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**Ultrastructure of Mitosis and Cytokinesis during Gametic
Differentiation in the Siphonous Green Alga
Pseudobryopsis hainanensis Tseng***

By
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Introduction

Ultrastructural details of the structural development of mitosis-cytokinesis and the architecture of the flagellar apparatus of motile reproductive cells play a considerable role in our understanding of the classification and phylogeny of the green algae. The knowledge has recently led to the recognition of four main evolutionary lines, the Charophyceae, the Ulvophyceae, the Pleurastrorphyceae, and the Chlorophyceae (MATTOX and STEWART 1984). At present, therefore, it appears that siphonous green algae should be classified in the Ulvophyceae by two characters (O'KELLY and FLOYD 1984): the flagellar apparatus with counterclockwise absolute orientation in *Bryopsis* (HORI 1977, MELKONIAN 1981), *Derbesia* (ROBERTS *et al.* 1981) and *Pseudobryopsis* (ROBERTS *et al.* 1982) and the mitotic and cytokinetic feature in *Bryopsis* (BURR and WEST 1970) and *Caulerpa* (HORI 1981). The latter is characterized by the appearance of a closed spindle and of a simple plasmamembrane furrow at cytokinesis associated with neither phycoplast nor phragmoplast microtubules. However, there is an optional case in the definition of the Ulvophyceae. Cytoplasmic microtubules may lead the centripetal ingrowth of wall septum in cellular (septated) ulvophycean algae (HUDSON and WAALAND 1974, MCDONALD and PICKETT-HEAPS 1976, LOKHORST and STAR 1983, LOKHORST 1986, SEGAAR and LOKHORST 1987). LOKHORST and STAR (1983) have proposed, in spite of conflict with the taxonomic status of the Ulvophyceae, that these cytoplasmic microtubules are the cytokinetic apparatus identical with the phycoplast defined by PICKETT-HEAPS (1972a).

Pseudobryopsis hainanensis TSENG, occurring in tropical and subtropical sea, is a siphonous green alga. The life history has been found to be a heteromorphic biphasic type by culture studies (KOBARA and CHIHARA 1978, OKUDA *et al.* 1979). Sexual reproduction is

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anisogamous. Gametes are produced in a macrothallic pinnate phase (a gametophyte). Microthallic filamentous phases derived from zygotes produce stephanokontan zoospores, which develop into macrothallic pinnate phases. The genera characterized by a heteromorphic life history and a stephanokontan zoospore, *Derbesia*, *Bryopsidella*, *Bryopsis* and also *Pseudobryopsis*, have a close relationship than to other genera in the order Caulerpales (VAN DEN HOEK 1981). However, *Pseudobryopsis* produces differentiated gametangia different from those in other related genera mentioned above. In *P. hainanensis* female and male gametangia are born on different pinnate branches (ramelli) in a monoecious gametophyte (OKUDA *et al.* 1979).

In *Pseudobryopsis hainanensis* the certain culture conditions in which the development of gametangia can be synchronously induced have been established by OKUDA and TATEWAKI (1982). To be brief, a sterile gametophytic plant is transferred into an inducible culture condition, which we called an induction treatment, not later than 12 hr after the onset of a light period. Subsequently, gametangia synchronously begin to develop on many ramelli and discharge gametes, at 18-21 hr and at 48-51 hr after the time of light-on just before an induction treatment, respectively. This indicates that *P. hainanensis* is an excellent organism to study the mechanism of gametic differentiation since we can know how and when cytological and physiological events proceed. It has already demonstrated that an endogenous circadian rhythm controls the timing of gametangium formation in this species (OKUDA and TATEWAKI 1982).

Using the synchronous culture system above mentioned, the developmental process of the gametangium in *Pseudobryopsis hainanensis* was previously examined by light and electron microscopy (OKUDA *et al.* 1987). The gametangia bud on ramelli and expand for 12 hrs to be a ovoid shape, introducing a part of the cytoplasm of the ramelli. Wall material is deposited inside the orifice between the gametangia and the ramelli and finally a plug is formed in the pore isolating the cytoplasm of the gametangia from that of the ramelli. A papillary outgrowth of wall is formed at the distal end of the gametangia and weakened so that the tip wall may disintegrate to be a liberation pore at gamete discharge. Thus, when the gametangia completely develop, a large vacuole occupies the basal portion of the gametangia and nuclei begin to divide in the cytoplasm.

The present study shows the ultrastructure of mitosis and cytokinesis during gametogenesis in *Pseudobryopsis hainanensis* in detail, confirming gametic differentiation process by using a synchronized culture system.

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Materials and Methods

Gametophytic plants of *Pseudobryopsis hainanensis* (clone MK-065) were used in the present study. This strain was collected at Ayamaru Point, Amami Oshima, Japan on June 2, 1977 and then maintained as the unialgal stock cultures grown in artificial medium ASP₁₂ (PROVASOLI 1963) at 22°C and a 14:10 hr L:D photoregime.

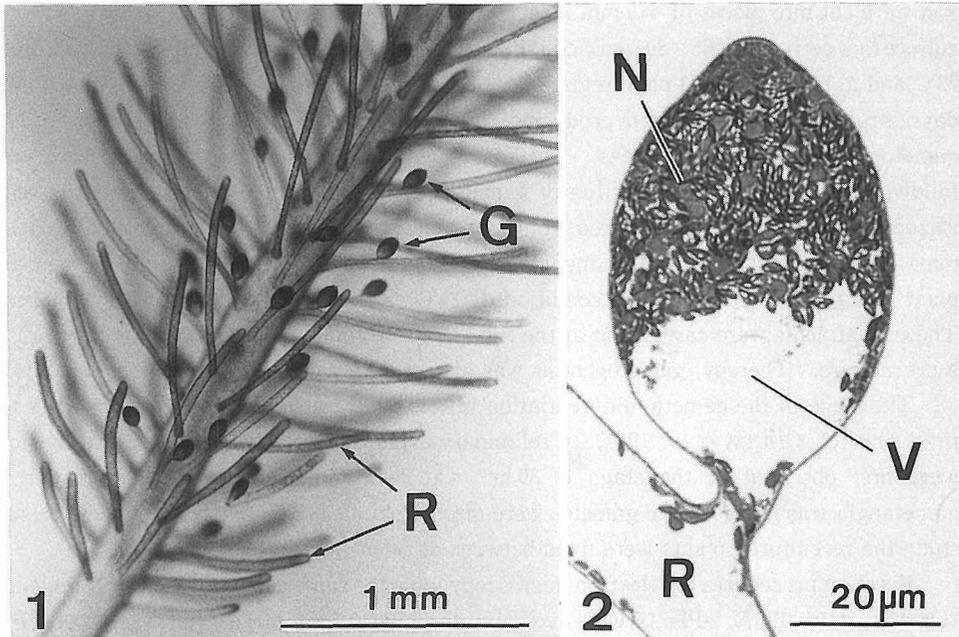
The methods of synchronized culture and treatments for inducing gametogenesis were essentially the same as those described previously (OKUDA and TATEWAKI 1982, OKUDA *et al.* 1987). Gametophytic plants without any ramelli were isolated from the stock cultures and were cut into pieces of 4-5 mm long. Fifteen of their fragments were grown as a pre-culture in a petri dish (65×80 mm) containing 150 ml ASP₁₂ medium under the condition of 22°C and a 14:10 hr L:D photoregime (ca. 3,000 lux). They erected themselves within 3 days after planting and began producing ramelli distally to develop into sterile, mature gametophytes for about 3 weeks. The plants grown in this manner were treated with following methods for the induction of gametogenesis: the plant removed from the basal rhizoids by scissors was transferred from a pre-culture petri dish into a test tube (18×135 mm) containing 10 ml of ASP₁₂ modified by OKUDA and TATEWAKI (1982). Then, the plant was re-cultured under the condition of 24°C and a continuous light (ca. 6,000 lux). These treatments were carried out at the time of the beginning of a light period (=0 hr) in a pre-culture. Thereby, gametogenesis was almost surely induced in re-cultured plants.

The stage of the gametic differentiation in this study was exhibited by hours after the time 0 hr (cf. OKUDA *et al.* 1987). Preliminary experiments showed that nuclear divisions were first observed at the stage of 30 hr (=at 30 hr) when the expansion growth of gametangia was finished, and gametes were discharged at 48 hr. Therefore, in the present study the re-cultured plants were fixed between at 30 hr and at 48 hr.

Fixation procedures for electron microscopy were the same as those described previously (OKUDA *et al.* 1987). Plants were fixed by dropping 0.5 ml of 10% glutaraldehyde into their growing pre-culture medium (final concentration is 0.5%). After 5 min. they were placed into 5% glutaraldehyde fixative containing 0.1 M sodium cacodylate pH 7.2 and 50% amount of major salt elements in ASP₁₂ medium for 25 min. at room temperature and

subsequently for 1.5 hr at 5°C. Following this, plants were post-fixed in 2% OsO₄ dissolved in 0.05 M sodium cacodylate pH 7.2 for 2 hr at 5°C after rinsing with 50% major salt solution several times. The ramelli bearing gametangia were cut off at the base with dissection scissors after briefly rinsing with a cold pure water. These ramelli were dehydrated slowly with an acetone series by 10% increments and finally embedded in Spurr resin. Thin sections were made with a diamond knife on a Sorvall Porter-Blum MT-1, mounted on formvar-coated slot mesh grids, and stained with 1% uranyl acetate solution in 50% ethanol for 5 min. followed by lead citrate (REYNOLDS 1963). Each section was observed by either a Hitachi H-300 or a JEOL JEM 100 U electron microscope.

Plants were fixed at seven stages, 30, 33, 36, 39, 42, 45, and 48 hr, and at least twenty gametangia at each stage were observed because the ultrastructural features of gametic differentiation appeared to change serially at each stage and not to be missed at intervals of three hours. In fact, the ultrastructure of gametangia at each stage was sometimes similar to that at the next stage, indicating that the process of gametic differentiation was thoroughly taken up.



Figs. 1-2 *Pseudobryopsis hainanensis*. Fig.1. Mature gametophyte producing gametangia (G) on lateral ramelli (R) at 27 hr. Fig.2. Longitudinal section of gametangium at 30 hr which is stained with toluidine blue. N, nucleus; V, vacuole; R, ramellus.

Results

Development of gametangium: After transferred into the condition inducing gametogenesis, the plant began producing gametangia on many ramelli at 18 hr, corresponding to the result reported by OKUDA *et al.* (1987). These gametangia developed synchronously (Fig. 1) and ceased their expansion growth at 30 hr. At this stage the cytoplasmic content assembled toward the distal side of the gametangium and a large vacuole was formed (Fig. 2). A pore between the gametangium and ramellus was plugged up with wall material by 39 hr.

Sexuality of gametangium: It was impossible to identify the sexuality of each gametangium till the developmental stage advanced at 33 or 36 hr. After 33-36 hr the male gametangia could be distinguished from the females. This was a result of the cytoplasmic content of the male gametangia changing from dark green to yellowish green in color under light microscopy (cf. OKUDA *et al.* 1979). However, the cytoplasmic content in females changed from dark green to brownish green after 42 hr due to the production of eye spots.

Reduction in nuclear size: The nuclear divisions began at 30 hr and finished by 39 hr in female gametangia and by 42 hr in male gametangia. Since the divisions successively occurred, the nuclear size considerably decreased.

According to PUISEUX-DAO (1966) who observed the zoosporogenesis of *Valonia*, reproductive mitosis occurs in such more rapid succession than vegetative mitosis that nuclei may not regain their normal dimensions. Similar decrease in nuclear size has been observed in gametogenesis of *Bryopsis* (RIETEMA 1975). If reduction in nuclear size in the gametogenesis of *Pseudobryopsis hainanensis* is brought without regaining in nuclear dimension, it may be assumed how many times nuclei divide because, as described later, the nuclear envelope is completely intact throughout mitosis.

Therefore, the volume of a mother nucleus was considered to be equal to the total volume of the daughter nuclei and to be distributed equally to daughter nuclei. Thus, the diameter of divided nuclei was approximately exhibited by the expression when all nuclei were regarded as a sphere:

$$R_n = R(0.794)^n,$$

where R_n , diameter of nuclei after divided n -times; R , diameter of original nuclei; n , frequency of nuclear divisions.

The diameter of the interphase nuclei at 30 hr which were sectioned about along the center was measured and an average value $3.0 \mu\text{m}$ was obtained. When the value $3.0 \mu\text{m}$ as the diameter of original nuclei was substituted for R in the equation, R_n could be calculated: $n=1$, $R_1=2.4 \mu\text{m}$; $n=2$, $R_2=1.9 \mu\text{m}$; $n=3$, $R_3=1.5 \mu\text{m}$; $n=4$, $R_4=1.2 \mu\text{m}$. The diameters of the nuclei in female and male gametangia at 42 hr when mitosis was finished, averaged $1.5 \mu\text{m}$ and $1.1 \mu\text{m}$, respectively. These measurements were very similar to the values of R_3 and R_4 . From this viewpoint, the nuclei were considered to divide successively

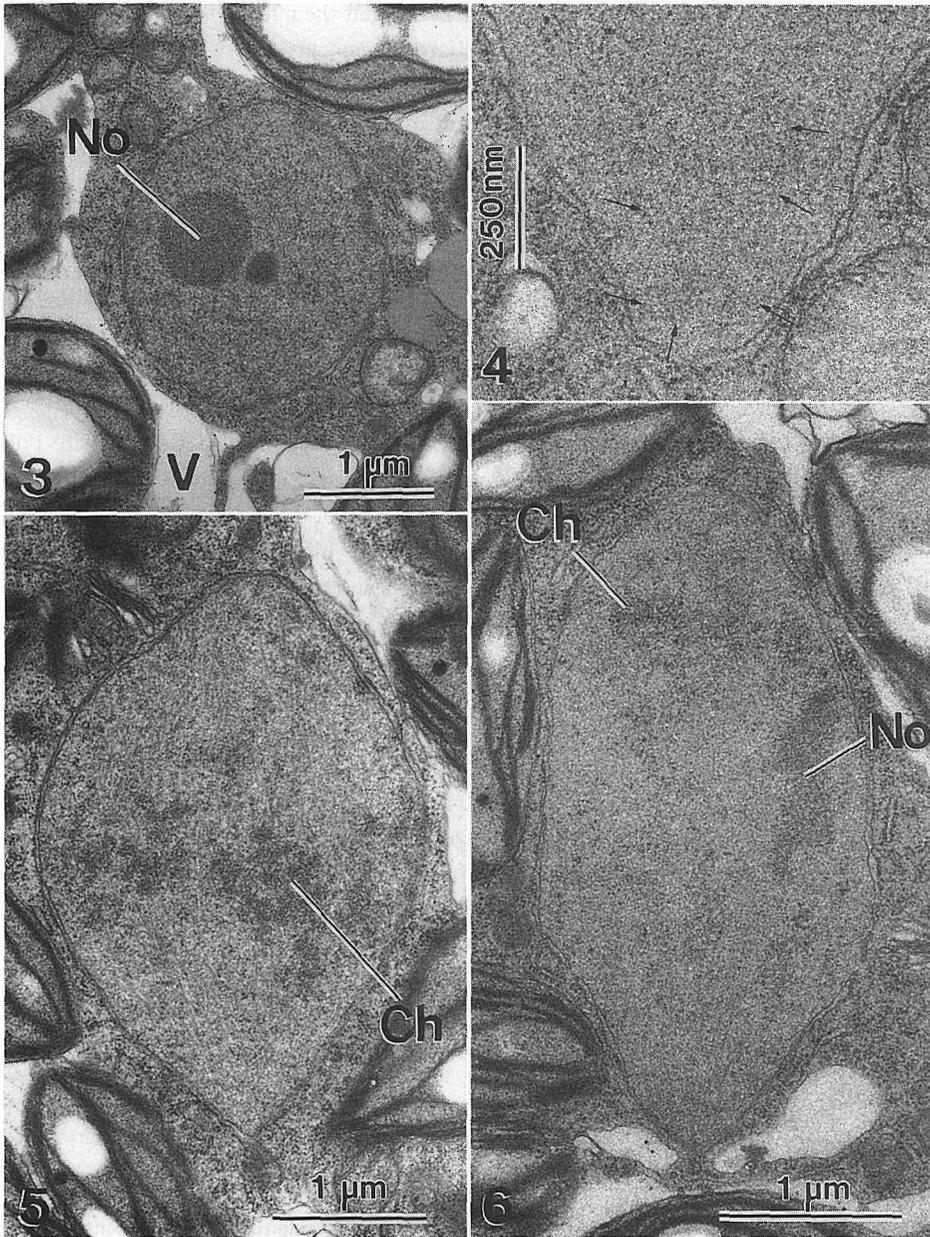
at least three and four times to produce female and male gametes, respectively.

Mitosis: Until the successive series of nuclear division finished, three types of mitoses different in the ultrastructural features were recognized. The first type appeared at 30-30 hr, the second type at 33-36 hr and the third type at 36-39 hr. Thus, the ultrastructural features of mitosis depended upon the stage of gametic differentiation.

1. Mitosis at 30-33 hr: It was noteworthy that the mitosis was acentric in this stage because centrioles appeared in the next stage. The interphase nuclei attained to the largest size (about $3\ \mu\text{m}$) in all stages. As the euchromatin homogeneously dispersed, a conspicuous nucleolus lay in about the central region of the nucleus (Fig. 3). The nucleoplasm of prophase nuclei became slightly less electron dense than that of interphase nuclei. At prometaphase, though the nuclear envelope was protruded in the polar regions and microtubules emerged, ill-defined chromatin masses scattered in the nucleus. Some microtubules, however, converged at the poles and fine granules were localized in the protruded nuclear envelope (Fig. 4). At metaphase, microtubules considerably proliferated and most of them extended from the poles to the chromosomes (Fig. 5). The terminals of bundled microtubules were attached to the chromosomal surface, but any differentiated kinetochore was not detected. A nucleolus migrated close to the equatorial region of the nuclear envelope during prometaphase and metaphase.

At early anaphase, the contour of the nucleus became elliptical and chromosomes separated (Fig. 6). During anaphase, the nucleus conspicuously elongated toward the opposite poles (Fig. 7). The electron density of chromosomes prominently reduced again at this period. Each of divided nucleolar masses (arrows in Fig. 7) also moved towards the opposite poles, slightly behind the separation of daughter chromosomes. Serial sections of the elongated anaphase nucleus showed that most of the continuous, pole-to-pole microtubules extended along the inner surface of the nuclear envelope (cf. Fig. 8). At telophase, the interzonal spindle that was filled with many long microtubules (Fig. 10) reached the maximum length as long as $10\ \mu\text{m}$ (Fig. 9). Thus, the interzonal spindle became a very narrow cylinder, resulting in squeezing out the nucleoplasm left in the interzonal spindle into both daughter nuclei. The interzonal spindle microtubules terminated in the entrance of the daughter nuclei where fine granules were aggregated (Fig. 11). In the daughter nuclei the chromatin had already been dispersed throughout and the nucleolus recovered its interphase profile (Fig. 9). The daughter nuclei were separated from the interzonal spindle by abscission.

At telophase some small bundles of microtubules were often projected into cytoplasm at a short distance (arrow in Fig. 9), but the nucleoplasm was never confluent with the cytoplasm, though the nuclear envelope was broken at the terminal of the projecting microtubules. This was assumed that these microtubules failed to incorporate with the interzonal spindle microtubules when the daughter nuclei were formed. Thus, the nucleus remained completely closed during the mitosis at this stage.



Figs. 3-6 Nuclear division at 30-33 hr. Fig. 3. Interphase nucleus with nucleolus (No). V, vacuole. Fig. 4. Showing polar region of prometaphase nucleus. Note the assemblages of fine granules (arrows). Fig. 5. Metaphase nucleus. Ch, chromosome. Fig. 6. Early anaphase nucleus. Ch, chromosome; No, nucleolus.

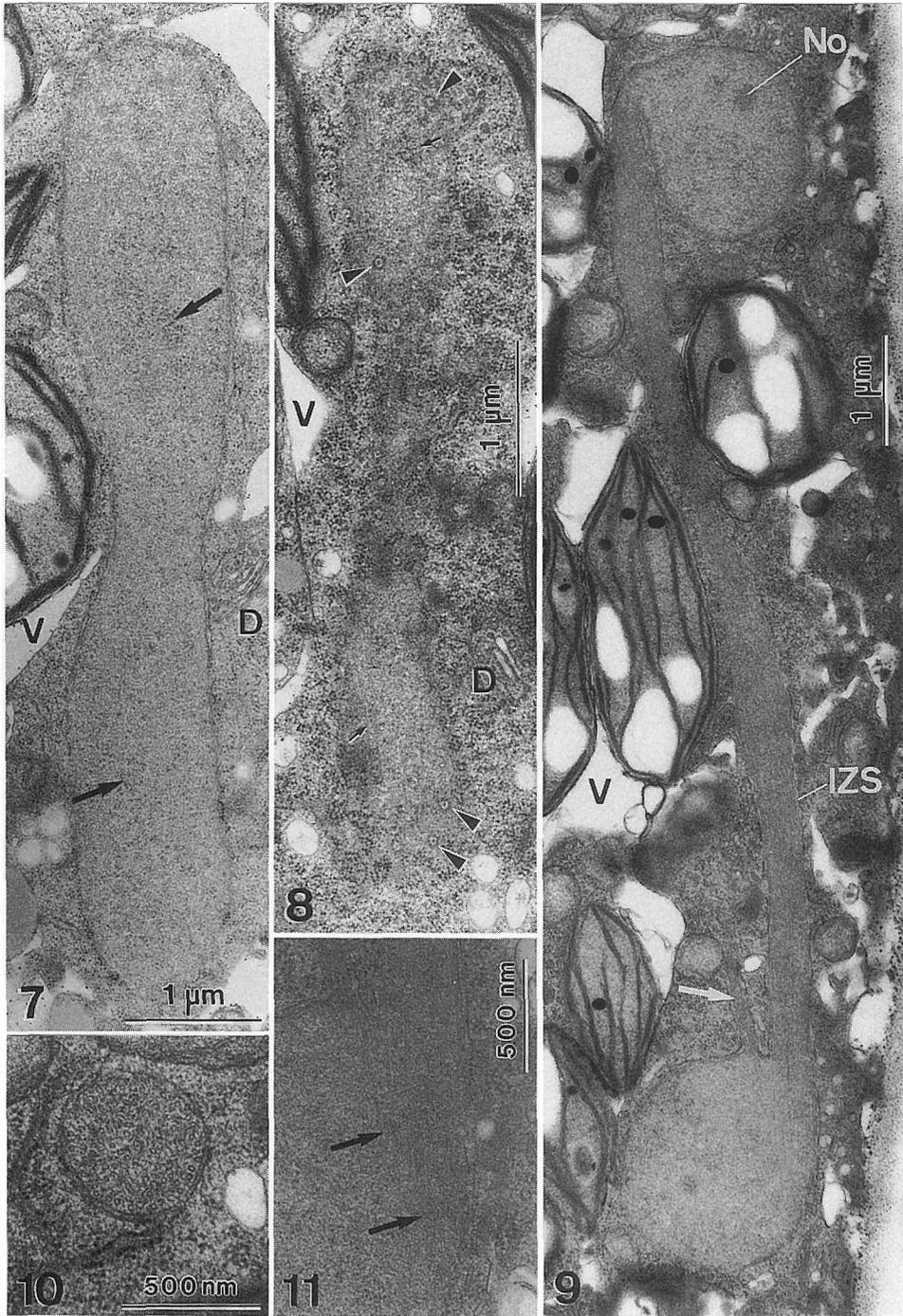
2. Mitosis at 33-36 hr : In this stage it was noted that the mitotic spindles were centric and cytoplasmic microtubules considerably proliferated, concurrently with the emergence of centrioles. Therefore, the centrioles were produced *de novo* around the interphase nuclei at the beginning of this stage since they were absent in the previous stage.

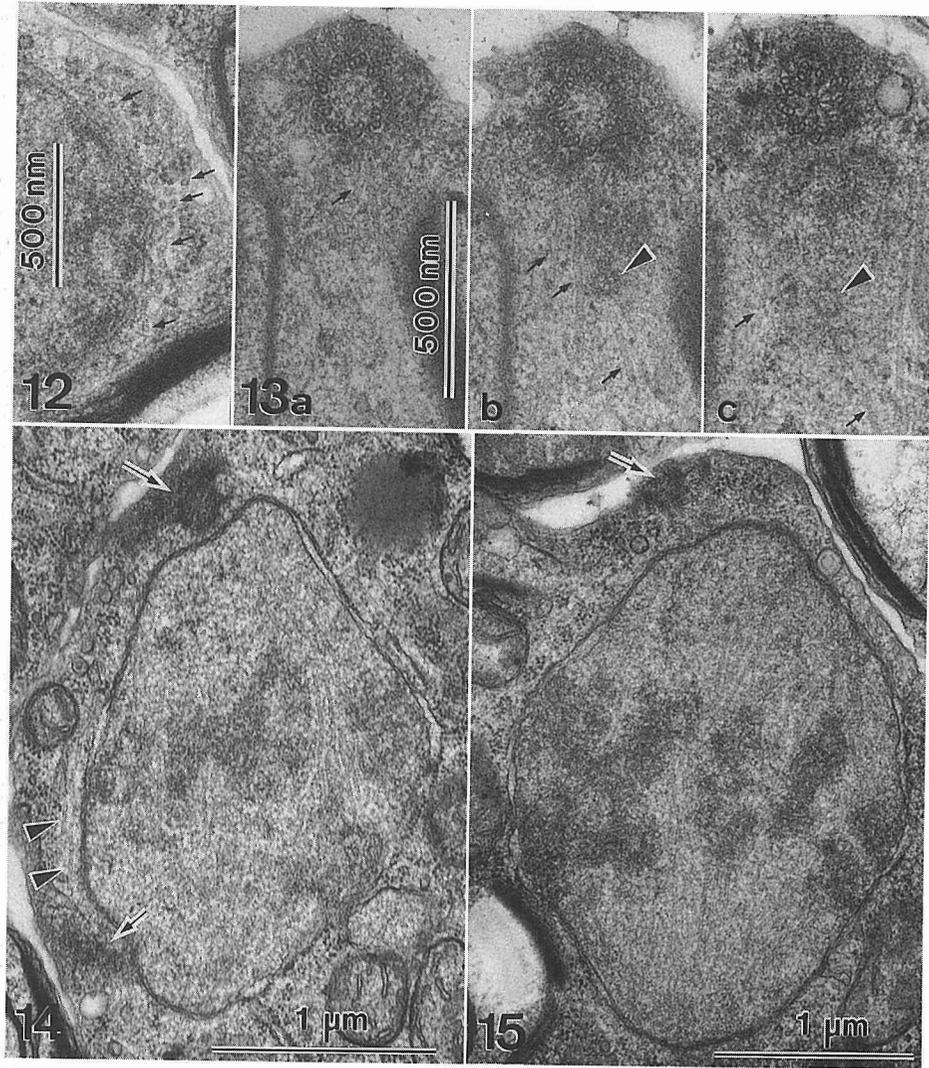
In the interphase nucleus with a pair of centrioles, the euchromatin was usually localized near the nuclear envelope, especially in the nuclei of male gametangia. A single nucleolus stood out in the nuclei. The nuclear envelope was distinctively depressed against the centrioles. It was evident that extranuclear microtubules lay adjacent to the nuclear envelope (Figs. 12, 13). Serial section analysis showed that about forty microtubules entirely ensheathed the nucleus with approximately parallel arrangement and extended from the vicinity of the centrioles to the opposite area of the nucleus (Fig. 35a, b). These microtubules seemed to constitute an extranuclear skeleton (see later section).

At prophase, the centrioles duplicated and each pair separated to the opposite pole. The chromatin was stained more densely than that in the acentric division and the nucleolus possibly dispersed. At prometaphase, the polar protrusion of the nuclear envelope occurred, but the centrioles situated laterally to the protrusions (Fig. 14). The position of both centriole pairs was biased to one half of the lateral side of the nucleus while the nuclear envelope in the other half side was often bulged. Although the spindle microtubules proliferated in the nucleus, no polar fenestrae were found in this alga unlike a closely related genus, *Bryopsis* (BURR and WEST 1970). At metaphase, the chromosomes lined up at the equator and many microtubules stretched between the chromosomes and pole (Fig. 15). The polar ends of the microtubules terminated in amorphous material in the nucleus. As in the previous stage, no evident kinetochore was found on the chromosomes.

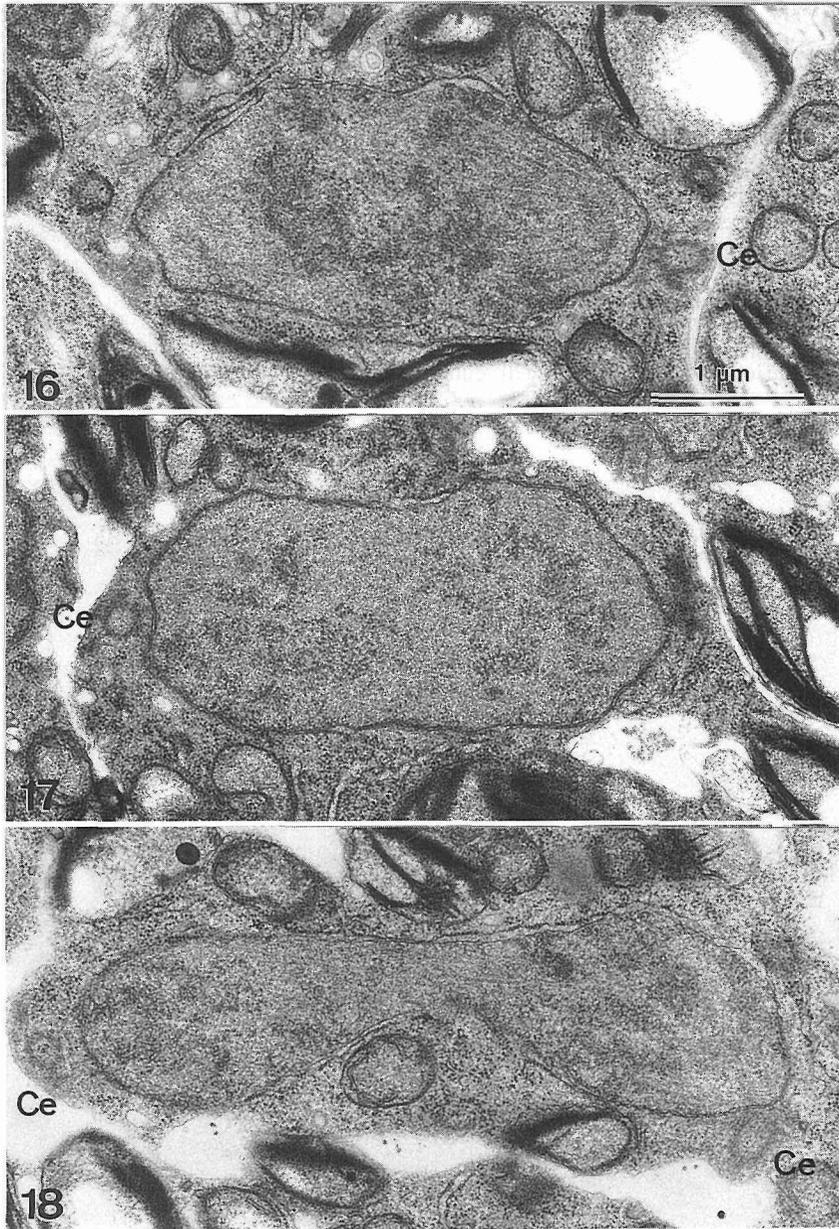
The separation of chromosomes during anaphase took two steps, spindle elongation and subsequent chromosome movement. At early anaphase, the nuclear shape changed into an ellipsoid (Fig. 16). Then, the distance between the chromosomes and pole was the same (ca. 1 μm) as that at metaphase, but the pole-to-pole distance increased from 2.4 μm to 3.5 μm (compare Fig. 15 with Fig. 16). This showed that the earliest separation of the chromosomes was caused by spindle elongation. Successively, the chromosome-to-pole distance

Figs. 7-11 Nuclear division at 30-33 hr. Fig. 7. Median longitudinal section of late anaphase nucleus. Arrows, nucleolar masses. V, vacuole; D, dictyosome. Fig. 8. Grazing longitudinal section of late anaphase nucleus. Showing the continuous microtubules (arrows) adjacent to inner nuclear envelope and nuclear pores (arrowheads). V, vacuole; D, dictyosome. Fig. 9. Telophase nucleus with interzonal spindle (IZS). Showing the 17th section selected from a series of 22 longitudinal sections in which the telophase nucleus is wholly cut. No, nucleolus. Arrow, some microtubules extruded from a daughter nucleus. Fig. 10. Cross section of interzonal spindle. Fig. 11. Enlargement of entrance of daughter nucleus. This is the adjacent section (18th) of the daughter nucleus shown below in Fig. 9. Arrows, the aggregations of fine granules at the terminal of interzonal spindle microtubules.





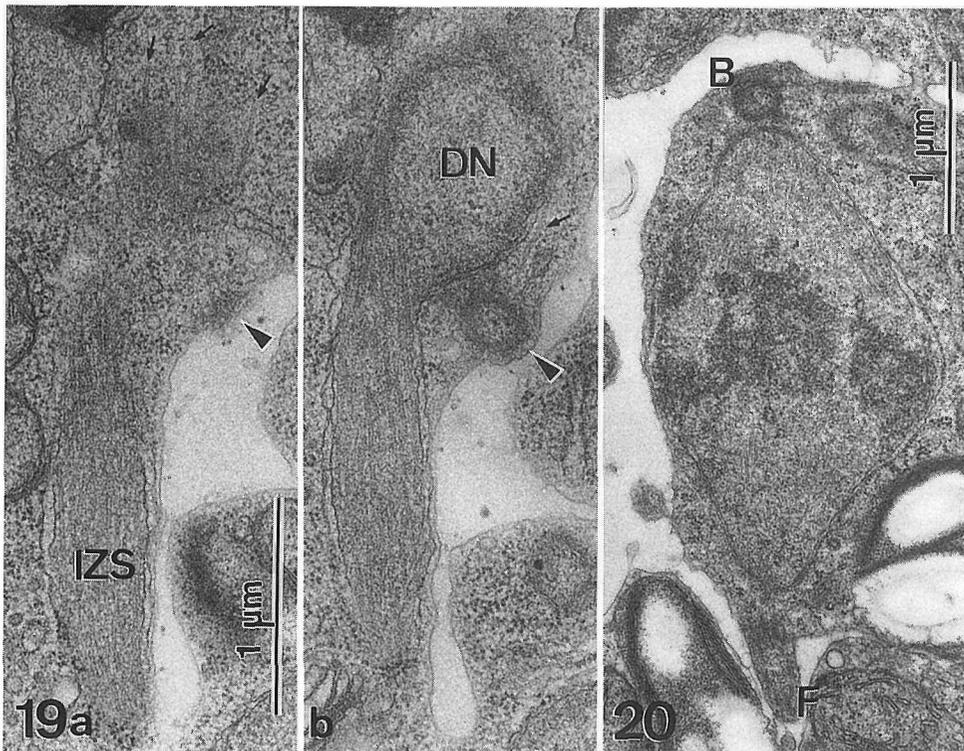
Figs. 12-15 Nucleus associated with centriole at 33-36 hr. Fig. 12. Showing the 8th section in a series of the cross sections of interphase nucleus which are cut perpendicularly to the direction from centrioles to nucleus. The microtubules (arrows) radiating from centrosomes ensheath the nucleus. Fig. 13a-c. Serial cross sections of the centrosomes as viewed from the distal end of the centrioles. The microtubules (arrows) extending from dense material around the centrioles lie adjacent to nuclear envelope. Arrowheads, nuclear pores. Magnification of Fig. 13a-c is the same. Fig. 14. Prometaphase nucleus with centrioles (arrows) at the lateral side of the pole. Arrowheads, the microtubules ensheathing the nucleus. Fig. 15. Metaphase nucleus. Arrow, centriole.



Figs. 16-18 Anaphase nucleus at 33-36 hr. Fig. 16. Early anaphase nucleus in male gametangium. Ce, centriole. Fig. 17. Mid anaphase nucleus in female gametangium. Ce, centriole. Fig. 18. Late anaphase nucleus in male gametangium. Ce, centriole. Scale in Fig. 16 also applies to Figs. 17 and 18.

decreased and the chromosomes moved towards the opposite poles (Fig. 17). The late anaphase spindle was dumb-bell shaped and the interzonal spindle microtubules proliferated (Fig. 18).

At telophase, both ends of the long interzonal spindle were tangentially linked to the daughter nuclei (Fig. 19a, b). The centrioles shifted their position between at anaphase and telophase, *i. e.* they made about a half revolution around the daughter nucleus which now looked like a head nodding (see later section). Extranuclear microtubules radiating from the vicinity of centrioles, however, ensheathed the daughter nuclei. Finally, the daughter nuclei were separated from the interzonal spindle by an acute constriction of the nuclear envelope, keeping intact. Thus, the mitosis at this stage was characterized by not so much nuclear conditions as the extranuclear conditions such as the presence of centrioles and microtubules.



Figs. 19-20 Centric nuclear division. Fig. 19a, b. Serial sections of telophase nucleus in male gametangium at 36 hr. The microtubules (arrows) extending from the centrosomes (arrowheads) ensheath the reforming daughter nucleus (DN). IZS, interzonal spindle. Magnification of Fig. 19a, b is the same. Fig. 20. Metaphase nucleus in male gametangium at 39 hr. B, basal body; F, flagellum.

3. Mitosis at 36-39 hr : The typical mitosis at this stage was observed only in the male gametangium. In the female gametangium the process of successive mitoses finished in a shorter period than in the male. This might show that total number of repeated divisions was larger in the male. Thus, this presumption well harmonized with the result obtained from reduction in nuclear diameter.

The nuclear conditions at this stage were the same as those at 33-36 hr, perfectly intact nuclear envelope ; dispersed nucleolus ; condensed chromatin even at interphase ; persistent interzonal spindle at telophase. However, in extranuclear condition, the extension of flagella from the centrioles was the most noticeable. The mitotic spindles were associated with the flagellar basal bodies (Fig. 20), but it was unclear whether these basal bodies duplicated before or after the extension of flagella.

In the female gametangia, also flagella began to extend at this stage (Figs. 37, 41), indicating the timing of the flagellar extension was programmed in both male and female gametic differentiation. Therefore, it was suspected that the flagellar extension was not essential for the mitosis at this stage and the mitosis only continued even when the flagellar extension commenced.

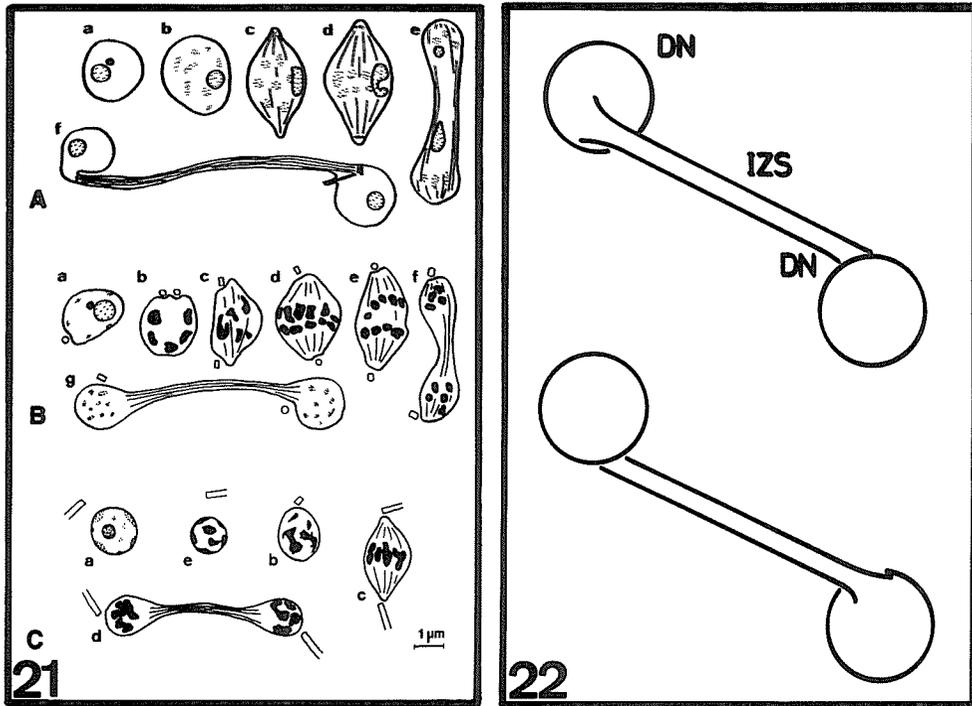
Other features changing from those at 33-36 hr stage were also noticed : The nuclear envelope was not remarkably depressed at the portion of the basal bodies and the microtubules which ensheathed the nuclear envelope were indistinguishable in the nuclei that had finished dividing. This interpretation is discussed later.

It was evident that there were three types of mitoses different in the ultrastructure. However, the truly closed spindle, no evident kinetochore and the presence of a persistent interzonal spindle at telophase were characteristics common to all the types. Figure 21 schematically represented the mitoses during gametogenesis in this species.

The most important characters were the centrioles and their associate extranuclear constituent, microtubules. They emerged at the definite stage of gametic differentiation, *i. e.* at the beginning of cytokinesis (see later section).

Telophase spindle shape : Serial section analysis in telophase spindles showed an interesting fact. The acentric telophase spindle showed in Fig. 9 was wholly cut into serial twenty-two sections and reconstructed three-dimensionally. For the typical model (Fig. 22) two other telophase spindles, reconstructed likewise, were also fit. As shown in Fig. 22, the interzonal spindle tangentially connected with the daughter nuclei, in other words, the telophase spindle bent through 90 degrees at the entrance of the daughter nuclei, so it seemed as if the daughter nuclei nodded. Then, the daughter nuclei were different from each other in the direction of their bending, *i. e.* when regarding the interzonal spindle as the axis of rotation, the direction in which one daughter nucleus bent was rotated through about 180 degrees around the axis.

Transformation occurred between at anaphase and telophase because the anaphase spindles were ellipsoid or dumb-bell in shape (Figs. 6, 7). In order to control this trans-



Figs. 21-22 Diagram of nuclear division. Fig. 21. Diagram of the mitosis during gametogenesis in *Pseudobryopsis hainanensis*. A: Acentric mitosis at 30-33 hr. Showing that the chromosomes (dots) are not electron denser and the profile of nucleolus is visible even during metaphase and anaphase. a, interphase; b, prophase; c, prometaphase; d, metaphase; e, late anaphase; f, telophase. B: Centric mitosis at 33-36 hr. Showing that the chromosomes become electron dense and the centrioles emerge near the nucleus. a, interphase; b, prophase; c, prometaphase; d, metaphase; e, early anaphase; f, late anaphase; g, telophase. C: Centric mitosis at 36-39 hr. Note nuclei with flagellar basal bodies and no division of the female nucleus. a, female gametic nucleus; b, interphase or prophase; c, metaphase; d, telophase; e, male gametic nucleus. Fig. 22. Diagrammatic reconstruction of the telophase nucleus. DN, daughter nucleus; IZS, interzonal spindle.

formation at least three mechanisms might be required: to squeeze nucleoplasm towards both poles and to form an interzonal spindle; to bend daughter nuclei; to determine the direction of bending.

The continuous microtubules at anaphase were assumed to be adequate to the first mechanism. These microtubules could be directly involved with the locomotion of nucleoplasm, or indirectly by narrowing down the interzone of the spindle.

To interpret the second mechanism, the fine granules at which microtubules terminate

were noticed. Although these fine granules were localized at intranuclear poles till early anaphase (cf. Fig. 4), they situated at the lateral sides of daughter nuclei at telophase (Fig. 11). When the continuous microtubules elongated, stress would act at the point of the inner nuclear envelope where the fine granules were localized since the anaphase nucleus might be stretched by the microtubules. At the same time, the nucleoplasm was accumulated around the intranuclear poles and daughter nuclei began forming (Fig. 7). But these daughter nuclei would be put back by some physical counteraction of surrounding cytoplasm because the continuous or interzonal spindle microtubules still elongated. Consequently, the fine granules came to both ends of the interzonal spindle and daughter nuclei bent (Figs. 9, 22).

However, no structure to control the direction of their bending could be found. It might be possible whether part of nuclear envelope was loose or spindles were twisted or wrenched around the axis of spindle elongation when daughter nuclei were formed.

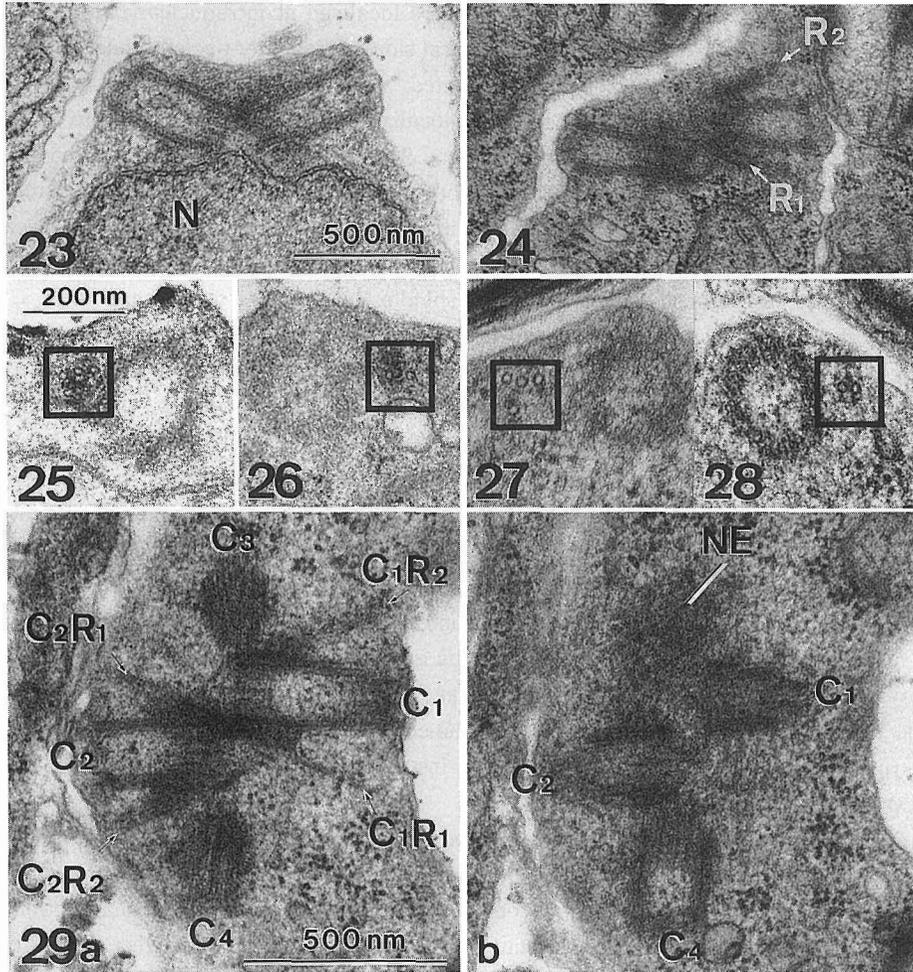
Also in the centric telophase spindle, the daughter nuclei bent (Fig. 19a, b). In this case it drew attention that the location of the centrioles on the nuclear envelope changed between at anaphase and telophase (see above section). If the centrioles at telophase were still attached on the nuclear envelope where they had situated at anaphase pole region, they were translocated by movement of the nuclear envelope. Therefore, by bending of the daughter nuclei the centrioles seemed to be rotated around the daughter nuclei passively, not actively.

It had been pointed out in other coenocytic green algae that the centrioles at telophase moved around daughter nuclei (MARCHANT and PICKETT-HEAPS 1970, SCOTT and BULLOCK 1976). In *Cladophora* translocation of the centrioles during telophase was ascribed to the rotation of daughter nuclei that might be instrumental in interzonal spindle separation (SCOTT and BULLOCK 1976).

Centrioles and rootlet templates: The centrioles were produced *de novo* at the out side of the nucleus during 33-36 hr, but their production process was unclear. Two centrioles lay in the V-shaped angle through about 120 degree in a longitudinal section and their proximal ends fronted toward the nuclear envelope (Fig. 23). However, if one viewed them from the out side of the nucleus, their proximal ends shifted counterclockwisely (Fig. 24).

At interphase, at least one of the centrioles certainly provided two different bundles of short microtubules at its inner (R_1) and outer (R_2) sides, respectively (Fig. 24). The bundles extended from electron dense material associated with the proximal periphery of the centriole. These microtubular bundles corresponded with the rootlet templates in *Oedogonium cardiacum* and *Tetraedron bitridens* observed by PICKETT-HEAPS (1975). Because both structures were the same in that they were covered with electron dense material and could act as some form of template for the extension of the flagellar rootlet microtubules (see below). In *Pseudobryopsis hainanensis* the bundles of the rootlet templates made an acute angle with the centriole and elongated somewhat longer than the centriole.

It was noticed that the number of the rootlet templates were discriminated not only



Figs. 23-29 Centrioles and rootlet templates at 33-36 hr. Fig. 23. Longitudinal section of centrioles associated with interphase nucleus (N). Fig. 24. Oblique section of centrioles. View is from centriole to nucleus direction. The rootlet templates, R_1 and R_2 lie at the inner and outer side of the centriole, respectively. Scale in Fig. 23 also applies to Fig. 24. Figs. 25-28. Cross sections of the rootlet templates (encircled by a square) as viewed from the distal end of the centriole. Figures 25 and 26 show the inner and outer rootlet templates in female, respectively. Figures 27 and 28 show the inner and outer rootlet templates in male, respectively. Scale in Fig. 25 also applies to Figs. 26-28. Fig. 29a, b. Two serial sections showing the duplication of centrioles. View from centriole towards nucleus. The parent centrioles, C_1 and C_2 are associated with the rootlet templates, C_1R_1 , C_1R_2 and C_2R_1 , C_2R_2 , respectively. C_3 and C_4 are daughter centrioles. NE, nuclear envelope. Magnification of Fig. 29a, b is the same.

between at the inner and outer sides of the centriole but between in female and male gametangia. Figures 25-28 showed cross sections of the rootlet templates. The inner templates were four membered, in a three over one configuration, in both female (Fig. 25) and male (Fig. 27) gametangia, but the outer templates were three in female (Fig. 26) while they were two in male (Fig. 28).

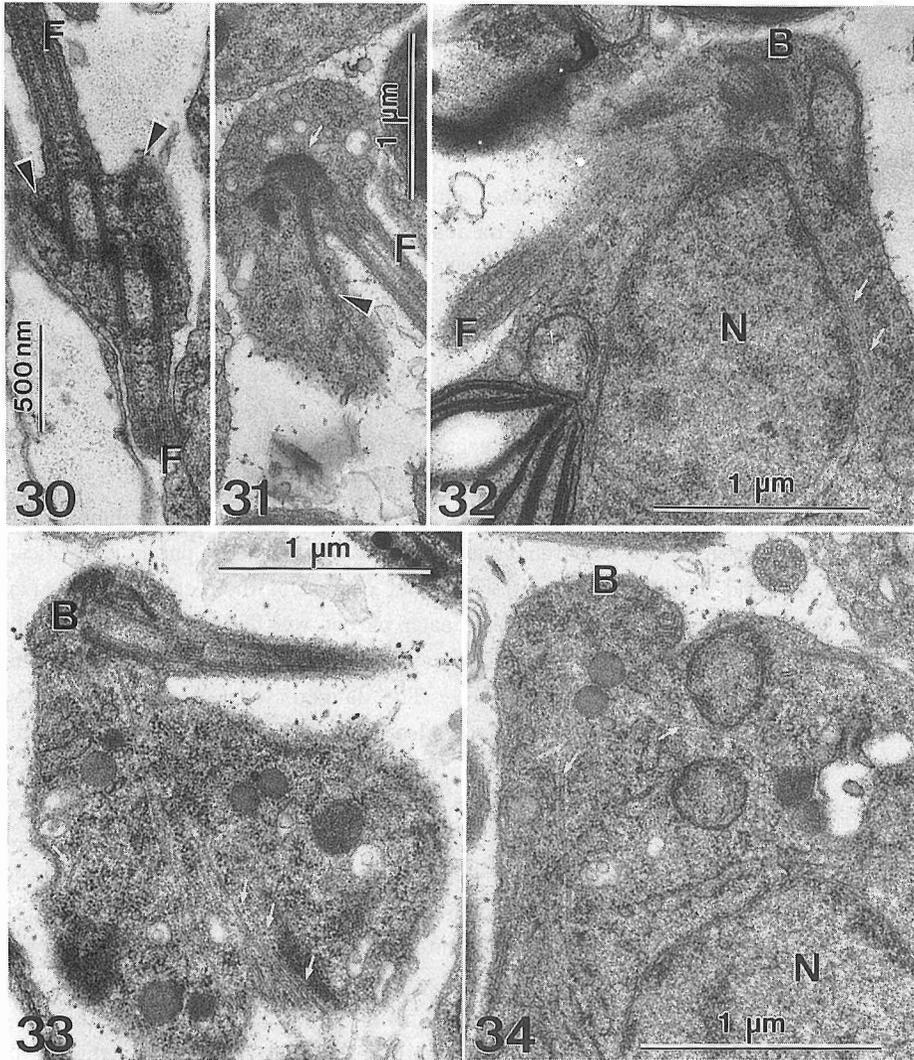
The number of these rootlet templates and their arrangement in differentiation, male and female gametangia were identical to the features of the flagellar rootlet microtubules in male and female gametes observed by ROBERTS *et al.* (1982) who used the same clone that were used in the present study. This was indirect evidence that the rootlet templates were prominent forms to function as microtubule-organizing centers (MTOCs) for the extension of the flagellar rootlet microtubules (PICKETT-HEAPS 1975) and therefore, the number and arrangement of the former determined those of the latter. Thus, it was suggested that the rootlet templates had to emerge with being regulated in their position and number and by this time the gametangia had already differentiated in their sexuality.

A sign of centriole duplication at prophase was the formation of short daughter centrioles at the proximal side of parent centrioles (Fig. 29a, b). The parent centrioles (C_1 and C_2) were found in close juxtaposition without change of their configuration, keeping counterclockwise arrangement. In Fig. 29a, each parent was linked to two sets of the rootlet templates: The C_1 centriole was to the sets of C_1R_1 and C_1R_2 , and likewise the C_2 centriole was to the sets of C_2R_1 and C_2R_2 . The daughter centrioles, C_3 and C_4 were without rootlet templates. The parent centrioles, C_1 and C_2 would be paired with the daughter centrioles, C_3 and C_4 , respectively. Then, at prometaphase these new pairs situated at opposite poles after their separation (Fig. 14). Thus, both centriole pairs were semiconservative.

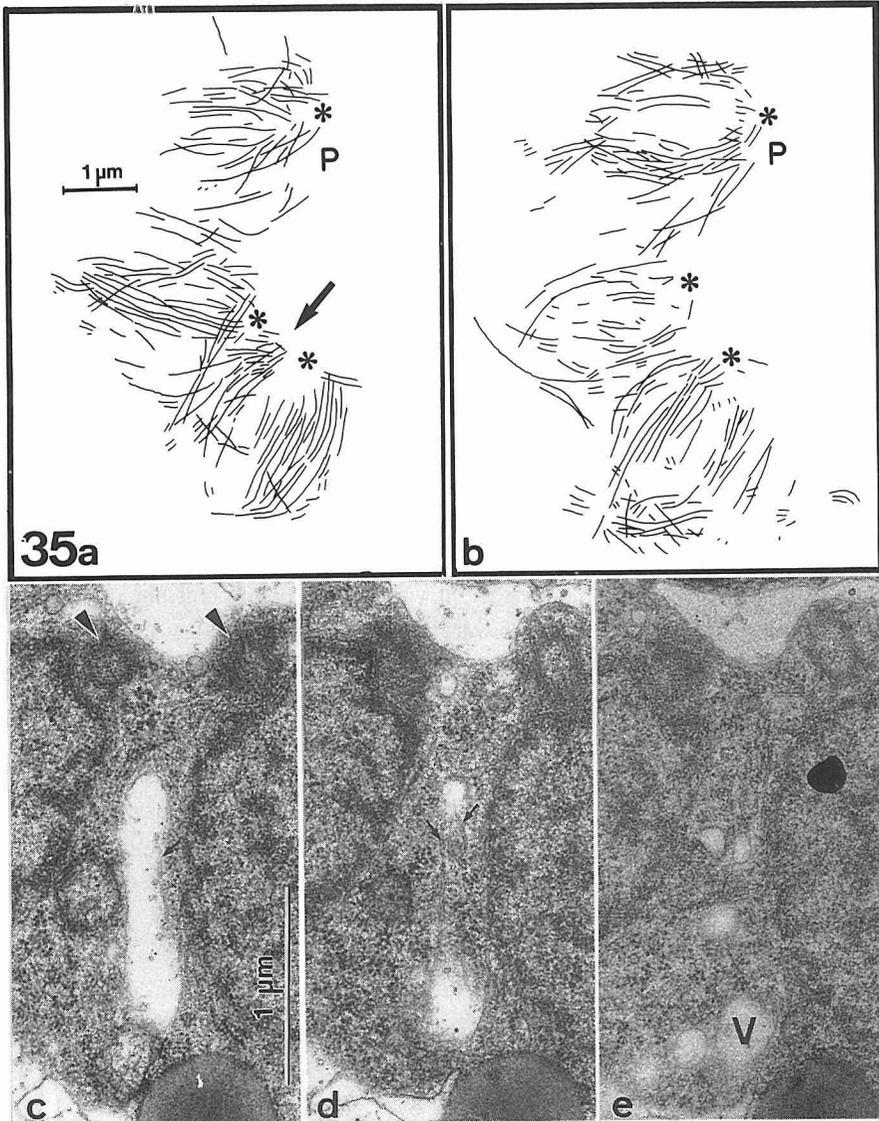
At the proximal side of one centriole (the daughter before) of the pair, rudimentary rootlet templates were generated at metaphase (not shown). They seemed to mature in time for centriole duplication in the next mitosis or the extension of flagellar rootlet microtubules.

The arrangement of paired centrioles changed during 39-42 hr when flagella had extended. Although the proximal ends of the centrioles had faced the nucleus during 33-36 hr (Fig. 23), they were now turned outward from the nucleus and slightly overlapped (Fig. 30). Along with the movement of the centrioles the rootlet templates also turned their direction and extended the flagellar rootlet microtubules from their distal ends (Fig. 31). It seemed to be pertinent that the centrioles were called the basal bodies after their movement. Subsequently during 42-45 hr such electron dense structures linking to the basal bodies as capping plates were formed to construct the flagellar apparatus (Fig. 31). The ultrastructure of the flagellar apparatus of the male and female gametes in this species had been examined by ROBERTS *et al.* (1982).

Microtubules radiating from centrosome : Cytoplasmic microtubules emerged at the



Figs. 30-34 Late stage of gametic differentiation. Fig. 30. Basal bodies at 39-42 hr in male gametangium. View from basal body towards nucleus. Arrowheads, flagellar rootlet microtubules. F, flagellum. Fig. 31. Flagellar apparatus at 45 hr in female gametic unit. Arrow, capping plate. Arrowhead, flagellar rootlet microtubule. Fig. 32. Female gametic unit at 42 hr. The microtubules (arrows) extending from the vicinity of the basal bodies (B) ensheath the nucleus (N). F, flagellum. Fig. 33. Female gametic unit at 45 hr. The microtubules (arrows) extending from the vicinity of the basal body (B) lie at the periphery of the gametic unit. Fig. 34. Female gametic unit at 45 hr. Note the nucleus (N) apart from the basal body (B). Arrows, cytoplasmic microtubules.



Figs. 35a-e Microtubules extending from centrosomes. Figures 35a and 35b show the reconstructions of microtubules of the first 13 and the sequent 14 serial sections, respectively. The lines show the microtubules distributed around three nuclei. Asterisks show the position of the centrosomes. The nucleus situates about in the ellipsoid area made by the lines (the contours of nuclei are not drawn). P, prophase nucleus. Arrow directs the place of Fig. 35c-e. Magnification of Fig. 35a, b is the same. Fig. 35c-e. The 10-12 th serial sections showing the cytoplasm between neighboring interphase nuclei. Arrows, microtubules. Arrowheads, centrosomes. V, vacuole or vesicle. Magnification of Fig. 35c-e is the same.

emergence of the centrioles and apparently radiated from the vicinity of the centrioles not from the centrioles (Fig. 13a-c). Thus, the structures functional in the organization of the microtubules were regarded as the centrosomes, by definition, rather than as the centrioles (cf. VOROBYEV and NADEZHINA 1987).

As mentioned in above section, some of the microtubules extending from the centrosomes ensheathed the nucleus. These microtubules lay tangentially adjacent to the outer surface of the nuclear envelope (Figs. 12, 13). In order to examine the whole arrangement and orientation of the cytoplasmic microtubules, twenty-seven serial sections were provided.

Figure 35a, b showed the reconstruction of the microtubules distributed around interphase and prophase nuclei at 36 hr in the male gametangium. The microtubules which ensheathed the nuclei converged into two foci, the centrosome side and the opposite side of the nucleus against the centrosomes. In this case it was remarkable to be no centrosomes at one of the two foci. In also metaphase, the distribution of the extranuclear microtubules were examined by serial sectioning (not shown). Although the microtubules which ensheathed the metaphase nucleus had two foci the same as in interphase and prophase, the centrosomes existed at both foci. Further, it was important to point out that the spindle axis and these ensheathing microtubules were approximately equivalent in direction though the centrosomes situated laterally to the intranuclear poles. This showed that the spindle microtubules would extend along the same direction as the ensheathing microtubules oriented. The ensheathing microtubules were constructed before the proliferation of the spindle microtubules and never intruded into the nucleus nor incorporated with the spindle microtubules. Therefore, the ensheathing microtubules were considered to form some extranuclear skeleton independent of the spindle microtubules forming the intranuclear skeleton. Two microtubular foci of the extranuclear skeleton at interphase were conceived to predetermine the position of future spindle poles.

After the duplication of centrioles, each centriole pair situated at the opposite pole at prometaphase (Fig. 14). On the basis of above conception, only one centriole pair migrated toward the other pole that had been predetermined by the extranuclear skeleton. Thus, the ensheathing microtubules which formed the extranuclear skeleton were considered to act as a centriole guiding system.

As mentioned above section, between at anaphase and telophase the centrosomes were rotated around the daughter nucleus by the deformation of the spindle. Then, the microtubules radiating from the centrosomes became to ensheath the daughter nuclei (Fig. 19).

The ensheathing microtubules extended from the vicinity of the basal bodies during 39-45 hr (Fig. 32). But, they disappeared during 45-48 hr (Fig. 34). The disappearance of the microtubules made the nucleus go away from the basal bodies concurrently with the disappearance of the depression of the nuclear envelope near the basal bodies (Fig. 34). This suggested that the ensheathing microtubules were instrumental in attaching the

centrosomes or basal bodies to the nucleus and such attachment was so tight that the nuclear envelope caved near the centrosomes or basal bodies. It was interesting to image that the ensheathing microtubules were reminiscent of the nucleus-associated system II flagellar root in *Batophora* (ROBERTS *et al.* 1984).

Figures 35a and b provided the evidence that the second microtubular system different in distribution was present. The microtubules, which were fewer than the ensheathing microtubules, extended away from the nuclei. Vacuoles or vesicles whose origin was unknown were embedded in the cytoplasm between neighboring nuclei (Fig. 35c-e). The microtubules often lay adjacent to the vacuoles and just under the plasmamembrane ingrowing around the centrosomes (Figs. 35c-e, 40a-c), suggesting their involvement in cytokinesis (see next section).

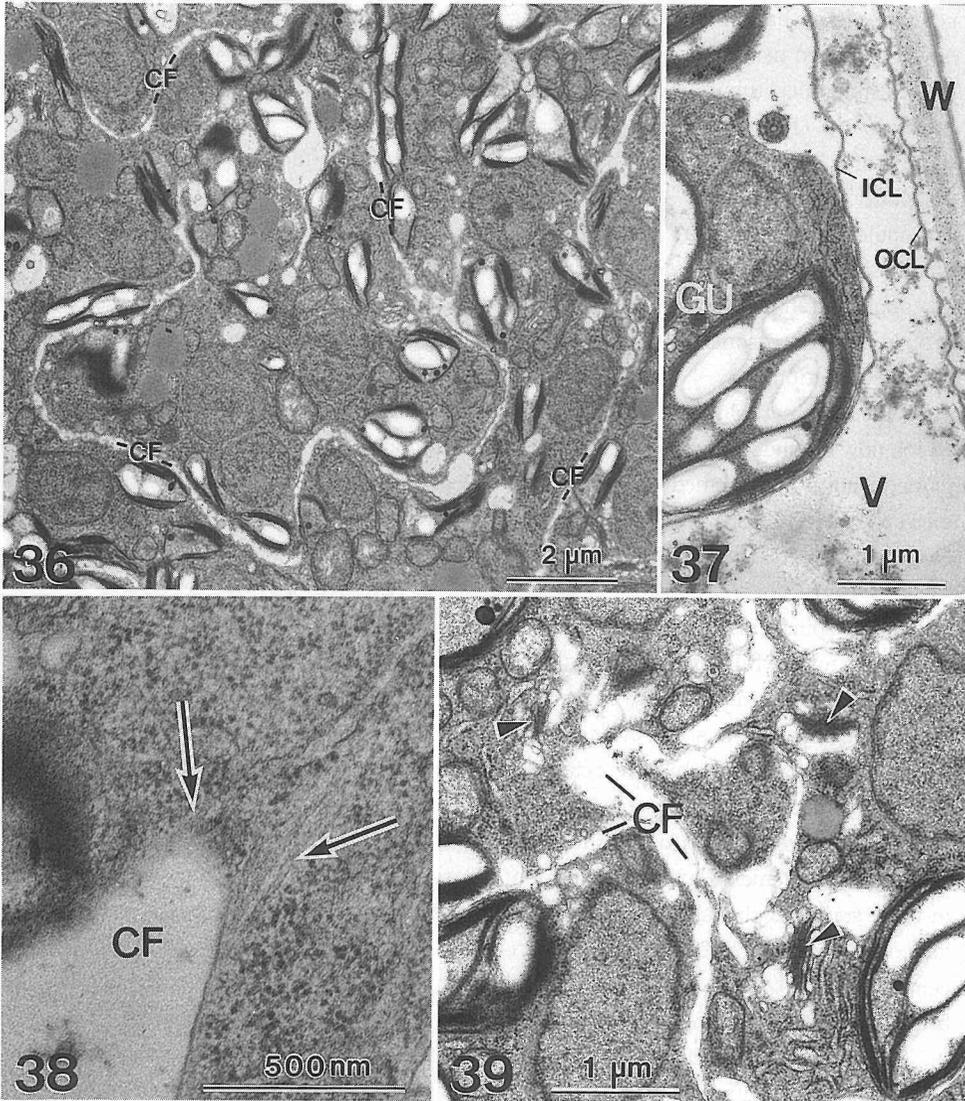
When the cytoplasmic cleavage almost established during 42-45 hr, the microtubules, which now extended from the vicinity of the basal bodies, lay under the plasmamembrane of gametic units and seemed to act as cytoskeletal elements similar to the flagellar rootlet microtubules (Fig. 33). Some microtubules, however, deeply intruded in the cytoplasm (Fig. 34).

Cytokinesis: Cytokinesis did not directly follow each mitosis. After telophase, the interzonal spindle separated from the daughter nuclei was left in the cytoplasm without collapsing for a while, but cytokinesis was not readily established in the region between the daughter nuclei. Although the cytoplasm around the remaining interzonal spindle pretended to grow inward at the stage of acentric nuclear division, it was caused by the intrusion of ramificated vacuole not by any cytokinetic activity because such cytoplasmic feature was seen in every part of the cytoplasm (Figs. 3, 7, 8, 9).

Attention was drawn to that the cytoplasm was subdivided by the furrows similar to long and narrow pavements after emergence of centrioles (Fig. 36). The furrows were different in their some regular shape and extensiveness of plain cytoplasmic frame work from the vacuolar system which irregularly intruded in the cytoplasm before the emergence of centrioles (Figs. 3, 9). Mechanism to regulate or transform the distribution of the membrane confining the cytoplasm had to occur after the emergence of centrioles.

Cleavage furrows involved microtubules. The microtubules were associated with the cleavage furrow (Fig. 38). In order to examine the distribution and orientation of the microtubules serial section analysis was carried out. The microtubules lay tangentially on the edge of the ingrowth of cytoplasm between the neighboring nuclei and most of them seemed to extend out of the nucleus (Fig. 40a-c). These microtubules were considered to lead the cleavage furrow.

As mentioned above section, there were two microtubular systems radiating from the centrosomes different in their distribution. The microtubules involved in the cleavage furrow, in this case, were considered to fit the second microtubular system away from the nucleus. However, not all microtubules of the other system was ignored in the involvement



Figs. 36-39 Cytokinesis. Fig. 36. Showing subdivided cytoplasm in male gametangium at 36 hr. CF, cleavage furrow. Fig. 37. Showing thin cytoplasmic layers in female gametangium at 39 hr. ICL, inner cytoplasmic layer; OCL, outer cytoplasmic layer; W, wall of gametangium; V, vacuole; GU, gametic unit. Fig. 38. The microtubules (arrows) associated with the edge of the cleavage furrow (CF) in male gametangium at 36 hr. Fig. 39. Dictyosomes (arrowheads) near cleavage furrow (CF) in female gametangium at 36 hr.

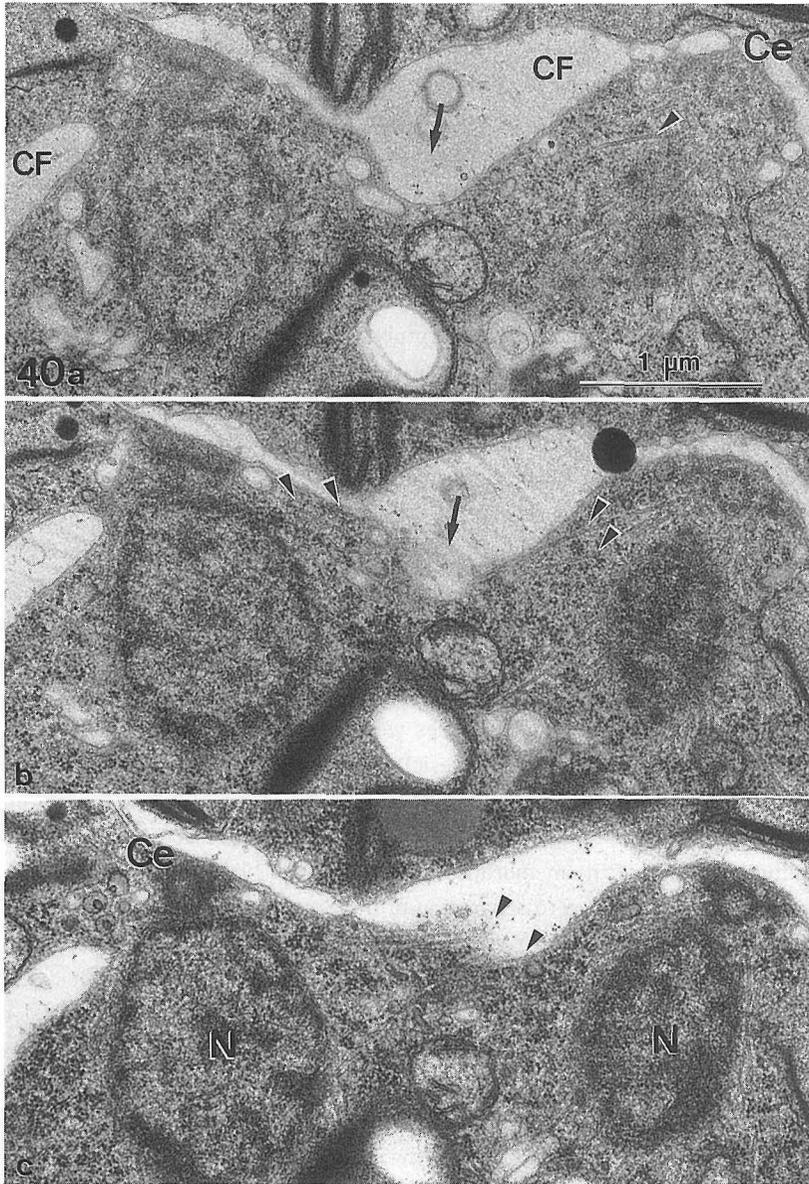


Fig. 40a-c Serial sections showing the ingrowth of cytoplasm towards the region between neighboring nuclei. Note the close association of the ingrowth of cytoplasm (arrows) with the microtubules (arrowheads) extending from the centrosomes (Ce). The microtubules lie tangentially to the edge of the ingrowth in Fig. 40c. This is the incipient cleavage furrow which proceeds towards the region between the neighboring nuclei. CF, cleavage furrow; N, nucleus. Magnification of Fig. 40a-c is the same.

of the cleavage furrow because distinction between two system was often ambiguous.

As the centrosomes were always present at the periphery of the subdivided cytoplasm, the early cleavage furrow seemed to trim the cytoplasm around the centrosomes (Fig. 40a-c). It was conceived that the microtubules might function as a partial cytoskeleton for adjustment of cytoplasmic frame work around each nucleus, resulting in the formation of some regular contour of the cleavage furrow.

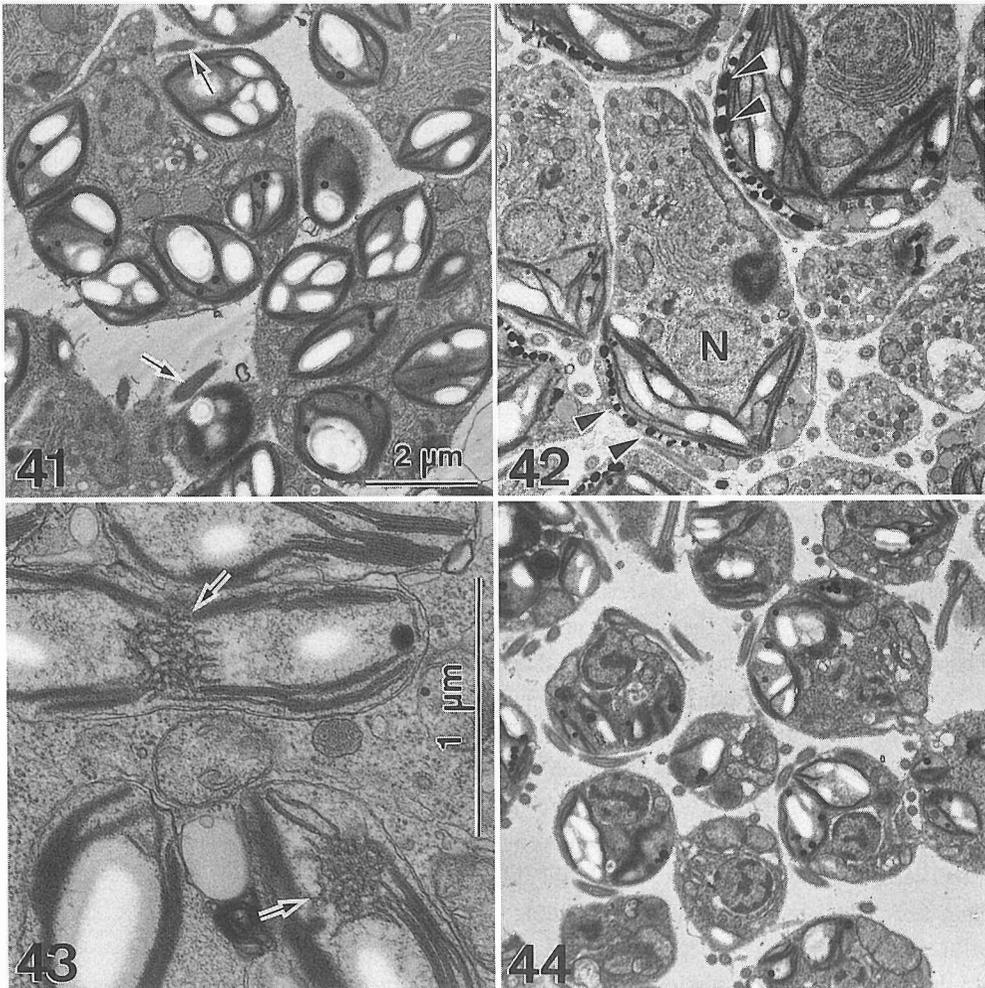
Of course, the microtubules were also considered to be involved in the extending of furrows. In front of the cleavage furrow, the microtubules were associated with vacuoles and vesicles (Fig. 35c-e). The furrow was assumed to be extended by their incorporating with these vacuoles under the lead of the microtubules and to cleave the cytoplasm between neighboring nuclei. The cytoplasm cleaved in the fashion contained one nucleus.

In the final stage of the cytokinesis, unfortunately the interpretation was difficult due to few microtubules on the leading edge of the furrow. However, the observation of abnormal zooids, which looked like that several normal zooids fused at each posterior end, in a septated coenocyte *Dictyosphaeria* observed by HORI and ENOMOTO (1978b) might give a hint on the interpretation. Similar abnormality often occurred in the gametes of the present species (not shown). According to HORI and ENOMOTO (1978b) a cause for such abnormal zooids production suggested failure in the final separation of zooids cytoplasm that followed the cleavage furrow initiating at the cytoplasm around basal bodies. In *Pseudobryopsis*, if any, the end of the cytokinesis might be delicate process not always enough to complete.

Dictyosomes and possibly derived vesicles were very often located near the cleavage furrow (Fig. 39). Whether or not these vesicles contributed to avail in addition of the furrow membrane was now pending by a reason for described below. Also, the origin of the furrow membrane, which especially led by phycoplast, had to be discussed after the use of selected staining methods of the membrane (cf. DOMOZYCH 1987).

On the other hand, just prior to separation of multinucleate cytoplasm into uninucleate units, two layers of thin cytoplasm were clearly separated near a vacuole located at the proximal side of the gametangium (Fig. 37). The outer cytoplasmic layer (OCL) lay adjacent to the wall of the gametangium and the inner cytoplasmic layer (ICL) tightly packed a whole group of gametic units. The ICL isolated the tonoplast facing the vacuole from the furrow membranes involved in cytokinesis. This was puzzled about the origin of the furrow membrane. Because the tonoplast which had organized the vacuolar system was considered to rest, at least partially, in the furrow membrane. Formation of a thin cytoplasmic layer similar to the ICL occurred in zoosporogenesis of *Hydrodictyon* (MARCHANT and PICKETT-HEAPS 1971) and *Characiosiphon* (STEWART *et al.* 1978) and in spermatogenesis of *Sphaeroplea* (CÁCERES and ROBINSON 1981) but did not in gametogenesis of the closely related genus, *Bryopsis* (BURR and WEST 1970).

The vacuole confined by the ICL and OCL, at gamete discharge, might be conceived to press the group of gametes by water suction and thereby the gametes were squeezed out



Figs. 41-44 Maturation of gamete. Fig. 41. Female gametic units with flagella (arrows) at 42 hr. Fig. 42. Female gametic units with a large chloroplast at 45 hr. Arrowheads, osmiophilic globules in the portion of eyespot. N, nucleus at the posterior of the gametic units. Fig. 43. Male chloroplasts with the tubular profiles (arrows) of thylakoid at 36 hr. Fig. 44. Male gametic units at 45 hr. Scale in Fig. 41 also applies to Figs. 42 and 44.

through a liberation pore.

Maturation of gamete: After at 33-36 hr, change in chloroplast structures conspicuously reflected the sexuality of the gametangia. Along with nuclear divisions, the chloroplasts also became small probably due to repeated divisions with no interim enlargement. Although the chloroplasts attained a size of 1.9-4.5 μm in length and 0.9-1.8 μm in breadth at

30 hr, they decreased to 1.0-2.0 μm and 0.5-0.9 μm in male gametangia and to 2.2-3.3 μm and 1.0-1.2 μm in female gametangia at 36 hr. At the same time, the number of thylakoid stacks was constant 6-11 in females while it decreased to 2-5 in males.

A tubular structure often appeared in the chloroplasts of the male gametangia during 33-36 hr (Fig. 43). It consisted of a central dense core formed of a net-work of short tubular-like elements and marginal tubular-like elements protruding outward. The tubular structure was similar to that of the chloroplasts in dark grown *Chlamydomonas* cells (FRIEDBERG *et al.* 1971) and of the chromoplasts during fruit ripening (SPURR and HARRIS 1968) and the prolamellar body of the higher plant etioplasts during the greening processes (GUNNING 1965).

The transformation of the chloroplasts in the male gametangia in the present species were accompanied by a lightening of the green color of the cytoplasmic content of the gametangia. The degradation of chlorophyll was assumed to occur selectively in the male chloroplasts.

The distinction in sexuality became clearer by transformation of the female chloroplast after 42 hr. The female chloroplasts began not only to form the eyespot (Fig. 42) but also to change morphologically. First, starch grains increased in number from 2-6 at 42 hr to 6-15 at 45 hr. Secondly, the number of thylakoid stacks also increased from 6-9 to 7-14. Thirdly, 42 hr-chloroplasts were spindle-shaped (Fig. 41), whereas 45 hr-chloroplasts were V- to U-shaped and lay along the posterior end of the gametic units (Fig. 42). The V- to U-shaped chloroplasts were constricted at their bending points, therefore not all thylakoid stacks extended from one end of the chloroplast to the other. Fourthly, the number of chloroplasts contained in a single gametic unit decreased during 42-45 hr. Fifthly, 45 hr-chloroplasts were larger than 42 hr-chloroplasts. This showed that several chloroplasts fused with one another to become a large chloroplast in the female gametic unit. Chloroplast fusion had been suggested in gametogenesis of *Acetabularia* cysts (WOODCOCK and MILLER 1973).

In addition to the possession of a large chloroplast, the female gametic unit was endowed with active dictyosomes and many vesicles derived from their cisternae near multi-folded ER stacks (Fig. 42). In contrast to the case of the female gametic unit, in the male gametic unit the chloroplasts without eye spots were small and dictyosomes seemed to be inactive (Fig. 44). Ultrastructural features in the male and female gametic units at the final stage of gametogenesis in *Pseudobryopsis hainanensis* were quite similar to those of the anisogametes in *Bryopsis hypnoides* (BURR and WEST 1970).

Discussion

Mitosis in *Pseudobryopsis*: In *P. hainanensis* the gametogenetic mitosis does not start till the gametangium formation ceases. The gametic nuclei are led by the successive series of mitoses, in which nuclear division might occur at least three and four times in the

female and male gametangia, respectively. During the successive nuclear divisions, the ultrastructural features of mitoses change especially in the extranuclear conditions which reflect the gametic differentiation. In the early mitosis neither centrioles nor extranuclear microtubules are present, but in the subsequent mitosis they appear around the nuclei. After that, in the male even when centrioles become basal bodies, continues the mitosis. Except for such extranuclear features, each mitosis is characterized by a complete closed spindle, no evident kinetochores and the presence of a persistent, long interzonal spindle at telophase.

Behavior of nuclear envelope: In septated and non-septated marine coenocytic green algae there are known three spindle types in the behavior of nuclear envelope. 1) A complete break down of the nuclear envelope at prophase occurs in *Urospora wormskioldii* (LOKHORST and STAR 1983). 2) Although the nuclear envelope is almost intact, the extranuclear microtubules extending from a region near centrioles intrude into the spindle through local disruption of the nuclear envelope at the poles (polar fenestrae) in *Urospora neglecta* (LOKHORST and STAR 1983), *Acrosiphonia spinescens* (HUDSON and WAALAND 1974), and *Bryopsis hypnoides* (BURR and WEST 1970). 3) As the case of the present species, the nuclear envelope entirely maintains its intact nature during the mitosis in *Cladophora glomerata* (MCDONALD and PICKETT-HEAPS 1976), *Cladophora flexuosa* (SCOTT and BULLOCK 1976), *Valonia ventricosa* (HORI and ENOMOTO 1978a), *Dictyosphaeria cavernosa* (HORI and ENOMOTO 1978c) and *Caulerpa brachypus* (HORI 1981).

The behavior of nuclear envelope is not always constant between species in the same genus (LOKHORST and STAR 1983) and even in the same species (ALDRICH 1969). This has been the reason why the significance of the nuclear envelope behavior as a taxonomic marker is reduced (LOKHORST and STAR 1983). HEATH (1980), however, has considered that the nuclear envelope behavior during mitosis is reasonably stable, with exceptions, and significant in consideration of spindle evolution.

In above two examples the nuclear envelope show a dimorphic behavior, the alternative of a disperse type or a polar fenestra type. These two types may be variable between them if the origin of cells different in the nuclear envelope behavior has close affinity each other. Because the mitotic spindles in both types are conceived to be equivalent in use of the extranuclear microtubules more or less. As against it, the completely closed spindle confines independent MTOCs enough for the assemblage of spindle microtubules within it (LOKHORST and STAR 1985). Therefore, the spindle which do not make use of the extranuclear MTOCs is conceived to be primitive or even conservative.

Persistent interzonal spindle: In *Pseudobryopsis hainanensis* the continuous spindles microtubules do not collapse soon after anaphase but proliferate and considerably extend to form a persistent interzonal spindle. The persistent interzonal spindle helps daughter nuclei to separate far away and prevents daughter nuclei from approaching one another (VAN DEN HOEK 1981).

The persistent interzonal spindle in *Pseudobryopsis hainanensis* is similar to that de-

scribed in *Cladophora flexuosa* (SCOTT and BULLOCK 1976), *Valonia ventricosa* (HORI and ENOMOTO 1978a), *Dictyosphaeria cavernosa* (HORI and ENOMOTO 1978c) and *Caulerpa brachypus* (HORI 1981). These persistent interzonal spindles are surrounded with nuclear envelope and remain for a while after separation of daughter nuclei (Fig. 20 in SCOTT and BULLOCK 1976). This nature is noteworthy (see below).

The persistent interzonal spindle occurs in two phylogenetic groups of the Charophyceae and Ulvophyceae (MATTOX and STEWART 1984). In the Charophyceae the continuous spindle microtubules which participated in spindle elongation and chromosome separation during anaphase persist between reformed daughter nuclei after telophase. These persistent interzonal spindle microtubules are involved in cytokinetic septum formation and are equivalent to phragmoplast microtubules typical of higher plants (PICKETT-HEAPS 1975). In the Ulvophyceae, species in which are classified have been studied by MATTOX and STEWART (1974) and SLUIMAN *et al.* (1983), a few microtubules persist between widely separated reforming daughter nuclei. These microtubules, however, are considered not to be involved in cytokinesis because a precocious cleavage furrow in cytokinesis always precedes the development of the interzonal spindle microtubules (MATTOX and STEWART 1974). The persistent interzonal spindle microtubules in both Charophyceae and Ulvophyceae mentioned above are not enclosed with the nuclear envelope after separation of daughter nuclei.

The persistent interzonal spindle in *Pseudobryopsis* is neither developed into phragmoplasts nor constricted by any cleavage furrow. The closed nature attributes to the completely closed spindle nature and therefore, the former seems to be no less primitive than the latter is.

Centrioles and centrosomes: In *Pseudobryopsis hainanensis* the centrioles are produced *de novo*. Since the mitosis dispenses with the centrioles at the early stage of the gametic differentiation and possible vegetative stage, the centrioles themselves are not essential for spindle formation.

The centrioles are, however, responsible for the formation of flagellar axonemal microtubules and differentiate into the basal bodies. PICKETT-HEAPS (1971) has considered the centrioles to be a highly structured and conspicuous form of MTOCs and to function as templates for the assemblage of axonemal microtubules. It is known that centrioles are absent in vegetative cells but appear before differentiation into flagellate forms of the cell (PICKETT-HEAPS 1968, MANTON *et al.* 1970 and this study). This shows that these no centriole cells have not lost the ability to form the centrioles and the emergence of the centrioles are required for their templating activity.

According to the concept formulated by PICKETT-HEAPS (1969), MTOC is the structure from which microtubules start their assembly and recognized as amorphous osmiophilic material. The complexes of the centrioles associated with the pericentriolar MTOCs are called the centrosomes, which organized the microtubules during both interphase and mitosis in general animal cells (VOROBYEV and NADEZHINA 1987). However, the absence of the

centrioles does not hinder the MTOC function which the centrosomes possess. In higher plants, which are lack of the centrioles in a full life cycle, the spindle microtubules are organized by the polar components identical with the pericentriolar MTOC (CLAYTON *et al.* 1985). The centrioles are considered to act as a rallying point for the MTOC components and to provide a focus for the microtubules nucleating (LLOYD and BARLOW 1982).

Rootlet templates: The rootlet templates were termed by PICKETT-HEAPS (1971). According to PICKETT-HEAPS (1971), the rootlet templates operate as some form of template for the extrusion of a specific spatial array of microtubules the same as centrioles and are considered as the conspicuous structures of MTOCs which the flagellar rootlet microtubules nucleate. In *Oedogonium* the rootlet templates arise *de novo* concurrently with the associated centrioles and are suspected to duplicate along with centrioles during mitosis (PICKETT-HEAPS 1975).

In *Pseudobryopsis hainanensis* the rootlet templates well correspond in structure and function to the description of PICKETT-HEAPS (1975): the short microtubules are covered with amorphous dense matrix adjacent to the centriole cylinder and extend the flagellar rootlet microtubules at the later stage. The duplication of the rootlet templates is semiconservative the same as that of the centrioles. Therefore, the rootlet templates, as the assumption of PICKETT-HEAPS (1975), are shared with daughter nuclei along with the centrioles.

The number of the rootlet templates occurring at the two sides of the centriole is always determined by the counterclockwise arrangement of the centriole pair in the present species. During the duplication of the centrioles, the daughter centriole pairs the parent centriole in the counterclockwise arrangement, so the two distinctive sides of the centrioles are always kept. It is assumed that the MTOCs which the rootlet templates nucleate at the two distinctive centriole sides, if any, control the number and the direction of the rootlet templates and receive even the information of sexuality.

The rootlet templates have been found in *Tetraedron* and *Oedogonium* (PICKETT-HEAPS 1975), *Microspora* (PICKETT-HEAPS 1973), *Sorastrum* (MARCHANT 1974a), *Pleurastrum* (MOLNAR *et al.* 1975), *Coelastrum* (MARCHANT 1977), *Golenkinia* (HEG- EWALD and SCHNEPF 1984), *Cylindrocapsa* (SLUIMAN 1985). The present study is the first description of the rootlet templates in siphonous green algae.

Extranuclear microtubules: In *Pseudobryopsis hainanensis* the extranuclear microtubules organized by centrosomes never incorporate with the intranuclear spindle microtubules. There are two microtubular systems different in their distribution: the first ensheathes the nuclear envelope, the second is involved with cytokinesis. The latter will be discussed later.

The term of extranuclear microtubules has been often used for the cytoplasmic microtubules radiating from the centrosomes. In some sense it may comprehend the phycoplast microtubules which are a certain cytokinetic apparatus. Confusion seems to be caused by

little knowledge with respect to the ontogeny and function of the microtubules generally termed extranuclear microtubules. Among green algae the centrosomes, except for the microtubules which are certainly involved in cytokinesis, constitute the microtubular system(s) that ensheathes nuclear envelope and/or that forms a centrosomal aster. The proposal that such the extranuclear microtubules may be classified into three categories by presumable function is made.

1) Incorporation with spindle microtubules: The microtubules ensheath the intact nuclear envelope between separated centrosomes at prophase in some chlorococcalean algae, *Kirchneriella* (PICKETT-HEAPS 1970), *Tetraedron* (PICKETT-HEAPS 1972b), *Scenedesmus* (PICKETT-HEAPS and STAEHELIN 1975), *Hydrodictyon* (MARCHANT and PICKETT-HEAPS 1970) and *Pediastrum* (MARCHANT 1974b). These microtubules are sandwiched between the nuclear envelope and perinuclear ER at prophase, though this condition does not always occur (DEASON and O'KELLEY 1979), but subsequently invade the nucleus during prometaphase or metaphase when broad polar fenestrae near the centrosomes start. In *Klebsorbidium* (LOKHORST and STAR 1985) also the microtubules extending from the polar centrosomes ensheath the nuclear envelope at prophase. As the nuclear envelope completely break down at prometaphase, these ensheathing microtubules invade the nucleus and form the spindle.

In above two examples the microtubules which ensheathed the nucleus completely incorporate with the spindles microtubules by metaphase and no further organization of them occurs later. Examples in *Chlorokybus* and *Pleurastrum* may partly come within this category (see also below). Though in *Carteria* (DOMOZYCH 1987) and *Draparnaldia* (LOKHORST *et al.* 1984) a few of the microtubules which ensheathed the prophase nucleus penetrate in the metaphase nucleus, these examples also, at present, are placed here.

2) Centrosome guiding system: In *Carteria* (DOMOZYCH 1987) the microtubules radiating from the centrosomes are sandwiched between the nuclear envelope and perinuclear ER at prophase the same as in above chlorococcalean algae, but during metaphase some of the microtubules remain intact though the others incorporate with the spindle microtubules. At anaphase the remaining microtubules present at the periphery of the nuclear envelope guide the centrosomes toward the interzone of the spindle.

In *Cylindrocapsa* (SLUIMAN 1985), unlike in *Carteria*, a few microtubules which intervened between the nuclear envelope and perinuclear ER at prophase disappear at metaphase. But at telophase the microtubules emerge at the periphery of the daughter nuclear envelope. The microtubules bring the centrosomes from the former spindle poles towards the region between the separate daughter nuclei.

The microtubular systems in *Chlorokybus* are further complex (LOKHORST *et al.* 1988). At preprophase the centrosomes lie on a future spindle equatorial plane between the nucleus and incipient furrow and organize the two microtubular systems: one ensheathing the nucleus and the other extending to the incipient furrow edge. At prophase two pairs of the

centrosomes following duplication separate towards future spindle poles away from both the nucleus and the furrow edge, but the two microtubular systems are still maintained. Therefore, the centrosome migration is conceived to be due to some pushing activity of their microtubules. At subsequent prometaphase the system ensheathing the nucleus forms the spindle as the nuclear envelope is completely dispersed (this falls under the first category).

In the present alga *Pseudobryopsis*, the first microtubular system organized by the centrosomes ensheathes the interphase nucleus. At prophase one of the centrosome pairs after duplication moves towards the opposite pole where is predetermined by the ensheathing microtubules focusing. Similar centrosome guiding system of microtubules is suggested also in *Cladophora* (SCOTT and BULLOCK 1976).

The centrosomes move either towards the interzone of spindle in which cytokinesis occurs (*Carteria* and *Cylindrocapsa*) or towards the spindle poles (*Chlorokybus* and *Pseudobryopsis*).

3) Anchor: In *Pleurastrum* (MOLNAR *et al.* 1975) the spindle formation is unusual (metacentric). After centrosome duplication at prophase two centrosome pairs do not separate and stay at the future spindle equatorial region between the nucleus and incipient furrow until telophase, unlike in *Chlorokybus*. The microtubules emanating from the centrosomes proliferate and overarch the late prophase nucleus. All of them participate in the spindle formation when the nuclear envelope is highly vesiculated at metaphase (these microtubules are placed under the first category). But the microtubules again extend from the centrosomes at telophase and ensheath the daughter nuclei. When cytokinesis proceeds between two centrosome pairs, the daughter nuclei are moored at their own centrosomes by the ensheathing microtubules, which keep one nucleus associated with one pair of the centrosomes.

In *Aphanochaete* (SEGAAR and LOKHORST 1988) the centrosomes form the polar microtubule asters at anaphase. When the interzonal spindle collapses at telophase, the asters keep the daughter nuclei in position, *i. e.* the nuclei are anchored. The anchoring system of microtubules also occurs in *Chlorokybus* (LOKHORST *et al.* 1988).

In *Pseudobryopsis* in this study the microtubules which ensheath the daughter nuclei at telophase are conceived to help the centrosomes to be associated with the daughter nuclei when the interzonal spindle considerably elongates. The centrosomes are anchored at the nucleus.

Although the extranuclear microtubules are classified into three categories in function mentioned above, possibility that these microtubules are involved in cytokinesis is omitted. Detailed investigation on the ontogeny of the extranuclear microtubules in various species is hoped.

Cytokinetic microtubular system: During gametogenesis in *Pseudobryopsis hainanensis* the cytokinesis does not immediately follow each mitosis. Vacuolar system, which is ramified irregularly and intrudes the cytoplasm near the nuclei, however, has no cytokinetic

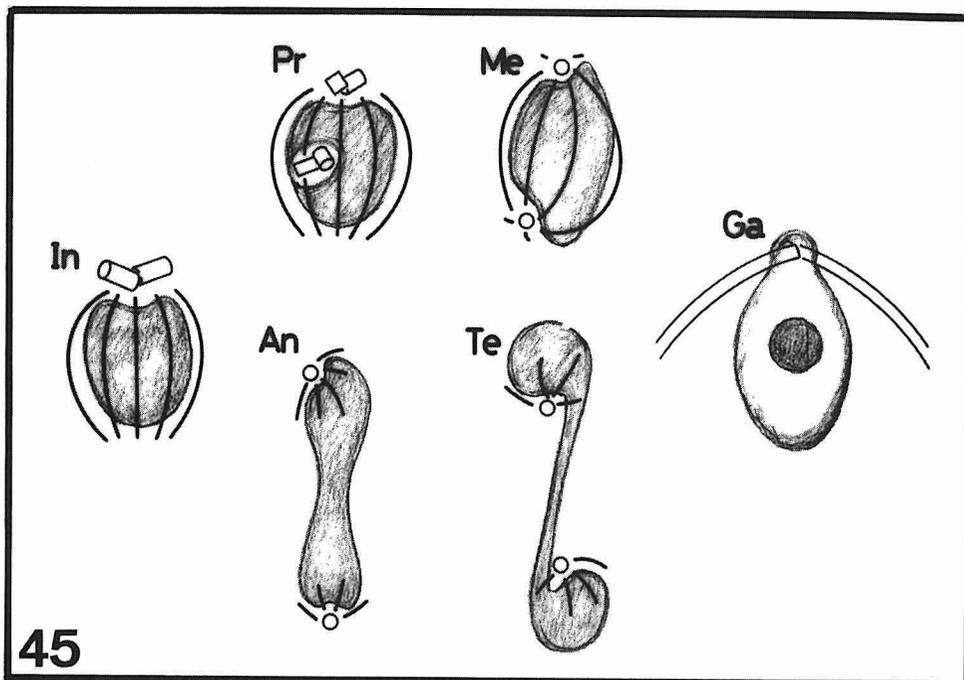
activity. The cytokinesis does not positively begin till the centrosomes emerge. At the early stage of the cytokinesis the subdivided cytoplasm is formed and it protrudes around the centrosomes. Consequently the cytoplasm between neighboring nuclei is depressed and the ingrowth of the membrane proceeds to the region of the cytoplasm between neighboring nuclei. Subsequently the ingrowing membrane incorporates with vesicles and vacuoles and develops into the cleavage furrow. Finally the cleavage furrow divides the cytoplasm into the uni-nucleate gametic units.

In *Pseudobryopsis hainanensis* the second microtubular system organized by the centrosomes is mainly involved in the cytokinesis. The microtubular system acts as a cytoskeleton and controls the cytoplasmic shape around the centrosomes. Consequently it causes the ingrowth of the membrane between neighboring nuclei. The microtubules lead the ingrowing membrane to vesicles and vacuoles. Thus, the cleavage furrow extensively develops. The microtubules tangentially lie to the cleavage furrow though they are not precisely parallel with the cleavage plane (*i. e.* mainly transverse direction). It is reasonable that the neighboring nuclei divided by the furrow is not always the daughter nuclei generated by the same mitosis.

The present study is the first evidence that the microtubular system is certainly involved in the cytoplasmic cleavage in siphonous green algae. It has been previously reported that no microtubules positively play a part in cytokinetic function in *Bryopsis* (BURR and WEST 1970) and *Caulerpa* (HORI 1981). This has been one of the cytological criteria to classify the siphonous green algae in the recent green algal systematics (MATTOX and STEWART 1984). According to O'KELLEY and FLOYD (1984) no involvement of microtubules at cytokinesis is the character which defines the order Ulvophyceae. In this regard the present species *Pseudobryopsis hainanensis* should be excepted from the Ulvophyceae.

PICKETT-HEAPS (1972) pointed out that the transverse arrays of microtubules between daughter nuclei which are involved in cytoplasmic furrow and cell plate formation are radically different from the phragmoplast and termed such transverse system of microtubules the phycoplast. Subsequently STEWART and MATTOX (1975) proposed to discriminate two phycoplast types in generation: a trochoplast which develops from a region near the centrioles and leads cleavage furrow; a mesoplast which develops among the remnants of the collapsed interzonal spindle and leads cell plate formation (STEWART *et al.* 1973). According to the definition the cytokinetic system of microtubules in *Pseudobryopsis hainanensis* is the trochoplast.

The broad definition of the phycoplast (PICKETT-HEAPS 1972) has given rise to some confusion in interpretation and identification as pointed out by SLUIMAN (1985). For example, both the microtubules which are involved in the centripetal ingrowth of wall septum (LOKHORST and STAR 1983, SEGAAR and LOKHORST 1987, LOKHORST *et al.* 1988) and the microtubules which ensheath daughter nuclei (MATTOX and STEWART 1984) may be regarded as the phycoplast. In addition, the trochoplast is used in vegetative cell division



Figs. 45 Diagram showing mechanism to ensure distribution of a pair of centrosomes to each nucleus during mitosis. In, interphase; Pr, prophase; Me, metaphase, An, anaphase; Te, telophase; Ga, gamete.

while the phycoplast which is neither the trochoplast nor the mesoplast is used in reproductive cell division in *Hydrodictyon* (MARCHANT and PICKETT-HEAPS 1970, 1971) and *Sphaeroplea* (CÁCERES and ROBINSON 1980, 1981). Whether what is, if any, the certain cyto-kinetic system of microtubules or if it is related with other microtubular systems such as extranuclear microtubules and cortical microtubules has not been properly answered due to little knowledge on the ontogenesis of these microtubular systems so far.

The role of centrosomes: In *Pseudobryopsis hainanensis* the gametogenesis is the process in which a multinucleate cell is transformed into a uninucleate cell. The centrosomes which emerge at this process play an important part in the gametic differentiation. The functions of the centrosomes are 1) to act as template MTOCs for flagellar axonemes; 2) to generate the rootlet templates; 3) to organize the microtubules which ensheath nuclei; 4) to organize the microtubules which are involved in cytoplasmic cleavage. How the centrosomes act in the mechanism to ensure the uninucleate cell formation of the multinucleate cell in this species is mentioned below.

Unless each pair of centrosomes was shared with each nucleus, no flagellar and multiflagellar gametes would be produced. One pair of the centrosomes is *de novo* formed

at each nucleus. As a nuclear division occurs, one pair of the centrosomes surely duplicates to be two pairs. Subsequently one pair of the centrosomes migrates to the opposite pole, guided by the microtubules which ensheath the nucleus. In spite of the considerable extension of the spindle, one pair of the centrosomes attaches to each daughter nucleus owing to the microtubules which ensheath each daughter nucleus. Thus, since one pair of the centrosomes is surely distributed to each nucleus, biflagellate gametes are generated. The scheme is represented in Fig. 45.

If multinuclear cytoplasm was incompletely divided, multinuclear gametes would be produced. As mentioned above, each nucleus has a pair of the centrosomes. The other microtubular system organized by the centrosomes trims the cytoplasm territorial around each pair of the centrosomes and extends the cleavage furrow into the cytoplasm between neighboring nuclei. Multinuclear cytoplasm is consequently partitioned into the cytoplasm which contains one nucleus and one pair of the centrosomes. The centrosomes finally differentiate into the flagellar apparatus of the biflagellar uninucleate gamete.

Summary

Mitosis, cytokinesis and gametic differentiation are studied in the siphonous green alga *Pseudobryopsis hainanensis* TSENG with transmission electron microscopy.

The successive series of mitoses continues for 9-12 hrs after gametangia attained to maximum size. The rate of reduction in a nuclear diameter indirectly shows that female and male gametic nuclei are produced by the successive series of at least three and four nuclear divisions, respectively.

Early mitosis dispenses with centrioles. Metaphase nucleus is a spindle shape with polar protrusions of nuclear envelope but chromosomes on the metaphase plate are not more electron dense. Anaphase nucleus is conspicuously elongated by pole-to-pole continuous microtubules and becomes elliptical and dumb-bell shaped. At telophase daughter nucleus is reformed at the lateral side of the end of an interzonal spindle and isolated from an interzonal spindle by sharp constriction of nuclear envelope.

Centrioles are produced *de novo* near an interphase nucleus before subsequent mitosis. They provide the rootlet templates which are generated in the same arrangement as the flagellar rootlet microtubules. The first microtubular system extending from centrosomes ensheathes nuclear envelope. The electron density of chromatin becomes higher in male gametangia. At prophase centrioles duplicate semiconservatively and one pair of centrioles migrates towards the opposite pole, presumably guided by the first microtubular system. Centrioles are situated laterally to poles at metaphase. At anaphase chromosomes are first separated by increasing in a pole-to-pole distance. Centrioles rotate around a daughter nucleus away from a normal spindle pole at telophase.

Mitotic activity continues in male gametangia even when centrioles extend flagella.

The characters of mitosis common to all nuclear divisions are completely intact nuclear envelope, no evident kinetochore and persistent interzonal spindle at telophase.

Cytokinesis does not immediately follow each nuclear division and does not occur till centrioles emerge. The second microtubular system which are organized by centrosomes leads the ingrowth of cytoplasm towards the region between neighboring nuclei. The microtubules lie tangentially to the edge of cleavage furrow. Cleavage furrow incorporates with the vesicles or vacuoles which are embedded in the cytoplasm between neighboring nuclei and divides multinucleate cytoplasm into uninucleate gametic units.

Ultrastructure in chloroplasts conspicuously reflects the sexuality during the process of gametic differentiation: the chloroplasts fuse with one another into a large chloroplast with an eye spot in female while they are reduced in size and often have a prolamellar body-like structure in male.

Following subjects are discussed: behavior of nuclear envelope; persistent interzonal spindle; centrioles and centrosomes; rootlet templates; extranuclear microtubules; cyto-kinetic microtubular system; role which centrosomes play in uninucleate cell formation in this coenocyte.

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