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CLASP Modulates Microtubule-Cortex Interaction during Self-Organization of Acentrosomal Microtubules

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CLASP proteins associate with either the plus ends or sidewalls of microtubules depending on the subcellular location and cell type. In plant cells, CLASP's distribution along the full length of microtubules corresponds with the uniform anchorage of microtubules to the cell cortex. Using live cell imaging, we show here that loss of CLASP in *Arabidopsis thaliana* results in partial detachment of microtubules from the cortex. The detached portions undergo extensive waving, distortion, and changes in orientation, particularly when exposed to the forces of cytoplasmic streaming. These deviations from the normal linear polymerization trajectories increase the likelihood of intermicrotubule encounters that are favorable for subsequent bundle formation. Consistent with this, cortical microtubules in *clasp-1* leaf epidermal cells are hyper-parallel. On the basis of these data, we identify a novel mechanism where modulation of CLASP activity governs microtubule-cortex attachment, thereby contributing to self-organization of cortical microtubules.

INTRODUCTION

Microtubule (MT) association with the cell cortex is crucial for cell polarization, outgrowth of cell extensions such as neuronal processes, and cell migration. MTs elongating from centrosomes can be stabilized upon plus-end interaction with anchoring sites in the cortex (Gundersen *et al.*, 2004). In contrast, in the acentrosomal cells of higher plants, the majority of interphase MTs are nucleated in a dispersed manner throughout the cell cortex and typically remain cortically anchored along their lengths for the duration of their lifespan (Hardham and Gunning, 1978; Wasteneys, 2002; Chan *et al.*, 2003; Shaw *et al.*, 2003; Ehrhardt and Shaw, 2006).

Despite the ubiquitous association of microtubules with the cell cortex in plant cells, little is known of the molecular components responsible for this linkage. Cross-bridges between MTs and the plasma membrane have been detected by transmission electron microscopy, but their identity remains unknown (Hardham and Gunning, 1978). The biochemical identification of a 90-kDa phospholipase D (PLD) from MT fractions led to a proposed role for PLD in membrane anchoring (Gardiner *et al.*, 2001). Consistent with this, treatment with the PLD drug *n*-butanol has been shown to disorganize cortical MTs (Gardiner *et al.*, 2003) or to detach them from the cell cortex (Dhonukshe *et al.*, 2003) when MTs are labeled by overexpression of the green fluorescent protein (GFP)-MBD MT reporter protein. Other studies, however, show a MT-destabilizing effect of *n*-butanol at higher concentrations (Hirase *et al.*, 2006). More recently *n*-butanol has been demonstrated to enhance the developmental reorientation of MTs from transverse to longitudinal in leek cells (Sainsbury *et al.*, 2008). The drug morlin has also been shown to induce detachment of cortical MTs from the membrane in combination with the overexpression of the GFP-MBD reporter protein, although the putative target(s) of this drug are unknown (DeBolt *et al.*, 2007).

We report here that the *Arabidopsis* CLASP protein is involved in mediating MT-cortex attachment. CLASP/Orbit/MAST proteins are important players in stabilizing subsets of MT plus ends in specific regions of the cell cortex (Akhmanova *et al.*, 2001; Lansbergen *et al.*, 2006). In response to cellular and morphogenetic cues, CLASPs localize preferentially to MT plus ends in the leading edge of migrating cells, where they form a complex with the cortex-associated proteins LL5β and ELKs, which, by forming a link between MT plus ends and the cortex, facilitates normal cytoskeletal polarization and cell migration (Drabek *et al.*, 2006; Lansbergen *et al.*, 2006). In migrating PtK1 epithelial cells, CLASP localization on MTs is also differentially regulated, exhibiting plus-end tracking in the cell body and MT sidewall association upon entry into the leading edge lamella (Wittmann and Waterman-Storer, 2005). In both cell types, the differential localization of CLASP is mediated by an important regulator of cell polarity, GSK3β, which reduces MT lattice-binding affinity in the cell body (Akhmanova *et al.*, 2001; Wittmann and Waterman-Storer, 2005).

The recently discovered CLASP homologue in higher plants has been shown to localize in several differentiated cell types along the full length of cortical MTs, with only a weak enrichment at MT plus ends (Ambrose *et al.*, 2007; Kirik *et al.*, 2007). This distribution is reminiscent of the MT sidewall distribution of GFP-CLASP seen in the leading edge lamellae of migrating PtK1 epithelial cells (Wittmann and Waterman-Storer, 2005), suggesting it is a functionally conserved regulatory mode of CLASP. Interestingly, the MT sidewall decoration of CLASP upon entry of MTs into the lamella appears to be accompanied by a drastic reduction in lateral MT mobility, presumably because of anchoring of the MTs along their length to the plasma membrane. On the basis of these observations, we hypothesized that the constitutive MT sidewall binding of CLASP in plant cells may play a role in the lateral association of MTs with the cell cortex and may represent a universal mechanism of linking MTs to the cortex.

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Using live imaging of *Arabidopsis* epidermal cells we show here that MTs in plants lacking CLASP display frequent and prolonged cortical detachments. Detached MTs exhibit large deformations and changes in orientation in response to the prevailing cytoplasmic stream. This enhanced lateral mobility of MTs in the absence of CLASP increases the frequency of interactions between MTs that result in bundle formation, and correlates with increased parallel ordering of MTs in leaf epidermal cells. These data reveal a new self-organizational mechanism for cortical MT arrays, wherein modulation of MT-cortex anchoring directs the outcome of MT–MT interactions.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Seeds were cold-treated after planting for 48 h to synchronize germination. Seedlings were grown aseptically on Hoagland's medium solidified with 1.2% agar at 23°C under continuous light. Cotyledons were excised from 7 to 12 d seedlings and placed in water between a slide and coverslip to enable imaging of the adaxial (top) surface at 24°C. The phenotypes described here were observable at all stages of development (4–12 d). No more than two cells were imaged per cotyledon to minimize phototoxicity. Wild-type seeds expressing GFP-β-tubulin 6 from *Arabidopsis* (Nakamura *et al.*, 2004) were obtained as a gift from Dr T. Hashimoto (Nara Institute of Science and Technology, Japan) and crossed to *clasp-1* plants (SALK accession no. salk_120061).

Tissue Preparation and Microscopy

Images were acquired on a Zeiss Axiovert (Zeiss, Göttingen, Germany) microscope equipped with an axiocam HRmCCD camera, driven by Axiovision software (Zeiss). Bandpass filters for GFP were 460–480-nm excitation and 505–530-nm emission. Typical light exposure regimens were 1–1.5-s exposures at 30% arc lamp intensity (Zeiss FluoArc), at 5-s intervals. Photobleaching was negligible under these conditions. Individual cells were observed for no more than 5 min, because MT detachment activity was sensitive to photodamage. Confocal imaging was performed with a 40× plan-apochromatic water immersion objective mounted on a Zeiss Pascal, using the 488-nm line from an argon laser. Typical scan times were 4 s, using a line averaging of two. Slice thickness was 1.5 μm. For latrunculin B treatments, a 20 μM solution in 1% DMSO was prepared from a 2 mM stock in 10% DMSO. Cessation of streaming was typically observed at 5–10 min after application. DMSO, 1%, alone had no obvious effect on detachment activity or MT organization.

Image Analysis and Statistical Analysis

Image analysis was performed using ImageJ software (http://rsb.info.nih.gov/ij/). Figures were assembled in Corel Draw (www.Corel.com; Corel Systems, Ottawa, ON, Canada). Statistical analysis was performed using Microsoft Excel (Microsoft, Redmond, WA).

RESULTS

Loss of CLASP Generates Hyper-Parallel Cortical MT Arrays That Exhibit Reduced Cortex Anchoring

To understand the role of CLASP in the spatial organization of cortical MT arrays, we compared MT behavior in wild-type and *clasp-1* mutant *Arabidopsis thaliana* plants, which contain a T-DNA insertion that knocks out expression of the single-copy *CLASP* gene (Ambrose *et al.*, 2007; Kirik *et al.*, 2007). In agreement with our previous immunofluorescence data reported for roots (Ambrose *et al.*, 2007), as well as for GFP-tubulin in hypocotyl cells (Kirik *et al.*, 2007), MTs in most *clasp-1* cells were well organized into parallel arrays. Analysis of MT orientation in living cells expressing GFP-tubulin (GFP-TUB6) revealed that in cotyledon and leaf epidermal cells, *clasp-1* MTs exhibited a greater degree of parallel order than those of wild type (Figure 1). In contrast to the varied MT orientations present within individual wild-type cells, *clasp-1* cells typically possessed one predominant MT orientation throughout the cell (Figure 1, A and B). Quantification of MT angles in individual cells showed a

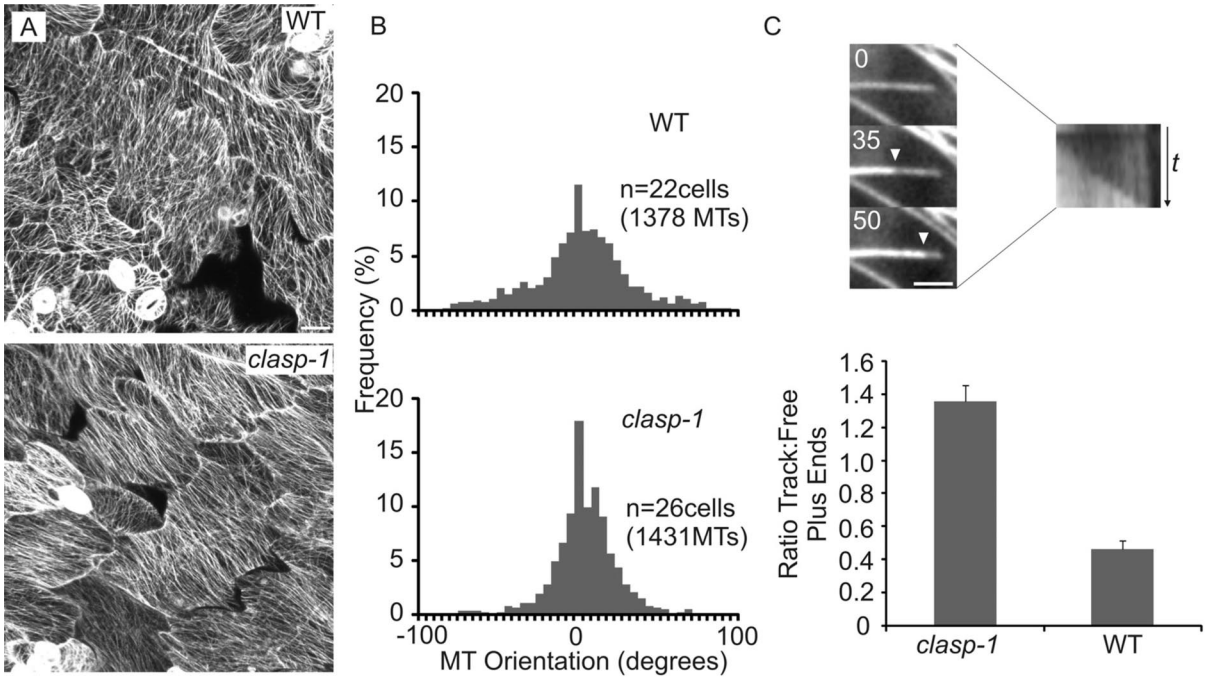

Figure 1. Cortical MTs in *clasp-1* cotyledon epidermal cells are hyper-parallel. (A) Confocal images of cotyledon adaxial epidermal cells in wild type and *clasp-1*. Images are Z-projections of stacks taken at the lower middle region of the leaf. (B) Histogram of MT angular distributions in wild-type and *clasp-1* cotyledon epidermal cells. Angles are normalized to each cell, where the 0° angle defines the predominant MT orientation. (C) A higher proportion of MT plus ends reside along other MTs in *clasp-1* (n = 976) compared with wild type (n = 837). The image series shows an example of an MT growing along another "track" MT. A kymograph is shown at right. Data for C is taken from 15 cells each. Bar, (A) 20 μm, (C) 2.5 μm. Times in C are seconds.

narrowing of the angular distribution in *clasp-1* compared with wild type (Figure 1B). Consistent with the increased MT parallelism, a higher proportion of MT plus ends were found to be growing along preexisting MT tracks in *clasp-1*. From time-lapse image series obtained with a high-resolution CCD camera, we determined that *clasp-1* mutants have an approximate threefold increase in the ratio of MT plus ends growing along other MT tracks compared with those growing freely (Figure 1C).

In *clasp-1* cells, MTs displayed a conspicuous behavior, wherein MTs partially detached from the cortex over large lengths and durations, deviating from their normally linear growth trajectories. The detached portions underwent extensive waving, bowing, and changes in orientation, particularly when exposed to the forces of cytoplasmic streaming (Figure 2).

These behaviors can be most easily appreciated in the accompanying time-lapse movies (see Supplementary Movies S1–S9). Figure 2A shows a typical reorientation, in which the leading end of a growing MT dissociates and undergoes a large change in orientation before reassociation with the cell cortex (see Supplementary Movie S4). MTs were frequently observed to partially reattach to the cortex in one or more locations. Figure 2B shows an example of this, in which the leading end of the detached region reassociates with the cortex and begins growth in a linear trajectory, whereas the middle segment of the MT remains free (Figure 2B; see Supplementary Movie S5). Extensive MT distortions between the two points of cortical anchoring are apparent (arrowheads). Partial reattachment points were often transient, or upon partial reattachment, the initial region of attachment was propagated along the MT, resulting in larger regions of attachment.

Cortex detachments were occasionally observed in wild-type cells, albeit much less frequently, and involving less extensive stretches of MTs than those observed in *clasp-1* cells (Figure 2, C–E). Specifically, MT detachments in *clasp-1* were 3.2 times more frequent, were 1.6 times longer in

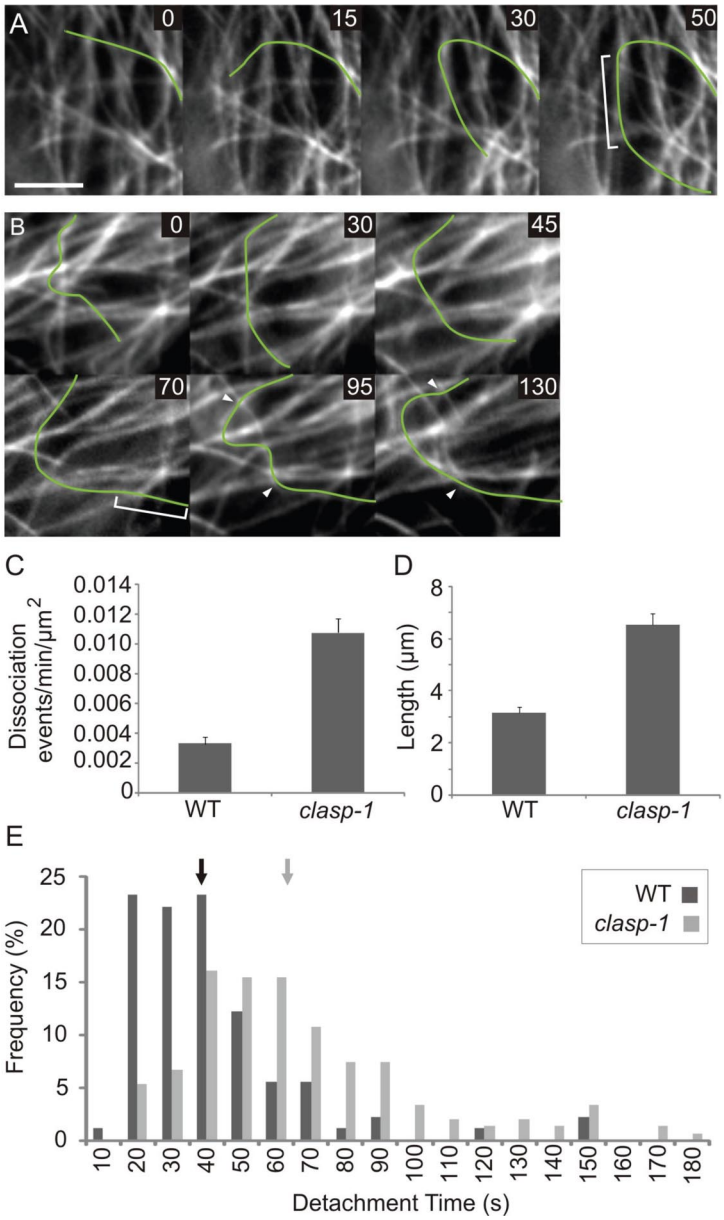

Figure 2. Cortical MTs in *clasp-1* cotyledon epidermal cells exhibit compromised cortex attachment. (A) Image sequence showing a typical MT detachment–reattachment event in *clasp-1*. On reassociation, the MT aligns with a cortex-bound MT (region of MT–MT overlap denoted by brackets). Corresponds to Supplementary Movie S4. (B) Very large MT detachment in *clasp-1*, accompanied by partial reattachment. Partial reattachment of the leading end occurs at 70 s (attached portion denoted by brackets). After partial reattachment, extensive MT distortions between the two points of cortical anchoring are apparent (arrowheads). Corresponds to Supplementary Movie S5. (C) Frequency of MT cortical detachments in wild-type and *clasp-1* cotyledon epidermal cells. Detachment was defined by the MT moving out of the focal plane at some point during observation and/or any lateral swinging of the MT. (D) Mean length (μm) of MT detachments in wild-type and *clasp-1* cotyledon epidermal cells. (E) MT detachment times (seconds) in wild-type and *clasp-1* cotyledon epidermal cells. Means are denoted by arrows. Data are means ± SEM. Bar, 5 μm.

Table 1. MT detachment parameters in wild-type and *clasp-1* cotyledon epidermal cells

	Wild type	*clasp-1*
Detachment frequency (events μm^{-2} min^{-1})	0.0033 ± 0.0005 (13, 136)	0.0108 ± 0.0010 (13, 485)
Detachment length (μm)	3.2 ± 0.3 (7, 44)	6.5 ± 0.4 (6, 58)
Detachment time (s)	40.0 ± 2.7 (12, 90)	64.7 ± 2.8 (17, 149)

MTs detach from the cortex more frequently, over larger MT lengths, and for longer durations. Values are means ± SEM, with number of cells and MTs, respectively, in parentheses. All differences between genotypes are statistically significant ($p < 0.001$).

duration, and the detached lengths were twice as long compared with those observed in wild-type cells (Figure 2, C–E; Table 1). The observed cortical detachments in wild type suggest that the *clasp-1* phenotype is an exacerbation of a normal behavior.

The enhanced cortical MT dissociation in *clasp-1* was observed in all cell types examined (hypocotyl epidermal, lateral root cap, and cotyledon epidermal) and was most easily documented in cotyledon epidermal cells, which are shown here. Although most MTs in *clasp-1* cotyledon epidermal cells resided within parallel arrays, most of the detachment behavior was observed in the subpopulation of MTs with discordant orientations relative to the predominant array. On the basis of the enhanced parallelism and the observation that MTs frequently realigned in the direction of the predominant cytoplasmic flow upon reattachment, we initially hypothesized that the enhanced parallelism observed in *clasp-1* mutants is due to cytoplasmic streaming-dependent flow alignment. The hypothesis, however, is not supported by the following data. First, in many cells, the prevailing direction of cytoplasmic streaming did not match the predominant MT orientation, often flowing at oblique or right angles to the MT alignment axis, or consisting of multiple flow vectors in different areas of the cortex. Second, MT arrays remained parallel upon elimination of acto-myosin–based cytoplasmic streaming with the drug latrunculin B, even after prolonged treatments of >24 h (Supplementary Figure S1). MTs in latrunculin B–treated *clasp-1* cells still detached from the cortex, although they did not show the characteristic lateral displacements observed in untreated cells (Supplementary Figure S1). Furthermore, streaming velocities did not appear to be affected in untreated *clasp-1* cells. These data show that streaming is not required for the maintenance of MT parallelism or that it plays only a minor role.

Partial Cortical MT Detachment Enables Interactions between MTs and Bundle Formation

Partially detached MTs in *clasp-1* often waved in the cytoplasmic stream until encountering another partially detached or a cortex-associated MT, resulting in the two MTs associating laterally to form a bundle (Figure 3, A and B; see also Figure 2A).

This search-and-capture–based MT bundle formation occurred in two distinct ways: 1) polymerization-dependent bundling, in which the detached plus-end encounters and then grows along another MT track (Figure 3A; see Supplementary Movie S6); and 2) polymerization-independent bundling, which can occur either by zippering, in which the detached segment (i.e., plus ends and/or interior portions) encounters and then progressively anneals behind the initial contact point (brackets in Figure 3, A and B, and brackets in Figure 2A), or by abrupt uniform lateral annealing of the two MTs (Figure 3C, brackets; see Supplementary Movie S8). The zippering mechanism is similar to the partial cortical reattachment described above, where cortex attachment is propagated from an initial point. Two unattached MTs frequently coaligned into a bundle upon encounter (Figure 3B; see Supplementary Movie S7). When two unattached MTs approached one another at different angles and interacted, the region of MT overlap typically straightened as the individual MTs grew together (Figure 3B, brackets). We also observed frequent detachment of MT bundles (as determined by filament width and fluorescence intensity) in *clasp-1* (Figure 3B). The example in Figure 3C shows a detached bundle "snapping" together with another MT.

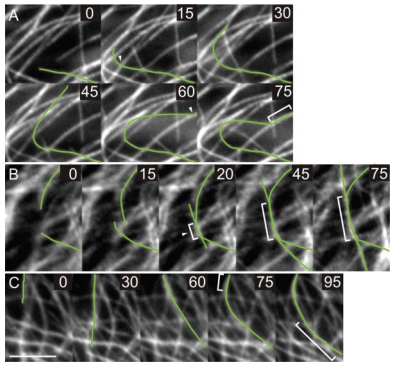

Figure 3. Cortical MT detachment behaviors often result in interactions between MTs in *clasp-1*. (A) Reattachment to a cortical MT upon reassociation. Arrowhead indicates the start of the detachment episode, which is coincident with encounter of the MT plus end with an obstacle MT. Brackets denote coalignment region between MTs after reattachment. Corresponds to Supplementary Movie S6. (B) Coalignment of two cortically detached MTs upon encounter. Note the gradual zippering and straightening of the region of overlap as it forms (brackets). Corresponds to Supplementary Movie S7. (C) MT bundle detachment. The bundle snaps into alignment with cortically associated MTs in two places (brackets). Corresponds to Supplementary Movie S8. Bar, 5 μm. Times are in seconds.

These conspicuous MT interactions in *clasp-1* prompted us to quantify MT bundle formation, which we define here as the annealing of two or more MTs into a single linear filament. To avoid erroneous inclusion of instances where MTs coincidentally grew in overlapping trajectories, bundling was only considered to have occurred when alignment was accompanied by a clear change in growth trajectory or position of one or both of the aligning MTs.

Our analysis identified a strong correlation between the frequency of MT detachment and MT bundle formation. Bundle formation events were roughly three times more frequent in *clasp-1* than in wild type (Figure 4A), which correlates closely with the 3.2-fold increase in detachment frequency (Figure 2C). Of these bundling events, 85% in *clasp-1* were associated with a detachment of one or both of

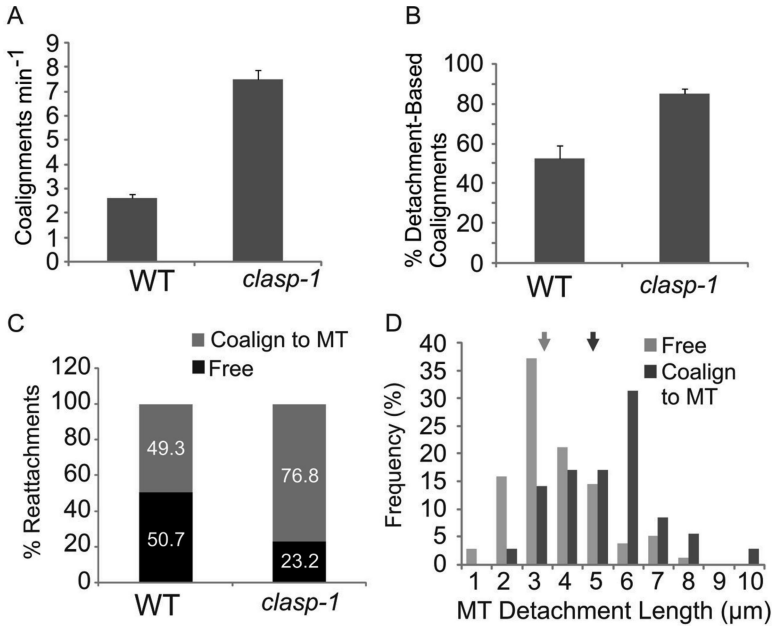

Figure 4. *clasp-1* mutants exhibit enhanced interactions between MTs, and greater MT detachment is associated with an increased ability to bundle with other MTs. (A) Bundling events per minute in a 20 × 20-μm box in *clasp-1* and wild type. (B) Percentage of MT bundle formations associated with a cortical detachment of one or both of the MTs. The proportion of detachment-associated bundling events is significantly higher in *clasp-1*. (C) Proportions of reattachments to cortex (free) or to another MT (bundle with MT) in *clasp-1* and wild type. MTs reattach to other MTs more frequently in *clasp-1*. (D) Longer MT detachment lengths result in bundling with other MTs more frequently than short detachment lengths in wild type. Means of each class are indicated by arrows. Data are means ± SEM. All comparisons are statistically significant ($p < 0.001$).

the aligning MTs, compared with 52.5% for wild type (Figure 4B). In the absence of CLASP, detached portions of MTs were also far more likely to reattach to the cortex via MT bundling rather than to reassociate freely with the cortex. In *clasp-1*, over three quarters of cortex reattachment events resulted in bundling upon reattachment, compared with roughly half in wild type (Figure 4C). Increased bundle formation in *clasp-1* is further supported by the observation that freely growing MTs, regardless of their cortical attachment status, spent less time before coaligning with other MTs in *clasp-1* compared with wild type (73.1 ± 4.1 s vs. 85.8 ± 4.4 s, $p = 0.018$, *t* test).

What is the basis for the increased prevalence of MT bundle formation in *clasp-1* cells? One possibility is that in the absence of CLASP, MTs bind each other more readily upon interaction, perhaps through increased availability of sites for cross-linking proteins. A second possibility is that the reduced cortical attachment in *clasp-1* enables MTs to explore a larger space, thereby increasing the incidence of MT encounters that are favorable for bundle formation. To differentiate between these two explanations, we quantified detachment events and bundling in wild type, where there is a natural range of detachment activity from cell to cell.

First, we drew a correlation between degree of detachment and bundle formation on a cell-to-cell basis. Wild-type cells that showed the highest degree of detachment activity also exhibited the highest frequency of bundling. The proportion of bundle formation events associated with MT detachment rose to as high as 80–90% in cells with the greatest degrees of detachment activity, compared with the above-noted wild-type average of 52.5%. Conversely, this proportion dropped to 10–20% in cells with the lowest detachment activity. Second, we were also able to draw a positive correlation between the degree of MT detachment and coalignment activity on an individual MT basis in wild-type cells. As shown in Figure 4D, longer MT detachment lengths are more likely to bundle with other MTs upon reattachment. Specifically, MTs that detached and bundled with other MTs were detached over a 31% greater MT length compared with those that did not bundle with other MTs upon cortex reassociation (Figure 4D).

Taken together, our data suggest an indirect role for CLASP in modulating MT–MT interactions, whereby maintaining MTs in close association along their entire lengths with the cell cortex reduces their exploratory capacity. When CLASP's function is reduced, larger partial MT detachment lengths allow exploration of larger swaths of cortical cytoplasm, thereby increasing the probability of finding and freely coaligning with other MTs.

CLASP Restricts the Angle of Incidence of MT Coalignment

On the basis of the findings that interactions between MTs may contribute to the self organization of noncentrosomal cortical MTs in plants and animals (Dixit and Cyr, 2004a; Reilein *et al.*, 2005) and the observations here of increased bundle formation in *clasp-1*, we next sought to determine whether the outcomes of MT–MT interactions are affected in *clasp-1* cells. As documented by others (Shaw *et al.*, 2003; Dixit and Cyr, 2004b), we also observed that growing MTs encountering an obstacle MT at small angles (<40°) frequently coalign to form a bundle. To distinguish this coalignment behavior from other types of bundling events, we define coalignment here specifically as the interaction and coalignment of a growing MT plus end with the obstacle MT it encounters. In wild-type cells this was the predominant mechanism of bundle formation, whereas in *clasp-1*, it was accompanied by the numerous detachment-associated mechanisms of bundle formation described above. Quantifying MT coalignments at first proved difficult in the steady-state arrays of *clasp-1* because many MTs were parallel to one another, thereby making collisions between freely growing plus ends and other MTs relatively infrequent. To achieve a situation wherein *clasp-1* was comparable with wild type, we rapidly depolymerized MTs with cold (−20°C, 4 min) and then observed their repolymerization upon return to room temperature (24°C). Immediately after cold treatment, MTs in *clasp-1* and wild type were completely depolymerized or retained a few short remnants of MT bundles (where bundles resided before chilling; Figure 5A; see Supplemental Movie S9). On return to room temperature, cells exhibited normal, rapid cytoplasmic streaming

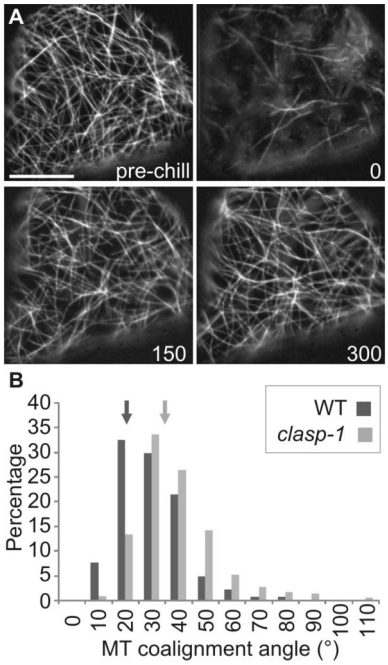

Figure 5. Cells lacking CLASP allow more permissive MT coalignment. (A) An example of the cold-depolymerization assay used to allow unbiased quantification of MT coalignment. Shown is a wild-type cell before and after chilling for 4 min at −20°C. MTs recover rapidly over the 300 s observation period. Most MTs are depolymerized at the start of recovery, and by 300 s most MTs have returned. Corresponds to Supplementary Movie S9. (B) Frequency distributions of wild-type and *clasp-1* MT coalignment angles in recovering cold-depolymerized cells. Arrows denote means that are significantly different ($p < 0.001$, *t* test). Bar, 10 μm.

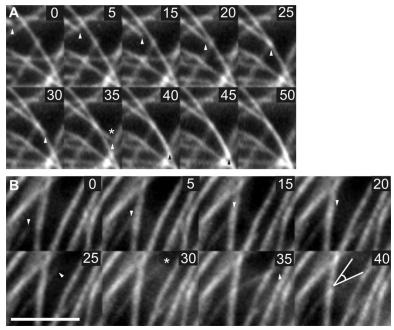

Figure 6. Encounter of MT plus ends with obstacle MTs can result in detachment from the cortex. (A) Detachment resulting in coalignment with the obstacle MT. This sequence is from a wild-type cell. Asterisk denotes the brief detachment that occurs upon encounter with the obstacle MT. Coalignment occurs with the obstacle MT after this dissociation, at 40 s. (B) Detachment resulting in reorientation to a new growth trajectory upon reattachment. This sequence is from a *clasp-1* cell. Bar, 10 μm.

and MT behavior and returned to full polymer status within <10 min, indicating the treatments were not detrimental to the cells (Figure 5A; see Supplementary Movie S9). At larger encounter angles, *clasp-1* MTs were more capable of coaligning than wild-type MTs (Figure 5B). MT coalignments in *clasp-1* occurred at up to 105° initial encounter angles, with a significantly higher mean coalignment angle of 35.2° for *clasp-1* and 23.8° for wild type (Figure 5B). Notably, large-angle MT coalignment events were also typically accompanied by partial detachment of the coaligning MT, consistent with our observations in non–cold-treated cells that MT detachment facilitates more promiscuous bundling activity.

Cortical MT Detachment Facilitates MT Coalignment

Previous reports have described MT coalignment between cortex-associated MTs (Shaw *et al.*, 2003; Dixit and Cyr, 2004b). We observed that in both wild type and *clasp-1*, MT coalignments are often associated with varying degrees of cortical detachment. In both wild type and *clasp-1*, encounter of a growing plus end with an obstacle MT is frequently followed by a brief dissociation from the cortex of the leading end of the growing MT. Importantly, for shallow angle encounters, this obstacle-associated detachment often results in coalignment, as the detached portion rapidly pivots into alignment with the obstacle MT (Figure 6A). Similarly, upon obstacle-based detachment, the MT may simply reassociate with the cortex and continue growth in the original trajectory or undergo reorientation during detachment and reassociate in a new growth path (Figure 6B). This was more typical of steep angle collisions, which have previously been shown to result in crossover of the obstacle MT (Dixit and Cyr, 2004b; Wightman and Turner, 2007).

In *clasp-1*, MT–MT encounters produced many of the large and prolonged detachment episodes described above, frequently resulting in coalignments with nearby nonobstacle MTs (Figure 3A). These data demonstrate that encounter of a growing MT plus end with an obstacle MT can lead to partial cortical detachment of the leading end, which subsequently allows increased angular freedom of the searching MT.

DISCUSSION

It has been 45 years since cortical MTs were identified in plant cells (Ledbetter and Porter, 1963). Here we provide compelling molecular-genetic evidence for the role of a microtubule-associated protein in MT-cortex attachment. On the basis of our findings, we present a hypothetical model for CLASP function in the spatial organization of cortical arrays (Figure 7). By modulating CLASP activity, plant cells can achieve varying degrees of MT-cortex lateral attachment. MTs attached along their full lengths grow in linear trajectories and thus can only "find" MTs in their one-dimensional growth path, as indicated by the yellow highlight. When CLASP's activity is reduced or absent, partial MT detachment allows the freed MT portions to explore a larger volume (yellow) of the cortical cytoplasm, thereby increasing the frequency of MT–MT encounters in orientations that can lead to bundling. Moreover, partial detachment allows MTs to anneal with other MTs at any point along the detached segment of the MT lattice (blue outline), whereas full-length anchoring restricts the anneal-able region to only the plus end. Thus partial detachment also greatly increases the "sticky" surface area of the searching MT, increasing the likelihood of encounters that favor bundle formation (i.e., shallow plus-end encounters or lateral encounters). Ultimately, MT searching capacity is defined by the combination of two factors: the search volume, and the MT surface area available for bundle formation. Furthermore, lateral uncoupling from the cell cortex also allows initial MT–MT contact points to propagate as MTs zipper together via polymerization-independent bundling (Figure 7, bottom).

In contrast to the increased parallel MT order in *clasp-1*, MT disorganization results when MTs are detached in the

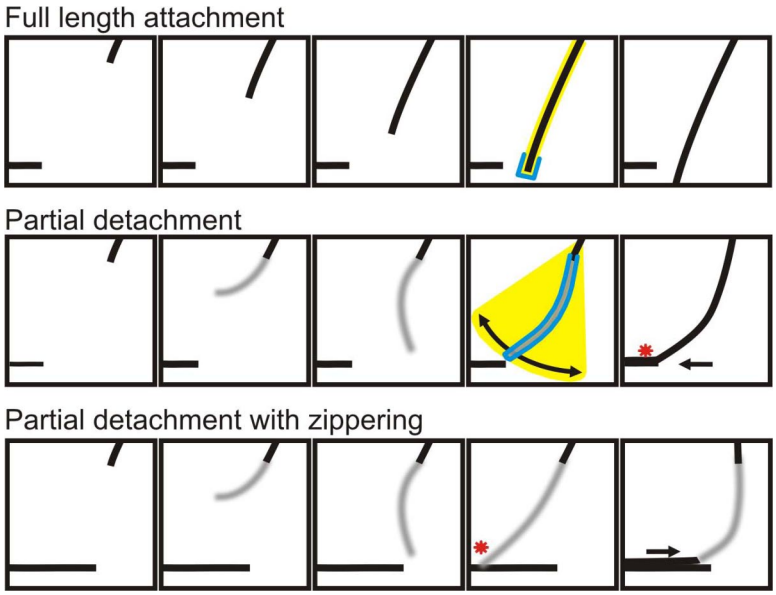

Figure 7. Hypothetical model for organization of cortical MT arrays. Top panel, a strongly anchored MT, and middle panel, a partially detached MT (gray region). Yellow shading indicates the area of exploration, which is increased by the partial detachment. Blue outline denotes the surface area available for bundle formation. Red asterisk indicates initial contact points. Partial detachment allows exploration of a larger volume with a greater surface area, and the MT therefore captures and bundles with another MT. Bottom panel, an example of zippering from an initial contact point. Cortical detachment permits initial MT–MT contact points to propagate as MTs zipper together in a polymerization-independent manner. We propose that these processes occur in all cells, but are enhanced in the absence of CLASP. In this way, CLASP indirectly affects MT–MT interactions via maintaining strong association of MTs along their length with the cell cortex. The fact that MTs remain partially attached in the absence of CLASP indicates the presence of other cortical anchoring factors.

presence of *n*-butanol or the drug morlin (during overexpression of the GFP-MAP4 reporter; Dhonukshe *et al.*, 2003; DeBolt *et al.*, 2007). The apparent contradiction of increased MT order in *clasp-1* cells is reconciled by the fact that cortex detachment is only partial in *clasp-1*, with MTs remaining bound along one or more regions, leaving the unattached regions free to explore the cortical cytoplasm in a restricted space. In contrast, complete dissociation of a MT from the cortex would result in internalization to the interior cytoplasm, which is sparsely populated with MTs. Furthermore, one cannot rule out possible nonspecific effects of these drugs, because the target(s) of morlin are unknown, and *n*-butanol likely affects many cellular processes.

Lower concentrations of *n*-butanol (0.2%) were recently shown to enhance the developmental reorientation of MTs from transverse to longitudinal in leek epidermal cells (Sainsbury *et al.*, 2008). Perhaps this concentration induces a *clasp-1*-like partial detachment of MTs. Interestingly, this MT reorientation in leek cells was shown to require the actomyosin-driven forces of cytoplasmic streaming, suggesting that detached MT sections may be prone to flow realignment, as in *Nitella* cells (Foissner and Wasteneys, 1999).

It is evident from the numerous instances of detached MT segments zippering and rapidly snapping into alignment with other MTs that MTs have an affinity for one another, presumably mediated by cross-linking MAPs of the MAP65 class (Chan *et al.*, 1999; Hussey *et al.*, 2002; Van Damme *et al.*, 2004; Wicker-Planquart *et al.*, 2004). Our data do not indicate a direct role for CLASP in MT cross-linking; however, a recent report demonstrates that CLASP plays a role in stabilizing overlapping MTs in fission yeast and that this function requires the MAP65 orthologue Ase1 (Bratman and Chang, 2007). MT bundles formed by overexpression of CLASP likely result from hyper-stabilization of MTs by CLASP, not via direct cross-linking of the MTs by CLASP (Maiato *et al.*, 2003; Ambrose *et al.*, 2007; Kirik *et al.*, 2007). The seemingly paradoxical finding reported herein of increased interactions between MTs in the absence of CLASP further supports this notion. Specifically, the fact that MTs retain their bundling capacity in the absence of CLASP is indicative of independent cross-linking by other factors. In the absence of CLASP protein, enhanced bundling is promoted not via hyper-stabilization, but rather by increasing the search capacity of MTs. We propose that by modulating the degree of MT-cortex anchoring in plant cells it is possible to achieve a wide range of organizational states ranging from net-like for wild type (strong attachment) to parallel in *clasp-1* (partial detachment) to complete disorganization of previously organized arrays (complete detachment). In this sense, keeping one part of the MT anchored while the rest probes for other cortical MTs is analogous to the search-and-capture behavior observed during spindle formation, wherein MTs nucleated and tethered to centrosomes dynamically probe the cytoplasm with their growing plus ends to interact with chromosomal kinetochores or MTs from the opposite pole.

Function of CLASP Sidewall Binding versus Plus-End Tracking

CLASP's role in cortex attachment may be conserved across eukaryotic taxa. Centrosome-dependent MT organization is typical in proliferating cells but many differentiated animal cell types have extensive noncentrosomal MTs as well as MTs that associate along their length with the cortex (Keating and Borisy, 1999; Bartolini and Gundersen, 2006). Interactions between MTs are important in the MT arrays found in association with the basal cortex of epithelial cells (Reilein and Nelson, 2005; Reilein *et al.*, 2005). Interestingly, in these cells the plus-end tracker EB1 also associates along the length of MTs, in contrast to its characteristic plus-end binding (Reilein and Nelson, 2005). In this manner, the regulation of MT plus-end versus sidewall binding of MAPs such as EB1 and CLASP represents a way to modulate cortical association of MTs (i.e., end-on attachment vs. lengthwise attachment).

The binding of CLASP to the full length of MTs in plants is informative as to the general role of CLASPs in eukaryotic cell MT-cortex association. The observation that the lattice binding of CLASP is differentially regulated in animal epithelial cells suggests that it may be an important determinant in regulating cortical association of MTs. In a simplistic model, sidewall labeling of CLASP (and other proteins such as EB1) results in lateral cortex association (as in higher plants and possibly in migrating cell leading-edge lamellae), whereas restricting CLASP to the plus ends favors the end-on MT-cortex association found in many animal cell types. Although we and others observed a slight enrichment

of CLASP at MT plus ends (Ambrose *et al.*, 2007; Kirik *et al.*, 2007), it remains to be seen whether this localization can be modulated in plants as well, perhaps in different cell types or stages of the cell cycle. One situation where one may predict a stronger plus-end association of CLASP in higher plants is after cell division, when MTs are nucleated on the nuclear envelope and grow radially through the cytoplasm toward the cortex.

In animal cells, CLASPs interact with two membrane bound proteins, LL5β and ELKS (Lansbergen *et al.*, 2006). BLAST searches indicate an absence of clear orthologues for these in the *Arabidopsis* genome. Identifying additional molecular components involved in plant cortical MT association will be of interest in the future.

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