VEGETATIVE MITOSIS IN THE MULTINUCLEATE GREEN ALGA, *CLADOPHORA FLEXUOSA*: AN ULTRASTRUCTURAL STUDY

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ABSTRACT

Transmission electron microscopy was utilized to examine the mitotic sequence in vegetatively dividing nuclei of Cladophora flexuosa. Mitosis is primarily restricted to nuclei of apical cells which undergo numerous concurrent asynchronous divisions. The nuclear envelope remains intact throughout the cycle. The spindle is exclusively intranuclear. Paired nucleus associated organelles of unknown function, described as "abbreviated centrioles" are absent during interphase but appear to arise de novo during prometaphase. The nucleolus persists in a diffuse, fragmentary form from middle prophase until regaining its compact structure at telophase. Spindle elongation plays a major role in the separation of daughter nuclei, shortening of the chromosome-to-pole distance being secondary. Separation of daughter nuclei from the interzonal spindle may be effected by nuclear rotation. Aside from minor variations, the sequence described for vegetative mitosis in C. flexuosa compares favorably with accounts of mitosis in other members of this genus as well as in other life history forms of this species. Current evolutionary schemes for the green algae are reviewed; however, placement of the Cladophorales among any particular line remains premature. Major ultrastructural features are discussed with respect to their use as phylogenetic indicators.

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INTRODUCTION

Mitosis has been a subject of scientific endeavor for over a century (Paweletz, 1964). Its continued longevity as a topic of research interest can be attributed to the intrinsic importance of mitosis as a process fundamental to cell reproduction, the complex nature of mitotic behavior manifested by numerous variations both among and within cell types, its value as a subject for the study of non-muscle motility, and its value in contributing to the determination of phylogenetic affinities.

The present paper is a report on the findings of an ultrastructural study of vegetative mitosis in the green alga, Cladophora flexuosa. The study was undertaken to provide a detailed ultrastructural account of the mitotic sequence in vegetatively dividing nuclei of this species, to note pertinent interspecific and intraspecific mitotic variations, and to discuss the significance of the observations with respect to phylogenetic considerations.

Cladophora flexuosa Dillw. Harv. is a marine green alga characterized by abundantly branched, uniseriate filaments composed of large multinucleate cells (Figs. 1, 2). Growth in this species is primarily apical, cytokinetic divisions occuring sometime after completion of numerous asynchronous mitotic divisions. Cytokinetic divisions perpendicular to the axis of the filament concomitant with cell growth increase filament length, while those occuring at

approximately 45° angles to the filament axis initiate branch formation (Figs. 1, 2).

C. flexuosa is assumed to exhibit an isomorphic alternation of generations (Fritsch, 1935; Scott & Bullock, 1976), a life history which is ideal for studying intraspecific cytological differences. This type of life history is uncommon in green algae. Although there are a small number of freshwater green algae, including Cladophora, that have isomorphic alternation of generations, most green algae exhibiting this life history are marine. Diploid individuals undergo reduction division to produce quadriflagellate zoospores which will develop into gametophyte generations. Haploid individuals can subsequently undergo mitotic divisions producing biflagellate gametes which fuse to form diploid zygotes that will mature to reconstitute the diploid sporophyte generation. Purely vegetative mitoses are primarily restricted to young plants of both haploid and diploid organisms.

Previous investigations of cell division in this genus include examinations of vegetative mitosis in C. fracta (Mughal & Godward, 1973), of mitosis during zoosporogenesis and vegetative cytokinesis in C. glomerata (McDonald & Pickett-Heaps, 1976), and of pregametangial mitosis and cytokinesis in C. flexuosa (Scott & Bullock, 1976). Some preliminary observations on meiosis preceeding zoosporogenesis in C. flexuosa have also been made (Scott, unpublished data).

MATERIALS AND METHODS

Four whole-plant specimens of C. flexuosa were collected during April at Sandy Point, Gloucester County, Virginia. Fixations were carried out immediately on portions of whole plants at field temperature for 2 hours in 4% glutaraldehyde in 0.1M phosphate buffer (pH 6.6) containing 0.15M sucrose. After several rinses in phosphate buffer (as above), the material was post-fixed in 1% osmium tetroxide (buffered as above) for 2 hours at 4° C. After 5 minutes in 50% acetone, specimens were stained en bloc in 70% acetone containing 2% uranyl acetate for 24 hours at 4° C. Dehydration was continued in an ascending acetone series the next day with three final changes in absolute acetone. Material was infiltrated with and embedded in Epon 812 (Luft, 1961). Prior to embedding, some selected apical regions were excised and flat embedded in inverted Beem capsules. The remaining filaments were flat embedded in disposable Petri plates from which selected areas were chosen and removed using a jewelers saw. Silver to silver-gold sections were cut with a diamond knife on either a Porter-Blum MT-2B ultramicrotome or an LKB III Ultrotome, from appoximately 30 selected areas. Twelve mitotic nuclei were serially sectioned throughout their entirety. Approximately 25 additional nuclei were partially serially sectioned. Numerous other mitotic nuclei were sampled without the advantages of serial sectioning.

Sections were stained with lead citrate (Sato, 1967), mounted on single slot formvar-coated copper grids, and examined using a Zeiss EM 9S-2 transmission electron microscope.

RESULTS

The interphase nucleus (Fig. 3) is essentially spherical and exhibits clumps of heterochromatin appressed to the inner membrane of the nuclear envelope and scattered throughout the nucleoplasm. The nucleolus is compact in structure and is characterized by an inner fibrillar area surrounded by a less dense granular region. Centrioles or any nucleus associated organelles (NAOs) are absent. Occasionally a few extranuclear microtubules (MTs) may be present in the surrounding cytoplasm.

During prophase, the chromatin condenses into chromosomes (Figs. 4, 5), MTs begin to appear in the nucleoplasm and are associated with both chromosomal and nucleolar components (Fig 5). Simultaneously the nucleolus begins to disperse (Figs. 4, 5). Dense granules of various sizes can be seen in the nucleoplasm and are present during all mitotic stages. The nuclear envelope is intact and remains so throughout the entire mitotic cycle.

By prometaphase the axis of the spindle is defined (Fig. 6). The nucleolus continues to disperse and migrate. Eventually it will usually occupy a position adjacent to the nuclear envelope, slightly poleward of the chromosomes, and become crescent in shape (Figs. 9, 16, 18). Chromosomes begin a congression to form a metaphase plate (Fig. 7).

Early metaphase nuclei demonstrate elongation in the region of the forming metaphase plate and also in polar directions (Figs. 7,

8, 9). Kinetochores, while not well-defined, are apparent (Figs. 8, 9). Paired NAOs at each pole are oriented at right angles to one another (Figs. 9, 10, 11). Chromosomes are unevenly distributed at the metaphase plate (Figs. 8, 16).

The paired NAOs appear to be "abbreviated centrioles" (ACs). Longitudinally sectioned, they reveal tubular elements surrounded by a ribosome-free halo (Fig. 12). Transversely sectioned, an internal structure consisting of a central hub surrounded by nine blades, at least some of which are composed of doublet tubules, is revealed (Fig. 13). The organelles measure approximately 140-170 nm in diameter and 140-150 nm in length. They appear to arise de novo during prometaphase in the absence of any structural precursor. Nuclear pores adjacent to ACs stain densely (Figs. 12, 13, 14). Other planes of sectioning through ACs show them to be situated in pit-like depressions of the nuclear envelope (Fig. 14) similar to nuclear pockets in fungi (Markey & Wilce, 1975). Numerous MTs radiate from them both toward the nuclear envelope and tangential to it (Figs 14, 15). MTs have never been observed to penetrate the nuclear envelope or to become channeled through nuclear pores.

Kinetochores become well-defined as metaphase proceeds (Figs. 16, 17). Structurally trilaminar, they consist of an outer kinetochore plate distal to the chromatid, attached to approximately 4 to 6 MTs, separated from an inner dense band attached to the chromatid, by an electron lucent zone.

Anaphase nuclei are distinguished by a progressive flattening of the former metaphase plate region accompanied by a shortening of the chromosome-to-pole distance and nuclear elongation as chromatids

migrate poleward in a staggered configuration. The result is a dumbbell-shaped nucleus with two bulbous ends separated by a microtubular spindle interzone (IZ) (Fig. 18). Extensive golgi activity, polyribosomal aggregates, and networks of rough endoplasmic reticulum are found associated with anaphase nuclei (Fig. 18).

Telophase culminates in the separation of daughter nuclei from the spindle IZ (Figs. 19, 23). By telophase, nucleoli have reacquired a compact appearance (Figs.21, 22). Depolymerization of MTs within daughter nuclei occurs from the poles toward the spindle IZ as nuclei gradually take on a spherical shape (Fig. 20).

The position and angle of the spindle IZ relative to daughter nuclei suggests nuclear rotation may play a role in the separation process (Fig. 22). Other observations on recently separated daughter nuclei reveal a few extranuclear MTs which appear to cradle the nucleus by means of an obtuse "V" formation (Fig. 23). Newly separated daughter nuclei are further isolated from each other by vacuolar intrusion into the surrounding cytoplasm (Fig. 24).

DISCUSSION

ABBREVIATED CENTRIOLES

The presence of centrioles or centriole-like structures at the poles in vegetative mitotic nuclei of C. flexuosa was unexpected. Because the small, paired ACs escaped detection for a considerable time during this study, the argument that because a flagellated form, i.e. zoospore or gamete, was not being produced, centrioles were unnecessary and therefore understandably absent was initially convincing. This postulate was reinforced by previous reports of acentric spindles in vegetative mitotic nuclei in C. fracta (Mughal & Godward, 1973), Chara (Pickett-Heaps, 1967), Oedogonium (Pickett-Heaps & Fowke, 1969a, 1969b), and Nitella (Turner, 1968), green algae which are characterized by centric spindles during spermatogenesis (Nitella, Turner, 1968; Chara, Pickett-Heaps, 1968), zoosporogenesis (C. glomerata, McDonald & Pickett-Heaps, 1968; Oedogonium, Pickett-Heaps, 1971a), and gametogenesis (C. flexuosa, Scott & Bullock, 1976). Other studies documenting both centric and acentric divisions during different stages of their life history or development include reports on diatoms (Lithodesmium, Manton et al., 1970), protozoa (Naegleria, Fulton & Dingle, 1971), slime molds (Physarum, Aldrich, 1969), bryophytes (Anthoceros, Marchantia, Moser & Kreitner, 1970), and mouse embryos (Szollosi et al., 1972).

The initial assumption that vegetative spindles of C. flexuosa

were acentric was proven to be both premature and erroneous by one fortuitous slice that revealed a longitudinally sectioned centriole-like pair at the division pole of a metaphase cell.

Examination of serial sections showed these organelles to occur in pairs, one oriented at right angles to the other, each consisting of an internal "cartwheel" structure measuring 140-170 nm in diameter, comprised of a central hub surrounded by a wall of nine tubular blades. The number of MTs per blade appeared to vary between one and two; however, numbers of MTs in other blades could not always be distinguished. Contrary to mature or complete centrioles, triplets were never observed.

In addition to differences in internal structure, an unmistakable size discrepancy in both diameter and length between these NAOs and classic centrioles was noted. These structures measured approximately 140-170 nm in diameter and 140-150 nm in length as compared to the 200-250 nm diameter and 300-350 nm length of classic centrioles. Although similar in size to the protocentrioles in the slime mold Labyrinthula described by Perkins (1970), Perkins & Amon (1969), and Porter (1971), the NAOs in C. flexuosa were characterized by definite tubular elements. The internal structure of C. flexuosa's polar organelles compared favorably with that of procentrioles described by Fulton (1971), Gall (1961), Mizukami & Gall (1966), and Stubblefield & Brinkley (1967), However, unlike procentrioles, which are normally found at right angles to and in association with the proximal end of preexisting mature centrioles, both members of the pair in C. flexuosa were arrested in development and did not mature into classic centrioles. The differences between classic centrioles,

protocentrioles, procentrioles, and the NAOs observed in this study are sufficient to warrant the use of the qualifying adjective "abbreviated" in conjunction with the term centriole to distinguish them from other centriolar types.

Structural analogs of ACs have been reported in the green alga, Kirchneriella (Pickett-Heaps, 1970b) and the mold Allomyces (Renaud & Swift, 1964). However, the ACs of vegetative C. flexuosa are distinguished from them by their seemingly permanent rather than temporary arrest in development, the total absence of any mature centriole in association with them, and their absence during interphase.

Unfornately, evidence of the origin, morphogenesis, or function of the ACs was not a product of this study. Failure to detect ACs in approximately twelve serially-sectioned interphase nuclei infers rapid assembly of ACs from structurally unrecognizable precursors or de novo synthesis.

A hypothesis for AC behavior based on extrapolations from patterns of normal centriole behavior (Gall, 1961; Murray et al., 1965; Robbins & Gonatas, 1964; Turner, 1968) suggests synthesis of ACs early in prophase followed by the relocation of each AC pair to its respective pole by late prophase. The earliest observations of ACs were found in prometaphase nuclei.

Regarding function, the relatively few MTs found emanating from and/or adjacent to ACs may indicate their role, if any, as MT organizing centers (MTOCs) is limited. Aside from this, inferences pertaining to AC function cannot be made.

In general, centriolar function during mitosis remains obscure. At one time, spindle formation was ascribed to centrioles (Brinkley

& Stubblefield, 1970); however, this view, based on animal cell studies, failed to explain the normal spindle formation displayed by acentric cells, most notably those of higher plants. A number of reports (Dietz, 1959, 1966; Hepler & Jackson, 1969; Mole-Bajer, 1967; Pickett-Heaps, 1969, 1971b; Szollosi, 1972) provided evidence refuting the spindle organizing role. Additional reports (Dietz, 1966; Friedlander & Wahrman, 1970; Pickett-Heaps, 1969, 1971b) have assigned the centriole the role of a passenger merely distributed by the spindle rather than effecting its formation.

Laser irradiation of the pericentriolar material in prophase nuclei of rat kangaroo cells, although failing to interfere with spindle formation, metakinesis, or cytokinesis, effectively blocked anaphase chromosome movements (Berns et al., 1977), indicating that the region adjacent to centrioles most likely is responsible for spindle morphogenesis. In a later study employing rat kangaroo cells, laserbeam irradiation of centrioles in prophase nuclei did not affect spindle formation, metakinesis, anaphase separation of chromosomes, or cytokinesis (Berns & Richardson, 1977). While Berns and Richardson (1977) could not dismiss the possibility of a passive role for centrioles during mitosis, their evidence strongly argued against any active role.

Centrioles are present during interphase and throughout mitosis is pregametangial and prezoosporangial C. flexuosa (Scott, unpublished data; Scott & Bullock, 1976). The fact that centriolar development is arrested in vegetatively dividing nuclei as opposed to being totally absent may imply some function, unknown to date, or, ACs could be interpreted as remnants in the process of being lost

over evolutionary time.

SPINDLE DYNAMICS

Despite the large number of cell types in which mitosis has been studied, a number of relevant questions remain unanswered, most of which concern spindle dynamics. Broadly speaking, spindle dynamics can be categorized into three phenomena: spindle formation, chromosome movement and spindle elongation, and spindle dissolution and separation of daughter nuclei from the spindle interzone. The discussion which follows is a summary of current ideas regarding these, accompanied by pertinent remarks concerning C. flexuosa.

Spindle Formation

McIntosh (1977) functionally defined the spindle as "the fibrous cellular machinery that segregates eukaryotic chromosomes at cell division." Structurally that "fibrous cellular machinery" is composed primarily of MTs with an associated matrix of ribosome-like particles, small vesicles, and ill-defined filamentous material. MTs, as major constituents of the spindle, and whose appearance and disappearance visually characterize the mitotic sequence, are the logical focal point for the study of spindle formation.

MTs are polymers of tubulin subunits which under physiological conditions exist as 6s dimers that vary from 110,000 to 120,000 MW (Borisy & Taylor, 1967; Snyder & McIntosh, 1976). Alpha and beta subunits of the tubulin dimers are thought to be the morphological units that make up protofilaments, 12 to 15 of which, arranged in a left-handed helix, constitute a MT wall (Behnke & Zelander, 1967; Burton et al., 1975; Erickson, 1974a, 1974b; Ledbetter & Porter,

1964; Nagano & Suzuki, 1975; Porter, 1966; Tilney et al., 1973). In vitro addition of tubulin to pre-existing tubules reveals that MTs display a structural polarity, with tubulin preferentially adding more rapidly to one end of the tubule than to the other (Allen & Borisy, 1974; Bergen & Borisy, 1980; Binder et al., 1975; Dentler et al., 1974; Rosenbaum et al., 1975; Summers & Kirschner, 1979). In vitro polymerization of tubulin, although exhibiting polar addition, spatially results in a random distribution of MTs (Borisy & Gould, 1977).

Although mechanisms of assembly/disassembly of mitotic MTs are not yet clearly understood, factors which have been shown to control MT assembly in vitro include pH and ionic strength (Olmsted & Borisy, 1973, 1975; Lee et al., 1974; Kuriyama, 1977), tubulin sulfhydryl groups (Kuriyama & Sakai, 1974; Mellon & Rebhun, 1976; Wallin et al., 1977), guanosine triphosphate (Weisenberg, 1972; Olmsted & Borisy, 1973; Lee et al., 1974), cysteine residues (Kuriyama & Sakai, 1974), ionic levels of calcium (Ca^{+2}) and magnesium (Mg^{+2}) (10^{-3}M Ca^{+2} provokes MT dissociation, 10^{-6}M Ca^{+2} stimulates MT polymerization, 10^{-3}M Mg^{+2} stimulates MT polymerization) (Borisy et al., 1975; Hepler & Palevitz, 1974; Harris, 1975; Timourian et al., 1974), Ca^{+2}-ATPase (Petzelt & vonLedebur-Villager, 1973; Harris, 1975), and other MT associated proteins (MAPs) (Weingarten et al., 1975; Burns & Pollard, 1974; Gaskin et al., 1974).

Ionic levels of the divalent cations Ca^{+2} and Mg^{+2} are considered likely candidates for the regulation of some aspects of tubule polymerization/depolymerization in vivo as well (Hepler, 1977). Recent immunofluorescence studies localizing a Ca^{+2}-dependent

regulator factor (CDR), calmodulin, at the spindle poles (Welsh et al., 1978) and subsequent work evidencing its in vitro ability to modulate MT Ca^{+2} sensitivity at physiological concentrations (Marcum et al., 1978), implicate CDR as a plausible regulator of MT polymerization/depolymerization in vivo.

Membranes, specifically endoplasmic reticulum and nuclear envelope, have been suggested as possible sites of control capable of shifting ion levels at the appropriate time so as to permit spindle MT assembly or disassembly (Hepler, 1977). Observations of well-developed endoplasmic reticulum commonly seen from middle anaphase through early telophase near vegetatively dividing nuclei of C. flexuosa may be significant in this regard.

After Mazia et al. (1972) reported the discovery of a Ca^{+2}-ATPase selectively present in the mitotic apparatus of sea urchin eggs, Petzelt and vonLedebur-Villager (1973) observed a close correlation between the activity of a Ca^{+2}-ATPase and spindle formation in parthenogenetically activated sea urchin eggs. This correlation led the authors to postulate that the enzyme was a contributing factor in controlling MT assembly via the regulation of Ca^{+2} ions. Later studies (Hepler & Palevitz, 1974; Harris, 1975) drew analogies between the endoplasmic reticulum-nuclear envelope of mitotic nuclei and the sarcoplasmic reticulum of muscle cells with respect to regulation of the ionic milieu by means of a Ca^{+2}-ATPase.

Another plausible role for the endoplasmic reticulum of dividing nuclei was suggested by Burgess and Northcote (1968). They proposed that the cisternal spaces of endoplasmic reticulum could tenably serve as a shuttle network for the transport of tubulin to

specific loci.

MAPs, specifically high molecular weight MAPS (HMW-MAPs) may govern spindle dynamics (Burns & Pollard, 1974; Gaskin et al., 1974; Sherline & Schiavone, 1978). Using indirect immunoflourescence on purified rat brain MT protein, Sherline and Schiavone (1978) were able to show that the distribution pattern of HMW-MAPs "is similar to that described for tubulin and corresponds to the known phases of mitosis."

Although assembly of MTs and conditions regulating their assembly are integral parts of spindle formation, mere assembly of MTs does not constitute a spindle. As noted previously, in vitro polymerization of tubulin results in a random distribution of MTs (Borisy & Gould, 1977). Conversely, a spindle reflects a spatially ordered array of MTs.

Spindle MTs commonly originate at the poles. These loci serve as both nucleating sites and as "microtubule organizing centers" (MTOCs) (Pickett-Heaps, 1969) which govern the spatial relationships of MTs. Studies employing lysed, dividing cells incubated with purified MT protein demonstrated centrosomes (i.e. centrioles plus associated pericentriolar material) were capable of MT nucleation in vitro (Weisenberg & Rosenfeld, 1975; McGill and Brinkley, 1975; Snyder & McIntosh, 1975). From their work with lysed Chinese hamster ovarian cells, Gould and Borisy (1977) were able to determine that pericentriolar material is the centrosomal component responsible for MT nucleation.

Amorphous osmiophilic masses associated with or in the absence of discrete NAOs may function as MTOCs (Pickett-Heaps, 1969). In

the case of higher plants, often no easily recognizable electron dense masses are apparent. Judging from the scarcity of associated MTs, the extranuclear centrosome of vegetative C. flexuosa appears to have a very limited role as an MTOC. Spindle MTs originate inside the nucleus in close proximity to the nuclear envelope. Contrary to reports from studies on other members of this genus (McDonald & Pickett-Heaps, 1976; Scott & Bullock, 1976), no masses of amorphous electron dense material are found at the poles of vegetative C. flexuosa. Despite the absence of any identifiable structure, clearly some MTOC is operating. Considering that the spindle is totally intranuclear, the concept of modified polar regions of the nuclear envelope acting as MTOCs would seen viable. The electron dense staining of nuclear pores in polar regions may be indicative of MTOC material.

Kinetochores have also been ascribed MT nucleating and organizing functions. In vitro experiments on dividing nuclei subjected to spindle fiber depolymerization from high hydrostatic pressure treatment (Pease, 1946) or ultraviolet microbeam irradiation (Inoue, 1964) revealed regeneration of chromosomal fibers at kinetochores. Incubation of lysed mitotic cells or isolated chromosomes with purified MT protein produced MTs specifically at kinetochore regions (Gould & Borisy, 1979; McGill & Brinkley, 1975; Summers & Kirschner, 1979; Telzer et al., 1975). While these results persuasively indicate nucleation at kinetochores, discrepancies among the aforementioned results and those from other studies (Snyder & McIntosh, 1975; Weisenberg & Rosenfeld, 1975) have prompted the argument that MT

binding, either as an alternative to or in conjunction with nucleation, must be considered (Pickett-Heaps & Tippit, 1978). Pickett-Heaps and Tippit (1978) have reported that kinetochores may "capture" elongating MTs in a developing spindle.

Spindle formation in C. flexuosa appears to exhibit a combination of MT nucleation occuring first at the polar regions adjacent to the inner membrane of the nuclear envelope and subsequently at kinetochores. Kinetochores and polar regions of nuclear envelope appear to demonstrate organizing influence. In addition, it is the author's opinion that self-assembly of free MTs in the nucleoplasm occurs concomitantly with site-initiated nucleation at poles and kinetochores. Eventually these free or self-assembled MTs seem to become associated with and governed by MTOCs. Crossbridging between free and organized MTs or kinetochore capture may be plausible association mechanisms by which free MTs are brought under the influence of an organizing center. The elaboration of MTs at nucleating sites other than organizing centers could allow for rapid build-up of MT machinery capable of becoming efficiently incorporated into the forming spindle. Although this idea contrasts the assumption that MT initiation in the mitotic apparatus (MA) probably occurs only at organizing centers (Luykx, 1970; Margolis et al., 1978, McIntosh et al, 1975; Pickett-Heaps, 1969), it would explain the presence and haphazard arrangement of MT fragments that do not appear to be associated with poles or kinetochores in the prometaphase nucleus. The possibility that MT fragments could have originated at an MTOC but were subsequently removed from that site by depolymerization should also be considered. The shortcomings of using static micrographs

derived from fixed cells to interpret dynamic processes in living cells are once again manifested.

Chromosome Movement and Spindle Elongation

Movement of chromosomes during mitosis leads to an eventual equal distribution of the genome between daughter nuclei at telophase. The random distribution of chromatin characteristic of interphase nuclei wanes as prophase chromatin condenses into chromosomes that begin a "congression" to the metaphase plate during prometaphase. Sometime following metakinesis, anaphase movements of chromatids toward the poles ensue.

Chromosomes of C. flexuosa nuclei undergoing vegetative mitosis apparently lack precision choreography during their redistribution. Rarely, if indeed ever, does one find a classic metaphase plate arrangement of chromosomes. Anaphase movements are asynchronous as well.

More important that the redistribution of chromosomes is the question of the motive force(s) responsible for such movement. Proposed models for anaphase elongation and chromosome movement include: the MT assembly/disassembly model, the sliding tubule model, movement by intrinsic MT behavior, the zipper hypothesis, and the contractile model.

Based on the dynamic equilibrium that exists between soluble tubulin dimers and assembled MTs, Inoue and colleagues (1964, 1967, 1975) have suggested chromosome movement can be attributed to the disassembly of MTs at the poles causing a shortening of kinetochore MTs and subsequent chromosome-to-pole movement. Additionally they

contend that spindle elongation is achieved by the addition of tubulin dimers to interpolar MTs, thus increasing the distance between poles. Although studies have confirmed chromosome-to-pole movement in response to MT depolymerization via spindle treatment with known MT poisons or as a result of experimentally induced spindle elongation (Inoue & Ritter, 1975; Salmon, 1975), evidence that MT assembly/disassembly per se provides the motive force for such movement is lacking (Nicklas, 1975; Pickett-Heaps & Bajer, 1977).

McIntosh et al. (1969) focused on MT crossbridges or arms as mechanochemical force producers for mitotic movement. According to their theory, simple lateral binding between spindle tubules is sufficient to exert a net force on chromosomes in the direction of the equator resulting in congression of chromosomes to the metaphase plate. Anaphase separation requires an active force derived from the shearing of mechanochemical couplings (i.e. crosslinkages between MTs) which presumably contain an enzyme capable of releasing energy from high energy compounds (ATP) and transducing a portion of that energy into mechanical work to move MTs and their associated material toward their respective initiating sites. Thus, antiparallel MTs (i.e. those of opposite polarity) slide over one another as a result of paraxial force production, whereas net force production from bridges between parallel tubules is zero. Tubule sliding accompanied by tubule disassembly of kinetochore MTs at the poles effects genome separation.

Tubule sliding is responsible for anaphase separation of the poles in diatom nuclei (McDonald et al., 1977; Pickett-Heaps & Bajer, 1977). MT-MT interactions responsible for powering anaphase

elongation, are assumed to occur in the overlap between interdigitating half spindles. Some chromosome movement may result from the sliding apart of the half spindles.

Margolis et al. (1978) have proposed a model for chromosome movement based on intrinsic MT behavior. Using ^{32}P-labelled guanosine triphosphate, Margolis and Wilson (1978) demonstrated that the rate of addition of subunits to the MT in vitro is equal to the rate of subtraction at the opposite end of the tubule effecting a slow directional flux of subunits along the tubule. Extrapolating from in vitro data, Margolis et al. (1978) have postulated MT growth occuring from the poles and at kinetochores during prometaphase until an equilibrium between subunits and tubules is achieved at metaphase. A constant poleward flow of MTs and material results from depolymerization of tubules at the polar ends accompanied by assembly at the midplane or at kinetochores to preserve the steady state. During anaphase, MT assembly at the kinetochores (i.e. MT heads or "+" ends of MTs, Bergen & Borisy, 1980) is blocked. Net disassembly occurs at the poles (i.e. MT tails or "-" ends of MTs, Bergen & Borisy, 1980), paired chromatids are freed from one another, and ATP-dependent sliding between interpolar MTs powers the separation of the poles. Disassembly during telophase occurs from the spindle pole region to the interzone.

The opposite end assembly-disassembly model of Margolis et al. (1978) infers mutually exclusive sites for polymerization and depolymerization of MT subunits. In vitro studies by Summers and Kirschner (1979) and by Bergen and Borisy (1980) have demonstrated that

assembly and disassembly occur at both ends of MTs. Using flagellar axonemes as seeds and porcine brain MT protein as subunits, Bergen and Borisy (1980) found that the end of the tubule with the larger polymerization (association) constant, i.e. the "+" end or head, was also the end possessing the larger dissociation constant, the converse being true for the "-" end or tail of the MT. A head-to-tail polymerization parameter, s, was calculated to measure the efficiency of the flux of subunits along the tubules under a given set of conditions. They reported a value of s=0.07 which is far below the value s=1.0 which would reflect the implied opposite end assembly-disassembly theory postulated by Margolis et al. (1978). The very low efficiency of fluxing reported by Bergen and Borisy (1980) raises questions as to the feasibility of this model for chromosome movement. They also state that "our result that both ends of a microtubule can add and lose subunits does not fit well with the idea of Margolis et al. (1978) that in anaphase the head is blocked allowing the tail end to disassemble."

Summers and Kirschner (1979) have reported differing rates of in vitro assembly and disassembly depending on the polarity of the MT and its nucleating center. They conclude that "by adjusting depolymerization conditions to selectively attack either the plus or minus end, the cell could also eliminate or preserve a specific set of microtubules. It is also conceivable that the cell could apply a block to existing microtubules, again being selective, for which end is free. Thus, microtubules of two different stabilities can be created from the same set of subunits based entirely on the polarity of the nucleation center."

Bajer (1973, 1977) contends that the motive force for chromosome movement is supplied by multiple zippings between MTs, each zipping being responsible for a minute displacement of the kinetochore. He defines zipping as "the coming together of two MTs which bond laterally and progressively along their length in such a way that the two ends come closer together." Interaction by zipping causes MT bending and a subsequent build-up of tension in the spindle. The tendency of the bent MTs to straighten results in thrust. MT breakage or breakage of the linkages between 2 MTs follows zipping. MT polarity is of no regard to the zipper hypothesis. Zipping is assumed to be capable of occurring both toward the poles and toward the equator. The incidence of zipping is assumed to be a function of the gradient of MT distribution; rate of zipping is determined by the rate of MT disassembly.

Mitosis, with respect to the zipper hypothesis, can be explained in terms of changes in spindle tension. Prophase-prometaphase is characterized by multiple zippings which tend to align MTs in a more parallel fashion. At metaphase, a stable parallel arrangement of MTs is achieved. Zipping in the direction of the equator (i.e. toward a higher density of MTs) predominates. The increased internal tension directed toward the metaphase plate manifests itself by a decrease in spindle length and an increase in spindle diameter. Anaphase reveals an increase in chromosome velocity and the parallel arrangement of MTs is lost as spindle tension is released. Zipping occurs predominantly poleward. During telophase, MT fragments, the products of zipping, zip together, elongate by assembly, and align in parallel

fashion, thus contributing to phragmoplast or stem body formation.

Localization of actin and myosin in chromosomal fibers of the MA has prompted speculation that they may function as a contractile unit to power chromosome movement. Ultrastructural localization using heavy meromyosin (HMM) which decorates actin with "arrowheads" (Huxley, 1963) has identified actin in meiotic spindles of crane fly testes (Behnke et al., 1971; Forer & Behnke, 1972), and in mitotic spindles of locust spermatogonia (Gawadi, 1971, 1974), neuroblastoma cells (Hinkley & Telser, 1974), and Haemanthus endosperm (Forer & Jackson, 1976). The fact that few papers have reported actin-size filaments in the MA of routinely-fixed cells (i.e. glutaraldehyde-osmium) (Bajer & Mole-Bajer, 1969; McIntosh et al., 1975; Muller, 1972; Sanger & Sanger, 1975) suggests classical EM fixation methods may not adequately preserve actin. Szamier et al. (1975) have reported the destruction of actin filaments by osmium when filaments are not stabilized with HMM or tropomyosin.

Experiments employing fluorescein-labelled HMM or myosin S-1 fragments to stain rat kangaroo cells prepared by a variety of methods for the presence of actin have revealed specific staining of kinetochore fibers (Sanger, 1975, 1977; Sanger & Sanger, 1976). Cande et al. (1977) used the fluorescent antibody technique to identify spindle actin in rat kangaroo cells. Their findings, concurring with those of Sanger & Sanger (1976), reported staining exclusively along kinetochore fibers. Furthermore, no actin was found in cells subjected to colcemid treatment prior to staining for actin (Cande et al., 1977; Sanger & Sanger, 1976), indicating a relationship between MTs and spindle actin. Fujiwara & Pollard (1976) used

fluorescently labelled antibody to myosin to demonstrate staining along kinetochore fibers indicative of myosin in chromosomal fibers of HeLa cells.

The agreement among findings from the variety of methods used to localize actin and myosin in spindles of cell models argues against post-vivo translocation and is, in the words of Sanger (1977) "compelling enough to affirm that actin and myosin are to be found in the area of the chromosomal spindle fibers." Whether they function as an actomyosin system in chromosome movement is undetermined. Kiehart et al. (1976) have found that myosin antibodies injected into marine oocytes fail to interfere with anaphase chromosome movement. Nevertheless, the presence of actin and myosin in chromosomal fibers, coupled with that of CDR at spindle poles (Welsh et al., 1978), and the presence of a vesicular system conjectured to be a sarcoplasmic reticulum equivalent noted among MTs and at polar regions in several cell types (Bajer & Mole-Bajer, 1969; Friedlander & Wahrman, 1970; Harris, 1962; Hepler, 1977; Robbins & Jentzsch, 1970) make the idea of a functional actomyosin system for chromosome movement attractive. Involvement of these contractile proteins in other types of spindle associated movements might also explain their presence (Bajer, 1967; Rebhun, 1963; Sanger, 1977).

If accomplishment of chromosome movement is attributable to a single mechanism, that mechanism should be able to account for all observations on chromosome movement in all cell types. To date, no single model has been proven to do so, due to either conflicting observations and/or the absence of direct tests on living cells. Arguing against the concept of a single unified theory that

"accommodates all observations on mitotic behavior," Pickett-Heaps and Bajer (1977) caution that the search for a universal mechanism may be "inhibitory in current efforts to understand mitosis." Rather than regarding diversity as a group of nonconforming flaws that serve to undermine whatever theories may be currently in vogue, the authors have promoted the idea of multiple mechanisms, the combination of which and the degree to which each operates being variables determined by evolution. The approach does not claim to simplify the task of mechanism determination. It merely precludes equating diversity with abnormality.

An answer to the question, "Which model or combination of models on chromosome behavior best fits that observed in C. flexuosa," is not within the scope of this purely descriptive ultrastructural study. The degree of spindle complexity found in C. flexuosa tends to deter one from even addressing the question. However, observations that appear to be compatible with several of the models discussed are noteworthy.

1. Polymerization/depolymerization of MTs characterizes the mitotic sequence in C. flexuosa. Evidence that chromosome movement is powered by the reversible tubulin reaction is not available.
2. Mitotic spindles of C. flexuosa exhibit a decrease in the chromosome-to-pole distance (i.e. kinetochore-MT length) and an increase in pole-to-pole distance during anaphase. An interpretation of MT disassembly at the poles accompanied by tubulin addition to interpolar MTs appears valid.

3. Sufficient numbers of MTs and adequate proximity of MTs to each other would permit lateral interactions of the types discussed in the sliding and zipping theories.
4. What appear to be MT fragments have been observed. Establishment that they are or are not products of zipping is virtually impossible considering spindle complexity in C. flexuosa.

Thus, observations made during the study appear to agree with some aspects of the polymerization/depolymerization theory, of the MT sliding and zipping theories, and of the intrinsic MT behavior theory.

Spindle Dissolution and Separation of Daughter Nuclei from the Spindle Interzone

The number of MTs comprising the spindle wanes as mitosis proceeds through anaphase. By early to mid-telophase, most of the remaining MTs are located near the nucleus-spindle IZ interface. Depolymerization appears to occur from the poles to the spindle IZ.

Nuclear rotation has been postulated as a plausible mechanism for separation of daughter nuclei from the spindle IZ in pregametangial C. flexuosa (Scott & Bullock, 1976), in Valonia (Hori & Enomoto, 1978a), and in Ulva (Braten & Nordby, 1973). The position and angle of the spindle IZ relative to daughter nuclei observed in the present study may also indicate nuclear rotation. Assuming rotation as a separation mechanism and recalling observations from the present study of obtuse "V" formations of extranuclear MTs which

appear to cradle daughter nuclei (Fig. 23), one could infer that these MTs were once associated with polar regions and that nuclear rotation has brought them to lie proximal rather than distal to the spindle IZ. Alternatively, they may represent a separate extranuclear MT system which contributes to separation of daughter nuclei from the spindle IZ by impinging on the nuclear envelope and spindle IZ complex at strategic locations.

EVOLUTIONARY CONSIDERATIONS

Ultrastructural features of cell division are considered to be valid criteria for deducing phylogenetic affinities (Fuller, 1976; Heath, 1974, 1975; Kubai, 1975; Pickett-Heaps, 1969, 1972, 1975a, 1975b; Pickett-Heaps & Marchant, 1972; Stewart & Mattox, 1975; Stewart et al., 1974). This premise, based on the assumption that a number of cell attributes are evolutionarily conservative and allow little opportunity for structural or physiological change, has provided the foundation for recent reclassification schemes proposed for the green algae (Mattox & Stewart, 1977; Pickett-Heaps & Marchant, 1972; Stewart & Mattox, 1975a, 1975b, 1978).

The advantages and disadvantages of using mitosis as a phylogenetic indicator have been enumerated by Oakley (1978) and Heath (1980). Briefly summarizing, the primary advantages of using mitosis as a phylogenetic indicator are that it enables comparisons among all eukaryotic cells, and due to the many parts which comprise the MA, convergent evolution of the entire MA is unlikely. Disadvantages include: convergent evolution of any single component, stemming from selection pressures both recognized and unrecognized, is probable,

the lack of observations comparable in quality and quantity make collation among organisms difficult, data collection for relevant features is often formidable, and interpretation of many observations is rarely clear-cut.

Ultrastructural accounts detailing the morphology of flagellated forms and the events of mitosis and cytokinesis have delineated at least two and tentatively three distinct evolutionary lines among multicellular and advanced unicellular green algae: the Chlorophyceae, the Charophyceae, and the Ulvaceae. All three lines are believed to be the products of early evolutionary divergence from a common ancestral scaly flagellate (Mattox & Stewart, 1977; Pickett-Heaps, 1975b; Stewart & Mattox, 1975a, 1978; Stewart et al, 1974).

The Chlorophyceae are distinguished by a "cruciate" rootlet system comprised of four or more microtubular rootlets extending downward from the region of the basal bodies. Collapse of the spindle IZ during telophase preceeds the formation of a phycoplast, a parallel array of MTs in the plane of cytokinesis unique to this group. Mattox and Stewart (1977) have theorized that the collapsing IZ region and phycoplast have evolved in cells whose cellular and, consequently, nuclear elongation during anaphase-telophase is spatially confined by rigid cell walls. Precise location of the cleavage furrow between the closely separated nuclei of such cells is thought to be determined by the phycoplast.

The Charophyceae possess rootlet systems termed "multilayered structures" (MLS), and display persistent telophase spindles and phragmoplasts. The MLS consists of a broad microtubular band in conjunction with a complex lamellar structure. The phragmoplast is

a parallel array of MTs perpendicular to the plane of cytokinesis between daughter nuclei.

A biochemical distinction between the Chlorophyceae and the Charophyceae based on the type of glycolate oxidizing enzymes present has been reported (Frederick et al., 1973). From limited observations it was inferred that those species evidencing collapsing telophase spindles (i.e. Chlorophyceae) possessed glycolate dehydrogenase, whereas those with persistent telophase spindles (i.e. Charophyceae) were said to possess glycolate oxidase. Floyd and Salisbury (1977) examined glycolate enzymes in nine species of prasinophycean algae, some of which possessed collapsing telophase spindles, the remainder of which did not. Glycolate dehydrogenase was found in all nine species, suggesting that "the evolutionary divergence of the glycolate enzyme came after the evolutionary divergence of the spindle features in the green algae."

The tentative third line proposed for the green algae is the Ulvaceae, a "problematical group" whose members possess neither MLSs, phycoplasts, or phragmoplasts (Mattox & Stewart, 1977; Stewart & Mattox, 1975a, 1975b, 1978).

Phycoplasts have never been observed in Cladophora. Observations of cytokinesis in prezoosporangial C. glomerata revealed several MTs in the vicinity of the cleavage furrow but none in the division plane (McDonald & Pickett-Heaps, 1976). Cytokinesis in pregametangial C. flexuosa was reported to occur by means of "progressive vacuolation" (Scott & Bullock, 1976). Similarly, no phycoplasts were found during preliminary studies on cytokinesis in vegetative

nuclei of C. flexuosa (Lewis, unpublished) and in post-meiotic nuclei of C. flexuosa (Scott, unpublished).

Although presumed to be cruciate, no documentation exists regarding the type of rootlet system that motile forms of Cladophora possess. This assumption, inferring the absence of a MLS, coupled with the absence of both a collapsing telophase spindle and of a phycoplast during cytokinesis, indicates tentative placement of Cladophora within the Ulvaceae. (In view of such scanty evidence, the term "tentative" cannot be emphasized enough).

Alternatively, the possibility that the Cladophorales represent a branch off of the Chlorophyceae, i.e. implying that the phycoplast in this group has been lost over evolutionary time, might also be considered. The multinucleate condition itself would appear unlikely to require a precise mechanism, like the phycoplast, for determining the location of the cleavage furrow. As in Cladophora, cytoplasmic cleavage in another multinucleate green alga, Dictyosphaeria cavernosa, does not employ the phycoplast (Hori & Enomoto, 1978b). The Chlorococcales, on the other hand, are coenocytic, form phycoplasts during cytokinesis, and are further characterized by a perinuclear envelope of endoplasmic reticulum (Pickett-Heaps, 1975b), a distinction Cladophora lacks. The question arises, "Is the multinucleate condition a product of convergent evolution, or, does the condition in Cladophora represent an evolutionary advancement whereby the phycoplast, appearing to be unnecessary, has been lost?"

In more general terms, i.e. without specific regard to green algal evolution per se, several other mitotic festures may be of evolutionary significance, including the nuclear envelope, nucleolus,

and NAOs. Mitosis in Cladophora occurs within the confines of a totally closed nuclear envelope, a condition considered primitive from an evolutionary standpoint. The totally closed spindle is uncommon in green algae. More often, fenestrations or gaps in the nuclear envelope are present at the poles. In addition to Cladophora (McDonald & Pickett-Heaps, 1976; Mughal & Godward, 1973; Scott & Bullock, 1976), the totally closed spindle has been reported in the green algae Bryopsis (Burr & West, 1970), Dictyosphaeria (Hori & Enomoto, 1978c), Euglena (Gillot & Triemer, 1978), Valonia (Hori & Enomoto, 1978a), Pedimonas (Pickett-Heaps & Ott, 1974), and Trentepohlia (Graham & McBride, 1978). An intact nuclear envelope throughout mitosis has also been reported for the golden green alga Vaucheria (Ott & Brown, 1972); protozoans Tetrahymena (Elliot, 1963) and Blepharisma (Jenkins, 1967); and numerous fungi (see Heath, 1980, for review).

Although the nuclear envelope is devoid of polar fenestrations throughout karyokinesis in C. flexuosa, an apparent increase in the number of nuclear pores exists at the poles. Spindle MT biogenesis appears to be exclusively intranuclear.

Patterns of nucleolar behavior during mitosis have been discussed by Pickett-Heaps (1970a) and more recently by Heath (1980), who has constructed a hypothetical scheme for the evolution of such behavior. Heath (1980) has identified five behavioral classes of nucleoli including the persistent (Pickett-Heaps, 1970b), discardive, fragmentary, associative, and dispersive types. The persistent nucleolus retains its interphase form during mitosis until constricted and divided at telophase into two comparable halves. Discardive

nucleoli are expelled in their intact form into the cytoplasm at prophase or telophase. The type of nucleolus found in Cladophora has been termed fragmentary, implying the breaking up of the nucleolus into fragments loosely strewn or scattered about the nucleus until telophase during which intact nucleoli reform. Associative behavior involves the coating of chromosomes with diffuse fragments of nucleolar material. The dispersive type of nucleolus completely dissociates into unrecognizable elements during prophase.

The tentative evolutionary scheme proposed by Heath (1980) based on the five behaviors discussed above recognizes the persistent nucleolus, functional throughout mitosis, as the most primitive type. The persistent condition could have evolved in two directions, one giving rise to the discardive nucleolus, a type either incapable of division or unable to export ribosomes; the other giving rise to the fragmentary condition, inactive during mitosis, requiring fragments for reformation of functional nucleoli. Stemming from the fragmentary behavior, the associative pattern is viewed as a precursor of the dispersive condition which is characterized by the presence of nuclear organizing regions on chromosomes, capable of reforming nucleoli.

It is difficult to discuss the evolutionary significance of an organelle whose function is unknown, e.g. C. flexuosa's AC. Excepting one study (presumable vegetative C. fracta, Mughal & Godward, 1973) in which the presence of NAOs may have been overlooked, other reports on Cladophora cite the presence of centrioles associated with interphase nuclei as well as mitotic nuclei (prezoosporangial

C. glomerata, McDonald & Pickett-Heaps, 1976; pregametangial C. flexuosa, Scott & Bullock, 1976). The absence of centrioles in interphase vegetative nuclei and the presumably de novo synthesis of structurally abbreviated centrioles during mitosis in vegetative C. flexuosa pose intriguing questions. Do the abbreviated centrioles and their transient nature represent evolutionary advancement allowing energy conservation in a phase of life history that does not produce motile forms (i.e. are these organelles remnants), or are they variant products of genetic drift?

As previously discussed, the function of the ACs is obscure. Micrographs from the present study reveal extranuclear MT organization, in the vicinity of the ACs, which appears to contribute structural support to dividing nuclei. Based on conclusions from other studies (Berns & Richardson, 1977; Berns et al., 1977), most likely the pericentriolar material, rather than the ACs, is responsible for this MT organization. The large number of nuclear pores in the vicinity of the ACs persuasively suggests some relationship between intranuclear events and the polar organelles or their surrounding material. ACs may, in some fashion, provide depolymerized MT subunits which can be shunted through nuclear pores to contribute to spindle formation.

Obviously, the answers to many questions must be found before any definitive conclusions can be made with respect to Cladophora's phylogenetic affinities. Elements other than those presented, including kinetochores, spindle characteristics, chromatin, membranes, and biochemical attributes should also be considered from the evolutionary standpoint. Broadly speaking,Cladophora possesses

"primitive" attributes and several unusual variations of unknown significance. Additional work, both within the species and on other Siphonaceous groups, will be necessary to advance the current understanding.

CONCLUDING REMARKS

Ultrastructural events of mitosis in vegetatively dividing nuclei of C. flexuosa closely parallel the mitotic behavior reported for other members of this genus and other life history forms of this species. One salient distinction, paired ACs, warrant further investigation to probe the origin, morphogenesis, and function of these unique NAOs. Continued work to complete the ultrastructural examination of all life history forms of C. flexuosa, particularly motile stages, will further contribute to the determination of the phylogenetic affinities of the Cladophorales.

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PLATE 1

Figure 1. Light micrograph of vegetative Cladophora flexuosa apical region, X 110.

Figure 2. Light micrograph of vegetative C. flexuosa apical region. Modified feulgen preparation reveals numerous densely staining nuclei. X 344.

Figure 3. Interphase nucleus. Nucleolus, n; chromatin, c, vacuoles, v. X 15,340.

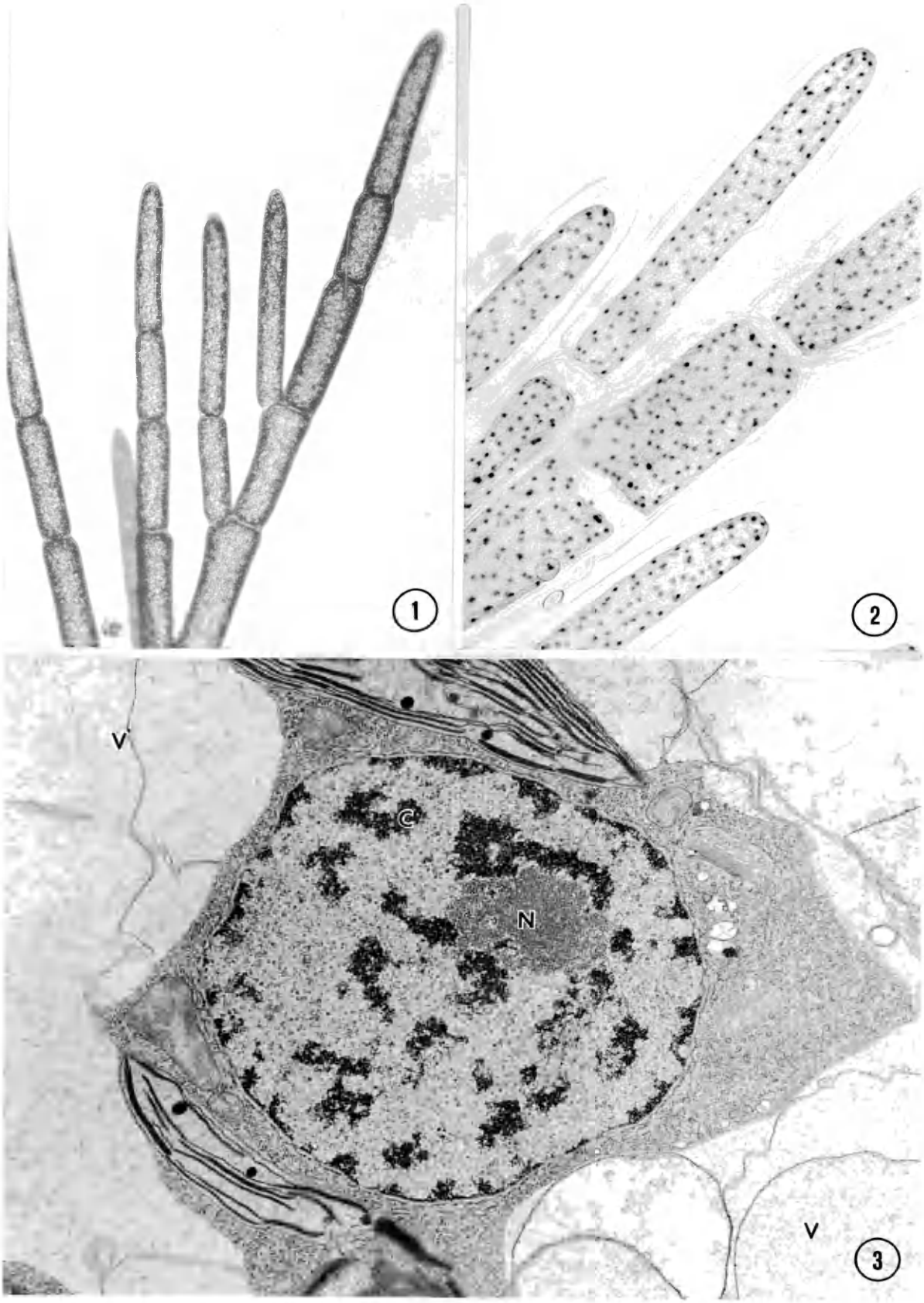
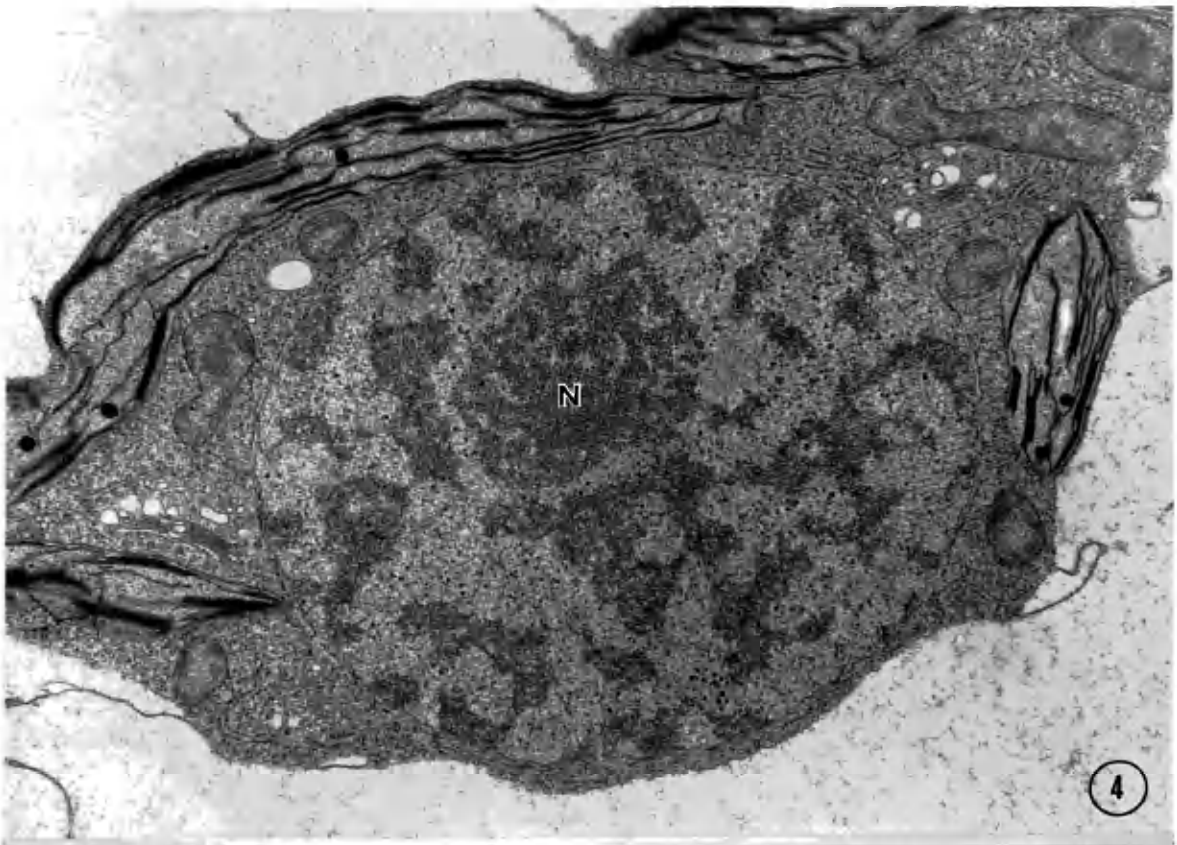

1
2
V
C
N
V
3

PLATE 2

Figure 4. Interphase-prophase transition in vegetative nucleus of C. flexuosa is marked by condensation of chromatin into chromosomes and initial nucleolar dispersion. X 14,300.

Figure 5. Late prophase nucleus. Note continued nucleolar dispersion. Microtubules (arrowheads) are present, associated with nucleolar and chrosomal components. X 18,200.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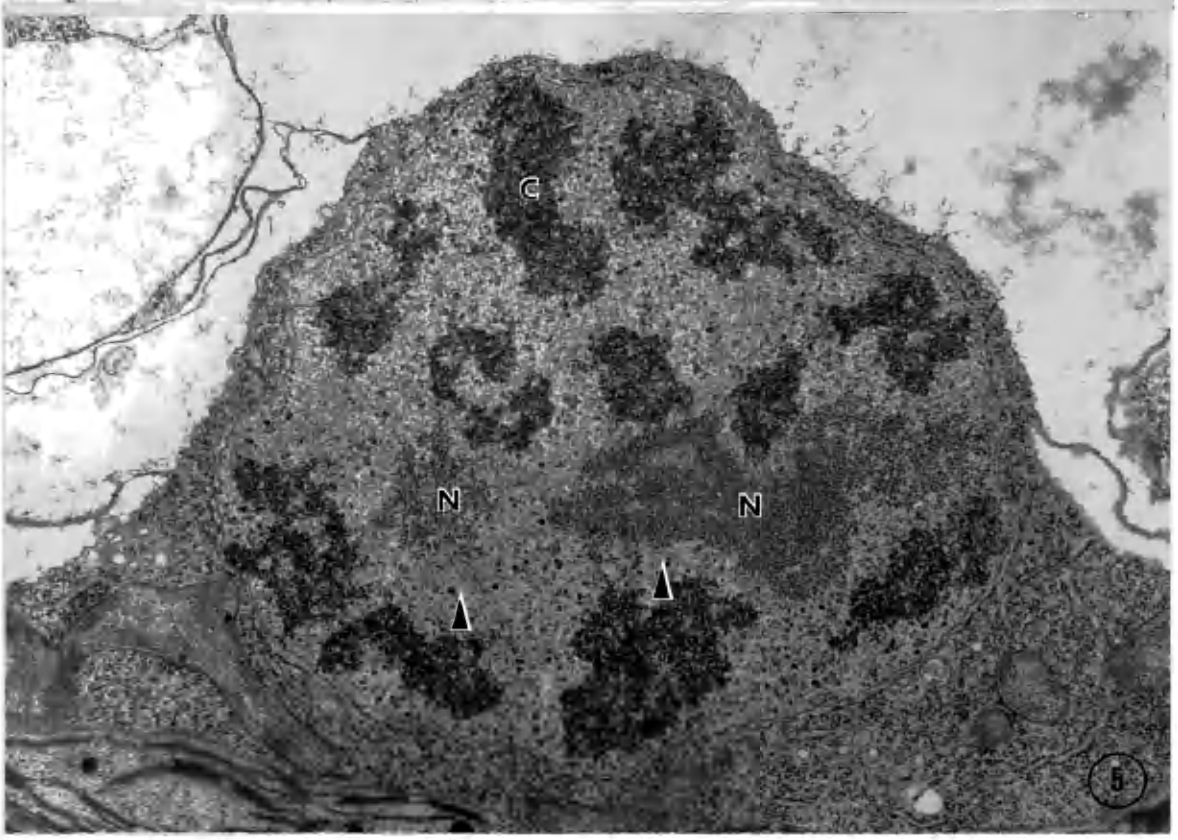
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PLATE 3

Figure 6. Prometaphase nucleus. The spindle axis is defined (between arrows). A kinetochore is visible (arrowhead). Cytoplasmic components include golgi apparatus, g; pyrenoid, p; mitochondrion, m; chloroplast, cl. X 21,700.


P
G
M
CL
6

PLATE 4

Figure 7. Prometaphase congression of chromosomes to the forming metaphase plate. Spindle is well-defined from pole to pole. X 12,700.

Figure 8. Metaphase nucleus. Chromosomes are aligned at a broad metaphase plate. X 18,330.

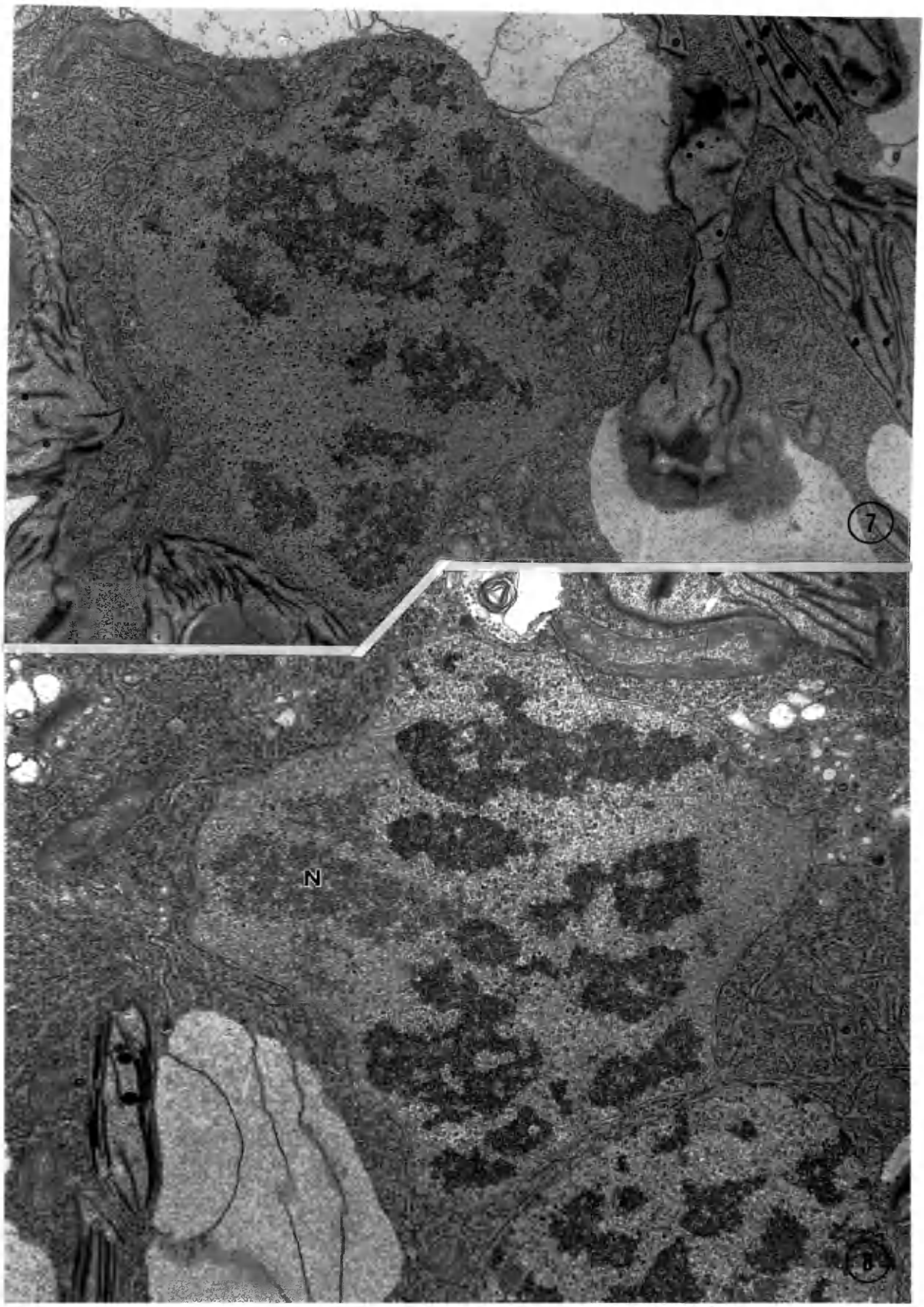
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PLATE 5

Figure 9. Metaphase nucleus with associated "abbreviated centrioles" at the poles. Nucleolus is very diffuse. X 26,125.

Figure 10. Higher magnification of left "abbreviated centriole" pair shown in Figure 9. X 48,750.

Figure 11. Higher magnification of right "abbreviated centriole" pair shown in Figure 9. X 48,750.

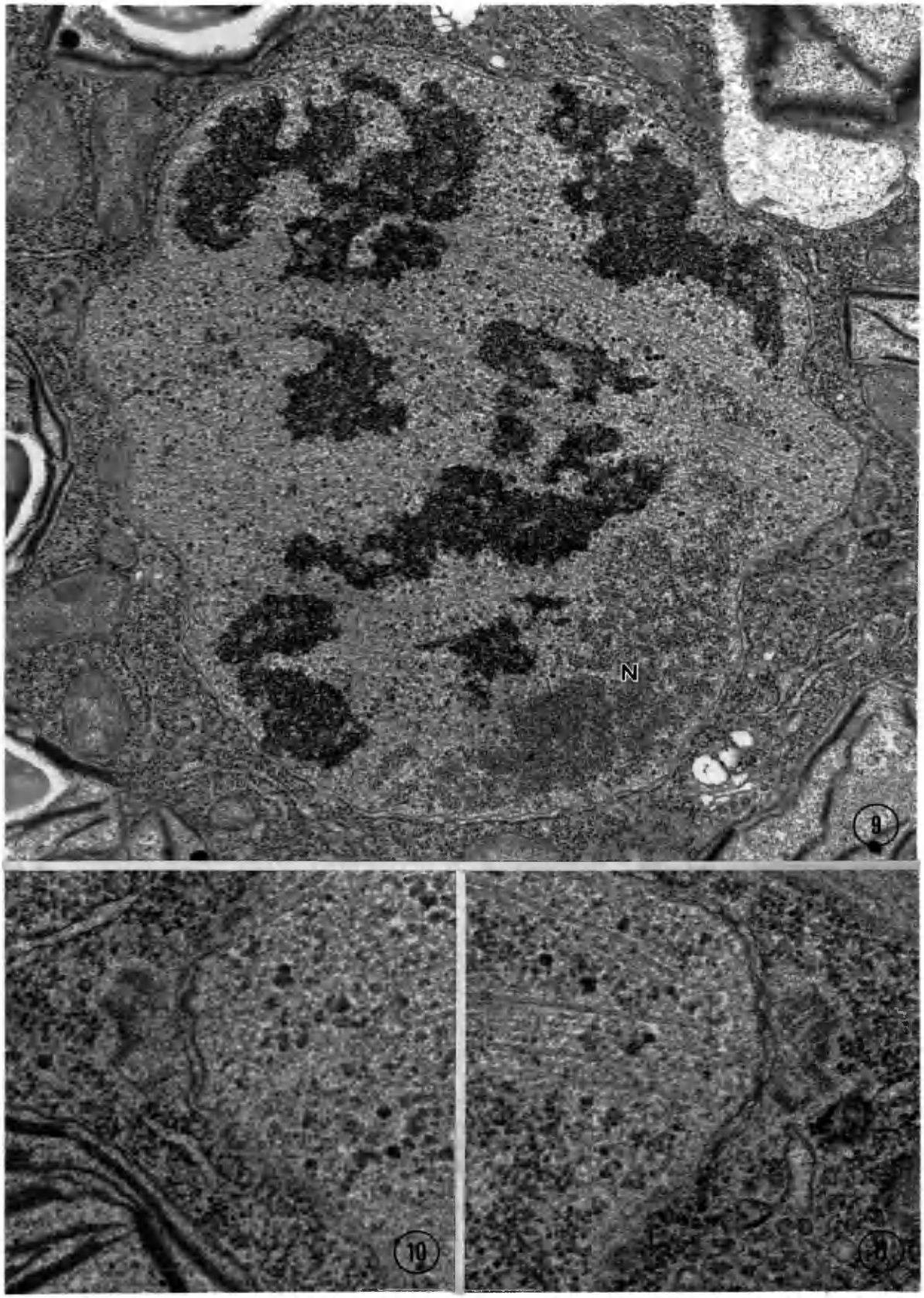
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PLATE 6

Figure 12. Slightly oblique plane of sectioning through an "abbreviated centriole" pair. Note the abundance of nuclear pores and their densely staining contents. X 65,700.

Figure 13. Cross-section through one member of an "abbreviated centriole" pair. Some blades are composed of doublet microtubules. X 61,300.

Figure 14. One member of an "abbreviated centriole" pair longitudinally sectioned, seen situated in a depression cup outlined by densely staining nuclear pores. X 50,000.

Figure 15. Tangential section through an "abbreviated centriole" pair and its associated microtubules. Nuclear pores are seen in cross-section. X 41,150.

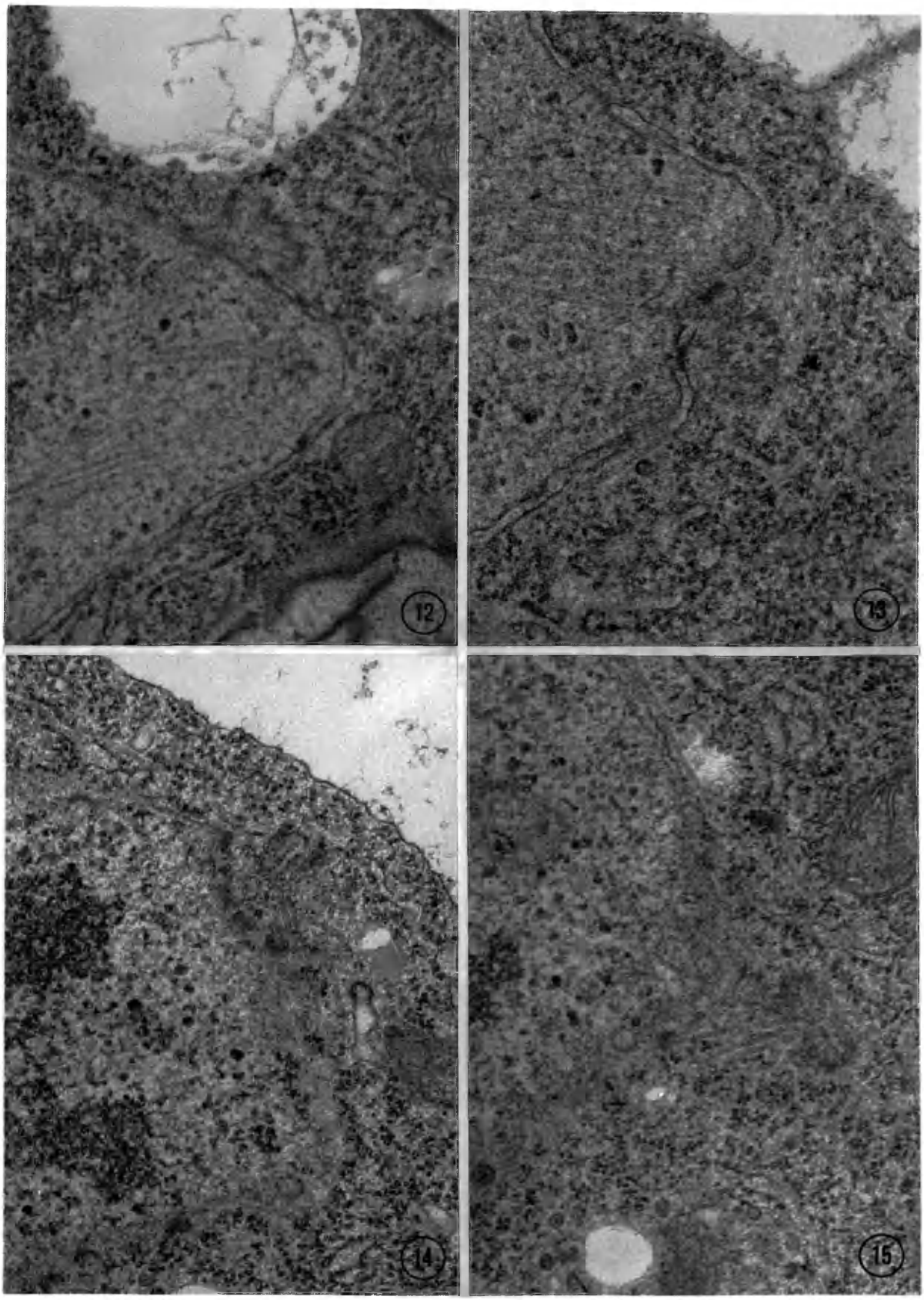
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PLATE 7

Figure 16. Late metaphase nucleus. Note maximally dispersed nucleolus and trilaminar kinetochores (arrowheads). X 17,900.

Figure 17. Enlarged view of a kinetochore and its kinetochore microtubules. X 73,200.


N
16

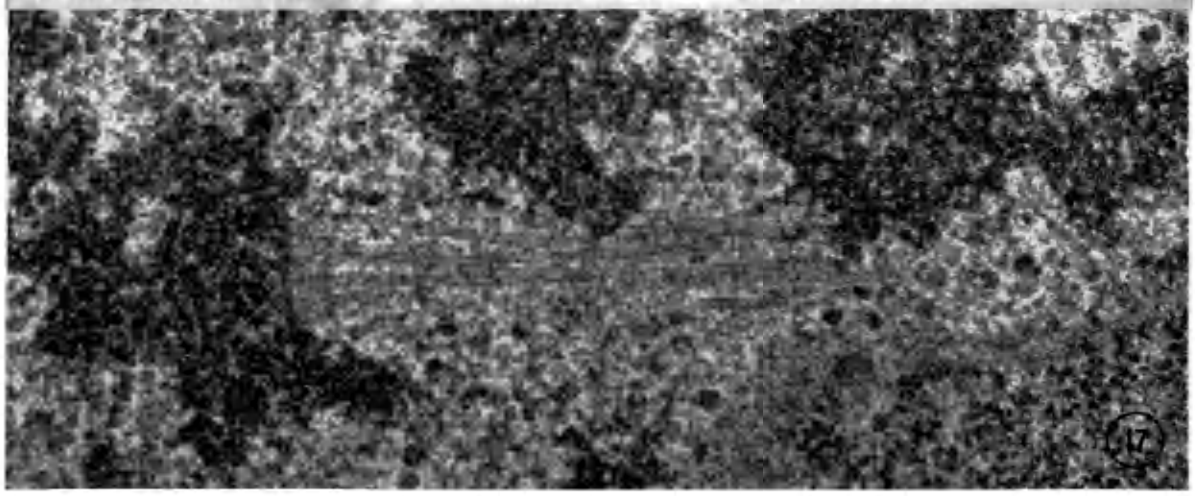
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PLATE 8

Figure 18. Anaphase nucleus with elongating spindle interzone. Note the very active golgi complexes and a preponderance of rough endoplasmic reticulum, er, in the surrounding cytoplasm. X 13,200.

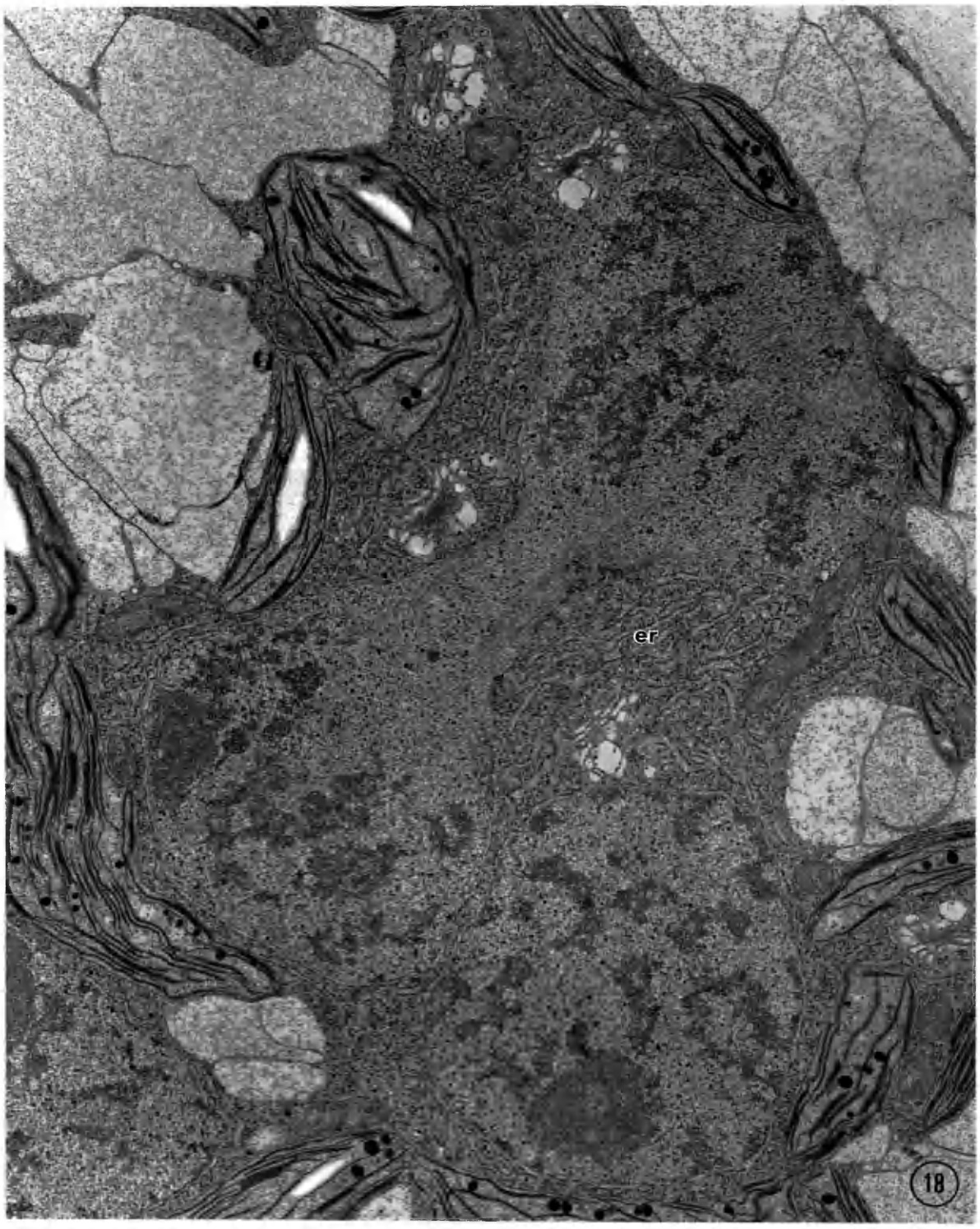
er
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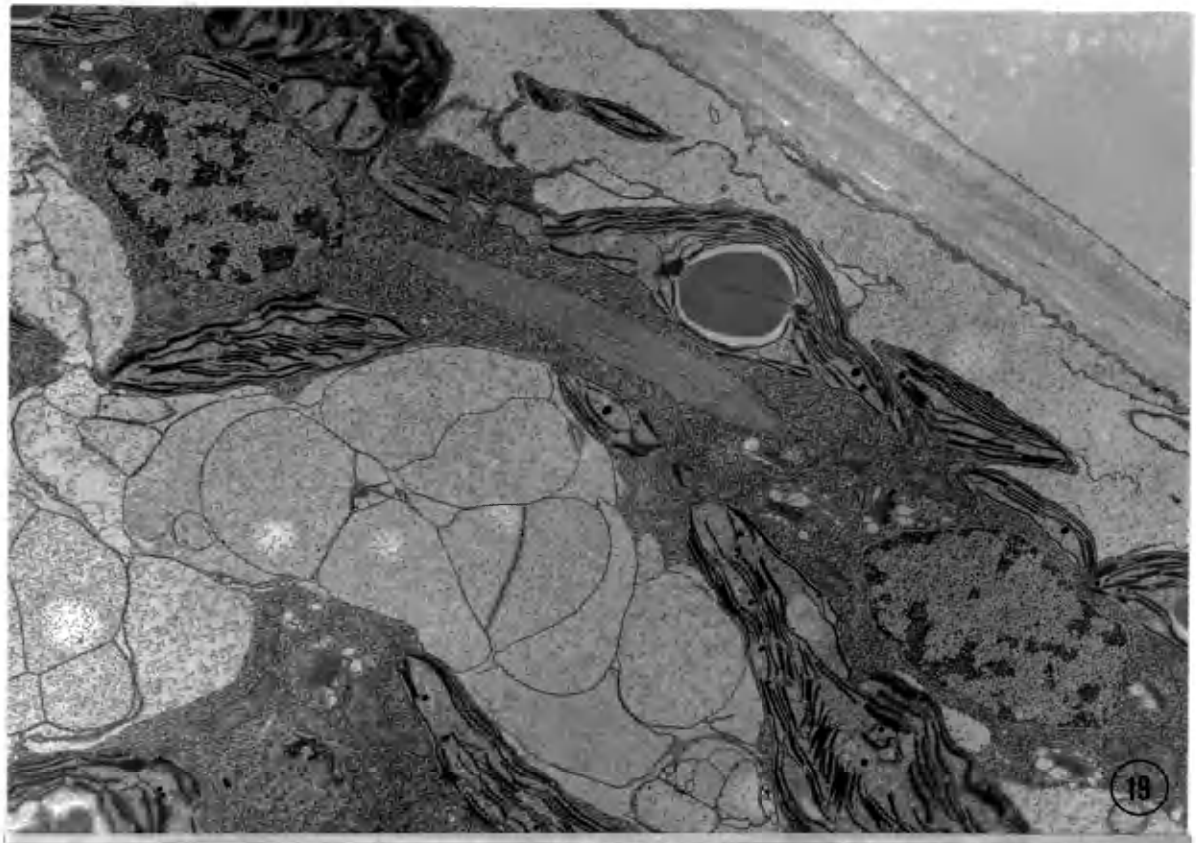
PLATE 9

Figure 19. Telophase nucleus. Although not apparent due to the plane of sectioning, the nucleus at the upper left in this micrograph is still attached to the spindle interzone. X 6,750.

Figure 20. Enlarged view of the microtubular spindle interzone shown in Figure 19. Some nucleolar fragments are visible. X 16,600.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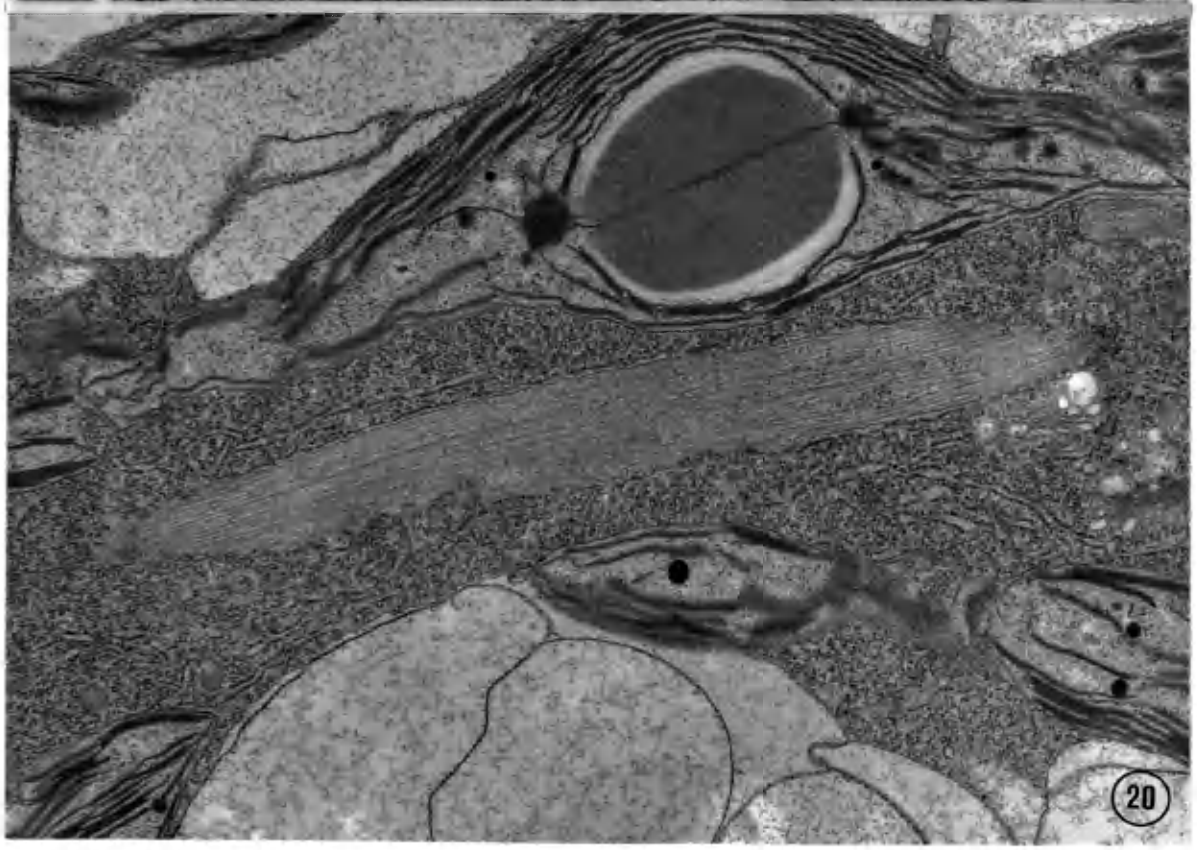
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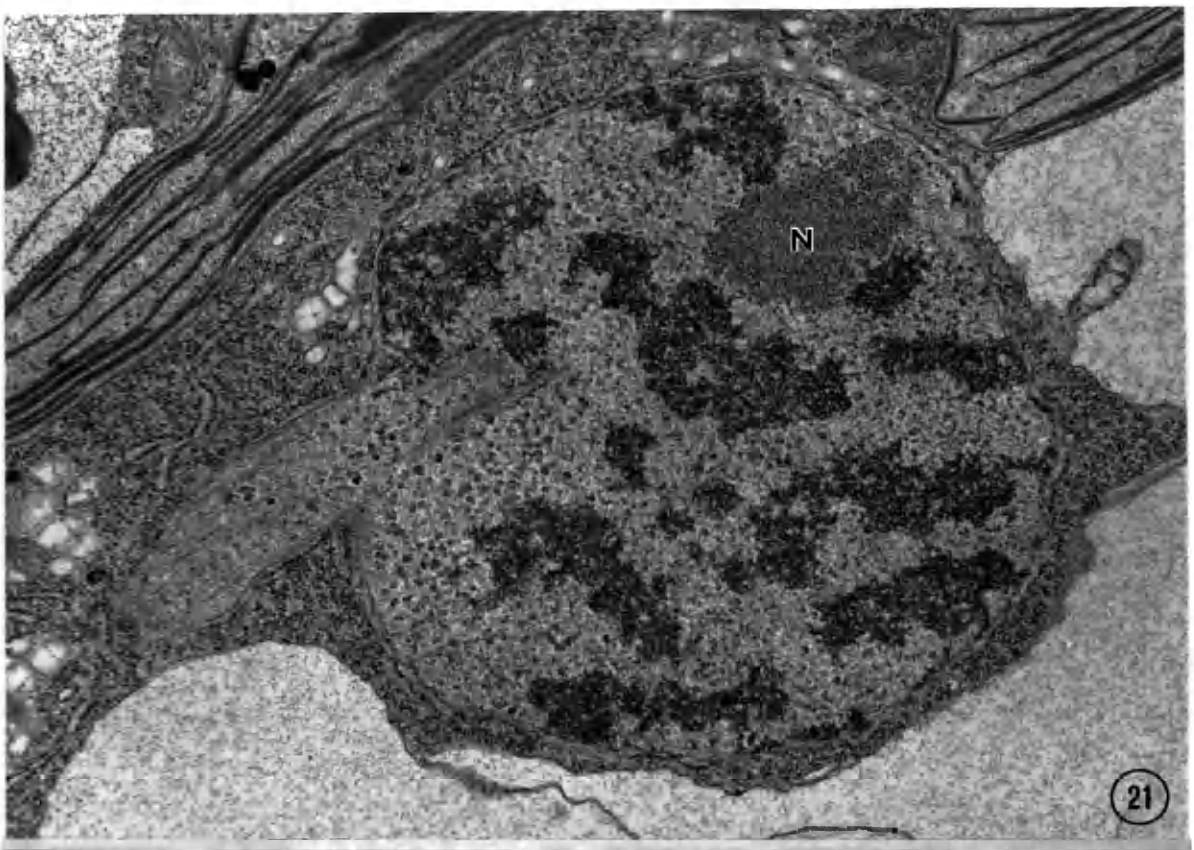
PLATE 10

Figure 21. Telophase daughter nucleus still attached to its spindle interzone. Nucleolus has regained its compact appearance. Microtubules have depolymerized into subunits except in the vicinity of the spindle interzone. X 26,700.

Figure 22. Telophase daughter nucleus. Position of the spindle interzone relative to the daughter nucleus may suggest nuclear rotation. X 19,000.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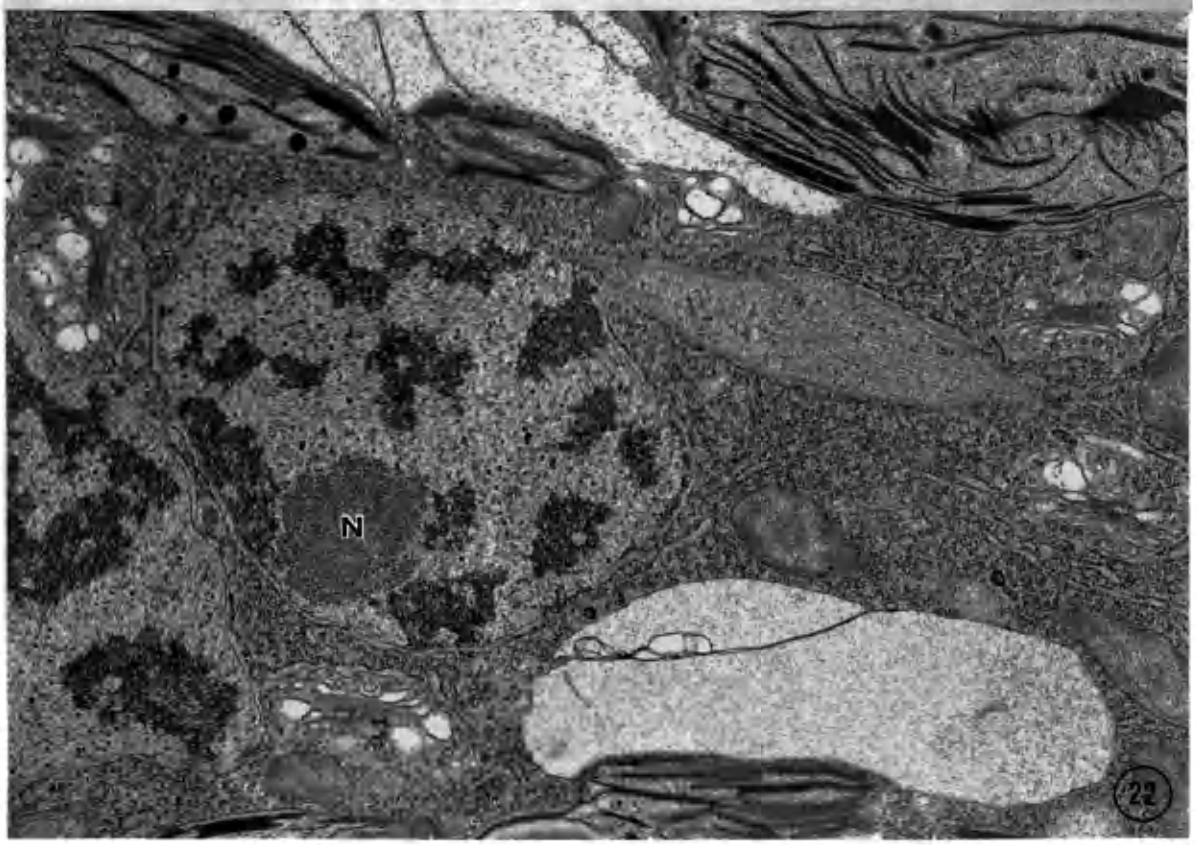
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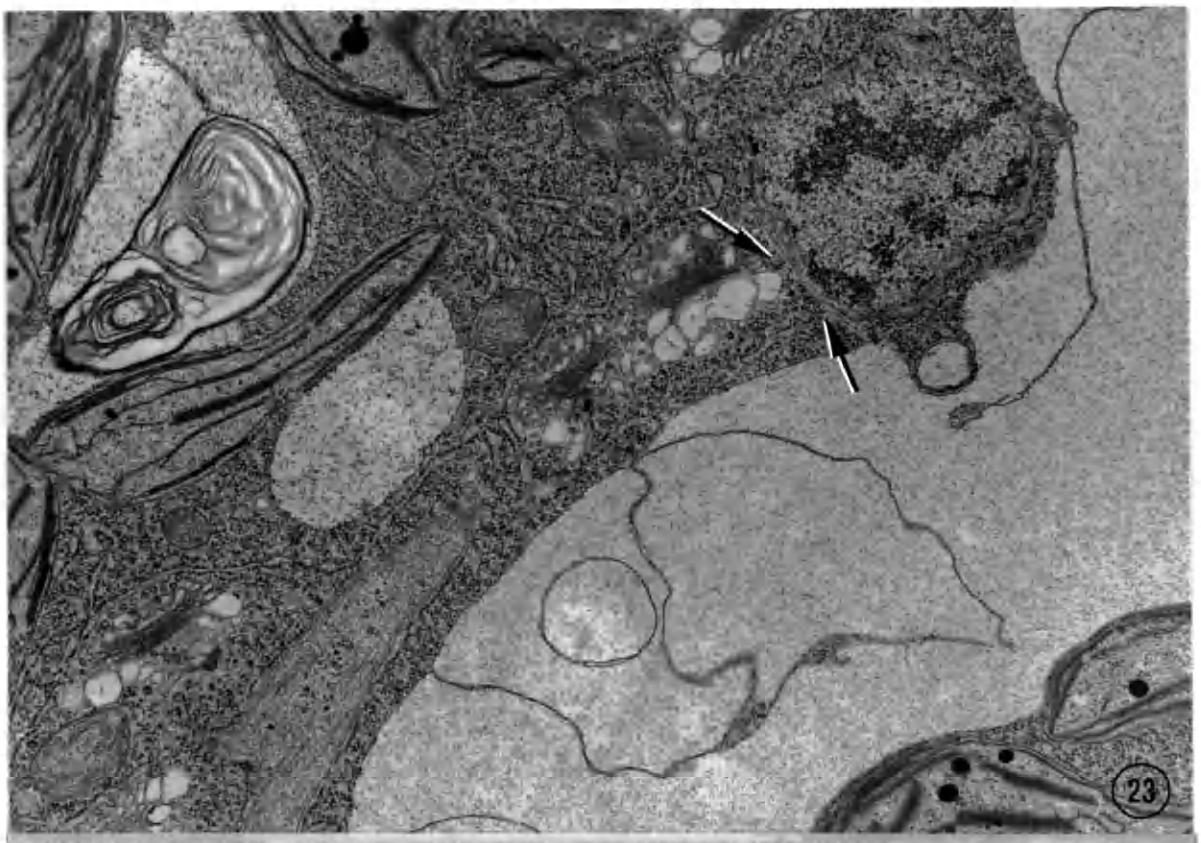
PLATE 11

Figure 23. Telophase daughter nucleus separated from its spindle interzone. Note converging microtubules (arrows) on the side of the nucleus proximal to the spindle interzone. X 16,250.

Figure 24. Vacuolar intrusion between two recently separated daughter nuclei. Spindle interzone remnant (arrow) still present in cytoplasm. X 8,100.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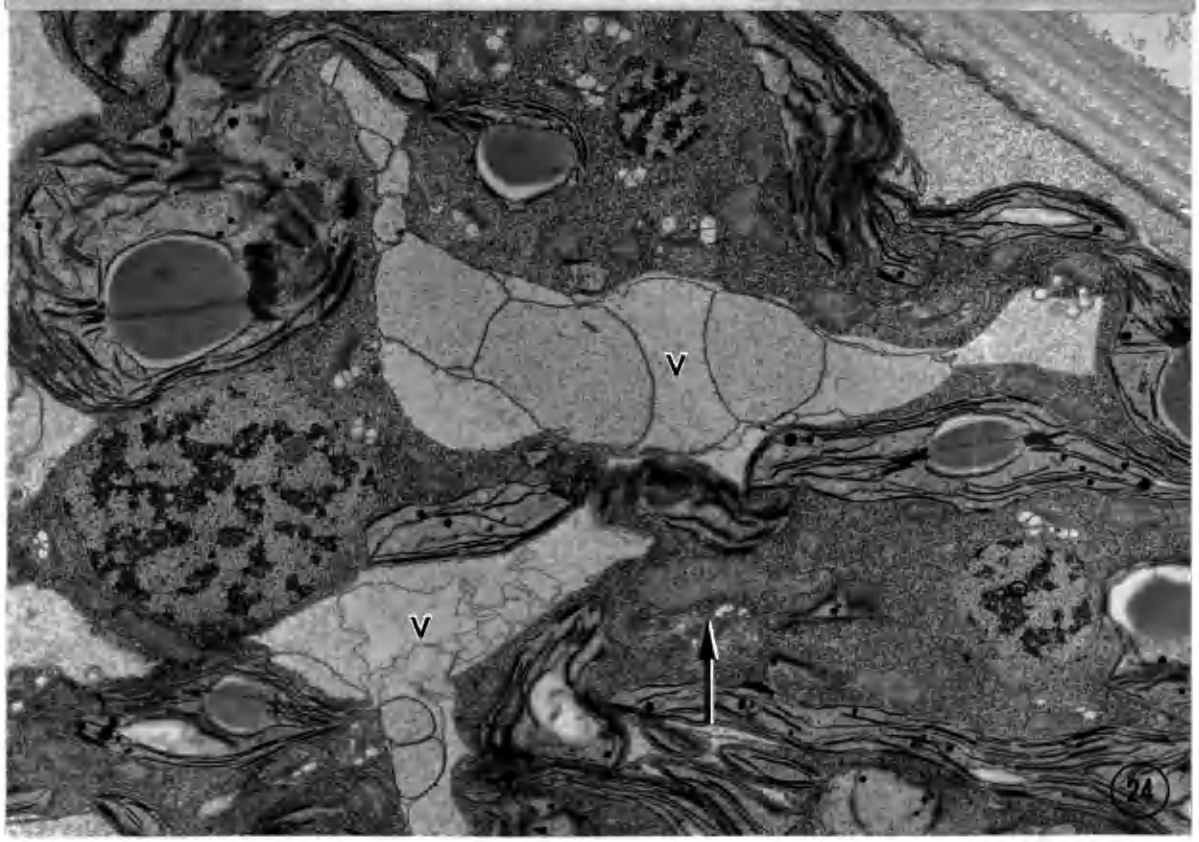
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V
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VITA

Ruth Schwab Lewis

Born November 10, 1950 in Dayton, Ohio. Undertook initial undergraduate studies at The Ohio State University, 1968-1969. Entered Oklahoma State University, 1970. In 1972, received undergraduate teaching assistantship in the Department of Zoology, Oklahoma State University. Received Bachelor of Science degree from Oklahoma State University, 1973, with a major in Zoology. Married and moved to Gloucester Point, Virginia where employed by the Virginia Institute of Marine Science until entering the College of William and Mary in Virginia in September, 1974, as a graduate teaching assistant in the Department of Biology. Accompanied family to College Station, Texas in 1976 where employed by Texas A & M University as an electron microscopist. Initial work on the degree Doctor of Philosophy was begun January, 1979 at Texas A & M University.