Jap. J. Phycol. (Sôrui) 33: 199-209. September 20, 1985

Ultrastructural studies on nuclear division in the sporophyte of *Carpomitra cabrerae* (CLEMENTE) KÜTZING (Phaeophyta, Sporochnales)

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MOTOMURA, T. and SAKAI, Y. 1985. Ultrastructural studies on nuclear division in the sporophyte of *Carpomitra cabrerae* (CLEMENTE) KÜTZING (Phaeophyta, Sporochnales), Jap. J. Phycol. 33: 199-209.

Nuclear division in cells of trichothallic hairs of *Carpomitra cabrerae* was studied with the electron microscope. A pair of centrioles was observed near the interphase nucleus. In early prophase, each pair of centrioles was derived from the duplication of the original, which had migrated to the poles, and the nucleus was depressed at both poles. Many microtubules radiated from electron dense material around the centrioles into the depression of the nuclear envelope. Chromatin condensed gradually and a nucleolus disappeared. In metaphase, chromosomes were arranged at the nuclear equator, and many spindle fibers were observed in the nucleoplasm, but kinetochores were not seen. The nuclear envelope was almost intact except for both polar fenestrate regions. In anaphase, separation of chromosomes was accompanied by increased distance between the poles, and disintegration of the nuclear envelope. In early telophase, the new nuclear envelope was formed around daughter nuclei in which the spindle fibers still remained. Large vacuoles were observed between daughter nuclei, and they were compressed by an increment in volume of daughter nuclei which was caused by the dispersal of chromatin and the regeneration of the nucleolus.

Key Index Words: Carpomitra cabrerae; *nuclear division*; *Phaeophyta*; *trichothallic hairs*; *ultrastructure*.

Ultrastructural investigations of algal mitosis have been reported recently. Some of these have suggested a phylogenetic scheme in the Chlorophyta. In the Phaeophyta, these works have been carried out in the Ectocarpales; *Pylaiella littoralis* plurilocular sporangia (MARKEY and WILCE 1975), Sphacelariales; *Sphacelaria tribuloides* apical cell (KATSAROS *et al.* 1983), Dictyotales; *Zonaria farlowii*, *Dictyopteris zonarioides*, *Padina pavonia*, *Dictyota dichotoma* apical cells (NEUSHUL and DAHL 1972), Cutleriales; *Cutleria hancockii* male gametangium (LA CLAIRE and WEST 1979), *C. cylindrica* trichothallic meristem (LA CLAIRE 1982), Fucales; *Fucus vesiculosus* antheridium (LEEDALE 1970), *F. vesiculosus* embryo (BRAWLEY *et al.* 1977), *F. serratus* antheridium (BERKALOFF and ROUSSEAU 1979), and *Hormosira banksii* embryo (FORBES and HALLUM 1979). Many of these observations were limited to one or a few stages of mitosis, usually metaphase. Detailed ultrastructural investigations on the whole process of mitosis is anticipated for the Phaeophyta.

More recently, LA CLAIRE (1982) and KATSAROS *et al.* (1983) investigated nuclear division in detail in the active dividing region of the trichothallic meristem of the *Cutleria cylindrica* gametophyte and the apical cell of *Sphacelaria tribuloides*.

The assimilatory hairs of the *Carpomitra cabrerae* sporophyte grow from a trichothallic meristem (SAUVAGEAU 1926). As the case

of *C. cylindrica*, it provides a good system for investigation of nuclear division, because 1) active cell divisions occur in one region (trichothallic growth), 2) the axis of nuclear division is one direction (haplostichy), and 3) the thallus grows actively in culture. In the present study, the process of nuclear division of the *Carpomitra cabrerae* sporophyte is reported in detail from electron microscope observations.

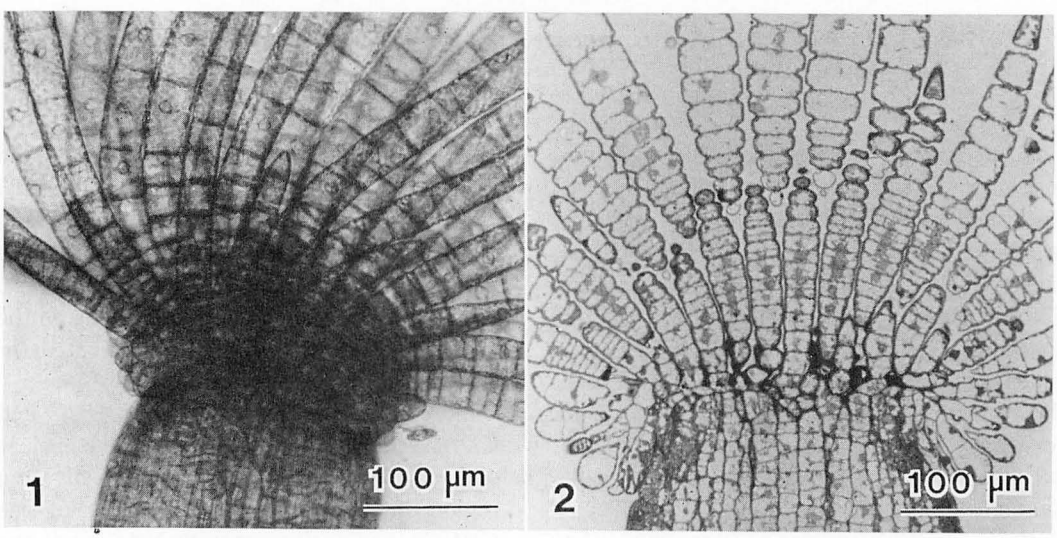
Materials and Methods

The strain used for the present observations was the same as the material used previously for the study of the life history of *Carpomitra cabrerae* (MOTOMURA *et al.* 1985). The medium used was PESI medium (TATEWAKI 1966). Vegetative gametophytes were maintained at 18°C, and illuminated with cool white fluorescent lamps (40–80 μmol $m^{-2}s^{-1}$), 14 : 10 LD cycles. These were transferred to 14°C, 10 : 14 LD under the same light conditions for the induction of maturation of gametophytes. After one month, monoecious gametophytes had matured and formed oogonia and antheridia. Fertilized eggs developed into sporophytes. Juvenile sporophytes were transferred again to a 18°C, 14 : 10 LD incubator for conducting active growth. Afterwards, the sporophytes reached 1–2 cm in height, and the apical regions, including trichothallic hairs were used for examining nuclear division.

Methods of fixation, dehydration and embedding were identical with those previously described for *Laminaria angustata* gametogenesis (MOTOMURA and SAKAI 1984). Thin sections were prepared on a Poter-Blum MT-1 ultramicrotome using glass and diamond knives, and they were double stained with uranyl acetate and REYNOLD'S lead citrate (REYNOLDS 1963), and observed with a Hitachi H-300 electron microscope. Serial sections were placed on formvar-coated slit grids.

Results

An interphase nucleus of cells of trichothallic hairs (Figs 1, 2) usually had one nucleolus and took spherical or ovoid form (Fig. 3). The nuclear envelope was intact and several Golgi bodies were present at the



Note: Legend abbreviations: C=centriole, CH=chromosome, Ch=chloroplast, CW=cell wall, ER=endoplasmic reticulum, G=Golgi body, M=mitochondrion, Mb=microbody, N=nucleus, Nu=nucleolus, V=vacuole.

Fig. 1. Apical region of *Carpomitra cabrerae*. Note many trichothallic hairs.

Fig. 2. Longitudinal section through the apex stained with 1% toluidine blue-O in 1% borax.

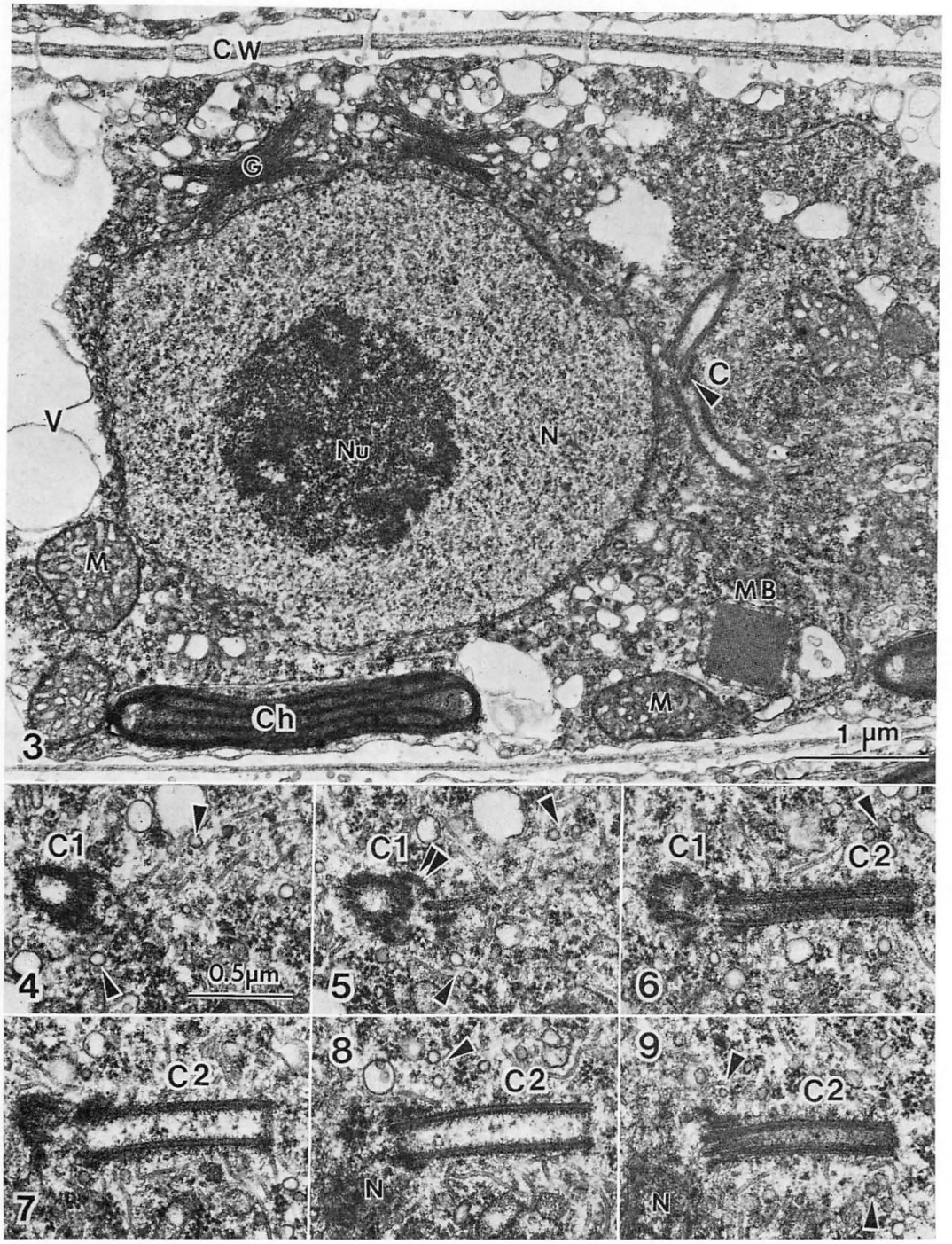


Fig. 3. Interphase nucleus with a nucleolus. A pair of centrioles exists. Arrow indicates the connecting structure between two centrioles.

Figs 4-9. Serial sections of a pair of centrioles (C1 and C2) in interphase. Note that many microtubules radiate from electron dense material around the centrioles. Small vesicles (arrow) exist around the centrioles. Double arrow in Fig. 5 indicates the connecting structure between two centrioles.

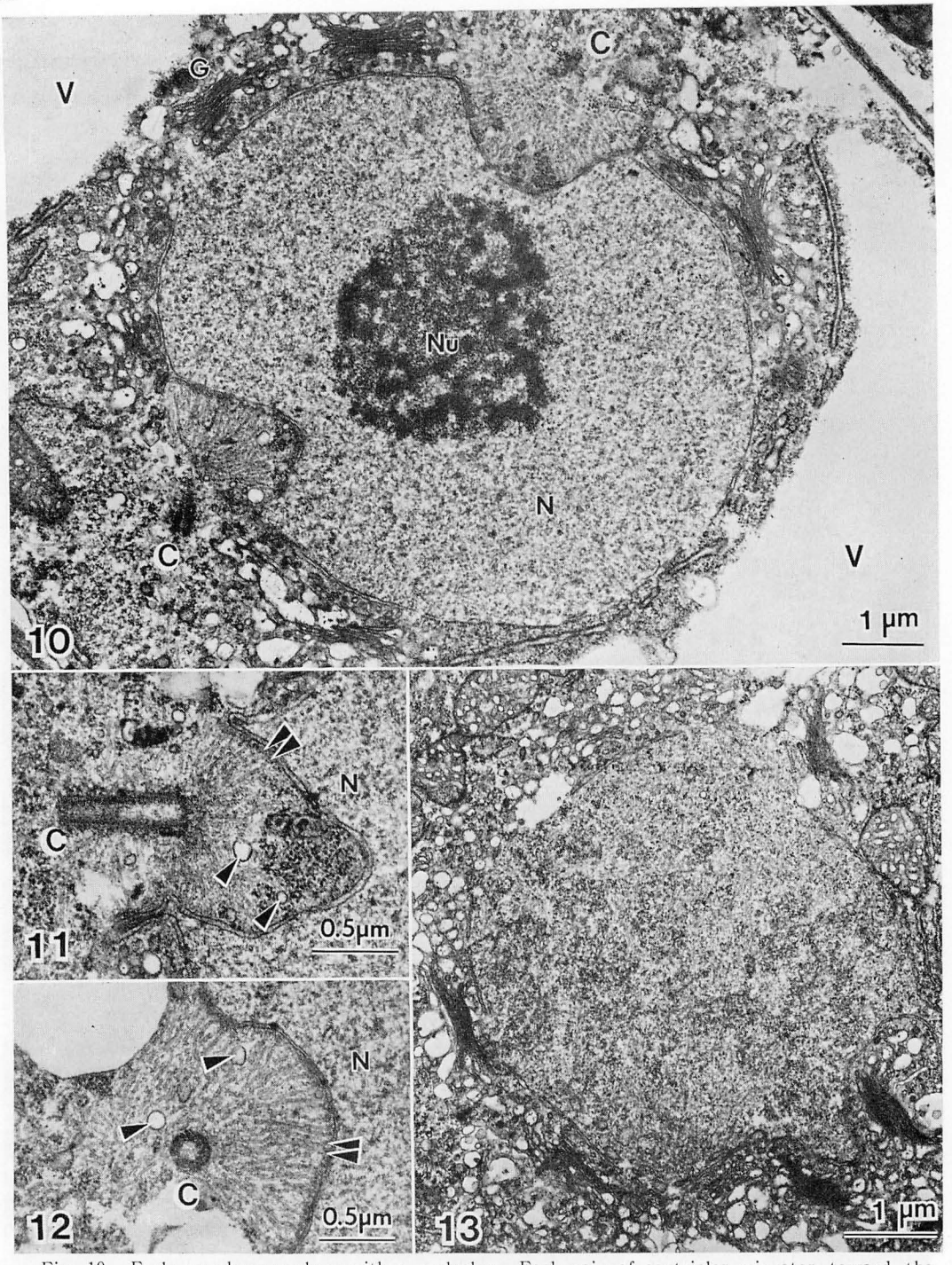


Fig. 10. Early prophase nucleus with a nucleolus. Each pair of centrioles migrates toward the both poles and the nucleus depresses at these regions.

Fig. 11. Polar depression of nucleus at the poles in prophase. Many microtubules radiate from the centriole to the nuclear depression. Electron opaque layer (double arrow) exists along the nuclear depression. Note several small vesicles (arrow) and many ribosomes in the nuclear depression.

Fig. 12. Another polar depression in prophase. Note electron opaque layer (double arrow) along the nuclear depression and several small vesicles (arrow).

Fig. 13. Late prophase nucleus. A nucleolus disappears and the chromatin condenses gradually.

perinuclear region. Examination of many thin sections of the interphase cells revealed that a pair of centrioles was situated at the side of the nuclear envelope perpendicular to the axis of nuclear division. Serial sections (Figs 4-9) showed that two centrioles were arranged at right angles to each other. Amorphous electron dense material existed either in spots or uniformly in some places around the centrioles. At the basal part of the centrioles, this amorphous material was thick. Occasionally, a structure which appeared to connect two centrioles was detected (Figs 3, 5). Many microtubules which radiated from the amorphous material were found in the cytoplasm, but not in the nucleoplasm. Small vesicles were present around the centrioles (Figs 4-9).

The first indication of early prophase must be the duplication of centrioles and their migration to the opposite poles. Although many sections were examined, these processes could not be followed in detail, therefore both processes must occur rapidly. Fig. 10 shows the early prophase nucleus. The nucleus was depressed at both poles, but the nuclear envelope was not ruptured. The nucleolus still existed. In the polar depression, many microtubules were found (Figs 11, 12). A layer of electron opaque material was present along the perinuclear region in the depression (Figs 11, 12), and microtubules terminated in this layer. The nuclear depression may be formed by the growth of microtubules. Several vesicles could be observed on the inside of the nuclear depression, and aggregation of ribosomes existed locally in the bottom of the depression (Figs 10, 11). In later prophase, a nucleolus dispersed and the chromatin began to condense. Polar fenestrae (the gap of the nuclear envelope at poles) developed, and microtubules began to enter into the nucleoplasm through the polar fenestrae (Fig. 13).

During metaphase, the nucleus turned into a spindle-shape, and chromosomes were arranged at the equator of the nucleus (Fig. 14). Polar fenestrae developed well. Many spindle fibers were observed in the nucleoplasm, but not in the cytoplasm. There was not apparent structure of kinetochores. The nuclear envelope, except for both polar fenestrate regions, was almost intact. In this period, several vesicles appeared in the nucleoplasm. Pole-to-pole distance was ca. 5 μm, and this value was the same as in a prophase nucleus.

At anaphase, daughter chromosomes separated toward the opposite poles. Fig. 15 shows the stage in which daughter chromosomes had almost migrated to the poles. The nuclear envelope was considerably broken, but remained at the vicinity of the chromosomes. Interzonal spindle fibers developed between the groups of daughter chromosomes. Pole-to-pole distance was ca. 7 μm, which was longer than those in prophase and metaphase nuclei. In later anaphase, the daughter chromosomes appeared to disperse a little and interzonal spindle fibers between the daughter chromosomes were hardly noticeable (Fig. 16). Golgi bodies had already moved toward the poles, and ER developed well along the axis of nuclear division. The nuclear envelope was gradually reformed, and ER was present nearby (Fig. 17).

In early telophase, the nuclear envelope was completely reformed around the group of daughter chromosomes and a pair of centrioles existed in the depression of the nuclear envelope (Figs 18, 19). Microtubules radiated from the electron dense material around centrioles to the cytoplasm. The daughter chromosomes dispersed gradually, and several spindle fibers were detected in daughter nuclei (Fig. 19). Large vacuoles appeared between two daughter nuclei (Fig. 18). Afterwards, the volume of the nucleus increased concomitantly with the dispersion of chromatin and with regeneration of the nucleolus (Fig. 20). Subsequently, the two daughter nuclei approached closely to each other. In early telophase, pole-to-pole distance was ca. 7 μm, which was the same as in the anaphase nucleus, but it gradually increased during cytokinesis.

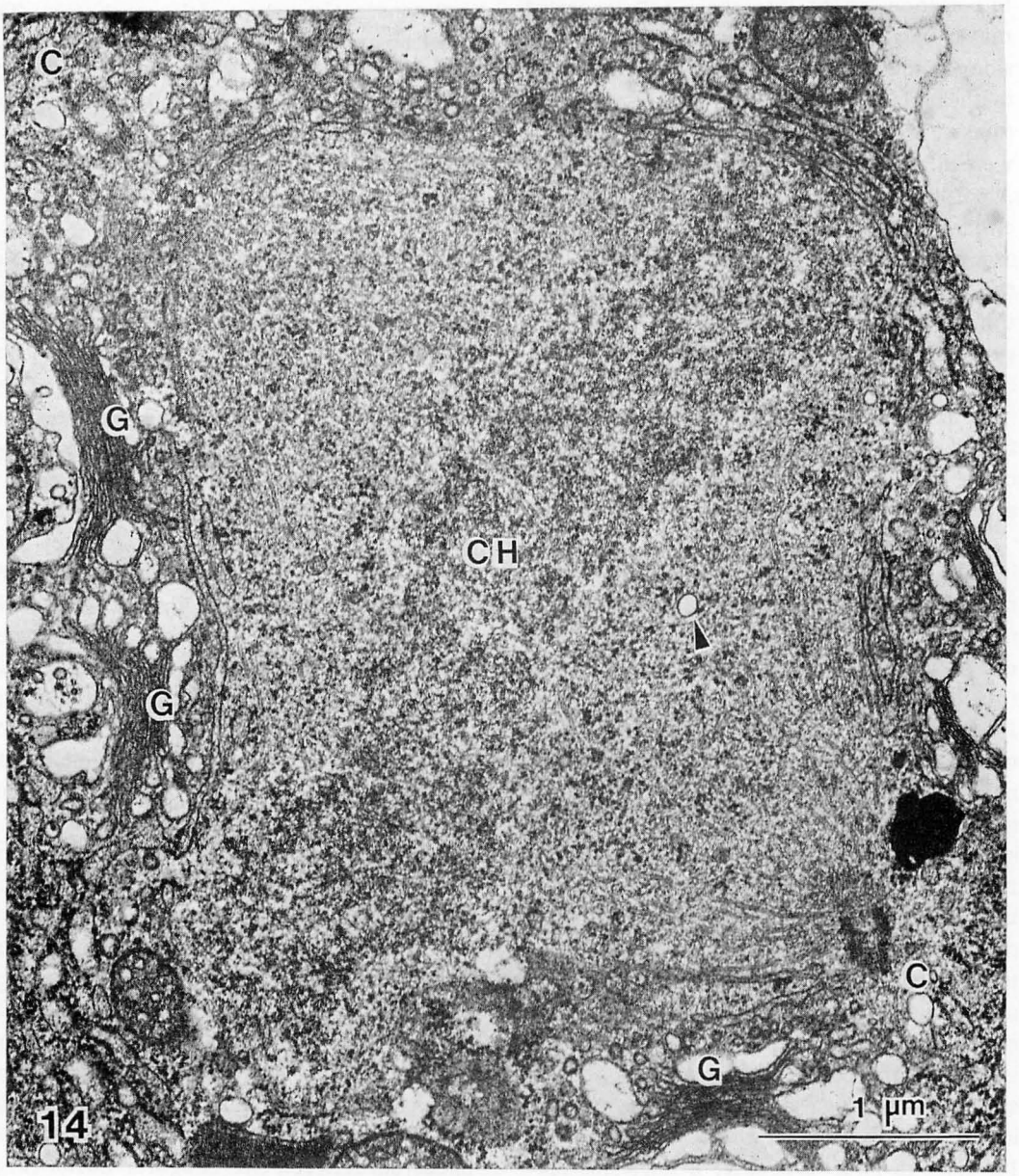


Fig. 14. Metaphase nucleus. Chromosomes arrange at the nuclear equator. The nuclear envelope seems intact except for the polar fenestrae. Many spindle fibers are noticeable from the electron dense material around the centriole to chromosomes, but kinetochores are not detected. Note several small vesicles (arrow) in the nucleoplasm.

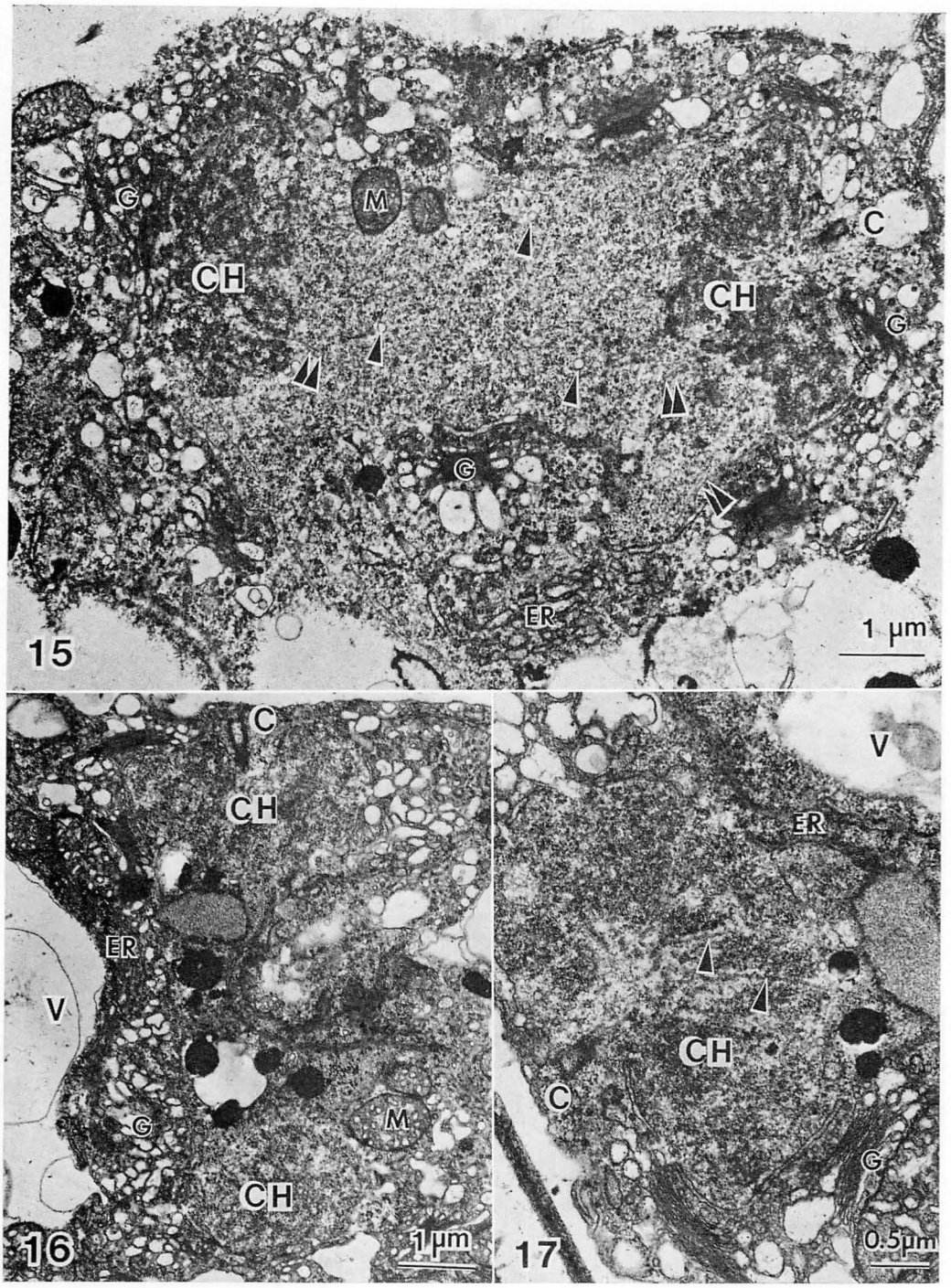


Fig. 15. Late anaphase nucleus. The mass of chromatin migrates almost to both poles. Nuclear envelope breaks down considerably. Note spindle fibers (double arrow) and small vesicles between the separated chromatin.

Fig. 16. More advanced late anaphase. ER developed well, and Golgi bodies migrate to the poles.

Fig. 17. Highly magnified figure of the upper nucleus in Fig. 16. Note spindle fibers in the nucleus (arrow).

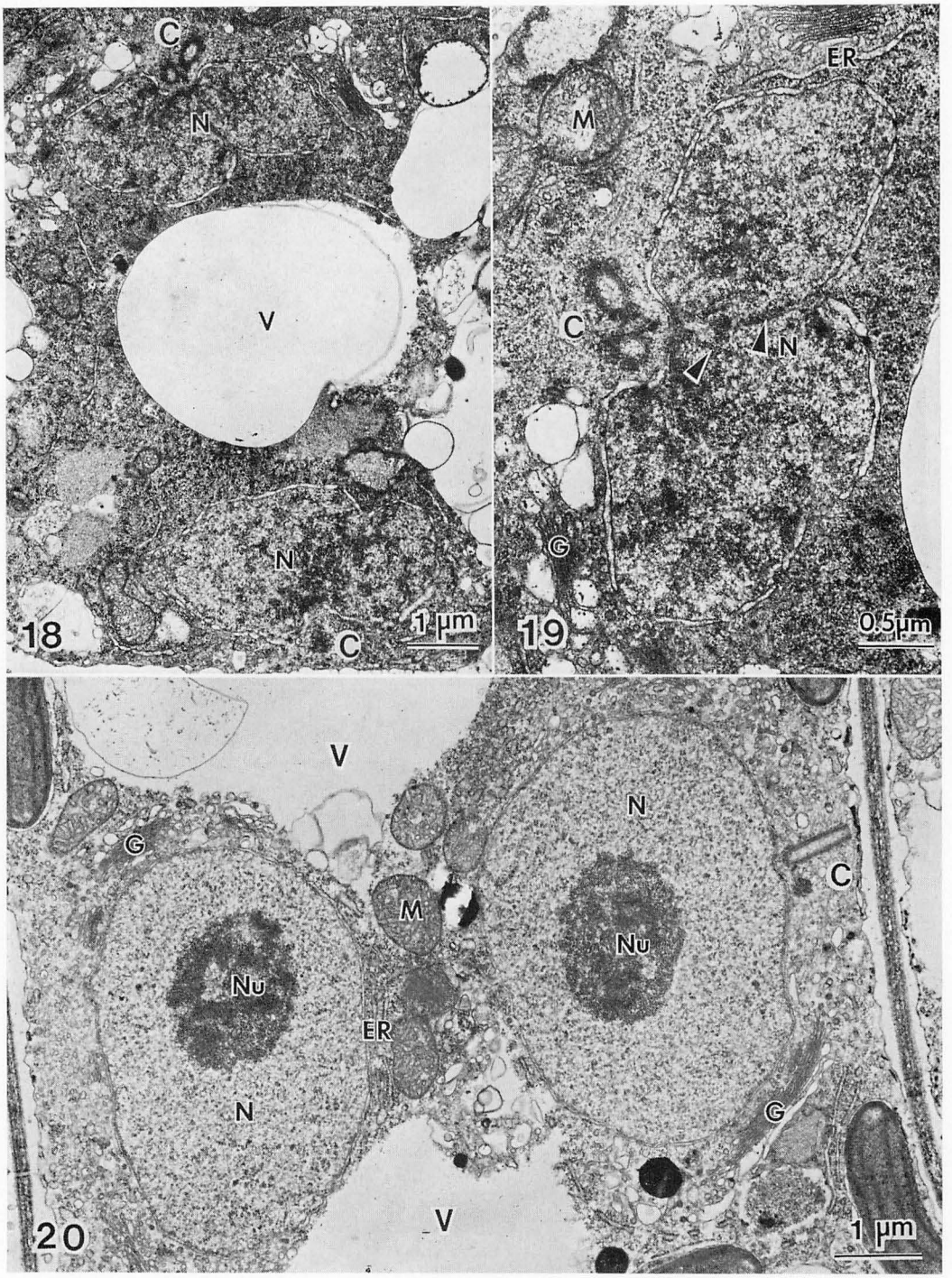


Fig. 18. Early telophase. Nuclear envelope is restored completely and condensed chromatin disperses gradually. Note large vacuole between daughter nuclei.

Fig. 19. Highly magnified figure of the upper nucleus in Fig. 18. A pair of centrioles exists at nuclear depression and several spindle fibers (arrow) are noticeable in the nucleus.

Fig. 20. Late telophase. The nucleolus regenerates in each daughter nucleus and the volume of nuclei increases with the dispersal of chromatin.

Discussion

There has been discussion as to whether a pair of centrioles existed throughout the whole cell cycle or whether they are formed *de novo* during mitosis in brown algae. According to recent studies using the electron microscope, it is evident that one pair of centrioles exists in interphase cells (LA CLAIRE 1982, KATSAROS *et al.* 1983). In *Carpomitra cabrerae*, a pair of centrioles is found easily in the interphase nucleus. The first indication of nuclear division is the duplication of centrioles and their migration to both poles. However, in the present study, the processes of duplication and migration of centrioles were not observed, in spite of observations on many sections. As suggested by LA CLAIRE (1982), these processes may be very rapid.

Polar depression of the prophase nucleus and a layer of electron opaque material along the depression have been observed in other species of Phaeophyta, *Pylaiella littoralis* (MARKEY and WILCE 1975) and *Cutleria cylindrica* (LA CLAIRE 1982). As compared with interphase, the number of microtubules increased in prophase. They radiated from the amorphous electron dense material around the centrioles to the layer of electron opaque material along the depression. The structures taking part in microtubule formation are reported as microtubule-organizing centers (MTOC) (PICKETT-HEAPS 1969, 1975), polar rings (MCDONALD 1972, SCOTT *et al.* 1980), and rhizoplast in *Ochromonas* (SLANKIS and GIBBS 1972, BOUCK and BROWN 1973) and *Tetraselmis* (=*Platymonas*) (STEWART *et al.* 1974). In several species of the Dictyotales (Phaeophyta), NEUSHUL and DAHL (1972) called the dark staining material around the centrioles the MTOC. Similar electron dense material is also observed in *Pylaiella* (MARKEY and WILCE 1975), *Cutleria* (LA CLAIRE 1982), *Sphacelaria* (KATSAROS *et al.* 1983), and *Carpomitra*. In these algae, microtubules radiated from this material, suggesting that it functions as the MTOC.

In the present observation, many ribosomes and several small vesicles are characteristically distributed in the polar depression of the prophase nucleus, although their function is not clear. With deepening of the polar depression, the number of microtubules which radiate from the electron dense material around the centrioles into the depression increases. As mentioned by LA CLAIRE (1982), it is possible that these microtubules affect the formation of the nuclear depression and the creation of the polar gap, and eventually function as the spindle fibers.

In Phaeophyta, two different types of nuclear envelope, especially in metaphase nuclei, have been reported. The first is the intact type of nuclear envelope with the polar gaps, and the other is the disperse type of nuclear envelope. The former type has been observed in *Pylaiella littoralis* plurilocular gametangium (MARKEY and WILCE 1975), *Sphacelaria tribuloides* apical cell (KATSAROS *et al.* 1983), *Zonaria farlowii*, *Dictyopteris zonarioides*, *Padina pavonia*, *Dictyota dichotoma* apical cells (NEUSHUL and DAHL 1972), *Cutleria cylindrica* trichothallic meristem (LA CLAIRE 1982), *Laminaria angustata* male gametophyte (MOTOMURA and SAKAI 1984), *Fucus* antheridium (LEEDALE 1970, BERKALOFF and ROUSSEAU 1979), and *Hormosira banksii* embryo (FORBES and HALLAM 1979). The latter type has been observed in *Cutleria hancockii* male gametangium (LA CLAIRE and WEST 1979) and *Fucus vesiculosus* embryo (BRAWLEY *et al.* 1977). In the present study, *Carpomitra cabrerae* shows the intact type of nuclear envelope in the metaphase nuclei but this disintegrates in anaphase. The behavior of the nuclear envleope in mitosis is one of the important aspects for considering phylogenetic relationships in Chlorophyta. In the case of *Fucus*, *Cutleria* and *Chara* (PICKETT-HEAPS 1967, 1968), however, the behavior of nuclear division may differ in different tissues, generations and developmental stages. ALDRICH (1969) suggested that there were two different types of nuclear division in the life cycle of *Physarum*, a genus of Myxomycetes.

However, as mentioned by LA CLAIRE (1982), in the present situation, any statements on the phylogenetic implications can not be made for Phaeophyta.

KATSAROS *et al.* (1983) are the only workers to report kinetochores in Phaeophyta (*Sphacelaria* apical cells), but other investigators have not confirmed it. In the present experiment, kinetochores could not be detected, but many spindle fibers existed near the chromosomes and some of them passed through the chromosomes.

In the ultrastructural investigations of mitosis of Phaeophyta, the stages of anaphase and early telophase have rarely been observed, because the periods of these stages progresses rapidly. MARKEY and WILCE (1975) reported that microtubles were not seen in the spindle region between the separated daughter chromosomes in *Pylaiella*. In the present study, however, the interzonal spindle fibers situated between the groups of daughter chromosomes could be detected as with the case of *Cutleria* (LA CLAIRE 1982). In early telophase, when the nuclear envelope was completely regenerated, they could not be detected. In these stages, ER developed gradually. As shown in Figs 17, 19, ER existed near the new nuclear envelope, therefore it seems to the present writers that involvement of the ER is implicated in the regeneration of the nuclear envelope.

Acknowledgements

We wish to express our thanks to anonymous reviewers for providing valuable criticisms, and Dr. P.M. BRADLEY of Northeastern University for his critical reading the manuscript.

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本村泰三・阪井與志雄：イチメガサ *Carpomitra cabrerae*（褐藻・ケヤリモ目）胞子体の核分裂の電顕的研究

イチメガサ胞子体の頂毛細胞の核分裂を電顕的に観察した。中間期の核はほぼ球形で，その周囲に1組の中心子が存在している。前期に中心子は複製し分裂極に移動する。核は極のところでくぼみ，多数の微小管が中心子のまわりの高電子密度物質から核のくぼみに伸びている。核小体は消失し始め，染色糸の凝縮が進む。中期には染色体が赤道面に並ぶが，動原体は観察されない。核膜は両極部分のみが開放し，中心子のまわりの高電子密度物質より紡錘糸が伸びる。後期に，核膜は徐々に破れる。両極間の距離は中期より増し染色体は両極へと移動する。二つの娘染色体塊の間には中間紡錘糸が観察される。この時期には分裂軸に沿って核領域の近くによく発達した小胞体が観察される。終期には二つの娘核は核膜で包まれ，核小体の再生・染色体の分散とともに核の体積が増す。 (051 室蘭市母恋南町 1-13 北海道大学理学部付属海藻研究施設)

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p. 225～232 の論文の和文要約.

L. ディック・R.E. ドゥブリード・D. ガーバリ：ブリティシコロンビアとカリフォルニアにおける紅藻 *Iridaea cordata*（スギノリ科）の配偶体と四分胞子体の出現と生活史

Iridaea cordata の配偶体と四分胞子体の出現状況を地理的分布を異にする個体群と波浪条件等を異にする個体群について調査した。両世代の藻体の識別は，藻体が含むカラゲーナンのタイプを知るために Craigie と Leigh (1978) が開発した resorcinol test を著者等が改変した方法によった。カナダ・ヴァンクーヴァー島とヴァンクーヴァー港の個体群では約60%が配偶体であったが，波の荒い地点から静かな地点にかけて配偶体数比が減少する傾向を示した。アメリカ・オレゴン州からカリフォルニア州中部にかけての太平洋沿岸12点の個体群では，北部では配偶体が約11%で少かったが，南部では78～90%と増加した。しかし，この出現比は地域により，また年により例外も多く見られた。両世代の出現比の違いがどのようにして生ずるかを知るには，多くの地点において出現状況を永年に亘って調査することが必要である。