Cytoskeleton and Morphogenesis in Brown Algae

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INTRODUCTION

Morphogenesis, according to the etymology of the term [Greek morphi (= shape) + genesis (= creation)], is a highly controlled process by which the enormous variability of shapes of organisms arises. Since the first research studies in the plant kingdom, the great variety of forms occurring in algae made them a precious source of model systems for basic research of both morphological and physiological importance.

Brown algae constitute a large group, which occupies a particular position in the evolutionary process. Their unique characteristics distinguish them from other classes within Heterokontophyta. The brown algal cell also shows particular characteristics in structure, cytoskeleton organization and division process. All the above make brown algae a very interesting model for many morphogenetical studies (Katsaros, 1995, 2001).

The role of the cytoskeleton in cell wall morphogenesis is well established in higher plants (Fowler and Quatrano, 1997; Baskin, 2001; Hasezawa and Kumagai, 2002; Smith, 2003; Wasteneys, 2004). Cortical microtubules (MTs) have been shown to determine directly (Burk and Ye, 2002) or indirectly microfibril (MF) orientation in higher plant cells, possibly defining the distribution of cellulose synthesizing enzymes in the plasmalemma (Tsekos, 1999; Baskin, 2001; Hasezawa and Kumagai, 2002). This is also the case in algae possessing cortical MTs (Mizuta, 1992; Tsekos, 1999). Contrary to the above, brown algal cells lack a cortical MT cytoskeleton and are characterized by centriole-bearing centrosomes, which function as microtubule organizing centres. Extensive electron microscope and immunofluorescence studies of MT organization in different types of brown algal cells have shown that MTs constitute a major cytoskeletal component, indispensable for cell morphogenesis. Apart from participating in mitosis and cytokinesis, they are also involved in the expression and maintenance of polarity of particular cell types. Disruption of MTs after Nocodazole treatment inhibits cell growth, causing bulging and/or bending of apical cells, thickening of the tip cell wall, and affecting the nuclear positioning. Staining of F-actin using Rhodamine-Phalloidin, revealed a rich network consisting of perinuclear, endoplasmic and cortical AFs. AFs participate in mitosis by the organization of an F-actin spindle and in cytokinesis by an F-actin disc. They are also involved in the maintenance of polarity of apical cells, as well as in lateral branch initiation. The cortical system of AFs was found related to the orientation of cellulose microfibrils (MFs), and to cell wall morphogenesis. This is expressed by the coincidence in the orientation between cortical AFs and the depositing MFs. Treatment with cytochalasin B inhibits mitosis and cytokinesis, as well as tip growth of apical cells, and causes abnormal deposition of MFs. Both the cytoskeletal elements studied so far, i.e. MTs and AFs are implicated in brown algal cell morphogenesis, expressed in their relationship with cell wall morphogenesis, polarization, spindle organization and cytokinetic mechanism. The novelty is the role of AFs and their possible co-operation with MTs.

Key words: Actin, cytoskeleton, microtubules, morphogenesis, Phaeophyceae, polarity, vegetative cells.

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POLAR ORGANIZATION OF THE PROTOPLAST: TIP GROWTH

Microtubule cytoskeleton

Cells frequently exhibit asymmetric distribution of organelles, proteins or cytoskeletal components along a particular axis. This internal organization is referred to as 'cell polarity' (Cove, 2000). Polarized cells exhibit different morphological and/or molecular characteristics at opposite ends aligned along an axis (Grebe et al., 2001; Baluska et al., 2003). The use of fucalean zygotes as models for the study of polarization mechanisms started very early (Knapp, 1931; Jaffe, 1958; Nuccitelli, 1978) and still continues (Kropf, 1994, 1997; Kropf et al., 1998; Brownlee and Bouget, 1998; Hable and Kropf, 1998, 2000, 2005; Robinson et al., 1999; Belanger and Quatrano, 2000a; Brownlee et al., 2001; Hable et al., 2003; Bisgrove and Kropf, 2004). The ultrastructural examination of the unfertilized egg revealed a symmetrical distribution of cell elements (Brawley et al., 1976a, b). The first signs of polarization, which is induced by environmental cues, become visible 8–10 h after fertilization by the secretion of adhesive material at the future growth site (Quatrano, 1990; Kropf, 1992a, b, 1994). Depolymerization of MTs does not affect zygote polarization or rhizoid emergence, meaning that MTs are not directly implicated in the establishment of polarity in fucalean zygotes (Brawley and Quatrano, 1979; Kropf et al., 1989). However, the asymmetrical division that follows is critical for the future development of the young embryo. Experimental alteration of the plane of this division leads to the formation of abnormal embryos and affects their viability (Bisgrove and Kropf, 1998). It is interesting that in a recent paper, Corellou et al. (2005), using microinjection of fluorescent tubulin into living Fucus zygotes, found a distinct cortical MT array that is reorganized during polarization and rhizoid emergence. According to these authors this MT configuration is crucial for the regulation of rhizoid growth. However, a similar MT organization was never reported in any of the earlier studies dealing with MT organization in fucalean zygotes (Brawley et al., 1976a, b; Kropf et al., 1990; Allen and Kropf, 1992; Bisgrove and Kropf, 1998, 2001, 2004; Nagasato et al., 1999b).

Contrary to the zygotes of Fucales, the tubular apical cells of Sphacelariales display a permanent polarity, clearly visible at a light microscope level (Fig. 1A). This polar organization is directly related to the particular growth pattern of these cells (Katsaros, 1980, 1995; Katsaros et al., 1983), which is similar to that of tip-growing cells like fungal hyphae, root hairs, pollen tubes, etc. It represents a gradual distribution of the cell elements along the longitudinal axis of the cell (Fig. 1B). The tip region is occupied by a large number of endoplasmic reticulum (ER) membranes associated with active dictyosomes and relatively small vacuoles, while the basal area is highly vacuolated. In this organelle gradient the nucleus occupies a particular position. The apical cell wall is continuously extending, and consists of two layers, a thin external one consisting of amorphous material, and an internal with a few randomly oriented...
MFs embedded in an amorphous matrix (Karyophyllis et al., 2000b). Subunits of cellulose synthases or entire terminal complexes (TCs) are probably transported via dictyosome vesicles to the plasmalemma of the tip region (Katsaros et al., 1996; Reiss et al., 1996). Recent freeze-fracture studies of apical cells of Syringodermia phinneyi have revealed an apico-basal gradient of TC distribution, with a higher density of TCs in the apical part, where more intense cellulose synthesis takes place. This seems to reflect the tip growth of the apical cells, suggesting a relationship between cellulose synthesis with the growth pattern (Schüssler et al., 2003).

Immunofluorescence labelling of tubulin showed that the interphase MT system in apical cells of Sphacelaria rigidula is strongly polarized. It extends from the two centrosomes towards the cortical cytoplasm, and forms a fine meshwork. The apical dome of the cell is traversed by numerous thin MT bundles, while the basal part is traversed by a few thick ones meandering among the vacuoles (Katsaros, 1992; Figs 1C and 2A). It can be assumed that they stabilize and mechanically support the cytoplasmic strands that are probably formed by the acto-myosin complex (see also Panteris et al., 2004). The plus ends of MTs in the apical cytoplasm frequently reach and probably contact the plasma membrane, where sometimes they almost curve (Fig. 1C). In animal and yeast cells they seem to interact with the cell cortex via plus end MT-associated proteins, like +TIPs (e.g. CLIPs, CLASPs, cytoplasmic dynein/dynactin, EB1, etc.; Mimori-Kiyosue and Tsukita, 2003). These proteins act mainly as microtubule-stabilizing factors and at the same time often link MTs to various cellular structures, such as the cell cortex (Carvalho et al., 2003; Bigsrove et al., 2004; Gundersen et al., 2004; Akhanova and Hoogenraad, 2005). The MT tip proteins interact with bridging proteins that guide the MTs to cortical receptors driven by AFs (Gundersen et al., 2004). On the other hand, interactions between MT tips and motors can cause translocation of vesicles along MTs. Therefore MT plus ends can serve as cargo-loading sites for minus-end-directed transport (Vaughan et al., 2002), or deliver cargos to particular cell regions (Felerbach et al., 2004). Similar activities can be attributed to the MTs of brown algal cells that bear centrosomes like animal cells, and to the MTs approaching the tip of the apical cell of S. rigidula where expansion takes place. Examination of the MT organization during mitosis and cytokinesis revealed that the above polar organization of MTs in apical cells is obvious during their whole cell cycle (Katsaros, 1992).

Treatment with taxol induced dramatic changes in MT organization in both interphase and dividing apical cells of S. rigidula. Massive assembly of MTs occurs in the cortical and sub cortical cytoplasm of interphase cells out of the centrosome areas. Mitosis and cytokinesis were inhibited and numerous MTs were found in the perinuclear cytoplasm of mitotic cells. It has been suggested that centrosome dynamics in MT nucleation varies during the cell cycle, and that in case centrosome activity is disturbed, the cortical/subcortical cytoplasm assumes the ability to assemble MTs (Dimitriadi et al., 2001).

Tubulin immunolocalization in isolated protoplasts of apical cells of Sphacelaria sp. revealed a different pattern of MT organization, both in interphase and during the first division. The cell becomes apolar and the MTs diverge symmetrically from the centrosomes towards the cell cortex, during wall regeneration (Rusig et al., 1993, 1994). The loss of polarity in MT organization after removal of the cell wall is retained for at least the first divisions of the regenerating protoplasts. This fact underlines the role of the cell wall in polarization of brown algal cells (Quatrano and Shaw, 1997; Ouichou and Ducreux, 2000; Bigsrove and Kropf, 2001). The polarity is re-established with the initiation of a new apical cell from a mass of about ten
undifferentiated cells (Ducreux and Kloareg, 1988). Similar behaviour characterizes the cytoskeleton of young and regenerating protoplasts of 

Macrocystis pyrifera gametophytes (V. Varvarigos, unpubl. res). After the first division of the germinating fucalean zygote, the rhizoid cell behaves like a tip-growing cell. The nature of tip growth is first manifested in wall structure. The previously uniform external cell wall becomes thinner at the tip compared with subapical regions (Allen and Kropf, 1992; Kropf et al., 1992). Experimental depolymerization of MTs using drugs like oryzalin, anti-prophos methyl, or Nocodazole (Nz) does not affect the fixation of the polar axis and the rhizoid germination in fucalean zygotes (Quatrano, 1973; Brawley and Quatrano, 1979; Kropf et al., 1990; Kropf, 1992a, b, 1994). However, MTs are involved in rhizoid morphogenesis, as in other tip-growing cells, e.g. moss protonemata (Doonan et al., 1985, 1988) or fungal hyphae (Raudaskoski et al., 1994; Robertson and Vargas, 1994; Rupes et al., 1995). When MTs are destroyed by anti-MT agents, the advanced developmental events are severely inhibited and the zygotes form aberrant rhizoids (Kropf, 1994). Similarly, in S. rigidula apical cells, treatment with Nz disturbs polarity and affects the growth pattern. The distribution of the organelles and particularly dictyosomes becomes more uniform, and the cell wall of the tip region appears thicker, while the cell sometimes bends. This suggests that MTs are probably involved in the transport of vesicles with wall materials to the tip region (Karyophyllis et al., 1997; Karyophyllis, 2003). Nagasato and Motomura (2002b) reported that Golgi-derived vesicles are transported along MTs towards the division plane of cytokinetic cells of Scytosiphon lomentaria. A detailed overview on the MT cytoskeleton in tip-growing plant cells is presented in a number of recent reviews (Mathur and Hulskamp, 2002; Sieberer et al., 2005; Smith and Oppenheimer, 2005).

MTs in tip-growing cells are also involved in the correct positioning of the nucleus. In most tip growing cells, MT depolymerization disturbs the nuclear positioning thus affecting the growth pattern (Doonan et al., 1985; Lloyd et al., 1987; Joos et al., 1994; Heath et al., 2000). Similar results have been obtained in apical cells of S. rigidula treated with Nz (Karyophyllis et al., 1997; Karyophyllis, 2003).

Actin filament cytoskeleton

The role of actin in establishment and maintenance of cell polarity has been well documented. The asymmetric spatial distribution and activity of the actin cytoskeleton, and the conserved signalling molecules that regulate it, play a role in cell polarity in metazoans, fungi (Johnson, 1999), amoebazoans (Chung et al., 2000) and higher plants (Yang, 2002). GTPases in the Ras superfamily, especially ones in the Rho family are such signalling molecules that regulate the actin cytoskeleton (Etienne-Manneville and Hall, 2002). Recently, cDNA that encodes a Rho family GTPase was isolated from Fucus distichous (Fowler et al., 2004). The small GTPase encoded (FdRac1) displays a polarized localization, being concentrated close to the growing tip of the rhizoid. This localization together with the evidence for some functional overlap with yeast Cdc42p, support the hypothesis that FdRac1 regulates algal cell polarity, possibly via actin cytoskeleton (Fowler et al., 2004).

In tip-growing cells of higher plants and fungi showing permanent polarity, like pollen tubes, root hairs or fungal hyphae, the actin cytoskeleton supports the polar architecture of the cytoplasm and determines the direction of cell expansion (Heath, 1990, 2000; Steer, 1990; Pierson and Cresti, 1992; Derksen et al., 1995; Miller et al., 1999; Emmons and de Ruijter, 2000; Geitman and Emmons, 2000; Vidali and Hepler, 2000; Hepler et al., 2001; Wasteneys and Galway, 2003). AFs traverse the cytoplasm of these tubular cells in an axial direction, possibly facilitating the transport of vesicles to the tip. However, actin is not always present at the very tip region of root hairs and pollen tubes (Miller et al., 1996, 1999; Emmons and de Ruijter, 2000; Vidali and Hepler, 2000; Lovy-Wheeler et al., 2005), while in developing fungal hyphae a dense meshwork of AFs is usually present at the tip (Heath, 1990, 2000). It must be noted that disruption of actin inhibits tip-growth in both root hairs and pollen tubes (Gibbon et al., 1999; Miller et al., 1999; Baluska et al., 2000).

The involvement of F-actin in the establishment of polarity in zygotes of Fucus or Pelvetia has been repeatedly underlined. Early studies using cytochalasin B (CB) have indicated that F-actin is involved in the conversion of a light gradient to a morphological gradient in these cells (Nelson and Jaffe, 1973; Quatrano, 1973). More recent studies using anti-actin agents like cytochalasin or lantrunculin further support the hypothesis that photopolarization of fucalean zygotes is actin-dependent (Hable and Kropf, 1998; Robinson et al., 1999; see also review in Fowler and Quatrano, 1997). During all these years enough efforts were made to visualize AFs (Brawley and Robinson, 1985; Kropf et al., 1989), since these fine structures are not well preserved after chemical fixation. These difficulties are commonly attributed to the high sensitivity of F-actin to aldehyde fixatives (Doris and Steer, 1996; Miller et al., 1996). However, more recent findings showed that rather than aldehyde fixation, some further steps in the procedures used for actin visualization are critical for preserving F-actin (Vitha et al., 2000). To overcome this problem, new approaches were developed, by introducing fluorescent phalloidin into living cells (Alessa and Kropf, 1999; Pu et al., 2000). These techniques have shown that F-actin is organized into cortical patches, which during photopolarization are localized at the rhizoid pole. In the young germinating rhizoid, these patches become a ring-like configuration that is located in the subapical zone of the elongating tip (Alessa and Kropf, 1999; Pu et al., 2000). Disruption of F-actin using lantrunculin B blocks photopolarization, probably by inhibiting the formation of cortical Ca2+ gradients (Pu et al., 2000). However, in all the above studies the staining usually revealed fluorescent patches, but not AFs.

Contrary to the zygotes of Fucus and Pelvetia, which are apolar cells with uniform distribution of cell elements and become polarized some time after fertilization, the apical cells of some filamentous brown algae like the genera
branch formation as in apolar zygotes. The F-actin ring that participates in the local softening of the cell wall, probably by transportation and exocytosis of some loosening factor via dictyosome vesicles. The subsequent cell division separates the apical cell of the young branch, which then behaves like the main apical cell of the thallus (Katsaros, 1992, 1995). Similar MT reorganization has been observed in cells of *Macrocystis pyrifera* gametophytes which give lateral branches. However, in this case the branch cell separated by the first division does not behave as an apical cell (Varvarigos et al., 2005).

**Actin filament cytoskeleton**

F-actin seems to be involved in the process of branch formation. Before any sign of proplast germination or thallus branching of *M. pyrifera* gametophytes, radial AF configurations are organized in the prospective sites of the wall outgrowth. These initially pointed radial AF structures change to radial-circular configurations located at the base of the emerging protrusion, while at more advanced stages they disappear. Treatment with latrunculin B resulted in the inhibition of germination and branching, suggesting that these radial AF configurations are involved in cell polarization (Varvarigos et al., 2004; Fig. 3). Similarly in *S. rigidula*, AFs are gathered in the sites of wall protrusion during branch formation. It is speculated that some motor protein(s) travel(s) along these actin structures transporting many types of cargo, including secretory vesicles. Polarized secretion of vesicles carrying cell wall remodelling enzymes and new cell wall constituents may promote local cell wall deformation and branch emergence. As described above, the first sign of branching is the formation of a wall outgrowth in a rather differentiated cell of the thallus. Rh-Ph staining revealed an increased F-actin fluorescence at the site of the bulge. At a later stage, when the outgrowth became a short tube with a dome-like tip, this F-actin reorganized and a dense fluorescence was observed at the base of the dome (Karyophyllis, 2003). This F-actin system is similar to that described by Allessa and Kropf (1999) (see also Kropf et al., 1998) and Pu et al. (2000) in germinating fucalean zygotes. It seems likely that AFs are involved in the establishment of a new polarity axis during branch formation as in apolar zygotes. The F-actin ring that
is formed at a later stage is involved in the morphogenesis of the tip-growing cell, and resembles the ring found in apical cells of *S. rigidula*. Similar F-actin rings have also been described in fern protonemata (Kadota and Wada, 1989; Quader and Schnepf, 1989) and fungal hyphae (Bachewich and Heath, 1998). The function of these rings is not yet completely understood. For those found in germinating fucoid zygotes it has been hypothesized that they are implicated in tip reinforcement in the boundary between the fragile apex and the subapical rhizoid domain (Henry et al., 1998; Alessa and Kropf, 1999). In this region a ring of tight adhesion between cortical F-actin, plasma membrane and the cell wall has been observed in *Pelvetia compressa* rhizoids (Henry et al., 1996). It has also been proposed that this actin ring participates in the maintenance of Ca$^{2+}$ ion pumps in the rhizoid tip (Pu et al., 2000). A different role of a transverse AF ring in cell wall morphogenesis of tip-growing cells is reported in apical cells of *S. rigidula*, where AFs have been found related to the orientation of the MFs (Karyophyllis et al., 2000b; Karyophyllis, 2003). They are probably related to the formation and maintenance of the tubular form of the cell.

**CELL CYCLE**

**Microtubule cytoskeleton**

The absence of cortical MTs, MT preprophase band and phragmoplast, as well as the presence of centrosomes in brown algal cells make them a quite interesting model for the study of MT organization during the cell cycle. However, the difficulties in the preservation of the fine structure in transmission electron microscope (TEM) studies resulted in an incomplete description of the organization of MTs in interphase and dividing brown algal cells (Neushul and Dahl, 1972; Galatis et al., 1973, 1977; Katsaros et al., 1983; Katsaros and Galatis, 1985, 1988, 1990; see also Katsaros, 1980, 1995). The particular composition of their cell wall also caused a considerable delay in the successful application of immunofluorescence staining of tubulin and in the detailed analysis of MT organization (Katsaros, 1992; Katsaros and Galatis, 1992; Rusig et al., 1993, 1994; Karyophyllis et al., 1997; Katsaros and Sala, 1997).

The organization of MTs during mitosis shows some differences between tip-growing apical cells, like those of *S. rigidula*, and other types of cells with more diffuse growth, like subapical cells of *S. rigidula*, or apical cells of *Dictyota dichotoma* (Katsaros, 1992; Katsaros and Galatis, 1992; Rusig et al., 1993, 1994). In tip-growing cells this becomes evident by the continuous coincidence between cytoplasmic polarity axis and the axis of the MT–centrosome system, as well as by the difference in the dynamics of the MTs of the apical part compared with those of the basal one. The latter is evident by the fact that the apical MTs disappear last with the entrance of the cell in mitosis and reappear first on anaphase (Katsaros, 1992, 1995; Rusig et al., 1994, 2001; Karyophyllis et al., 1997).

By the completion of anaphase, the spindle reaches its longest size, therefore the daughter nuclei are formed at a distance from each other, which is longer in elongated cells. In brown algal vegetative cells, contrary to higher plants, cytokinesis starts long after the completion of telophase. In the meantime daughter nuclei assume a completely interphase appearance (Katsaros, 1980, 1992, 1995; Katsaros et al., 1983; Katsaros and Galatis, 1992). At this stage spindle MTs are depolymerized and cytoplasmic MTs reassemble from the two oppositely placed centrosomes. The daughter nuclei approach each other and the ‘cytokinetic’ MTs form two cage-like configurations, surrounding the daughter nuclei and overlapping at the midplane (Katsaros and Galatis, 1992). At a later stage the daughter nuclei move apart again, probably by interaction of the two interdigitating MT systems via motor proteins. In this way, two nuclear-cytoplasmic domains (NCDs) or ‘cytoplasts’ are organized (Porter and McNiven, 1982; Porter et al., 1983; Pickett-Heaps et al., 1999). In transverse divisions of elongated cells, a clear space free of MTs is formed between the two NCDs. The cytokinetic diaphragm is then developed in the mid-plane between the two NCDs, by the fusion of vesicles and cisternae (Katsaros and Galatis, 1992; Nagasato and Motomura, 2002b). The NCD concept was described, although not termed, in sporangia of *Halopteris filicina* and in dividing vegetative cells of *D. dichotoma* (Katsaros and Galatis, 1986, 1992). Treatment with anti-MT or anti-actin agents inhibits cytokinesis, meaning that both MTs and AFs are involved in this process (Karyophyllis et al., 1997, 2000a; Bisgrove et al., 2003). However, although the study of cell division in brown algae started quite early, the cytokinetic mechanism is still a matter for discussion (Galatis et al., 1973; Rawlence, 1973; Markey and Wilce, 1975; Brawley et al., 1977; LaClaire, 1981; Katsaros et al., 1983; Belanger and Quatrano, 2000b; Nagasato and Motomura, 2002b; Bisgrove and Kropf, 2004).
The organization of AF cytoskeleton was studied in vegetative cells of *S. rigidula* during the whole cell cycle (Karyophyllis *et al.*, 2000a; Karyophyllis, 2003). The interphase organization of AFs was described above. During mitosis the F-actin cytoskeleton is completely reorganized. The cortical F-actin system remains well organized until telophase. At prophase the fluorescence of the endoplasmic AFs becomes weak, and the perinuclear AFs predominate. In parallel, a bipolar spindle of AFs starts forming, that is disorganized after anaphase. Although at these stages the filamentous character of AFs is not always visible, it is clear that their organization resembles that of spindle MTs. The poles of the F-actin spindle coincide with those of the MT spindle but they cover a more broad area. At metaphase, AFs extend from the poles towards the chromosomes, and during anaphase interzonal AFs are visible. By the progression of telophase, the daughter nuclei move apart from each other. The perinuclear AF system is still intense, but the fluorescence in the interzonal area temporarily weakens. At advanced telophase the interzonal AF system becomes gradually more intense, and when the two daughter nuclei have gained an interphase organization, all the other F-actin systems are gradually reduced, except for the interzonal one. Finally, before any sign of cytokinetic diaphragm formation, a complete, dense F-actin disc is formed in the plane of the future division. The assembly of an actin disc midway between daughter nuclei was also confirmed in the diaphragm has been proposed in zygotes of brown algae (Bisgrove and Kropf, 2004). It must be noted here that, in vacuolated cells of *M. pyrifera* gametophytes, a unique pattern of F-actin organization participates in the control of cytokinesis. This involves an F-actin ring organized in the cortical cytoplasm which develops inwards to form the F-actin disc (Varvarigos *et al.*, 2005). TEM examination of similar stages revealed vesicles and cisternae gathering in the area of the F-actin disc. Fusion of these patches gives rise to the membranous diaphragm separating the daughter cells. Although a centrifugal development of the diaphragm has been proposed in zygotes of brown algae (Nagasato and Motomura, 2002b; Bisgrove and Kropf, 2004), this seems not to be the case in vegetative cells examined so far (see also Karyophyllis *et al.*, 2005a).

The above data show that F-actin is involved in mitosis and cytokinesis of brown algal vegetative cells, possibly cooperating with spindle and cytokinetic MTs. Evidence in favour of this hypothesis was provided by experiments using CB. Cells treated with CB at a premitotic stage did not complete mitosis, but were blocked at a stage resembling metaphase. The absence of anaphase stages after treatment implied that AFs possibly participate in chromosome movement. Similar results have been reported by Forer (1985, 1988) in animal cells, where disruption of AFs by cytochalasin also inhibited cytokinesis. The mechanism of cytokinesis in brown algal cells is not yet fully understood. However, a possible scenario would be drawn by correlation of the above results with the available ultrastructural and MT-immunofluorescence data (Galatis *et al.*, 1973; Rawlence, 1973, Markey and Wilce, 1975; Brawley *et al.*, 1977; LaClaire, 1981; Karyophyllis *et al.*, 1983; Karyophyllis, 1992; Karyophyllis and Galatis, 1992; Karyophyllis *et al.*, 1997; Belanger and Quatrano, 2000b; Nagasato and Motomura, 2002b; Bisgrove and Kropf, 2004). Starting at a post-telophase stage, when the daughter nuclei have gained a fully interphase appearance, the possible steps of the cytokinetic process would be:

(a) Interaction of cytokinetic MTs between each other via motor proteins, as well as interaction with AFs (and possibly associated proteins), guides the daughter nuclei to move apart and/or take their final positions.

(b) Cortical AF cytoskeleton disappears, AF spindle is disorganized and F-actin is gathered in the mid-area between the daughter nuclei.

(c) A cytokinetic AF disc is formed at the plane that is determined by the two interdigitating MT systems. In vacuolated cells, an AF ring is initially organized in the periphery of the cell, before the formation of the AF disc. In elongated cells, where the daughter nuclei move away from each other, a clear zone is left between them.

(d) Vesicles of dictyosome origin and thin, peculiar cisternae of unknown origin, appearing tube-like in sections, are aligned along this plane, possibly stabilized by F-actin. This stage probably coincides with stage (c).

(e) Fusion of these vesicles and cisternae results in the formation of the membranous cytokinetic diaphragm.

(f) The F-actin disc disappears and the daughter cells gain the interphase actin organization.

(g) Wall deposition from both daughter cells is continued until the completion of the new cell wall.

**CELL WALL MORPHOGENESIS**

*Microtubule cytoskeleton*

The mechanical properties of the cell wall that control its expansion are mainly determined by the orientation of MFs. In higher plant cells, cortical MTs have been proven to be involved in the orientation of MFs, probably by guiding cellulose synthases to the cell cortex (Williamson, 1991; Sonobe and Takahashi, 1994; see review in Baskin, 2001; Smith and Oppenheimer, 2005). Thus, MTs constitute the main cytoskeletal elements participating in cell wall morphogenesis of higher plants.

The absence of cortical MTs in brown algal cells suggests that a different mechanism of cell wall morphogenesis may operate in them. Experiments using MT inhibitors like oryzalin did not reveal any effect in the development or the strength of the cell wall depositing in young zygotes of Fucales (Kropf, 1992a, b, 1994; Bisgrove and Kropf, 1998). However, in tip-growing rhizoids of fucalean zygotes, as well as in apical cells of *Sphacelaria*, MTs are involved in cell wall morphogenesis. Their disruption causes abnormal growth and thickening of the wall of the tip region. It seems possible that MTs and/or AFs are implicated in the transport of wall material to the extending wall (Karyophyllis *et al.*, 2005a).
1997; Belanger and Quatrano, 2000b; Karyophyllis, 2003). Moreover, MTs seem to be implicated in cytokinesis of S. lomentaria zygotes, by transporting vesicles containing wall material to the developing diaphragm (Nagasato and Motomura, 2002b).

Application of antibodies against α-actinin, vitronectrin and integrin in Sphacelaria, as well as in zygotes of Fucus has shown that cortical sites involving transmembrane connections between the cytoskeleton and the extracellular matrix are crucial for the establishment of cell polarity (Quatrano and Shaw, 1997; Ouichou and Ducreux, 2000; Brownlee et al., 2001).

**Actin filament cytoskeleton**

The discovery of a cortical F-actin system in vegetative cells of brown algae was a strong stimulus for the investigation of the possible role of these AFs in cell wall morphogenesis. At first this was checked in the apical cells of S. lomentaria and S. tribuloides (Karyophyllis et al., 2000b). Thin sections passing through selected regions of the cell wall (Figs 2C and 4A and B) were examined under TEM. This wall displays a multilayered structure, similar to that described very early by Dawes et al. (1960, 1961; see also Prud’homme van Reine and Star, 1981; Quatrano, 1982; Tamura et al., 1996). The mature cell wall consists of four layers, named L₁, L₂, L₃ and L₄, going from the external surface inwards (Fig. 2C). L₁ consists of amorphous material and covers all the cell wall externally. L₂ is a thin layer consisting of a few randomly distributed MFs embedded in an amorphous matrix. L₃ is also a thin layer rich in MFs, which form a ring oriented transversely to the long axis of the cell. L₄ is the innermost and thickest layer bearing numerous MFs with an axial or fishbone-like arrangement (Figs 2C and 4A).

It was particularly interesting that the above layers are gradually depositing along the apical cells, i.e. the cell wall of the tip area of the cell is very thin and consists only of L₁ and L₂. L₃ starts depositing at the base of the hemispherical dome of the apex, and L₄ at the area where the shape of the cell becomes cylindrical (Figs 2C and 4A). The above-described MF arrangement coincides with the organization of AFs in this cell as found after Rh-Ph staining of F-actin (see above, Fig. 1D).

In order to examine whether the above relationship is a general phenomenon in brown algal cells, the organization of MFs and the underlying AFs was investigated by TEM and Rh-Ph staining, respectively, in a variety of species and cell types, i.e. subapical and differentiating cells of S. rigidula, apical cells of D. dichotoma, subapical cells of Choristo-carpus tenellus and meristematic cells of D. dichotoma. In all cases a cortical AF system was present, always oriented parallel to the depositing MFs of the innermost wall layer (Karyophyllis et al., 2000b; Katsaros et al., 2002). This coincidence suggests a possible involvement of AFs in the orientation of MFs.

To confirm the above hypothesis, F-actin was disrupted with CB, in order to ascertain whether MF deposition would be affected. Treatment of S. rigidula and D. dichotoma thalli with 100 μg mL⁻¹ CB for 24–36 h caused a gradual disruption of AFs. TEM examination of treated material showed that the MFs of the internal wall surface did not exhibit the clear parallel arrangement observed in normal cells. Instead, a rather random orientation of loose and thin MFs was found (Fig. 4B). It must be noted that the cell wall of fully differentiated cells was normal. Cytochalasin affected only cells where wall deposition was in progress during the treatment (Karyophyllis et al., 2000b; Katsaros et al., 2002). A similar suggestion has been made by Mizuta et al. (1994), who reported that the helicoidal pattern of MF orientation in the giant green alga Boergsensia was disrupted after cytochalasin treatment.

Although the coincidence of AF–MF orientation has not been confirmed until now by direct observation under TEM, the mutual alignment between cortical AFs and MFs is strong evidence that AFs are involved in cell wall morphogenesis of brown algae. This means that a different mechanism of cell wall morphogenesis is functioning in this group. In this mechanism, AFs seem to be involved, playing a role similar to that of cortical MTs in higher plant cells.
CENTROSOME

Structure: centrosomal proteins

Vegetative cells of brown algae bear detectable centrosomes during their whole cycle; in this they differ from higher plants and resemble animal cells. Centrosomes in brown algae were found very early by light microscopy (Strasburger, 1897; Swingle, 1897; Mottier, 1900; Yamanouchi, 1909), and described in more detail by electron microscopy (Berkaloff, 1963; see also Manton and Clarke, 1950; Manton, 1957). Each centrosome consists of two centrioles that lie at about right angles to one another and in close proximity at one end (proximal). In animal cells, pericentriolar material (PCM) surrounds the centrioles and is in part organized by them (Bobinnec et al., 1998). PCM is the site of MT nucleation (Wheatley, 1982; Balczon, 1996; Doxsey, 2001) through γ-tubulin containing complexes (Moritz and Agard, 2001), although other proteins also appear to be involved in this process. The centrosome comprises hundreds of proteins, several of them serving as docking sites for a number of regulatory and other activities (Doxsey et al., 2005).

The Ca²⁺-modulated contractile protein centrin was, at first, found associated with basal bodies of green flagellates (Salisbury et al., 1984; Huang et al., 1988a, b). After its first localization and identification, centrin was found in a great variety of eukaryotic cells, in relation with basal bodies, mitotic spindles, spindle poles, centrosomes, etc. In higher plants and fungus, zygotes and vegetative cells of brown algae (Katsaros et al., 1984; Huang et al., 1988a, b), and in close proximity at one end (proximal). In animal cells, pericentriolar material (PCM) surrounds the centrioles and is in part organized by them (Bobinnec et al., 1998). PCM is the site of MT nucleation (Wheatley, 1982; Balczon, 1996; Doxsey, 2001) through γ-tubulin containing complexes (Moritz and Agard, 2001), although other proteins also appear to be involved in this process. The centrosome comprises hundreds of proteins, several of them serving as docking sites for a number of regulatory and other activities (Doxsey et al., 2005).

In brown algae, centrin was detected in the basal apparatus of motile male gametes (Melkonian et al., 1992; Katsaros et al., 1993). It was localized in protein structures connecting the basal bodies to each other or to the nucleus (nucleus–basal body connector) (Katsaros et al., 1993). This connection is quite strong and extraction of motile cells using detergents does not separate basal bodies from the centrosome during their whole cycle; in this they differ from higher plants and resemble animal cells. Centrosomes in brown algae were found very early by light microscopy (Strasburger, 1897; Swingle, 1897; Mottier, 1900; Yamanouchi, 1909), and described in more detail by electron microscopy (Berkaloff, 1963; see also Manton and Clarke, 1950; Manton, 1957). Each centrosome consists of two centrioles that lie at about right angles to one another and in close proximity at one end (proximal). In animal cells, pericentriolar material (PCM) surrounds the centrioles and is in part organized by them (Bobinnec et al., 1998). PCM is the site of MT nucleation (Wheatley, 1982; Balczon, 1996; Doxsey, 2001) through γ-tubulin containing complexes (Moritz and Agard, 2001), although other proteins also appear to be involved in this process. The centrosome comprises hundreds of proteins, several of them serving as docking sites for a number of regulatory and other activities (Doxsey et al., 2005).

The identification in brown algae of complexes containing γ-tubulin and its precursors is an interesting task from an evolutionary perspective, since the heterocont group diverged early in eukaryotic evolution (Baldauf, 2003). These structures are present not only in animals and fungus (Zheng et al., 1995; Jeng and Stearns, 1999; Hynes and Zheng 1999; Hynes and Zheng 1999) but also in plants (Stoppin-Mellet et al., 2000; Dryková et al., 2003).

Centrosome cycle

During interphase, brown algal centrosomes are located close to the nucleus, sometimes in a shallow depression of the nuclear envelope (Bouck, 1965; Neushul and Dahl, 1972; Katsaros, 1980; Katsaros et al., 1983; Motomura, 1991, 1994; Katsaros and Galatis, 1992). MTs radiate out of the pericentriolar material, some of them surround the nucleus, while others traverse the cytoplasm towards the cell cortex.

At prophase the activity of the two oppositely located centrosomes appears to be increasing, and numerous short MTs or MT bundles are assembled, forming aster-like configurations. At this stage the centrosomes, in the presence of the nuclear envelope, are closely associated with the nuclear envelope, interacting with an

S. lomentaria was isolated and analysed. The protein deduced exhibited 84% homology to the Chlamydomonas

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electron-dense material that appears at its cytoplasmic face. This interaction probably causes the breakage of the already weakened nuclear envelope at the poles. After the formation of the polar gaps, spindle is formed by the extension of MTs from the centrosomes towards the chromosomes.

Duplication of centrosomes usually occurs during the S phase of the cell cycle, and the daughter centrosomes separate and migrate to opposite sides of the nucleus (Kuriyama and Borisy, 1981). In most cases of brown algal cells examined so far, two oppositely placed centrosomes (two centriole pairs) are visible during most of interphase, since duplication and separation of centrosomes occurs soon after cytokinesis, and thus they are in place before any sign of the onset of mitosis (Katsaros and Galatis, 1992; Rusig et al., 1994). MTs seem to be involved in the separation and migration of the daughter centrosomes to the poles. Depolymerization of MTs after Nz treatment in S. rigidula did not affect centrosome duplication, but disturbed their separation. Prolonged treatment caused mult centrosomal nuclei (up to four in one nucleus). This means that MT disruption inhibits cell division, but not the centrosome cycle, that proceeds independently (Karyophyllis et al., 1997; Karyophyllis, 2003). This observation is in agreement with the idea that the centrosome is not under the cell cycle control, rather it exerts control over the cell cycle (Doxsey et al., 2005). However, since anti-centrin immunofluorescence was used for centrosome identification, the possibility that the centrin spots represent separated centrioles or PCM fragments and not complete centrosomes should not be excluded (Karyophyllis et al., 1997). Interestingly, centrosome separation was recently found associated with Golgi apparatus. Unexpectedly, disorganization of Golgi after treatment with brefeldin A seems to inhibit centrosome movement to the opposite poles (V. Varvarigos et al., unpublished).

Apart from centrosome separation, MT dynamics seem to play a significant role in the structure and function of centrosomes in dividing cells of brown algae. In apical cells of S. rigidula, after Nz treatment, centrin was localized at cytoplasmic sites far from the centrosomes. It was suggested that disruption of MTs probably caused fragmentation of centrosomes or dispersal of centrosomal material, or even shift of centrosomes from their correct position (Karyophyllis et al., 1997; Karyophyllis, 2003). In cells recovering from Nz treatment, a pair of long, parallel, rod-like structures, showing positive centrin reaction appeared at the centrosome sites (Karyophyllis et al., 1997; Karyophyllis, 2003). These structures probably represent abnormally differentiated centrioles, similar to ‘primary cilia’ described by Wheatley et al. (1996). A similar phenomenon was described by Alieva and Vorobjef (1991) in cultured cells of pig embryo. These authors observed quite elongated centrioles (1.5–5 times longer than the usual).

Recent studies, using polyspermic zygotes of the brown alga S. lomentaria, have shown that in brown algae the cytokinetic plane is determined by the position of the centrosomes after mitosis (Nagasato et al., 1999b; Nagasato and Motomura, 2002a) and not by the spindle as it was proposed earlier (Bisgrove and Kropf, 2001). Similar results have been reached by Bisgrove et al. (2003) in asymmetric division of fucoid zygotes, where it was suggested that the division plane is determined by the telophase nuclei.

**CONCLUDING REMARKS: FUTURE PERSPECTIVES**

The study of both fine structure and cytoskeleton organization shows that the cytoskeletal elements studied so far are implicated in brown algal cell morphogenesis. The novelty is the role of AFs and the probable co-operation between them and MTs. The dominating role of cytoskeleton, and especially AFs, in morphogenesis of brown algal cells is expressed in the following processes:

(a) The structural and functional interconnection between the cytoskeleton, the plasma membrane and the extracellular matrix (= cell wall). This is analogous to the ‘cell periphery complex’ proposed for higher plant cells (Baluska et al., 2000) in which cortical MTs are involved. In both model systems of brown algae studied, namely zygotes and vegetative cells, a cortical AF cytoskeleton seems to play a critical role. This scheme has been considered as a more ‘primitive’ complex that has been replaced by the cortical MTs in higher plant cells (Baluska et al., 2000, 2001).

(b) Polarization. The implication of radial F-actin configurations in cell polarization seems to be a general phenomenon in brown algae, as it is in a great variety of other organisms.

(c) Organization of the mitotic spindle. Co-operation or at least coexistence occurs between spindle MTs and AFs during mitosis. The inhibition of anaphase after treatment with CB indicates a direct or indirect role of F-actin in mitosis of brown algal cells.

(d) Cytokinesis. Fluorescence microscopy of both normal and experimentally treated material has revealed an involvement of F-actin in the cytokinetic process. This finding, combined with the absence of any of the known cytokinetic systems (phragmoplast, phycoplast or cleavage furrow) and the presence of centrosomes, showed that a particular cytokinetic mechanism operates in brown algal cells.

All the above need further investigation. For the first, the mechanism by which the ‘cell periphery complex’ regulates the deposition and orientation of the nascent MFs remains to be clarified. Several studies using freeze fracture have shown putative cellulose synthase complexes (TCs) on the plasma membrane of brown algal cells (Peng and Jaffe, 1976; Katsaros et al., 1996; Reiss et al., 1996; Tamura et al., 1996; Schüssler et al., 2003). Similar approaches, combined with rapid freeze-fixation, could possibly give information on the relationship between the cortical F-actin cytoskeleton and the transmembrane synthase complexes. An analogous relationship between cortical MTs and rosette-type TCs has been suggested for cellulose synthesis in higher plants (Mizuta, 1992; Sonobe and Takahashi, 1994).
The role of F-actin and the centrosome in the cytokinetic process is also a very stimulating issue that needs further study. Together with it, the investigation of the mechanism determining the cell division plane is also a rather attractive target.

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**LITERATURE CITED**


