



## The first spindle formation in brown algal zygotes

Taizo Motomura\* & Chikako Nagasato

Muroran Marine Station, Field Science Center for Northern Biosphere, Hokkaido University,

Muroran 051-0003, Japan

\*Author for correspondence; E-mail: motomura@bio.sci.hokudai.ac.jp

**Key words:** anisogamy, brown algae, centriole, centrosome, fertilization, isogamy, mitotic spindle, oogamy

### Abstract

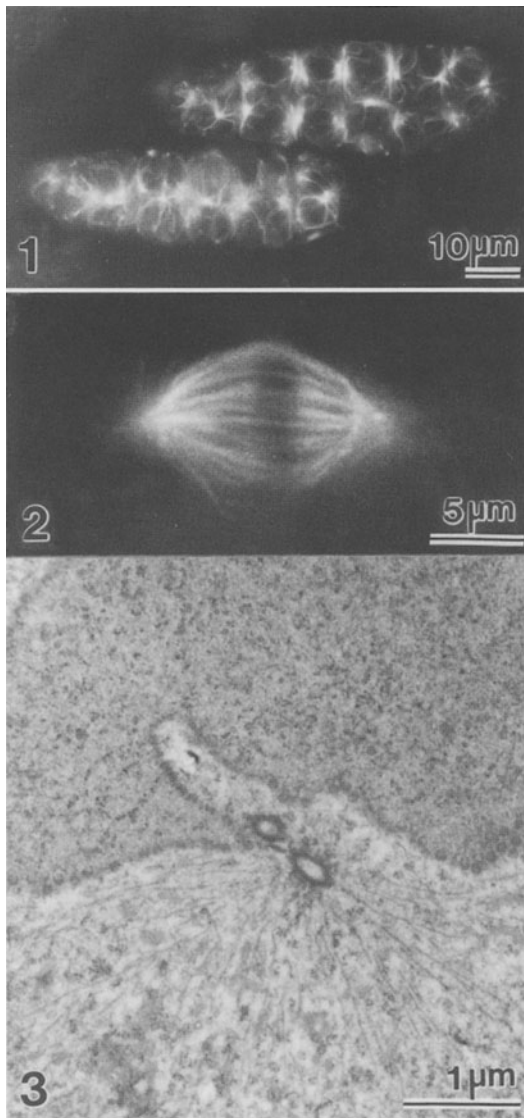
Regulation of the first spindle formation in brown algal zygotes was described. It is well known that there are three types of sexual reproduction in brown algae; isogamy, anisogamy and oogamy. Paternal inheritance of centrioles can be observed in all these cases, similar to animal fertilization. In isogamy and anisogamy, female centrioles (= flagellar basal bodies) selectively disappear and male centrioles remain after fertilization. In a typical oogamy (e.g. furoid members), liberated egg does not have centrioles, and sperm centrioles are introduced in zygote. Participation of sperm centrioles to the spindle formation in zygotes was also described using *Fucus distichus* as a model system. Sperm centrioles function as a part of centrosome, namely microtubule organizing center, in zygote. Therefore, they have a crucial role in the spindle formation. Observations on the spindle formation in polygyny and karyogamy-blocked zygotes strongly suggest that egg nucleus can form a mitotic spindle by itself without centrosome, even though the resulting spindles are of abnormal shapes.

### Introduction

Mitosis in brown algae has been observed in detail by using electron microscopy; for example, plurilocular sporangia of *Pylaiella littoralis* (L.) Kjellm. (Markey & Wilce, 1975), apical cells of *Sphacelaria tribuloides* Meneghini (Katsaros et al., 1983), *Halopteris filicina* (Grateloup) Kützing (Katsaros & Galatis, 1990), *Zonaria farlowii* S. and G., *Dictyopteris zonarioides* Farlow, *Padina pavonia* (L.) Gaill., and *Dictyota dichotoma* (Hudson) L. (Neushul & Dahl, 1972), vegetative cells of *Dictyota dichotoma* (Katsaros & Galatis, 1985) and *Dictyopteris membranacea* (Stackh.) Batt. (Katsaros & Galatis, 1988), male gametangia of *Cutleria hancockii* Dawson (La Claire & West, 1979), trichothallic meristem of *Cutleria cylindrica* Okamura (La Claire, 1982) and *Carpomitra cabreræ* Kützing (Motomura & Sakai, 1985), and antheridium of *Fucus serratus* L. (Berkaloff & Rousseau, 1979). It becomes apparent that a pair of centrioles exists in interphase cells of brown algae, and it duplicates and each of them migrates to both poles prior to mitosis. Moreover, recent immunofluorescence studies using anti-tubulin antibody (Motomura, 1991; Katsaros, 1992; Katsaros & Galatis, 1992; Rusig et

al., 1993; 1994) have clearly shown that microtubule (MT) cytoskeleton in brown algal cells is quite different from that of green algae and land plants (Lloyd, 1991). Characteristic MT arrays like cortical MTs during interphase and preprophase band just before mitosis in land plant cells are not detected. Almost all MTs elongate from the defined centrosome, which is composed of a pair of centrioles and pericentriolar materials, and located near to the nucleus (Figs 1–3). Therefore, in this respect, centrosome structure and MT cytoskeleton in brown algal cells are similar to those in animal cells (Wheatley, 1982; Kimble & Kuriyama, 1992; Balczon, 1996).

Different from the vegetative cells, a pair of centrioles changes into flagellar basal bodies in swimmers in the brown algae, i.e. gametes and zoospores. Characteristic, well-regulated MT flagellar rootlets (O'Kelly, 1989) are differentiated, instead of the random MT radiation from the centrosome in vegetative cells. Therefore, it can be speculated that several regulations including centrosome activation must have occurred before the mitosis in zygote after fertilization of male and female gametes. In this paper, we will discuss the regulation of the first spindle formation in brown algal zygotes with regards to centriole and centrosome.



Figures 1–3. Microtubule (MT) cytoskeleton, mitotic spindle and a pair of centrioles in brown algal cells. **1.** MT cytoskeleton of two juvenile sporophytes of *Laminaria angustata*. Note that almost all MTs elongate from centrosome. **2.** Mitotic spindle in metaphase of *Dictyopteris divaricata* cell. Spindle MTs converge to centrosomes at both poles. **3.** A pair of centrioles just nearby a nucleus in *Fucus distichus* zygote. Note that numerous MTs radiate from the periphery of centrioles.

### Paternal inheritance of centrioles in the fertilization of brown algae

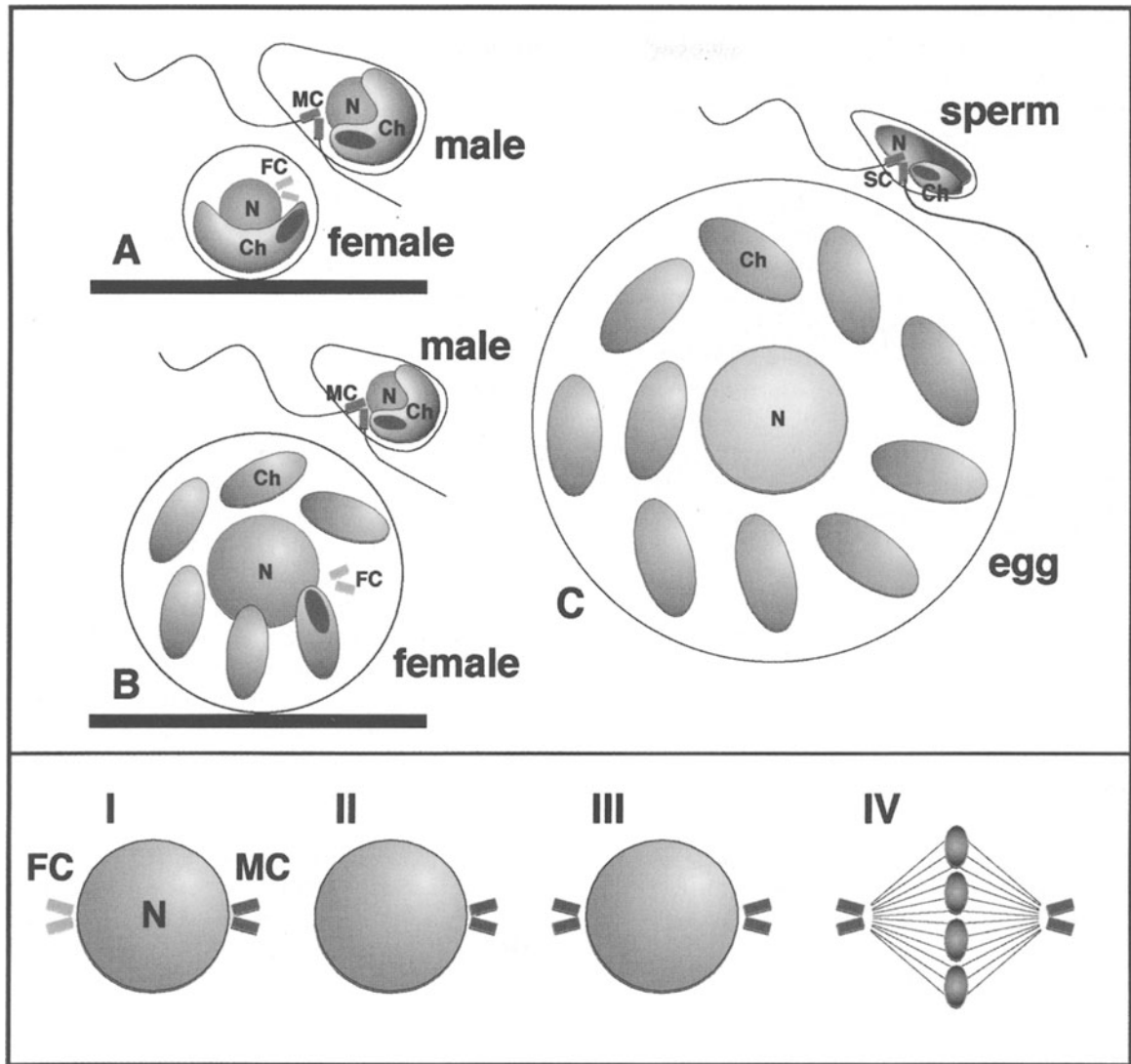
It is well known that in most animal fertilization, centrioles in zygote are derived from sperm (Schatten, 1994). In oogamy in animals, including those in sea urchin, *Drosophila*, *Xenopus*, and mammals,

centrioles disappear in the process of oogenesis. For example, in starfish oocytes, each division pole in meiosis I has a pair of centrioles and each in meiosis II has only one centriole (Sluder et al., 1989; Kato et al., 1990). Contrary to animal fertilization, it is a characteristic feature that three types of sexual reproduction have been confirmed in the brown algae: isogamy, anisogamy and oogamy (Wynne & Loiseaux, 1976). We have examined fertilization in the brown algae and the zygote development using electron and immunofluorescence microscopy, and recently confirmed that paternal inheritance of centrioles is universal in brown algae (Fig. 4; Nagasato et al., 1998), similar to animal fertilization.

In isogamous brown algae, e.g. *Colpomenia bulbosa* (Saunders) Yamada and *Scytosiphon lomentaria* (Lyngbye) Link (Scytosiphonales), one of two pairs of centrioles (= flagellar basal bodies) disappeared in zygote after plasmogamy. In isogamy, it is difficult to distinguish the cell organelle including nucleus, chloroplast, mitochondria and centrioles between male and female gametes. But observations on the polyspermic zygotes showed that only one pair of centrioles disappeared. Therefore, we concluded that centrioles from female gamete selectively disappeared (Motomura, 1992).

In anisogamous brown algae, e.g. *Cutleria cylindrica* Okamura (Cutleriales), one of two pairs of centrioles disappeared after plasmogamy as well as isogamy. In anisogamy, sizes between male and female gametes are quite different. Therefore, we can distinguish male and female nuclei; male nucleus is a little smaller and the chromatins are more condense than those of the female. From observations using electron and immunofluorescence microscopy on *C. cylindrica* zygotes, in which karyogamy had not yet occurred, we confirmed that the centrioles near the male nucleus remained and the ones near the female nucleus disappeared (Nagasato et al., 1998).

In the extremely anisogamous *Laminaria angustata* Kjellman (Laminariales) (because eggs have vestigial flagella, Motomura & Sakai, 1988), there are two pairs of centrioles derived from egg and sperm in zygote. We are able to distinguish egg centrioles and sperm centrioles in the zygote since the sperm flagellar basal bodies (= centrioles) are associated with a characteristic electron dense connecting band (Motomura, 1989). Observation using serial sections on electron microscopy clearly showed that egg centrioles selectively disappeared and sperm centrioles remained in the zygotes (Motomura, 1990).



**Figure 4.** Schematic representation showing the paternal inheritance of centrioles in the fertilization of brown algae. (A) isogamy (e.g. *Colpomenia* and *Scytosiphon*), (B) anisogamy (e.g. *Cutleria*) and (C) oogamy (e.g. *Fucus* and *Pelvetia*). In isogamy and anisogamy, after female gamete settles on substratum and drew the flagella, male gamete approaches the female gamete as it is attracted by the sexual pheromone, and plasmogamy occurs. Therefore, in these cases, two pairs of centrioles (= flagellar basal bodies) from female and male gamete exist in the zygote at first (I). Afterwards, centrioles from female gamete disappear and ones from male gamete remain selectively (paternal inheritance of centrioles, II). In oogamy, egg does not contain centrioles originally, therefore, only a pair of centrioles from sperm exists in the zygote (II). A pair of centrioles from sperm duplicates and migrates to both mitotic poles (III). Finally, bipolar spindle with centrioles at both poles is formed (IV). N, nucleus; Ch, chloroplast; FC, female centrioles; MC, male centrioles; SC, sperm centrioles.

In the typical oogamy (Fucales), e.g. *Fucus distichus* Linnaeus and *Pelvetia fastigiata* (J. Ag.) De Toni, centrioles disappear during oogenesis. They do not exist originally in mature eggs (Motomura, 1994; Bisgrove et al., 1997; Nagasato et al., 1999). Therefore, centrioles in zygotes are introduced as the sperm flagellar basal bodies as that occurring during fertilization in most animals (Schatten, 1994). Of course,

judging from the recent molecular phylogenetic data, we must remind that the typical oogamy in Fucales is not directly derived from isogamy (Scytosiphonales) and anisogamy (Cutleriales and Laminariales).

At present, it is clear that karyogamy does not affect the selective disappearance of female centrioles in *Colpomenia bullosa* (Motomura, 1992) and *Cutleria cylindrica* (Nagasato et al., 1998), but the

morphological, physiological and biochemical differences between female and male centrioles are still obscure. We are now proposing that, in the case of isogamy and anisogamy, plasmogamy would trigger a signal that induces the selective disappearance of female centrioles, because they always disappear 4–8 h after plasmogamy in *C. bullosa* and *C. cylindrica*. Moreover, unfertilized female gametes of *C. cylindrica* can develop parthenogenetically as well as zygotes (Kitayama et al., 1992), and centrioles do not disappear and a normal bipolar spindle is formed during parthenogenetic development of the female gametes (Nagasato et al., 1998). While, in extremely anisogamous *L. angustata*, centrioles disappear in the parthenogenesis of unfertilized eggs. This causes abnormal spindle formation including the monopolar and multipolar ones (Motomura, 1991). Most of these parthenogenetic sporophytes develop abnormally and abort at the early stage (Nakahara, 1984; Motomura, 1991; Lewis et al., 1993).

**Spindle formation in (I) polyspermic zygotes, (II) zygotes from multinucleate eggs, and (III) karyogamy-blocked zygotes in *Fucus distichus***

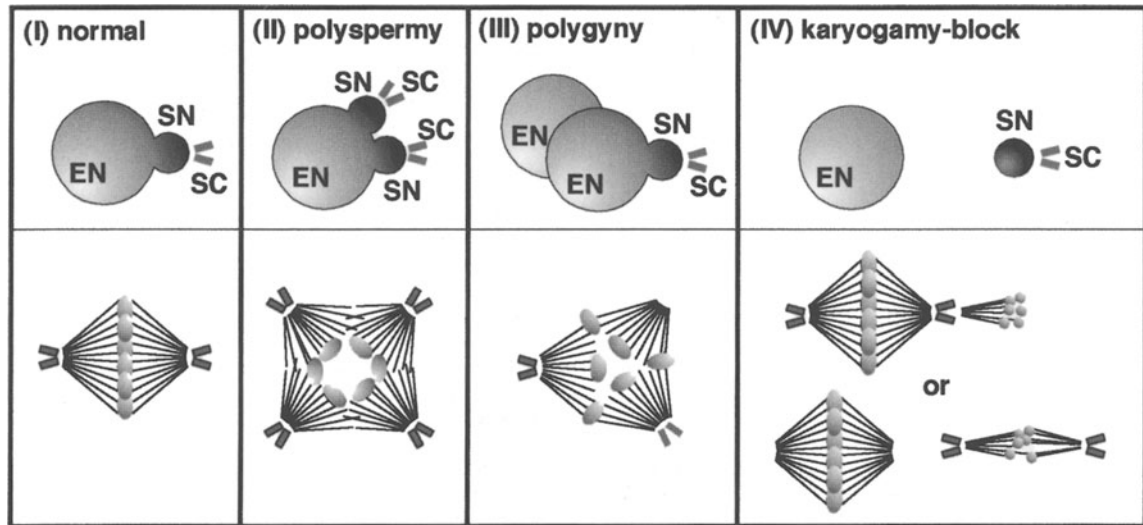
When a normal fertilization occurs in *Fucus distichus*, each of the centrioles derived from sperm flagellar basal bodies separates, migrates to the mitotic poles and duplicates. A normal bipolar spindle (I) is formed and chromosomes are arranged at the equator (Motomura, 1994; Bisgrove et al., 1997). Contrary to the normal fertilized zygotes, the mitotic spindles in (II) polyspermic zygotes, (III) zygotes from multinucleate eggs (polygyny) and (IV) karyogamy-blocked zygotes possess unusual forms as shown in Figure 5 (Nagasato et al., 1999). Using these experimentally induced systems in *Fucus distichus*, we tried to clarify in details the participation of several factors, including sperm centrioles, in the spindle formation.

In polyspermic zygotes, all sperm nuclei that are incorporated into an egg fuse to the egg nucleus and a multipolar spindle is formed. Each sperm brings a pair of centrioles, and each of these centrioles duplicates, migrates to the mitotic poles and participates in spindle formation. Therefore, in the case of polyspermy in *Fucus*, as well as in animal polyspermy (Holy & Schatten, 1991; Navara et al., 1994), the number of mitotic poles is double that of the number of sperm that is incorporated into an egg.

Eight eggs are formed in an oogonium after meiosis and one mitosis in *Fucus*. However, final cytoplasmic cleavage occurs abnormally or not at all, producing multinucleate eggs. When sperms inseminated these multinucleate eggs (polygyny), a sperm nucleus fuse with one of the egg nuclei. The mitotic spindle possesses a multipolar, eccentric form in many cases. Although centrioles derived from the sperm exist at two mitotic poles in the spindle, several supplementary mitotic poles without centrioles exist. As a result, chromosomes cannot be arranged at the equator.

When karyogamy between sperm and egg nuclei is blocked by inhibiting the migration of sperm nucleus with colchicine (Brawley & Quatrano, 1979; Swope & Kropf, 1993; Motomura, 1995), we can see two types of the spindle formation in the egg nucleus. First, the sperm nucleus is located close to the egg nucleus, and sperm centrioles are drawn towards the egg nucleus from the sperm nucleus. In this case, a normal mitotic spindle is formed in the egg nucleus, and the associated MTs elongate from one pole of the spindle towards the scattered sperm chromosomes. Second, the egg and the sperm nuclei are sufficiently separated, and sperm centrioles cannot be drawn to the egg nucleus. In this case, monopolar or bipolar barrel-shaped (this means that spindle MTs do not converge at a spot on both mitotic poles) spindles without centrioles are formed at the egg nucleus. A normal bipolar spindle is not formed on the sperm nucleus, even though two pairs of centrioles are located and MTs radiate from there. Motomura (1995) reported that when karyogamy is blocked, the egg chromosomes condense after DNA synthesis but the sperm chromosomes condense prematurely without DNA synthesis. Therefore, the absence of a normal bipolar spindle on the sperm nucleus might be related to the premature condensation of the sperm chromosomes.

These observations strongly suggest that sperm centrioles certainly have a crucial role in the mitotic spindle formation in *Fucus distichus* zygotes. In contrast, monopolar or bipolar barrel-shaped spindles without centrioles in the egg nucleus of karyogamy-blocked zygotes and several supplementary mitotic poles without centrioles in polygyny are characteristic features. This indicates that the egg nucleus in *Fucus* can form mitotic spindle by itself, even though the shapes are abnormal, irrespective of the participation of centrioles from sperm. We have confirmed that  $\gamma$ -tubulin, which serves as the nucleation of MT growth at the centrosome in eukaryotic cells (Oakley et al., 1990; Joshi et al., 1992), is not localized at



**Figure 5.** Schematic representation showing the spindle formation in (I) normal, (II) polyspermy, (III) polygyny and (IV) karyogamy-block with colchicine in *Fucus distichus*. In normal fertilization, after karyogamy between egg nucleus (EN) and sperm nucleus (SN) and duplication of a pair of centrioles from sperm, a bipolar spindle is formed. In polyspermy, incorporated sperm nuclei fuse to egg nucleus, and a multipolar spindle is formed. In this case, each of the mitotic poles contains a pair of centrioles. In polygyny, sperm nucleus fuses to one among several egg nuclei, and a multipolar spindle is formed. In this case, two mitotic poles contain a pair of centrioles, while the others do not. In the case of karyogamy-block using colchicine, there are basically two types of spindle formation. When centrioles migrate to egg nucleus from sperm nucleus, a normal bipolar spindle is formed at egg nucleus and MTs elongate to sperm nucleus from one mitotic pole. When centrioles do not migrate to egg nucleus, barrel-shaped bipolar or monopolar spindle without centrioles is formed at egg nucleus. Spindle-like MT array is formed at sperm nucleus. EN, egg nucleus; SN, sperm nucleus; SC, sperm centrioles.

these mitotic poles without centrioles (Nagasato et al., unpublished data). Recently, it is becoming clear that non-centrosomal proteins, NuMA (nuclear protein associated with a mitotic apparatus), dynactin and cytoplasmic dynein, are essential in organizing the minus ends of MTs for spindle formation (Gaglio et al., 1997; Heald et al., 1997; Compton, 1998). Therefore, we are now considering that the formation of the mitotic spindle poles without centrosomes in the egg nucleus of *Fucus distichus* would be induced by these noncentrosomal proteins which are hidden behind the luster of the centrosomes.

### Acknowledgements

We express our thanks to C. S. Vairappan, Hokkaido University for his critical reading of the manuscript. This study was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan (11874121 and 12440232). Chikako Nagasato was supported by the Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists.

### References

- Balczon, R., 1996. The centrosome in animal cells and its functional homologs in plant and yeast cells. *Int. Rev. Cytol.* 169: 25–82.
- Berkaloff, C. & B. Rousseau, 1979. Ultrastructure of male gametogenesis in *Fucus serratus* (Phaeophyceae). *J. Phycol.* 15: 163–173.
- Bisgrove, S. R., C. Nagasato, T. Motomura & D. L. Kropf, 1997. Immunolocalization of centrin during fertilization and the first cell cycle in *Fucus distichus* and *Pelvetia compressa* (Fucales, Phaeophyceae). *J. Phycol.* 33: 823–829.
- Brawley, S. H. & R. S. Quatrano, 1979. Effects of microtubule inhibitors on pronuclear migration and embryogenesis in *Fucus distichus* (Phaeophyta). *J. Phycol.* 15: 266–272.
- Compton, D. A., 1998. Focusing on spindle poles. *J. Cell Sci.* 111: 1477–1481.
- Gaglio, T., M. A. Dionne & D. A. Compton, 1997. Mitotic spindle poles are organized by structural and motor proteins in addition to centrosomes. *J. Cell Biol.* 135: 399–414.
- Heald, R., R. Tournebise, A. Habermann, E. Karsenti & A. Hyman, 1997. Spindle assembly in *Xenopus* egg extracts: Respective roles of centrosomes and microtubule self-organization. *J. Cell Biol.* 138: 615–628.
- Holy, J. & G. Schatten, 1991. Spindle pole centrosomes of sea urchin embryos are partially composed of material recruited from maternal stores. *Dev. Biol.* 147: 343–353.
- Joshi, H. C., M. J. Palacios, L. McNamara & D. W. Cleveland, 1992.  $\gamma$ -tubulin is a centrosomal protein required for cell cycle-dependent microtubule nucleation. *Nature* 356: 80–83.

- Kato, K. H., S. Washitani-Nemoto, A. Hino & S. Nemoto, 1990. Ultrastructural studies on the behavior of centrioles during meiosis of starfish oocytes. *Dev. Growth and Differ.* 32: 41–49.
- Katsaros, C., 1992. Immunofluorescence study of microtubule organization in some polarized cell types of selected brown algae. *Bot. Acta* 105: 400–406.
- Katsaros, C. & B. Galatis, 1985. Ultrastructural studies on thallus development in *Dictyota dichotoma* (Phaeophyta, Dictyotales). *Br. Phycol. J.* 20: 263–276.
- Katsaros, C. & B. Galatis, 1988. Thallus development in *Dictyopteris membranacea* (Phaeophyta, Dictyotales). *Br. Phycol. J.* 23: 71–88.
- Katsaros, C. & B. Galatis, 1990. Thallus development in *Halopteris filicina* (Phaeophyceae, Sphacelariales). *Br. Phycol. J.* 25: 63–74.
- Katsaros, C. & B. Galatis, 1992. Immunofluorescence and electron microscope studies of microtubule organization during the cell cycle of *Dictyota dichotoma* (Phaeophyta, Dictyotales). *Protoplasma* 169: 75–84.
- Katsaros, C., B. Garatis & K. Mitrakos, 1983. Fine structural studies on the interphase and dividing apical cells of *Sphacelaria tribuloides* (Phaeophyta). *J. Phycol.* 19: 16–30.
- Kimble, M. & R. Kuriyama, 1992. Functional components of microtubule-organizing centers. *Int. Rev. Cytol.* 136: 1–50.
- Kitayama, T., H. Kawai & T. Yoshida, 1992. Dominance of female gametophytes in field populations of *Cutleria cylindrica* (Cutleriales, Phaeophyceae) in the Tsugaru Strait, Japan. *Phycologia* 31: 449–461.
- La Claire, J. W., II, 1982. Light and electron microscopic studies of growth and reproduction in *Cutleria* (Phaeophyta). III. Nuclear division in the trichothallic meristem of *C. cylindrica*. *Phycologia* 21: 273–287.
- La Claire, J. W. II & J. A. West, 1979. Light- and electron-microscopic studies of growth and reproduction in *Cutleria* (Phaeophyta). II. Gametogenesis in the male plant of *C. hancockii*. *Protoplasma* 101: 247–267.
- Lewis, R. J., B. Jiang, Y. M. Neushul & X. G. Fei, 1993. Haploid parthenogenetic sporophytes of *Laminaria japonica* (Phaeophyceae). *J. Phycol.* 29: 363–369.
- Lloyd, C. W., 1991. The Cytoskeletal Basis of Plant Growth and Form. Academic Press, London: 330 pp.
- Markey, D. R. & R. T. Wilce, 1975. The ultrastructure of reproduction in the brown alga *Pylaiella littoralis*. I. Mitosis and cytokinesis in the plurilocular gametangia. *Protoplasma* 85: 219–241.
- Motomura, T., 1989. Ultrastructural study of sperm in *Laminaria angustata* (Laminariales, Phaeophyta), especially on the flagellar apparatus. *Jpn. J. Phycol.* 37: 105–116.
- Motomura, T., 1990. Ultrastructure of fertilization in *Laminaria angustata* (Phaeophyta, Laminariales) with emphasis on the behavior of centrioles, mitochondria and chloroplasts of the sperm. *J. Phycol.* 26: 80–89.
- Motomura, T., 1991. Immunofluorescence microscopy of fertilization and parthenogenesis in *Laminaria angustata* (Phaeophyta). *J. Phycol.* 27: 248–257.
- Motomura, T., 1992. Disappearance of centrioles derived from female gametes in zygotes of *Colpomenia bulbosa* (Phaeophyceae). *Jpn. J. Phycol.* 40: 207–214.
- Motomura, T., 1994. Electron and immunofluorescence microscopy on the fertilization of *Fucus distichus* (Fuciales, Phaeophyceae). *Protoplasma* 178: 97–110.
- Motomura, T., 1995. Premature chromosome condensation of the karyogamy-blocked sperm pronucleus in the fertilization of *Fucus distichus* (Fuciales, Phaeophyceae). *J. Phycol.* 31: 108–113.
- Motomura, T. & Y. Sakai, 1985. Ultrastructural studies on nuclear division in the sporophyte of *Carpomitra cabreriae* (Clemente) Kützinger (Phaeophyta, Sporochneales). *Jpn. J. Phycol.* 33: 199–209.
- Motomura, T. & Y. Sakai, 1988. The occurrence of flagellated eggs in *Laminaria angustata* (Phaeophyta, Laminariales). *J. Phycol.* 24: 282–285.
- Nagasato, C., T. Motomura & T. Ichimura, 1998. Selective disappearance of maternal centrioles after fertilization in the anisogamous brown alga *Cutleria cylindrica* (Cutleriales, Phaeophyceae): paternal inheritance of centrioles is universal in the brown algae. *Phycol. Res.* 46: 191–198.
- Nagasato, C., T. Motomura & T. Ichimura, 1999. Influence of centriole behavior on the first spindle formation in zygotes of the brown alga *Fucus distichus* (Fuciales, Phaeophyceae). *Dev. Biol.* 208: 200–209.
- Nakahara, H., 1984. Alternation of generations of some brown algae in unialgal and axenic culture. *Sci. Pap. Inst. Algal. Res. Fac. Sci. Hokkaido Univ.* 7: 77–194.
- Navara, C. S., N. L. First & G. Schatten, 1994. Microtubule organization in the cow during fertilization, polyspermy, parthenogenesis, and nuclear transfer: the role of the sperm aster. *Dev. Biol.* 162: 29–40.
- Neushul, M. & A. L. Dahl, 1972. Ultrastructural studies of brown algal nuclei. *Am. J. Bot.* 59: 401–410.
- Oakley, B. R., C. E. Oakley, Y. Yoon & M. K. Jung, 1990.  $\gamma$ -tubulin is a component of the spindle pole body that is essential for microtubule function in *Aspergillus nidulans*. *Cell* 61: 1289–1301.
- O'Kelly, C. J., 1989. The evolutionary origin of the brown algae: information from studies of motile cell ultrastructure. In Green, J. C., B. S. C. Leadbeater & W. L. Diver (eds), *The Chromophyte Algae. Problems and Perspectives*. Oxford University Press, Oxford: 55–278.
- Rusig, A. M., H. Le Guyader & G. Ducreux, 1993. Microtubule organization in the apical cell of *Sphacelaria* (Phaeophyceae) and its related protoplast. *Hydrobiologia* 260/261: 167–172.
- Rusig, A. M., H. Le Guyader & G. Ducreux, 1994. Dedifferentiation and microtubule reorganization in the apical cell protoplast of *Sphacelaria* (Phaeophyceae). *Protoplasma* 179: 83–94.
- Schatten, G., 1994. Centrosome and its mode of inheritance: the reduction of the centrosome during gametogenesis and its restoration during fertilization. *Dev. Biol.* 165: 299–335.
- Sluder, G. F., F. J. Miller, K. Lewis, E. D. Davidson & C. L. Rieder, 1989. Centrosome inheritance in starfish zygotes: Selective loss of the maternal centrosome after fertilization. *Dev. Biol.* 131: 567–579.
- Swope, R. E. & D. L. Kropf, 1993. Pronuclear positioning and migration during fertilization in *Pelvetia*. *Dev. Biol.* 157: 269–276.
- Wheatley, D. N., 1982. *The Centriole: A Central Enigma of Cell Biology*. Elsevier Biomedical Press, Amsterdam.
- Wynne, M. J. & S. Loiseaux, 1976. Recent advances in life history studies of the Phaeophyta. *Phycologia* 15: 435–452.