Influence of the centrosome in cytokinesis of brown algae: polyspermic zygotes of *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyceae)

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Summary

We examined the relationship between the spindle orientation and the determination site of cytokinesis in brown algal cells using polyspermic zygotes of *Scytosiphon lomentaria*. When two male gametes fuse with one female gamete, the zygote has two pairs of centrioles derived from male gametes and three chloroplasts from two male and one female gametes. Just before mitosis, two pairs of centrioles duplicate and migrate towards the future mitotic poles. Spindle MTs develop and three or four spindle poles are formed. In a tri-polar spindle, one pair of centrioles shifts away from the spindle, otherwise, two pairs of centrioles exist adjoining at one spindle pole. Chromosomes arrange at several equators of the spindle. As a result of these multipolar mitoses, three or four daughter nuclei developed. Subsequently, these daughter nuclei form a line along the long axis of the cell. Cell partition always takes place between daughter nuclei, perpendicular to the long axis of the cell. Three or four daughter cells are produced by cytokinesis. Some of the daughter cells after cytokinesis do not have a nucleus, but all of them always contain the centrosome and chloroplast. Therefore, the number of daughter cells always coincides with the number of centrosomes or microtubule organizing centers (MTOCs). These results show that the cytokinetic plane in the brown algae is determined by the position of centrosomes after mitosis and is not dependent on the spindle position.

Key words: Brown algae, Centrosome, Chloroplast, Cytokinesis, Freeze-substitution, Microtubules, *Scytosiphon lomentaria*, Spindle formation

Introduction

In eukaryotic cells, the mitotic apparatus participates in the equal separation of duplicated genomes. Animal cells have a discernible centrosome [a pair of centrioles and pericentriolar material (Kimbel and Kuriyama, 1992; Balczon, 1996)] that functions as a microtubule organizing center [MTOC (Pickett-Heaps, 1969)]. Duplication of the centrosome occurs during S phase (Kuriyama and Borisy, 1981) in many cases, and each centrosome migrates to the opposite pole of nucleus. Spindle microtubules (MTs) elongate from the centrosome towards the chromosomes, and a fusiform bipolar spindle is formed. Land plants have quite different MT arrays from animal cells, such as interphase cortical MTs, the preprophase bands and phragmoplasts, instead of distinctive MTOCs such as centrosomes. During mitosis in land plant cells, MTs around the nucleus proliferate and form a barrel-shaped spindle (Baskin and Cande, 1990).

After separation of genomes, cytokinesis must occur for accurate distribution of genomes to the daughter cells. For cytokinesis in animal cells there is an apparatus called the contractile ring that consists of microfilaments (MFs) and myosin in cortical cytoplasm at the equator of the cell. This structure appears during late anaphase and the cleavage furrow occurs at this site (Satterwhite and Pollard, 1992; Fishkind and Wang, 1995). In land plants the phragmoplast, which consists of MTs, MFs and Golgi-derived vesicles, appears at the mid-plane of the cell during late anaphase (Zhang et al., 1993;

Staehelin and Hepler, 1996). Golgi-derived vesicles are transported by phragmoplast MTs to the cell division plane, where they accumulate, fuse with each other and form a cell plate. This cell plate grows centrifugally and, eventually, cytokinesis is completed by the deposition of cell wall material.

There is also a difference in the determination period of the cell division site in animals and land plants. In animal cells, the orientation of the division plane is determined by the position of the spindle (Rappaport, 1986), and the positioning of the spindle is mediated by the astral MTs and MFs at the cell cortex (Lutz et al., 1988; Hyman, 1989; Strome, 1993; Waddle et al., 1994; White and Strome, 1996). However, the spindle is not directly involved in furrowing (Rappaport, 1986). Although the mechanism for induction of the cleavage furrow is not completely understood, the spindle appears to stimulate the accumulation of molecules such as actin and myosin involved in the contractile ring at the cell cortex (Rappaport, 1986; Satterwhite and Pollard, 1992). By contrast, in land plants, the experimental displacement of the spindle by centrifugation indicates that the position of the spindle does not relate to the cell division plane (Galatis et al., 1984). In land plants, the preprophase band (PPB) consisting of MTs is transiently observed at the equator of pre-mitotic nuclei, the PPB disappears with progression of mitosis, and generally the cell cortex is predetermined at this site (Mineyuki et al., 1988; Gunning and Sammut, 1990; Mineyuki, 1999). The phragmoplast and resulting cell plate are formed at the site of the PPB.

Brown algal cells have a centrosome that always functions as the MTOC (Katsaros and Galatis, 1992; Bisgrove et al., 1997; Motomura, 1991; Nagasato et al., 1999a). Similar to animal cells, the centrosome plays a crucial role in spindle pole formation. Also, fluorescence-labeled phalloidin showing MFs (Brawley and Robinson, 1985; Karyophyllis et al., 2000) revealed that the future cell division plane occurs after mitosis. Treatment with cytochalasin B and D indicates that MFs act during cytokinesis (Brawley and Robinson, 1985; Kropf et al., 1990; Karyophyllis et al., 2000). However, this actin-based structure in brown algal cytokinesis is quite different from the actin contractile ring of animal cells, because it is observed as a disk-like structure over the future cell division plane, and remains there during and soon after cytokinesis (Karyophyllis et al., 2000).

Recently, we described cytokinesis in the brown alga, *Scytosiphon lomentaria*, using freeze substitution techniques, and clarified that cytokinesis is never accomplished by the cleavage furrow of the plasma membrane (Nagasato and Motomura, 2002). After completion of mitosis, MTs from centrosomes elongate to the mid-point between two daughter nuclei and apparently transport Golgi-derived vesicles to the future division plane (Nagasato and Motomura, 2002). The vesicles fuse with tubular cisternae that simultaneously appear there, and membranous flat sacs grow towards the plasma membrane. After the new cell partition reaches the plasma membrane, cell wall material is deposited between the plasma membranes. Therefore, the formation of a new cell partition in brown algae partially resembles cell plate formation in land plants (Samuels et al., 1995; Verma and Gu, 1996), although there is no specialized structure such as the phragmoplast.

Brown algal cells have a combination of features similar to centrosomal spindle formation in animal cells and cell plate formation in mitosis and cytokinesis in land plants. In brown algae, the conclusive factor that determines the cytokinetic plane is still unclear because there is no prominent structure, such as the contractile ring in animals and the preprophase band in land plants, associated with it. In *Scytosiphon* zygotes, cytokinesis always takes place perpendicular to the growth axis of the cell (Nagasato et al., 2000; Nagasato and Motomura, 2002). *Scytosiphon* is a very simple type of brown algae; for example, each cell contains only one chloroplast and one or two Golgi bodies that are always close to the centrosome (Clayton and Beakes, 1983). In this study, using polyspermic zygotes of *S. lomentaria*, we tried to clarify when and how the cytokinetic plane is established, emphasizing the orientation of the spindle and the affect of the centrosomes on the cell division sites.

Materials and Methods
Culture
Mature male and female gametophytes of *S. lomentaria* (Lyngbye) Link were collected from March to May 1999-2001, at Charatsunai, Muroran, Hokkaido (42° 19′ N, 140° 59′ E). Liberation of the male and female gametes and induction of plasmogamy have been described previously (Nagasato et al., 1999b; Nagasato et al., 2000). Female gametes were first inoculated into a drop of PESI medium (Tatewaki, 1966) on coverslips, or gel support films (ATTO, Japan) that were previously washed with distilled water, cut into a less than 1 cm side-length triangle, and attached to the petri dishes by adhesive tape. After female gametes had settled, the male gametes were added.

When excess male gametes were added to the settled female gametes, polyspermy took place frequently. After 10 minutes, motile male gametes were washed out with sterilized seawater and zygotes were cultured in PESI medium under fluorescent lamps (30-40 μmol/m^2/s photon flux density) at 14°C long-day condition (14 hours light: 10 hours dark).

Fluorescence microscopy
Polyspermic zygotes on coverslips, 32-34 hours after fertilization, were fixed and stained with sea water containing 1% glutaraldehyde and 1 μg/ml DAPI (4′-6-diamidino-2-phenylindole) for 10 minutes. Chloroplasts could be simultaneously observed as red by their autofluorescence of chlorophylls.

In preparation for immunofluorescence microscopy, zygotes on coverslips were fixed for 1 hour at room temperature in 3% paraformaldehyde and 0.5% glutaraldehyde in PHEM buffer (60 mM Pipes, 25 mM Hepes, 10 mM EGTA, 2 mM MgCl$_2$, pH 7.4) with 3% NaCl. After fixing, they were washed several times with modified PBS (513 mM NaCl, 2.7 mM KCl, 4.9 mM Na$_2$HPO$_4$, 1.5 mM KH$_2$PO$_4$, pH 7.4) and gradually changed into PBS (137 mM NaCl, 2.7 mM KCl, 4.9 mM Na$_2$HPO$_4$, 1.5 mM KH$_2$PO$_4$, pH 7.4). The zygotes were slightly squashed between slide and coverslip to facilitate penetration of antibodies. Samples were treated with PBS containing 5% Triton X-100 for 60 minutes at room temperature and washed with PBS several times. Then they were incubated for 30 minutes in PBS containing 1 mg/ml NaBH$_4$, followed by several rinses in PBS. Samples were incubated for 30 minutes at 30°C in blocking solution (2.5% nonfat milk, 5% normal goat serum and 0.05% NaN$_3$ in PBS). Afterwards they were incubated overnight at 22°C with a polyclonal anti-centrin antibody (a gift from M. Melkonian, University of Cologne, Germany) diluted 1:250 in PBS. After rinsing in PBS, the samples were incubated for 60 minutes at 30°C with an anti-β-tubulin antibody (Amersham Pharmacia, Uppsala, Sweden; diluted 1:50 in PBS), rinsed again in PBS and incubated for 60 minutes at 30°C with rhodamine-conjugated goat anti-rabbit IgG (Bio Source International, Camarillo, CA; diluted 1:50) and FITC-conjugated goat anti-mouse IgG (Bio Source International; diluted 1:50). They were washed with PBS several times, stained for 10 minutes at room temperature with DAPI (4′-6-diamido-2-phenylindole; 0.5 μg/ml in PBS), and rinsed with PBS. Finally, the samples were mounted in Mowiol 4-88 mounting medium containing 0.2% *p*-phenylendiamine (Osborn and Weber, 1982).

Samples were observed on an Olympus epifluorescence microscope (BX50WI-FLA) equipped with differential interference contrast (DIC). Photographs were taken on Tri-X film (Kodak) at ISO 1600 and developed with Super Prodole (Fuji Photo Film). They were then converted to PhotoShop 6.0 format (Adobe Systems) for the final image presentation.

Electron microscopy
Zygotes on the gel support films were rapidly frozen by putting the films with samples into liquid propane previously cooled to –180°C by liquid nitrogen; they were immediately transferred into liquid nitrogen. They were then transferred into cooled acetone (–85°C) containing 2% osmium tetroxide, and were stored at –85°C for about 2 days. After that, samples were kept at –20°C for 2 hours and 4°C for 2 hours. Finally the temperature of the fixative was gradually allowed to rise to room temperature. The samples were then washed with acetone at room temperature several times and embedded in Spurr's epoxy resin on dishes of aluminium foil. When samples were embedded in the resin, the surface of the gel support films with the samples was placed upside-down on the upper surface of the resin. Serial sections were cut using a diamond knife on a Porter-Blum MT-1 ultramicrotome and mounted on formvar-coated slot grids. Sections were stained with uranyl acetate and lead citrate, and observed with

a Hitachi H-300 electron microscope. In this experiment we examined almost all serial sections of each zygote.

Results

Sexual reproduction in *S. lomentaria* is nearly isogamous; the male gametes are slightly smaller than the female gametes, and each of the gametes has only one chloroplast (Nakamura and Tatewaki, 1975; Nagasato et al., 1999b). First, female gametes settle on the substratum; male gametes are attracted by the sexual pheromone and fuse to the female gametes. However, there are no differences in the size and morphology of nuclei and chloroplasts in male and female gametes. When two male gametes fertilize one female gamete (polyspermy), two male nuclei fuse with one female nucleus. Just after fertilization, three chloroplasts and three pairs of centrioles, from two male gametes and one female gamete, can be seen in the zygote. After 6 hours, the zygote starts to germinate and the pair of centrioles derived from the female gamete disappears (Nagasato et al., 1998; Nagasato et al., 2000). As a result, only two pairs of centrioles derived from the two male gametes remain in the polyspermic zygote.

In normal zygotes, in which a single male gamete fertilizes a female gamete, mitosis and cytokinesis could be observed 32-34 hours after fertilization in polyspermic zygotes. Just before mitosis, the two centrosomes (namely, two pairs of centrioles) derived from the male gametes were located at the same side on the long axis of the oblong nucleus, as in normal fertilization (Nagasato et al., 2000), and each of these two centrosomes (Fig. 1A,B, marked by anti-centrin antibody as red dots) duplicated at this site. Each of the four centrosomes migrated to the future mitotic poles (Fig. 1C,D) and spindle MTs developed from them. In polyspermic zygotes, more than two mitotic spindle poles were formed, either four (not shown) or three (Fig. 1E-H). When the tripolar spindle was formed, one of the four centrosomes did not create a discrete spindle pole. Rather, two of the four centrosomes joined as one mitotic pole (Fig. 1E,F), or one centrosome was located away from the spindle (Fig. 1G,H). In the latter case, the isolated centrosome was connected to the spindle by MTs. Chromosomes were arranged at the equators of the spindle, which formed between the centrosomes.

Fig. 2A-C shows ultrastructural images coinciding with the immunofluorescence of Fig. 1H. One of the four centriole pairs was located away from the spindle (Fig. 2C) and chromosomes were arranged at the equators between the other three spindle poles (Fig. 2A-C). MTs from centrosomes elongated towards the chromosomes, and the nuclear envelope remained intact, except at the poles. A Golgi body was always observed in the vicinity of the centrioles in *Scytosiphon* cells.

At anaphase, masses of daughter chromosomes separated towards the centrosomes. As a result of having multipolar spindles, three or four daughter nuclei were formed (Fig. 3A,B). Daughter nuclei were lined up along the growth axis and cytokinesis followed. Cell division planes are perpendicular to the growth axis. The following three patterns of cytokinesis in the polyspermic zygotes of *S. lomentaria* were discernible. First, three cell division planes were formed and four daughter cells were produced. Each cell contained a nucleus and a centrosome (Fig. 3C,D). Second, two cell division planes were formed and three daughter cells were produced. Each cell contained a nucleus and a MT focus, but one of the three cells contained two centrosomes, which were marked by the anti-centrin antibody as two spots (Fig. 3E,F). However, the centrosomes were close to each other and functioned as one MTOC. Third, three cell division planes were formed and four cells were produced. Although, one of the four cells did not have a nucleus, a centrosome was present in all four daughter cells (Fig. 3G,H). Therefore, in all patterns of cytokinesis in polyspermic zygote, each daughter cell always contained a centrosome.

In normal fertilization of *Scytosiphon*, two chloroplasts, one from the male and one from the female gamete, are donated to the zygote. In the first cell cycle, chloroplast division did not occur in the zygote, and each chloroplast is distributed into daughter cells by cytokinesis (Nagasato et al., 1999b; Nagasato et al., 2000). In polyspermic zygotes, when two male gametes fuse with one female gamete, three chloroplasts are found in the zygote. We examined the distribution of three chloroplasts into four daughter cells after the first mitosis of a polyspermic
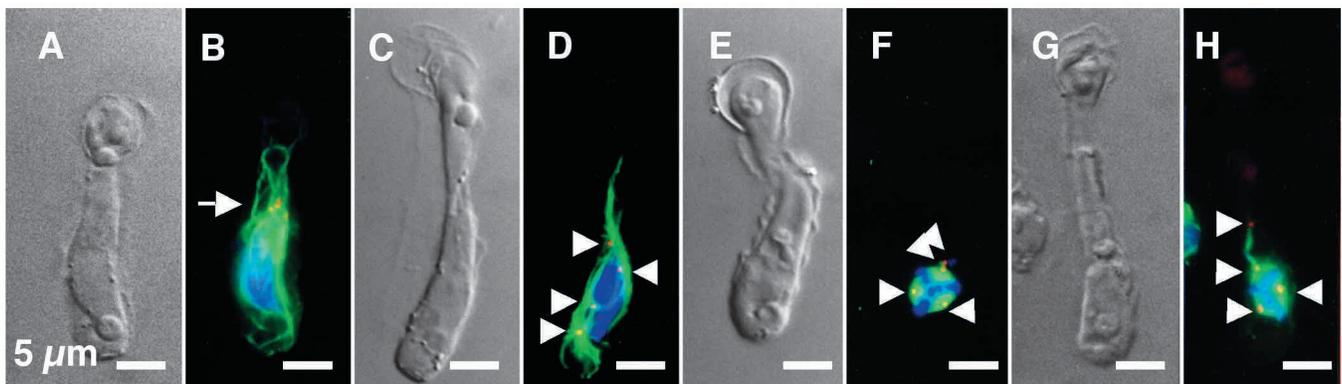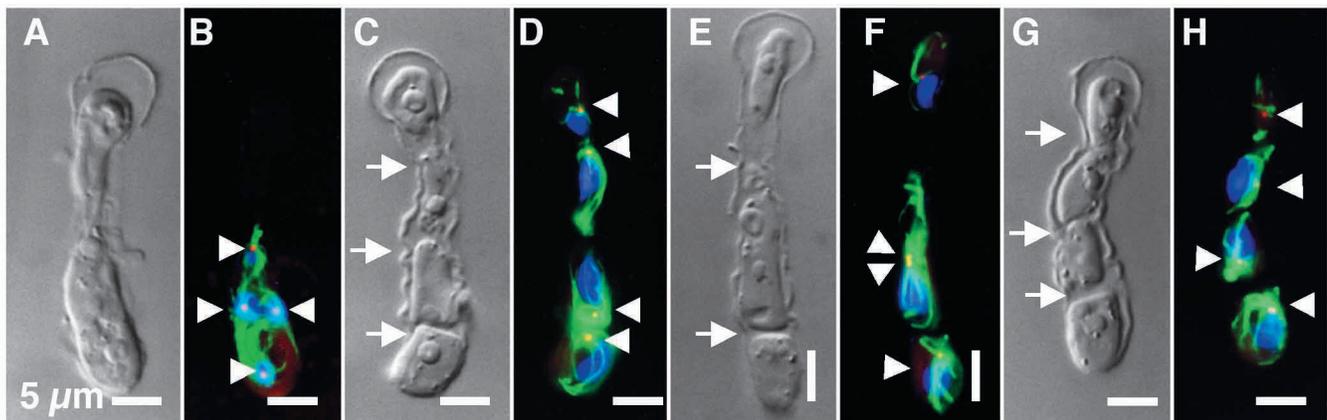

Fig. 1. Immunofluorescence images of spindle formation in polyspermic zygotes of *S. lomentaria*. (A,C,E,G) DIC images; (B,D,F,H) merged images of immunolocalization of β-tubulin (green), centrin (red) and DAPI staining (blue). (A,B) Just before mitosis. Two pairs of centrioles derived from male gametes locate at one side of the nucleus and duplicate there. Therefore, four anti-centrin-positive spots can be observed (arrow in B). (C,D) Migration of four centrosomes to the future mitotic poles. Arrowheads in D show anti-centrin-positive spots. (E,F) Tri-polar spindle is formed and one of the three poles has two centrosomes. Arrowheads in F show four anti-centrin positive spots. (G,H) Tri-polar spindle is formed. One of the four centrosomes is positioned away from the spindle. Arrowheads in H show four anti-centrin-positive spots.

zygote. The three chloroplasts did not show any change during mitosis (Fig. 4A-C). All four daughter cells contained a chloroplast even though one cell is produced without a nucleus (Fig. 4D,E). This shows that one of the three chloroplasts must be irregularly divided into two and distributed to the daughter cells.
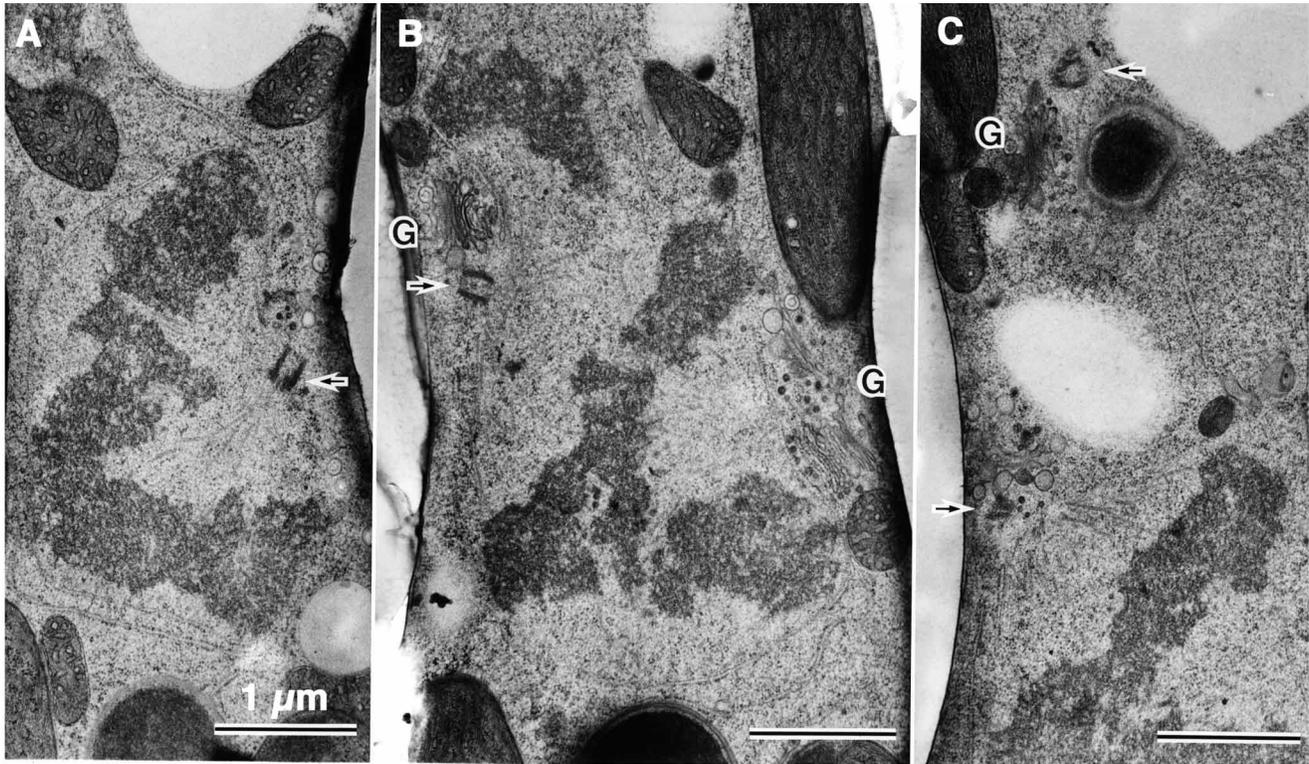
Fig. 2. Ultrastructural images of a multipolar spindle in a polyspermic zygote of *S. lomentaria*. Three sections are shown from the consecutive serial sections of the same cell. The cell is at the same point in mitosis as shown in Fig. 1H. Arrows show centrioles. (A) One pole of the tripolar spindle. Spindle MTs from around the centriole (arrow), elongate towards the chromosomes and become arranged at equators of the multipolar spindle. The nuclear envelope is almost intact, except at the spindle poles. (B) Another spindle pole (arrow). Golgi bodies (G) exit at nearby centrioles. (C) One centrosome is located away from the spindle. The Golgi body is close to the centrosome. The bottom arrow shows the same mitotic pole as in B.

Fig. 3. Immunofluorescence images of cytokinesis in polyspermic zygotes of *S. lomentaria*. (A,C,E,G) DIC images; (B,D,F,H) merged images of immunolocalization of β-tubulin (green), centrin (red) and DAPI staining (blue). (A,B) Anaphase of a multipolar spindle. Four daughter nuclei are formed. Arrowheads in B show four anti-centrin-positive spots. (C,D) Four daughter cells are produced. Each of the four daughter cells contains one nucleus and one centrosome. Arrows in C show cytokinetic planes and arrowheads in D show anti-centrin-positive spots. (E,F) Three daughter cells are produced. Each of the three daughter cells contains one nucleus. One of the three cells contains two centrosomes. These centrosomes are close to each other and function as one MTOC. Arrows in E show cytokinetic planes and arrowheads in F show anti-centrin-positive spots. (G,H) Four daughter cells are produced. Each of the four daughter cells contains one centrosome. The top cell has no nucleus. Arrows in G show cytokinetic planes and arrowheads in H show anti-centrin-positive spots.

Cytokinesis of polyspermic zygotes was observed by TEM. Three or four daughter cells were produced in polyspermic zygotes. Therefore, cytokinesis occurred at two or three cell division planes, but cytokinesis did not initiate and proceed simultaneously in each division plane (Fig. 5A). In one division plane, new cell partition by fusion of vesicles extended to the plasma membrane, while in another plane, there was no fusion of vesicles (Fig. 5A-C). As in the previous study (Nagasato and Motomura, 2002), cytokinesis of *S. lomentaria* proceeded by centrifugal growth of a membranous cell partition combined with fusion of Golgi-derived globular vesicles and tubular cisternae (Fig. 5B). As mentioned above, each of four daughter cells in polyspermy had a chloroplast; therefore, one of three chloroplasts must have been bisected. Fig. 5C,D shows a bisecting chloroplast accompanied by cell plate formation. In these figures there is a pair of centrioles near the pyrenoid at the edge of the chloroplast, Golgi-derived globular vesicles and tubular cisternae are gathered at the future cytokinetic plane, and the chloroplasts are positioned across this cytokinetic plane. Finally, the chloroplast was bisected by cytokinesis. Therefore, in polyspermic zygotes that divide into four daughter cells, one of the three chloroplasts is bisected during progression of cytokinesis and is distributed into the daughter cells.

Fig. 6 shows the mitosis and cytokinesis patterns of polyspermic zygotes of *S. lomentaria* and the behavior of the nucleus, the chloroplast and the centrosome after fertilization.

Discussion

The mechanism of cytokinesis in brown algae, especially when and how the cytokinetic plane will be determined, has been unclear. Brown algal cells have no characteristic cytoplasmic structures participating in cytokinesis, such as the PPB and phragmoplast in land plants or the contractile actin ring in animal cells. There have been several reports that the final position of the mitotic spindle may define the plane of cell division in brown algae, using the fucoid algae *Fucus* and *Pelvetia* (Kropf et al., 1990; Allen and Kropf, 1992; Shaw and

Quatrano, 1996; Bisgrove and Kropf, 1998; Bisgrove and Kropf, 2001). In these brown algae, early zygotes are spherical; afterwards, a rhizoid emerges during gemination, and the cell division plane always forms perpendicular to the cell growth axis. Bisgrove and Kropf examined the orientation of the centrosomes before and during mitosis in relation to the cell growth axis in *Pelvetia* (Bisgrove and Kropf, 1998). Duplicated centrosomes migrate to the opposite sides of the nucleus before mitosis. At this time, the centrosomal axis is randomly aligned perpendicular to the growth axis; however, the spindle finally becomes aligned with the growth axis during mitosis. If misorientation of the spindle position occurs, the cytokinetic plane is never perpendicular to the cell growth axis (Shaw and Quatrano, 1996; Bisgrove and Kropf, 1998). Bisgrove and Kropf suggested that centrosomal rotation is necessary to move the mitotic spindle position to ensure the correct cytokinetic plane, because cytokinesis takes place through the middle plane of the spindle, as in animal cells (Bisgrove and Kropf, 1998; Rappaport, 1986; Satterwhite and Pollard, 1992).

Cytokinesis in brown algae starts after completion of mitosis, as shown by ultrastructural studies (Rawlence, 1973; Markey and Wilce, 1975; Brawley et al., 1977; La Claire, 1982; Katsaros et al., 1983; Katsaros and Galatis, 1988; Katsaros and Galatis, 1992; Nagasato and Motomura, 2002). Therefore there is no spatial and temporal relationship between the mitotic spindle and cytokinesis in brown algae. In this study, we examined the spindle formation and cytokinesis using polyspermic zygotes of *S. lomentaria*. As a result, we have evidence that the spindle orientation does not participate in the determination of the future cytokinetic planes. First, the cytokinetic plane is always perpendicular to the cell growth axis, irrespective of the spindle orientation. Even when a multipolar spindle was formed, two or three cytokinetic planes were produced after the daughter nuclei were rearranged along the long axis of polyspermic zygotes. Second, the cytokinetic planes were always positioned between each centrosome even when one centrosome was located away from the spindle. Therefore, the determination of the cytokinetic plane depends
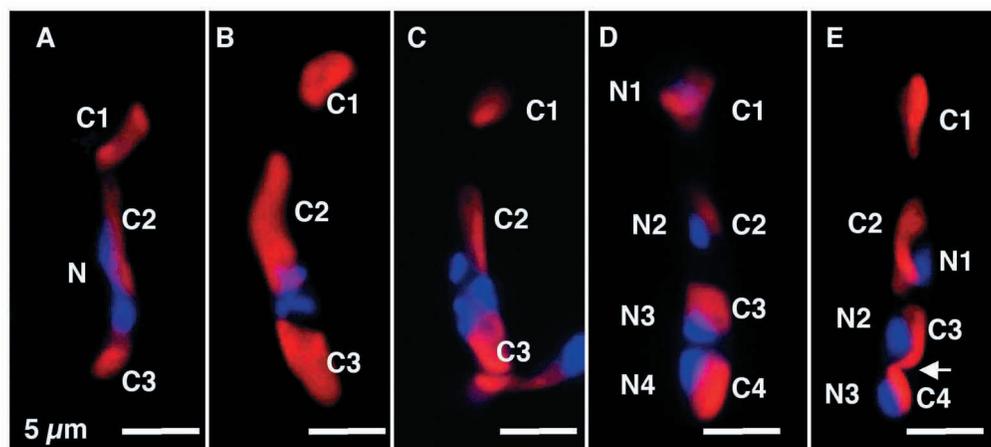
Fig. 4. Fluorescence images of chloroplasts and nuclei in polyspermic zygotes. Red shows autofluorescence of chlorophyll in chloroplasts and blue shows DNA staining with DAPI. (A) Before mitosis; there are three chloroplasts (C1-C3) and a pre-mitotic nucleus (N). (B) Metaphase. (C) Anaphase; three daughter chromosome masses are observed. Three chloroplasts do not show any change. (D) Four daughter cells are produced. Each of the cells has a nucleus and a chloroplast. (E) Three daughter nuclei are produced. One of the three chloroplasts (arrow) is constricted and there are two nuclei near it. There is no nucleus next to the top chloroplast (C1).
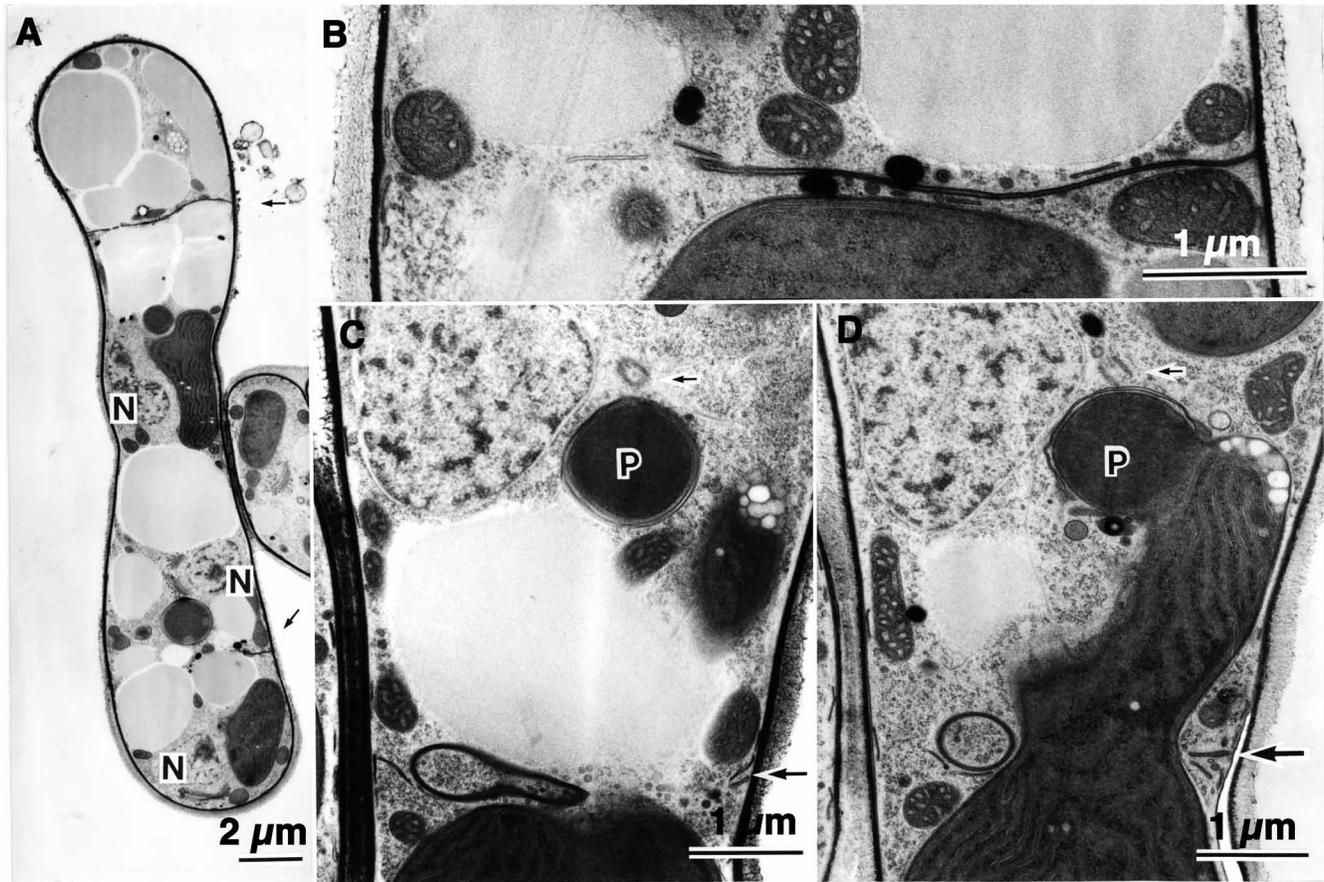
Fig. 5. Ultrastuctural images of cytokinesis in a polyspermic zygote of *S. lomentaria*. These sections are shown from the consecutive serial sections of the same cell. (A) This polyspermic germinate has four nuclei after mitosis, and three nuclei (N) are observed in this section. Cell divisions (arrows) do not proceed simultaneously; cytokinesis proceeds further at the upper position than at the lower one. (B) Magnified image of the nascent membranous cell partition seen at the lower arrow in another section (A). Golgi-derived globular vesicles and flat tubular vesicles can be observed near the membranous cell partition. Note that one edge of the cell partition is connected to the plasma membrane. (C,D) The other cell division plane. Vesicle accumulation and fusion during cytokinesis progress add one chloroplast.

on the centrosomal position after mitosis, rather than the mitotic spindle orientation.

We conclude that the cytoplasmic plane is not determined during mitosis in brown algae. The spindle orientation does not affect the subsequent cytokinetic plane, as in zygotes of *Sargassum confusum* (Tahara and Shimotomai, 1926; Nagasato et al., 2001). *Sargassum confusum* is a member of the Fucales, and the mature egg characteristically contains eight nuclei located around its periphery. After plasmogamy, the sperm nucleus fuses with one of the eight nuclei, and the first spindle of metaphase is formed there. Afterwards, each daughter nucleus migrates longitudinally to the opposite sides of the zygote during anaphase and telophase, and cytokinesis occurs at the middle plane of both daughter nuclei.

It is well known that cytokinesis in the brown algae is completely inhibited by cytochalasin B and D (Brawley and Robinson, 1985; Kropf et al., 1990; Karyophyllis et al., 2000). Recently, it was confirmed that Golgi-derived vesicles are involved in the progression of cytokinesis of *Fucus* by using brefeldin A, which blocked Golgi-mediated secretion (Shaw and Quatrano, 1996; Belanger and Quatrano, 2000). By electron microscopy using freeze substitution we previously showed that cytokinesis of *Scytosiphon* is initiated between daughter nuclei and expands by fusion of Golgi vesicles transported by MTs from centrosomes and tubular cisternae; there is no furrowing of the plasma membrane (Nagasato and Motomura 2002). Karyophyllis et al. observed the MF array during cytokinesis of *Sphacelaria* using rhodamine-phalloidin staining (Karyophyllis et al., 2000). They reported that the actin filaments were organized into the disk structure, where the new cell partition will be formed. They also observed that, in cells that were treated by cytochalasin, cytokinesis was inhibited and the calcofluor-stained wall was accumulated as a ring at the periphery of the possible cytokinetic plane. Based on these observations, we argue that the cytokinetic plane of brown algae is primarily determined by MTs from both centrosomes after mitosis, where MTs from the centrosomes interact (Karyophyllis et al., 2000; Nagasato and Motomura, 2002). Golgi vesicles and tubular cisternae accumulate at the cytokinetic plane by these MTs. Further, MF as a disk structure will be necessary to transport these vesicles and cisternae to the central part of the cytoplasm between daughter nuclei, where the vesicles and cisternae fuse together. In addition, the MF would have a role in the expansion of the new cell partition.

Lastly, we must mention the distribution of chloroplasts in the polyspermic zygotes of *Scytosiphon*. Motomura et al.
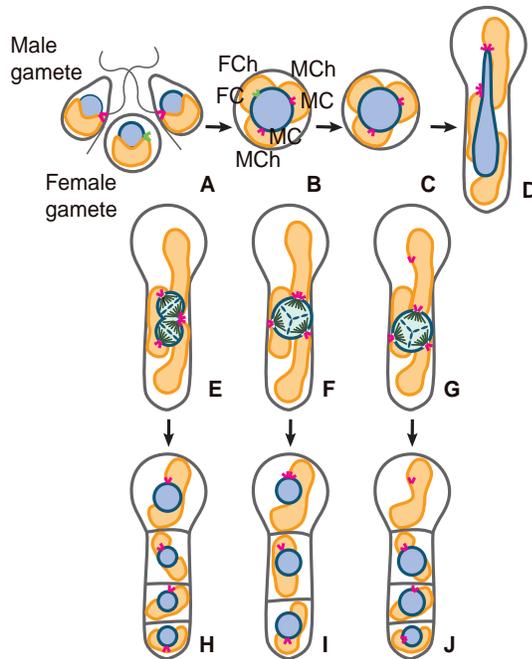
Fig. 6. The patterns of spindle formation and subsequent cytokinesis in polyspermic zygotes of *S. lomentaria* after fertilization. (A-D) Fertilization and germination. (A) Two male gametes fuse to one female. (B) Just after fertilization, there are three chloroplasts and three pairs of centrioles. (C) Female centrioles disappear and two pairs of centrioles from male gametes remain. (D) Before mitosis, the two pairs of centrioles duplicate into four. (E-G) Spindle formation in polyspermic zygotes. (E) Tetra-polar spindle. (F) Tri-polar spindle. One of the three poles contains two pairs of centrioles. (G) Tri-polar spindle. One of the four pairs of centrioles locates away from spindle. (H-I) Cytokinesis in polyspermic zygotes. (H) Four daughter cells are produced. Each cell contains a nucleus, a centrosome and a chloroplast. (I) Three daughter cells are produced. Each cell contains a nucleus and a chloroplast. All cells have a centrosome, and one cell contains two centrosomes. At that time, two centrosomes adjoin and function as one MTOC. (J) Four daughter cells are produced. Each cell contains a centrosome and a chloroplast. Sometimes, a cell is produced without a nucleus. FC, centrioles derived from female gamete; FCh, chloroplast from female gamete; MC, centrioles derived from male gamete; MCh, chloroplast from male gamete.

reported that each chloroplast becomes adjacent to each nucleus with mediation of a centrosome, after meiosis in zoosporogenesis of *Laminaria angustata* (Motomura et al., 1997). Subsequently, the ratio between chloroplasts and nuclei is maintained at 1: 1, and chloroplast division invariably occurs before mitosis. Each centrosome is always located adjacent to a chloroplast and, before mitosis, it duplicates and migrates to either side of the chloroplast. *Toxoplasma gondii*, an Apiconplexan parasite, has a single non-photosynthetic plastid (apicoplast). Striepen et al. confirmed that the centrosome is always close to the apicoplast throughout the cell cycle (Striepen et al., 2000). Moreover, it is well known that, during mitosis and meiosis of monoplastidic cells in lower land plants that do not have the centrosomes, the mitotic spindle directly develops from both tips of the plastid (Brown and Lemmon, 1984; Brown and Lemmon, 1990; Brown and Lemmon, 1997). In these cases, the mitotic poles associate with plastids directly or by mediation of centrosomes, and ensure the precise

distribution of plastids into daughter cells during nuclear division. In this study, we observed that one of the three chloroplasts was bisected when four daughter cells were produced by polyspermy. In contrast to the above three cases, the chloroplast appeared to be bisected during cytokinesis in polyspermic zygotes of *S. lomentaria*, not by autonomous division of the chloroplast. Cells of the Scytosiphonaceae have only one chloroplast with a pyrenoid, which is a taxonomic character of this family (Clayton and Beakes, 1983). We believe that a physical relationship between centrosome and chloroplasts occurs after mitosis in *S. lomentaria* and, in some cases, one chloroplast is associated with two centrosomes in polyspermic zygotes. As a result, bisection of chloroplasts would be caused by cytokinesis.

In conclusion, this study shows that the cytokinetic plane in brown algal cells is determined by MTs from centrosomes after mitosis, and not by the orientation of the mitotic spindle. These MTs could transport Golgi vesicles to the future cytokinetic plane. Therefore, the position of centrosomes after mitosis will influence the position of the cytokinetic plane, without specialized structures such as the PPB band in land plant cells and contractile ring in animal cells.

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