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Article

Rho GTPase Signaling Activates Microtubule Severing to Promote Microtubule Ordering in *Arabidopsis*

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Summary

Background: Ordered cortical microtubule (MT) arrays play a critical role in the spatial control of cell division and expansion and are essential for plant growth, morphogenesis, and development. Various developmental, hormonal, and mechanical signals and a large number of MT-associated proteins are known to impact cortical MT organization, but the underlying mechanisms remain poorly understood. Our previous studies show that auxin signaling, which is mediated by the ROP6 Rho GTPase and its effector RIC1, promotes the ordering of cortical MTs in pavement cells, but it is unknown how RIC1 controls the organization of cortical MTs into well-ordered arrays.

Results: Our genetic screens identified the conserved MT-severing protein katanin (KTN1) as a downstream component of the ROP6-RIC1 signaling pathway leading to well-ordered arrangement of cortical MTs. KTN1 and RIC1 proteins displayed overlapping localization. In vivo and in vitro studies showed that RIC1 physically interacts with and promotes the MT-severing activity of KTN1. Live-cell imaging reveals a role for RIC1 in promoting detachment of branched MTs that is known to rely on KTN1.

Conclusion: We have demonstrated that a Rho GTPase signaling pathway regulates katanin-mediated MT severing in plant cells and uncovered an explicit regulatory mechanism underpinning the alignment and ordering of cortical MTs in plants. Our findings provide new insights into regulatory mechanisms underlying growth stimuli such as auxin promote the organization of cortical MTs into parallel arrays in plants.

Introduction

Dynamic microtubule (MT) arrays play pivotal roles in the regulation of many fundamental cellular processes, such as mitosis, intracellular transport, cell motility, and cell expansion. In plants, highly ordered cortical MTs are formed to locally restrict cell expansion and are essential for both anisotropic cell expansion and the erect stature of plants. Cortical MTs have been shown to direct the insertion of cellulose synthase in the plasma membrane [1, 2] and to guide the orientation of cellulose microfibrils [3], which in turn are proposed to restrict cell expansion to the direction perpendicular to their predominant orientation [2, 4–8]. Developmental signals and other stimuli, such as light, hormones, and mechanical stress, have been shown to influence MT ordering, suggesting a morphogenetic feedback loop among MT ordering, the generation of growth anisotropy, and the alteration of various developmental and environmental signals [9–12]. However, the signaling mechanisms underlying the organization of cortical MTs are poorly understood, and their elucidation is crucial for understanding the molecular basis of plant morphogenesis and pattern formation.

Live-cell imaging has revealed that cortical MTs in plant cells are initially formed at disperse nucleation sites in the cell cortex [13, 14]. Randomly oriented individual MTs have been proposed to self-organize into ordered arrays through MT dynamics and polymer interactions in plant cells [6, 14–21]. Previous studies have also shown that most newly nucleated MTs are branched at an angle of 40°–45° from the sides of existing MTs in plant cells [22–26], while the detachment of minus ends from their nucleation complexes by katanin-mediated severing is implicated in the ordering of these branched cortical MTs [25]. However, the biological significance of the branching and detaching behaviors of cortical MTs and the molecular mechanisms regulating these behaviors are unknown.

A molecular pathway leading a hormonal signal to MT ordering in plant cells was recently revealed [12, 27, 28]. Through a cell-surface receptor, ABP1, auxin activates a member of the Rho GTPase family, ROP6, which promotes the organization of cortical MTs into well-ordered parallel arrays by activating its effector RIC1 [12, 27, 28]. In this study, we have demonstrated that the ROP6-RIC1 signaling pathway activates the detachment of branched cortical MTs. Our results suggest that RIC1 directly binds KTN1, the p60 subunit of katanin in *Arabidopsis*, to promote KTN1's MT-severing activity. These findings reveal an explicit regulatory mechanism contributing to the alignment and ordering of cortical MTs in plants, as well as a signaling pathway that regulates katanin-mediated MT severing in eukaryotic cells.

Results

Mutations in *KTN1* Altered the Pavement Cell Phenotype Induced by *ROP6* Overexpression

To dissect the ROP6-RIC1 pathway in the control of cortical MT organization and cell expansion in pavement cells (PCs), we generated mutants using ethyl methane sulfonate (EMS) in an *Arabidopsis* line moderately overexpressing *ROP6*, *ROP6-OX*. Compared with highly wavy wild-type (WT) cells with a puzzle-piece shape, *ROP6-OX* pavement cells exhibit narrower necks and shallower lobes (Figures 1A–1D). Our initial screen yielded two recessive allelic mutants with severely swollen PCs in cotyledons (Figure 1A–1D). Map-based cloning revealed point mutations in the katanin p60 catalytic subunit *KTN1* for both independent alleles (Figure S1B available online). We named

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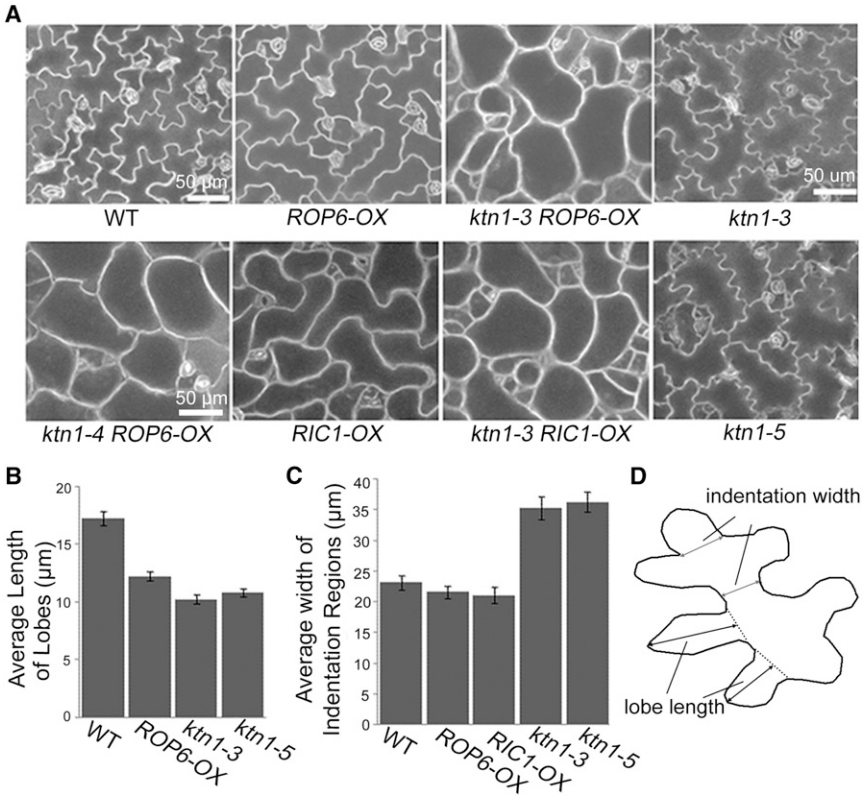

Figure 1. Phenotype Analysis of *ktn1* Mutants in Comparison to *ROP6-OX* or *RIC1-OX* Transgenic Plants

(A) Pavement cell (PC) shapes of WT, *ROP6-OX*, *RIC1-OX*, *ktn1-3* (a mutant contains a Pro^{257}-Leu point mutation in KTN1 protein), and *ktn1-5* (a T-DNA insertion null mutant of *KTN1*) and double mutants *ktn1-3 ROP6-OX*, *ktn1-3 RIC1-OX*, and *ktn1-4 ROP6-OX* (a mutant contains a Ser^{460}-Leu point mutation in KTN1 protein in *ROP6-OX* background).

(B) Quantitative analysis of lobe lengths of cells from the WT, *ROP6-OX*, *ktn1-3*, and *ktn1-5*. The mean lobe length of *ktn1-3* cells was similar to that of *ktn1-5* cells ($p > 0.05$, Student's t test), whereas it was significantly shorter than that of cells from the WT and *ROP6-OX* ($p < 0.05$, Student's t test). All data are represented as mean ± SD.

(C) Quantitative analysis of indentation neck widths of cells shows that *ktn1-3* and *ktn1-5* mutant cells had significantly wider neck widths than did WT, *ROP6-OX*, or *RIC1-OX* cells ($p < 0.05$, Student's t test). All data are represented as mean ± SD.

(D) A cartoon depicting how the neck widths and the lobe lengths were measured. A dashed line segment was drawn at the base of each lobe, and the distance between the segment midpoint and the vertex of the lobe was decided as the lobe length.

See also Figure S1.

the two alleles *ktn1-3 ROP6-OX* and *ktn1-4 ROP6-OX*, respectively (Figures 1A–1D), since Nakamura and his colleagues have named their *KTN1* mutants *ktn1-1* and *ktn1-2* [25]. Both *ktn1-3 ROP6-OX* and *ktn1-4 ROP6-OX* displayed mutations in *KTN1*'s AAA (*A*TPases *a*ssociated with various cellular *a*ctivities) domain (Figure S1B), which did not affect the messenger RNA transcript level of *KTN1* in these mutants [29] (Figure S1C).

ktn1-3 ROP6-5 was backcrossed to the WT to obtain the *ktn1-3* homozygous single mutant. Interestingly, PCs in *ktn1-3* cotyledons differed from those both in the WT and in *ktn1-3 ROP6-OX*, exhibiting much wider necks and more numerous but shallower lobes than those observed in WT cells (Figure 1A). A transfer DNA (T-DNA) insertion null mutant *ktn1-5* displayed PC shape and seedling phenotypes identical to those observed in *ktn1-3* (Figures 1 and S1). Transformation of *pKTN1::KTN1* into *ktn1-3* complemented the mutant PC phenotype in five individual lines (two displayed in Figures S2A–S2C). Taken together, these results demonstrated that the abnormal cell shape in *ktn1-3* cells was due to loss of KTN1 function.

KTN1 Genetically Interacts with ROP6 and RIC1

Reduced lobe expansion and narrower necks in PCs overexpressing either *ROP6* (*ROP6-OX*) or *RIC1* (*RIC1-OX*) are associated with increased ordering of cortical MTs over the pavement cell cortex [12, 28], while isodiametric cell shapes in the *ktn1-3 ROP6-OX* and *ktn1-3 RIC1-OX* lines (Figure 1) would be consistent with their severe disorganization. We hypothesized that KTN1 acts downstream of the ROP6-RIC1 pathway and thus is required for the hyperordering of cortical MTs induced by ROP6 or RIC1 overexpression. As a first step in testing our hypothesis, we created *ktn1-3 ric1-1* and *ktn1-3 rop6-1* double mutants and measured indices of PC shape. An increase in PC neck widths is characteristic of *rop6-1* and *ric1-1* PCs due to reduced ordering of cortical MTs [27, 28]. Quantitative analysis of neck widths and lobe lengths of PCs from *rop6-1*, *ric1-1*, *ktn1-3*, and their double mutants revealed significant wider neck regions and shorter lobes in these mutants compared to WT PCs. Compared to *rop6-1* and *ric1-1* cells, the mean length of lobes in *ktn1-3* was even shorter, and the mean width of neck regions was much wider (Figures 2B and 2C). The PC phenotype in *ktn1-3 rop6-1* or *ktn1-3 ric1-1* double mutants was similar to that of the *ktn1-3* single mutant (Figure 2). These observations support a genetic and functional interaction between KTN1 and the ROP6-RIC1 pathway for the local restriction of cell expansion in the neck regions of PCs.

KTN1 Is Required for ROP6- and RIC1-Dependent MT Ordering

We next assessed whether KTN1 plays a role in ROP6- and RIC1-mediated ordering of cortical MT arrays. In *ktn1-3* cells, PCs contained much more extensive network-like cortical MTs of lower labeling intensity than were observed in the WT (Figures 3 and S3). By contrast, *ROP6-OX* or *RIC1-OX* PCs contained well-ordered parallel MTs all arranged in perpendicular to the long axis of PCs (Figure 3) [27, 28]. When either *ROP6* or *RIC1* was overexpressed in the *ktn1-3* background, cortical MTs were highly disorganized, resembling those in *ktn1-3* PCs (Figure 3). Thus, the ROP6 and RIC1 induced MT ordering was suppressed by the *ktn1-3* mutation. These results indicate that KTN1 is required for normal microtubule ordering in PCs and for the hyperordering caused by ROP6 or RIC1 overexpression. Compared to *ktn1-3* cells, however, cell shape defects were much more severe in *ktn1-3 ROP6-OX* or *ktn1-3 RIC1-OX* cells (Figure 1). These results indicate

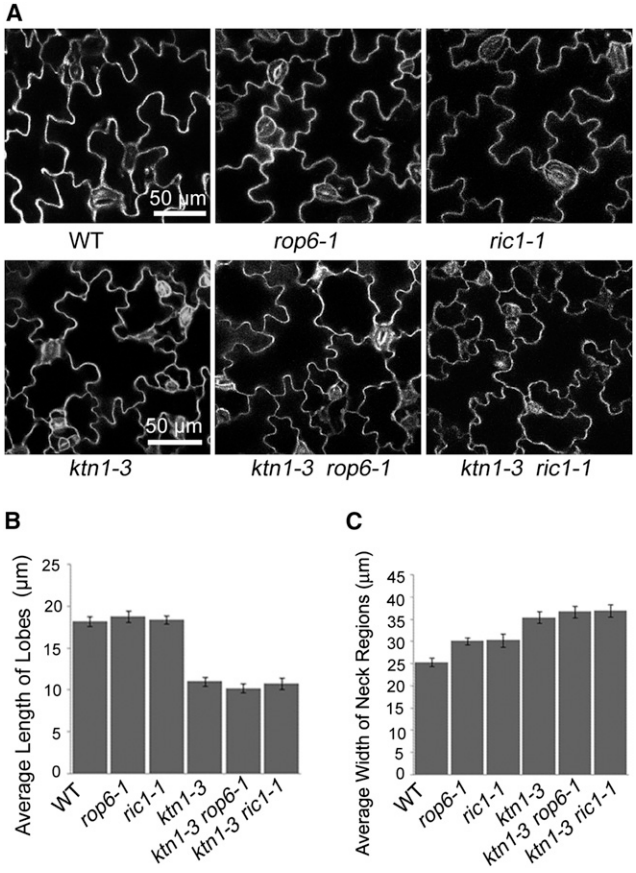

Figure 2. Phenotype Analysis of the *ktn1-3* Single Mutant in Comparison to the *ktn1-3 rop6-1* or *ktn1-3 ric1-1* Double Mutants

(A) PC shapes of WT, *rop6-1*, *ric1-1*, and *ktn1-3* and double mutants *ktn1-3 rop6-1*, *ktn1-3 ric1-1*.

(B) Quantitative analysis of lobe lengths of cells indicated that cells from the double mutants *ktn1-3 rop6-1* and *ktn1-3 ric1-1* were similar to those from *ktn1-3* cells, which had significantly shorter lobes than did WT cells or *rop6-1* and *ric1-1* single-mutant cells ($p < 0.05$, Student's t test). All data are represented as mean ± SD.

(C) Quantitative analysis of neck widths of cells indicates that cells from the double mutants *ktn1-3 rop6-1* and *ktn1-3, ric1-1* were similar to those from *ktn1-3* cells, which had significantly wider neck regions than did WT cells or *rop6-1* and *ric1-1* single-mutant cells ($p < 0.05$, Student's t test). All data are represented as mean ± SD.

See also Figure S2.

that not all of the changes caused by ROP6 or RIC1 overexpression are mediated through KTN1 function.

KTN1 Physically Interacts with RIC1

To test whether KTN1 is a direct target of RIC1, we investigated whether they directly interact with each other. It has been demonstrated that GFP-RIC1 is associated with cortical MTs [27], and KTN1-GFP frequently labels MT branching sites and MT intersections [25]. Dual immunostaining with anti-KTN1 and anti-RIC1 antibodies showed that an average of 78.6% ± 8.9% of anti-KTN1 signal was colocalized with anti-RIC1 signal in a punctate fashion (Figure S4). Then we performed in vitro pull-down assays with affinity-purified proteins and found that His-tagged KTN1 was pulled down by MBP-tagged RIC1, but not by MBP alone (Figure 4A). We further precipitated RIC1 from plant extracts with the anti-RIC1 antibody, and the KTN1 protein was coprecipitated from extracts prepared from WT but not *ktn1*-5 seedlings (Figure 4B), indicating that these proteins form a (common) complex or structure in vivo. These results demonstrate that RIC1 physically interacts with KTN1.

RIC1 Promotes the MT-Severing Activity of KTN1 In Vitro

Since MT-severing activity of KTN1 may be critical for the organization of cortical microtubule arrays [25, 30–32], we first tested whether RIC1 directly regulates the severing activity of KTN1 in vitro, using rhodamine-labeled prepolymerized MTs as substrates. In the absence of His-RIC1 and His-KTN1, 83% of MTs were longer than 5 μm, whereas only 16.5% of MTs were 1–5 μm in length and short MTs (<1 μm) were rare (0.5%) (Figures 5A and 5B). Similarly, when treated with 150 nM RIC1, 75% of MTs were longer than 5 μm, and the remaining 25% of MTs were 1–5 μm in length (Figures 5A and 5B). As expected, treatment with 300 nM KTN1 reduced the proportion of longer MTs (>5 μm) to 13% and increased the frequency of shorter polymers such that 77% of MTs were between 1 and 5 μm in length and the remaining 10% were less than 1 μm (Figures 5A and 5B). When His-KTN1 (300 nM) and His-RIC1 (150 nM) were mixed together prior to the addition to the MT preparations, only 10% of MTs were between 1 and 5 μm, and the remaining 90% were below 1 μm in length (Figures 5A and 5B). Thus, addition of His-RIC1 greatly enhanced the ability of His-KTN1 to shorten MT lengths.

To investigate whether the shift in the MT length distribution resulted from KTN1-mediated severing instead of MT depolymerization, we performed time-lapse imaging to monitor the dynamics of the prepolymerized rhodamine-labeled MTs under a spinning-disk confocal microscope. Over a 400 s time period, no MT severing event was observed in the control (a total 215 MT filaments from three independent experiments) (Figure 5C and Movie S1). In the presence of KTN1 alone, gap formation in the middle of MT filaments (a total 167 MT filaments from three independent experiments) was observed, indicating the occurrence of severing events that were made visible by erosion of the exposed polymer ends (Figure 5C and Movie S2). When both RIC1 and KTN1 were added, severing events were greatly increased, and the progressive shortening of MTs was more efficient, as revealed by the quantified kinetics analysis (Figures 5C and 5D and Movie S3). Consequently, the MTs incubated with both RIC1 and KTN1 (a total 169 MT filaments from three independent experiments) were cut into much shorter and more numerous fragments compared to those incubated with KTN1 only (Figures 5C and 5D and Movie S3). At the end of 400 s in this assay, the majority of MT fragments (66%) were less than 4 μm in length, and only 20% of them were longer than 6 μm when both RIC1 and KTN1 were present (Figure 5E). In contrast, 57% of MT fragments were more than 6 μm in the presence of KTN1 alone (Figure 5E). In control, 86% MT fragments were more than 6 μm (Figure 5E). These results clearly demonstrate that RIC1 functions in promoting the MT-severing activity of KTN1 in vitro.

RIC1 Promotes the Detachment of Branched MTs In Vivo

Since RIC1 promotes the ordering of cortical MTs in vivo [27, 28], we next explored whether and how RIC1 stimulation of KTN1-based MT severing could lead to MT ordering. New MTs in the cortex of plant cells are primarily generated through nucleation from existent MTs, with nucleations that branch at an angle from the mother polymer being about twice as common as those that occur in parallel to it in both *Arabidopsis*

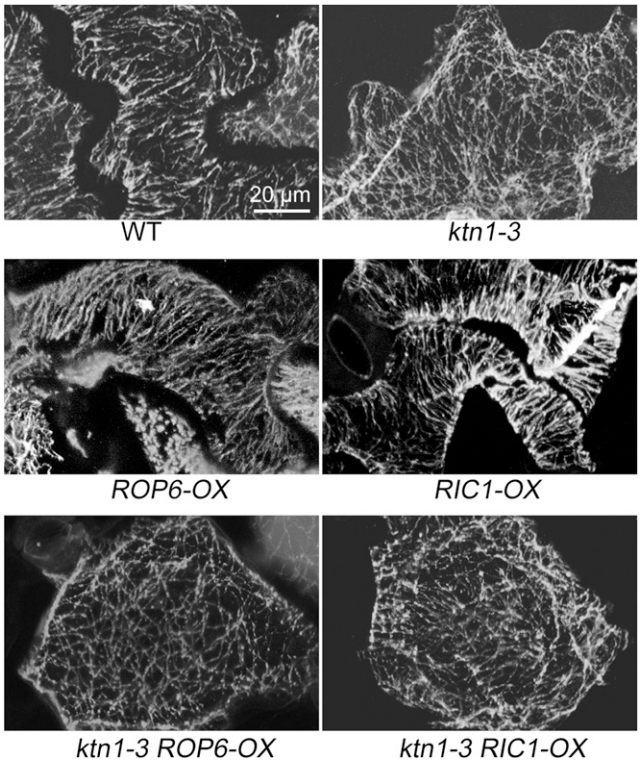

Figure 3. KTN1 Is Required for MT Ordering

Immunofluorescence microscopy was used for visualization of cortical MT organizations in PCs of WT, *ktn1-3*, *ROP6-OX*, and *RIC1-OX* and the double mutants *ktn1-3 ROP6-OX* and *ktn1-3 RIC1-OX*. Overexpression of ROP6 or RIC1 promoted the formation of highly ordered transverse MTs all over the cell. However, a point mutation in KTN1 caused a randomly organized cortical MT network, no matter whether it was in the WT background or in the *ROP6-OX* or *RIC1-OX* background. See also Figure S3.

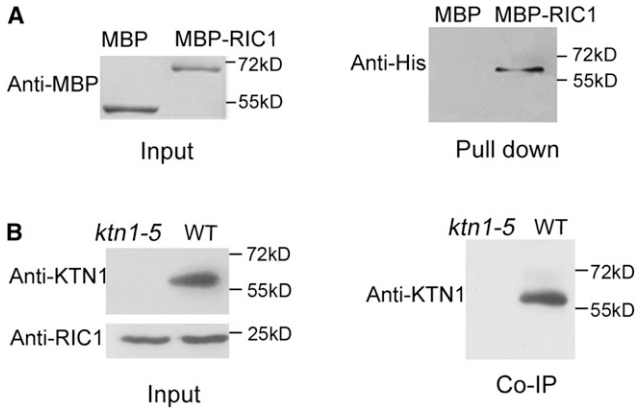

Figure 4. KTN1 Physically Interacts with RIC1

(A) In vitro pull-down assay was performed with MBP-RIC1 and His-KTN1. His-KTN1 pulled down by MBP-RIC1 was detected by anti-His antibody.

(B) KTN1 coimmunoprecipitated with RIC1. Proteins isolated from WT plants were immunoprecipitated with anti-RIC1 antibody and analyzed by western blotting with an anti-KTN1 antibody. The *ktn1-5*-null mutant was used as a negative control.

See also Figure S4.

hypocotyl and pavement cells [25, 26]. Furthermore, evidence suggests that KTN1 regulates MTs ordering by severing at the minus end of branching MTs [25], thus releasing nascent MTs for translocation and freeing nucleation complex proteins for recruitment to new locations. Direct interaction between RIC1 and KTN1 hints at an intriguing possibility that the ROP6-RIC1 pathway promotes KTN1-mediated severing at the branching site to detach newly formed MTs from their mother MTs. Therefore, we compared rates of detachment of branched MTs between WT, the *RIC1-OX* line, and the *ric1-1* mutant expressing GFP-tagged tubulin (Figures 6A and 6B and Table S1). Because of the difficulties in live imaging of cortical MTs in PCs due to high surface curvature in this cell type, we monitored MT severing in *Arabidopsis* hypocotyl cells, of which the cell expansion is regulated by ROP6-RIC1-MT pathway as well [28]. As predicted, the rates of minus-end detachment from branching nucleation sites were significantly increased in *RIC1-OX* cells compared to WT cells (Figure 6 and Table S1). Among the 80 branching nucleation events observed in eight WT cells, 32 detachments were detected over the interval of observation, a frequency of 40%. By contrast, in *RIC1-OX* cells, 73 detachment events were detected in 138 branched nucleations, a frequency of 53%. The expected number of detachments in the mutant population based on the WT detachment frequency would be 55. The observed value of 73 events is significantly different by binomial test at $p < 0.0013$. These results, together with RIC1's ability to promote KTN1's MT severing activity, indicate that RIC1 can promote severing of MTs from branched nucleation sites.

Branch site severing was essentially similar in *ric1-1* cells (28 severing events in 83 branching nucleations, 34%, from eight cells) as compared to WT cells (Figure 6B and Table S1). The lack of a reduction in severing in the *ric1-1* mutant may be due to the technical difficulty in detecting all of the minus-end-severing events since some MT branches were sheltered by thick MT bundles. Alternatively, RIC1 might activate a repressor of KTN1, as well, or might be genetically redundant with a RIC1 homolog or a different pathway.

Discussion

Arrangements of cortical MTs regulate cell growth, morphogenesis, and division patterns in plants, and yet the regulatory systems underlying the organization of cortical MTs remain poorly understood. In the present study, we have demonstrated that the ROP6-RIC1 signaling pathway promotes MT ordering by activating katanin-mediated detachment of branched MTs. Our work has also established a signaling pathway that regulates the MT-severing activity of katanin, a conserved protein complex that is critical for MT organization in eukaryotic organisms. Given our recent data showing that auxin activates the ROP6-RIC1 pathway to promote MT ordering in pavement cells [12], the RIC1-based regulatory system may serve as a paradigm for understanding how growth stimuli such as auxin promote the organization of cortical MTs into parallel arrays in their regulation of cell elongation and morphogenesis in plants (Figure 6C).

The ROP6 GTPase Signaling Pathway Activates Katanin-Dependent MT Severing for Organizing Paralleled Cortical MT Arrays

Katanin severs MTs in an ATP-dependent fashion and carries out an important role in MT organization for a wide range of cellular processes in eukaryotes. In animal cells, the MT-stimulated ATPase activity of the katanin p60 subunit is regulated by the WD40-containing p80 regulatory subunit. Evidence

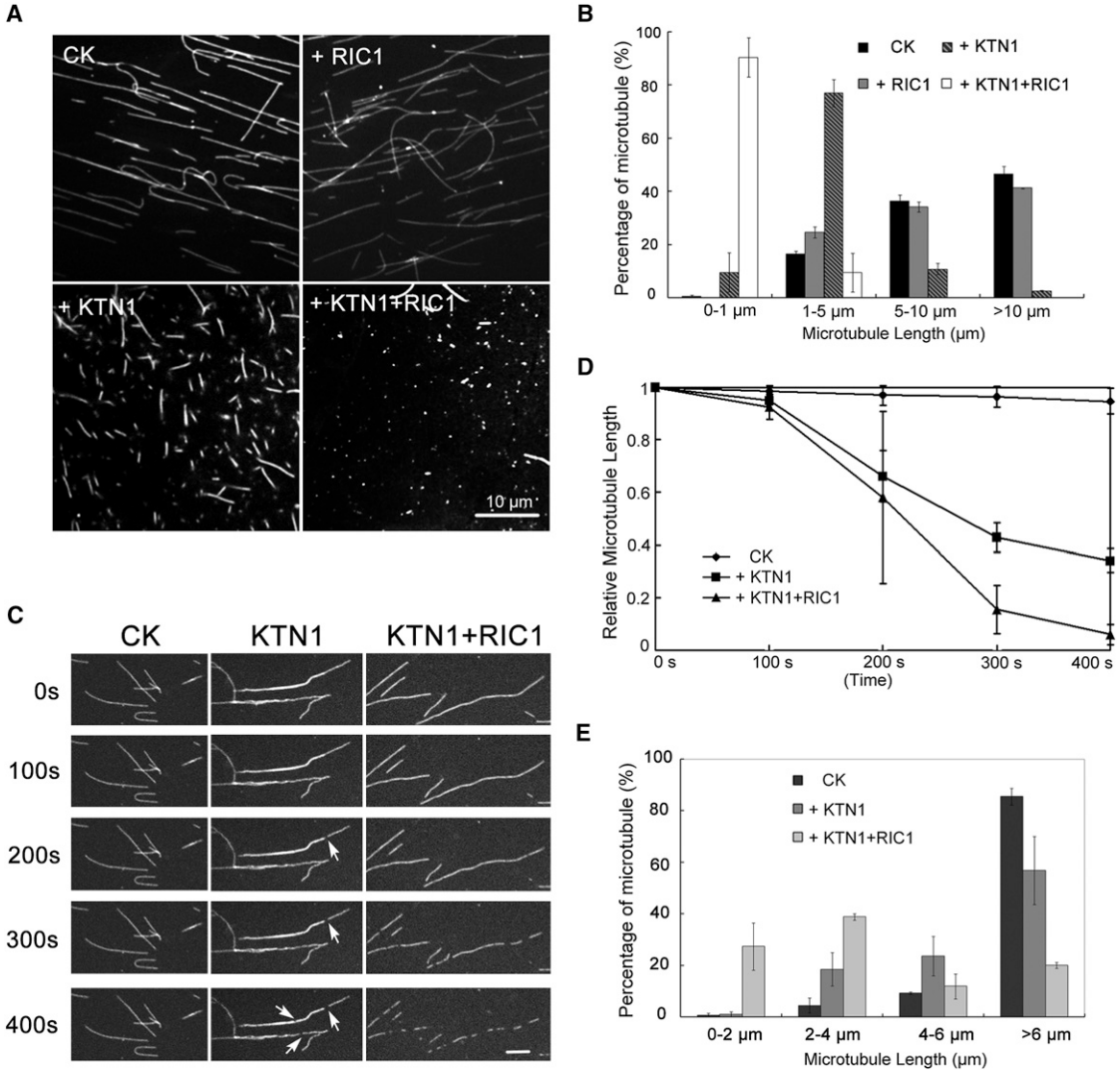

Figure 5. RIC1 Promotes the MT-Severing Activity of KTN1 In Vitro

(A) RIC1 promotes the MT-severing activity. KTN1 severs taxol-stabilized MTs polymerized from rhodamine-labeled tubulin into fragments. Premixing of 150 nM RIC1 with 300 nM KTN1 before incubation with 1 μM prepolymerized MTs causes much shorter dots in the same reaction system. The scale bar represents 10 μm.

(B) Quantitative analysis of the length of MTs prepolymerized from rhodamine-labeled tubulin in the presence of 300 nM KTN1, 150 nM RIC1, or both of them in comparison to control. Data were collected from three independent experiments for each treatment. A total of 191 MTs in control, 206 MTs in the presence of RIC1, 558 MTs in the presence of KTN1, and 1477 MTs in the presence of both KTN1 and RIC1 were measured. All data are represented as mean percentage ± SD.

(C) Time series of prepolymerized and taxol-stabilized rhodamine-labeled MTs. Over a 400 s time period, MTs were cut into fragments after 300 nM KTN1 protein was introduced (middle panel; arrows indicate where severing occurred). Stronger severing activity of KTN1 was seen when 150 nM RIC1 was added together with KTN1; severing events increased, and MTs were cut into much shorter fragments (right panel), whereas in control system, MTs were rarely changed (left panel). The scale bar represents 10 μm.

(D) Quantified kinetics indicating the progressive MTs shortening. A total of 100 MTs in control, 99 MTs treated with KTN1, and 94 MTs treated with the mixture of KTN1 and RIC1 were traced. Relative MT lengths at each time point were normalized by the initial length of the MTs. Data were collected from three independent experiments for control and each treatment. All data are represented as mean ± SD.

(E) Quantitative analysis of the length of MTs at the end of the real-time imaging showed in (C). Data were collected from three independent experiments for control and each treatment. A total of 215 MTs in control, 167 MTs treated with KTN1, and 169 MTs treated with the mixture of KTN1 and RIC1 were observed. All data are represented as mean percentage ± SD.

See also Movie S1, Movie S2, and Movie S3.

suggests that p80 recruits katanin to the severing site of MTs (centrosome) [33, 34]. Other proteins such as cyclinB/cdk1, a polo-like kinase (Plx1), and XMAP4 have also been implicated in the regulation of the katanin catalytic activity in animal cells [35]. In plant cells, KTN1 shows ATPase-dependent MT severing activity that is also regulated by its binding to MTs [36]. A putative plant homolog of katanin p80 subunit has been identified, but its function has not been determined [9]. Interestingly, KTN1/LUE1 impacts MT dynamics regulated by ethylene and gibberellins [9] and is required for cells' responses to mechanical forces generated by growth [37]. These findings suggest that hormonal and mechanical signals may regulate MT dynamics via katanin. However, no signaling pathways had been linked to the regulation of katanin in eukaryotic

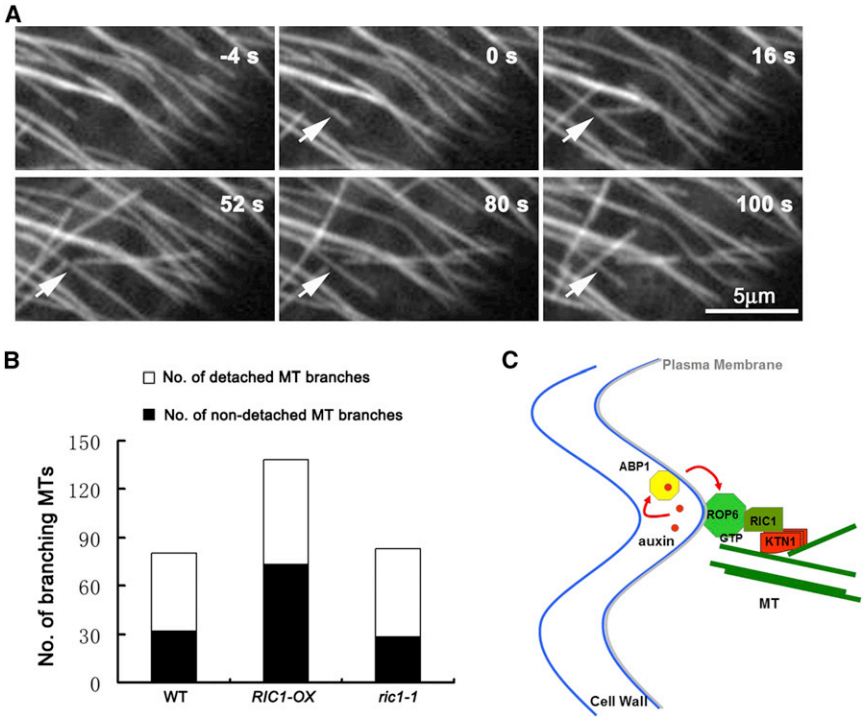

Figure 6. RIC1 Promotes the Katanin-Based MT Detachment

(A) A time serial of a part of a hypocotyl epidermal cell in the WT. Arrows indicate a daughter MT elongated after a MT-dependent branching nucleation. The release of the daughter MT was due to the severing at its minus end.

(B) Quantitative analysis of the minus-end-severing events of branched MTs in WT, *RIC1-OX*, and *ric1-1* cells. In the observed eight WT cells, 32 out of 80 newly formed MT branches were detached from mother MTs, a frequency of 40%. By contrast, in six *RIC1-OX* cells, 73 detachment events were detected in 138 branched MTs, a frequency of 53%. The expected number of detachments in the mutant population based on the WT detachment frequency would be 55. The observed value of 73 events is significantly different by binomial test at $p < 0.0013$. Branch site severing was similar in *ric1-1* cells (28 severing events in 83 branching MTs, 34%, from eight cells) as compared to WT cells.

(C) A working model for the ROP6-RIC1 signaling pathway in regulation of MT ordering. ROP6-RIC1 is activated by the ABP1 perception of auxin and signaling to MTs [15]. RIC1 regulates the MT-severing activity of KTN1. KTN1-dependent MT detachment frees the newly formed MT branches to facilitate MT self-organization and eventually leads to MT ordering.

See also Table S1.

cells. Our recent work shows that the ROP6 Rho GTPase promotes the ordering of parallel cortical MTs by activating its effector RIC1 (also a plant MAP) [27, 28]. In this report, our combined genetic, biochemical, and cell biological analyses have convincingly demonstrated that the ROP6-RIC1 signaling pathway promotes the formation of well-ordered MTs through the direct activation of katanin-based MT severing. This establishes the first signaling pathway directly leading to the regulation of katanin to control MT organization in eukaryotic cells (Figure 6C).

Signal-Dependent MT Severing Produces Ordered MTs from Branched MTs

The revelation of the ROP6-RIC1 signaling pathway that promotes katanin's severing activity has an important implication in our understanding of the mechanism that regulates the organization of cortical MTs in plant cells. In plant cells, cortical MTs are primarily generated by branching from extant cortical MTs at an angle of 40°–45° [14, 23–25, 38]. However, cortical MTs form parallel arrays to affect signal-dependent cell elongation, cell morphogenesis, and the spatial pattern of cell division [3, 5, 6]. The katanin-based severing is critical for ordered alignment of cortical MTs [9, 30–32]. The severing was proposed to induce a densely packed and aligned array by removing nonaligned MTs and increasing MT numbers [14, 23, 31, 38]. However, a recent study by Nakamura and his colleagues suggests that the katanin-mediated severing at MT minus ends is critical for the release of nascent MTs from mother MTs [25]. Here we show positive evidence indicating that RIC1 promotes local MT ordering by positively regulating katanin-mediated minus-end release, further confirming the importance of this katanin-mediated process in the ordering of cortical MTs, although we cannot exclude the possibility that RIC1-activated katanin might also remove nonaligned MTs as previously proposed [14, 23, 31, 38].

The ROP6-RIC1 Pathway Links Cytoplasmic Auxin Signaling to the Regulation of Cortical MT Organization and Cell Elongation

Our finding that the ROP6-RIC1 pathway regulates MT branch detachment also provides important insights into how various signals rapidly regulate MT organization in plant cells. For a long time, it has been known that the organization/orientation of cortical MTs responds to growth stimuli, including multiple phytohormones [39]. However, little is known about the perception and transduction mechanisms by which such signals regulate MTs. Auxin is one of the most investigated phytohormones and is essential for almost every aspect of plant growth and development [40, 41]. Auxin activates ROP6 through ABP1 to modulate RIC1's association with cortical MTs and to promote their ordering in the indentation region in PCs [12]. Our findings support the model for a ROP-based cytoplasmic auxin signaling pathway in the modulation of cortical MT orientation (Figure 6C). In addition, this model implies that Rho GTPase regulation of katanin-based MT-detachment might serve as a common mechanism for MT self-organization in response to growth stimuli that regulate plant cell elongation. We anticipate that more regulators and signaling pathways are involved in activation of katanin-severing activity and MT organization during cell development. Therefore, uncovering these new regulators and signaling pathways will be an exciting direction in cell and development biology in the near future.

Experimental Procedures

Full experimental details are provided in the Supplemental Experimental Procedures.

Accession numbers

Sequence data from this research can be found in the GenBank/EMBL data libraries under the following accession numbers: AT4G35020 (*ROP6*), AT2G33460 (*RIC1*), and AT1G80350 (*KTN1*).

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, four figures, one table, and three movies and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.01.022.

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