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## **Cell Division in the Multicellular Marine Red Alga *Agardhiella subulata***

Karel Joan Klepacki  
*College of William & Mary - Arts & Sciences*

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CELL DIVISION IN THE MULTICELLULAR MARINE RED ALGA  
*AGARDHIELLA SUBULATA*

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A Thesis  
Presented To  
The Faculty of the Department of Biology  
The College of William and Mary in Virginia

In Partial Fulfillment  
Of the Requirements for the Degree of  
Master of Arts

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by  
Karel Joan Klepacki  
1994

APPROVAL SHEET

This thesis is submitted in partial fulfillment of  
the requirements for the degree of  
Master of Arts

Karel. Joana Klepacki  
Karel Joan Klepacki

Approved, May 1994.

Joseph L. Scott  
Joseph L. Scott

Sharon T. Broadwater  
Sharon T. Broadwater

Stanton F. Hoegerman  
Stanton F. Hoegerman

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## ABSTRACT

Observations of interphase and mitotic cells were made, with the transmission electron microscope, on the multicellular marine red alga *Agardhiella subulata* (C. Agardh) Kraft et Wynne (Solieriaceae, Gigartinales, Rhodophyta), marking the first documented account of a mitotic study of a species in the Gigartinales. The spherical interphase nucleus of *Agardhiella* develops a spindle shape by prophase. The outer membrane of the nuclear envelope (NE) extends through a typical polar ring and becomes continuous with an endoplasmic reticulum cisterna, a feature found also in *Sarcodiotheca gaudichaudii* (Gigartinales) and *Lomentaria baileyana* (Rhodymeniales). Two microtubule-filled channels develop at each pole, at least one of which extends from pole to pole.

By prometaphase, these channels rupture, producing two big gaps with one large NE remnant between them along each of the two broad, flat metaphase division poles. A typical metaphase plate forms across the wide nuclear midregion. Chromatids separate to opposite nuclear poles in anaphase and an interzonal midpiece (IZM) forms between them as the nucleus acquires a dumbbell shape. By telophase, the daughter nuclei are separated by vacuoles, starch, and the newly detached IZM. The IZM is surrounded by swirls of the two to four layers of perinuclear endoplasmic reticulum which had encased the nuclear sides without capping the polar regions from prophase through telophase. A cleavage furrow forms in the cell membrane to complete the division into two daughter cells.

Considered to lie somewhere between the orders of Batrachospermales and Rhodymeniales in taxonomic complexity, it is not surprising that *A. subulata* was found to have mitotic features of both these orders. Previously, five basic mitotic types were established to aid in classification. *Agardhiella subulata*, along with several other species, does not fit neatly into any one of these types. The revision of the five type system was in order, and was recently accomplished by recognizing variations within two new main classes - the polar gap type and the polar fenestrations type. *Agardhiella subulata* should be considered a member of the latter type.

CELL DIVISION IN THE MULTICELLULAR MARINE RED ALGA  
*AGARDHIELLA SUBULATA*

## INTRODUCTION

Detailed light microscopic accounts of the morphology, as well as the vegetative and reproductive development, of the marine red alga *Agardhiella subulata* have been published by Gabrielson and Hommersand (1982). Other descriptions at the light microscopic level, though many of these conflict and use various superseded nomenclatures, can be found in Harvey (1853), Osterhout (1896), Kylin (1928), Taylor and Rhyne (1970), Wynne and Taylor (1973), Ganesan (1981), and Coomans (1986), and thus need be only briefly reviewed here.

In habit, *A. subulata*, the type species of this genus, is a fleshy, terete, inter- to subtidal plant found along most coasts of the North American Atlantic, Gulf of Mexico, and Caribbean Sea. The plant main axis grows erect from an attachment disc and the branches, which are generally irregular-alternate, commonly taper toward both the apices and bases of attachment. Reportedly, thallus characteristics vary with environmental parameters (Gabrielson and Hommersand, 1982). The plant may grow to 40 cm tall with axes 4 mm in diameter and with four to five orders of branching when in shallow, more sheltered waters, 10 cm tall and narrower in the deep subtidal, and shorter

and thicker intertidally. Thalli have been found to range from yellow to pink, red, or reddish brown.

Thalli of *A. subulata* are multiaxial (more than one apical cell per axis) with varying reports of four to ten obliquely dividing apical cells giving rise to the indeterminate filaments (Gabrielson and Hommersand, 1982; Bold and Wynne, 1985; Coomans, 1986).

The mature thallus consists of several cell layers organized into an outer cortex, inner cortex, and medulla. A single layer of small, ellipsoidal cells with their long axes perpendicular to the thallus surface comprise the outermost surface layer, the outer cortex. These cells are tightly packed, uninucleate, and pigmented. The four to five layers included in the inner cortex consist of multinucleate, spherical to ovoid cells that get progressively larger approaching the thallus center. Inner cells of the lateral filament and rhizoid systems, along with cells of the axial file (the two are indistinguishable in the mature thallus), make up the medulla. These multinucleate, generally longitudinally oriented and highly elongated medullary cells constitute 60-70% of the axis diameter.

With the exception of surface cortical cells, all cells in *A. subulata* form secondary pit connections. Magne (1964) noticed that higher Rhodophyta tend to maintain a fairly constant ratio of nuclear volume to cell volume. This can be the result of karyokinesis sans cytokinesis (Goff and

Coleman, 1986) and/or an increase in size of a single nucleus (Coomans and Hommersand, 1990). The evolution of secondary pit connections provided a third avenue by which this balance could be secured. These connections, common in most higher Rhodophyta, arise from the fusion of small conjuctor cells with adjacent vegetative cells, and result in increasing the nuclear number in each receiving cell by one. (Besides secondary pit connections, Coomans and Hommersand (1990) expect that fusion with certain multinucleate cells, analogous to conjuctor cells, contribute to the highly multinucleate state of axial cells in *Agardhiella*.) Secondary connections not only give further integrity to the thallus structure, but provide new opportunities for vegetative thallus modifications (Gabrielson and Hommersand, 1982; Coomans, 1986; Coomans and Hommersand, 1990).

The bulk of this study involves ultrastructural observations on mitotic stages of vegetative apical cells, from both determinate and indeterminate axes of *A. subulata*. Although there are no absolute boundaries to the beginning or ending of cycle stages, as mitosis is a continuous process, the following account will categorize the general fine structure and overall appearance of cells in the various recognized main phases of cell division.

Mitotic studies, in particular, have been invaluable in untangling lineage mysteries in the Chlorophyta (Stewart and Mattox, 1975) and fungi (Heath, 1980a). To date, studies of

this type have been published on 15 red algal species, included in six rhodophycean orders. It is hoped that these studies collectively will yield similar evidence of phylogenetic associations among the Rhodophyta. The investigated species include *Porphyridium purpureum* (Bronchart and Demoulin, 1977; Schornstein and Scott, 1980, 1982), *Rhodella violacea* and *R. maculata* (Patrone et al., 1991), *Dixoniella grisea* (Scott et al., 1992), *Flintiella sanguinaria* (Scott, 1986), *Compsopogon coeruleus* (Scott and Broadwater, 1989), *Batrachospermum ectocarpum* (Scott, 1983), *Lomentaria baileyana* (Davis and Scott, 1986), *Griffithsia flosculosa* (Peyriere and Heim, 1971), *Dasya baillouviana* (Phillips and Scott, 1981), *Apoglossum ruscifolium* (Dave and Godward, 1982), *Membranoptera platyphylla* (McDonald, 1972), *Polysiphonia denudata* and *P. harveyi* (Scott et al., 1980), and most recently, *Bossiella orbigniana* (Broadwater et al., 1993) (Table 1). Present work on *A. subulata* is unique in representing the first mitotic study in the order Gigartinales.

By examining these studies, five tentative mitotic "types" have been established (Scott and Broadwater, 1990). Features of *A. subulata* will be compared and contrasted to these types to determine where this plant's mitotic system would be best included. Evolutionary considerations will then briefly be considered. In addition, recently, another publication on red algal mitosis has been completed detailing a newly proposed mitotic typing (Broadwater et

al., 1993). A brief assessment of the placement of *Agardhiella* within this new scheme will be included in addendum (see ending note of Discussion).

As in all microscopic studies employing fixatives, caution should always be used in extrapolating observations to describe the live condition. In more recent years, the employment of new and/or varied fixation and specimen preparation techniques, embedding media, staining techniques, microtome designs, knives, and buffer types and concentrations for electron microscopy, have all reduced the possibility of "misinterpretation from technique-induced artifact" (Wischnitzer, 1981). However, even though the results of electron microscopic preparation can often be duplicated, and micrographs are generally considered to adequately reflect the live condition, it must be remembered that the state of specimen preservation is somewhat non-objective, and that observations are still subject to deviations from nature (artifact) through osmolaric, staining, or other fixation or equipment problems. Also, reservation must be kept regarding the accuracy of interpreting a dynamic process such as mitosis, including analysis of 3-dimensional structures, through a series of mostly unrelated, 2-dimensional still photographs, or at best, from whole or sketchy serial thin section photography (Leedale, 1964).

Due to these problems, countless authors have wisely insisted on correlating as much electron microscopic



findings as possible with light microscopy of the organism. With respect to this view, cursory light microscopic observations of mitosis in *A. subulata* have been included. Furthermore, over 30 different electron microscopic fixations were performed, some with a number of variations in protocol, in an attempt to recognize unnatural conditions and attain better fixation quality. To minimize descriptions of artifact, several hundred cells of each fixed thallus were viewed, vastly increasing the sample size. All considered, explicit confidence in the following cellular and fine structural details can not be guaranteed, but can reasonably be expected and adopted as reflecting the live cell condition, provided the aforementioned warnings are kept in mind. To assist in this, many areas of possible or probable artifact have been indicated in the following text.

## METHODS AND MATERIALS

*Agardhiella subulata* (C. Agardh) Kraft et Wynne (Solieriaceae, Gigartinales, Rhodophyta) was collected from its natural habitat, both at the York River near Yorktown, Virginia, and from the rock pilings of the Engineering Pier at Fort Monroe, Virginia. Collections were made over the years 1986 and 1987 during the months of March through September though most of the mitotic figures found and studied were from the mid to late summer months. Healthy drift specimens were used primarily since sublittoral attached plants were scarce.

Initial monitoring for mitotic activity was done by examining material collected from sunrise up to 6 h afterwards. These times were chosen since it was already known that some other macroscopic algae of the more advanced red algal orders had division peaks at approximately 2 h into the light cycle (Scott et al., 1980; Phillips and Scott, 1981; Davis and Scott, 1986). Fixed branch tips of *A. subulata* that were stained with 4'-6-Diamidino-2-phenylindole (DAPI) and monitored with fluorescence microscopy (procedure discussed later) also showed a slight peak of mitotic activity starting approximately 2 h into the light cycle; thus, branch tips cut from plants were

routinely field-fixed at 15-30 min intervals from 1-4 h after sunrise.

Field fixations were used exclusively since plants collected and acclimated for several days to controlled laboratory conditions in an attempt to establish light-dark synchronized cell division (Lloyd et al., 1982) resulted in a marked increase in starch, vacuoles, and a granular cytoplasmic appearance, making the search for dividing cells even more difficult. Large branches of each plant were brought back to the lab to later be identified, when possible, as either tetrasporophytic or gametophytic plants.

Tips were fixed for 2 h in 2% glutaraldehyde in 0.1 M phosphate buffer at pH 6.6 with 0.15 M sucrose added to adjust for osmolarity of Yorktown specimens, and 0.25 M sucrose for Fort Monroe plants. Tips were rinsed three times for 15 min each in 0.1 M phosphate buffer. Post-fixation was in 1% osmium tetroxide in the same phosphate buffer for 2 h at 4 °C. Dehydration began at room temperature with one 20 min wash each in 50% and 70% acetone, then left overnight at 4 °C in 70% methanol with 2% uranyl acetate. Dehydration continued with 30 min washes each in 70% acetone, 90% acetone, then three changes of 100% acetone.

Infiltration of tips was done at room temperature for 1 h each in a series of 2:1, 1:1, then 1:2 parts 100% acetone:epoxy resin embedding medium, then left overnight in pure resin. Results presented here are exclusively of Embed

812 embedding medium though Epon 812 was tried. Resin was changed two times a day for at least another two days, then tips were embedded in fresh resin, one tip per flat embedding mold to ensure correct orientation. Resin was allowed to polymerize in a 60 °C oven for at least three days before sectioning.

Gold thin sections (90-130 nm thick) were cut with a Dupont diamond knife on a Sorvall Porter-Blum MT-2B ultramicrotome and picked up on empty one-hole copper slot grids, post-stained 1 min with Sato's lead, then rinsed 5 s on each of 13 water droplets. In an attempt to reduce lead contamination, NaOH pellets were occasionally placed in the covered staining dish. Thin sections were then transferred to formvar-coated one-hole copper slot grids for examination and photography with a Zeiss EM 109 electron microscope using Ilford Pan-F film.

Thick sections of apical branch tips (200-250 nm) were cut with glass knives and transferred to glass slides where some were stained with 1% toluidine blue O (a metachromatic cationic dye which binds to negatively charged groups) in 1% sodium borate for approximately 1 min over low heat. Stained and unstained thick sections were examined with a Zeiss Photomicroscope II using bright field and Nomarski differential interference optics and photographed with 35 mm Panatomic-X film.

Tips were prepared for fluorescence microscopy (modified from Goff and Coleman, 1984) by fixing for 1-3 h

in 3:1 95% ethanol:glacial acetic acid or in 5% glutaraldehyde. After rinsing in distilled water or buffer, tips were transferred to a glass slide and stained with  $0.5 \text{ ug} \cdot \text{mL}^{-1}$  DAPI. A highly specific DNA fluorochrome, DAPI binds to adenine-thymine rich DNA regions quantitatively with respect to the amount of DNA present (Coleman et al., 1981). The apical ends of tips were squashed before adding a cover slip. Alternatively, 200-250 nm thick epoxy sections were prepared and similarly stained. Both were examined with an Olympus BH-2 microscope equipped for epifluorescence using an HBO 100-W mercury lamp for excitation. An Olympus UV filter combination was used giving a peak excitation between 334 and 365 nm, resulting in a characteristic whitish-blue fluorescence.

## RESULTS

This ultrastructural study of cell division exclusively employed vegetative apical cells from the thallus tips of *Agardhiella subulata*. The pattern of vegetative development of branch apices predicted that only these cells would have a rate of mitotic activity high enough to lend practicality to the undertaking of this study. Thus, apical cells of both indeterminate and determinate filaments, from axial and periaxial files respectively, were used for observation and photography. Whenever possible, female gametophytes were chosen for fixation, as their conspicuous raised cystocarps were easier to visually identify in the field. Upon return to the lab, some others were identified by light microscopy as tetrasporophytes by the presence of zonate tetrasporangia scattered among the surface cortical cells. No noticeable light microscopic or fine structure differences in either quiescent or various mitotic cells were observed between determinate or indeterminate filaments, or between haploid female gametophytes, diploid tetrasporophytes, or the many plants of unidentified life history stages. Therefore, it is suggested that mitosis in apical cells of both axial and periaxial files, and in all life history stages of this triphasic isomorphic plant, is similar or identical, and

thus will be described together in the following account of cell division.

#### LIGHT MICROSCOPIC OBSERVATIONS

Mitotic light microscopic results are presented here as a supplement to observations of mitotic ultrastructure. These findings are in accordance with the much more detailed work of Coomans (1986), who, using different methods, completed a light microscopic study of mitosis in an array of selected simple to more complex rhodophycean genera, including *Agardhiella subulata*.

Finding dividing cells using light microscopy was almost as frustrating as doing similar work at the electron microscopic level. Much of this difficulty arose from working at relatively low magnifications, complicated by a lack of great contrast between the chromatin and the rest of the cell, and by the problem of fluorescence bleaching by ultraviolet (UV) light. Few obviously dividing cells were discovered; these were found exclusively with the fluorescent microscope, and though most were in metaphase, a few suspected anaphase cells were spotted.

As in the electron microscopic study which comprises the bulk of this report, only the tapered apical tips (Fig. 2) of this multi-branched alga (Fig. 1) were used for light microscopic observation. While both thick section and

squash techniques were used, the increased number of apical cells in the squashes resulted in increased findings of division. These preparations, however, did not allow for determination of division pole orientation with respect to the thallus tips.

In agreement with electron microscopic findings, though, other observations at the light microscopic level were indeed possible. The three cell types previously described, (outer cortex, inner cortex, and medulla) were clearly distinguishable in the thick sectioned thallus (Fig. 2), as well as in the squash preparations (Fig. 3). Stained with DAPI, the autofluorescence of chloroplast and mitochondria DNA appeared almost as a dusting of tiny sparkles within the cells, adding to the incredible beauty of the preparations.

In dividing apical cells, chromatin of each cell in metaphase had condensed onto a plate that appeared as an almost linear band (0.5-1.5 X 4-7  $\mu\text{m}$ ) extending across most of the cell's width (Fig. 4). At anaphase, sister chromatids migrated to opposite poles in the form of two flattened bands. These bands fluoresced with somewhat less intensity than did the metaphase plates. A large vacuole (or vacuoles) could usually be perceived between the two chromosome bands, and the suggestion of an annular ingrowth or furrow was often seen forming on one or both sides of anaphase cells (Fig. 5).



## ELECTRON MICROSCOPIC OBSERVATIONS

### General Aspects of Interphase Cells

Apical cells were typically ellipsoidal (7-8 X 12-15 um). Two different types of apical cells were initially examined (Fig. 6). Those of short cortical branches were oriented around the thallus periphery in such a way as to be perpendicular to the thallus long axis. These gradually angled parallel to the thallus long section at the thallus apex, where the other type of apical cells (those which produce the elongate medullary filaments from which arise the cortex) were located. Observations in this report were restricted to the latter type of apical cells, as cell division was not found with enough frequency in the former type to include them.

In all cases, as they were sectioned, fixed branch tips pulled away from the resin block at the point where the resin met the plant's outer cell wall (Fig. 6). This presumably was due to a lack of complete infiltration of the bulky tips, resulting in a separation of materials of different consistencies (resin versus cell wall).

The cell wall appeared as a region of irregularly concentric, layered fibrous material, 0.5-2.5 um thick, which surrounded each individual cell (Figs. 8, 13, 14). On occasion, electron dense particles were found scattered within the otherwise electron translucent wall. It should be noted, however, that cell wall appearance may be highly

unpredictable, as pointed out by Duckett and Peel (1978) and Brawley and Wetherbee (1981), who concluded that its thickness, density, fiber orientation, and composition may vary greatly even within the same species or thallus with respect to many factors including geography and habitat, length and nature of fixation, and life history phases. Craigie (1990) added that cell wall composition is also affected by the age of tissue, and by seasonal and site-specific variations, including growth temperatures and the availability of fixed nitrogen. In the case of *Agardhiella*, wall thickness was expected to be partly artifactual due to osmolaric fixation problems causing cell shrinkage and thus a seemingly thicker wall. A slight transparency could be seen in the area surrounding most cells, which may mark the natural location of the cell and wall positions (Fig. 6).

Though cells of the inner thallus often were highly invaginated and irregular, the plasma membrane of the outer layer of apical cells were well preserved and appeared as a generally smooth, thin, continuous double line surrounding the cells. As mentioned, the plasma membranes seemed shrunken from their natural positions, and were at their probable original cell boundaries only at places where they met and were continuous between other cells at pit connections (Fig. 7). Each connection consisted of an aperture, filled by a central, electron dense, double-lens shaped septal core, or pit plug, assumed to be of the variety possessing no plug caps, as were all other studied

members of the order Gigartinales (Pueschel and Cole, 1982). Cap membranes, already demonstrated to exist in the Gigartinales, were occasionally discernible in *Agardhiella*, on one or both sides of the pit plugs. These membranes were continuous with the plasmalemma and were associated with each end of the plug in a way that resulted in the plug core being extracellular (Pueschel, 1987).

Scattered mostly around the cell periphery, 7-9 chloroplast profiles per thin section could be seen (Figs. 8, 14, 30, 46). In contrast to the several to many small, discoid chloroplasts supposedly typical of the subclass Florideophycidae (Bold and Wynne, 1985), *A. subulata* possessed a parietal chloroplast with no pyrenoid. The chloroplast was nearly whole in apical cells but soon after divided to form an "irregular network of interconnected bands" (Coomans, 1986). Thin sectioned chloroplast profiles varied greatly in size. Since these seemingly independent profiles may represent sections through a smaller number of chloroplasts which interconnect in different planes or levels, the true size and number of chloroplasts per cell remains unclear.

The chloroplast envelope consisted of two membranes. There were 4-10 single, unstacked, basically parallel thylakoids in the stroma of each chloroplast. As expected in most orders of the Rhodophyceae (Pueschel, 1990), peripheral thylakoids, or "peripheral inner limiting discs", were apparent in the chloroplasts (Figs. 8, 14, 30). At

higher magnifications, phycobilisomes, electron dense 19-28 nm diameter particles expected to contain the water soluble photosynthesizing accessory pigments allophycocyanin, R-phycocyanin, and phycoerythrin, (Gantt and Lipschultz, 1972), were seen arranged in a typical alternating pattern on opposite, outer (stromal) sides of the thylakoid membranes (Figs. 16, 21, 25). Phycobilisomes are composed of acidic phycobiliproteins bound together by basic linker proteins that properly orient the chromophores, and though most are disc-like, the exact size and shape of these complexes is known to vary among species (Gantt, 1990).

In some sections, small electron transparent areas (85-170 nm diameter) could be seen scattered in the chloroplast stroma, as could small, dark globules (40-80 nm diameter) (Figs. 14, 21, 30). The transparent areas might reasonably be identified as genophores, areas where DNA fibrils are thought to be attached to the thylakoid membrane, whereas the electron dense stroma globules might be lipid granules or plastoglobuli, since they appeared to resemble similar structures reported in chloroplasts of higher plants, and may function in lipid synthesis and storage (Brawley and Wetherbee, 1981).

Though mitochondria were found throughout the cytoplasm of interphase cells, they seemed to be concentrated around the nucleus (Fig. 8). Also, as will be discussed subsequently, there were always mitochondrial profiles in association with the forming, or cis face of all dictyosomes

(Fig. 9). Spherical to ovoid mitochondrion profiles, commonly 0.4-0.5  $\mu\text{m}$  in diameter and 0.7-0.9  $\mu\text{m}$  long, ranged in number from five to nine per thin section. Occasionally, elongated, irregular, winding shapes were observed, adding to the probability that some or most of the discrete profiles actually belonged to an interconnected network of only a few organelles. Although this supposition has been demonstrated true in only one red alga to date (*Rhodella reticulata* = *Dixoniella grisea*), it is indeed the case in many other organisms (Broadwater and Scott, 1986).

The fine structure of mitochondria was generally not well preserved, with membrane swelling in some areas and fuzziness in others. In profile, cristae appeared as 200-300 nm long and 20 nm in diameter structures, typically projecting from all sides along the inner membrane and spaced at 20-100 nm intervals. Within the same cell, depending upon planes of section, some profiles showed cristae to be of random orientation, some somewhat parallel, and others decidedly so. Extreme variation, possibly artifactual or due to sectioning plane, also occurred in cristae density, with some mitochondria within the same cell almost devoid of these structures.

Located throughout the ground cytoplasm or cytosol, 2-3 Golgi body (dictyosome) profiles per median thin section could be seen. Each was composed of approximately 6-10 parallel, stacked cisternae formed from smooth surfaced membranes. Cisternae were thin and flat in the center and

dilated at the periphery, with an intercisternal space of about 10 nm (Fig. 9).

At the cis or forming face, cisternae were generally smaller (0.4-0.5  $\mu\text{m}$  in diameter) and flatter compared to the rest of the organelle. Rough endoplasmic reticulum (RER) could be seen in the cis region, and as in most all red algal orders studied to date, a mitochondrial profile was always found in close association (Fig. 9) (Pueschel, 1990). In contrast, on the opposite side of the cisternal stack, the maturing or trans dictyosome face was formed of larger, more concave cisternae, 0.5-1  $\mu\text{m}$  in diameter. The trans region is thought to be responsible for the many small 85-110 nm, membrane-bound vesicles clustered near the cisternal margins. These electron transparent vesicles were irregularly spherical and often contained some fibrous or membranous material.

Highly electron transparent floridean starch grains ranged from spherical to ellipsoidal (0.5-1 X 0.6-1.5  $\mu\text{m}$ ) and varied greatly in number from cell to cell and from fixation to fixation, with none to over 20 visible per thin section (Figs. 8, 14, 16, 28, 41). Generally, aged cells and inner thallus cells had a greater accumulation of starch (Fig. 6). These storage polysaccharides, existing more often in groups rather than as individuals, were only found free in the cytoplasm and seemed not to be confined to any particular cellular area, though were often seen in association with RER. Floridean starch grains, the main

photosynthetic reserve product in the Rhodophyta, appeared to have no internal structure.

Several other types of membrane-bound grains, vesicles, or vacuoles of varying electron densities could be found in outer cortical cells of *A. subulata*, presumably housing a variety of secretory or storage products. The most worthy of mention in the context of this study is the electron transparent vacuole. In interphase apical cells, from one to a few small, often highly irregularly shaped vacuoles were usually found (Fig. 8). These commonly had an excess of membranous material which appeared tangled within, or on occasion, seemed to partition an otherwise larger vacuole. The actual number of vacuoles per cell was unclear, as the possibility existed that some or all might have been continuous within other planes of section.

Rough endoplasmic reticulum (RER) was seen in thin section as single-membraned, convoluted cisternae, 20-30 nm thick in most places with areas of swelling throughout giving an irregular appearance. Configurations of 2-6 cisternae aligned almost parallel in some areas and more randomly in others were seen more frequently than single cisternae. Though not obviously excluded from any particular cellular area, cisternae were observed in greater number toward the cell center (Fig. 8). These cisternae, however, did not encircle the nucleus or run parallel to the nuclear envelope for any extended length. In addition, a peripheral RER system, seemingly of a single, irregularly

shaped band of ER, was often visible just under the cell membrane (Figs. 14, 29, 30). Connections of this peripheral RER to the cell's internal ER system may have existed, though were not clearly seen.

At higher magnification, ribosomes could not only be seen attached along the cytosolic side of RER membranes (Figs. 9, 15), but also as free ribosomes seen in uniform concentration throughout the ground cytosol, giving it a generally granular appearance.

Interphase apical cells possessed a single, centrally located spherical to ovoid nucleus (3-5  $\mu\text{m}$  in diameter) that showed no noticeable polarity in most sections (Fig. 8). The nucleus was encased by an intact nuclear envelope (NE) which consisted of two parallel unit membranes, one surrounding the other, with a perinuclear space of 15-20 nm. In some sections, several small, electron dense particles, presumably ribosomes, were present on the cytosolic side of the outer nuclear membrane (not shown). Penetrating the NE were nuclear pores, electron dense 80 nm diameter areas which appeared to be somewhat evenly spaced throughout the NE. Clear attachment of ER and NE was not observed at this stage of the cell cycle.

Areas of differing compositions and electron densities could be seen within the interphase nucleus. The following descriptions of nuclear components and characteristics were drawn from composite micrographs and observations of many



sections and cells. The nucleus in Fig. 8 is representative of most of these descriptions.

The nucleoplasm, a region with an electron transparent, coarsely granular and fibrous appearance, occupied most of the nuclear volume. Immediately adjacent to and all along the inner nuclear membrane in some cells were small diffuse clumps of electron dense, granular material presumed to be heterochromatin. In cells assumed close to the onset of mitosis, this condensed, peripheral chromatin appeared even darker staining and was noticeably more concentrated as it joined in some areas to form branching aggregates that extended deeper into the nucleoplasm. In these cells, condensed masses of chromatin were often found just outside the nucleolus, and on occasion, this perinucleolar chromatin (or material of similar staining and make-up) seemed to penetrate fairly deeply into the nucleolar volume.

The large prominent, central nucleolus (1.2-1.7  $\mu\text{m}$  in diameter), was formed of mostly granular and some fibrous, moderately electron dense components. A few spherical areas (0.2-0.4  $\mu\text{m}$  in diameter) of electron density and granular make-up similar to the nucleoplasm, were seen within the diffuse, spheroidal nucleolus in some cells.

The nucleus associated organelles (NAOs) in *Agardhiella subulata* were ring shaped and have been referred to as "polar rings" (PRs) (Scott and Broadwater, 1990). Under high magnifications and in fortuitous planes of sectioning, PRs could be visualized as two separate structures (Fig.

11), each composed of a single electron dense "doughnut" or ring (the proximal and distal portions). The two rings, always in close association with the nucleus, lay one on top of the other and were consistently oriented perpendicular to the nuclear surface so that the stacked doughnut holes faced the NE (Figs. 10-12).

The proximal and the distal portions of the PR each measured 100-120 nm in diameter, and were separated from each other by an electron transparent zone of 15-20 nm. Continuous electron dense material was observed only at the periphery. The area penetrating the combined doughnut holes was often lighter staining, whereas a diffuse, more electron dense granular buildup was often found external to the sides of the PR that were lateral and distal to the NE (Figs. 11, 12). In some sections, it appeared that the proximal PR portion may have been attached to the outer NE by very fine filaments or struts (Fig. 11).

Though MTs were assumed to persist to some degree in all cells, they were not seen in large numbers or in definite configurations at interphase. Their relative absence is only mentioned here to point out contrasts with later mitotic stages.

### Cell Division

Subsequent text describes the impressive dynamics of cell division. Many of the aforementioned cellular components did not vary in structure or description through

the course of mitosis, thus only those structures or organelles that changed from the interphase state will be mentioned.

Prophase. Although the vast majority of thallus cells were in interphase, an estimated 35 cells were sectioned close enough through their polar areas to be recognizable as being in early prophase. One is cautioned that the features of prophase in *Agardhiella*, as for most red algae, do not coincide precisely with those outlined for prophase of other eukaryotic organisms (see Discussion; Prophase). With this in mind, perhaps the single most striking sign of the onset of prophase in *A. subulata* was the elongation of the previously spherical nucleus into an unmistakably more oblong, spindle shape (Figs. 13, 14). This elongation gave a defined polarity to the nucleus, and thus to the cell.

By chance, three early prophase cells were each sectioned longitudinally through their respective nuclei, as well as through both their PRs (e.g. Fig. 14). Notably, all three cells showed PRs already situated at opposite nuclear ends as PR migration and the establishment of division poles had already taken place. The PR pairs remained in this position at opposing nuclear poles throughout this stage; they were not observed in cells in post-prophase stages.

The PRs were surrounded by small, ribosome free, zones of exclusion (Figs. 13-15). These were seen as lighter staining areas of a granular nature, devoid of structure

except for some poorly defined MTs. Some of these MTs emanated from the zones of exclusion and extended longitudinally from the PR area toward and alongside the NE, sandwiched between it and the PER (discussed shortly) (Figs. 18-20). These parallel, extranuclear MTs terminated at varying but short distances of less than halfway along the lateral nuclear sides. No continuous, extranuclear, pole-to-pole MTs were discovered.

Cellular polarity increased as the nucleus continued to progressively elongate, and its polar areas often approached opposite sides of the plasma membrane. Meanwhile, bulges or swellings in the NE formed and enlarged at each pole. Out of each of these polar convexities arose a small, often sharply pointed, projection of the nucleus, located immediately beneath, though not directly touching, its associated PR (Figs. 10, 11, 15). These nuclear envelope protrusions (NEPs) are typically found in mitotic cells of the higher Rhodophyta (Scott and Broadwater, 1990) and give the NE the appearance of being pulled outward at that point. This condition contributed to the typical and pronounced spindle shaped nucleus mentioned above, which seemed to become more pronounced as prophase progressed.

By mid-prophase, the outer NE membrane actually extended directly through the center of the PR, while the inner NE membrane remained intact (Figs. 18, 19). In cross section, the outer membrane of the NE and the PR, respectively, were seen as a 35-50 nm diameter single

membraned ring within a larger 100-110 nm diameter, more electron dense and thicker ring. The inner membrane ring never directly contacted the PR inner wall (Fig. 20). On occasion, the PR-penetrating NE membrane appeared to be continuous with a single RER cisterna (Figs. 18, 19), which transversed the otherwise ER-barren exclusion zone.

Within the nucleus, the nucleolus appeared unchanged from its interphase state, while chromatin began to condense into electron dense granular clumps with occasional fibrous components. Most of the inner NE was lined with condensed peripheral chromatin and chromatin globules were usually seen in the NEPs (Figs. 11, 15). Nuclear pores were still relatively evenly concentrated throughout most of the NE at this time, including the polar areas (Fig. 17), but were excluded from the membranes of the NEPs. Occasionally, a few mitochondrial profiles were seen in polar regions.

By mid-prophase, a developed perinuclear endoplasmic reticulum (PER) system had been established. Though a few micrographs classified as very early prophase showed some PER development, either as short ER segments adjacent only to some areas of the nucleus (excluding the midregions) (Fig. 14), or as already matured systems (Fig. 13), all micrographs, without exception, revealed an extensive PER system running the entire length of the nucleus by mid-prophase (Fig. 16).

Generally, stacks of 2-4 parallel cisternae were located along the nuclear sides, the most proximal of which

remained at an approximate equidistant 50-100 nm from the NE along much of its length. These profiles abutted the exclusion zones but usually extended only slightly beyond the level of the PR and never enclosed or capped the polar areas (Figs. 13, 14, 16). The PER most distal from the NE often appeared less ordered and more tangled (Figs. 14, 16), and at times terminated in close proximity to the plasma membrane. Mid-prophase PER perhaps aided in creating a separate environment within the cell, containing the nucleus, zones of exclusion, and MTs.

In a glancing section through the polar region of one nucleus, PER appeared to radiate outward and thereby produced interesting swirled configurations (Fig. 17). It is not certain from which mitotic stage this swirled pattern was photographed; however considering the long-section "tangled" ER arrangement that surrounded the zones of exclusion in the *A. subulata* cells mentioned above, prophase seemed a likely possibility. Somewhat similar ER patterns, termed nuclear-ER (NER), have been described by Peel et al. (1973) in various plant cells, including some fertilized eggs, developing spores, encysting zoospores, developing and germinating pollen, differentiating sieve elements, and post-meiotic tetraspore mother cells of *Corallina officinalis* L., though since all these describe highly active meiotic divisions, functional similarities seem improbable.

Late prophase was distinguished by the formation of invaginations in the NE immediately adjacent to the NEP; one on either side of each PR (Figs. 21, 22). Of the 30-35 late prophase cells found, most were sectioned through only a single indent, though micrographs depicting two cavities were occasionally procured. Sometimes the two invaginations at each pole seemed to grow into one another, leaving a common channel opening, which separated into two tunnels further into the nucleus (not shown).

The channel center contained a granular matrix, similar in nature to the ribosome filled cytosol found outside the exclusion zones (Figs. 21-24). Up to seven MTs per thin section were positioned longitudinally in the channel, and several nuclear pore cross sections could be seen in its NE lining (Figs. 22-24). Small amounts of ER were sometimes found in the center of the "mouth" area and a short distance into the tunnel entrance (Figs. 22, 24).

Although NE-lined tunnels were seen in late prophase, very few cells showed evidence of a continuous nuclear channel transecting most or all of the nucleus (Fig. 25). This paucity of pole-to-pole examples was naturally complicated by plane of section, and presumably by the ephemeral nature of the tunnels. The number of channels penetrating the nucleus in this way could not be discerned, as even tangentially sectioned photographs revealed no more than two channels per nucleus (Fig. 26). Thus, various possibilities existed, including two channels from each pole

joining to form two transecting tunnels, or independently creating four channels, which may have grown at varying rates.

The polar areas of the late prophase nucleus sometimes still displayed the pointed, spindle shaped nuclear poles of early prophase (Figs. 21, 23), and other times appeared more rounded. The NE covering the entire polar area, including the depressions, still appeared intact, though tangentially sectioned cavity sides were most likely viewed more often than the actual deepest points of the invaginations. The NEP, when seen, was still drawn sharply toward the PR center, but was usually no longer seen penetrating the PR, or associating itself with an ER cisterna (Fig. 21). (Only one example of the outer NE membrane still penetrating the PR was found.) Though they may have existed in other planes of section, NEPs were not generally seen in cells with deeper channels.

Microtubules were numerous. As mentioned, MTs were visible between the zonal area and the forming cavities. Though these MTs were seen within the NE depressions, it was not clear whether they terminated at or before reaching the lining membrane. Additionally, several extranuclear MTs remained in a parallel, lengthwise alignment between the NE and PER (Figs. 21, 23, 24, 26). Fewer, shorter MTs were visible on the side of the zone distal to the nucleus, in no apparent definite arrangement (Fig. 21).



In late prophase, the nucleolus was no longer seen in some sections (Figs. 25, 26), while nucleolar fragments were still visible in others (not shown). Dark staining chromatin was condensed into granular aggregates scattered throughout the nucleus. Perinuclear ER was present along parts of the NE (Figs. 25, 26), and was significantly reduced in quantity relative to early prophase or later mitotic stages. As in early prophase, PER did not cap the polar areas (Figs. 21, 25, 26).

Prometaphase. Prometaphase has been defined in eukaryotes (Wolfe, 1993) as the stage in which a cell is undergoing the processes of: completion of spindle assembly and movement of the spindle to the nuclear area, attachment of chromosomes to the spindle MTs by kinetochores, and congression (movement of the chromosomes to the spindle midpoint). However, the onset of prometaphase in *Agardhiella subulata* is perhaps easier to recognize by the termination of prophase, which is marked by the breakdown of the NE.

After pole-to-pole (or nearly so) channel formation, the NE which had lined the late prophase tunnels ruptured. Thus, prometaphase nuclei contained fragments of membrane scattered throughout the nuclear center and among the condensing chromatin (Figs. 27-29). These fragments were of varying lengths, and existed singly, or branched. Often, they appeared to emerge directly from the surrounding NE

itself (Fig. 27), giving further support to the assumption that the membrane origin was indeed late prophase nuclear channels.

Channel rupture presumably ended MT containment, and allowed for the release of MTs into the nucleoplasm. This could not be verified, however, as MTs were not well preserved in the three prometaphase cells found. Similarly, PRs were not seen, even though some sections of these cells revealed exclusion zones.

Prometaphase nucleolar material had either dispersed completely (Figs. 27, 29), or was barely distinguishable within the nucleus (Fig. 28). Chromatin aggregated into perhaps 13-15 individual, approximately equal sized masses, which are assumed to be chromosomes. This number, however, may be an underestimate as contiguous chromosomes may have been counted as single entities, and other chromosomes may not have been in the plane of section. Some sections showed these masses beginning to concentrate in the nuclear center for metaphase plate formation (Fig. 29). One to four layers of PER were present around parts of the nucleus, although without knowing the polar orientation of many of the thin sections obtained, PER patterns can not be noted with certainty.

Metaphase. Generally, nuclei seemed more centrally located in metaphase cells than they did during prophase and prometaphase (Fig. 30). Nuclear poles were flattened

significantly in the 9-10 metaphase cells obtained, while the mid-region, which commonly spanned 2.2-2.6  $\mu\text{m}$  at prophase and 2.4-3.0  $\mu\text{m}$  at prometaphase, expanded up to 4.0-4.5  $\mu\text{m}$  wide (Figs. 30-32). The metaphase nucleus, therefore, dramatically changed dimensions and became approximately twice as broad as its pole-to-pole length. (Pole-to-pole length averaging 1.9-2.3  $\mu\text{m}$  was measured as the distance between the remaining large polar membrane segments.)

Failure to find sections showing the zonal areas at prometaphase resulted in a lack of knowledge of the fate of the poles as channels ruptured. However, by metaphase, two large gaps, 700-900 nm each, were clearly evident in the NE at each of the extremely flat and wide metaphase poles. Between these two gaps remained a rather large segment of NE (0.9-1.0  $\mu\text{m}$  long), which often appeared to have extraneous membrane (possibly ruptured channel membrane) associated with it (Figs. 30-33). The rest of the gap area was generally devoid of membranes, though some sections showed small NE fragments along the former nucleoplasm/exclusion zone interface (Figs. 30, 31, 33). Elsewhere around the nucleus, the NE remained intact.

Zones of exclusion were extremely narrow and long, as if accommodating the metaphase polar dimensions. Each zone was of a finely granular nature and was still devoid of organelles. The NE remnants and the exposed nucleoplasm at the gaps bordered the zone on one side, while various

organelles closely bordered the other. Most commonly, large vacuoles, sometimes still with internal membranous partitions, accumulated immediately adjacent to exclusion zones (Figs. 30, 31). Several mitochondria (Figs. 31-33) or starch grains were also common, either themselves directly proximal to the zones, or found among the large bordering vacuoles. To a lesser degree, chloroplasts (Fig. 32) and an occasional Golgi body were also spotted.

The PER and intact NE surrounding the nuclear mid-regions were orientated in seemingly identical patterns. The two were therefore indistinguishable, except that the NE was the most proximal of the 2-4 parallel membranes encircling most of the nucleus. Near the poles, PER veered outward somewhat, encompassing the various aforementioned polar associated organelles into a nuclear environment (Figs. 30, 31). Although some sections seemed to show PER capping the poles (Figs. 30, 31), it was assumed that these cells were simply not sectioned directly through their polar centers. Occasionally, parallel membranous swirls, assumed to be PER, were seen penetrating the nucleoplasm from the polar area (Fig. 32).

Within the nucleus, the nucleolus had disappeared, although sometimes granular components were present (Figs. 30-32), which might represent nucleolar fragments. Across the breadth of the nuclear mid-region were fully formed, granular chromosomes. The electron dense chromosomes had taken on a fairly crisp, almost rectangular appearance, and

were closely aligned along a typical metaphase plate. Very small spaces existed between some of the chromosomes, while others were so close that they appeared to touch or merge. It was apparent that if the nucleus had not become so broad at its mid-region, chromosomes would not have fit across its width as evidenced in metaphase photographs. Though a count should still be considered quite inexact, 7-11 metaphase chromosomes (or condensed clumps of chromosomes) were typical per thin section.

Large numbers of MTs were seen oriented in a lengthwise direction within the nucleus (Figs. 30, 31, 33). Several of these were attached from each chromosome to the corresponding polar area, though clearly defined kinetochores were not visible. Microtubules converged broadly, with no specific focal point, across the entire expanse of the poles. Those directed toward the single NE remnant located between the gaps terminated upon meeting it. Similarly, MTs stopped at the former level of the NE elsewhere along the poles, without passing through the NE gaps into the bordering zone of exclusion (Fig. 33). Since the NE had ruptured across such a broad polar expanse, leaving only small broken ends diverging from the PER (Fig. 31), the MTs found between the NE and PER in earlier stages were no longer present. Also not found at this stage were PRs.

Anaphase. Fine-structural, cellular differences between the stages of metaphase and early anaphase existed almost exclusively in the nucleus. As in metaphase, the nucleus remained in a generally central location within the cell (Fig. 34). Still surrounding the nucleus were 2-4 layers of PER, which again, due to the plane of section, occasionally gave the mistaken appearance of capping the polar regions (Fig. 35). As before, mitochondria, starch, and vacuoles, were persistent immediately outside the exclusion zones.

Within the NE, however, major changes were evident. Early anaphase occurred with the initial separation of sister chromatids toward their respective poles (anaphase A) (Fig. 34). Traveling in two almost linear bands (one toward each pole), chromatin masses were more irregularly shaped than the rectangular formations seen in metaphase. Ten or more chromosome profiles could be seen in some sections but obvious kinetochores were not observed.

A narrow zone of exclusion still stretched along the entire polar area (Fig. 34). The smaller, fine granules of the zones, usually lighter staining and casting a more uniform appearance, were apposed to coarser, more irregularly aggregated (probably nucleolar) granules throughout the darker staining nucleoplasm matrix. Occasionally, small NE fragments were found between the zonal area and the nucleoplasm (not shown). Not located was the single large NE remnant seen at each metaphase pole,

possibly due to incomplete sectioning of the three early anaphase cells found.

A short time after the chromatin bands traveled nearer the poles, the poles themselves began to separate (compare Figs. 35, 36). Anaphase chromosomes, not as individually distinguishable as metaphase chromosomes, remained proximal to the zonal areas throughout this late anaphase polar movement (anaphase B), so that only a thin strip of nucleoplasm could be seen between the bands and the exclusion zones (Figs. 36, 37). Thus by mid-anaphase, the entire nucleus had elongated into a "dumbbell" shape, possessing two broad, terminal, bulbous areas that accommodated the linear chromosome bands, and an interzonal midpiece (IZM) between them that grew longer and narrower as anaphase progressed.

Nothing can be said about the MTs of early anaphase, as they were poorly preserved. With some difficulty, MTs of mid-anaphase could be discerned within the thin nucleoplasm layer between the chromatid bands and the exclusion zones of their targeted poles (Fig. 38). As in metaphase, several MTs seemed to be associated with each chromosome. These MTs remained broadly focused along the entire polar area, and as expected, appeared to terminate at the nucleo/cytoplasmic interface without penetrating the exclusion zone itself. Other MTs were located lengthwise within the narrowing IZM between the two separating chromosomal bands (Figs. 36-38). It was not determined whether these MTs were continuous from

one pole to the other or if they represented overlapping half spindles.

Perinuclear ER retained its position along the nuclear sides, as 3-4 parallel cisternae were clearly evident along the IZM (Figs. 36, 37). As they neared the poles, cisternae either ended abruptly, continued a short distance to terminate closer to the plasma membrane, or seemingly connected to the general cytosolic ER system. In any case, polar regions remained uncapped.

Starch, mitochondria, and vacuoles continued to closely associate themselves with at least one of the mid-anaphase polar regions (Figs. 36, 38). Often, the other pole was found too near the plasma membrane to allow for extensive organelle association. Zones of exclusion kept their characteristic appearance along the broad anaphase poles, and several fragments of ER were visible at the nucleoplasm/exclusion zone border. On occasion, the single large NE remnant between the two polar gaps was located (Fig. 37).

At late anaphase, the IZM began to detach from the forming daughter nuclei. In the single late anaphase cell observed, the plane of section may have created a potentially misleading appearance. Though the NE and its accompanying PER appeared to be bisecting the nucleus at the IZM (Fig. 39), it seemed more reasonable that *Agardhiella* followed the same pattern as other higher Rhodophyta examined for details of mitosis (Scott and Broadwater,



1990): three separate nuclear sections should be formed as the NE membrane pinched in around both ends of the chromatin-containing "dumbbell", resulting in the isolation of the IZM between them. (Further evidence of this is seen in the telophase IZM and PER patterns of Fig. 40, discussed shortly).

Few MTs could be distinguished in the chromatin-polar areas, while, perhaps due to dismantling of the spindle apparatus coupled with poor preservation, none were discernible in the IZM. Two to four layers of PER continued to surround and pinch into the nucleus at the IZM along with the NE. Although it was difficult to distinguish NE from PER, it appeared that two gaps and one large NE segment were still present at each pole (Fig. 39). Polar areas remained relatively flat, with close association of mitochondria, starch, and vacuoles. Large vacuoles were also externally juxtaposed to the NE indentation forming in the IZM. While exclusion zones were present in the same band-like form as in metaphase and earlier anaphase cells, they were perhaps not as clearly defined in texture or staining properties as in these previous stages. Chromatin masses, still in bands, seemed less condensed and less crisply shaped than they appeared earlier in anaphase. Nucleolar material was not seen.

Telophase. By telophase, nuclear membranes had reformed around each set of daughter chromosomes, resulting

in distinct nuclei located on opposite sides of the cell (Fig. 40). Although expected to persist to this time, spindle or other MTs were not discovered in any of the 8-12 telophase cells located. Instead, a remnant of the IZM separated the two nuclei. This IZM segment was detached from and located between the two daughter nuclei and was enveloped by layers of PER from anaphase (Fig. 40). Also between the daughter nuclei were large partitioned vacuoles bestrewn with starch granules (Figs. 40-42). Often, these came to occupy most of the cell's center, with nuclei and other organelles (visually dominated by chloroplasts) located at the periphery.

Chromosomes were no longer found in the band-like arrangement of metaphase and anaphase. Telophase cells revealed uncoiled and decondensed chromatin scattered throughout the nucleus. In addition, these cells often showed reformation of the nucleolus (Figs. 40, 41), which was generally centrally located. Thus the state of chromatin in telophase nuclei and during cytokinesis (discussion following) was reminiscent of that already described for interphase and prophase. However, while the contents of the nucleus resembled other mitotic phases, the nuclei's usual position (diagonally situated against opposite walls of the plasma membrane) and shape (usually curved on the side where the IZM had detached, and flattened on the side against the plasma membrane) were unique to telophase.

Cytokinesis. Cytokinesis, the physical division of the cell into two daughter cells, began before the termination of telophase. Since these cell division events overlapped, only changes not previously discussed will here be presented.

Early cytokinesis was distinguished by the formation of cleavage furrows in the plasma membrane of telophase cells (Figs. 40-42). No structures were visible in the furrows, which contained only fibrous material that resembled the make-up of the surrounding cell wall. In addition, cytosolic regions immediately adjacent to the innermost furrow regions showed no special differentiation.

Though cell orientation with respect to the thallus could not be verified with the light microscope in this study due to the problems discussed earlier (see Light Microscopic Observations), electron microscopic findings suggested that the overall position of the daughter nuclei depended upon the cell's position within the thallus. Combining observations from various stages (prophase through cytokinesis), a pattern could be discerned. Apical initials of axial files formed tilted axes, as prophase PRs were often orientated at angles of  $30-45^{\circ}$  away from the vertical. Daughter nuclei separated along this axis. In outer cortical cells, the cytokinetic furrow was usually formed along a diagonal axis, dividing the cell in a plane roughly perpendicular to the position of the nuclei (Fig. 41), (although this was not always the case (Fig. 42)).

The forming furrow continued to pinch deeper into the cell so that, by late cytokinesis, two separate cells were distinguishable, each of similar size and with approximately equal amounts of organelles. As the attachment of daughter cells grew to a minimum (Fig. 43), ER profiles (four shown here) were found oriented lengthwise in the connecting area (septum) between the two cells (Fig. 44). These profiles, usually described with associated electron dense material, are common in higher Rhodophyta, and present as part of the formation of pit plugs (Davis and Scott, 1986; Scott et al., 1980; Aghajanian and Hommersand, 1978; Ramus, 1969), structures previously described (see General Aspects of Interphase Cells). Within the deepest point of the cleavage area, several fibers were aligned somewhat perpendicular to the aforementioned ER while parallel to the furrow. The remainder of the furrow contained anastomosing fibers in an otherwise electron transparent space (Fig. 44).

Nuclei were found along opposite sides of the cleaving cell. Two to four residual PER profiles often encompassed a region devoid of organelles on the side of these nuclei where the PER had lined the once attached IZM (Fig. 43). The vacuoles and starch that were seen in metaphase and anaphase were also persistent. The IZM was not found in any cell at this stage. It is therefore assumed to disappear sometime before the completion of cytokinesis.

Residual PER patterns were also discovered in some cells which had completed telophase, yet in which no

cleavage furrow had formed. These cells contained decondensed chromatin (not shown here), reformed nucleoli, central vacuoles, and other features expected at the completion of karyokinesis, while signs of cytokinesis were absent (Fig. 45). In these cases, binucleate cells result, initiating the transition to the multinucleate state common in the inner cortex and medullary cells of *Agardhiella* (Coomans, 1986).

At the completion of cytokinesis, still sometimes with residual PER visible (Fig. 46), cells physically further distanced themselves from one another, smoothed the side of their plasma membrane left irregular where the cleavage furrow had penetrated, achieved a more evenly curved shape, then remained in a state of interphase until the entire mitotic process, here detailed, began once again.

## DISCUSSION

Several features discernible with the electron microscope are thought to be of possible use in determining the evolutionary relationships of the Rhodophyta (Scott, 1984). Among these are chloroplast ultrastructure (Hara and Chihara, 1974), pit connections (Pueschel, 1990; Pueschel and Cole, 1982), dictyosome (Golgi body) associations (Scott, 1972), and patterns of mitosis and cytokinesis (Scott and Broadwater, 1990). (Although meiotic features have also been looked at (Sheath et al., 1987; Broadwater et al., 1986a, 1986b; Scott and Thomas, 1975), only one species has been satisfactorily studied to date (*Dasya baillouviana*; Broadwater et al., 1986a, 1986b); therefore, the systematic value of these studies is still unclear.) These ultrastructural features, coupled with macroscopic, biochemical, and molecular characteristics are valuable tools in re-evaluating the current taxonomic scheme.

To further this objective, this mitotic study of *Agardhiella subulata* was undertaken. It is the first mitotic study done in the order Gigartinales. Similar work has been completed on 15 other rhodophycean species (listed in Table 1). Most important in relation to this study are the publications on *Batrachospermum ectocarpum* (Scott, 1983)

and *Lomentaria baileyana* (Davis and Scott, 1986) in the orders Batrachospermales and Rhodymeniales respectively. According to the currently accepted classification scheme, Gigartinales is considered somewhere intermediate in development and complexity between these two orders. Likewise, the fine structure and patterns of mitosis in *Agardhiella* might be considered somewhat intermediate between those described for these two orders.

With the exception of the several mitotic studies done on the less advanced forms of Rhodophyta, and the above mentioned three algae (*Batrachospermum*, *Agardhiella*, *Lomentaria*), all other rhodophycean species examined for details of mitosis belong to the order Ceramiales (Table 1). From the comparisons discussed below, it seems a probable pattern of mitotic trends might be drawn among all four of these higher orders (Batrachospermales, Gigartinales, Rhodymeniales, Ceramiales). As work on representative species of the remaining orders is completed, a gradient of many of these features seems likely to be established. At a minimum, the mitotic features of *Agardhiella*, compared with those of other higher red algal species already studied, have neither discounted nor contradicted this as a possibility. Following are discussions of some of these more notable features.

## NAOs

Heath (1980b) has stated that the nucleus associated organelle (NAO) and its surrounding electron opaque amorphous material (NAO-associated material) likely function as a microtubule organizing center (MTOC), and suggested that the role of the NAO in some organisms may involve the organization of the spindle MTs, perhaps by acting as a template to control their formation. However, he noted that in other organisms (*Polysiphonia*, in particular), the NAO is rather small with respect to a large polar area, and therefore may not function in spindle organization, or may not even be associated with mitosis at all. Rather, the NAO may be located at the nuclear poles simply to ensure equal distribution into daughter cells. This view is not shared by all researchers though, as Broadwater et al. (1992) maintain that rhodophycean NAOs do appear to be involved in the mitotic process.

Also according to Heath (1980b), morphological variations of NAOs in the lower eukaryotes are thought to be caused by functional or evolutionary factors. Though they are fairly uniform within most members of this group, red algal NAOs can exist in a variety of types and sizes (Table 2). When comparing the morphological and dimensional trends of rhodophycean NAOs, the doughnut shaped, intermediate sized polar rings (PRs) of *Agardhiella subulata* were not unexpected.



The unique "ring within a ring" PR structure of *Batrachospermum* contrasted with the variously sized "ring upon a ring" PR configurations of *Rhodella*, *Dixoniella*, *Flintiella*, *Compsopogon*, *Agardhiella*, *Lomentaria*, *Dasya*, *Membranoptera*, and two *Polysiphonia* species (Scott and Broadwater, 1990). The "polaire corpuscles dense" of *Griffithsia* (Peyriere and Heim, 1971) and the "polar organelles" of *Apoglossum* (Dave and Godward, 1982) were also common bipartite PRs. In fact, to date, the only other deviation from the typical, equal sized, superimposed, proximal and distal subunit, PR-type NAO reported in a red alga was found in *Porphyridium purpureum* of the lower Rhodophyta, and, though still bipartite, consisted of a large proximal granule with a smaller distal disc-like granule upon it (Schornstein and Scott, 1982).

Dimensions of NAOs ranged from the small 16 X 29-35 nm PRs of *R. violacea*, to the 70 X 160-190 nm PRs of *M. platyphylla*. The PRs of *Agardhiella* measured 25-40 X 100-120 nm, which was slightly larger than the typical PRs of the lower Rhodophyta, though somewhat smaller than those of the higher reds. Though not a perfect correlation, the few measurements available so far generally showed increasingly larger PR dimensions in increasingly more advanced rhodophycean orders. (Notably, the unique NAOs of *P. purpureum* and *B. ectocarpum* were excluded from comparison.)

Although the PRs of *Agardhiella* were only seen in interphase and prophase, they are expected to persist

throughout the cell cycle, as suggested by work on mitotically inactive vegetative cells of *P. harveyi* (Scott et al., 1981). In three out of four *A. subulata* cells where both PRs were found, they were located on opposite sides of the early prophase nucleus (single exception described shortly). This was evidence that PR migration and establishment of poles had already been completed at or before early prophase (see Discussion; Prophase). The fact that these cells featured both PRs already on opposing nuclear sides, rather than in stages of migration, seemed to imply that PR migration was a rather rapid process.

The only observation of mitotic cells in *Agardhiella* that did not disclose the PR pair at opposite poles, showed one PR in its expected position at the opening of a late prophase channel, while the other was seen in a different section at approximately  $90^\circ$  further along the NE, thus not at the opening to an opposing channel. As only a single such cell was found, it is impossible to judge whether this PR location was chronologically significant, or whether an unnatural condition or atypical cell was being observed.

Polar ring behavior differs among species. In the pre-prophase stages of *Lomentaria*, possibly *Flintiella*, and in the Ceramiales, including *Dasya*, and *Polysiphonia*, the NAOs were found close together and both attached to the NE. With the onset of prophase, one PR migrated  $180^\circ$  to form the opposite division pole. During prometaphase, the PR halves "snapped apart", or detached, resulting in the positioning

of the distal PR portion above and lateral to the proximal. Previous to this separation, nuclear envelope protrusions (NEPs) (discussion following) were formed. Though the two PR portions remained disjoined throughout most of mitosis, by late telophase the distal section moved closer to the NE and again existed alongside the proximal portion, just as originally observed in pre-prophase (Scott et al., 1980).

*Porphyridium*, with its unique NAO, did not follow this pattern of PR behavior. Rather, after one NAO migrated to form an opposing pole at prophase, a depression (pocket) formed under each NAO. The proximal portion of the NAO then became diffuse, took an irregular form, then dispersed. This left only the distal portion present for most of mitosis. At this time, the NE of the pocket broke down leaving a large gap, in the center of which the distal NAO subunit took residence at the level where the NE once existed (Schornstein and Scott, 1982).

Little was learned of PR migration and behavior in many of the other previously studied Rhodophyta, including *Agardhiella*. Reportedly, the NAOs of *R. violacea* completed their migration in prophase (Patrone et al., 1991). Since the PRs of *Compsopogon* were already in opposite exclusion zones by interphase, the authors concluded these division poles were prepared soon after daughter cells were formed, and not at prophase (Scott and Broadwater, 1989). More recently, however, at least one of these authors considers this interpretation to be incorrect and believes that PR

migration in *Compsopogon* needs to be re-evaluated (Broadwater, personal communication). Similarly, not much is known of NAO behavior in *Batrachospermum*, though Scott (1983) mentioned that PRs had been observed in stages of migration during division pole establishment. Though early-mid prophase was indeed found to be the usual time of PR migration for most eukaryotes (Heath, 1980b), clearly, more research is needed in order to positively determine timing of PR migration in the Rhodophyta.

In light of the PR morphology and size in *A. subulata*, and of the formation of prophase NEPs (see Discussion; Prophase Poles), one might expect the PRs of *Agardhiella* to be similar in behavior to those of *Lomentaria* and the Ceramiales: close proximity of PRs in interphase, PR migration at prophase, then separation of PR portions at prometaphase. However, this could neither be verified nor disputed as so few cells showing both PRs were located. Since no PRs were found past late prophase, and migration or "snapping apart" of PRs was not observed, perhaps a more exhaustive search to locate and study additional examples of the PRs of *A. subulata*, and the opportunity to re-examine their behavior, would be in order before conclusions are drawn.

#### PER

Another noteworthy feature is the behavior of perinuclear endoplasmic reticulum (PER). The presence and

amount, or the absence, of these membranes was constant within each species studied. When present in a red alga, PER occurred in association with the nucleus during mitosis, in single or multiple cisternal profiles, and was suspected to aid in karyokinesis by separating the nuclear environment from the remainder of the cell (Phillips and Scott, 1981).

*P. purpureum*, *F. sanguinaria*, *R. violacea*, *R. maculata*, and *Dixoniella grisea*, uninucleate unicells of the lower Rhodophyta, did not form PER during cell division, though ER cisternae were found at the metaphase and anaphase poles of *Porphyridium* (Schornstein and Scott, 1982), as well as alongside the nucleus during anaphase and telophase in *Flintiella* (Scott, 1986). In *Rhodella violacea*, either smooth ER or NE fragments were often seen in the NE gap formed in metaphase cell poles, and ER cisternae were sometimes associated with the IZM NE at anaphase (Patrone et al., 1991). Perinuclear ER was also present in reduced quantities and did not encase the nucleus at the poles in the filamentous red alga *Compsopogon* (Scott and Broadwater, 1989), and in the freshwater species *Batrachospermum* (Scott, 1983), whose PER cisternae had dispersed by anaphase or telophase.

The PER pattern of *A. subulata* and *Lomentaria* seemed almost identical. Both had an extensive PER buildup. Though not found in interphase, 2-4 PER profiles were found from early prophase through telophase in *Agardhiella* (though perhaps reduced somewhat in late prophase). These profiles

were oriented lengthwise from pole to pole, though at no time did they cap the polar regions. Even at late cytokinesis, 2-4 whole or partial, residual PER profiles could sometimes be found. Similarly, the PER in *Lomentaria* was observed in extensive quantities from prometaphase to telophase, and it, too, never formed a cap over the poles (Davis and Scott, 1986).

In contrast, the five studied species in the Ceramiales (*D. baillouviana*, *A. ruscifolium*, *M. platyphylla*, *P. harveyi*, and *P. denudata*) were homogeneous in that the PER was observed in much more extensive quantities than that seen in the lower orders, and that the PER did indeed encase the entire nucleus by forming a cap around the polar areas (Scott and Broadwater, 1990). The extensive presence of PER in *Agardhiella*, therefore, seemed to be in keeping with evolutionary trends among the red algal orders. Whereas some of the simpler Rhodophyta studied to date (unicellular and usually uninucleate) did not amass PER at all during their cell cycles, the available information showed an increase in PER presence coinciding with the more complex (multicellular and mostly multinucleate) rhodophycean orders. This correlation gives further credence to the supposition that PER may function in keeping multinucleate nuclear domains separate during mitosis (Patrone et al., 1991).

## Prophase

Classically, prophase is known as the mitotic stage involving condensation of chromatin, nucleolus dispersal then disappearance, development of mitotic spindle, division then migration of NAOs to mark the division poles, and finally the breakdown of the NE, which marks the termination of this stage (Raven and Johnson, 1988; Wolfe, 1993). There is a problem relating these criteria to the red algae, as the Rhodophyta seem not to progress through the cell cycle in the same manner as do most eukaryotes.

The work of Goff and Coleman (1990) exemplifies this. These researchers used fluorescent stains (fluorochromes) specific for double stranded DNA to examine sample species with epifluorescence microscopy. Since the stain bound quantitatively, the amount of stain was proportional to the amount of DNA present. Measuring the light emitted from nuclei prepared in this way therefore allowed for the determination of ploidy levels of these nuclei, without the tedious and often error laden task of counting chromosomes.

Typically, eukaryotic cells spend most of their life-span in the gap, or first growth ( $G_1$ ) phase of the cell cycle and are involved in synthesizing proteins, carbohydrates, and lipids. This is followed by the DNA synthesis (S) phase in which the  $G_1$  macromolecules continue synthesis and the entire DNA genome, along with the chromosomal proteins, is duplicated. The usually brief gap, or second growth ( $G_2$ ) phase follows, which concludes with

the synthesis of all proteins needed to complete the mitotic process. These three phases constitute the interphase state.

The short mitosis (M) phase follows, with the formation of two daughter nuclei. Overlapping the completion of mitosis, the even more rapid cytokinesis (C) progresses and results in the formation of two daughter cells. These typical cells remain in interphase (in  $G_1$ ) until the "restriction (or start) point" in late  $G_1$  is passed, after which, a cell is committed to go through the cell division cycle again.

Goff and Coleman (1990) discovered that, in stark contrast to most other eukaryotes, nearly all the rhodophycean species they examined had at least two copies of their nuclear genome present during most of interphase, and that nuclear polygenomy was especially common in the more advanced rhodophycean orders. This finding led to the supposition that the Rhodophyta are primarily " $G_2$  organisms", that is, the cells spend their life-span predominantly in the  $G_2$  phase of the cell cycle. Though *A. subulata* was not one of the species chosen for testing, the closely related species *Sarcodiotheca gaudichaudii*, obtained from California, was categorized as a  $G_2$  organism, as were all the tested specimens of the higher orders of the Rhodophyta, and many of the lower forms too (Goff and Coleman, 1990). Interestingly, other life forms, such as



the protozoan *Amoeba*, have also been shown to be  $G_2$  organisms (Wolfe, 1993).

The length of time a eukaryote cell remains in the  $G_1$  phase of the cell cycle determines how quickly or slowly the cell will divide. Depending upon the species and tissue type, this stage can last from eight hours up to years (Alberts et al., 1983). Though time variations in  $G_1$  account for the differences in interphase lengths of typical cells, the combined time most cells take to get through the S,  $G_2$ , M, and C phases is fairly constant (Wolfe, 1993). The fact that most Rhodophyta, in contrast, have a very reduced  $G_1$  phase and spend the major portion of their cell cycle in the  $G_2$  phase (or more cautiously worded, somewhere between the  $G_2$  and early prophase stage), makes the mitotic stage of prophase atypical and rather difficult to define in the red algae.

Since interphase cells in most Rhodophyta are in  $G_2$ , they already have undergone genome replication and possibly even the establishment of division poles by PRs, leaving the interphase cell in a state ready for mitosis (Scott and Broadwater, 1990). It has been suggested that PR migration in red algal cells may parallel that of animal centrioles by migrating during or immediately after the S phase. Since  $G_2$  cells have already completed the S phase, the positioning of PR pairs already opposing one another by early prophase might be expected (Scott et al., 1992), though again, more research is needed to establish this.

As a final note, red algae further defy the classical definition of prophase in that their NEs remain intact throughout mitosis except at the division poles. Hence the spindle apparatus, although assembled, will not associate with the nucleoplasm until prometaphase (Broadwater et al., 1992).

### Prophase Poles

In early prophase, a nuclear envelope protrusion (NEP) began to form at each pole in *A. subulata*. This outward bulging of NE immediately subjacent to the NAO is common in the higher red algal orders, as NEPs are also found in *Lomentaria*, *Dasya*, *Apoglossum*, and *Polysiphonia*. *Membranoptera* likewise produced NEPs at its prophase poles, although this fact was revealed by Scott et al. (1980), after re-examining McDonald's (1972) work on this species.

Scott and Broadwater (1990) suggested that the formation of NEPs was evidence that PR behavior, in the higher Rhodophyta, creates a physical tension. The PR is anchored in place in the zone of exclusion by MTs, and its proximal portion is attached to both the NE and to its distal portion. In late prophase, as the nuclear poles begin to flatten, the tension created draws the NE (linked to the proximal PR portion by fine struts) into an NEP. The eventual "snapping apart", or disjunction, of the proximal and distal NAO portions at prometaphase is thought to be a result of this strain on the NE during prophase.

Later in prophase, the NEPs of *Agardhiella* became more pronounced until the outer NE membrane of the protrusion actually extended through the PR and was continuous with a single swollen RER cisternae. These NEP penetrations of the PR and connections to RER seemed especially important in light of evolutionary considerations, since the same NEP extension and associated RER were previously found in only a single other red alga: *Lomentaria* of the Rhodymeniales (Davis and Scott, 1986). In addition to this report on *Agardhiella*, though not included, cursory observations of *Sarcodiotheca gaudishaudii*, another species in the Gigartinales, were made for comparison. Though late prophase photographs of this species were not resolved with great satisfaction, they do appear to show the same penetration of the NEP's outer membrane through the PR, along with the expected association of an individual RER cisterna. As representative species in other orders are studied, perhaps this feature will be found limited to only certain closely related orders and thus help resolve lineage relations.

Among the lower orders, NEPs do not form. Though *Porphyridium* displayed pointed polar regions, a slight NE depression rather than a protrusion was seen under each of its prophase NAOs (Schornstein and Scott, 1982). After the PRs of *R. violacea* completed their migration, the NE under their prophase poles was flattened (Patrone et al., 1991). Like *Porphyridium*, the prophase nuclei of *Flintiella* were

also typically elongated, though rather than an elevation or depression of the polar NE in this species, a blunt flattened region similar to that described in *R. violacea* existed (Scott, 1986). An absence of NEPs was also noted in *Compsopogon* and *Batrachospermum*, as the poles of the former were flat and slightly invaginated (Scott and Broadwater, 1989), while two shallow depressions were formed at each pole in the latter (Scott, 1983).

#### Late prophase/Prometaphase Poles

The poles of red algal late prophase and prometaphase cells varied among the different species studied. In the lower Rhodophyta, variations were seen even among the unicells.

At about the time of the dispersion of the proximal PR portion, subjacent to each of its remaining distal PR subunits, one pocket was formed at each late prophase pole in *Porphyridium*, which did not subsequently develop into a tunnel. Instead, the nuclear pocket opened, or ruptured, leaving a single gap in the NE at each pole (Schornstein and Scott, 1982). *R. violacea* shows a similar breakdown of the NE at the base of a single polar pocket that formed at each of its poles. As in *Porphyridium*, this breakdown, too, occurred before channels developed, and left *R. violacea* with a single gap at each pole, sometimes with smooth ER or NE fragments present (Patrone et al., 1991).

*Dixoniella grisea* also developed one polar pocket which grew into a single MT-filled depression at each of its poles before breaking down. After rupturing, one small gap "plugged" with MTOC material was left at each opposing pole (Scott et al., 1992). Two late prophase pockets developed at each nuclear end in *Flintiella*. These grew into MT-filled tunnels, but extended, if not fully, then at least almost, from pole to pole. As the channels ruptured, mirroring the other unicells here mentioned, one large polar gap was created at each nuclear end (Scott, 1986).

Scott and Broadwater (1989) admit that no positively identified prometaphase cell was found in the filamentous red alga *Compsopogon*. However, late prophase cells displayed a flattening and invagination of the polar regions, and a containment of MTs within narrow cytoplasmic tunnels. By metaphase, many small fenestrations were found at the poles, seemingly due to rupture of these channels.

The late prophase poles of the more advanced genus *Batrachospermum* seemed remarkably similar to those already described for *Flintiella*. Also similar, were the poles of late prophase in *Agardhiella*. In all three of these algae, two nuclear pockets, growing into pole-to-pole, MT-containing channels, were seen at each nuclear end. It was not certain how many tunnels extended through each cell before rupturing in any of these species, due to difficulty with planes of section and serial sectioning (Scott, 1983, 1986). Associated with the tunnel opening in *Agardhiella*

and *Batrachospermum*, ER was often seen, and occasionally a single cisterna extended a bit deeper into the channels.

Rather than the single polar gap commonly created from tunnel rupture in the lower orders, each prometaphase pole of *Agardhiella* (as in *Lomentaria*) instead formed two gaps at each mitotic pole, separated by a persistent, centrally located NE segment which was located subjacent to the expected, if not actually seen, PR position (Davis and Scott, 1986). Also commonly found in both algae were NE fragments scattered along the nucleoplasm/cytoplasm interface, in varying sizes and amounts. On occasion, remnants of NE were also located among the chromosomes and elsewhere in the nucleoplasm in *Agardhiella* and *Lomentaria*, as well as in *Batrachospermum*.

Despite the similarity of later stages in *Agardhiella* and *Lomentaria*, late prophase in *Lomentaria* contrasted starkly with that of *Agardhiella*, as well as *Flintiella* and *Batrachospermum*. Instead of forming two NE-lined, MT containing, nucleus transecting tunnels at each pole, the two polar pockets that formed in *Lomentaria* did not contain MTs, and ruptured when they were only shallow invaginations (Davis and Scott, 1986). Thus, it seems that the mitotic description of late prophase and prometaphase in *A. subulata*, as expected, falls somewhere between those reported for *Batrachospermum* and *Lomentaria*.

Within the Ceramiales, homogeneity was apparent. Of the studied species in this order, none developed pockets or

channels. Rather than describing the formation of various types of gaps (as found in the lower orders), the mitotic accounts of *Dasya*, *Apoglossum*, *Membranoptera*, and *Polysiphonia* all reported polar NE interruptions due to numerous fenestra and NE fragments.

In all the red algal species chosen for mitotic study to date, spanning the unicells to the Ceramiales, the NE remained intact throughout the entire mitotic process, except at the poles. At prometaphase, the NE was interrupted either by large gaps, small fenestrations with NE fragments, or some combination of the two. It has been postulated that the forming MTs may put pressure on the nuclear poles, thus initiating pocket formation (Davis and Scott, 1986). Although discontinuities in the NE, often caused by rupture of such pockets, have been shown to allow for MT entrance into the nucleoplasm in the fungi (Heath, 1978), Davis and Scott (1986) caution that NE interruptions in some Rhodophyta may not follow suit. In particular, MTs were not observed in the polar pockets of *Porphyridium* or *Lomentaria*, and were only seen in nuclei after pocket rupture.

## Conclusion

There are, at present, five mitotic "types" tentatively described by Scott and Broadwater (1990). These are the *Porphyridium*, *Flintiella*, *Batrachospermum*, *Lomentaria*, and *Polysiphonia* types (summarized in Table 3). Several mitotic

studies have been completed on different species in the order Ceramiales to date. It is not surprising that mitotic features in all of these algae were sufficiently similar to be included in the *Polysiphonia* type of mitosis. Thus far, each of the other four types include only the single studied species in the genus indicated by the type name. This, too, is no surprise, since all four genera belong to different families or orders, and thus could be expected to be more distantly related, mitotically and otherwise.

Porphyridiales is the only other order in which more than one species has been studied. However, as more information is compiled on the algae in this group, it seems increasingly more apparent that this is a heterogeneous order that is in need of taxonomic reform (Patrone et al., 1991).

The heterogeneity of the Porphyridiales is reflected in the mitotic typing of this order. Though *Porphyridium* is grouped in its named type, several features made the authors of works on that taxon reluctant to include *R. violacea* and *R. maculata* in the *Porphyridium*, or any other previously established mitotic type (Patrone et al., 1991). Scott et al. (1992) never mentioned the five type system in their mitotic work on *Dixoniella*. However, the fact that the mitotic features of *D. grisea* were found to be so similar to those of *R. violacea* and *R. maculata* imply that the authors would have had the same reservations in associating *D. grisea* with any of the types.



Mitosis in *F. sanguinaria*, also of the Porphyridiales, proved unique enough to warrant establishment of a new type of its own (Scott, 1986). Likewise, *Compsopogon coeruleus*, of the Compsopogonales, is another species not decidedly fixed into one of the five types. Though Scott and Broadwater (1989) stated that its mitotic characteristics most closely resembled those of *Polysiphonia*, certain features of its mitosis were not consistent with this type.

The information gained in this study of *Agardhiella* also did not neatly coincide with any one of the five types. Descriptions of the PRs, the extensive PER buildup except at the poles, the eventual penetration of the NEP outer membrane through the PR, the formation of two polar prometaphase gaps separated by a central NE segment, the broad convergence of the metaphase MTs at the poles, and the separation of daughter nuclei by vacuoles in *A. subulata* were all nearly identical to those described for *Lomentaria*. However, the extension of the two polar late prophase pockets into NE-lined, MT-containing, pole-to-pole channels in *Agardhiella* were most similar to the *Batrachospermales* mitotic type. All these listed features prevent *Agardhiella* from firmly identifying with either the *Batrachospermales* or the *Lomentaria* mitotic types. Instead, *Agardhiella* seems to lie somewhere in between, sharing traits of both.

It seems then that these five types serve as an excellent preliminary guideline of mitotic features with which to compare and contrast newly studied species and

orders. However, as more information is gained, it seems inevitable that more and more "types", or variations thereof, will be found, perhaps eventually making it fruitless to label specific types and more productive to look for similarities and taxonomic trends in mitotic characters among the orders. In fact, this is precisely what has been decided and is presently being done in current and ongoing research in red algal mitosis (Scott, personal communication).

Admittedly, there are still some mysteries surrounding the mitotic process in *Agardhiella*. Among these are the exact nature of the PR (behavior, migration, and replication), the true number of nucleus-transecting tunnels per late prophase cell, the exact chromosome number, and the MT/kinetochore relationship at metaphase. Some facts of mitosis have similarly been left undiscovered in the other already studied rhodophycean species, and, more importantly, many red algal orders have not yet even been explored.

Understanding the full scope of red algal mitotic patterns and their evolutionary implications demands that additional work be done on a broader selection of red algal species; certainly at least one representative sample from each of the orders. Only in this way can a more complete and decisive knowledge of the mitotic relationships existing among the Rhodophyta, and existing between this group and other organisms, be known.

Subsequent to the writing of the preceding, another study on mitotic features was published which has great impact on the points made above. Work completed on *Bossiella orbigniana*, the first mitotic study in the order Corallinales (Broadwater et al., 1993), is of the utmost relevance to, and therefore necessarily must be included in this study. Though the Corallinales is considered difficult to place taxonomically (Garbary and Gabrielson, 1990), *B. orbigniana* has already been included in Tables 1-2 of this paper, in what might be a reasonable assessment of its relative level of complexity.

Based on the information gained from *Bossiella*, in conjunction with knowledge accumulated from other mitotic studies, Broadwater et al. (1993) have formalized the ideas alluded to in this work that obsolesce Scott and Broadwater's (1990) five mitotic types. Enough species have now been studied for these authors to propose a new system of "typing" the distinctions and trends found in rhodophycean mitosis. Based upon observations mainly from the prophase and metaphase stages of cell division, the polar gap (PG) and polar fenestrations (PF) types have been introduced (summarized in Table 4). The authors suggest that more sense can be made of mitotic features in the red algae by reclassifying species into these two major types, while recognizing that both types contain variations.

The PG type includes mostly the less complex species, and has a smaller PR embedded in obvious MTOC material. It

develops polar invaginations which break down to form a single gap in prometaphase, upon which the spindle narrowly focuses. Little or no PER is present. Conversely, the PF type generally possesses a larger PR, forms a prophase NEP, and undergoes a partial breakdown of polar NE resulting in the production of numerous fenestra. (Shallow invaginations are present before this NE breakdown in only some species). Spindle MTs are focused broadly, and well developed PER is also typical.

Within this new scheme, *Agardhiella subulata* identifies most readily with the PF type. In fact, based on the authors' descriptions (Table 4), only two areas of difficulty arise. One of these is that not one micrograph of *A. subulata* showed distinguishable kinetochores, whereas kinetochores in the PF type are said to be well developed. This variance, however, could be the result of fixation techniques that left the kinetochores in *Agardhiella* poorly preserved. Alternatively, the lack of clearly defined kinetochores could be explained as a minor variation of the PF type.

The other problem arises from the fact that PF types are defined as undergoing partial breakdown of polar NE at prometaphase producing numerous polar fenestrations. This discrepancy is perhaps a bit more serious, in that *A. subulata* developed not just shallow invaginations (as Broadwater et al., (1993) pointed out existed in *Lomentaria*), but indeed pole to pole channels at

prometaphase before these ruptured, resulting not in numerous fenestrations, but instead in two gaps, with one central large (and sometimes a few small) NE remnant. This feature of the channel in *A. subulata* is almost identical to those of *Flintiella* and *Batrachospermum*, both considered members of the PG group. Surely *Agardhiella* embraces more (indeed most) features associated with the PF type, although the variations of developing MT-filled tunnels in the prometaphase nucleus, and never developing the numerous fenestra that the PF type is named after are inconsistencies not able to be explained away by poor fixation quality.

Thus, admittedly, there are problems even with this new type grouping. As stated earlier in reference to the now defunct five type system, completion of additional mitotic studies (especially in the orders where no mitotic work has yet been done), analyzed in conjunction with other descriptive and biochemical research would be required to determine the usefulness of this new PG/PF typing. It may prove to adequately reflect taxonomic and evolutionary relations by further subdividing these two major groups into several subgroups. Or perhaps lineages will be best identified simply by recognizing the unique and the shared patterns within and among each separate order (in effect, order-typing). In either case, if a taxon is found to have major features not conforming to those of the taxa with which it is classified, the disparity would probably signify

that a closer look need be taken at the current taxonomic arrangement.

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TABLE 1. Rhodophycean species that have been used for mitotic studies.

**PORPHYRIDIALES**

PORPHYRIDACEAE

- Porphyridium purpureum* ..... Bronchart & Demoulin, 1977.  
 Schornstein & Scott, 1980 & 1982.  
*Rhodella violacea* ..... Patrone, Broadwater, & Scott, 1991.  
*Rhodella maculata* ..... Patrone, Broadwater, & Scott, 1991.  
*Dixoniella grisea* ..... Scott, Broadwater, Saunders, Thomas, & Gabrielson, 1992.

PHRAGMONEMATAACEAE

- Flintiella sanguinaria* ..... Scott, 1986.

**COMPSOPOGONALES**

COMPSOPOGONACEAE

- Compsopogon coeruleus* ..... Scott & Broadwater, 1989.

**BATRACHOSPERMALES**

BATRACHOSPERMACEAE

- Batrachospermum ectocarpum* . Scott, 1983.

**CORALLINALES**

CORALLINACEAE

- Bossiella orbigniana* ..... Broadwater, Scott, Field, Saunders, & Thomas, 1993.

**GIGARTINALES**

SOLIERIACEAE

- Agardhiella subulata* ..... Klepacki, this study.

**RHODYMENIALES**

LOMENTARIACEAE

- Lomentaria baileyana* ..... Davis & Scott, 1986.

**CERAMIALES**

CERAMIACEAE

- Griffithsia flosculosa* ..... Peyriere & Heim, 1971.  
*Dasya baillouviana* ..... Phillips & Scott, 1981.

DELESSERIACEAE

- Apoglossum ruscifolium* ..... Dave & Godward, 1982.  
*Membranoptera platyphylla* .. McDonald, 1972.

RHODOMELACEAE

- Polysiphonia harveyi* ..... Scott, Bosco, Schornstein, & Thomas, 1980.  
*Polysiphonia denudata* ..... Scott, Bosco, Schornstein, & Thomas, 1980.

TABLE 2. Types and dimensions of NAOs in selected species.

<b>SPECIES</b>	<b>NAO TYPE</b>	<b>DIMENSIONS (in nm) (height X diameter)</b>
<i>P. purpureum</i>	disc over granule	45-50 X 65-70 over 55 X 140
<i>R. violacea</i>	PR	16 X 29-35
<i>R. maculata</i>	PR	13 X 38-40
<i>D. grisea</i>	PR	30 X 70
<i>F. sanguinaria</i>	PR	40 X 80-90
<i>C. coeruleus</i>	PR	40 X 80-90
<i>B. ectocarpum</i>	PR (ring within ring)	30 X 70 within 120 X 150
<i>B. orbigniana</i>	PR	25 X 120
<i>A. subulata</i>	PR	25-40 X 100-120
<i>L. baileyana</i>	PR	40-60 X 120-140
<i>D. baillouviana</i>	PR	35-45 X 120-140
<i>A. ruscifolium</i>	PR	56-69 X 100-144
<i>M. platyphylla</i>	PR	70 X 160-190
<i>P. harveyi</i>	PR	50-60 X 130-150
<i>P. denudata</i>	PR	50-60 X 130-150

TABLE 3. *Agardhiella subulata*, as compared to the five tentative mitosis "types" described in Scott and Broadwater, 1990.

Mitosis type	Porphyridium-type	Flintiella-type	Batrachospermum-type	<i>Agardhiella subulata</i>	Lomentaria-type	Polysiphonia-type
NAO	small distal granule upon large proximal granule	PR; ring upon ring	PR; ring within ring	PR; ring upon ring	PR; ring upon ring	PR; ring upon ring
PER	absent	absent	present in reduced quantities; does not cap poles	present in extensive quantities; does not cap poles	present in extensive quantities; does not cap poles	present in extensive quantities; caps poles
interphase and prophase poles	no NE extension	no NE extension	no NE extension	extension of outer NE membrane through PR	extension of outer NE membrane through PR	no NE extension
late prophase pole	one polar pocket with no MTs	2 MT-filled tunnels that completely transect nucleus	2 MT-filled tunnels that completely transect nucleus	2 MT-filled tunnels that completely transect nucleus	2 shallow pockets; MTs possibly absent	no pockets or channels
prometaphase and anaphase poles	one wide gap in NE	one wide gap in NE	one wide gap in NE	2 small gaps	2 small gaps plus many fenestrations	many small fenestrations

TABLE 4. Polar gap and polar fenestrations mitotic types based on observations of the prophase and metaphase polar regions. Established by Broadwater et al., 1993.

<b>PG</b> <b>POLAR GAP TYPE</b>	<b>PF</b> <b>POLAR FENESTRATIONS TYPE`</b>
.....	.....
most bangiophycid and lower florideophycid	most higher florideophycid
.....	.....
NAO usually smaller ring upon ring	NAO usually larger ring upon ring
.....	.....
prophase NAO usually embedded in obvious MTOC cloud; MTOC probably extracellular	extranuclear MTOC material not observed; MTOC probably intracellular
.....	.....
no NEPs develop at prophase poles	pore-free NEPs usually develop at prophase poles
.....	.....
prometaphase polar invaginations	prometaphase shallow invaginations in some species
.....	.....
prometaphase invaginations break forming single polar gap	prometaphase partial breakdown of polar NE forming small gaps and numerous fenestra
.....	.....
PER absent or poorly developed	PER present from prometaphase through telophase, often capping poles
.....	.....
kinetochores poorly defined	kinetochores well defined
.....	.....
spindle MTs usually narrowly focused at polar gap, terminating at MTOC	spindle MTs usually broadly focused at fenestrations, terminating at NE or interface



PLATE 1.

- Figure 1. Habit photograph of the multibranched alga *Agardhiella subulata*. This female gametophyte is identified by the many conspicuous raised cystocarps (arrowheads) on its branches. Branches taper toward both their apices and their bases of attachment. X 0.9.
- Figure 2. Light micrograph of a thick sectioned apical tip stained with toluidine blue O. The three cell types (outer cortex, inner cortex, and medulla) can be seen. X 200.
- Figure 3. Squash preparation of a thallus tip stained with DAPI and photographed with the fluorescence microscope. Three cell types are shown. Outer cortex cells (arrowhead) are small, ellipsoidal, and uninucleate. Inner cortex cells (double arrowhead) are larger, spherical, and multinucleated while filaments (arrow) included in the medulla are elongated and multinucleated. Autofluorescence of chloroplast and mitochondria DNA (shown red) is visible. X 325.
- Figure 4. Fluorescence micrograph of a metaphase cell stained with DAPI and using squash technique. Chromatin has condensed into a typical linear band metaphase plate. X 1425.
- Figure 5. Fluorescence micrograph of an anaphase cell stained with DAPI and using an accessory green filter. Two flattened bands of daughter chromatids are visible as is the vacuole (v) separating them and a forming cleavage furrow (arrowhead). Squash preparation. X 1425.

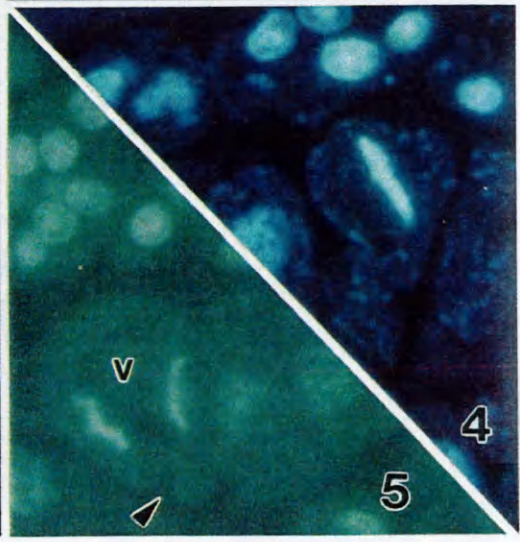
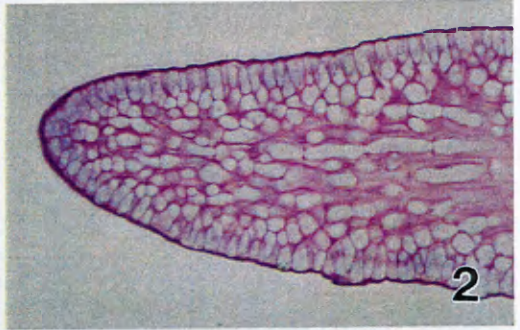
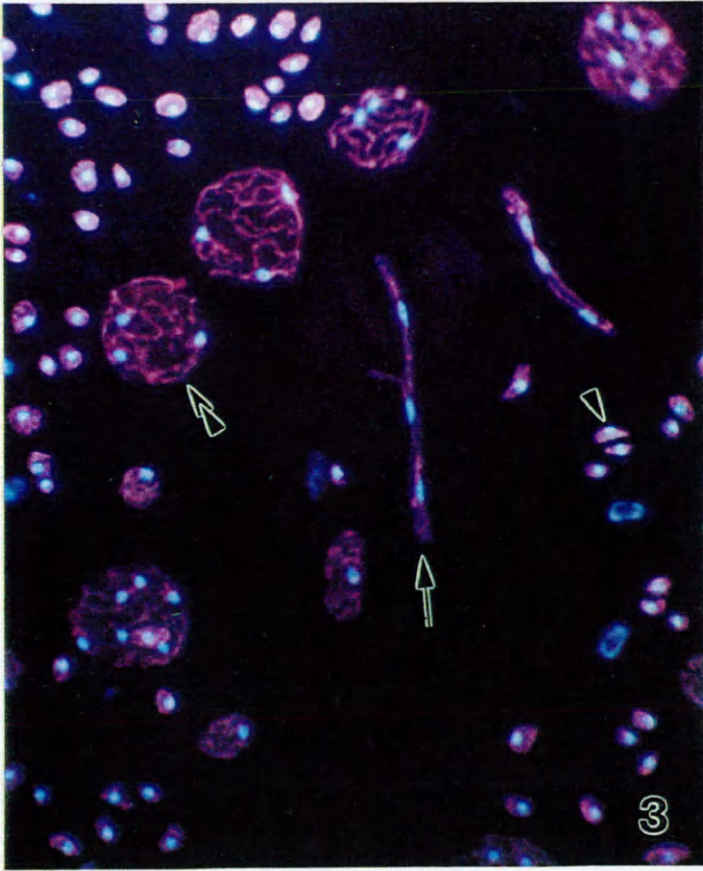
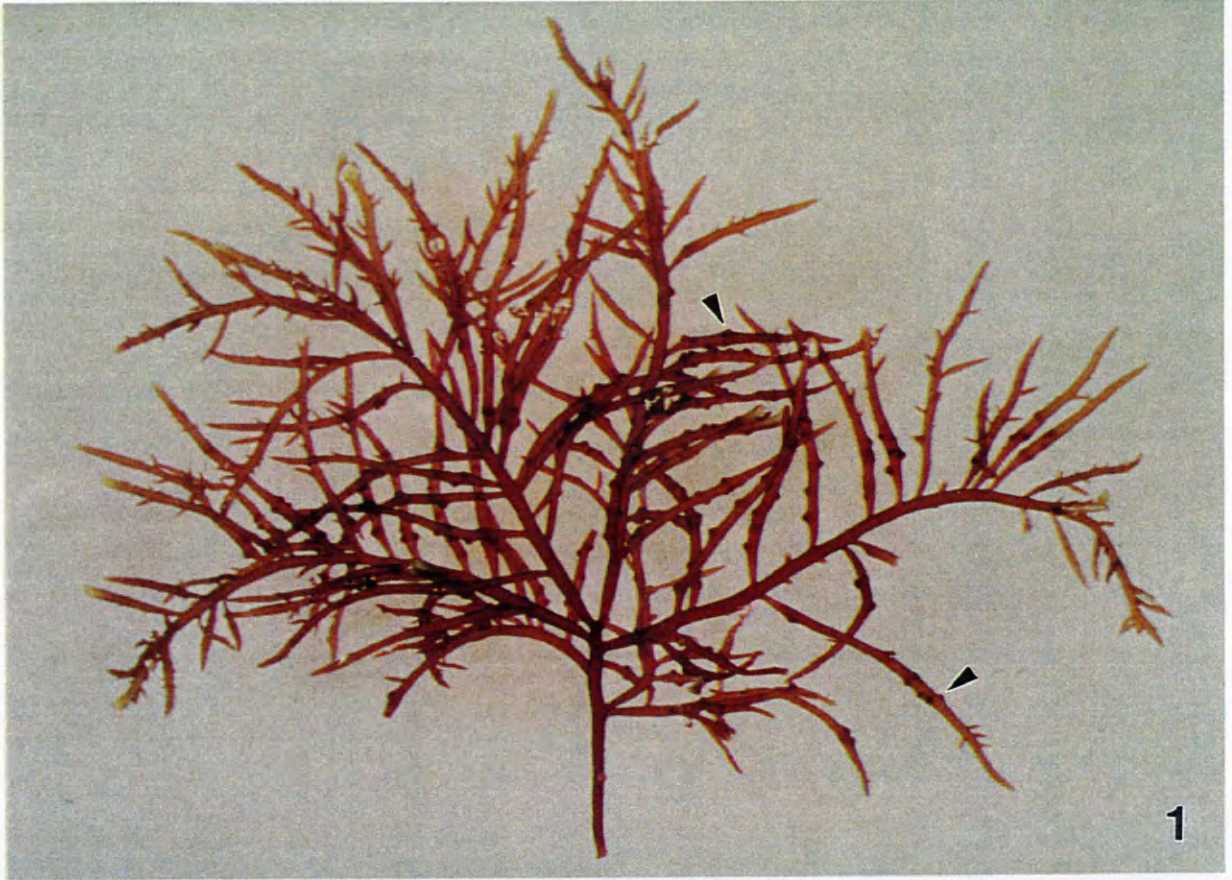


PLATE 2.

- Figure 6. Transmission electron micrograph of an apical tip of *A. subulata* showing the apical cells used in this study and the more irregularly shaped inner thallus cells. Files of several cells can be seen and used to trace cell lineages. Lighter staining areas surrounding cells (arrowhead) may mark natural cell wall positions. The fixed thallus tip has separated from the resin block (double arrowhead). X 825.
- Figure 7. Electron micrograph of a pit connection. The electron dense septal core (pit plug) has no plug caps although a cap membrane can be seen (arrowhead). X 63600.
- Figure 8. Interphase cell. Chloroplasts (c) with unstacked thylakoids and peripheral inner limiting discs are located mainly around the cell periphery, whereas mitochondria (m) and RER (arrowhead) are concentrated mainly around the nucleus. Profiles of starch (s), vacuoles (v), and Golgi (double arrowhead) are also visible. The centrally located spherical nucleus shows typical heterochromatin configurations and a nucleolus (asterisk). X 14250.
- Figure 9. Mitochondrial association with the forming (cis) face of a dictyosome. Some RER segments (arrowhead) are also present in the cis region. A second Golgi is visible in glancing section. X 33100.

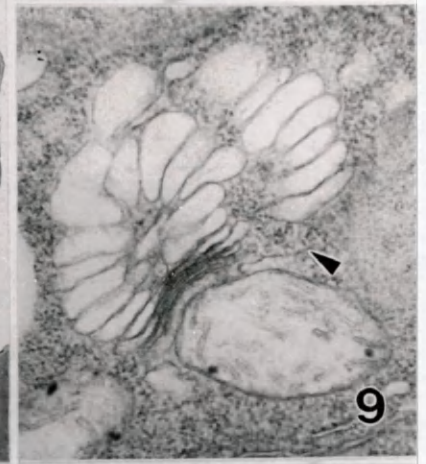
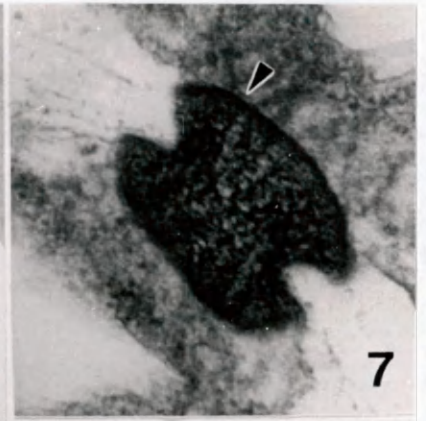
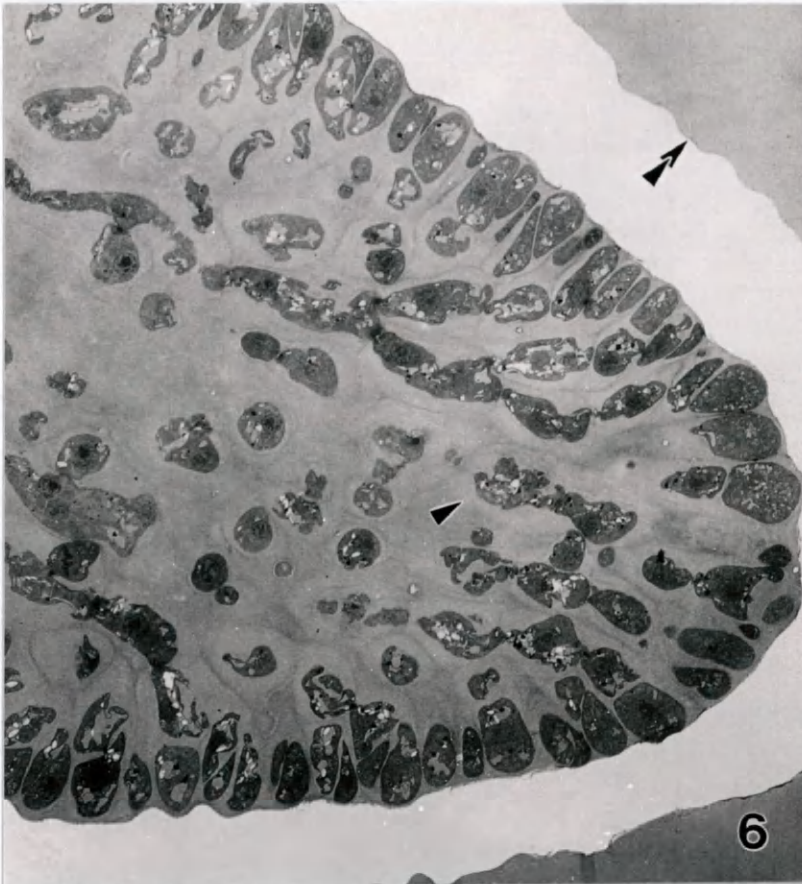


PLATE 3.

- Figure 10. Glancing section through a PR. Electron dense material (double arrowhead) is present on the PR sides lateral and distal to the nucleus. Microtubules (arrowhead) are visible in the zone of exclusion. Nuclear pores are not seen on the NEP. X 76850.
- Figure 11. Section through a PR and the associated NEP. The bipartite structure of the PR is evident. A lighter staining area exists in the ring center, with darker staining material to the lateral and distal sides. Some chromatin is located in the NEP (asterisk). The outer membrane of the NE has broken and hints at the possibility that it extends through the PR and attaches to an ER profile (double arrowhead). Microtubules (arrowhead) are visible in the zone of exclusion. X 74200.
- Figure 12. Cross section through a PR. The center of the PR is more electron transparent than the area immediately exterior to it. A zone of exclusion surrounds the ring, and irregular ER patterns and ribosomes can be seen surrounding the zone. X 46350.
- Figure 13. Early prophase. The nucleolus (asterisk) persists in the now ellipsoidal nucleus. A PR (arrowhead) with a surrounding zone of exclusion can be seen on one end of the nucleus. PER has already matured as several layers can be seen on lateral sides of the nucleus (double arrowhead). X 20150.
- Figure 14. Early prophase. This spindle shaped nucleus is sectioned longitudinally through both PRs. Layers of PER (arrowheads) can be seen except at the nuclear midregion and at the poles. Toward the poles, ER profiles become more irregularly oriented. X 28525.

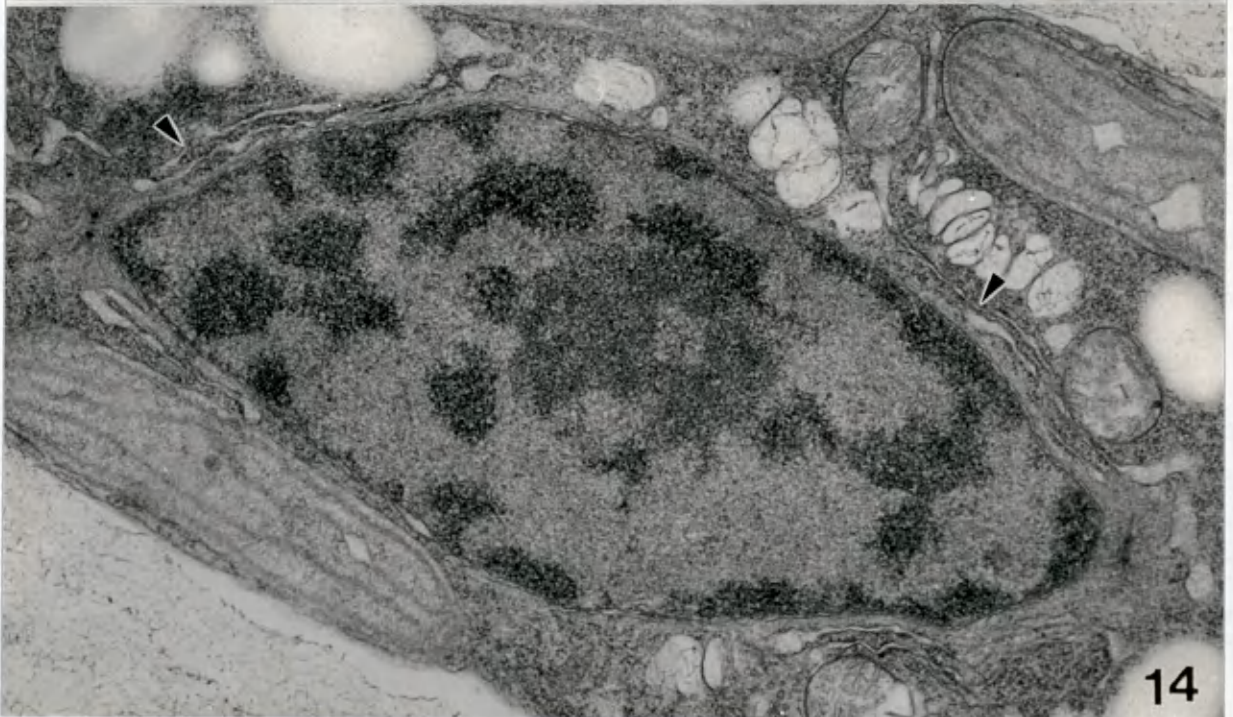
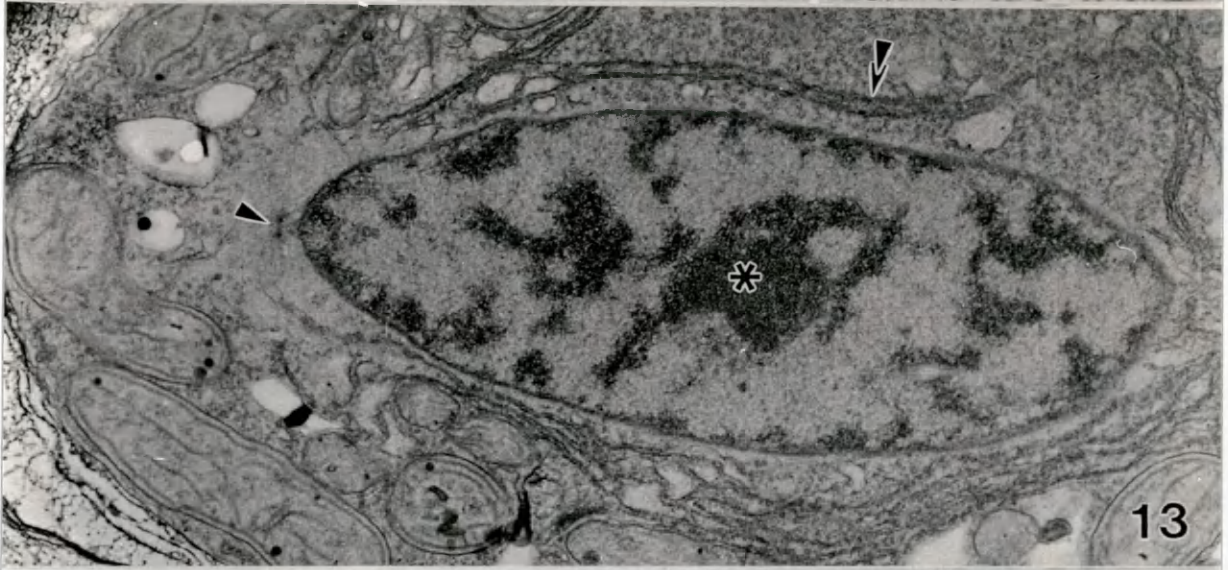
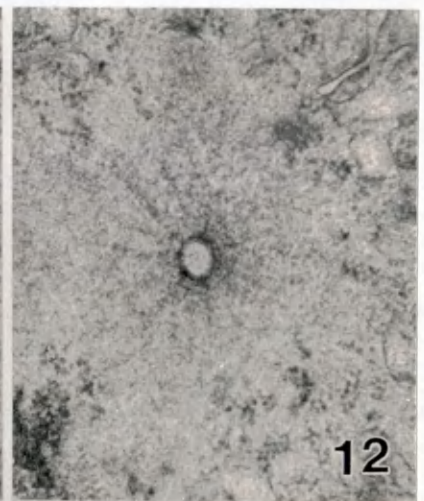
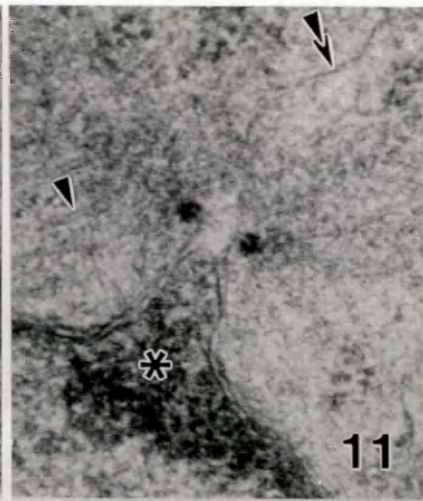
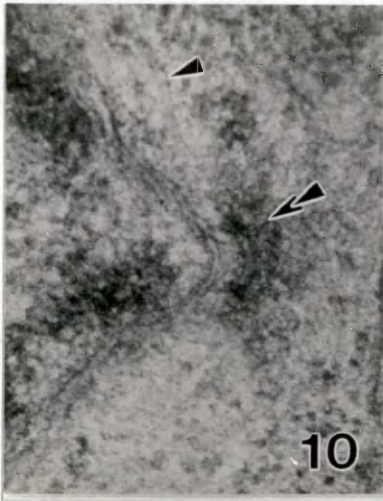


PLATE 4.

- Figure 15. PR with the surrounding zone of exclusion. PER profiles lined with ribosomes (double arrowhead) end at the zone border. Chromatin can be seen in the NEP (asterisk). Though the outer NE membrane of the NEP (arrowhead) does not extend through the PR, it extends further toward it than does the inner NE membrane. X 64550.
- Figure 16. Mid-prophase nucleus sectioned through only one PR. The spindle shaped nucleus, with the nucleolus still present, is encased by layers of PER extending the entire length of the nucleus though not capping the poles. Nuclear pores (arrowheads) can be seen on all areas of the NE except in the NEP region. Phycobilisomes (double arrowhead) are visible in an alternating pattern on opposite stromal sides of the thylakoid membrane. Layers of fibrillar material are present in the cell wall (cw). X 34725.
- Figure 17. Glancing section through the polar region of a nucleus where PER displays a radiating pattern. Several nuclear pores (arrowhead) can be seen in the NE. X 36950.

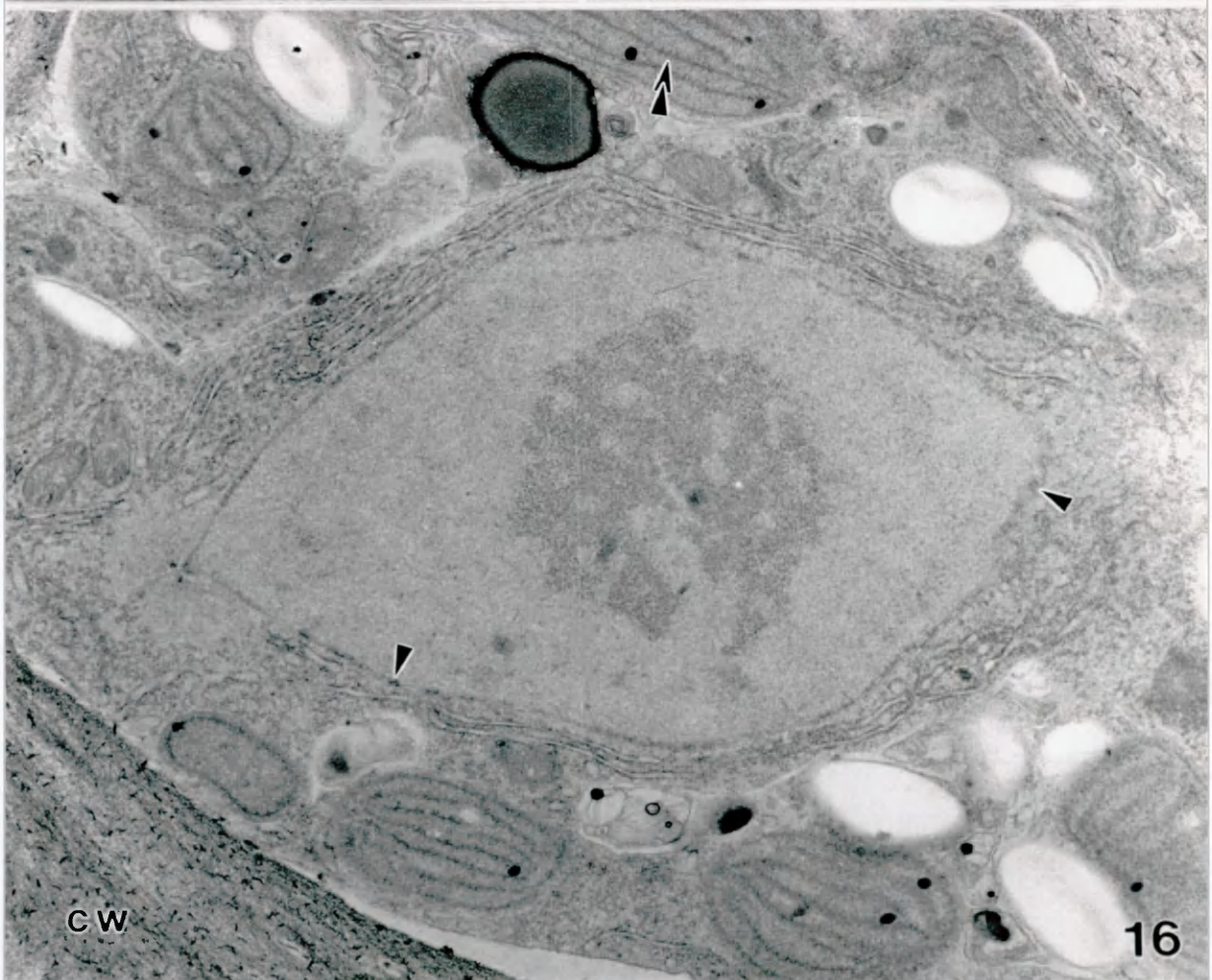
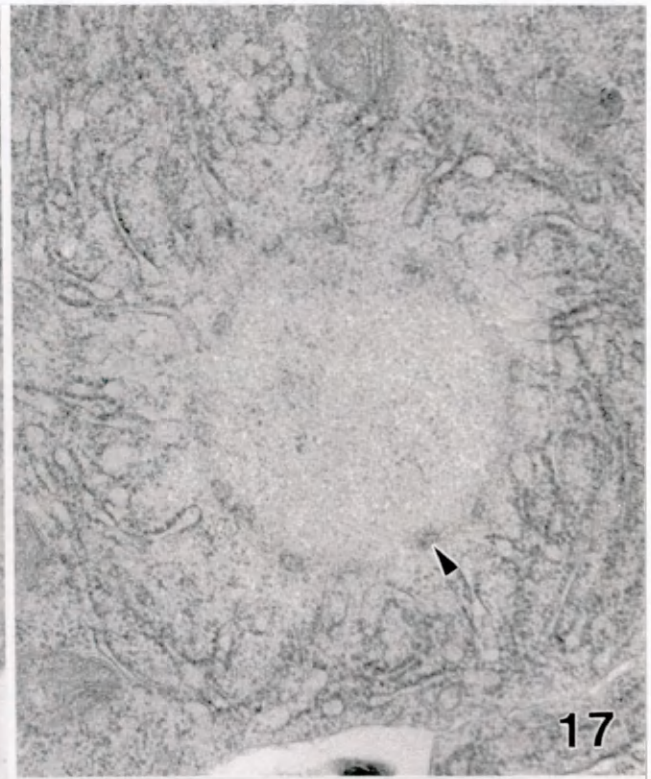
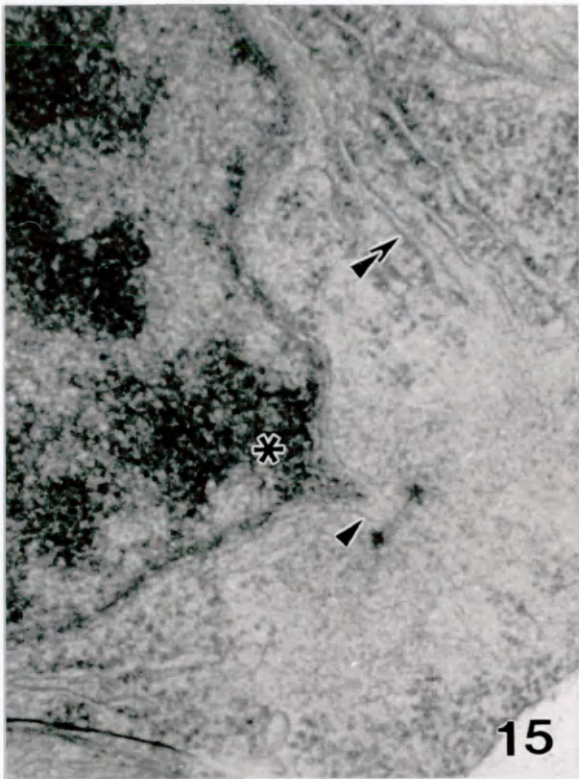




PLATE 5.

- Figure 18. Section of a mid-prophase pole showing the outer membrane of the NEP extending through the PR (arrowhead) and possibly continuous with RER. As is common, chromatin is present in the NEP and nuclear pores are absent. Some poorly defined MTs (double arrowhead) are present in the zone. Ribosomes are clearly seen on the PER. X 90100.
- Figure 19. Mid-prophase polar area showing the outer membrane of the NEP extended through the bipartite PR and clearly continuous with a swollen ER cisternae (double arrowhead). Several lengthwise and a few cross sectioned MTs (arrowheads) are visible in the zone. X 82150.
- Figure 20. Cross section of the situation photographed in Figs. 18, 19. A tubular extension of the outer membrane of the NEP extends through the center of a mid-prophase PR. Many MTs can be seen radiating from the zonal area (arrowhead). X 52950.
- Figure 21. Late prophase pole showing invaginations in the NE (arrowheads) adjacent to the NEP. MTs are present in the zonal area as well as longitudinally between the nucleus and the PER. X 56250.
- Figure 22. Glancing section through a PR of a late prophase nuclear pole. The tubular NE extension is again seen in the PR center and two channels have developed under the PR on either side of the extension. Fragments of ER (double arrowhead) and several MTs (arrowhead) can be seen in the channels. X 40000.

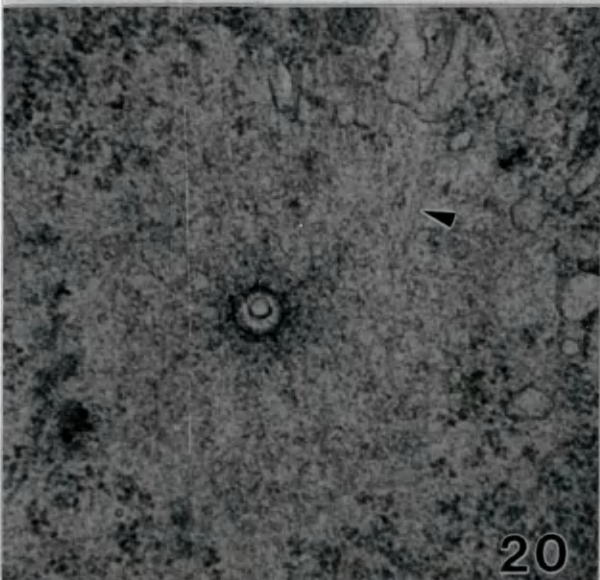
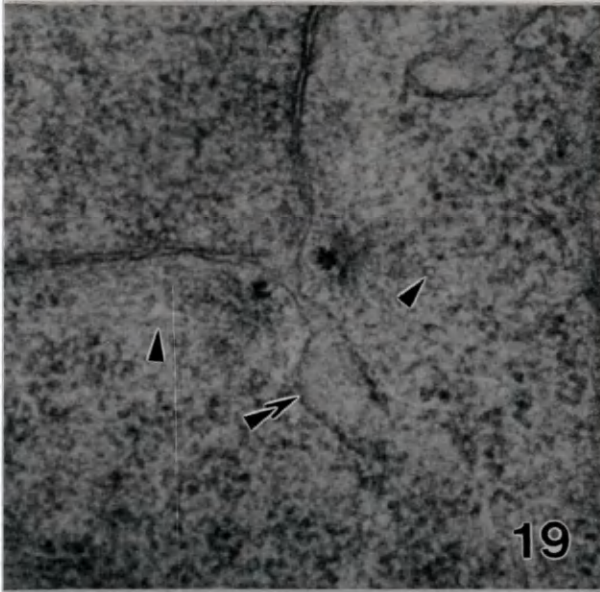
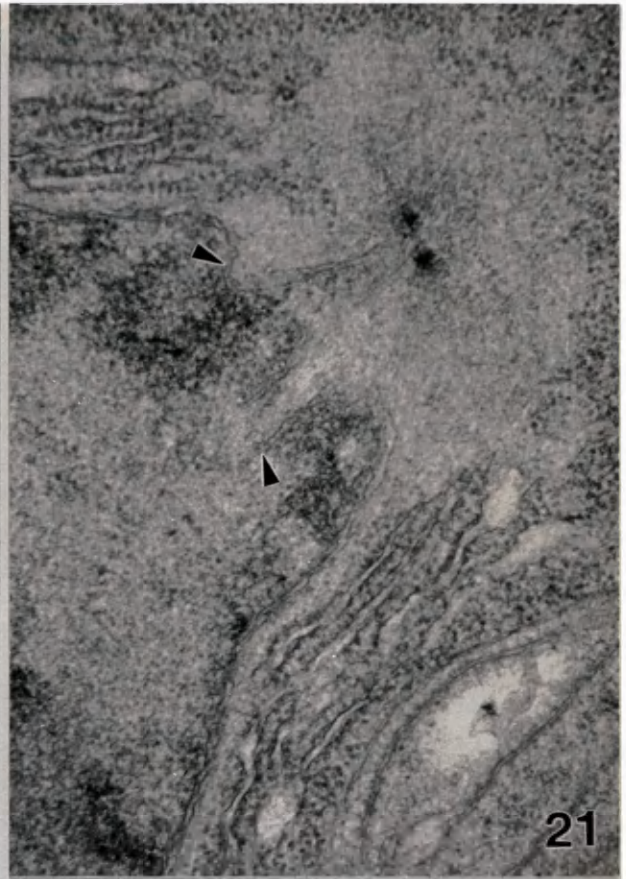
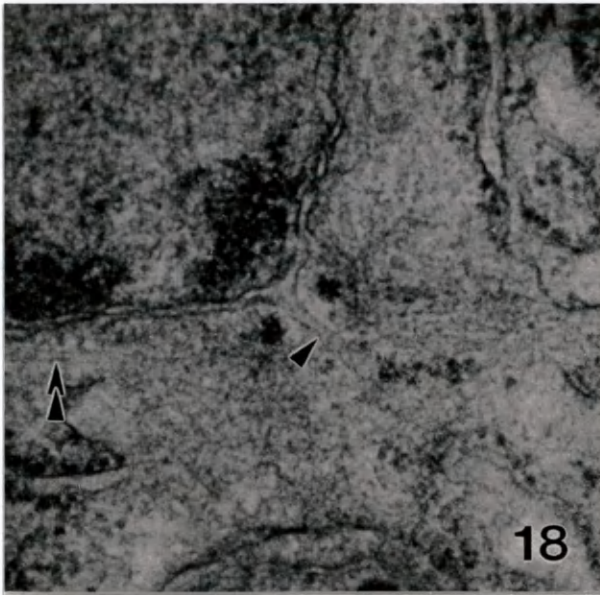
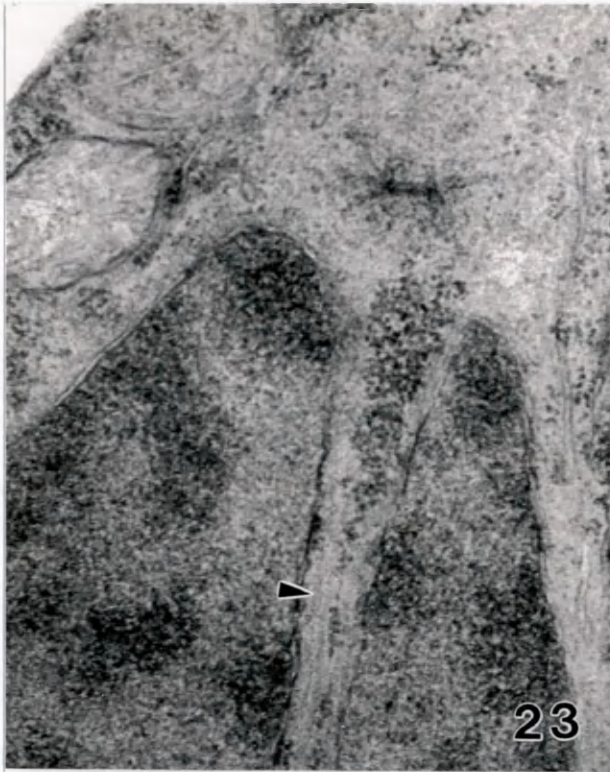


PLATE 6.

- Figure 23. Longitudinal section through a late prophase nuclear pole. The channel shown is filled with MTs (arrowhead) and contains some granular material. A PR is shown in glancing section at the opening of the channel. X 51525.
- Figure 24. Late prophase. Many MTs (arrowheads) are aligned longitudinally in the channel as well as arranged lengthwise in the area between the NE and the PER. Fragments of ER are located in the tunnel opening. Cross sections of nuclear pores (double arrowhead) can be seen in the NE lining the channel. X 57925.
- Figure 25. Longitudinal section of a late prophase nucleus depicting one channel completely transecting the nucleus. Fragments of ER and MTs are evident in the channel. The NE appears to be intact throughout the channel as well as around the entire nucleus. The nucleolus has dispersed and chromatin is concentrated in clumps. PER appears in reduced quantity. X 36575.



25% COTTON CONTENT

PLATE 7.

- Figure 26. Tangential section through a late prophase nucleus. One PR is visible with an MT-filled channel adjacent to it. Glancing sections of two channels can be seen within the nucleus (arrowheads), one of which contains MTs and granular material. PER is reduced in quantity. X 22950.
- Figure 27. Section of a prometaphase nucleus showing ruptured NE within the nucleus. The fragments, one of which is branched, emerge from the NE itself. Several layers of PER surround the nucleus. X 19850.
- Figure 28. Prometaphase nucleus. The NE fragments from the ruptured NE-lined channels (arrowheads) are scattered among the condensing chromatin. The nucleolus (asterisk) is barely visible. Several starch grains (s) are located around the nucleus. X 23550.
- Figure 29. Prometaphase nucleus. Condensed chromatin has begun to concentrate in the nuclear center to form a plate. Ruptured NE fragments (arrowheads) are still evident scattered within the nucleus. X 22325.

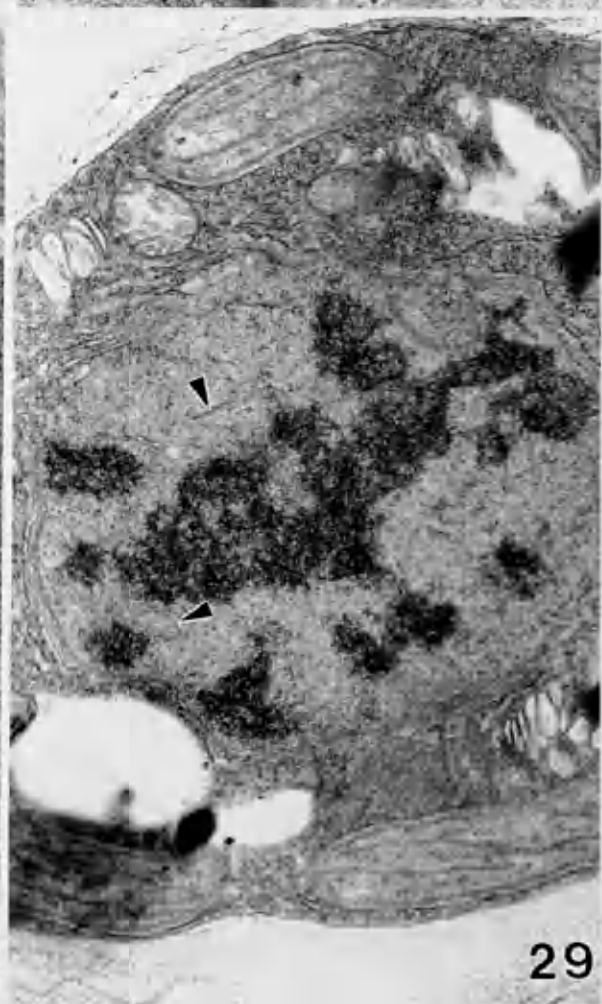
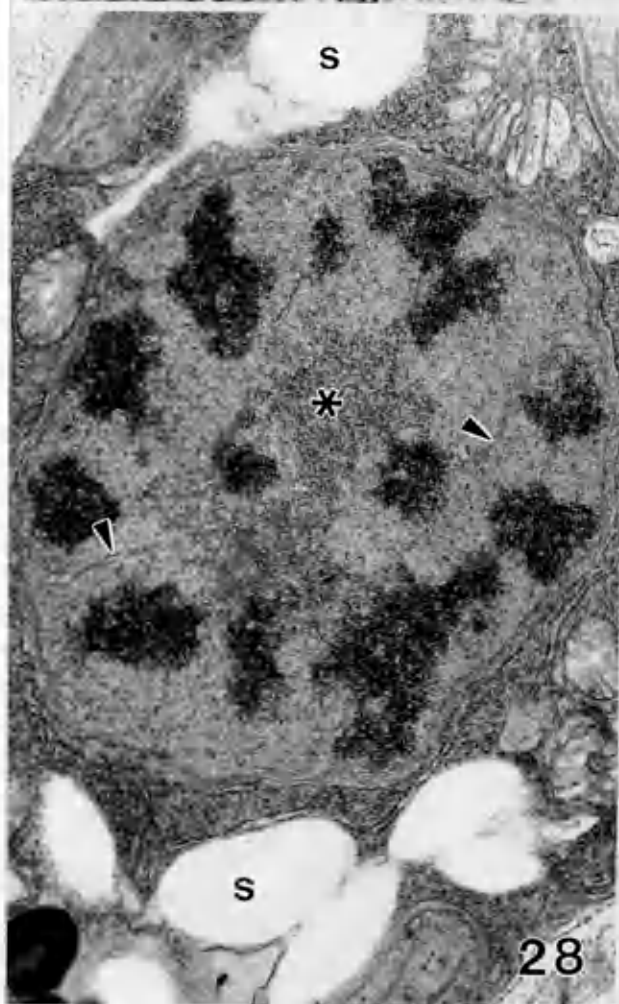
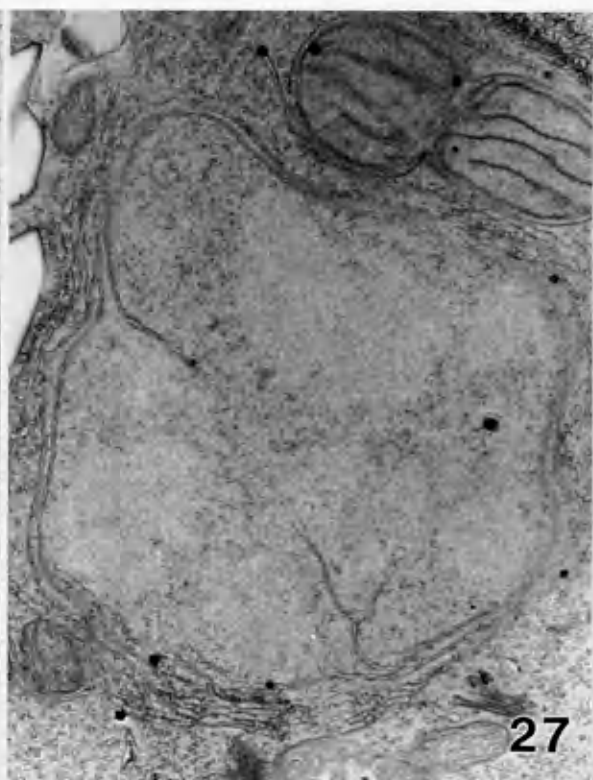
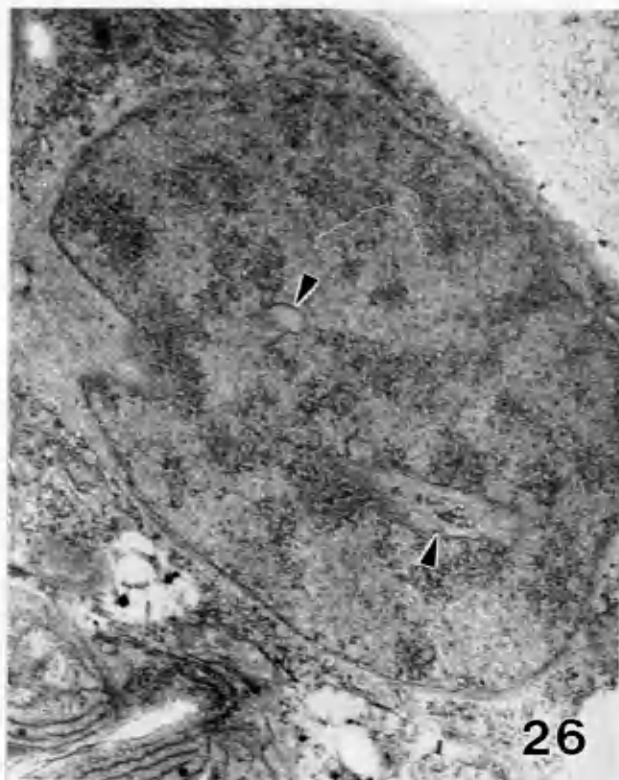
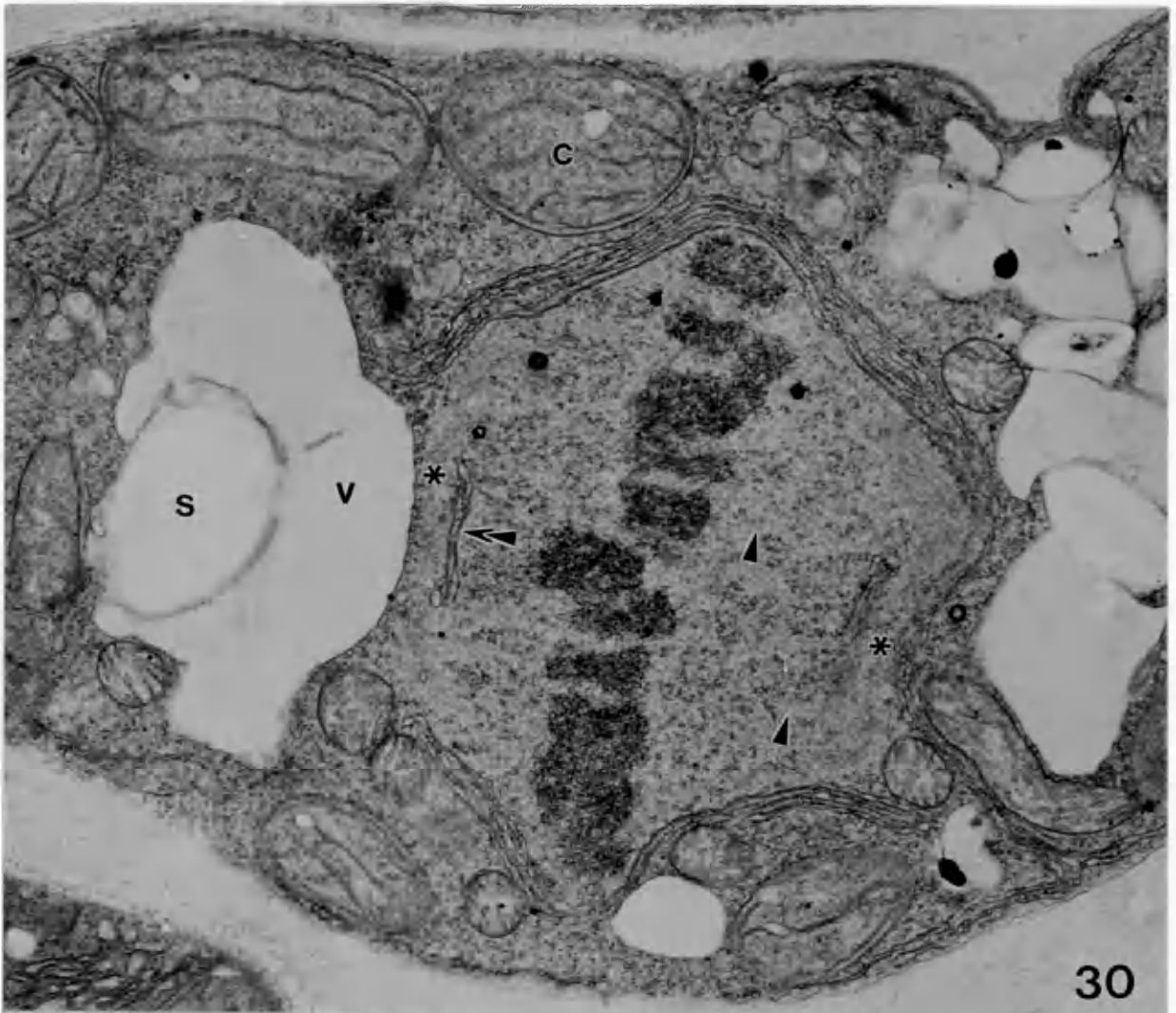


PLATE 8.

Figure 30. Longitudinal section through a metaphase nucleus. Zones of exclusion (asterisks) are shown at both broad flat poles. The nuclear midregion has grown wide and accommodates a linear arrangement of chromosomes in a typical metaphase plate. Layers of PER surround the nucleus except at the poles. Though the NE remains intact at the nuclear midregions, there are two large gaps in the NE at each pole. One large central NE remnant (double arrowhead) and several small membranous fragments remain at each former polar NE level. Numerous MTs (arrowheads) are visible in the nucleus. These are focused broadly at the poles and either terminate at the NE remnants or at the former level of the NE. Vacuoles (v) and starch (s) have accumulated at the poles. The peripheral inner limiting thylakoid and genophores are evident in the chloroplasts (c). X 19450.

Figure 31. Metaphase nucleus showing flattened poles and a broad midregion. Many MTs are evident, focused broadly at the polar region. One of the large polar NE remnants (double arrowhead) is shown at one pole, while due to tangential sectioning, only several small NE fragments and a broken end of NE which diverged from the surrounding PER (arrowhead) can be seen at the other. Mitochondria and vacuoles abut the zonal nuclear sides. X 17350.

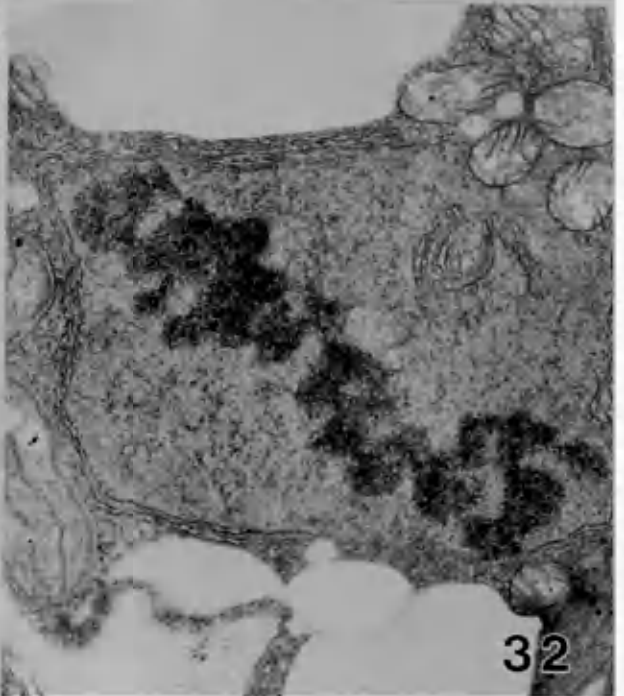
Figure 32. Glancing section through a metaphase nucleus. Mitochondria, starch, and vacuoles are found adjacent to the nuclear poles. Membranous fragments and swirls are located at the polar sides. X 16750.



30



31



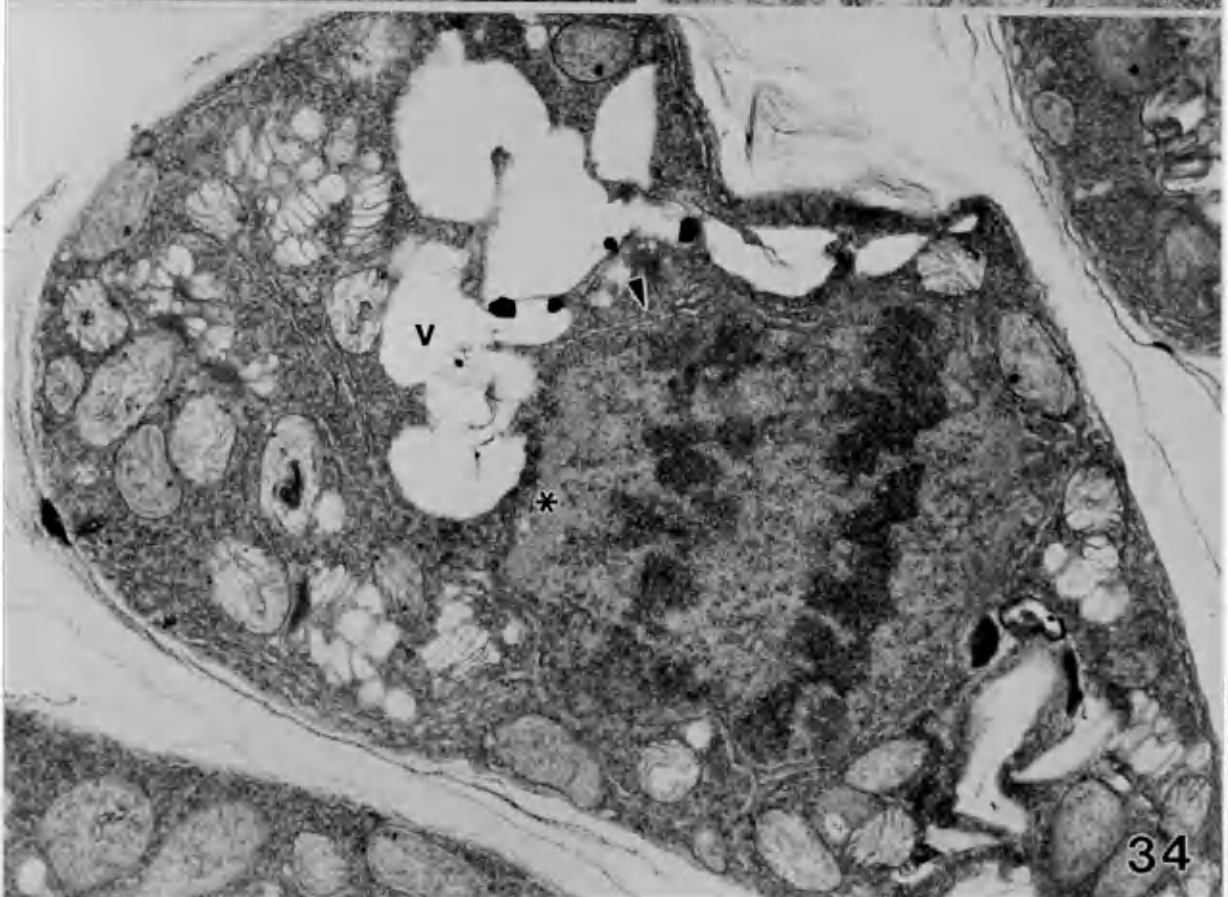
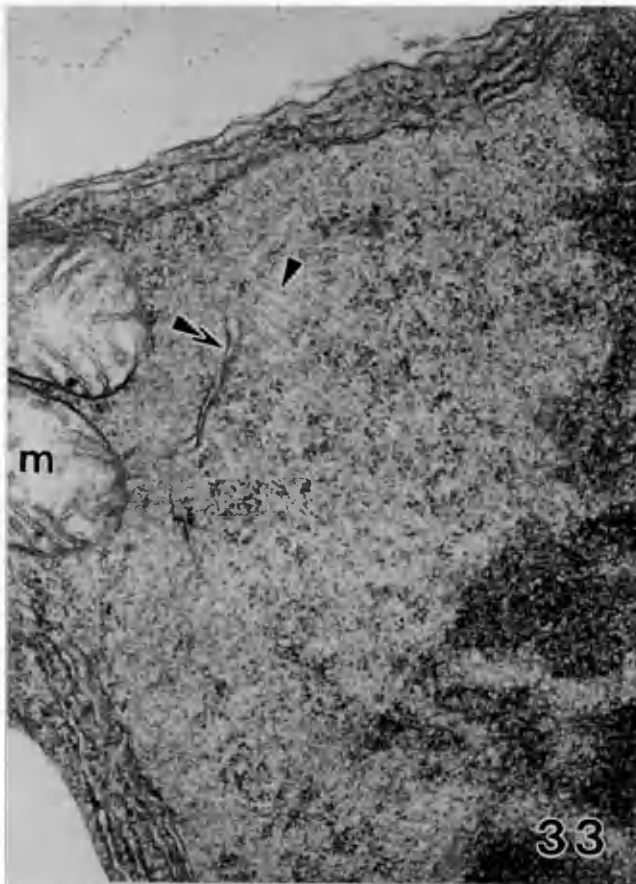
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*Periplasma*



PLATE 9.

- Figure 33. Thin section through a metaphase nuclear pole showing the large NE remnant (double arrowhead) and other membranous fragments typical at the former level of the NE. Several MTs (arrowhead) are shown; these terminate at the level of the former NE. Mitochondria (m) have accumulated in the polar region. X 38075.
- Figure 34. Early anaphase nucleus. The centrally located nucleus has begun to elongate as sister chromatids have started separating toward their targeted poles. Several layers of PER (arrowhead) surround the nucleus, and vacuoles (v) are located at the poles as well as along the nuclear midregion. A narrow zone of exclusion (asterisk) can be seen at one of the poles. X 15650.
- Figure 35. Early anaphase section showing sister chromatids further advanced toward their respective poles. The typically associated organelles are visible at the polar regions. X 27275.



*Penicillium*

PLATE 10.

Figure 36. Sister chromatids abut the broad zonal areas (asterisks) of this mid-anaphase nucleus. The poles themselves have begun to separate from one another giving the nucleus a characteristic dumbbell shape. The single large NE remnant (arrowheads) and smaller fragments can be seen at each pole. Several layers of PER run concentrically with the narrowing and elongating nuclear midregion, or IZM, and vacuoles and mitochondria are located nearby. X 21250.

Figure 37. Mid-anaphase nucleus showing the large NE polar remnant (arrowheads) between the chromatids and the zone of exclusion at each broad pole. Some MTs are visible in the IZM (double arrowhead). Three to four layers of PER encase the IZM while vacuoles (v) can be found exterior to them. X 27900.

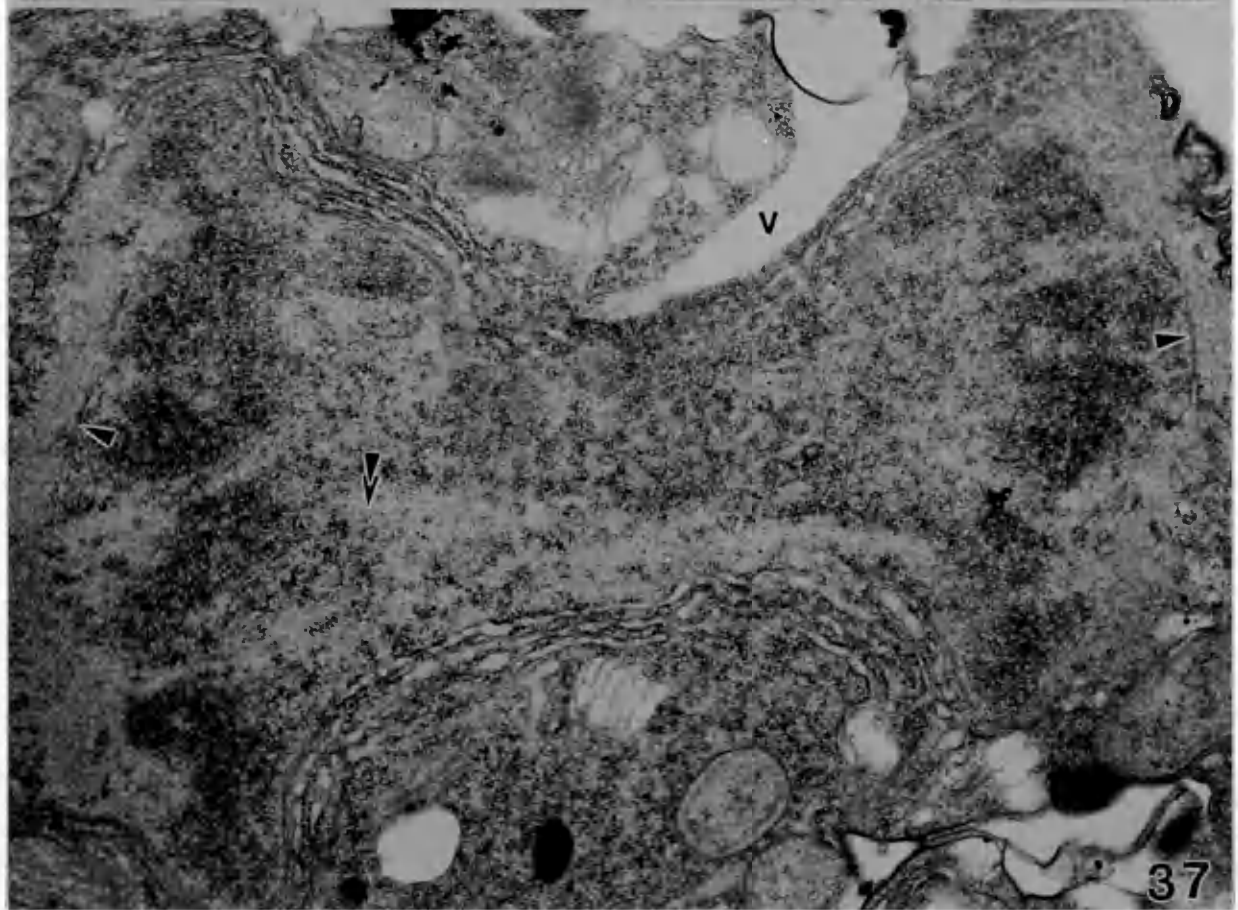
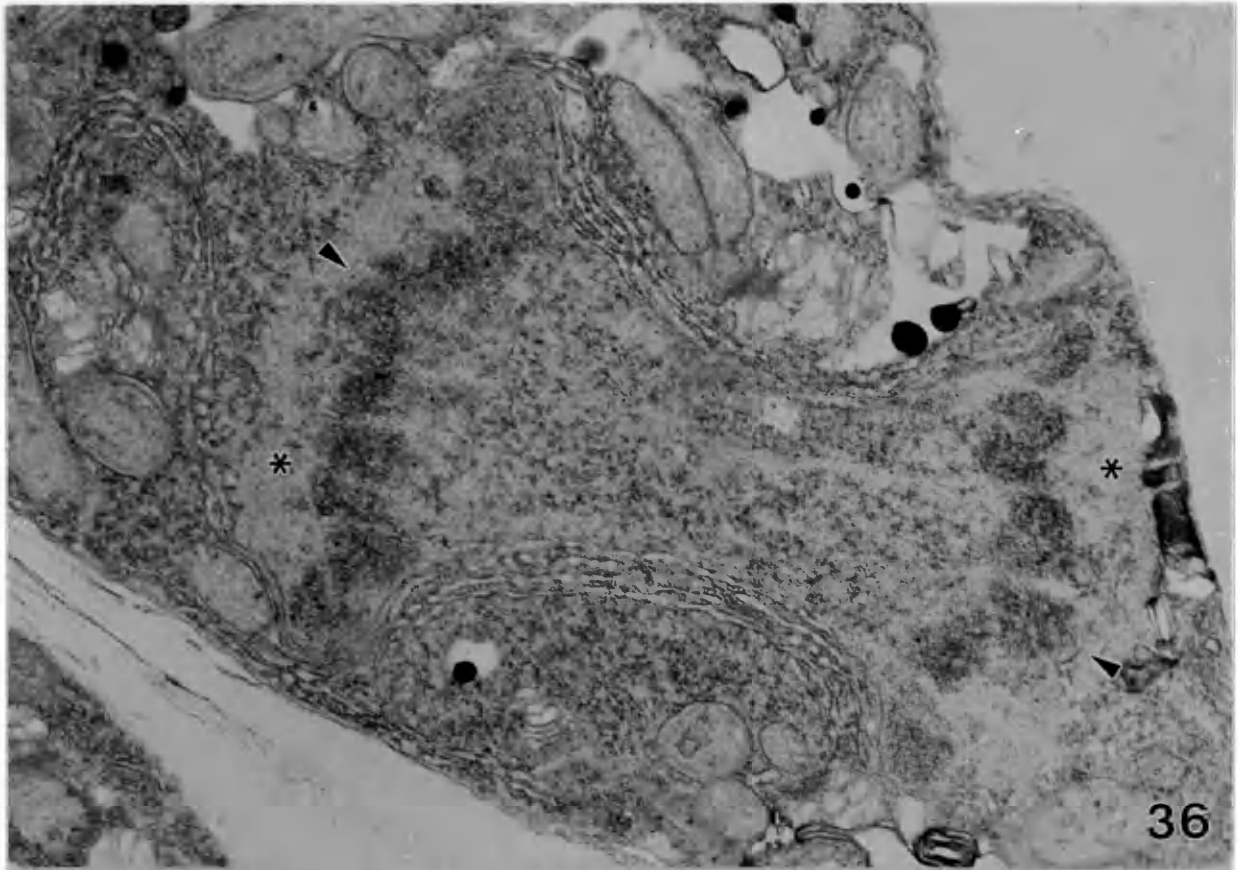


PLATE 11.

- Figure 38. Tangential section through a mid-anaphase nuclear pole. Fragments of NE (arrowheads) are found between the chromosomes and the poles. Several MTs can be seen per chromosome. Layers of PER surround the lateral side of the dumbbell shaped nuclear end, while vacuoles (v) and mitochondria (m) are found at the poles and lateral to the IZM. X 35850.
- Figure 39. Micrograph of a late anaphase nucleus. The chromosomes have separated to opposite poles of the nucleus where zones of exclusion, two polar gaps, and the large NE polar remnant between them (arrowheads) are still visible at each pole. The process of separating daughter nuclei from the IZM has begun by the infurrowing of NE and PER (double arrowheads). Chromatin has begun to decondense, and vacuoles are seen adjacent to the forming furrows in the IZM. X 23550.
- Figure 40. Whole cell in telophase/early cytokinesis. Nuclear membranes have reformed around the two daughter nuclei where chromatin has decondensed and a reformed nucleolus (n) is visible in one. The IZM (asterisk) has been detached from the daughter nuclei and remains isolated between them, encased in several layers of PER. The nuclei are located on opposite sides of the cell, separated by the IZM, vacuoles, and starch. Cleavage furrows (arrowheads) are visible on both sides of the cell. X 18000.

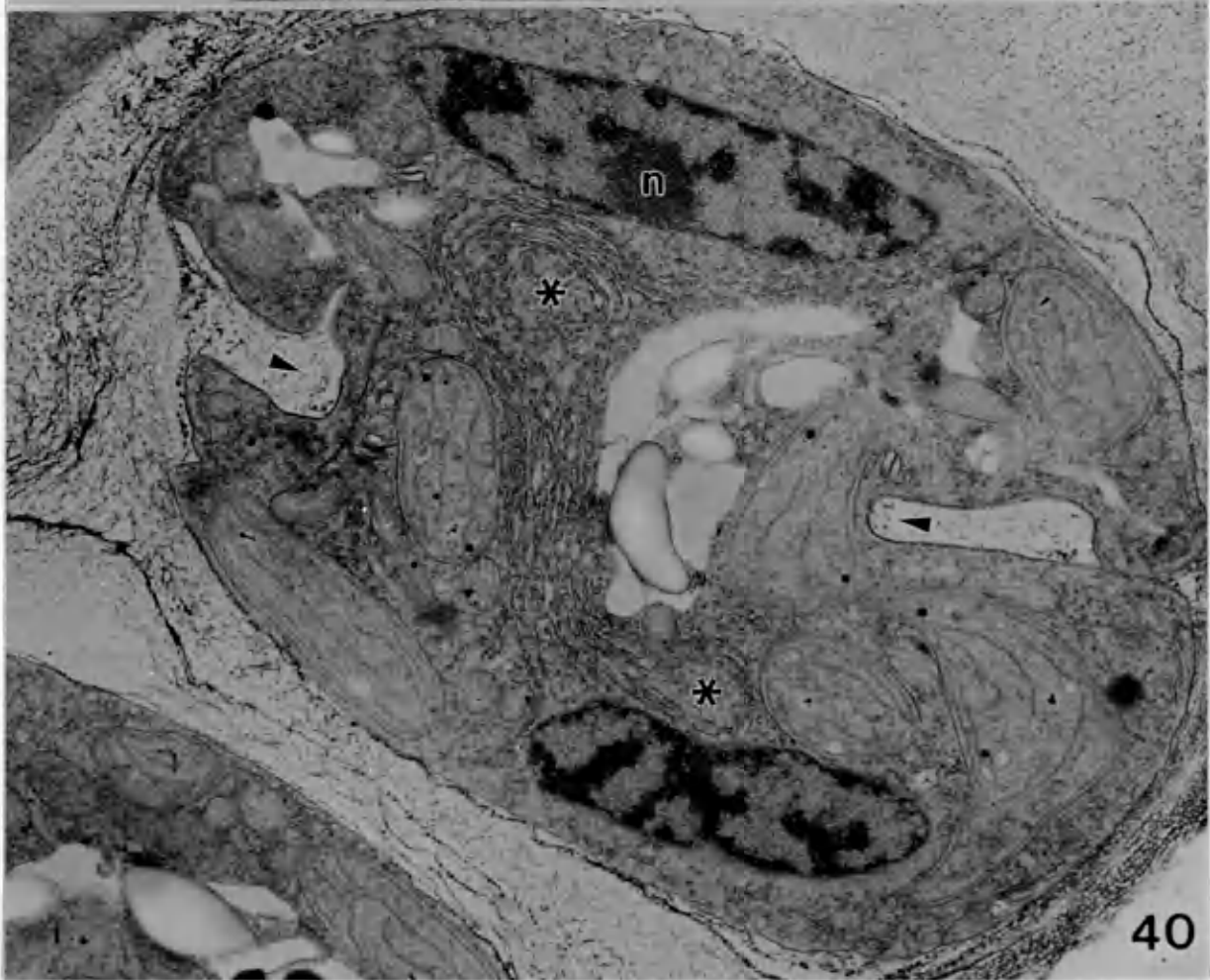
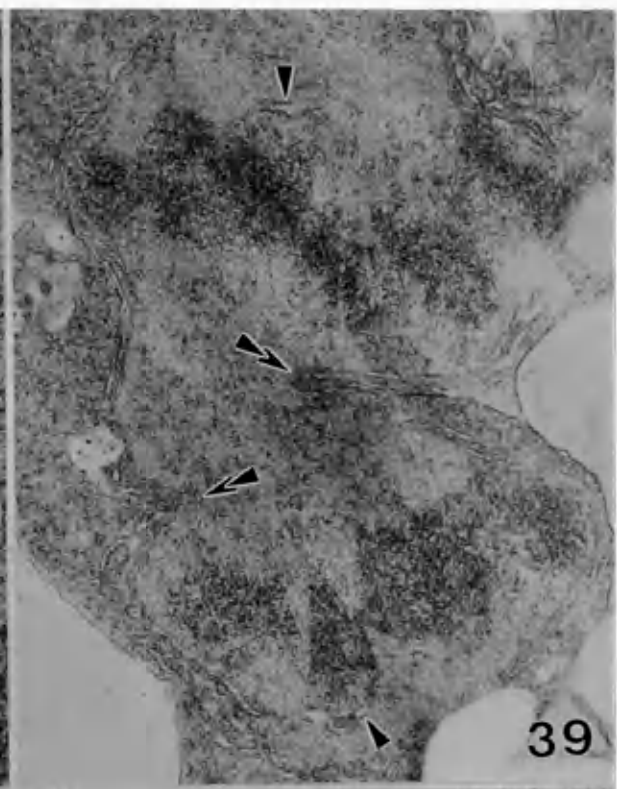
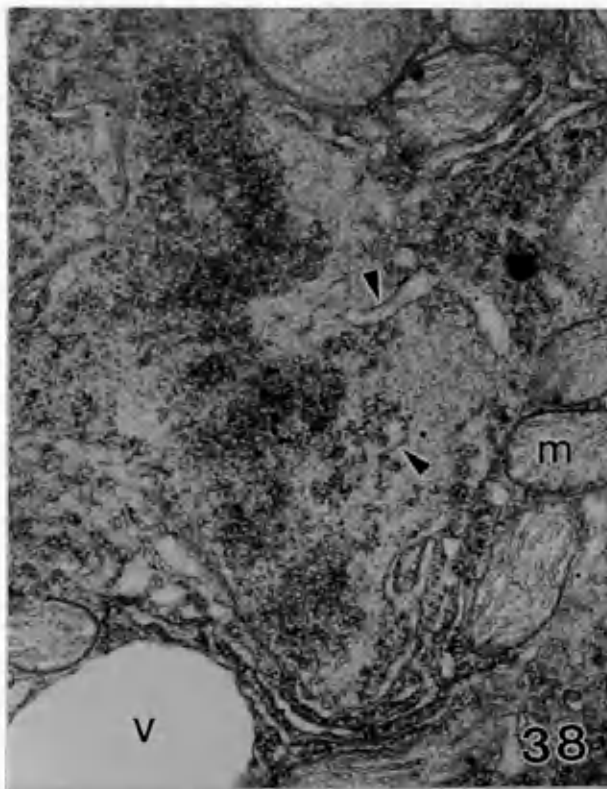


PLATE 12.

- Figure 41. Daughter nuclei in their typical telophase/early cytokinesis position on opposite Tangential sides of the cell. Note the NE is relatively flattened against the plasma membrane while the opposite nuclear side is more rounded and is still associated with several RER layers. Nuclei are separated by vacuoles and starch, and cleavage furrows (arrowheads) can be seen penetrating inward from the plasma membrane in a division plane which is perpendicular with respect to the nuclei. X 9700.
- Figure 42. Cell in telophase/early cytokinesis. Vacuoles and starch separate the daughter nuclei. Though somewhat less typical, cleavage furrows here are forming lengthwise rather than tangentially in the cell (arrowheads). X 12200.
- Figure 43. A single nucleus is shown in this glancing section of a late cytokinesis cell. Associated residual PER can still be seen on the side of the nucleus where the IZM once was (arrowhead), and vacuoles (v) and starch (s) separate it from the other forming daughter cell. Cleavage furrows have almost completely divided the cell in two; only a small connection remains between the daughter cells. X 17275.

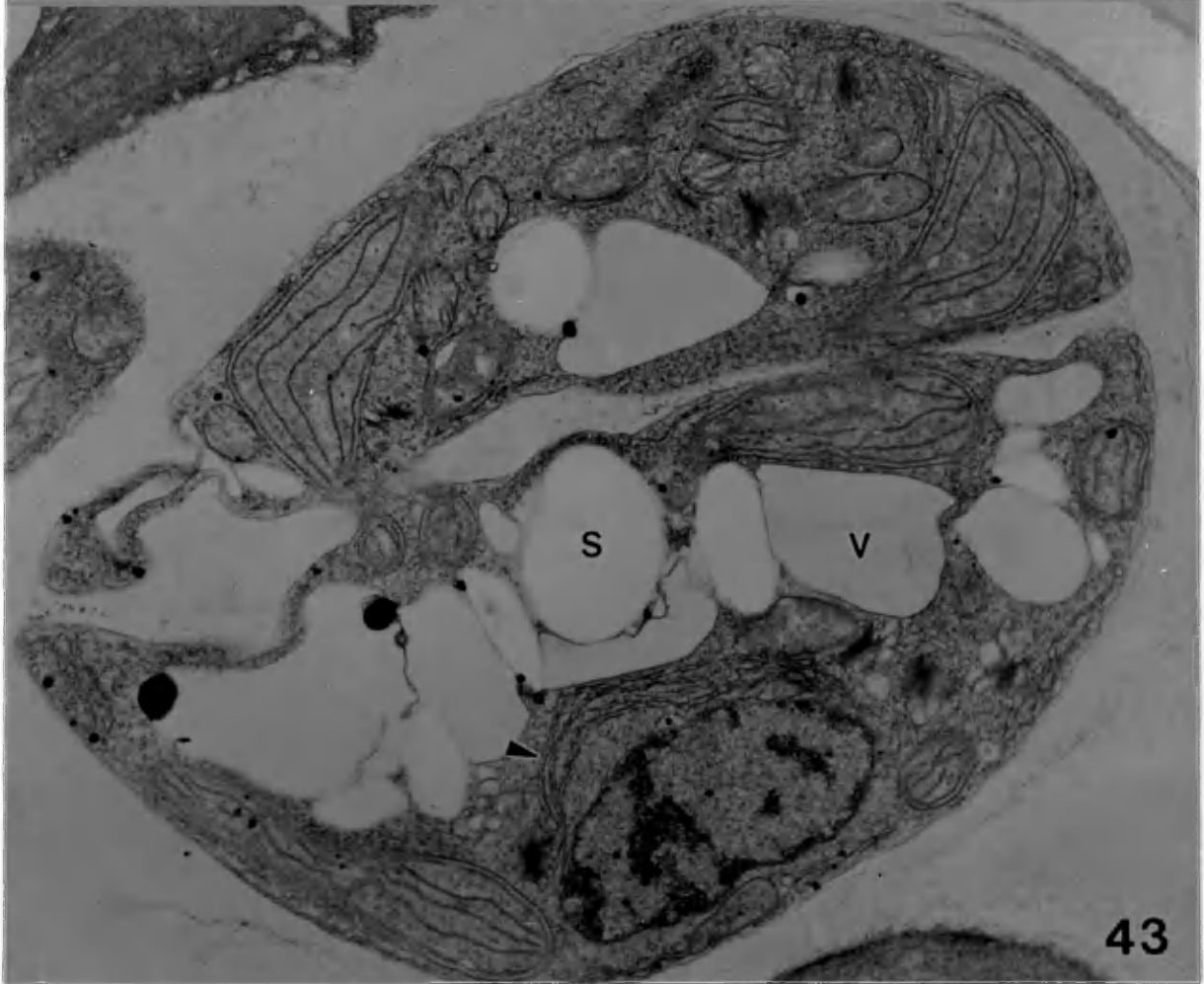
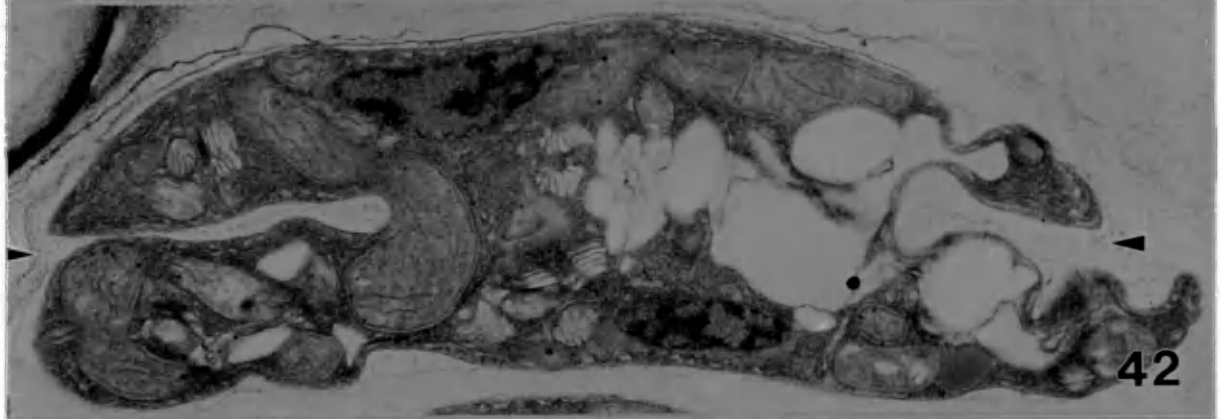
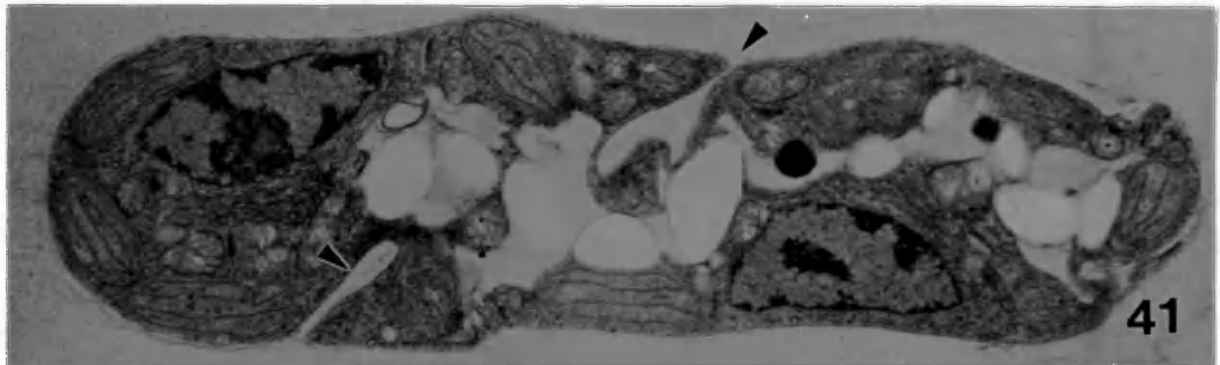
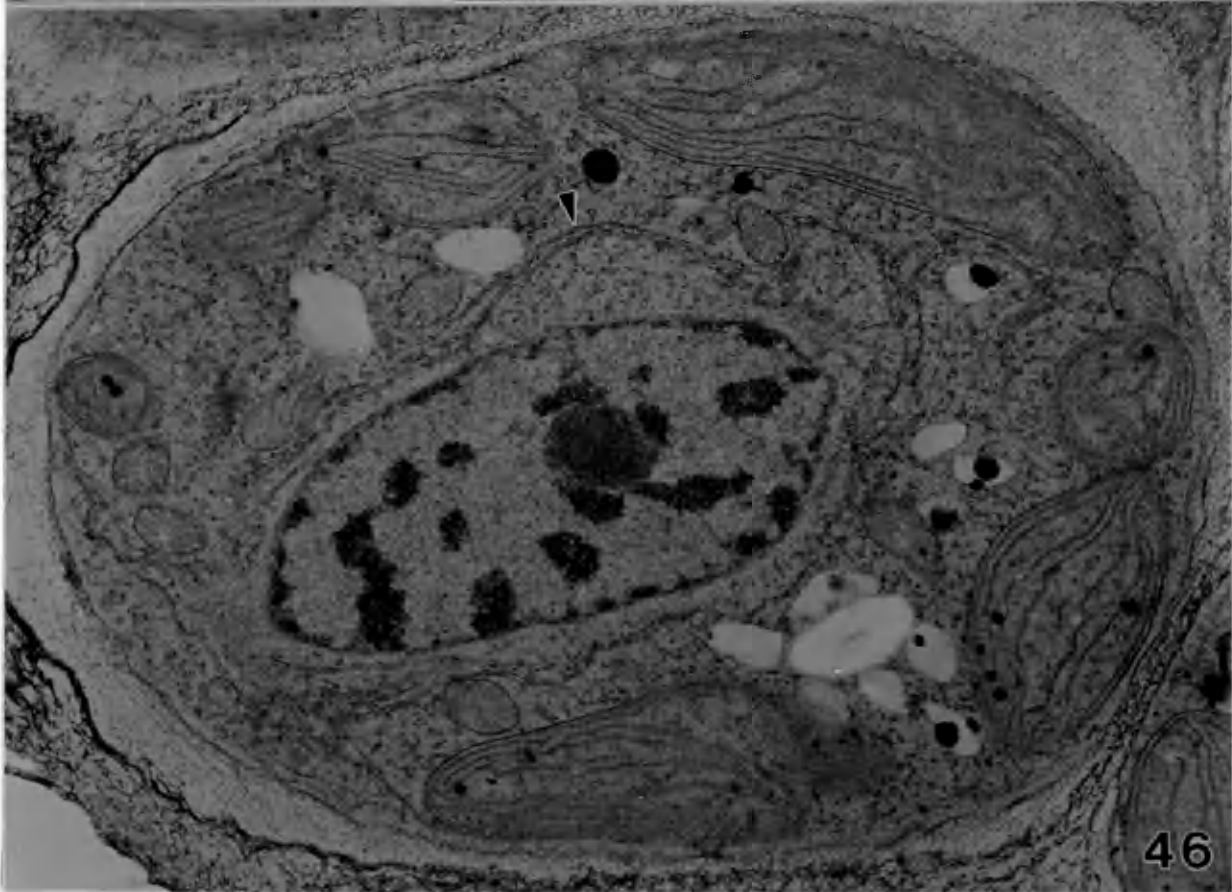
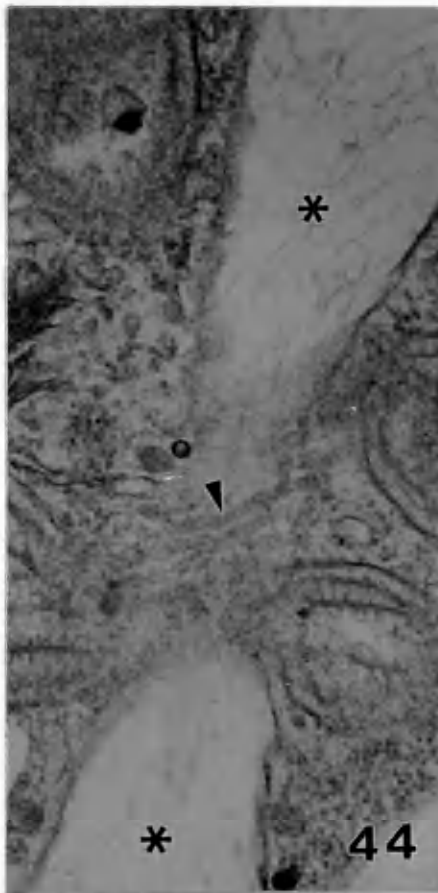




PLATE 13.

- Figure 44. Enlarged micrograph of the connection remaining between the late cytokinesis daughter cells shown in Fig. 43. Note the four ER profiles running between the two cells (arrowhead) and the fibrous nature of the cleavage furrow (asterisk). X 56275.
- Figure 45. Interphase cell with residual PER (arrowheads) surrounding the two daughter nuclei. Though telophase has completed, and a vacuole (v) separates the nuclei, they are not located on opposite cell walls and cleavage furrows have not been formed. Thus, a binuclear cell apparently has been created. X 12950.
- Figure 46. A newly formed, relatively round, interphase cell showing the nucleus centrally located in the cell. Some residual PER (arrowhead) still surrounds the nucleus. X 19800.



## VITA

Karel Joan Klepacki

Born 02 September 1959, in Brooklyn, New York.  
Graduated from Valley Stream Central High School, Valley Stream, New York, June 1977. Attended New York University, New York, New York, September 1977-June 1979. Inducted into Alpha Phi Sigma Honorary Fraternity, April 1980, Chi Beta Phi Scientific Honorary Fraternity, March 1981. B.A. in Biology, and Teacher's Certification in Biology and General Science, Mary Washington College, Fredericksburg, Virginia, May 1982. Entered Masters Program in Biology, the College of William and Mary, Williamsburg, Virginia, August 1984. Accepted teaching assistantship, January 1985.