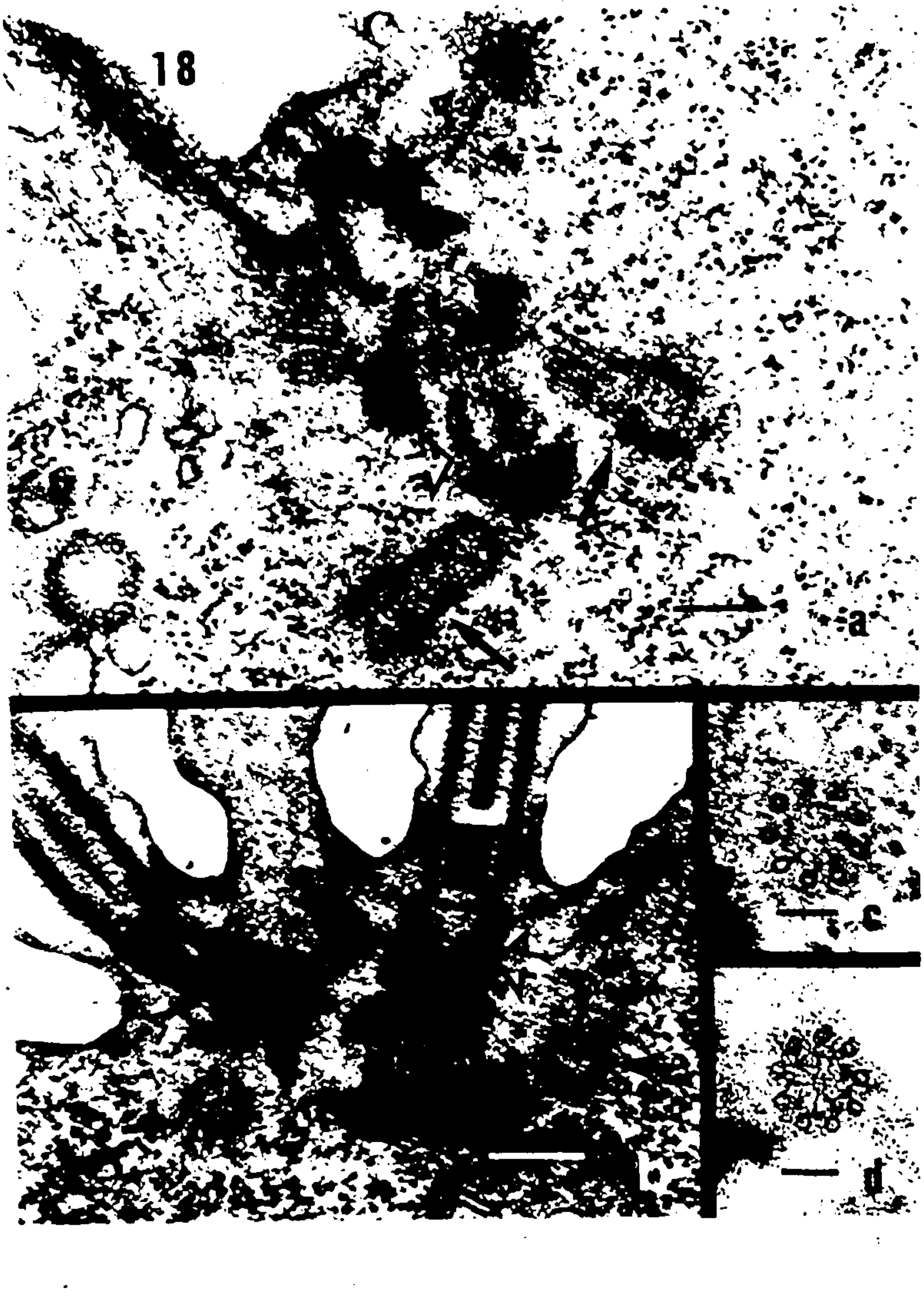

18
a
b
c
d

Figure 19 Longitudinal section through a late telophase cell with two nuclei showing forming basal body (arrow) at the anterior end of the cell. X 18,000 Bar: 0.5 µm

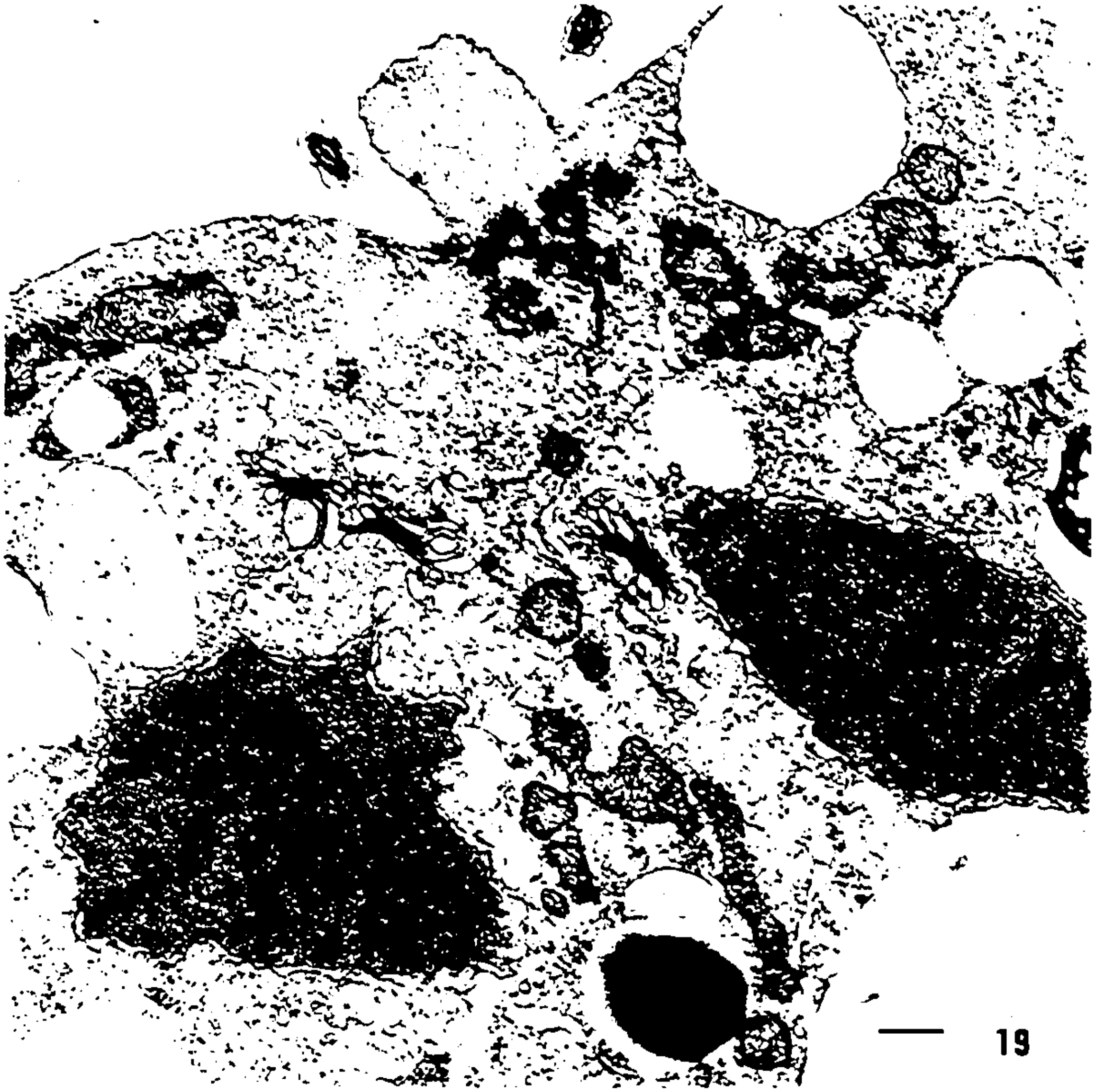


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Figure 20 Non-adjacent serial sections through the papilla region of the cell shown in Figure 19. The complete set of four forming basal bodies can be observed (arrows 1 to 4).
X 20,000 Bar: 0.5 µm

20

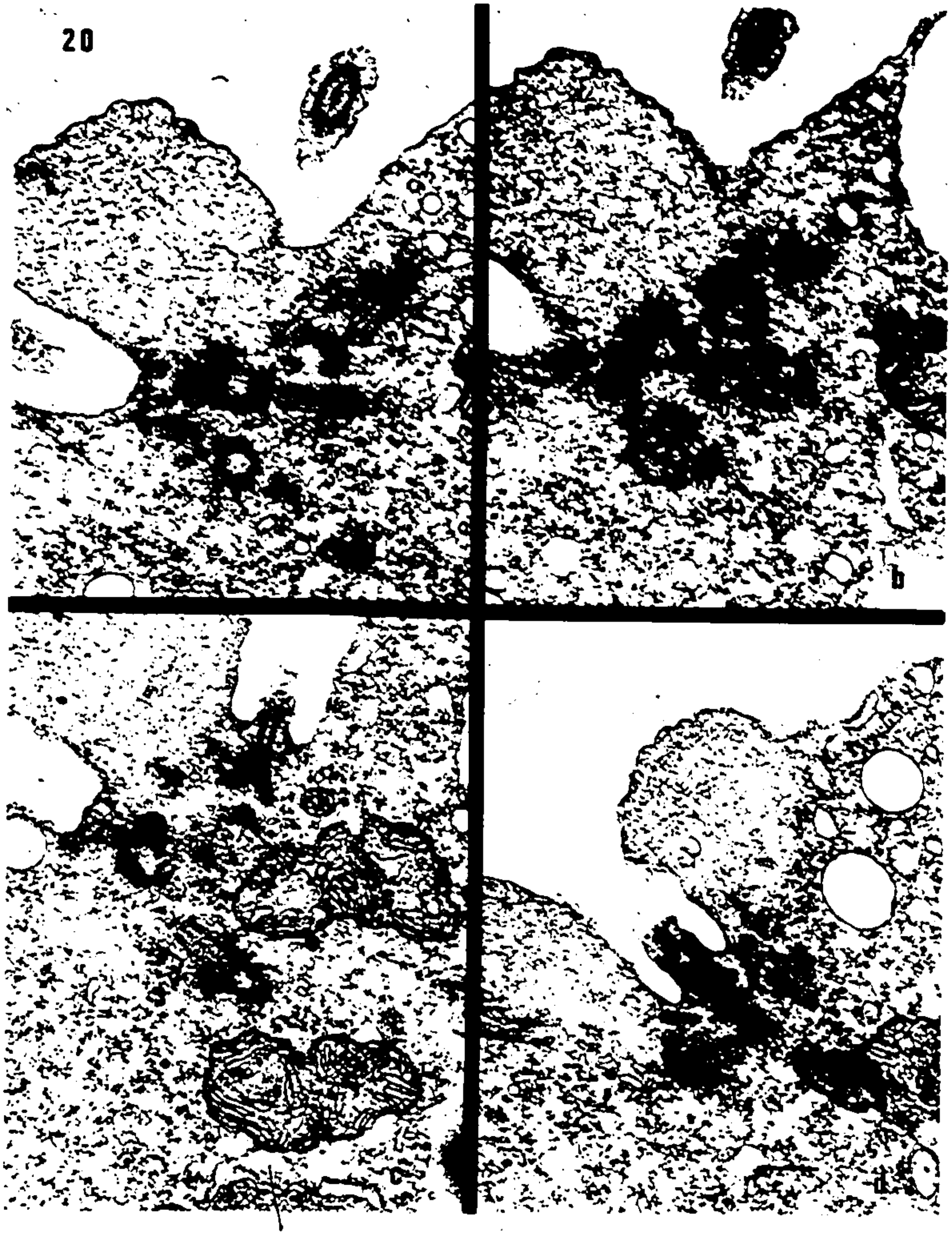


Figure 21

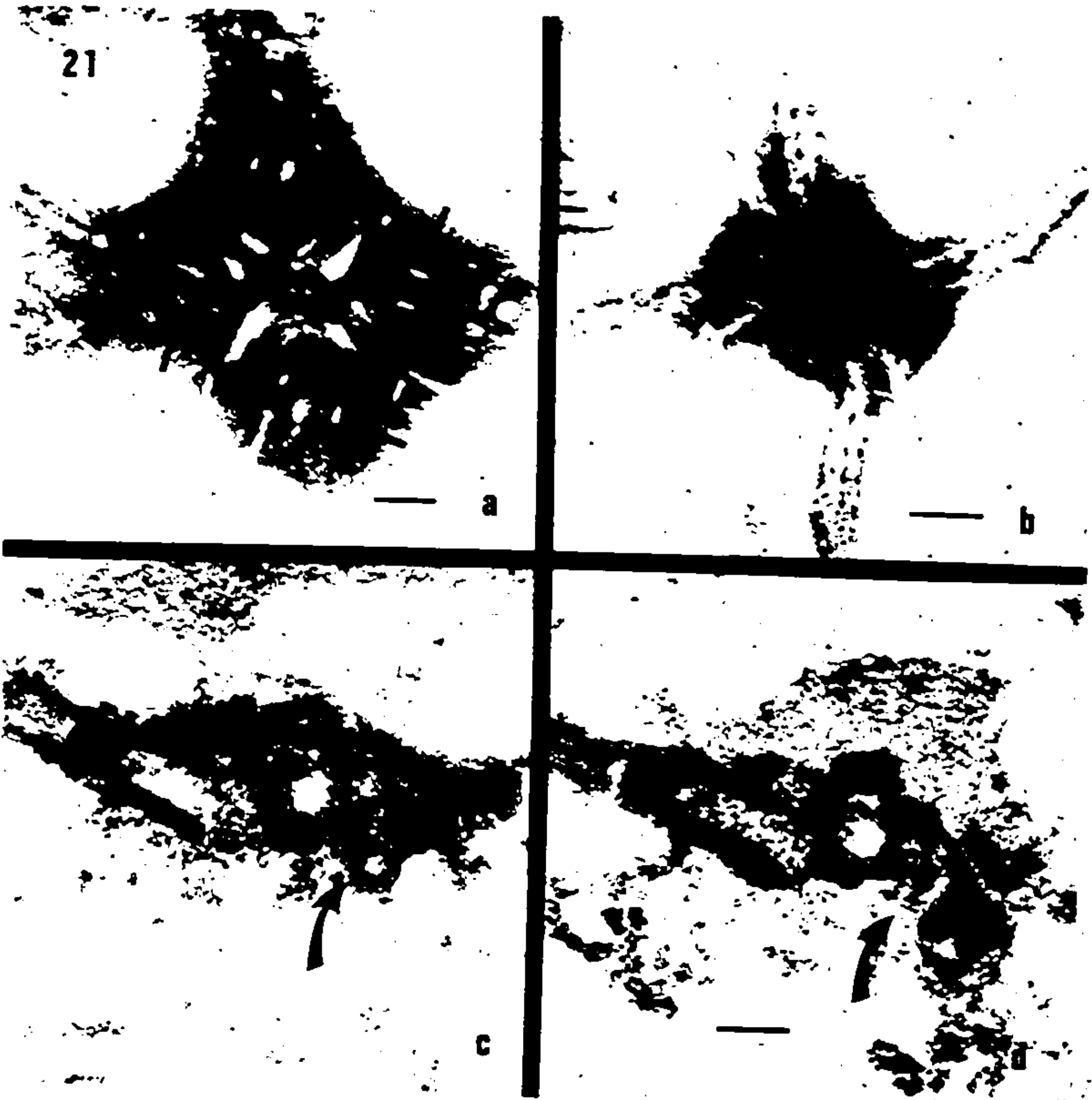
a) Isolated basal body-rootlet complex, 40 minutes after the termination of the cold shock, showing forming basal bodies (arrows).
X 17,000 Bar: 0.5 µm

b) Isolated basal body-rootlet complex, from an expomentially growing culture, showing the absence of forming basal bodies.
X 20,000 Bar: 0.5 µm

c) Thin section of isolated basal body-rootlet complex, 40 minutes after the termination of the cold shock, showing a forming basal body in the singlet stage (arrow). X 33,000

d) Thin section of isolated basal body-rootlet complex, isolated immediately after the termination of the cold shock, showing a forming basal body (to the right of the arrow).
X 33,000 Bar: 0.3 µm

21



DISCUSSION

The totally closed spindle displayed by *Polytomella agilis* has also been reported for *Chlamydomonas moewusi* (Triemer and Brown, 1974) and *Asteromonas gracilis* (Floyd, 1978). Other closely related organisms such as *Chlamydomonas reinhardi* (Johnson and Porter, 1968) display polar fenestrae during mitosis. A possible role for these polar fenestrae is to allow the entry of cytoplasmic microtubules (Johnson and Porter, 1968) or MTOCs (Heath, 1980) into the nucleoplasm in order to form the mitotic spindle. If such is the case, it would appear as if organisms which lack polar fenestrae (ie. *Polytomella*), maintain an intranuclear MTOC responsible for mitotic organization. Whether or not disruptions occur in the nuclear envelope, most unicellular green algae maintain their nuclear envelope throughout mitosis (see Table 1 in the Introduction).

The collapsing telophase spindle in *Polytomella agilis* is a characteristic feature displayed by all the chlorophycean algae in Table 1. Unlike these algae, *Polytomella agilis* does not appear to have a phycoplast. The phycoplast in the Chlorophyceae is always associated with the cleavage apparatus as it bisects the cell between the two adjacent nuclei. This has led Mattox and Stewart (1977) to postulate that the phycoplast appeared at the same time as the collapsing telophase spindle to ensure equal distribution of the nuclei within the crowded cytoplasm of walled cells. Based on this assumption, Polytomella agilis would not require a phycoplast since it lacks a cell wall and its nuclei appear to be already widely separated prior to cleavage of the cytoplasm (Figure 17g).

In most of the Chlorophyceae, the nucleus approaches the basal bodies from preprophase to prophase, a considerable number of microtubules appear in the cytoplasm between the basal bodies and the nucleus, and as the nucleus gradually elongates into a crescent-shaped spindle, the basal bodies are distributed to opposite parts of the cell. In Polytomella agilis, the nucleus appears to remain in its interphase position during mitosis, microtubules are not found between the basal bodies and the nucleus, and the basal bodies do not separate nor detach from the flagella, but remain at their usual interphase position, obviously removed from the nuclear poles throughout mitosis. Based on Pickett-Heaps' (1969) view that the distribution of the basal bodies in these algae is ensured by the elongating mitotic spindle, two tentative conclusions have been drawn from these mitotic differences: 1) since Polytomella lacks both centrioles and cytoplasmic microtubles near its nucleus, it may be that these microtubules, seen only during mitosis in other Chlorophyceae, are part of a structural system responsible for the distribution of the centrioles; 2) the distribution of basal bodies in Polytomella is ensured by a different mechanism than that in the other Chlorophyceae.

The duration of cytokinesis when the cell is placed on a slide is much slower (roughly 20 minutes) than when it is in the culture medium, as indicated by the difficulty of finding dividing cells in a log phase culture. This process of cell division thus appears to be sensitive to environmental influences such as temperature changes and confinement in P. agilis

A different type of cytokinesis, where the cleavage furrow begins at the posterior end and proceeds to the anterior end, has been reported for Polytomella caeca by Lewis et al. (1974). This report has made us question whether our observations were artifacts of the cold shock. We have rejected this hypothesis based on the fact that cell division is a conservative process and a change in temperature would not likely cause a completely different type of cytokinesis. This leaves us with a perplexing situation where the genus Polytomella exhibits two types of cytokinesis.

Stages of basal body formation in Polytomella are similar to those reported for Chlamydomonas (Cavalier-Smith, 1974). Several forming basal bodies consisting of nine singlets surrounding the cartwheel structure have been observed. The cartwheel structure

(Figure 18d) is located at the base of the basal body (Ringer, 1967). Gould (1975) has reported that forming basal bodies in Chlamydomonas develop from a ring of triplets and that the observed stages of singlets were sections through the elongating tip of the basal body. Since the nine singlets have been found surrounding a cartwheel for Chlamydomonas (Cavalier-Smith, 1974), Polytomella and other organisms, it seems unlikely that these rings of singlets are views of the elongating tip. Our observations support the view of Cavalier-Smith (1974) that basal bodies are formed by the assembly of additional microtubules unto the singlets to form the characteristic cylinder of triplets in the mature stage.

Basal body formation in Polytomella appears to be closely associated in time to mitosis, although the exact timing could not be determined. Triemer and Brown (1974) have proposed that basal body formation occurs early (preprophase) in Chlamydomonas while Pickett-Heaps (1973) has proposed that it occurs later (telophase) in Tetraspora.

Basal bodies isolated from homogenized cells remain firmly attached to each other in Chlamydomonas (Cavalier-Smith, 1974). Isolation of basal body complexes consisting of four new and four old basal bodies in Polytomella (Figure 21a) indicates a strong attachment of not only the old basal bodies but also of the new set to the old one. Observation of the eight original rootlets in these complexes also suggests that basal body elongation is completed before the new rootlets are assembled.

Marano (1976) has stated that the cleavage furrow in *Dunaliella* separates the basal bodies into pairs of one old and one new basal body. The observation of a shorter pair of flagella in each set during migration of the flagellar complexes during early cytokinesis suggests a similar semiconservative distribution of basal bodies in *P. agilis*.

A close relationship between *Polytomella* and *Chlamydomonas* has already been suggested (Brown et al, 1976c) by the similarity in their flagellar apparatuses: the connecting fibers between the A pair of basal bodies in *Polytomella* are identical to those of *Chlamydomonas* (Goodenough and Weiss, 1978) and the rootlets in both organisms consist of identical compound and microtubular rootlets, in a cruciate arrangement. The same type of rootlets have been shown in *Dunaliella* (Eyden, 1975). In the classifications of Fritsch (1935) and Smith (1950), *Dunaliella* and *Polytomella* are one family (Polyblepharidaceae) removed from *Chlamydomonas* (Chlamydomonadaceae) because of the absence of a cell wall which has likely been lost (Fritsch, 1935).

This thesis has shown a similarity in cell division (i.e. collapsing telophase spindle) between Polytomella, Dunaliella and Chlamydomonas which supports the classification of Polytomella within the Chlorophyceae. The difference in the basal body distribution and the lack of phycoplast indicates that Polytomella diverged from a chlamydomonad-like ancestor early in its history.

Studies of cell division in other phytomonads and biochemical studies of the type carried out in Tolbert's laboratory (Frederick et al., 1973) should contribute to further elucidate the phylogeny for this heterogeneous group of organisms.

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