Plant Cell Physiol. 46(4): 671–675 (2005)
doi:10.1093/pcp/pci068, available online at www.pcp.oupjournals.org
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Short Communication

Aberrant Cell Plate Formation in the *Arabidopsis thaliana microtubule organization 1* Mutant

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MICROTUBULE ORGANIZATION 1 **encodes a microtubule-associated protein in *Arabidopsis thaliana* but different alleles have contradictory phenotypes. The original mutant *mor1* alleles were reported to have disrupted cortical microtubules, swollen organs and normal cytokinesis, whereas other alleles, embryo-lethal *gemini pollen 1* (*gem1*), have defective pollen cytokinesis. To determine whether MOR1 functions generally in cytokinesis, we examined the ultrastructure of cell division in roots of the original *mor1-1* allele. Cell plates are misaligned, branched and meandering; the forming cell plates remain partly vesicular, with electron-dense or lamellar content. Phragmoplast microtubules are abundant but organized aberrantly. Thus, MOR1 functions in both phragmoplast and cortical arrays.**

Keywords: *Arabidopsis thaliana* — Cell division — Cortical microtubules — Phragmoplast — Roots

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Microtubules are able to participate in diverse cellular functions by virtue of microtubule-associated proteins. A powerful way of identifying proteins responsible for a given activity is through developmental genetics. An *Arabidopsis thaliana* mutant recovered by screening the integrity of the cortical array was called *microtubule organization 1* (*mor1*; Whittington et al. 2001). This is a temperature-sensitive mutant, appearing indistinguishable from wild type at the permissive temperature (20°C) and expressing an altered phenotype at the restrictive temperature (30°C). At 30°C, the mutant has cortical arrays with fewer and disorganized microtubules in roots, hypocotyls and leaves. Concomitantly, these organs tend to grow isotropically. The protein identified by this mutation is a member of the XMAP215 family of microtubule-associated proteins, present throughout eukaryotes and known in animals and fungi to play a prominent role in mitosis and cell division (Gard et al. 2004).

The characterization of *mor1* stated that the aberrant phenotype was specific to cortical microtubules, with spindle and phragmoplast being unaffected (Whittington et al. 2001). This is puzzling for several reasons. First, in a screen for pollen development, alleles of *MOR1* called *gemini pollen1* (*gem1*) were recovered that have manifestly defective pollen cytokinesis (Park et al. 1998, Twell et al. 2002). The two *gem1* alleles recovered are embryo lethal and so it is not known whether they would lead to cytokinetic anomalies in sporophytic tissue. Second, two independent antisera against MOR1 stain the phragmoplast brightly (Twell et al. 2002, Hamada et al. 2004), which is consistent with a function in cytokinesis. Finally, a phragmoplast function for MOR1 is suggested by biochemical experiments with the tobacco homolog, TMB200 (Yasuhara et al. 2002, Hamada et al. 2004).

For these reasons, we re-investigated the cytokinetic phenotype of *mor1-1* using electron microscopy to determine whether defects in cell division were apparent at the ultrastructural level. We show here that cell plate formation in the *mor1-1* root is strikingly aberrant.

The process of cell plate formation appeared to be unaffected in root meristems of *mor1-1* grown at the permissive temperature (Fig. 1A, B), and indistinguishable from the wild type (not shown). Notably, the cell plate formed rather symmetrically in the cell, roughly equidistant from each side wall, and from its inception the plate ran more or less straight across the cell from one edge to the other. Particularly at the edges of the forming cell plate, phragmoplast microtubules were abundant (Fig. 1B). In contrast, many cell plates were abnormal in *mor1-1* exposed to the restrictive temperature, even for 6 h (Fig. 1C–H). Anomalies seen included off-center phragmoplasts (Fig. 1C), wavy cell plates (Fig. 1E, G) and branched cell plates (Fig. 1F). At the edges of the forming cell plate, only a few microtubules could be observed (Fig. 1D). Furthermore, the membranous compartments of the forming cell plate, although sometimes electron lucent as at 20°C (Fig. 1E), were typically electron dense, often extremely so (Fig. 1D, F). The lumens were often swollen, containing amorphous or multi-lamellar material (Fig. 1H).

With exposure to the restrictive temperature for 24 h, the cell plate anomalies became more frequent and severe (Fig. 2). Cell plates were incomplete, asymmetric, wandering, branched and electron dense. Multi-lamellar bodies occurred frequently around the cell plate or even around presumptive sites of wall formation (Fig. 2E). The process that leads to a regular coales-

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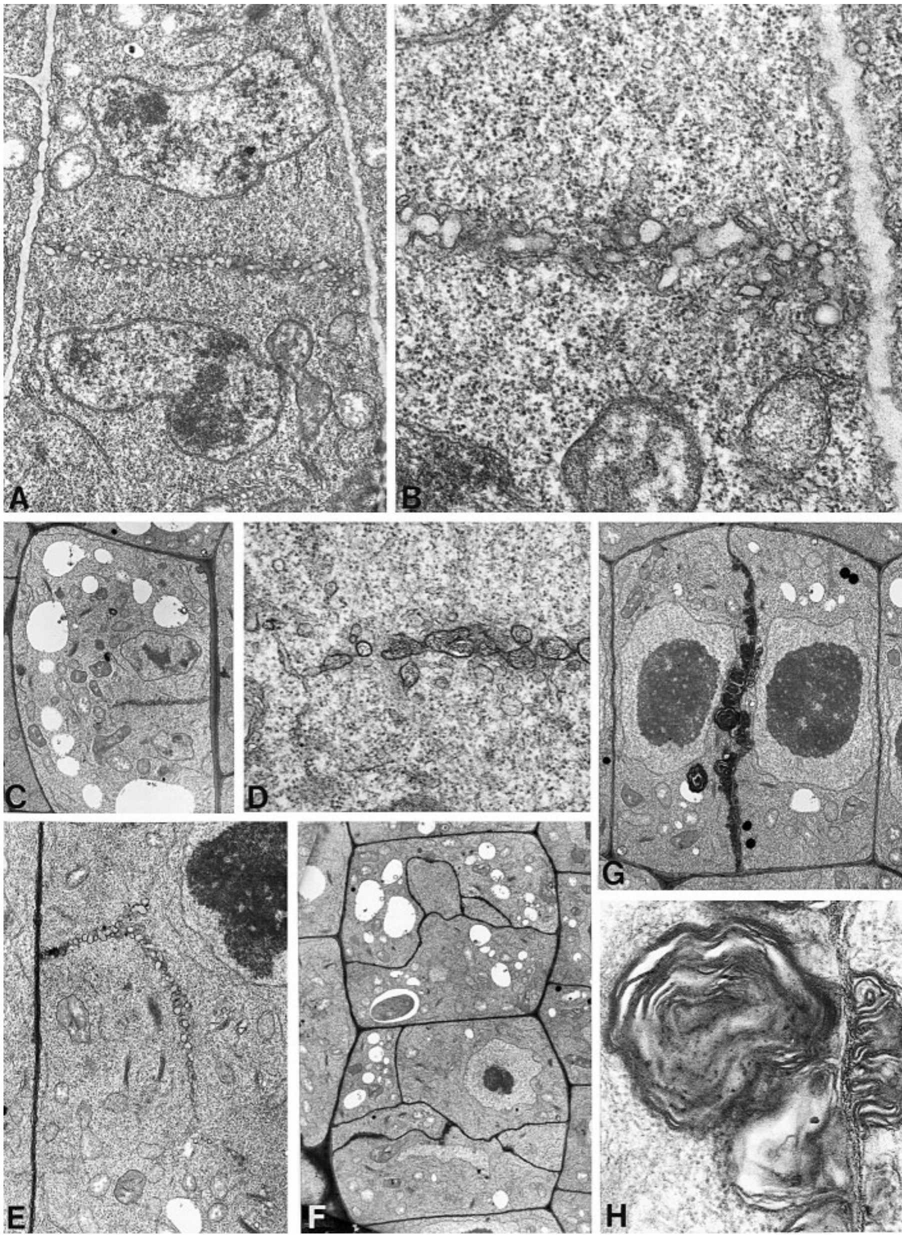

Fig. 1 Electron micrographs of the *mor1-1* mutant of *A. thaliana* either (A, B) exposed to the permissive temperature (20°C) for 6 d or (C–H) exposed to 20°C for 5 d followed by 6 h at the restrictive temperature (30°C). (A) Developing cell plate. ×10,400. (B) Higher magnification view of the right end of the cell plate shown in (A). Phragmoplast microtubules can be detected. ×29,600. (C) Half-completed cell plate attaching to the external wall of a meristematic epidermal cell. ×2,500. (D) Higher magnification view of the left edge of the cell plate in (C). Only a few microtubules can be detected. ×23,600. (E) Wandering cell plate in an epidermal cell. ×4,700. (F) Recently divided epidermal cells with bifurcated cytokinetic walls. ×2,000. (G) Recently divided cell with darkly staining material within and around the cell plate. ×3,100. (H) Higher magnification view of the cell plate in (G) showing multi-lamellar bodies. ×30,700.

cence of the cell plate vesicles into an integral layer seemed prone to disruption, with the cell plates having the appearance of independent aggregates of vesicles. At the edges of the cell plate, microtubules were scarce if not entirely absent (Fig. 2G).

In the electron micrographs of *mor1-1* at the restrictive temperature, microtubules associated with the phragmoplast were rare. To assess the microtubule organization in the phragmoplast, we turned to light microscopy and immunocytochemistry. We cryofixed roots in liquid propane to maximize preservation because microtubules in the mutant might be quite labile. Sections were double stained for cell walls and microtubules. At the permissive temperature, cell walls in *mor1-1* were organized with predictable geometrical regularity (Fig. 3A), and indistinguishable from wild type (not shown). In contrast, after 24 h at the restrictive temperature, anomalous cell walls pervaded the root meristem (Fig. 3B). Nearly every cell appeared affected in epidermis and cortex as well as many cells interior to these tissues. At the permissive temperature, microtubules in the phragmoplast were well organized, on either side of the forming cell plate (Fig. 3D). In contrast, after 24 h at 30°C, phragmoplast microtubules were often disrupted. In Fig. 3E and F, a cell is shown with an apparently branched cell plate and several states of microtubule organization typical of the images. The upper cell plate fragment has relatively well structured microtubules with the typical unstained region in the middle; however, the other areas of cell plate have micro-

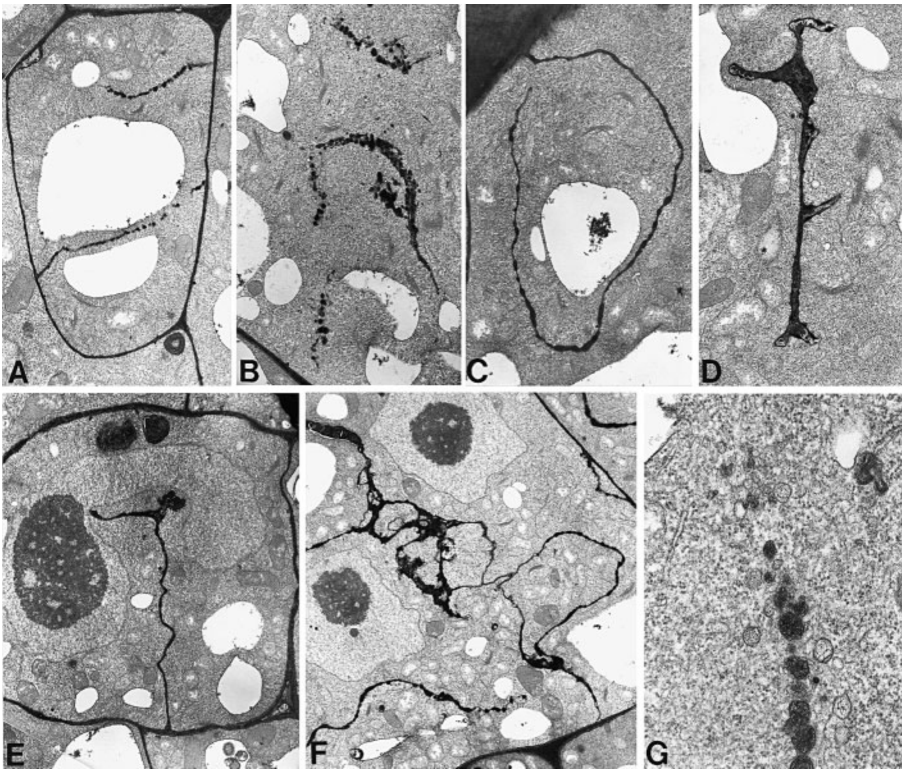

Fig. 2 Electron micrographs of *mor1* exposed to 20°C for 5 d followed by 24 h at (30°C). (A) Dividing epidermal cell with two cell plates connected to opposite parental walls. ×3,400. (B) Central part of a dividing epidermal cell illustrating several independent, irregular aggregations of cytokinetic vesicles. ×3,500. (C) Wandering cell plate. ×4,000. (D) Branched cytokinetic wall in the middle of a cell without evident connection to lateral parental walls. ×5,300. (E) Non-divided nucleus curving around the branched end of an incomplete wall. At the opposing site where normally the wall was expected to fuse, there are aggregations of multilamellar bodies. ×3,250. (F) Highly irregular cytokinetic walls meandering in the cytoplasm and separating daughter nuclei. ×2,300. (G) One edge of a cell plate consisting of densely staining vesicles. Vesicles are granular, while phragmoplast microtubules are absent. ×17,500.

tubules whose organization is unusual. In some cells, the only disturbance to the phragmoplast appeared to be its orientation, for example running obliquely across a cell (not shown).

We show here that *mor1-1* has a substantial, temperature-sensitive defect in cytokinesis. The wandering, bifurcated cell plates and irregular membrane profiles seen here in the root closely match those reported in *gem1* pollen (Park and Twell 2001), linking the phenotypes of these distinct alleles of *MOR1*. Evidently, the properties conferred on microtubules by MOR1 are required by the cortical array as well by the phragmoplast.

Previously, *mor1-1* was reported to have wild-type cytokinesis (Whittington et al. 2001, Sugimoto et al. 2003), although neither study showed cell plates or phragmoplasts. Cytokinesis in *mor1-1* could be sensitive to a particular growth condition, although our nutrient medium, temperatures, light intensity and time course match closely those of the previous reports. One exception is that we grew plants under yellow filters, used to remove ultraviolet light (Stasinopoulos and Hangarter 1990). Alternatively, the authors of the previous publications focused on cortical microtubules; in whole-mount preparations where only the cortical microtubules against the outer tangential cell wall are observed, cell plate status may be difficult to appreciate.

At the electron microscope level, microtubules in all arrays were difficult to see in *mor1-1* at the restrictive temperature. Because microtubules in the cryofixed material were abundant in spindles and phragmoplasts, we ascribe our failure to see them in electron micrographs to poor preservation. In other cell types, XMAP215 has microtubule-stabilizing activity (Gard et al. 2004) and so a mutant at this locus could be expected to destabilize microtubules. Indeed, it has been shown recently that destabilizing microtubules in low concentrations of the inhibitor of polymerization, oryzalin, leads to fewer and disorganized microtubules that require cryofixation for clear imaging (Baskin et al. 2004). Caution is required when using chemical fixation on material in which microtubule stability is compromised. Nevertheless, the product of microtubule activity, namely the cell plate, is revealed clearly at the electron microscope level and shown to be remarkably aberrant when *mor1-1* is grown at the restrictive temperature.

In *mor1-1* at 30°C, not only was the structure of the cell plate altered but so too was its orientation. The disoriented cell walls link *mor1-1* to several other mutants that have aberrant root morphogenesis. In *rsw2* (Lane et al. 2001), *rsw4* and *rsw7* (Wiedemeier et al. 2002), root swelling is associated with incomplete and grossly misaligned cell walls, similar to the patterns seen in *mor1*. The gene product of *RSW2* is the endoglucanase, KORRIGAN, thought to participate in cellulose synthesis (Doblin et al. 2002). Although cell walls are aligned properly in *rsw1* (Williamson et al. 2001), the common association between root swelling and misaligned cell walls suggests a causal relationship. Given that *rsw4* and *rsw7* expand to the same extent whether or not cell division is blocked (Wiedemeier et al. 2002), it is reasonable to conclude that stimulated radial expansion can interfere with the process of cell wall alignment. However, a deleterious influence of swell-

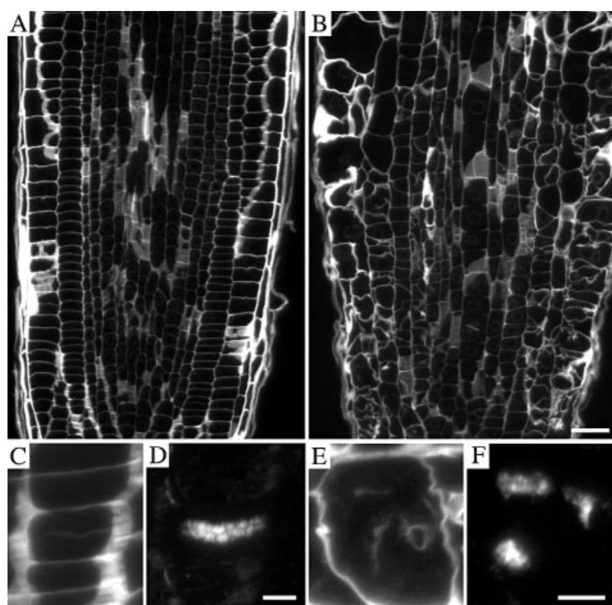

Fig. 3 Light micrographs of *mor1-1* exposed to the permissive temperature (20°C) for 6 d (A, C and D) or exposed to 20°C for 5 d followed by 24 h at 30°C (B, E and F). Images are methacrylate sections surveying cell wall alignment (A and B; calcofluor staining) or higher magnification views of cell plate formation in sections double-stained for cell walls (C and E) and tubulin (D and F). Bars = 20 µm in A, B; and 5 µm for C–F.

ing is insufficient to explain the cell plate abnormalities seen in *mor1-1*. Cell wall formation in *rsw7* and *rsw4*, although misaligned, was otherwise similar to wild type (our unpublished observations), in contrast to the wandering, bifurcated and vesicular cell plates of *mor1-1*.

Instead, the anomalies in the phragmoplast and cell plate seen for *mor1* are likely to result from decreased microtubule function. These anomalies generally resemble those observed in roots exposed to herbicides that target microtubules, particularly when moderate concentrations are used so that some microtubules remain (Vaughn and Lehnen 1991, Hoffman and Vaughn 1994). Specifically, meandering and vesicular cell plates with electron-dense contents, similar to those shown here, were seen in wheat and onion roots exposed to the herbicide, dithiopyr (Armbruster et al. 1991, Lehnen and Vaughn 1991). This compound has little affinity for tubulin but instead binds directly to a putative microtubule-associated protein of 67 kDa (Armbruster et al. 1991). Interestingly, dithiopyr treatment fragments and shortens microtubules, effects that are similar to those reported for *mor1-1* (Whittington et al. 2001, Sugimoto et al. 2003), suggesting that dithiopyr targets a protein in the same pathway as MOR1.

In vitro, the tobacco homolog of MOR1, TMBP200, cross-links and bundles microtubules in a manner reminiscent of that in the phragmoplast (Yasuhara et al. 2002), and in addition drives microtubule polymerization (Hamada et al. 2004). We show here that the phragmoplast as well as the cortical array is disrupted in *mor1-1*; future work will reveal which activity, bundling or polymerization, or both, is required for the functions of cell division and the control of directional cell expansion.

Materials and Methods

Seeds of the *Arabidopsis thaliana* L (Heynh) Columbia wild type and of the temperature-sensitive mutant, *mor1-1* (Whittington et al. 2001), the generous gift of Geoffrey Wasteneys (University of British Columbia), were used. Seed was sterilized in dilute bleach, germinated on agar-solidified growth medium in Petri dishes that were sealed with air-permeable bandage tape, and seedlings were grown vertically under continuous yellow light (150 µmol m^{-2} s^{-1}) and a constant temperature of 20°C (permissive temperature) for 6 d in a growth chamber, as described previously (Wiedemeier et al. 2002). To cause expression of the swelling phenotype, plates containing 5-day-old seedlings were transferred to 30°C (non-permissive temperature) under continuous, yellow light (80 µmol m^{-2} s^{-1}) for up to 48 h.

For electron microscopy, fixative was poured on the seedlings on the plates, which were gently rocked for 2 h at room temperature. The fixative contained 2% (v/v) glutaraldehyde, 50 mM PIPES (pH 7.05), 5 mM $CaCl_2$, and in some cases 0.1% (w/v) tannic acid (Mizuhira and Futaesaku 1972) or 2 mM ethylene glycol bis-sulfosuccinimidylsuccinate (Lovy-Wheeler et al. 2005). After rinsing in buffer containing 50 mM PIPES and 5 mM $CaCl_2$ for 45 min (three changes), root tips were excised and sandwiched between Formvar films on wire loops as described by Baskin and Wilson (1997). Root tips were then post-fixed in 1% OsO_4 similarly buffered, for 2 h at room temperature. Alternatively, some roots were post-fixed in the same medium containing 0.8% potassium ferricyanide (Hepler 1980). After rinsing in buffer, they were stained with 2% aqueous uranyl acetate for 2 h, dehydrated through an acetone series and embedded in Epon/Araldite epoxy resin. Ultrathin sections were collected on 698 mesh uncoated copper grids and examined with a Jeol 100S electron microscope after double staining with 2% aqueous uranyl acetate and lead citrate for 10 + 10 min.

For light microscopy, seedlings were cryofixed in liquid propane, freeze-substituted in acetone, embedded in butyl-methyl-methacrylate, sectioned (1.75 µm), and immunostained for tubulin as described previously (Baskin et al. 1996). In the final rinses, sections were also incubated in 0.01% Calcofluor White for 10 min to stain cell walls.

Acknowledgments

We thank Geoffrey Wasteneys (University of British Columbia) for seeds of *mor1-1*. This project was partially funded by a grant to T.I.B. from the U.S. Department of Energy (award No. 03ER15421), which does not constitute endorsement by that Department of views expressed herein. E.P.E. thanks the Aristotle University of Thessaloniki for supporting his sabbatical leave.

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(Received January 3, 2005; Accepted February 4, 2005)