Dynamics of microtubule reassembly and reorganization in the coenocytic green alga *Ernodesmis verticillata* (Kützing) Børgeesen

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Abstract. The dynamics of microtubule (MT) disassembly and reassembly were studied in the green alga *Ernodesmis verticillata*, using indirect immunofluorescent localization of tubulin. This alga possesses two distinct MT arrays: highly-ordered, longitudinally-oriented cortical MTs, and shorter perinuclear MTs radiating from nuclear surfaces. Perinuclear MTs are very labile, completely disassembling in the cold (cells on ice) within 5–10 min or in 25 μM amiprophos-methyl (APM) within 15–30 min. Although cortical MTs are generally absent after 3 h in APM, it takes 45–60 min before any cold-induced depolymerization is apparent, and some cortical MTs persist after 6 h of cold treatment. The extent of immunofluorescence of cytoplasmic (depolymerized?) tubulin is inversely proportional to the abundance of cortical MTs. Recovery of MT arrays upon warming or upon removal of APM occurs within 30–60 min for the perinuclear MTs, but the cortical arrays take much longer to regain their normal patterns. The cortical MTs initially reappear in a random distribution with respect to the cell axis, but within 3–4 d of warming (or 24–36 h of removing APM) they are nearly parallel to each other and to the cell's longitudinal axis. Thus, although the timing differs, the actual patterns of depolymerization and recovery are similar, irrespective of whether physical or chemical agents are used. Longer-term treatments in 1 μM APM indicate that despite the rapid disappearance of perinuclear MTs, a loss of the uniform nuclear spacing occurs gradually over 1–6 d. Similar disorganization of nuclei is obtained with long-term treatment with 1 μM taxol, where a gradual loss of perinuclear MTs is accompanied by an increased abundance of mitotic spindles. This implies that perinuclear MTs can disassemble in vivo in the presence of taxol, and that they are not the sole components involved in maintaining nuclear spacing in these coenocytes. The results indicate that both nuclear and cortical sites of MT nucleation may exist in this organism, and that MT reassembly and re-organization are temporally distinct events in cells that have highly-ordered arrays of long MTs.

Key words: Chlorophyta – *Ernodesmis* – Immunofluorescence – Microtubule (reassembly) – Tubulin

Introduction

Microtubules (MTs), which are highly dynamic structures in plant cells, are involved in organizing, positioning and effecting cell division in addition to other functions. In higher plants, these cell-cycle events are accompanied by progressive changes in the organization of the MT cytoskeleton. Four discrete MT arrays typically occur in a predictable order: the cortical MT array, the preprophase band of MTs, the mitotic apparatus, and the phragmoplast (Lloyd et al. 1985; see Seagull 1989 for a review). After each array has functioned, its MTs disappear prior to or simultaneously with formation of the next MT array in the cycle (e.g. Wick and Duniec 1984; Lloyd et al. 1985). Thus, MT nucleation is presumably a critical and tightly-regulated step in this complex process.

Little is currently known about the structure or composition of MT-organizing centers (MTOCs) in plant cells, whose counterparts in animal cells are capable of MT nucleation in vivo and in vitro (Gould and Borisy 1977). (The actual organization or ordering of MTs into discrete arrays, may or may not be associated with MTOCs in different cell types, so the term “MTOC” may be misleading (Seagull 1989).) In addition to direct ultrastructural observation of potential nucleating sites (Gunning et al. 1978), one approach toward identifying MTOCs in plants has been an immunological one. The human autoimmune serum 5051 recognizes pericentriolar material in a variety of animal cells, and it has been used to identify potential MTOCs in higher plant cells (La Claire and Goddard 1989, for review). It was deter-
mined later that certain controls are essential for the proper interpretation of labeling by serum 5051 in plant cells (Busby and Gunning 1988; Harper et al. 1989; La Claire and Goddard 1989), raising some questions about its utility in identifying higher-plant MTOCs. In the coenocytic green alga *Ernodesmis*, serum 5051 specifically labels pericentriolar material, which is thought to be involved in the nucleation of the intranuclear spindle MTs (La Claire and Goddard 1989). Similarly, a monoclonal antibody that recognizes human pericentriolar material labels the spindle poles in *Chlamydomonas* (Harper et al. 1990). However, these antibodies have failed to identify any other forms of MTOCs in either of these algae.

Another widely-used approach toward locating and identifying MTOCs in plant cells has been the examination of MT recovery patterns after cold-, pressure- or inhibitor-induced depolymerization (Cleary and Hardham 1990, for review). The fact that increased concentrations of monomers can promote reassembly from incompletely depolymerized MTs, for example, indicates that this approach has its limitations (Galway and Hardham 1989; Flanders et al. 1990). Some multinucleate green algae (including *Ernodesmis*) have two spatially separate and morphologically distinct sets of MTs throughout the cell cycle: a dynamic array associated with each nucleus, and a highly ordered cortical array consisting of parallel MTs associated with the plasma membrane (La Claire 1987; Shihira-Ishikawa 1987; McNaughton and Goff 1990). The latter array does not exhibit any obvious structural changes during the cell cycle and appears to be more stable than the perinuclear MTs (La Claire 1987). Consequently, MT nucleation patterns in these coenocytic green algae may be very different from those in higher-plant cells; and despite their limitations, MT-recovery studies may provide an indication of MTOC locations in cells of coenocytic green algae.

A search for cortical nucleating sites in *Ernodesmis* was undertaken using MT-recovery studies since no cortical labeling with serum 5051 was observed in these cells, despite their elaborate cortical MT arrays (La Claire and Goddard 1989). Also, observation of MT patterns in untreated cells has provided no information on the nucleation or establishment of cortical MTs in this alga. Since these cells have such highly-organized cortical arrays, they also provide favorable systems for investigating the relationship between MT nucleation and MT ordering in stable (cortical) and more dynamic (nuclear) arrays.

Material and methods

Cultures. Unialgal cultures of *Ernodesmis verticillata* (Kützing) Børsgesen were maintained as described in La Claire (1982). The large-cell isolates (La Claire 1987) were used throughout the course of this study. Note: the term "cell" is used herein to denote the multinucleate entity that contains a single central vacuole and is surrounded by a common cell wall.

Cold and drug treatments. Groups of 8–10 *Ernodesmis* cells were submerged directly in Petri dishes (50 mm diameter, 15 mm high) containing 10 ml of culture medium pre-chilled on ice, and separately pre-incubated for 8, 30, 60, 90 min, 2, 4, 6, 8 and 10 h. After removal of each cell was then cut transversely with microdissecting scissors to promote fixation and handling, and the cells were immediately transferred to 10 ml fixative at room temperature. Wounding has been shown previously to have no effect on any MT arrays in *Ernodesmis* over the short time period between cutting and fixation (La Claire 1987). For recovery studies, cells were pre-incubated as above in the cold for 6 h (the minimum time necessary for complete depolymerization of MTs), and transferred to 10 ml fresh culture medium at room temperature. After allowing groups of cells to recover for 15, 30, 60 min, 2, 4, 12, 24, 48 and 90 h, cells were cut and fixed as above.

Amiprophos-methyl (APM) (Mobay Chemical Corp., Kansas City, Mo., USA) was prepared as a 10-mM stock solution in dimethylsulfoxide (DMSO) for the shorter-term experiments. For each group of 8–10 cells, 25 μl stock solution was diluted into 10 ml culture medium for a final concentration of 25 μM APM and 0.25% DMSO. Groups of cells were pre-incubated separately for 5, 10, 15, 30, 45, 60 min, 2, 3 and 4 h. Control treatments contained only 0.25% DMSO. For recovery studies, cells were pre-incubated as above in APM for 3 h (the minimum time required for complete depolymerization of MTs), briefly incubated in 75 ml culture medium, and transferred to 10 ml of fresh medium. To wash out the APM, the medium was subsequently changed every 30 min for the first 2 h, and changed daily thereafter in longer-term recovery tests. Cells were allowed to recover for 15, 30, 60 min, 2, 4 and 48 h, and subsequently prepared for microscopy as noted above. For longer-term treatments, a 1-nM stock solution was prepared in DMSO, and diluted to a final concentration of 1 μM APM (+ 0.1% DMSO) in culture medium. The cells were pre-incubated as above for 24, 48, 96 and 140 h, and were carefully transferred to fresh APM-containing solutions daily. Control treatments consisted of 0.1% DMSO alone in culture medium.

Taxol (Natural Products Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md., USA; No. NSC 125973) was also dissolved in DMSO (1 mM) and diluted to 1 μM in culture medium. Cells were treated identically to those in the longer-term APM treatments.

Figs. 1–10. Time course of MT breakdown in *Ernodesmis* during cold treatment. All micrographs of cortical MTs have the longitudinal axes of cells oriented from top to bottom of page. × 1100; bars = 20 μm. Fig. 1. Tubulin immunofluorescence of cortical MT array in untreated control, showing that MTs are predominantly parallel to each other and to the longitudinal axis. Fig. 2a, b. Tubulin immunofluorescence a and DIC b of same region showing corresponding nuclei. Many MTs radiate from a bright immunofluorescent halo surrounding each nucleus. Fig. 3. After 8 min on ice the cortical MTs remain intact, as seen in this plasma membrane "peel". Fig. 4a, b. Immunofluorescence a and DIC b of the same region showing perinuclear arrays have completely disappeared within 8 min on ice. No further changes in them are apparent in longer treatments. Fig. 5. Most cortical MTs appear unchanged in "peels" after 30 min on ice. Fig. 6. Far fewer cortical MTs are present after 60 min on ice, and more MT ends are apparent as seen in this "peel". Fig. 7. Following 90 min on ice fewer cortical MTs remain, and cytoplasmic fluorescence is evident. Fig. 8. Very few cortical MTs of varying lengths are present after 2 h on ice. Fig. 9. After 4 h on ice the number of cortical MTs is greatly reduced, and the reticulate pattern of cytoplasmic labeling is apparent in this "peel". Those remaining are still oriented parallel to the longitudinal axis. Fig. 10. Generally all cortical MTs are absent following 6 h on ice, but regions can be found in some cells where a few remain. There is also much cytoplasmic labeling, including that surrounding the chloroplasts.