CELL DIVISION IN *GRIFFITHSIA PACIFICA* KYLIN

by

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ABSTRACT

Mitosis and cytokinesis in the coenocytic, marine red alga Griffithsia pacifica Kylin were studied using light and electron microscopy. In the meristematic apical cells, a wave of mitosis passes through the nuclei; the apical nuclei complete division before the basal nuclei begin. During mitosis, the nuclear envelope remains intact but is fenestrated at the poles from prophase, when the nucleolus breaks down, until telophase; the nucleolus does not reform until after the daughter nuclei have separated. A single polar ring appears at each pole during prophase and remains until prometaphase. At metaphase each nucleus is enclosed within a sheath of perinuclear endoplasmic reticulum, and kinetochores are visible on the chromosomes. During anaphase the spindle elongates while the chromosome-to-pole distance decreases. As the daughter nuclei separate at telophase, cytoplasm occupies the interzone which is devoid of microtubules. Cytokinesis is effected by a centripetal furrowing of the plasmalemma and is accomplished without microtubules. The formation of a septal plug, which separates the cytoplasm of adjacent cells, completes cytokinesis. A proliferation of tubular endoplasmic reticulum is associated with septal plug formation. Cell division in G. pacifica is compared with other algae.

INTRODUCTION

Recent ultrastructural investigations of cell division in algae have unexpectedly led to the development of some new theories regarding cell division and evolution. First, by comparing the ultrastructural accounts of algal cell division (Table 1, p. 7), we may gain insight into the mechanisms involved in the two quite separable events of cell division: mitosis or nuclear division and cytokinesis. In addition, certain of the described systems, such as the diatoms, are suitable for experimental studies on the physiology and mechanics of cell division. Second, by comparing the differences within the mitotic and cytokinetic apparatus of different organisms, we might obtain additional information as to how the eukaryotic condition arose (Pickett-Heaps, 1972a, 1975a). This second approach is based on the idea that the mitotic event, which is functionally a method of segregating two identical genomes equally into two daughter nuclei, is conservative within eukaryotes, but that certain minor features of mitosis have become altered throughout evolution. These minor variations may be conserved in certain taxonomic levels, such as an order or family. Finally, comparative analyses of cell division have resulted in some new phylogenies which may be further tested by direct observation (Pickett-Heaps and Marchant, 1972; Stewart and Mattox, 1975; Pickett-Heaps, 1975a). These phylogenies extend our knowledge concerning evolution and complement other conventional schemes based on morphology, cytology, and physiology (Klein and Cronquist, 1967; Scagel et al., 1965).

Higher land plants have long been thought to have originated from green algal progenitors. Both groups often share many significant taxonomic characteristics, such as the chlorophylls a and b, certain accessory pigments, multi-thylakoid chloroplast grana, starch storage, similar cell wall composition, and plasmodesmata. Cell division studies have not only corroborated this basic hypothesis but also refined it by suggesting more precisely the pathways of cytological evolution. The first refinement occurred in 1967, when Pickett-Heaps reported that persistent interzonal microtubules and vesicles, analogous to the phragmoplast in higher plants, effected cytokinesis in the advanced green alga Chara, thereby linking this alga and higher plants through a conservative feature (at least in higher plants) of cytokinesis. Subsequent studies of cell division revealed that not all green algae divided using the same cytokinetic system as Chara, and that at least two major lines evolved in the green algae: one group having the phragmoplast and other previously described characteristics similar to higher plants, and a second group dividing either by a centripetal cleavage or by a novel system of microtubules called a phycoplast (Pickett-Heaps, 1972a).

While new phylogenies based, in part, on ultrastructural criteria have been established for green algae (Pickett-Heaps and Marchant, 1972; Stewart and Mattox, 1975; Pickett-Heaps, 1975a), the novel criteria used have not been widely applied to other classes of algae. Do other algal classes exhibit diversity in cell division as do the green algae? If so, is this diversity useful in understanding relationships within and between the different taxa? Table 1

summarizes a literature survey to determine if these questions are answerable on the basis of available information. It is clear that there are disappointingly few relevant studies apart from the green algae, and certainly not enough to determine the extent of cell division diversity within each class. This thesis attempts to extend the knowledge about the ultrastructure of cell division and to apply that information to current theories of evolution and cell division.

The Enigmatic Position of the Red Algae

Various phylogenetic affinities among the algal classes have been proposed (Scagel et al., 1965; Fott, 1965; Klein and Cronquist, 1967; and many others), and most classes have diagnostic features which have been used in various combinations to link them together. However, the red algae possess many characteristics which exclude them from all but distant phylogenetic affinities with other algae:

1. Red algae entirely lack flagella and basal body/centrioles. While certain taxa within other algal classes may also lack flagellated stages (e.g., the Conjugales and certain of the Chlorococcales in the green algae, and the Pennales in the diatoms), these latter exceptions are considered reduced forms, presumably derived from flagellated ancestors.
2. The physiology and biochemistry of photosynthesis in red algae are quite different from any other examined eukaryotes. Whereas two different types of chlorophyll molecules function in the photoreaction centers in other eukaryotic plants, only chlorophyll a has been demonstrated *in vivo* in red algae (Meeks, 1974). In addition, red algae and Cryptophyceae are

the only eukaryotes possessing the phycobiliprotein accessory pigments. Only the prokaryotic blue-green algae exhibit the same photosynthetic machinery as red algae.

3. Recently, Howland and Ramus (1971) demonstrated that red algae possess ribosomal RNAs (rRNA) intermediate in size between the 0.56 X 10^6 and 1.07 X 10^6 dalton prokaryotic rRNAs and the 0.69-0.73 X 10^6 and 1.27-1.75 X 10^6 dalton eukaryotic cytoplasmic rRNAs. The values of 0.54-0.58 X 10^6 and 1.21 X 10^6 daltons for cytoplasmic rRNAs in red algae indicate the uniqueness of this class even at the molecular level.
4. Life cycles in the red algae are among the most complex and perplexing in the plant kingdom. Asexual reproduction is common in the lower forms, but the more advanced species exhibit a tri-phasic life cycle with one haploid and two diploid phases.

The red algae offer a variety of model systems, but they have been used only sparingly in experimental investigations (Duffield, Waaland, and Cleland, 1972). Techniques for laboratory culture of red algae have been developed, and certain species have completed their life cycle under laboratory conditions (West, 1966). Several species which grow well under simple culture conditions are commercially available (Starr, 1964). In spite of the availability and culturability of some red algae, the ultrastructure of cell division has been little investigated. This lack of information is especially surprising considering the numbers (about 400 genera and 2500 species; Smith, 1955) and diversity within the class Rhodophyceae, which is composed of two sub-classes, the primitive Bangiophycidae and the more advanced

Florideophycidae. These two sub-classes exhibit a range of diversity in their morphology, cytology and physiology that correspondingly suggests that the mechanisms of cell division may also vary between them. However, the only ultrastructural account of cytokinesis in the Bangiophycidae is for Porphyridium cruentum (Gantt and Conti, 1965), and mitosis has not been observed with the electron microscope. In the Florideophycidae there are two ultrastructural reports of mitosis: McDonald (1972a) described mitosis in Membranoptera platyphylla, and Peyrière (1971) gave a brief account of prophase and metaphase in Griffithsia flosculosa. Various descriptions of cytokinesis in the Florideophycidae have been given for Pseudogloiophloea confusa (Ramus, 1969b), Levringiella gardneri (Kugrens and West, 1972), Ptilota hypnoides (Scott and Dixon, 1973), and Erythrocystis saccata (Kugrens and West, 1974).

Griffithsia as a System

In the present study, mitosis and cytokinesis in Griffithsia pacifica Kylin (Order Ceramiales, Family Delesseriaceae) are described. Several features make G. pacifica a useful system for studying cell division. G. pacifica has simple culture requirements and grows rapidly with one or two cell divisions per day in the apical meristematic cells. It has an uncorticated, uniseriate, indeterminately-branched thallus composed of upright shoot cells and prostrate rhizoidal cells. Vegetative cells are coenocytic and range in size from 0.1 mm in young apical cells to almost 3 mm in older cells. New cells may be formed in one of four ways:

1. The most common mode of cell formation is by apical cell division (there is no intercalary meristem).
2. Cells below the apex may initiate lateral branches.
3. Rhizoidal cells may divide to form either new rhizoidal cells or new, upright shoot cells.
4. Intercalary cells which have been injured may be regenerated by the concurrent growth of a new apical cell and rhizoidal cell by the two cells abutting on the injured cell. The two new cells may fuse to form a replacement cell, or the thallus may fragment, producing a new somatic thallus (Waaland and Cleland, 1974).

In his classic work, Lewis (1909) described mitosis in G. flosculosa as a "wave" passing along the cell. Apical nuclei initiated mitosis, and basal nuclei were the last to finish. Lewis also estimated that each apical cell contained 12 to 75 nuclei. Conceivably, then, at a given moment during a "mitotic wave," all of the stages of mitosis should be exhibited in the same cell.

Since mitosis has been studied in detail for only one other red alga (McDonald, 1972a), further investigations are necessary to establish whether comparison of mitosis might yield useful information on the affinities of various red algae. Finally, a comparison of mitosis in G. pacifica with the few mitotic features described for G. flosculosa (Peyrière, 1971) would indicate to what extent mitosis is conservative within this genus.

Table 1. Summary of Ultrastructural Descriptions Primarily Concerned with Mitosis and/or Cytokinesis in Algae.

CLASS[a]	NO. OF GENERA[b]	GENERA AND REFERENCES	% GENERA DESCRIBED
Bacillario-phyceae	170	Diatoma (Pickett-Heaps, Tippit and McDonald, 1976) Lithodesmium (Manton, Kowallik, and von Stosch, 1969, 1970) Melosira (Tippit, McDonald, and Pickett-Heaps, 1976)	1.8
Charophyceae	6	Chara (Pickett-Heaps, 1967, 1968) Nitella (Turner, 1968)	33.3
Chloromonadaceae	6	Vacuolaria (Heywood, 1973)	16.7
Chlorophyceae	425	Acrosiphonia (Hudson and Waaland, 1974) Bulbochaete (Pickett-Heaps, 1973c) Chlamydomonas (Johnson and Porter, 1968; Coss, 1974; Triemer and Brown, 1974) Chlorella (Wilson, Wanka, and Linskens, 1973) Cladophora (McDonald and Pickett-Heaps, 1976) Closterium (Pickett-Heaps and Fowke, 1970) Coleochaete (Marchant and Pickett-Heaps, 1973) Cosmarium (Pickett-Heaps, 1972e) Cylindrocapsa (Pickett-Heaps and McDonald, 1975) Eudorina (Gottleib and Goldstein, 1975) Fritschiella (McBride, 1970) Hydrodictyon (Marchant and Pickett-Heaps, 1970, 1972) Kirchneriella (Pickett-Heaps, 1970a) Klebsormidium (Floyd, Stewart, and Mattox, 1972b; Pickett-Heaps, 1972b) Microspora (Pickett-Heaps, 1973b)	7.5

Table 1, Continued

CLASS	NO. OF GENERA	GENERA AND REFERENCES	% GENERA DESCRIBED
Chlorophyceae (continued)		Mougeotia (Bech-Hansen and Fowke, 1972) Oedogonium (Hill and Machlis, 1968; Pickett-Heaps and Fowke, 1969; Pickett-Heaps, 1970b) Pediastrum (Marchant, 1974b) Pleurastrum (Molnar, Stewart, and Mattox, 1975) Pseudendoclonium (Mattox and Stewart, 1974) Raphidonema (Pickett-Heaps, 1976) Scenedesmus (Nilshammar and Welles, 1974; Pickett-Heaps and Staehelin, 1975) Schizomeris (Stewart, Mattox and Floyd, 1973) Sorastrum (Marchant, 1974a) Spirogyra (Fowke and Pickett-Heaps, 1969a,b) Stichococcus (Pickett-Heaps, 1974) Stigeoclonium (Floyd, Stewart, and Mattox, 1972a) Tetraedron (Pickett-Heaps, 1972d) Tetraspora (Pickett-Heaps, 1973a) Trichosarcina (Mattox and Stewart, 1974) Ulothrix (Floyd, Stewart and Mattox, 1971, 1972a) Ulva (Løvlie and Bräten, 1970) Volvox (Deason and Darden, 1971)	
Chrysophyceae	70	Ochromonas (Slankis and Gibbs, 1972; Bouck and Brown, 1973) Prymnesium (Manton, 1964)	2.8
Cryptophyceae	12	Chroomonas (Oakley and Dodge, 1973)	8.3
Dinophyceae	125	Amoebophryidae (Cachon and Cachon, 1970) Blastodinium (Soyer, 1971) Gyrodinium (Kubai and Ris, 1969) Haplozoon (Siebert and West, 1974)	5.6

Table 1, Continued

CLASS	NO. OF GENERA	GENERA AND REFERENCES	% GENERA DESCRIBED
Dinophyceae (Continued)		Peridinium (Tippit and Pickett-Heaps, 1976) Syndinium (Ris and Kubai, 1974) Woloszynskia (Leadbeater and Dodge, 1967)	
Euglenophyceae	25	Euglena (Leedale, 1968; Pickett-Heaps and Weik, pers. commun.) Phacus (Pickett-Heaps and Weik, pers. commun.)	8.0
Phaeophyceae	195	...	0.0
Prasinophyceae	15	Pedinomonas (Pickett-Heaps and Ott, 1974) Platymonas (Stewart, Mattox, and Chandler, 1974) Pyramimonas (Pearson and Norris, 1975)	20.0
Rhodophyceae	400	Griffithsia (Peyrière, 1971) Membranoptera (McDonald, 1972a)	0.5
Xanthophyceae	75	Vaucheria (Ott and Brown, 1972)	1.3
Uncertain Affinities	...	Cyanophora (Pickett-Heaps, 1972c)	...

a Classification ("class") of the algae after Christensen (1962).
b Number of genera after Smith (1955).

MATERIALS AND METHODS

Live Material

Culture Methods

Tetrasporic plants of Griffithsia pacifica Kylin were collected from Punta Pelacano near Puerto Peñasco, Sonora, Mexico by James R. Norris in March, 1975. Unialgal cultures were established in 200 ml of Provasoli's Enriched Seawater (Provasoli, 1968) in Pyrex #3250 culture dishes at 19 C. Illumination was supplied by "cool white" fluorescent lamps at approximately 2500 lux on a 14:10, light:dark photoregime. Subcultures were made every 2-4 wk by excising either individual shoot cells or thallus fragments of 2-10 cells. A single shoot cell would yield a multicellular thallus with 4-7 dividing apical cells within 1 wk. The overall growth rate and pattern was essentially the same as described by Duffield, Waaland, and Cleland (1972).

Light Microscopy

Living cells were observed with either a Zeiss Universal Microscope equipped with Nomarski Differential Interference Phase Contrast optics or with a Nikon Stereo Microscope. Photomicrographs were taken on Kodak Panatomic X film (ASA 32) with a Nikon M35 camera connected to a Nikon Automatic Microflex AFM exposure meter. All film was developed in Kodak Microdol-X (1:3).

Fixed Material for Light and Electron Microscopy

Fixation Methods

Entire thalli (up to 5 cm in length) were fixed for 1/2-2 h at room temperature (22 C) using a wide variety of primary and post-fixation techniques. The primary fixations were as follows:

Schedule 1. 2.5% glutaraldehyde and 0.1 N sodium cacodylate made up in the culture medium at pH 7.0.

Schedule 2. 1.8-2.5% glutaraldehyde made up in the culture medium at pH 7.0.

Schedule 3. 2.5% glutaraldehyde, 0.1 N sodium cacodylate and 1% tannic acid made up in the culture medium at pH 7.0.

Schedule 4. 2.5% glutaraldehyde, 0.1 N sodium cacodylate and 0.01% ruthenium red (ICN Pharmaceuticals, Inc.) made up in the culture medium at pH 7.0.

Schedule 5. 2.5% glutaraldehyde, 0.1 N sodium cacodylate, and 1% cetyl pyridinium chloride (ICN Pharmaceuticals, Inc.) made up in the culture medium at pH 7.0.

Schedule 6. 2.5% glutaraldehyde, 0.1 N sodium cacodylate, and 0.05% cetyltrimethyl ammonium bromide (ICN Pharmaceuticals, Inc.) made up in the culture medium at pH 7.0.

Schedule 7. 2.5% glutaraldehyde and 0.1 N sodium cacodylate made up in the culture medium at pH 7.0, fixed for 30 seconds before the addition of enough 2% osmium tetroxide to give a final concentration of 0.5% osmium tetroxide. This "semi-simultaneous" fixation was allowed to proceed for 30 min.

In all cases the material was washed five times in the buffer with decreasing amounts of salts until a final wash in distilled water. All schedules, except Schedule 7, were then post-fixed in 1% osmium tetroxide made up in distilled water for 1/2-1 hours before a triple rinse in distilled water.

Dehydration and Embedding

Dehydrations were performed on ice over two or three days by the slow, drop-wise addition of a graded series of acetone-water solutions (10%, 25%, 50%, 75%, 95%) and finally changed three to five times in 100% acetone which had been stored over a molecular sieve to remove trace amounts of water. The material was embedded over a three-day period in Spurr's low viscosity resin "hard" formula (Spurr, 1969). Resin-acetone series of 10%, 25%, 50%, 75%, and 90% were added drop-wise until the resin concentration was about 75%. The remaining acetone was allowed to evaporate overnight, and the material was then transferred to fresh 100% resin which was changed once again before final embedding. Thin embedding was accomplished by placing the material in 15 X 60 mm aluminum foil dishes and covering it with about 2 mm of resin or by the alternate method of sandwiching the material between two glass microscope slides coated with Dri-Slip (3M Co.) along with a small amount of resin, thereby forming a plastic wafer a few tenths of a millimeter thick. Embedded material was placed in a 75 C oven and allowed to polymerize overnight.

Sectioned Material

Selected cells from thin embedded material were cut out with either a razor blade or a jeweler's saw and mounted on plastic blocks with Epoxy 907 adhesive (Miller-Stephenson Chem. Co.). All sections were cut with glass knives on a Reichert OM-U2 ultramicrotome. Thick sections (0.25 μm) for light microscopy were stained with 1% toluidine blue in a 1% aqueous borax solution and photographed as described under Light Microscopy. Thin sections in the silver and gold interference range (corresponding to 60-90 nm: Peachey, 1958) were mounted on 0.25% Formvar-coated, slotted, copper grids and stained 3 min with a 1% uranyl acetate: 1% dimethyl sulfoxide: methanol solution, rinsed briefly in methanol, and post-stained for 1/2-1 min in lead citrate. Thin sections were examined with a Philips 200 electron microscope at 60 kv and photographed on DuPont Graphic Arts Film which was developed in Kodak Dektol.

RESULTS

Light Microscopy

Interphase Morphology

Cells of Griffithsia pacifica Kylin contain a single, large central vacuole with a thin layer of cytoplasm (varying from about 1-10 μm) distributed between the vacuole and cell wall (Fig. 2). The coenocytic cells maintain an even distribution of nuclei and chloroplasts in this layer. Older cells appear cylindrical while the younger apical cells are hemispherical in shape (Fig. 1, 3). (All figures, 1-45, are collected for convenience in handling at end of Discussion, pp. 37-49.)

Pre-Mitotic Events

While cells of G. pacifica do not synchronously divide in culture, they do exhibit an endogenous circadian rhythm (Waaland and Cleland, 1972). Therefore, divisions are periodic rather than random. Most divisions occur between 2-4 h after onset of the dark cycle (Fig. 1).

Imminent mitosis in apical cells is signaled by the prominent concentration of cytoplasm toward the tip of the cell resulting in a dense dome (Fig. 3, 4, 5). The concentration at the tip is enriched with nuclei (Fig. 5), and requires about 2 h to form.

Mitosis

Direct observation of mitosis in situ is hampered by the minute size of the nuclei which are 4-5 μm in diameter (Fig. 4, 5, 6, 8), the concentration of cytoplasm at the cell apex, and the thickness of the

cells (about 200 μm) which decreased the resolution. However, mitotic nuclei were observed in the rest of the cytoplasm below the apical concentration (Fig. 4). Mitosis proceeds as a wave through the nuclei with the apical nuclei completing division before the basal ones begin. Many mitotic nuclei were thus observed by following this division wave down a single cell. All nuclear divisions are complete by about one hour after initiation. All of the nuclei in a dividing cell undergo mitosis, which takes about 30 min to complete in a given nucleus.

Interphase nuclei, each with a single, prominent nucleolus (Fig. 8) are distributed throughout the cytoplasm and separated by a distance of several micrometers (Fig. 4). However, the nuclei at the tip of a cell are closer together as the cytoplasm concentrates at the apex prior to mitosis (Fig. 5).

Prophase lasts for about 7 min and includes the disappearance of the nucleolus and the concomitant condensation of the chromosomes, which appear randomly distributed throughout the nucleoplasm (Fig. 6, 9). The nucleus then begins to assume a spindle shape as the chromosomes undergo metakinesis to form the metaphase plate which remains stationary for up to 12 min (Fig. 6, 10). Anaphase includes both chromosome-to-pole motion and spindle elongation (Fig. 11). This phase is quite rapid, lasting about 2 min. By the end of anaphase the nucleus is dumbbell-shaped, the chromosomes are indistinguishable in the nucleoplasm, and the surrounding cytoplasm begins to obscure viewing of the nucleus (Fig. 12). Once separated at telophase, the daughter nuclei move apart, and chloroplasts enter into the interzone

(Fig. 13). The nucleolus does not immediately reappear, and the daughter nuclei are, at this point, quite obscure in the cytoplasm.

Cytokinesis

Before the mitotic wave reaches the basal nuclei, a centripetally ingrowing septum forms immediately below the cytoplasmic concentration at the tip of the cell. Details of the formation of this septum are difficult to observe with the light microscope, as are the events in the development of the pit or septal plug which occupies the central region of the septum. As the septum completes its annular ingrowth, thin, membranous strands, some of them with swellings along their length, focus on the central region where the septal plug will appear (Fig. 7, 45).

Cell division in G. pacifica results in two unequal, multinucleate daughter cells. The newly formed apical cell is small (about 50-75 μm in length) with a dense cytoplasm, and the larger subapical cell (about 100-400 μm in length) maintains a large central vacuole and an evenly distributed thin layer of cytoplasm characteristic of interphase cells.

Electron Microscopy

Fixation

Of the numerous fixations performed on G. pacifica, the most satisfactory cytoplasmic preservation was obtained with Schedule 2 (1.8%-2.5% glutaraldehyde in the culture medium. See section on Fixation Methods for details). Cell wall material preserved poorly

(see especially Fig. 31) unless stabilized by the addition of ruthenium red, cetylpyridinium chloride, or cetyl trimethylammonium bromide; however, the cytoplasm did not preserve so well with these additional fixatives or with the semi-simultaneous fixation (Schedule 7). All electron micrographs are of cells fixed using the procedures outlined in Schedules 1-3.

Ultrastructure of Mitosis

Interphase. Figure 14 shows a typical interphase nucleus and its associated cytoplasmic components. The nucleus exhibits characteristics common to most eukaryotic plant cells: a double-membrane nuclear envelope with evenly dispersed nuclear pores, a single prominent nucleolus with granular ribosome-sized particles (12-15 nm), and heterochromatin which is distributed around the periphery of the nucleoplasm near the nuclear envelope. Nuclei are circular or slightly ovoid in section and surrounded by a complement of cytoplasmic organelles and inclusions including chloroplasts, mitochondria, Golgi bodies, endoplasmic reticulum, polyribosomes, and starch granules. Cytoplasmic microtubules were not observed in any interphase material.

Prophase. Impending mitosis is characterized by several nuclear and cytoplasmic events. The nucleus assumes a spindle shape, and nuclear pores begin to concentrate at the poles (Fig. 15, 17). Elements of the endoplasmic reticulum begin to ensheath the nucleus, separating it from other cytoplasmic organelles (Fig. 16). This perinuclear endoplasmic reticulum develops gradually throughout prophase until the nucleus is completely surrounded at metaphase (Fig. 25). Microtubules appear in

the cytoplasm just outside of the nuclear envelope and radiate away from the poles, either around the nucleus or out into the cytoplasm (Fig. 15-17).

The Polar Ring. For a brief period during prophase a distinct structure appears just outside of the nuclear envelope at the poles (Fig. 18, 19, 21, 22). This structure is cylindrical, measuring about 150 nm in diameter and about 50 nm in height, and will be referred to in subsequent discussion as the polar ring. The cytoplasm around the polar ring is quite dense, making detailed observations on it difficult (Fig. 18, 21). Microtubules are usually associated with the polar ring and appear to focus on it; however, none have been observed directly connected to this structure. In some serial sections, the nuclear envelope appears to bulge out toward the polar ring (Fig. 20, 21). Figure 22 shows the polar ring immediately adjacent to the nuclear envelope. Both Figures 20 and 22 suggest that the nuclear envelope and polar ring may somehow interact, but the details of this implied interaction were not discernable.

The polar ring has been observed only rarely, and then only in association with the nucleus during prophase. The origin and fate of the polar ring are unknown in spite of attempts to locate it during other stages of mitosis and the cell cycle.

Pro-Metaphase. This stage is characterized by two distinct features: the formation of polar fenestrae and the appearance of microtubules within the nucleus (Fig. 23). The polar fenestrae are large gaps (up to 1 μm in diameter) in the nuclear envelope, appearing at pro-metaphase and closing during telophase. These polar fenestrae are

capped with a differentiated cytoplasm which excludes ribosomes and other organelles. Membranous fragments are interspersed in the "cytoplasmic caps," and may represent either remnants of the nucleus resulting from polar fenestra formation or cisternae of the pER. Mitochondria often lie immediately adjacent to the cytoplasmic caps. Microtubules within the nucleus extend between the polar fenestrae and the chromosomes. A few microtubules remain outside the nuclear envelope, but the polar rings are conspicuously absent.

Metaphase. The metaphase nucleus is shortened along its polar axis so that the metaphase plate is wider than the pole-to-pole distance. The pER is fully developed, and few microtubules remain external to the nucleus (Fig. 24, 25).

Two types of microtubules are distinguishable within the nucleus. The spindle microtubules apparently extend between the poles, but the curvature of these tubules and the difficulty of tracking them via thin sections make their exact displacement uncertain. Figure 26 shows a cross section of a metaphase nucleus, approximately in the region of the metaphase plate. Microtubules predominate in the nucleoplasm between the chromosomes and nuclear envelope; these are presumably the spindle microtubules. The second set of microtubules, the chromosomal microtubules, are probably attached to the chromosomes at the kinetochore (Fig. 24, 26). Both sets of microtubules focus on the polar fenestrae, but do not extend out into the cytoplasm.

Anaphase-Telophase. Of the several hundred nuclei observed in the study, very few were captured during early anaphase (Fig. 27) indicating the rapidity of chromosomal movement. The chromosomes lose their

contrast with the nucleoplasm, making them difficult to discern during late anaphase (Fig. 28, 29). This same feature was also noticeable with the light microscope. The nucleus elongates and a centripetal constriction begins to pinch it in half. No specialized arrangements of microtubules or microfilaments have been observed associated with nuclear cleavage. The pER remains quite pronounced (Fig. 28), and the polar fenestrae are still apparent. Changes in the length and arrangement of spindle microtubules, which might suggest a mechanism for anaphase movement, could not be determined.

As the nucleus cleaves into two daughter nuclei at telophase, the polar fenestrae close and the cytoplasmic caps disappear. The pER remains, but mitochondria and other cytoplasmic organelles enter into the interzone between the daughter nuclei (Fig. 30). However, no microtubules have been observed in the interzone. The chromosomes are indistinct, and the nucleolus is not apparent until the nuclei are well separated.

Cytokinesis

Septum Formation. About the time the apically located nuclei have completed division, cytokinesis begins with an annular infurrowing of the plasma membrane (Fig. 31). The cleavage furrow initiates near where the cytoplasm concentrates at the tip of the cell. Formation of the septum is rapid with the furrow complete after about 30 min. Microtubules are not associated with the ingrowing cleavage furrow. However, Golgi bodies appear to function in septum formation in two ways. First, what are interpreted as flattened Golgi cisternae are usually appressed

to the leading edge of the centripetally ingrowing furrow. Second, Golgi bodies and their, presumably, derived vesicles are observed along the furrow as it forms. The vesicles appear to fuse with the plasmalemma, discharging their contents into the septum (Fig. 32).

As the septum grows inward, it cuts through the central vacuole, pinching off a smaller vacuole which passes to the apical daughter cell. Membrane-bound, crystalline bodies are often segregated into daughter cells by this means (Fig. 33, 34). Cytokinesis is completed by the formation of a septal plug.

The Septal Plug. The cleavage furrow ceases growing inwards, leaving a small aperture in the center of the septum (Fig. 35). This aperture then fills with cytoplasm which soon accumulates a massive proliferation of tubular endoplasmic reticulum (Fig. 36, 37). The cytoplasm containing this tubular ER becomes extremely electron opaque (Fig. 38), presumably due to the accumulation of septal plug material. The plug itself forms rapidly as no intermediate formative stages have been observed.

The completed plug is lens shaped with the two convex faces abutting on the adjacent cells. The upper face is slightly smaller than the lower one. An equatorial groove lies between the faces along the plane of the septum (Fig. 39, 41).

The septal plug is not uniform in structure, as at least four distinct zones may be discerned. First, two thin caps line the plug face on either side. In longitudinal sections, a cap may be resolved into an outer dark band approximately 11 nm thick and an inner light band, also about 11 nm thick. The outer dark band was occasionally

lined with electron opaque particles about 40 nm in diameter (Fig. 41). Immediately beneath the caps is an amorphous second zone which is electron dense. This second zone is between 115 nm and 200 nm thick (Fig. 40). A third zone consists of an ordered array of hexagonal chambers resembling a honeycomb when viewed in cross section. Each chamber is about 40 nm in diameter, and its lumen is occupied by a dense fiber about 17 nm in diameter (Fig. 42-44). The chambers extend into a fourth zone, the central matrix, which appears as an amorphous band across the equatorial groove.

The cytoplasm adjacent to the septal plug is devoid of ribosomes and usually contains an abundance of endoplasmic reticulum (Fig. 40, 41). In younger cells, the ER focuses on the faces of the septal plug while, in older cells, the ER assumes a more random orientation. The density of the plug, especially at the rims, obscured the finer details of membrane association with the plug. For example, whether the ER actually penetrates into the caps could not be discerned. This would seem unlikely, however, as Figure 43 shows a slightly oblique cross section through a septal plug, including part of the cap: the extreme density of the cap would argue against its penetration by the ER. The displacement of the plasmalemma with respect to the septal plug is also uncertain, as the plasmalemma was invariably disrupted in regions near the plug during preparation for electron microscopy (Fig. 39-41).

DISCUSSION

There is only one previous ultrastructural account of cell division in a red alga (McDonald, 1972a), and but a few reports of various mitotic or cytokinetic features in other selected genera (Gantt and Conti, 1965; Ramus, 1969b; Peyrière, 1971; Kugrens and West, 1972; Scott and Dixon, 1973; Kugrens and West, 1974). With such a paucity of information, a comparative analysis of cell division cannot yet be used in the construction of phylogenies based upon ultrastructure as has been done in the green algae (Pickett-Heaps and Marchant, 1972; Stewart and Mattox, 1975; Pickett-Heaps, 1975a). However, by comparing information from the few available accounts of cell division in red algae, certain correlations begin to emerge.

Mitosis in Red Algae

The present investigation and others (Peyrière, 1971; McDonald, 1972a) clearly demonstrate that mitosis in red algae is not similar to that in higher plants as was previously supposed from light microscopic observations (Magne, 1964). The complete breakdown of the nuclear envelope during prophase, as in higher plants, does not occur in red algae studied so far. Also, the red algae do not form a preprophase band of microtubules which appear in the cytoplasm near the nucleus of higher plant cells (Pickett-Heaps and Northcote, 1966) or in certain green algae (Pickett-Heaps, 1974) and indicate the eventual plane of cytokinesis. Red algae possess at least two other features not present in higher

plants: a system of perinuclear endoplasmic reticulum and the polar ring.

All mitotic studies in red algae have so far been confined to the same order, the Ceramiales. This is the largest and most diverse order in the Rhodophyta and is composed of four families. Two families are represented in these mitotic studies: Membranoptera platyphylla (McDonald, 1972a) is a member of the Delesseriaceae, and both Griffithsia pacifica Kylin and G. flosculosa (Peyrière, 1971) are members of the Ceramiaceae.

The Polar Ring

McDonald (1972a) describes a short, cylindrical structure measuring 160-190 nm in diameter by 70 nm in height at the poles of the prophase nucleus in Membranoptera platyphylla. For this new structure he suggests the term "polar ring," as it includes both a structural and positional description. In Griffithsia flosculosa, Peyrière (1971) reports a "dense body" measuring 100 nm in diameter by 50 nm in height at the poles of the nucleus during prophase and metaphase. The present investigation also demonstrates a structure measuring about 150 nm in diameter by 50 nm in height near the prophase nuclear poles of G. pacifica. I have adopted the term "polar ring" after McDonald (1972a) and suggest use of this term in future investigations where a structure of similar dimensions and behavior to those described here is encountered.

The polar ring is almost invariably associated with microtubules or embedded in a dense matrix near where microtubules are found in later

mitotic stages. This association suggests that the polar ring may function as part of a microtubule organizing center (Pickett-Heaps, 1969). If so, then the elongation of the anaphase spindle must be an event independent of the polar ring, which is noticeably absent from each pole at anaphase (Fig. 28, 29). The proximity of the polar ring to the nuclear envelope may be significant and will be discussed later. The precise role of the polar ring awaits elaboration, and its existence in other red algae verified by future ultrastructural studies.

Polar Fenestrae

Another consistent mitotic feature is the maintenance of an intact nuclear envelope at metaphase in Membranoptera platyphylla and Griffithsia. Polar fenestrae are present in both M. platyphylla and G. pacifica, but Peyrière (1971) reported a completely closed nuclear envelope at metaphase in G. flosculosa. It is not clear whether this obvious discrepancy between two species of the same genus is significant. One possibility is that Peyrière misinterpreted his data; while claiming that G. flosculosa has a completely closed spindle, Figure 4 of Peyrière (1971) does not unequivocably show the continuity of the nuclear envelope at the pole. Furthermore, that author did not support his conclusion with more compelling evidence such as a set of serial sections through the metaphase poles.

If, however, G. flosculosa proves to lack polar fenestrae, this would demonstrate a rare case of either interspecific diversity or a diversity within different cell types of the same plant. Both possibilities have precedents in other organisms. First, Chlamydomonas is

the only other algal genus for which different morphologies in the mitotic apparatus have been observed in two separate species. Johnson and Porter (1968) and Coss (1974) report polar fenestrae in C. reinhardi, while Triemer and Brown (1974) describe a completely closed spindle in C. moewusii. Finally, Peyrière worked with tetraspore mother cells while the present study was conducted on somatic cells of tetrasporophytes. Although radical differences in mitosis have been described for cells in different life cycle stages for the slime mold Physarum flavicomum (Aldrich, 1969), equivalent differences have not yet been found in algae. The possible differences in mitosis between G. flosculosa and G. pacifica will be resolved only when definitive investigations on identical cell types are compared.

The perinuclear Endoplasmic Reticulum (pER)

The presence of an enclosing layer of endoplasmic reticulum around mitotic nuclei has been reported for some fungi (e.g., McNitt, 1973) and certain chlorococcalean genera: Kirchneriella (Pickett-Heaps, 1970a), Hydrodictyon (Marchant and Pickett-Heaps, 1970), Tetraedron (Pickett-Heaps, 1972d), Sorastrum (Marchant, 1974a), Pediastrum (Marchant, 1974b), and Chlorella (Wilson, Wanka, and Linskens, 1973). It is now apparent that this pER also evolved in the red algae. Both M. platyphylla and G. pacifica exhibit pER with only subtle differences between the two. The pER is also visible in G. flosculosa (Peyrière, 1971; see his Fig. 4) but was not specifically mentioned by that author. In M. platyphylla, the pER is well formed during prophase and envelops a system of perinuclear microtubules (McDonald, 1972a, Fig. 10, 11).

Some of these perinuclear microtubules persist outside the nucleus even after the polar fenestrae and spindle have formed. G. pacifica does not exhibit a complete sheath of pER until metaphase, although increasing amounts of ER lie near the nucleus during prophase (Fig. 16). Few microtubules exist between the pER and the nuclear envelope once the pER has formed. Microtubules which are oriented roughly pole-to-pole outside of the nucleus during early prophase do not persist once the polar fenestrae form at later prophase. The significance of the pER and the minor variations in its behavior are not clear, but its presence may be of phylogenetic importance (Pickett-Heaps, 1972a).

Cytokinesis in Red Algae

Cytokinesis in G. pacifica occurs by an annular membrane infurrowing. Golgi bodies appear to be associated with the ingrowing furrow and with deposition of new wall material inside the septum (Fig. 32). Neither microtubules nor microfilaments were observed in the cytoplasm at any time near the septum during its formation. Cytokinesis by membrane infurrowing has been reported in the florideophycidean algae M. platyphylla (McDonald, 1972a and personal communication), Ptilota hypnoides (Scott and Dixon, 1973), and Levringiella gardneri (Kugrens and West, 1972). In P. hypnoides, as in G. pacifica, the membrane furrow initiates as an invagination of the plasmalemma. Soon thereafter, cisternae of the ER (instead of Golgi cisternae in G. pacifica) are closely associated with the furrow, and presumed Golgi-derived vesicles appear to deposit new cell wall material into the septum.

In the Bangiophycidae, there is only one account of cytokinesis. Gantt and Conti (1965) report that cells of Prophyridium cruentum cleave by an infurrowing of the entire cell cortex (plasmalemma plus wall).

The above accounts of cytokinesis and the apparent absence of cytoplasmic microtubules in interphase cells of ultrastructurally examined red algae suggest that microtubule-mediated cytokinesis may not have arisen in this group. This speculation, however, awaits verification from future investigations.

The Septal Plug

One of the most intensely studied features of red algae is the septal plug. Previously called pit plugs, the older terminology was modified by Meyers et al. (1959) who proposed the terms "aperture" for the hole in the septum left by incomplete cytokinesis, and "plug" for the structure filling the aperture. Ramus (1969b) adopted this terminology and later emphasized that pit connections in the red algae bear no structural or biochemical resemblance to the true pits in xylem tissue of higher plants (Ramus, 1971). To avoid further confusion, Ramus used the term "septal plug" rather than the inaccurate "pit plug." In addition, I propose use of the term "septal complex" in reference to the septal wall, aperture, and plug. The term "pit connection" connotes a misleading analogy with higher plants, and its use should be discouraged.

Until recently, septal plugs were thought to occur exclusively in the red algae, but analogous structures have since been reported in

the fungi for certain ascomycetes (Carroll, 1967; Kreger-Van Rij and Veenhuis, 1969; Furtado, 1971; Setliff, MacDonald, and Patton, 1972; Hammill, 1974) and basidiomycetes (Heath and Heath, 1975). Investigations on the Conchocelis phases of the bangiophycidean genera Porphyra (Lee and Fultz, 1970; Bourne, Conway, and Cole, 1970; Lee, 1971) and Bangia (Sommerfeld and Leeper, 1970; McDonald, 1972b) have revealed septal plugs, which were long regarded absent in this sub-class. While the septal plug is a consistent feature of the Florideophycidae, its ultrastructure is highly variable (Meyers et al., 1959; Bouck, 1962; Bischoff, 1965; Bisalputra, Rusanowski, and Walker, 1967; Ramus, 1969a; Bourne et al., 1970; Feldman and Feldman, 1970; Lee and Fultz, 1970; Lee, 1971; Tripodi, 1971) and may exhibit variation within the same plant (Feldman and Feldman, 1970; Tripodi, 1971). A discussion of the morphological variation in septal plugs would serve little purpose here, although Lee (1971) proposed that certain features of the plug may be phylogenetic indicators. The general morphology of the plugs is basically the same; they are pulley-shaped with a cap on each face and contain a central matrix of variable density.

A major structural question about septal plugs concerns their relationship with cytoplasmic and plasma membranes. Many authors agree that the plasma membrane is continuous from cell to cell around the rim of the septal plug (Ramus, 1969a; Sommerfeld and Leeper, 1970; Bourne et al., 1970; Lee, 1971). In addition, Bisalputra et al., (1967), Bourne et al., (1970), Ramus (1969a), Lee (1971), and Pueschel (1975) have also described a membrane which covers the plug caps, but whether the plug is completely membrane-bound, and thus totally isolated from the cytoplasm,

remains questionable. McDonald (1972b) points out that, unfortunately, incontrovertible evidence on plug-membrane relationships has not been available in any report. Neither does the present study provide a definitive answer to this problem, since the plasmalemma near the septal plugs fixes poorly, and the cap material is too dense to resolve continuous membranes on its surface (Fig. 39-41).

Septal Plug Formation

The ontogeny of the septal plug is described for Pseudogloiophloea confusa (Ramus, 1969b) and for G. pacifica in the present work. McDonald (1972b) also describes certain features of plug formation in Membranoptera platyphylla. In all three cases, a proliferation of longitudinally oriented, tubular endoplasmic reticulum fills the cytoplasmic aperture before the plug is secreted. Tubular cisternae are also present in a similar array in the "pore apparatus" of the ascomycetes Ascodesmis sphaerospora and Saccobolus kerverni (Carroll, 1967) and Sordaria fimicola (Furtado, 1971). The next observable event of plug formation in both P. confusa and G. pacifica is the accumulation of electron opaque material to form the nascent plug, while elements of the ER (or "flattened vesicles" in P. confusa) still extend away from the aperture. These membranous components appear to be involved in the formation of the plug, but their exact role cannot be determined by electron microscopy alone. In G. pacifica and some more primitive red algae (Lee, 1971), the ER persists in a zone of cytoplasm which excludes other cell organelles and lies adjacent to the completed plug (Fig. 40, 41). Whether or not any of this ER traverses the plug is uncertain. Lomasomes similar to those

described in P. confusa are not observed at any time during plug formation in G. pacifica. Furthermore, recent work by O'Brien et al., (1973) suggests that lomasomes are probably fixation artifacts or injuries to living cells.

Septal Plug Function

Despite the numbers of reports concerning septal plugs, their function remains unclear. Lee (1971) relates their occurrence to the genetic complement of an organism, pointing out that when plugs occur in the Bangiophycidae, they appear only in diploid cells. However, he does not provide a corresponding explanation for their presence in both the haploid and diploid cells in the Florideophycidae. Ramus (1971) suggests that the plug is a preformed "weakness" in the septum and may dissolve away during gonimoblast development, thus allowing for the unobstructed flow of nutrients within this fusion tissue. The presence of the plug in vegetative cells, then, simply represents a vestigal role. Kugrens and West (1974) argue alternatively that the plugs are points of wall strengthening, cementing cells together in the absence of a middle lamella. Neither explanation is particularly compelling, nor have they been subsequently tested experimentally.

Defining a specific function for such an apparently inert structure as the septal plug will require information from biochemical and physiological experiments. The presence of septal plugs in only one phase of a life cycle and their absence in another, as in certain of the Bangiophycidae, suggests that these are systems which can be useful in defining such functions.

Cell Division and the Coenocytic Habit

Prior to division in Griffithsia pacifica, the cytoplasm concentrates in the apical, lateral, or basal portion of a cell, depending on the type of cell to be formed. This cytoplasmic movement is done in the absence of cytoplasmic streaming. A similar event occurs in the coenocyte Acrosiphonia spinescens (Hudson and Waaland, 1974) where nuclei, chloroplasts, and other organelles aggregate to form a localized division "band." However, in contrast to Griffithsia where all of the nuclei in a given cell divide, only the nuclei in the division band of A. spinescens divide; the other nuclei in the cell remain in interphase. Cytokinesis in A. spinescens occurs in the region of the division band, and an ingrowing cleavage furrow is preceded by a "hoop" of microtubules in the cytoplasm. In G. pacifica, microtubules are not associated with cytokinesis. Acrosiphonia and Griffithsia are the only two observed algae for which a large concentration of cytoplasm precedes division, yet both organisms use quite different methods to achieve nuclear segregation and cytokinesis.

Coenocytes also display some diversity in the components of their mitotic apparatus. Perinuclear endoplasmic reticulum is present in Griffithsia and in the green alga Hydrodictyon reticulatum (Marchant and Pickett-Heaps, 1970). Centrioles are also present in Hydrodictyon reticulatum (Marchant and Pickett-Heaps, 1970), Acrospiphonia spinescens (Hudson and Waaland, 1974), Cladophora glomerata (McDonald and Pickett-Heaps, 1976), Vaucheria littorea (Ott and Brown, 1972), and Bryopsis hypnoides (Burr and West, 1970), and lacking only in Griffithsia. One feature common to all coenocytes so far is the presence of an intact

nuclear envelope during mitosis. Even so, subtle differences exist between these "closed" spindles. The nuclear envelope may be perforated by polar fenestrae of variable size in Hydrodictyon, Acrosiphonia, and Griffithsia, while the poles remain completely closed in Cladophora, Vaucheria, and Bryopsis. The presence of closed spindles in coenocytic organisms of three different algal classes may be an important indicator for one possible function of membranes during mitosis. Ross (1968) proposes that a closed spindle is a necessity in multinucleate cells undergoing synchronous mitosis; otherwise, open spindles might have a tendency to fuse, resulting in polyploid or aneuploid nuclei. By comparison, completely closed spindles are not necessarily essential for unicellular, uninucleate algae. Such spindles have only been reported for Chlamydomonas moewusii (Triemer and Brown, 1974) and Pedinomonas minor (Pickett-Heaps and Ott, 1974).

Polar Rings, Nuclear Pores, and the Evolution of Mitosis

Certain features of mitosis in red algae stimulate speculation concerning their distinctive taxonomic position. All red algae observed so far possess an intact nuclear envelope during mitosis. Such a closed spindle is regarded as evolutionarily primitive (Leedale, 1970; Pickett-Heaps, 1972a, 1975b). Also, the presence of a polar ring, which is so far unique to red algae, and nuclear pore behavior during mitosis are probably additional indicators of primitiveness.

Recent reviews by Heath (1974), Pickett-Heaps (1975b), and Kubai (personal communication) discuss the events necessary for the development of advanced, eukaryotic mitotic mechanisms from primitive, prokaryotic

genome separation systems. It has been suggested that the eukaryotic condition arose from an ancestral prokaryote possessing a genome separation mechanism similar to that of present-day bacteria. Extant bacteria have a specialized region of the plasma membrane which functions as a genome attachment site (Ryter, 1968). Segregation of replicated bacterial genomes is accomplished by partitioning the attachment sites (which presumably replicate along with the genome) by the cleavage furrow so that each daughter cell receives one genome. Hypothetically, an intermediate organism whose genome was enclosed by a primitive nucleus would maintain the specialized attachment sites in the nuclear envelope. Reliable segregation of a nuclear-enclosed genome is most easily envisioned if a system of microtubules (the continuous spindle) is generated between the genome (chromosome) attachment sites, which now additionally function as microtubule organizing centers (MTOCs). Further refinements occurred with the development of the kinetochore, which may also have arisen when chromosomes attached to MTOCs in the nuclear envelope became detached from the envelope, taking part of the MTOCs with them. Finally, MTOCs became "externalized" from the nucleus, resulting in the formation of basal bodies, centrioles, and "open" spindles independent of membrane-mediated mitosis.

Mitosis in red algae appears to be at an intermediate stage between a primitive, completely closed ("internalized") spindle and a more advanced, open spindle ("externalized") with polar centrioles. That is, the red algae have achieved externalization without centriolarization. No centrioles have been reported for any red alga yet examined, and in the light of the consistency with which the polar ring appears

(only during the spindle formative stage of mitosis), I doubt that future investigations will reveal any basal bodies or centrioles in these organisms. The microtubule in red algae perhaps remains a slave of mitosis and does not perform any of its diverse functions displayed by other algae and higher plants (e.g., cytokinesis, cell wall deposition and morphogenesis). Notably, the inability of red algae to utilize microtubules in such diverse cell functions may be instrumental in explaining processes unique to red algae, such as bipolar cell wall deposition (Waaland, Waaland, and Cleland, 1972).

If the polar ring is part of an MTOC which became externalized from the nuclear envelope, some behavior of the polar ring and nuclear envelope during mitosis may indicate this relationship. Serial sections through the pole of a prophase nucleus in Griffithsia pacifica (Fig. 20, 21) show the polar ring with a portion of the nuclear envelope projecting towards it. In addition, Figure 23 shows the nuclear envelope and polar ring in apparent contact. Peyrière (1971) reports that during prophase, the polar ring is connected to the nuclear envelope. In both instances, the polar ring seems to have an intimate association with the nucleus. Furthermore, polar rings have never been found in distal regions of the cytoplasm such as occupied by the MTOC in the diatom Diatoma vulgare (Pickett-Heaps, Tippit, and McDonald, 1976). Whether the polar ring functions exclusively as part of an MTOC or in some other, as yet unknown, manner remains a mystery. The presence or absence of polar rings in lower red algae (e.g., the Bangiophycidae) would certainly be significant, especially if the polar ring is demonstrated as a universal feature of red algae.

The redistribution of nuclear pores towards the poles of mitotic nuclei in Griffithsia pacifica and Membranoptera platyphylla (McDonald, 1972a) may play a role in spindle formation by permitting externally formed microtubules or tubulin subunits to enter into the nucleus. Peyrière (1971) reported that the nuclear pores of G. flosculosa lacked their dense cores at metaphase, indicating a possible change in nuclear pore function during mitosis. Examples of apparently modified nuclear pores have been reported in other organisms. Burr and West (1970) report that in the green coenocyte Bryopsis hypnoides spindle microtubules pass through the nuclear pores of the completely closed spindle and terminate in the cytoplasm near the polar centrioles. In the protozoan Trichonympha agilis (Kubai, 1973), the mitotic spindle is formed completely outside of the nuclear envelope. Modified nuclear pores allow the chromosomes, which are connected to the inner nuclear membrane at specialized attachment sites, to attach to the externalized spindle.

Most likely, nuclear pores play little or no part in the formation of advanced, open mitotic spindles. However, future investigations are necessary to determine if nuclear pore concentration at the poles during mitosis is a universal feature of red algal mitosis.

All figures are of Griffithsia pacifica Kylin. Figures 1-13 and 45 are light micrographs. Figures 14-44 are electron micrographs.

Figure 1. Entire vegetative thallus. -- Both shoot cells (s) and rhizoidal cells (r) compose the thallus. Previous injury to the plant results in wound response (arrow) with production of regenerative cells. This photograph was taken three h into the dark cycle. Note that most of the apical cells exhibit cytoplasmic concentrations characteristic of incipient mitosis. X 3.2.

Figure 2. Median optical section through a rhizoidal cell. -- The dark bud (b) will give rise to a new shoot cell. A thin layer of cytoplasm lies between the vacuole and cell wall (arrowheads). X 260.

Figure 3. Apical segment of a vegetative thallus. -- Apical cells in various stages of division are shown. Interphase cells (i) maintain a uniform cytoplasm, while mitotic cells (m) form a dome of concentrated cytoplasm at the apex. Cytokinesis results in the formation of a small, dense apical cell and a larger supapical cell (c). X 27.

Figure 4. Dividing apical and subapical cells. -- The cytoplasmic concentrations which will form new apical (a) and branch (b) cells are shown. Nuclei (n) are visible in areas where the cytoplasm has not concentrated. X 260.

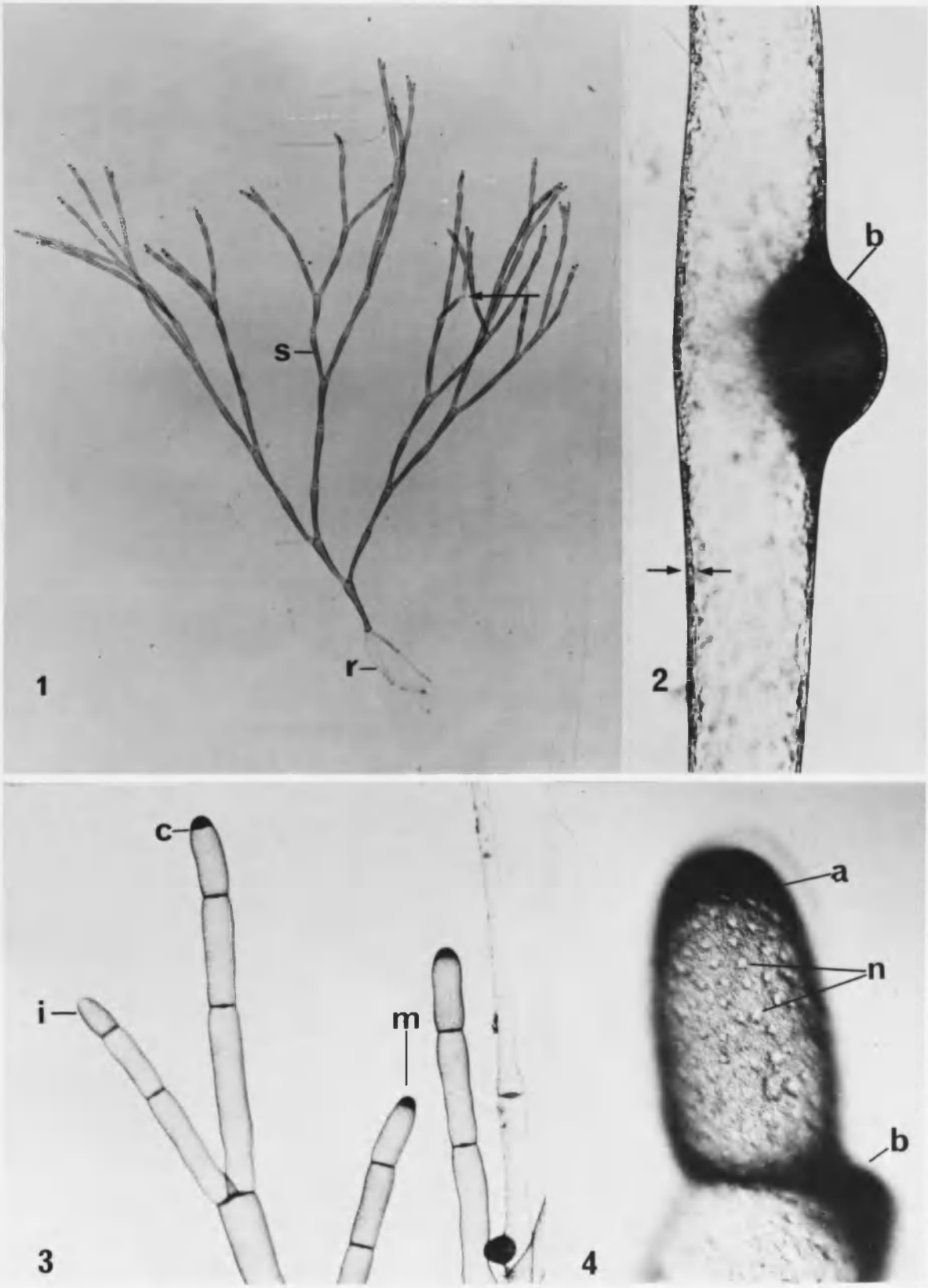
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Figure 5. Longitudinal thick section of incipient cell division in an apical cell. -- The cytoplasmic concentration at the apex is especially rich in nuclei (n). A crystalline body (arrow) is often present near the tip of the cell. The cell was disrupted slightly during the fixation process, as the cytoplasm is pulled away from the cell wall in certain areas (arrowheads). Toluidine blue stain. X 450.

Figure 6. Thick section of a mitotic apical cell. -- Metaphase nuclei (m) are visible with a dark band of chromosomes forming the metaphase plate. The condensing chromatin of a prophase nucleus (p) is also apparent. Toluidine blue stain. X 940.

Figure 7. Median optical section through a cytokinetic cell. -- Thin, membranous strands with swellings (arrow) proliferate in the aperture left by incomplete septation. The septum (arrowheads) forms by an annular infurrowing of the plasmalemma. X 490.

Figures 8-13. Sequential stages of mitosis in living cells. Nomarski optics. All micrographs X 2,470.

Figure 8. Interphase. -- Nucleus containing a single large nucleolus.

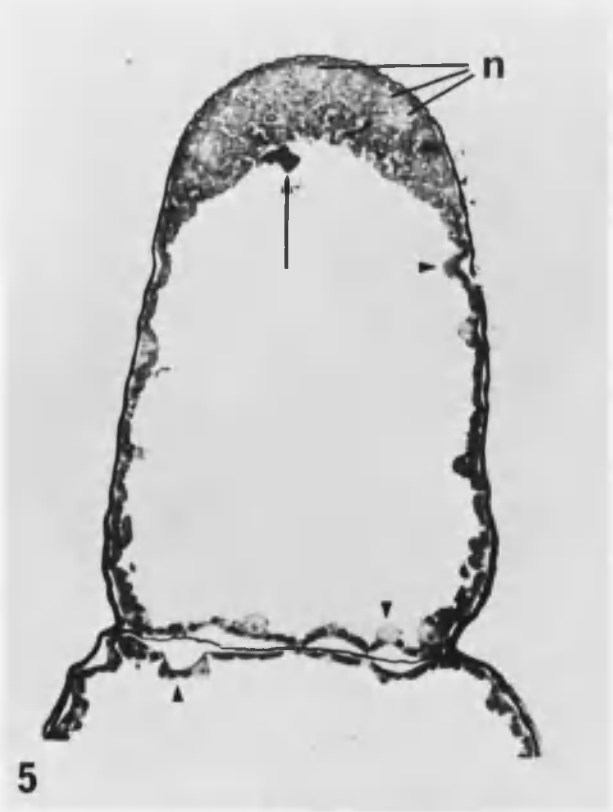
Figure 9. Prophase. -- Chromatin conensation and simultaneous nucleolar disappearance.

Figure 10. Metaphase. -- Chromosomes aligned at metaphase plate.

Figure 11. Early anaphase. -- Chromosome separation as spindle begins to elongate.

Figure 12. Late anaphase. -- Chromosomes lose contrast with nucleoplasm as nucleus begins to cleave.

Figure 13. Telophase. -- Cleavage into daughter nuclei. Nucleoli have not yet reformed.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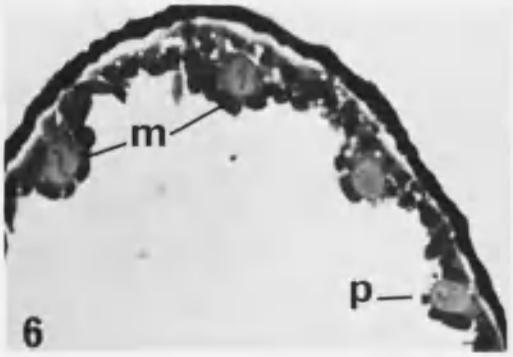
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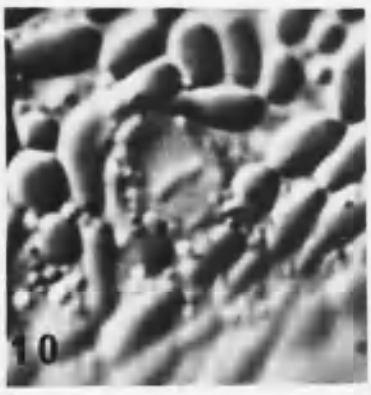
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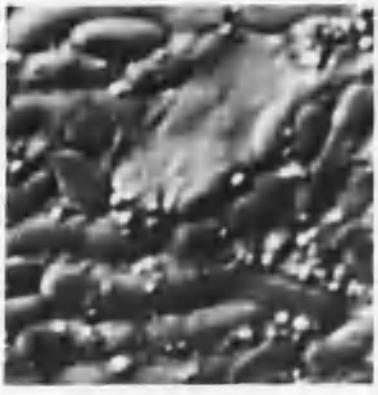
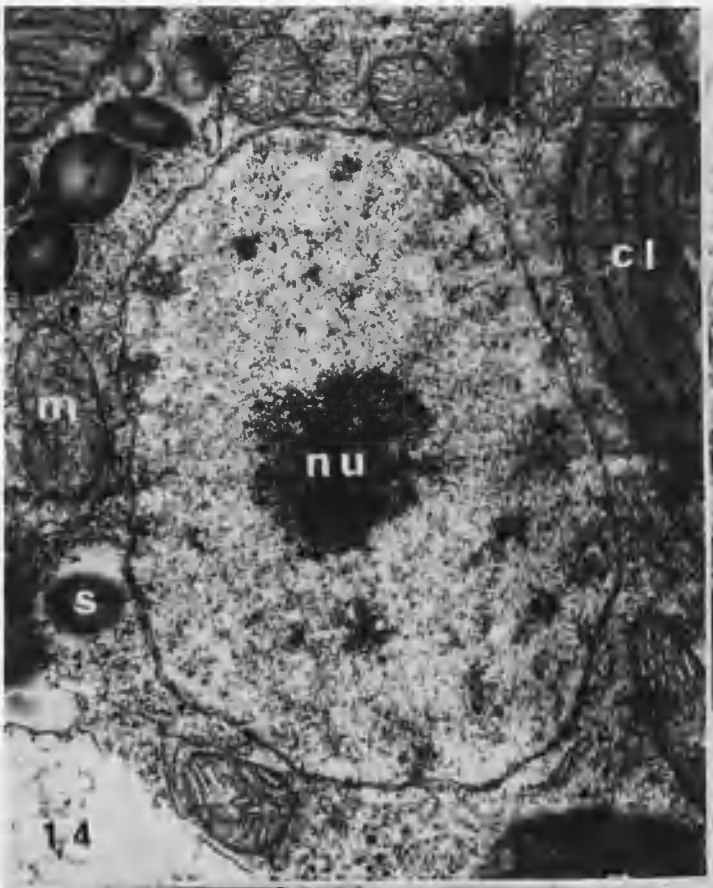


Figure 14. Interphase nucleus. -- A single granular nucleolus (nu) occupies the center of the nucleus which is surrounded by a typical complement of cytoplasmic organelles, such as mitochondria (m), chloroplasts (cl), and floridean starch granules (s). X 20,700.

Figure 15. Prophase nucleus, longitudinal section. -- The nucleus assumes a characteristic spindle shape during prophase, and nuclear pores (arrows) appear more abundant toward the poles. A few microtubules (arrowheads) appear outside of the nucleus. X 20,500.

Figure 16. Prophase nucleus, cross section. -- Perinuclear endoplasmic reticulum (arrows) begins to collect around the nucleus. Cross sections of longitudinally-oriented microtubules (arrowheads) are also visible. X 16,700.

Figure 17. Prophase nucleus, polar region. -- Microtubules (t) focus on the dense polar area (p) and radiate either longitudinally around the cell or out into the cytoplasm. Nuclear pores (arrows) concentrate toward the poles. X 23,000.



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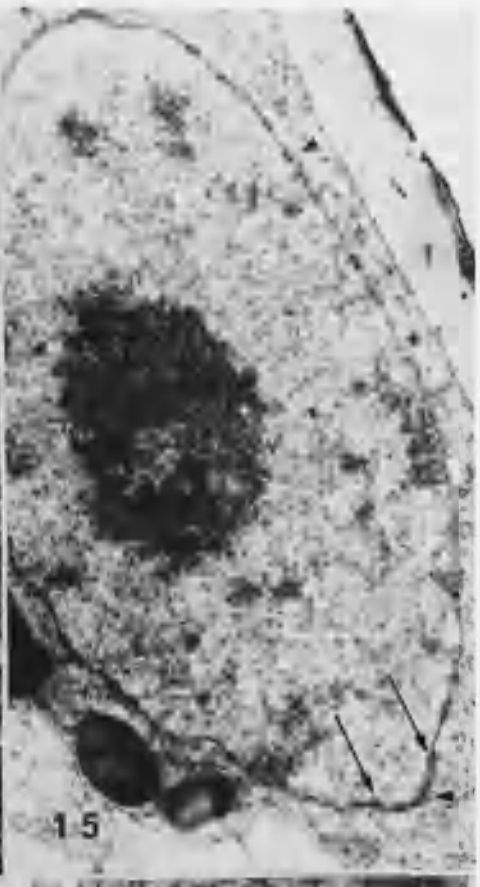

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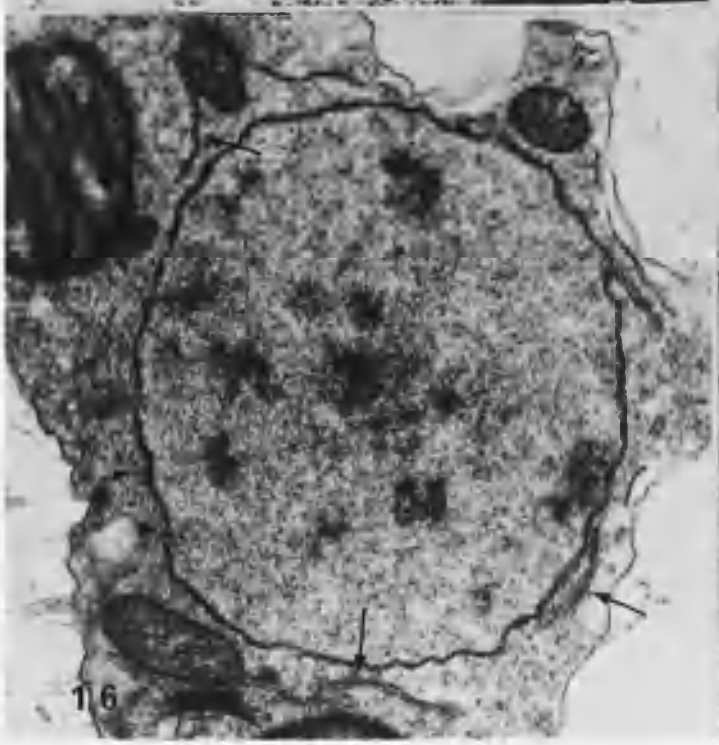

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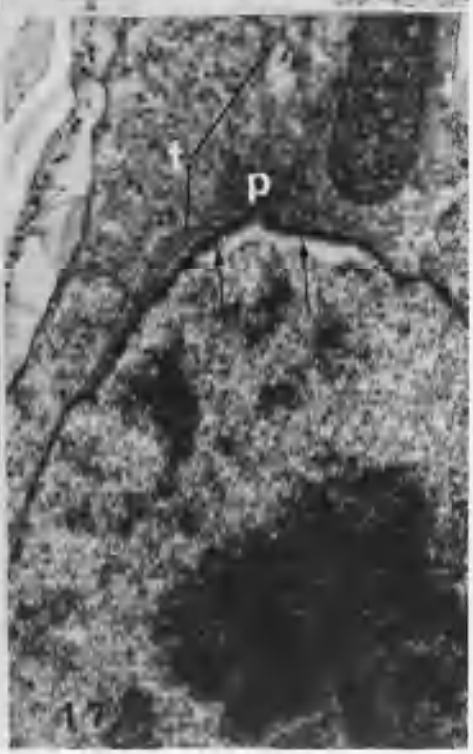

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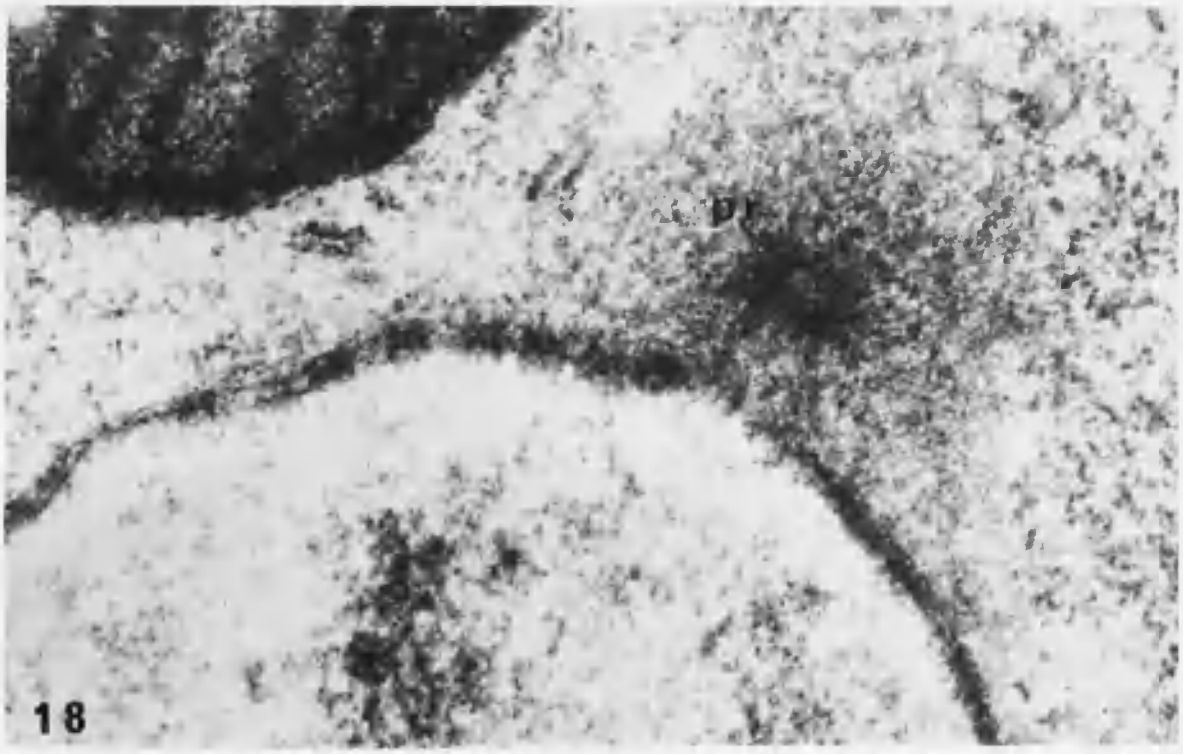
Figure 18. Oblique section of polar ring near prophase nucleus. -- The polar ring (pr) is surrounded by dense cytoplasm. X 77,300.

Figure 19. Longitudinal section of polar ring. -- The dense cytoplasm around the polar ring (pr) excludes ribosomes and other organelles. A transverse section through a nuclear pore is also visible (arrow). X 77,300.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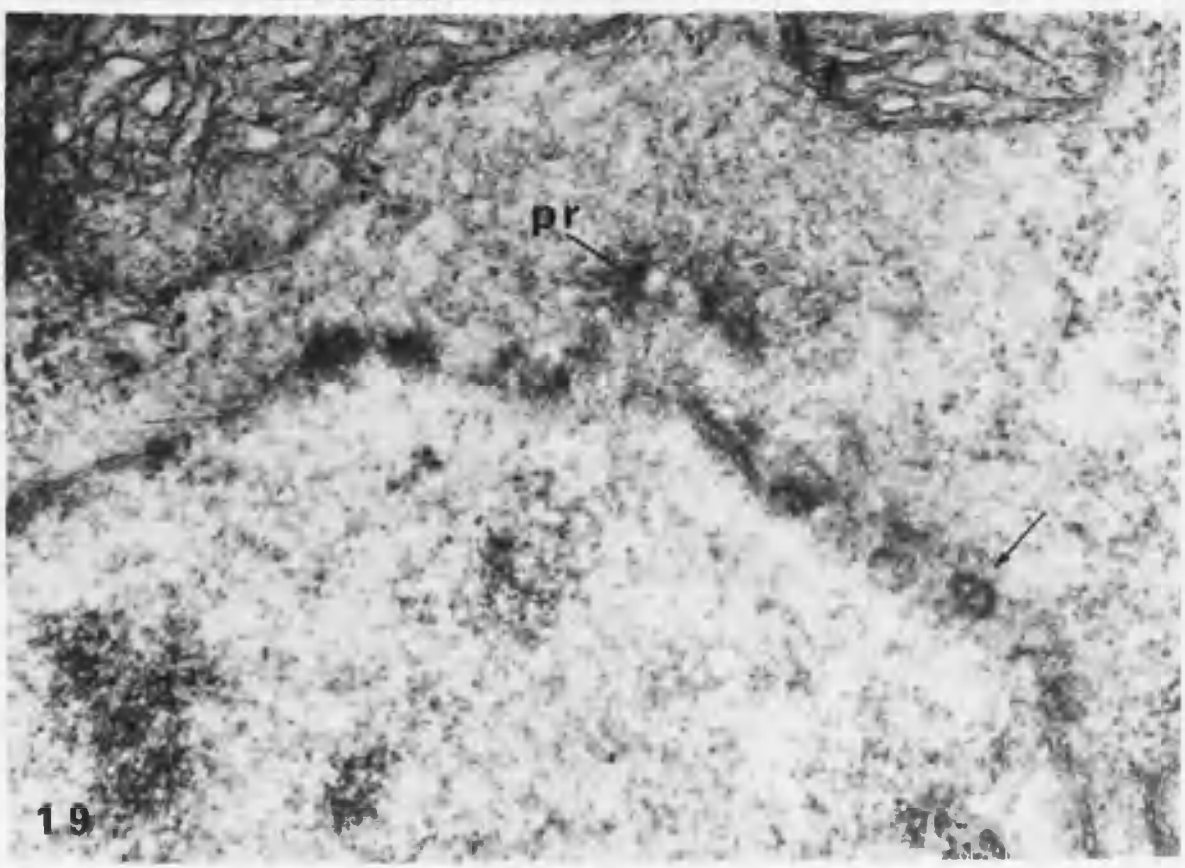
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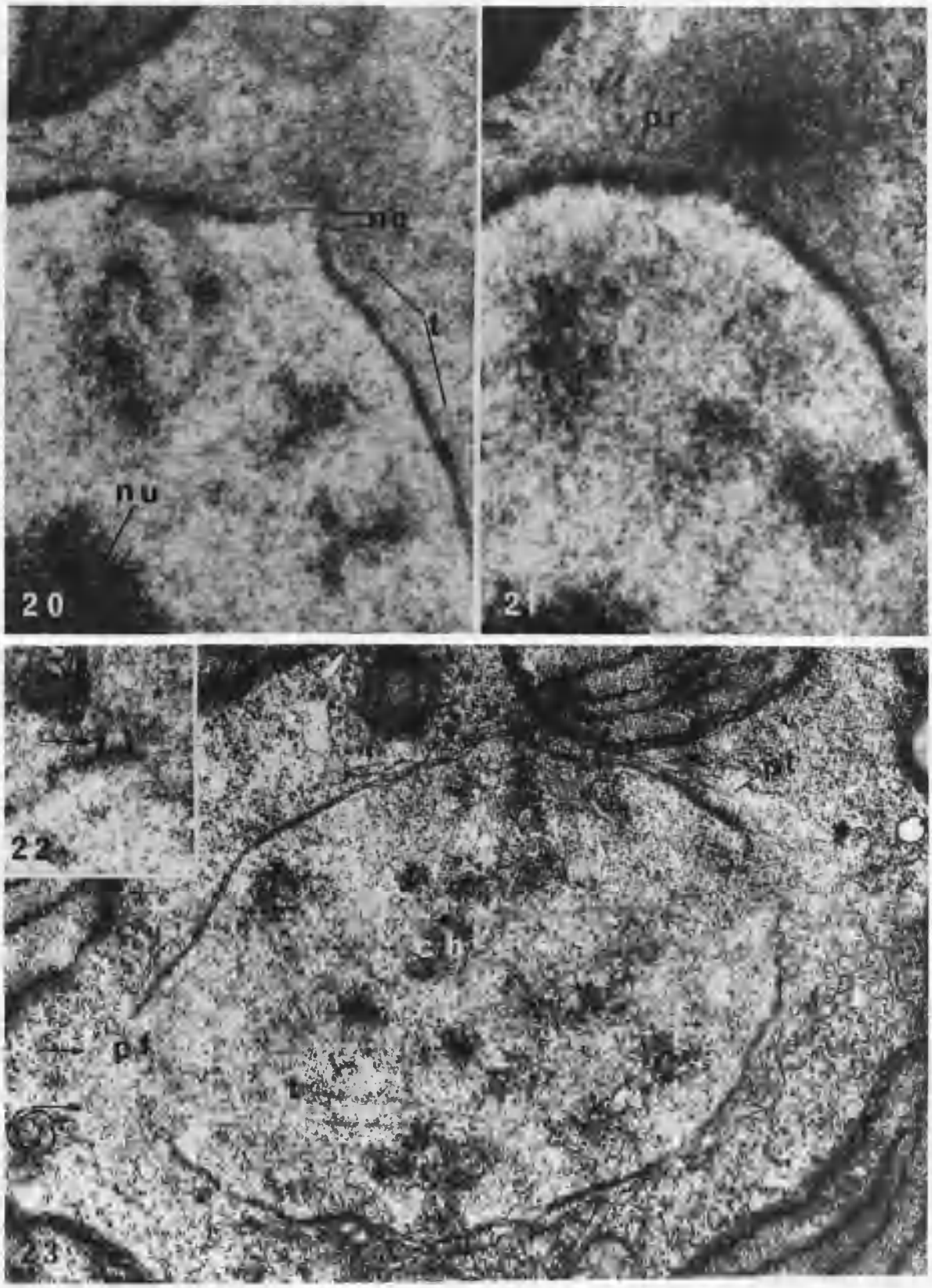
Figure 20. First of two serial sections through a pole of a prophase nucleus. -- The nuclear envelope (ne) projects out into a dense, amorphous area from which microtubules (t) appear to radiate. Chromatin is beginning to condense, but the nucleolus (nu) is still present. X 57,600.

Figure 21. Second of two serial sections through a pole of a prophase nucleus. -- The polar ring (pr) is shown in the dense amorphous cytoplasm near where the nuclear envelope projects in Figure 20. X 57,600.

Figure 22. Longitudinal section of polar ring in close association with nuclear envelope. -- The polar ring (arrow) appears appressed to the nuclear envelope. X 45,200.

Figure 23. Pro-metaphase, longitudinal section. -- Polar fenestrae (pf) form and are covered by "cytoplasmic caps" containing membranous fragments (arrows) and excluding ribosomes. Spindle microtubules (t) extend from the polar fenestrae into the chromosomes (ch). Some perinuclear microtubules (pt) are sandwiched between the perinuclear endoplasmic reticulum and nuclear envelope. X 30,200.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pl
ch
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23

Figure 24. Metaphase nucleus. -- Chromosomes aligned at metaphase plate with kinetochores, from which chromosomal microtubules radiate. The nuclear envelope is clearly discontinuous (arrows) at the polar fenestrae, and a mitochondrion lies adjacent to the polar region. X 29,000.

Figure 25. Metaphase nucleus, oblique section. -- The nucleus is surrounded by a layer of perinuclear endoplasmic reticulum (per), and the nuclear envelope remains completely intact (arrow) in areas not disrupted by the polar fenestrae. X 29,000.

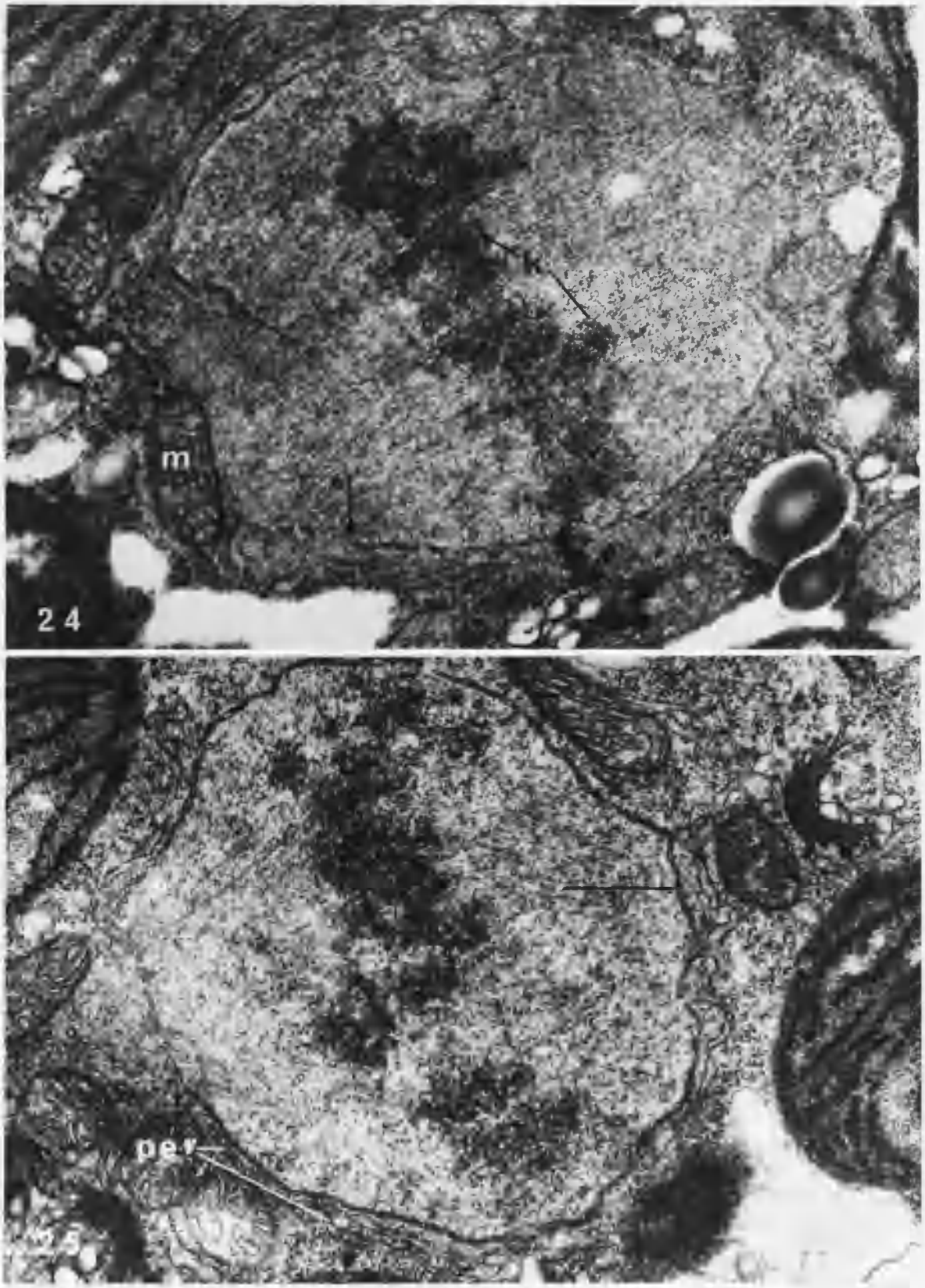
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Figure 26. Metaphase, cross section. -- Microtubules (t) appear in the region between the chromosomes (ch) and the nuclear envelope, suggesting that the continuous spindle lies external to the chromosomal microtubules. X 21,800.

Figure 27. Early anaphase nucleus. -- Kinetochores (arrows) are visible as chromosomes begin to move toward the poles. The nucleus is elongated from its metaphase configuration. X 19,300.

Figure 28. Mid-anaphase nucleus. -- Elongating nucleus cleaves centripetally as chromosomes lose contrast within nucleoplasm. The perinuclear endoplasmic reticulum (per) has formed at least two layers, especially around cleavage area. Mitochondria (m) are situated near the poles, which remain open. X 24,500.

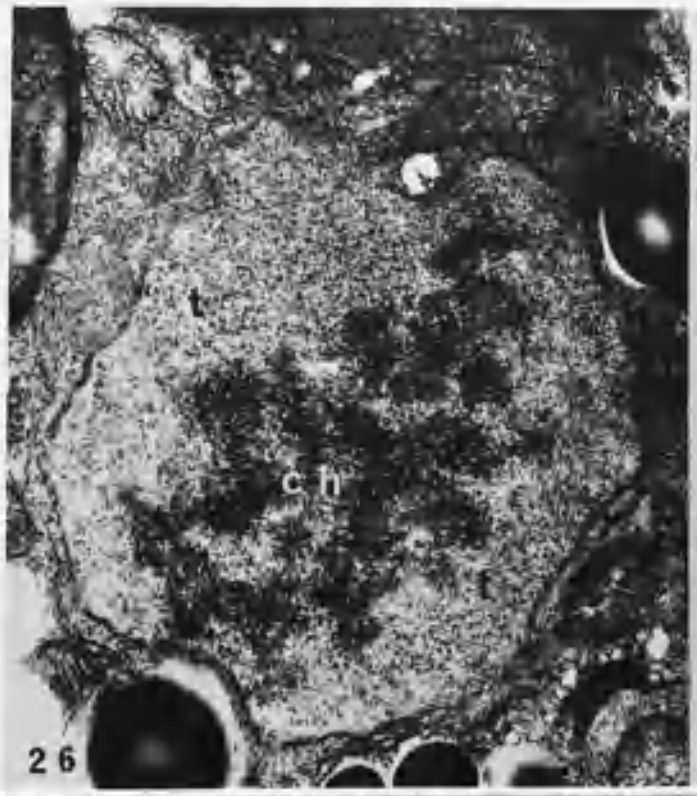

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Figure 29. Late anaphase nucleus. -- Nuclear cleavage is nearly complete. Polar fenestrae (arrows) are still present at this late stage, and kinetochores (arrowheads) are also visible. X 26,600.

Figure 30. Telophase nuclei. -- Daughter nuclei separate, allowing mitochondria and other organelles to enter interzone (i). Perinuclear endoplasmic reticulum (per) still remains around each daughter nucleus. X 26,600.


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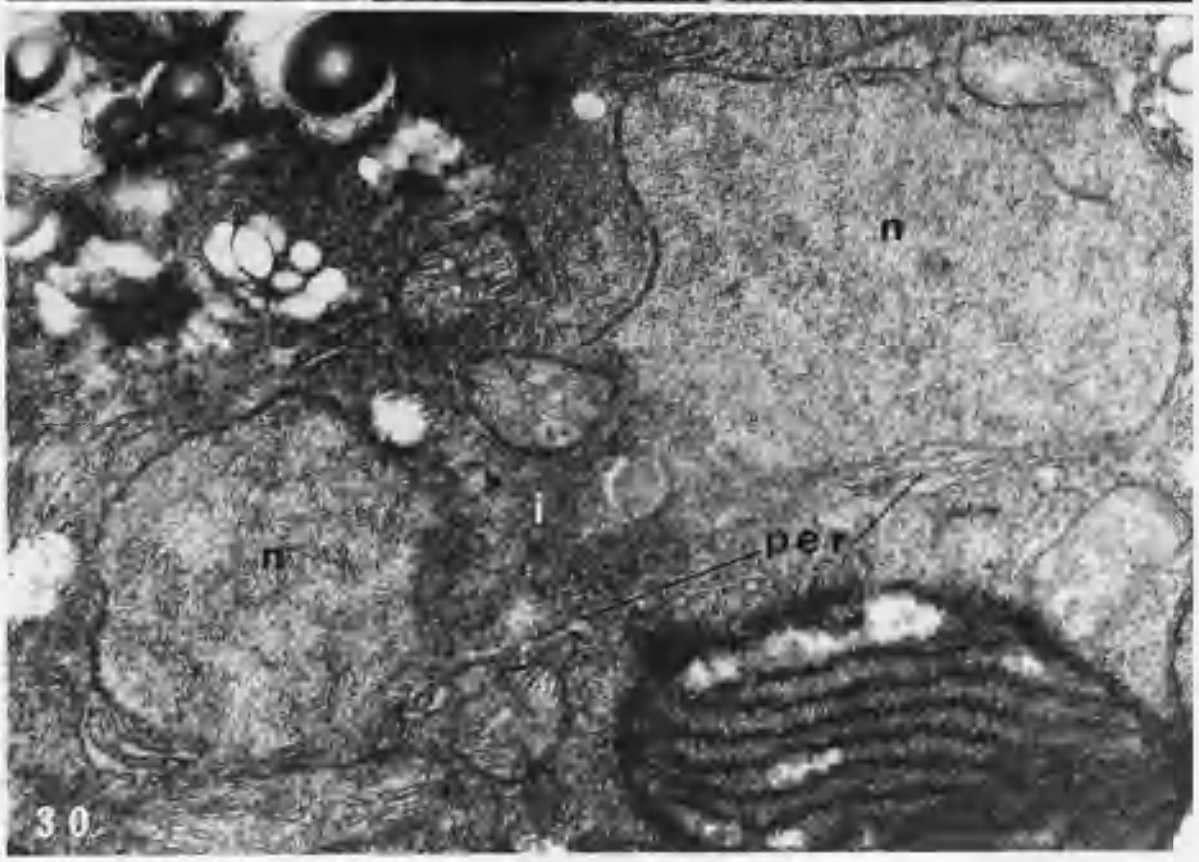
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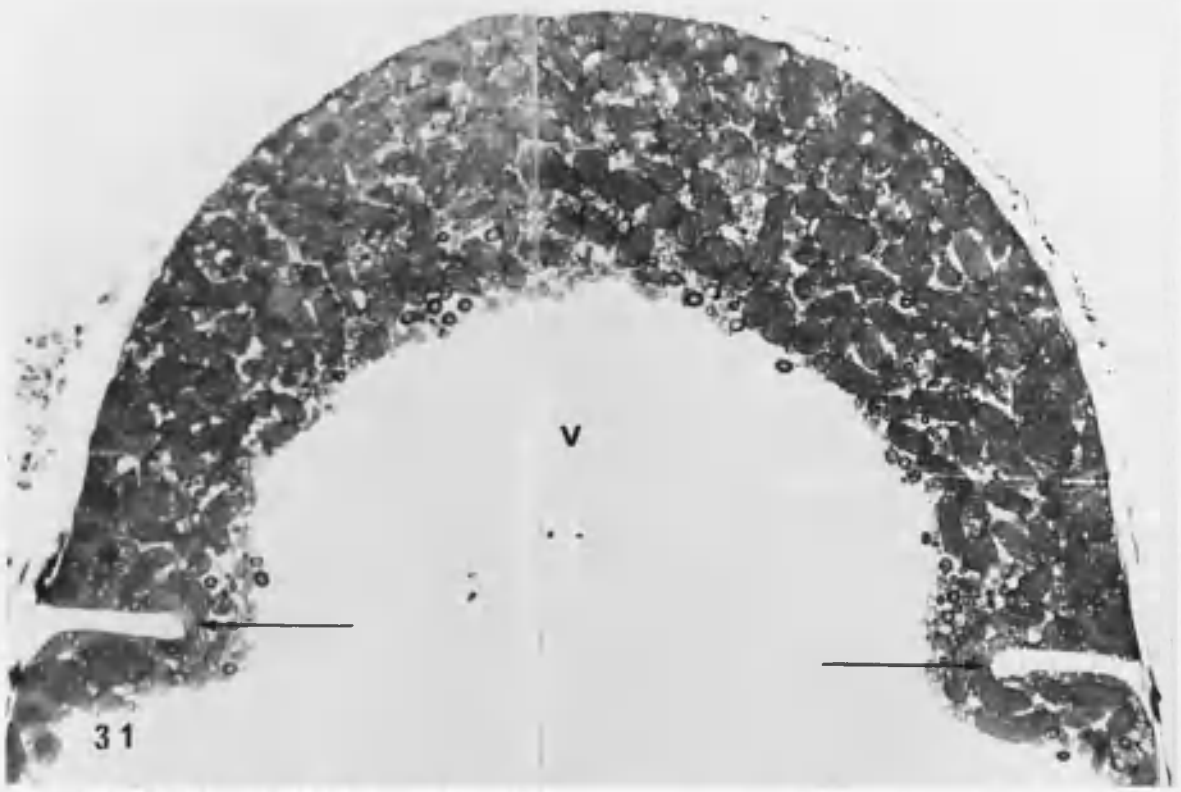
Figure 31. Cytokinesis. -- Ingrowing annular furrow (arrows) forms just beneath cytoplasmic concentration and begins to cleave the central vacuole (v). X 2,000.

Figure 32. Higher magnification of cleavage furrow. -- Golgi bodies (g) lie near the growing edge of the furrow and along the septum. Numerous vesicles (v) lie near the plasmalemma at the septum and probably discharge their contents into it. X 8,100.

Figure 33. Cytokinesis, tangential section through septum. -- Segregation of membrane-bound crystalline bodies (x) into daughter cells occurs as the septum (s) cleaves the central vacuole. X 2,000.

Figure 34. Crystalline body. -- High magnification of membrane-bound crystalline body shows the regular pattern within the lattice. The arrow points to the membrane. X 40,000.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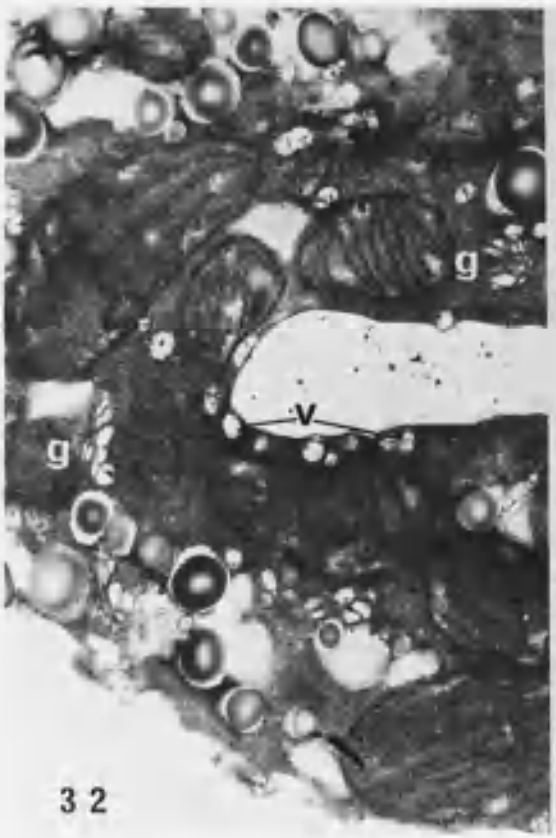
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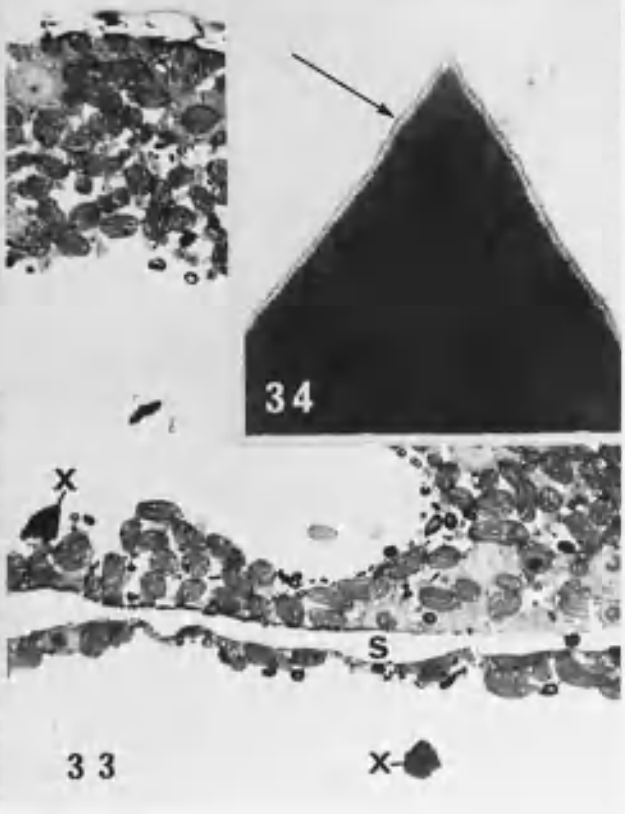
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X
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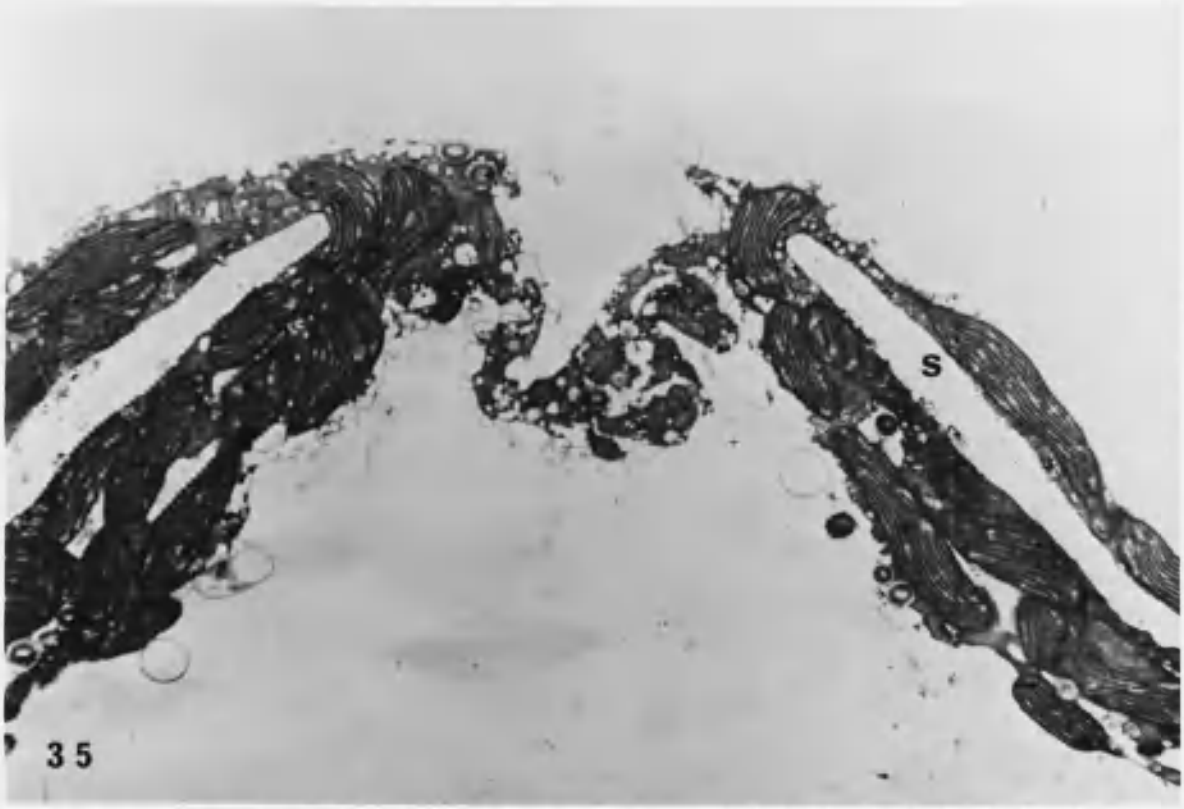
Figure 35. Cytokinesis, septal complex formation. -- The septum (s) has completely separated the upper and lower vacuoles, but leaves a small aperture where cytoplasm collects. X 4,000.

Figure 36. Septal plug formation, early stage. -- A large amount of tubular endoplasmic reticulum (er) proliferates in the cytoplasm which collects in the aperture left by incomplete cleavage by the septum (s). X 75,800.



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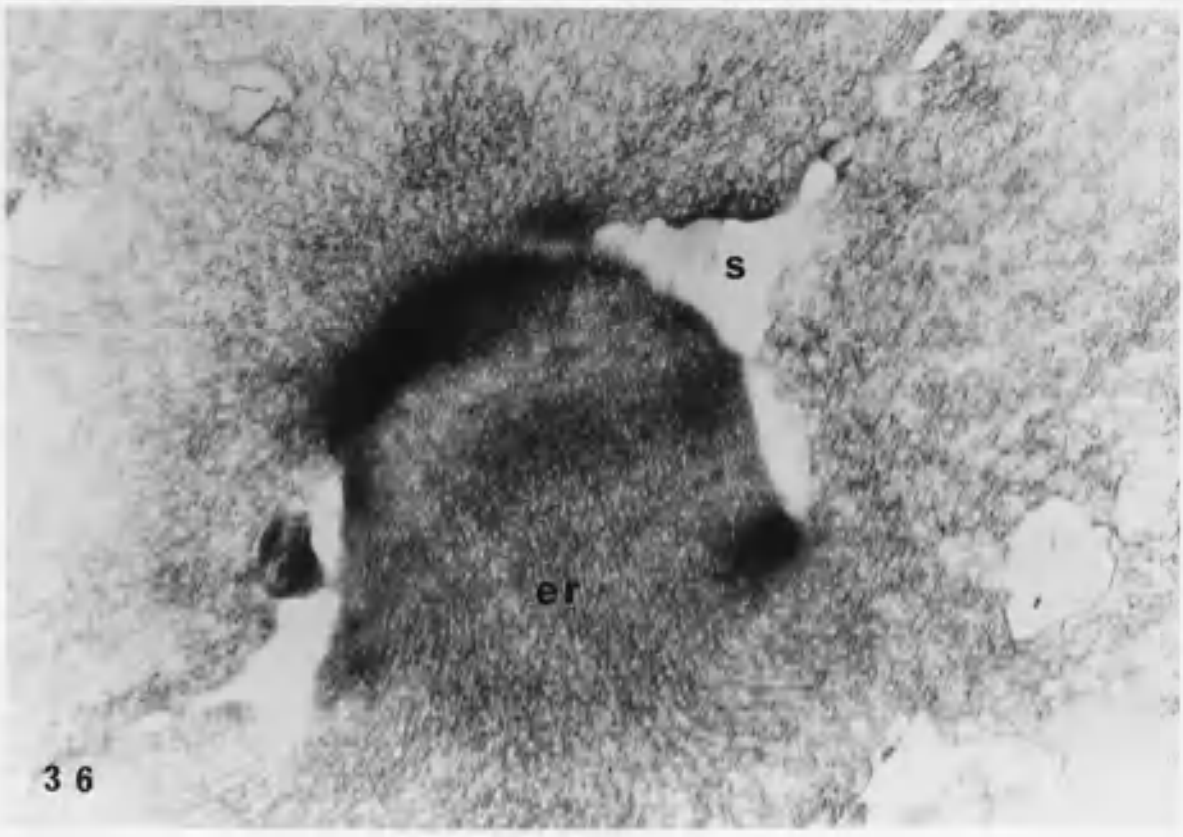

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Figure 37. Septal plug formation, oblique section near aperture. -- Both longitudinal and cross sections through the tubular endoplasmic reticulum (er) are visible. Note that all other organelles, including ribosomes, are excluded by the endoplasmic reticulum. X 56,700.

Figure 38. Septal plug formation, later stage. -- Electron dense material fills aperture and obscures the tubular endoplasmic reticulum. X 26,100.

Figure 39. Completed septal plug, median longitudinal section. -- Some endoplasmic reticulum (er) remains near plug (p). Differences in cytoplasmic density are apparent between apical (a) and subapical cells. Septal wall material (s) has been disrupted during preparation. X 8,800.

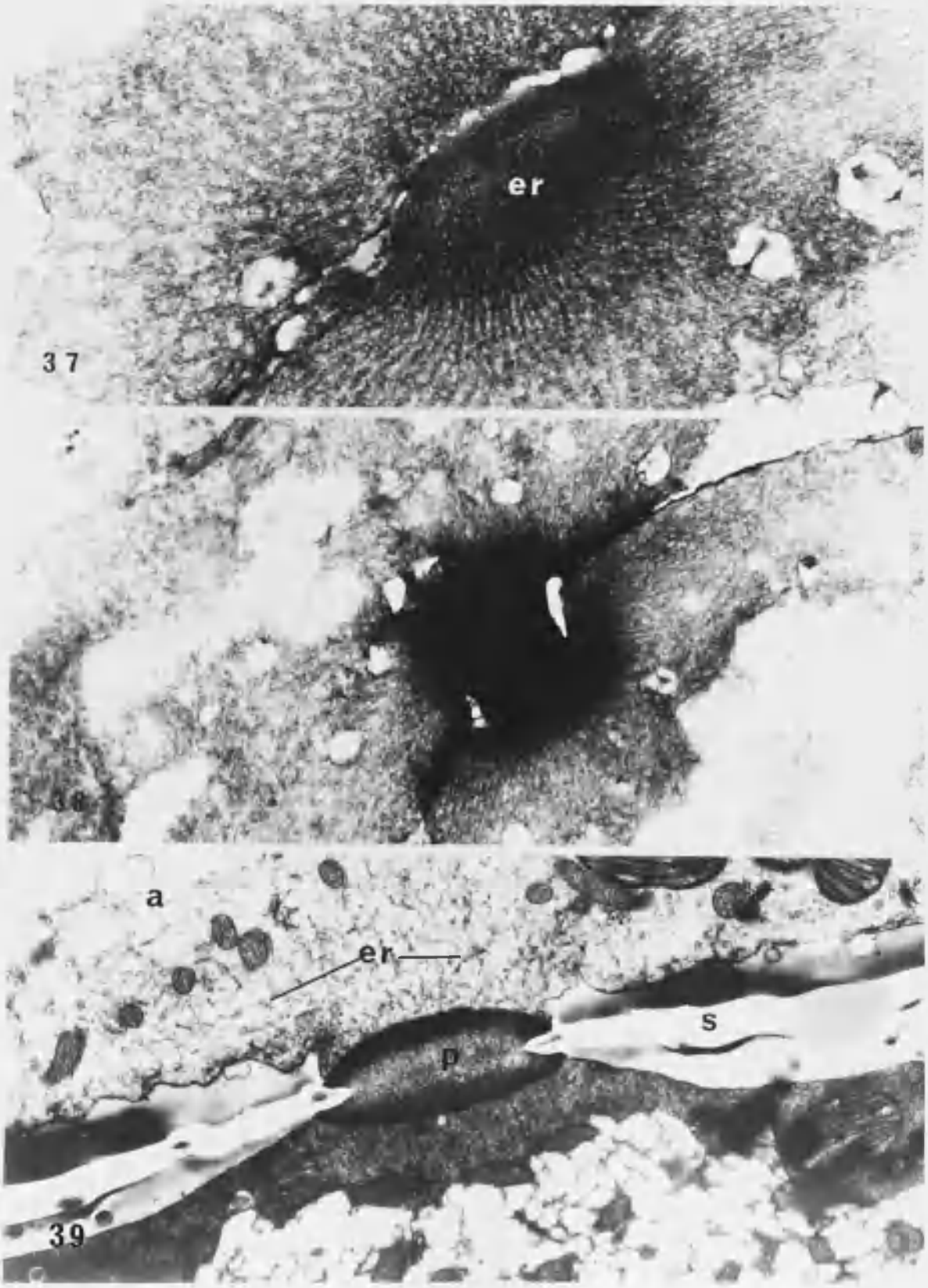
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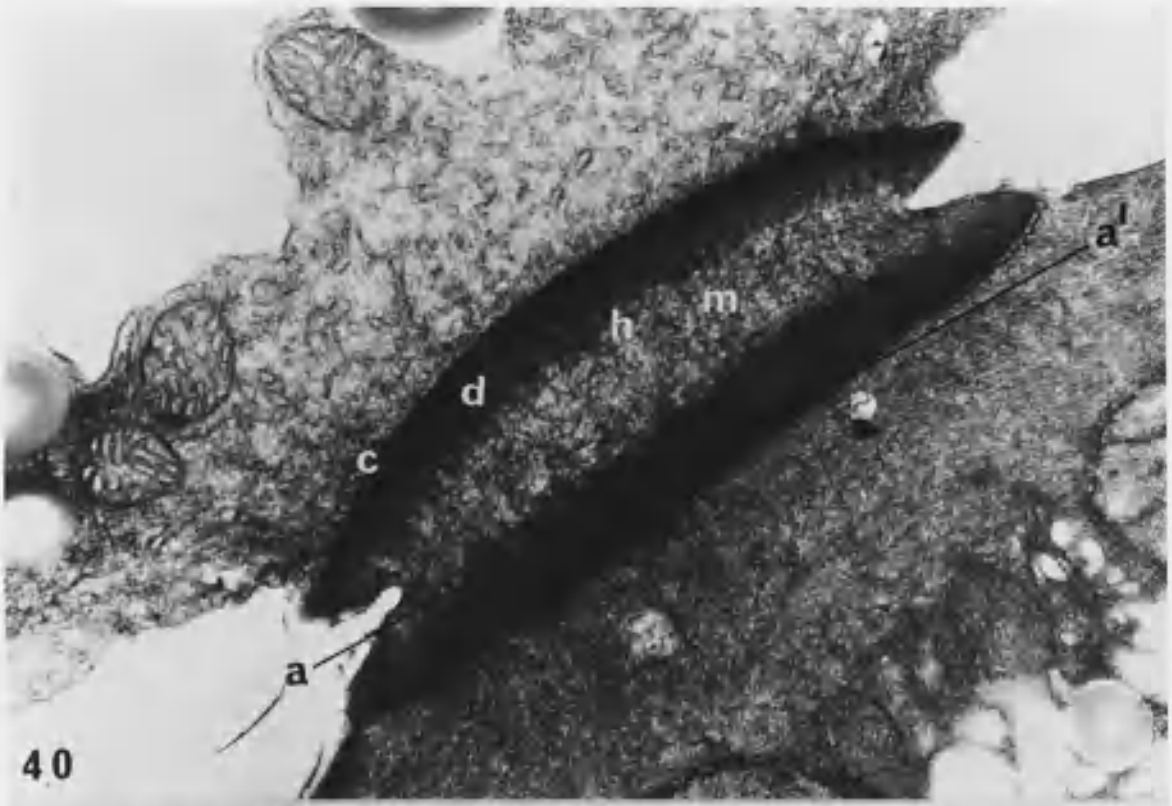
Figure 40. Septal plug, high magnification. -- The septal plug consists of four major zones: both cytoplasmic faces are covered with a plug cap (c); an electron dense zone (d) lies immediately beneath the caps; the third zone consists of a parallel tubular network (h) appears as a honeycomb in cross section; and the central matrix (m) is the fourth and center zone. See text for discussion. Line a-a' denotes plane of section in Figure 43. X 26,100.

Figure 41. Mature septal plug. -- Lower cap is lined with dense particles about 40 nm in diameter. X 26,100.



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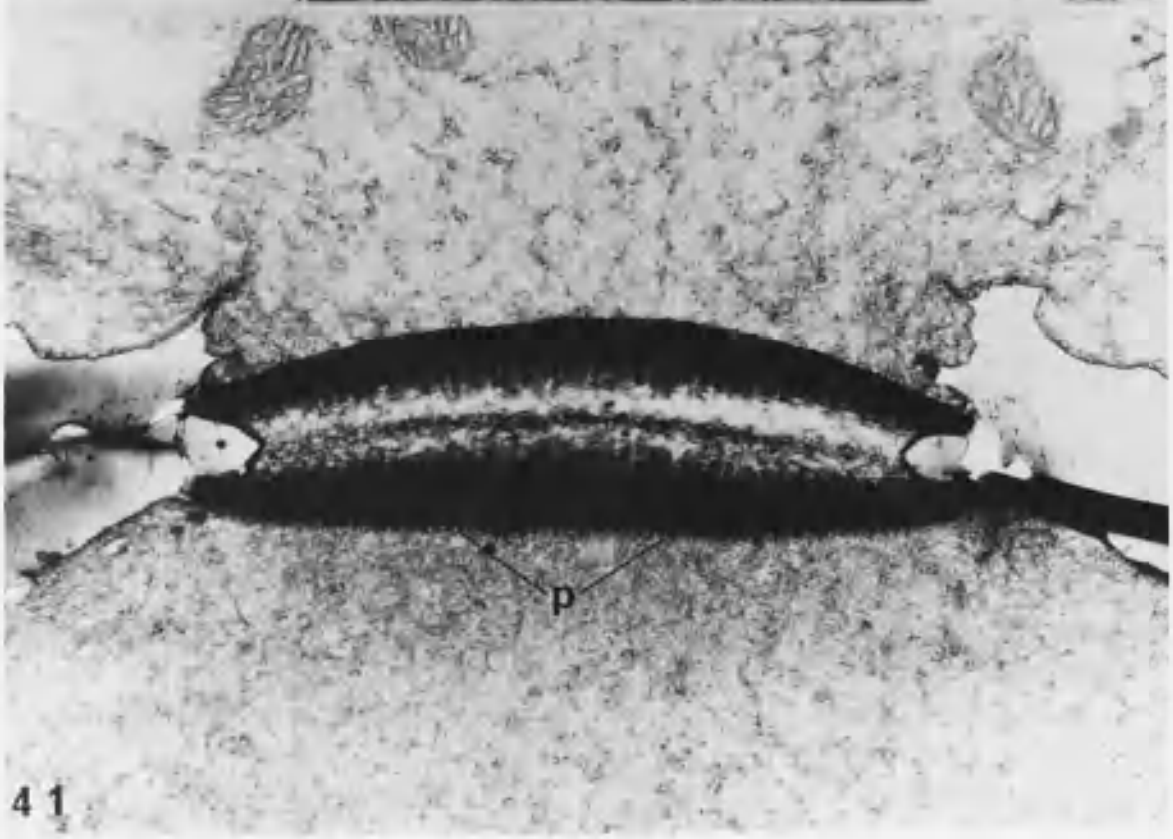

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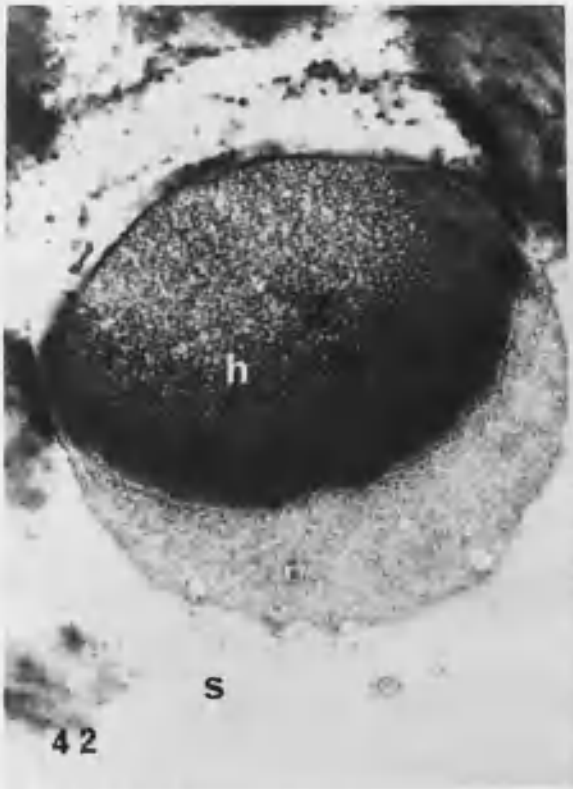
Figure 42. Septal plug, slightly oblique cross section. -- The parallel tubular zone shown in Figure 40 appears here as a well-ordered honeycomb network (h). Septal wall material surrounds the plug. X 7,600.

Figure 43. Septal plug, slightly oblique cross section through several zones. -- The plane of section is represented in Figure 40 as line a-a'. The honeycomb network (h), dense cap and amorphous zones (c) are visible. X 17,400.

Figure 44. Honeycomb network, high magnification. -- Fibrils (f) occupy the lumen of each hexagonal chamber. X 57,400.

Figure 45. Septal plug, light micrograph of a cross section. -- Centrally located septal plug (arrow) is surrounded by cytoplasm. Numerous nuclei (n) are shown. Toluidine blue stain. X 394.



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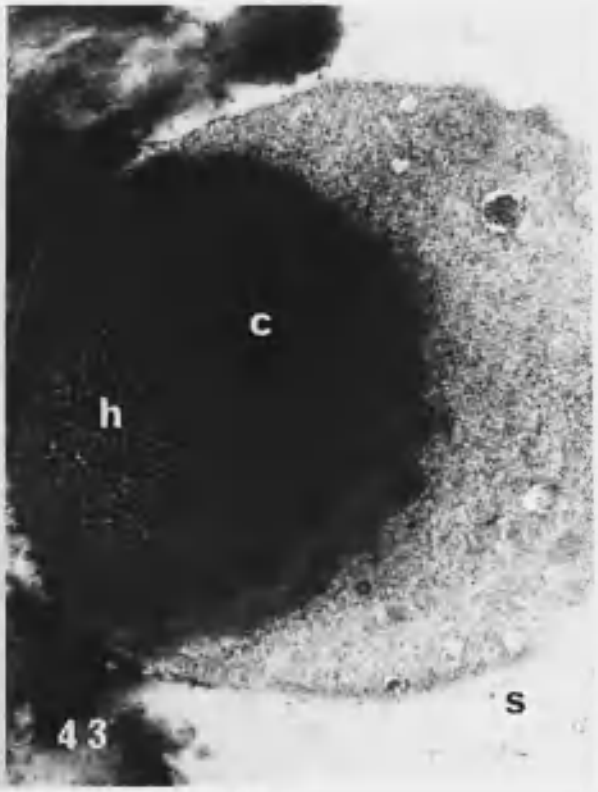

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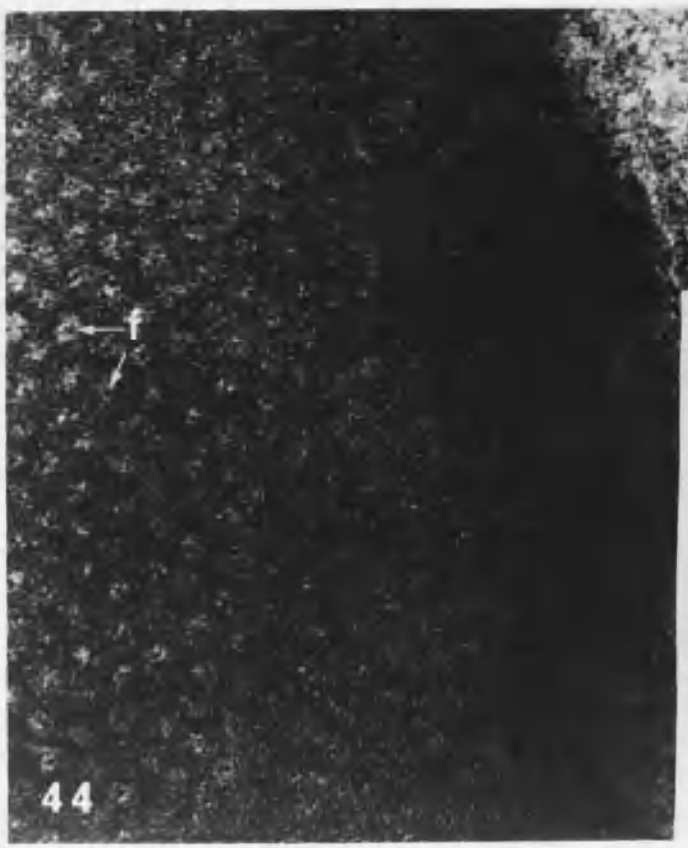

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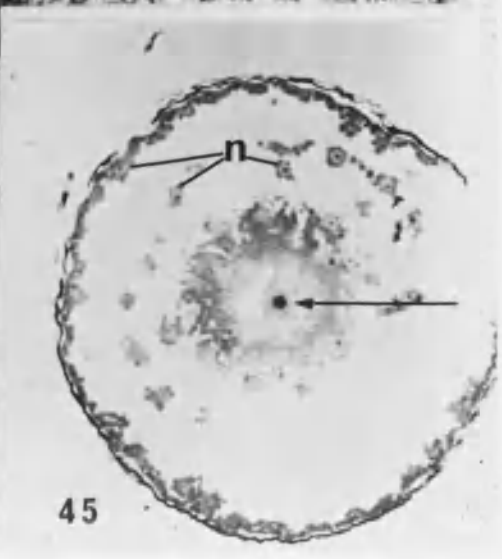

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