Plant Signaling & Behavior 7:1, 1–3; January 2012; © 2012 Landes Bioscience

Aberrant microtubule organization in dividing root cells of p60-katanin mutants

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Keywords: *Arabidopsis thaliana*, cell division, katanin, microtubules, TPX2

Aberrant microtubule organization has been recently recorded in dividing root cells of *fra2* and *lue1* p60-katanin *Arabidopsis thaliana* mutants. Here, we report similar defects in the *bot1* and *ktn1-2* mutants of the same plant, proposing that they constitute a consistent phenotype of p60-katanin mutants. In addition, we show that the Targeting Protein for Xklp2 (TPX2) protein co-localizes with microtubules on the surface of prophase nuclei of the mutants, probably participating in multipolar spindle assembly. As microtubule organization defects are not observed in metaphase/anaphase spindles and initiating phragmoplasts, we also discuss the putative association of the observed aberrations with the nuclear envelope and we emphasize on the mechanism of bipolar metaphase spindle organization in the mutants. It seems that chromosome-mediated spindle assembly, probably minimally dependent on microtubule severing by p60-katanin, dominates after nuclear envelope breakdown, restoring bipolarity.

Microtubule Organization Defects in Dividing Root Cells of p60-Katanin Mutants

Microtubule severing by p60-katanin has been proven crucial for cortical microtubule organization and cell morphogenesis.[1,2] We have shown recently that it is also essential for the organization of microtubules in dividing root cells.[3] The following defects were recorded in dividing root cells of *fra2*[4,5] and *lue1*[6] p60-katanin *Arabidopsis thaliana* mutants: (1) Early preprophase bands consisted of poorly aligned microtubules, while few mature preprophase bands were asymmetrically organized. In addition, preprophase band microtubules persisted during prometaphase. (2) The prophase spindles were multipolar. (3) The microtubules of expanding phragmoplasts were long, bended and attached to the daughter nuclei, exhibiting a particular "double-arrow" configuration. The frequently observed oblique cell divisions in the mutant roots were mainly attributed to the multipolarity of prophase spindles.[3]

Microtubule organization defects, similar to those of *fra2* and *lue1* dividing root cells, were also recorded in dividing root cells of two more p60-katanin mutants, *bot1*[7] and *ktn1–2*[8] (**Fig. 1**). It can therefore be assumed that they are universal among p60-katanin mutants of *Arabidopsis thaliana*. Studies on the *dgl1*[9] mutant of *Oryza sativa* might further establish these observations as a consistent phenotype among katanin mutants of diverse plant species.

TPX2 and Multipolar Prophase Spindle Organization

In wild-type prophase *Arabidopsis thaliana* cells, perinuclear microtubules exhibit uniform "meridian-like" orientation, perpendicular to the division plane, while in the p60-katanin mutants, microtubules with various orientations persist around the prophase nucleus. We have suggested that in the wild type, any microtubules that do not follow the "meridian" arrangement are removed and/or reoriented by severing, resulting in bipolar prophase spindles. As unaligned microtubules are not severed in the mutants, they converge in multiple poles at any side of the prophase nucleus.[3] TPX2, a central regulator of spindle assembly in vertebrate cells, has been shown to participate in spindle organization also in plants, exported from the nucleus during prophase, interacting with Aurora kinases and probably initiating microtubules.[10] Importantly, TPX2 was immunolocalized in the multipolar prophase spindles of p60-katanin mutants (**Fig. 2**). It seems, therefore, that TPX2 cannot *per se* achieve prophase spindle bipolarity. TPX2 may rather participate in the convergence of aligned perinuclear microtubules of wild-type cells or unaligned perinuclear microtubules of mutant cells to bipolar or multipolar spindles, respectively.

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Submitted: 09/09/11; Revised: 10/06/11; Accepted: 10/10/11
http://dx.doi.org/10.4161/psb.7.1.18358

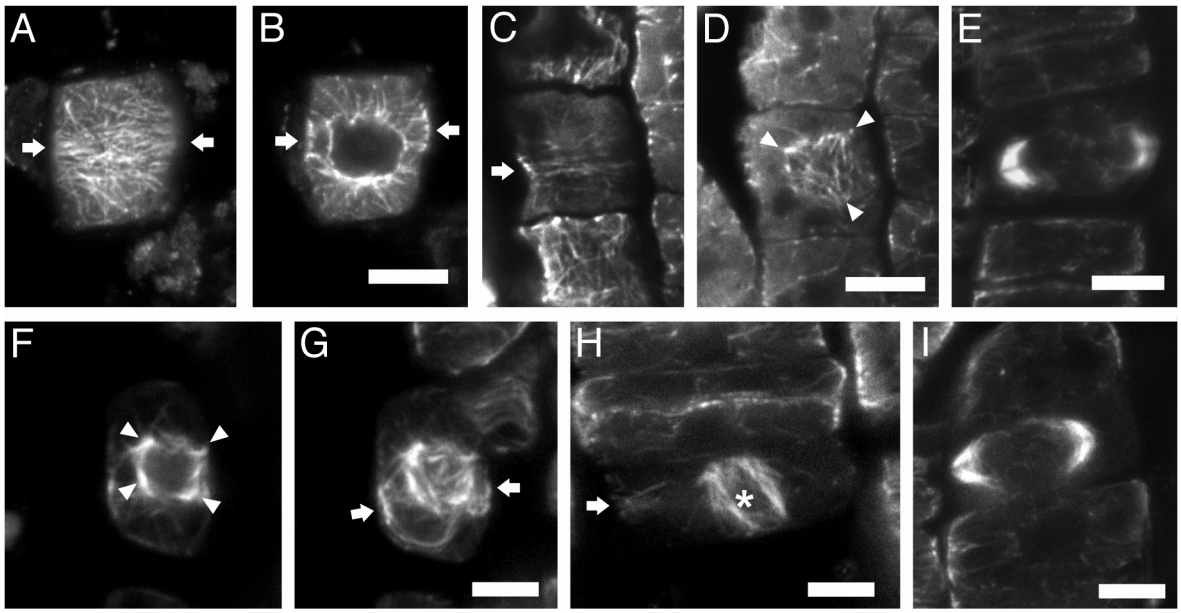

Figure 1. CLSM sections after α-tubulin immunostaining, as described in ref. 3, of *bot1* (A–E) and *ktn1–2* (F–I) roots. The seeds of *bot1* were purchased from NASC, while those of *ktn1-2* mutant were offered by Dr. Masayoshi Nakamura and Prof. Takashi Hashimoto (Graduate School of Biological Sciences; Nara Institute of Science and Technology; Japan). (A, B) Early preprophase cell, at cortical (A) and intranuclear (B) section. The arrows point to the division plane. The preprophase band consists of poorly aligned microtubules (A), while the nucleus is already surrounded by numerous microtubules (B). (C, D) Late preprophase/prophase cell CLSM sections, through the cortical cytoplasm (C) and at the surface of the nucleus (D). The preprophase band is loose (C, arrow), while perinuclear microtubules exhibit various orientations (D), converging to multiple foci (arrowheads in D). (E) "Double-arrow" shaped expanding phragmoplast in cytokinetic *bot1* cell. (F, G) Late preprophase/prophase cell at intranuclear CLSM section (F) and maximum projection of 8 successive sections (G). The arrows in (G) point to the division plane. The perinuclear microtubules exhibit various orientations, converging to multiple foci (arrowheads in F). (H) Prometaphase cell (the asterisk indicates the spindle) with preprophase band remnants at the cell cortex (arrow). (I) "Double-arrow" shaped expanding phragmoplast in cytokinetic *ktn1–2* cell. Scale bars: 5 μm.

Metaphase Spindle Assembly Seems to be Independent from p60-Katanin Function

As reported by GFP-katanin localization,[3] p60-katanin is associated with every cell cycle-specific array. Interestingly, the microtubule arrays that assemble after nuclear envelope breakdown, at late prophase, until its reassembly at telophase, i.e., metaphase/anaphase spindle and early phragmoplast, are typical in organization in the p60-katanin mutants. On the contrary, nuclear envelope-associated microtubules, i.e., unaligned perinuclear microtubules at prophase and nuclear envelope-connected minus ends of phragmoplast microtubules at late telophase, are not severed, resulting in the observed aberrations. It seems thus that severing by p60-katanin is necessary on and/or around the nuclear envelope for normal microtubule organization. Accordingly, although microtubule-dependent microtubule nucleation by γ-tubulin complexes may occur almost anywhere in the plant cell[8,11,12] it seems that the nuclear envelope may possess some special centrosomal properties (see also ref. 13). In this context, its engagement with severing by p60-katanin seems to represent an analog to the centrosome-katanin duet of animal cells. Experiments interfering with nuclear envelope assembly/disassembly are required to further elucidate this point.

Spindle assembly in plants may be achieved by a combination of nuclear envelope- and chromosome-mediated pathways.[14] Our observations support that, as prophase spindle multipolarity does not influence metaphase spindle bipolarity, chromosome-mediated spindle assembly, probably minimally dependent on microtubule severing by p60-katanin, may take control after nuclear envelope breakdown, as an alternative mechanism, to correct any previous spindle defect. This also resembles to the chromosome-mediated spindle assembly in acentrosomal animal cells,[15] supporting that in angiosperms metaphase spindle may not originate exclusively by the prophase spindle.[16,17]

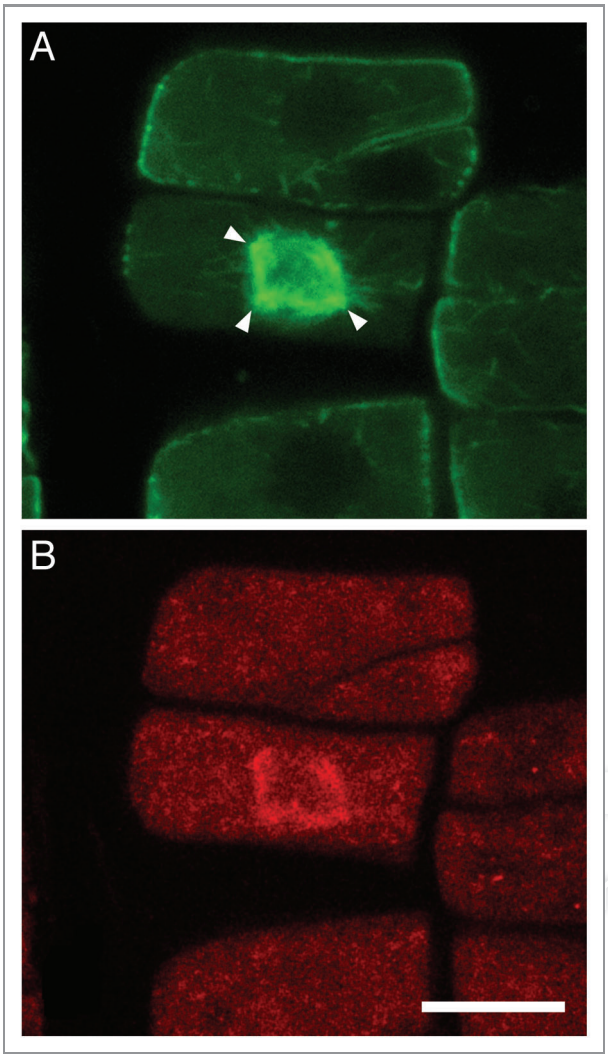

Figure 2. Co-localization of microtubules (A) and TPX2 (B) at the prophase spindle of a *fra2* root cell. The anti-TPX2 antibody was a generous gift of Prof. Anne-Catherine Schmit (Institut de Biologie Moléculaire des Plantes, CNRS, Strasbourg, France). Double immunostaining was performed as described in references 3 and 18 (with the addition of a 30 min and a successive overnight step of incubation with 0.5% $NaBH_4$ before enzyme treatment, and a 20 min step in cold methanol just afterwards) with monoclonal anti-α-tubulin and polyclonal anti-AtTPX2.[10] The prophase spindle, at intranuclear CLSM section, appears to consist of microtubules converging to more than two poles (arrowheads in A). TPX2 signal (B) is coincident with the prophase spindle microtubules (compare with A). Scale bar: 10 μm.

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