Title

Microtubule Disassembly: When a Sleeper Is Activated

Journal

Current Biology, 23(20)

ISSN

0960-9822

Author

Liu, Bo

Publication Date

2013-10-01

DOI

10.1016/j.cub.2013.08.019

Peer reviewed

7. Kwon, J.Y., Dahanukar, A., Weiss, L.A., and Carlson, J.R. (2007). The molecular basis of CO_2 reception in *Drosophila*. Proc. Natl. Acad. Sci. USA *104*, 3574–3578.
8. Miyamoto, T., Slone, J., Song, X., and Amrein, H. (2012). A fructose receptor functions as a nutrient sensor in the Drosophila brain. Cell *151*, 1113–1125.
9. Miyakawa-Naito, A., Uhlén, P., Lal, M., Aizman, O., Mikoshiba, K., Brismar, H., Zelenin, S., and Aperia, A. (2003). Cell signaling microdomain with Na, K-ATPase and inositol 1,4,5-trisphosphate receptor generates calcium oscillations. J. Biol. Chem. *278*, A50355–50361.
10. Liu, J., Ward, A., Gao, J., Dong, Y., Nishio, N., Inada, H., Kang, L., Yu, Y., Ma, D., Xu, T., *et al*. (2010). *C. elegans* phototransduction requires a G protein-dependent cGMP pathway and a taste receptor homolog. Nat. Neurosci. *13*, 715–722.
11. Xiang, Y., Yuan, Q., Vogt, N., Looger, L.L., Jan, L.Y., and Jan, Y.N. (2010). Light-avoidance-mediating photoreceptors tile the *Drosophila* larval body wall. Nature *468*, 921–926.
12. Thorne, N., and Amrein, H. (2008). Atypical expression of *Drosophila gustatory receptor* genes in sensory and central neurons. J. Comp. Neurol. *506*, 548–568.
13. Gallio, M., Ofstad, T.A., Macpherson, L.J., Wang, J.W., and Zuker, C.S. (2011). The coding of temperature in the *Drosophila* brain. Cell *144*, 614–624.
14. Hamada, F.N., Rosenzweig, M., Kang, K., Pulver, S.R., Ghezzi, A., Jegla, T.J., and Garrity, P.A. (2008). An internal thermal sensor controlling temperature preference in *Drosophila*. Nature *454*, 217–220.
15. Viswanath, V., Story, G.M., Peier, A.M., Petrus, M.J., Lee, V.M., Hwang, S.W., Patapoutian, A., and Jegla, T. (2003). Opposite thermosensor in fruitfly and mouse. Nature *423*, 822–823.
16. Sato, K., Pellegrino, M., Nakagawa, T., Nakagawa, T., Vosshall, L.B., and Touhara, K. (2008). Insect olfactory receptors are heteromeric ligand-gated ion channels. Nature *452*, 1002–1006.
17. Wicher, D., Schafer, R., Bauernfeind, R., Stensmyr, M.C., Heller, R., Heinemann, S.H., and Hansson, B.S. (2008). *Drosophila* odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. Nature *452*, 1007–1011.
18. Sato, K., Tanaka, K., and Touhara, K. (2011). Sugar-regulated cation channel formed by an insect gustatory receptor. Proc. Natl. Acad. Sci. USA *108*, 11680–11685.
19. Benton, R., Sachse, S., Michnick, S.W., and Vosshall, L.B. (2006). Atypical membrane topology and heteromeric function of *Drosophila* odorant receptors in vivo. PLoS Biol. *4*, e20.
20. Zhang, H.J., Anderson, A.R., Trowell, S.C., Luo, A.R., Xiang, Z.H., and Xia, Q.Y. (2011). Topological and functional characterization of an insect gustatory receptor. PLoS ONE *6*, e24111.

Neuroscience Research Institute and the Department of Molecular, Cellular and Developmental Biology, University of California Santa Barbara, Santa Barbara, CA 93106, USA.
E-mail: craig.montell@lifesci.ucsb.edu

http://dx.doi.org/10.1016/j.cub.2013.09.026

Microtubule Disassembly: When a Sleeper Is Activated

Tubulin modifications are emerging as interesting mechanisms to regulate microtubule dynamics. A new study shows that phosphorylation of α-tubulin by the atypical kinase PHS1 leads to rapid disassembly of cortical microtubules in plant cells that are under hyperosmotic stresses.

Bo Liu

Plant cells form a network of parallel microtubules (MTs) at the cell cortex that are aligned perpendicular to the growth axis. Cortical MTs provide tracks for the plasma membrane-associated cellulose synthase complex to take linear trajectories when synthesizing cellulose microfibrils that are deposited in the nascent cell wall [1]. Thus, these MTs directly influence the growth and form of plants. Compared to animal cells in which interphase MTs typically have turnover rates over 10–20 times slower than those in dividing cells, cortical MTs turn over at rates comparable to those in mitotic and cytokinetic arrays in plant cells [2]. So exactly what are the advantages for sessile plant cells to establish such a highly dynamic MT array when they are not dividing? The answer may have to do with the fact that plant cells typically cannot escape from various biotic and abiotic stresses imposed on them by the ever-changing environment.

When plant cells are challenged by biotic or abiotic stresses, rapid disassembly of cortical MTs often is an immediate response [3,4]. There are two possible consequences following MT depolymerization. The cells may undergo programmed cell death, for example, in the process of hypersensitive responses to pathogens. Alternatively, the cells may be at an early phase of adaptation to sudden changes of the surrounding environment by remodeling the MT network. For example, hyperosmotic treatment of wheat root cells results in the formation of endoplasmic MT bundles after transient disappearance of the cortical MT network [5]. Another study demonstrated that α-tubulin was rapidly phosphorylated in response to hyperosmotic stresses in both rice and *Arabidopsis thaliana* [6]. So what is the consequence of tubulin phosphorylation? Which enzyme acts as the tubulin kinase in plant cells? In this issue of *Current Biology*, Fujita *et al.* [7] have answered these questions by characterizing the PHS1 (Propyzamide HyperSensitive1) protein in *A. thaliana*.

The semidominant *phs1-1* mutant exhibits hypersensitivity to low doses of the MT-depolymerizing herbicide propyzamide [8]. The primary amino acid sequence PHS1 indicates that it harbors a mitogen-activated protein kinase (MAPK) phosphatase domain toward its carboxy terminus. Indeed, the phosphatase activity of PHS1 was confirmed *in vitro* on the terminal MPK18 kinase of a MAPK cascade [9]. However, later experiments performed by Hashimoto and colleagues showed that the *phs1-5* null mutant formed seedlings that were morphologically indistinguishable from wild type when challenged by propyzamide [10]. The study also showed that PHS1 has a diffuse localization pattern in the cytoplasm. This is rather unusual, as most proteins that regulate MT dynamics would show localization either along MTs or at the ends of the filaments.

The disproval of a direct impact of the phosphatase domain on MT stability drew the authors' attention to the rest of the 922 amino acid PHS1 polypeptide. At first, they found that a truncated PHS1 lacking the phosphatase domain was sufficient to induce MT depolymerization *in vivo*. Such an effect could be blocked by the co-expression of PHS1's phosphatase domain [7]. The authors then pinpointed a central domain which bears limited sequence homology to actin-fragmin kinase in the slime mold *Physarum*. Previously, α-tubulin was found to be phosphorylated at Thr349 when under hyperosmotic stresses or by challenges to MT dynamics [6].

Voila, PHS1 is indeed a tubulin kinase! Thr349 is an absolutely conserved residue among eukaryotic α-tubulins and resides in the contact site facing β-tubulin, between tubulin heterodimers within the protofilament. Fujita *et al.* confirmed that the phosphomimetic TUA6^{T349D} mutant protein was incompetent for MT polymerization *in vitro* [7], recapitulating the *in vivo* data published earlier [6]. Therefore, phosphorylation of α-tubulin leads to the conversion of polymerization competent α-tubulins into incompetent ones, and consequently the reduction of unphosphrylated α-tubulin in the cytoplasmic tubulin pool. Because plant cortical MTs turn over rather rapidly, α-tubulin phosphorylation can be rapidly translated into the disassembly of these MTs in interphase cells.

Common posttranslational modifications of tubulins are acetylation of α-tubulin, glutamylation and glycosylation of both α- and β-tubulins, and detyrosination of α-tubulin [11]. These modifications often lead to stabilization of MTs. For example, MTs with acetylated α-tubulin represent a subpopulation of stable MTs [12]. Conversely, it has also been shown that phosphorylation of β-tubulin, by the cyclin-dependent kinase, would inhibit the incorporation of the dimers containing such a modified tubulin subunit into polymerizing filaments [13]. Although a phosphatase-inactive PHS1 mutant could induce depolymerization of MTs in mammalian cells upon ectopic expression as it did in plant cells [7], currently it is unclear whether animals produce an analogous tubulin kinase to regulate MT turnover. PHS1 homologs can be found among photosynthetic organisms from green algae to angiosperms. Thus, PHS1 takes part in a conserved regulatory mechanism for MT turnovers among these organisms.

Lastly, the authors tested whether PHS1 indeed functioned as an effector enzyme when cells perceive hyperosmotic stresses. The null *phs1-5* mutant became convenient in their experiments. Not only was α-tubulin no longer phosphorylated when the *phs1-5* seedlings were treated with high concentrations of NaCl or sorbitol, cortical MTs also became resistant against these challenges in the mutant cells [7]. Therefore, the authors concluded that PHS1 decodes the hyperosmotic stress signal for MT depolymerization.

Hyperosmotic stresses, like other abiotic stress, typically lead to the activation of MAPK cascades before plant cells take further action [14]. PHS1 contains a kinase-interacting motif (KIM) toward its amino terminus [10], and indeed it can directly interact with MPK18 [9]. In addition, the null *mpk18-1* mutation can suppress the propyzamide hypersensitive phenotype caused by the *phs1-1* mutation. While MPK18 can be a substrate of PHS1 as a phosphatase, it is unclear whether PHS1 is phosphorylated by MPK18 or other MAPK cascades. Furthermore, it is yet to be tested whether MPK18 is activated after cells respond to hyperosmotic stresses.

It should be noted that PHS1 is not the only protein that regulates MT assembly when plant cells are under salt stresses. SPIRAL1 (SPR1), a plant-specific MT plus end-tracking protein, antagonizes the depolymerization challenge to cortical MT organization brought about by salt stresses [15]. When plant cells are exposed to high concentrations of NaCl, SPR1 is rapidly degraded *via* the 26S proteasome pathway [16]. Elevated SPR1 expression would lead to hypersensitivity to salt stresses [16], possibly because cortical MTs failed to be disassembled promptly for remodeling. It would be interesting to learn whether PHS1 and SPR1 are directly connected when plant cells remodel the cortical MT network in response to imbalanced salt.

Among other remaining questions, a critical one is how the Arg64-to-Cys substitution in the KIM domain, caused by the *phs1-1* mutation, alters the function of PHS1 *in vivo* so that cortical MTs become hypersensitive to MT-depolymerizing agents. Furthermore, it is particularly intriguing how stress signals lift the inhibitory effect imposed by PHS1's intramolecular phosphatase domain in order to activate this atypical tubulin kinase.

In summary, the authors have demonstrated that α-tubulin phosphorylation by PHS1, as a consequence of hyperosmotic stress perception by plant cells, causes rapid disassembly of cortical MTs.

References

1. Lloyd, C., and Chan, J. (2008). The parallel lives of microtubules and cellulose microfibrils. Curr. Opin. Plant Biol. *11*, 641–646.
2. Hush, J.M., Wadsworth, P., Callaham, D.A., and Hepler, P.K. (1994). Quantification of microtubule dynamics in living plant cells using fluorescence redistribution after photobleaching. J. Cell Sci. *107*, 775–784.
3. Hardham, A.R. (2013). Microtubules and biotic interactions. Plant J. *75*, 278–289.
4. Nick, P. (2013). Microtubules, signalling and abiotic stress. Plant J. *75*, 309–323.
5. Komis, G., Apostolakos, P., and Galatis, B. (2002). Hyperosmotic stress induces formation of tubulin macrotubules in root-tip cells of Triticum turgidum: their probable involvement in protoplast volume control. Plant Cell Physiol. *43*, 911–922.
6. Ban, Y., Kobayashi, Y., Hara, T., Hamada, T., Hashimoto, T., Takeda, S., and Hattori, T. (2013). α-Tubulin is rapidly phosphorylated in response to hyperosmotic stress in rice and arabidopsis. Plant Cell Physiol. *54*, 848–858.
7. Fujita, S., Pytela, J., Hotta, T., Kato, T., Hamada, T., Akamatsu, R., Ishida, Y., Katsuna, N., Hasezawa, S., Nomura, Y., *et al.* (2013). An atypical tubulin kinase mediates stress-induced microtubule depolymerization in *Arabidopsis*. Curr. Biol. *23*, 2063–2070.
8. Naoi, K., and Hashimoto, T. (2004). A semidominant mutation in an Arabidopsis mitogen-activated protein kinase phosphatase-like gene compromises cortical microtubule organization. Plant Cell *16*, 1841–1853.
9. Walia, A., Lee, J.S., Wasteneys, G., and Ellis, B. (2009). Arabidopsis mitogen-activated protein kinase MPK18 mediates cortical microtubule functions in plant cells. Plant J. *59*, 565–575.
10. Pytela, J., Kato, T., and Hashimoto, T. (2010). Mitogen-activated protein kinase phosphatase PHS1 is retained in the cytoplasm by nuclear extrusion signal-dependent and independent mechanisms. Planta *231*, 1311–1322.
11. Wloga, D., and Gaertig, J. (2010). Post-translational modifications of microtubules. J. Cell Sci. *123*, 3447–3455.
12. Westermann, S., and Weber, K. (2003). Post-translational modifications regulate microtubule function. Nat. Rev. Mol. Cell Biol. *4*, 938–947.
13. Fourest-Lieuvin, A., Peris, L., Gache, V., Garcia-Saez, I., Juillan-Binard, C., Lantez, V., and Job, D. (2006). Microtubule regulation in mitosis: tubulin phosphorylation by the cyclin-dependent kinase Cdk1. Mol. Biol. Cell *17*, 1041–1050.
14. Rodriguez, M.C., Petersen, M., and Mundy, J. (2010). Mitogen-activated protein kinase signaling in plants. Annu. Rev. Plant Biol. *61*, 621–649.
15. Shoji, T., Suzuki, K., Abe, T., Kaneko, Y., Shi, H.Z., Zhu, J.K., Rus, A., Hasegawa, P.M., and Hashimoto, T. (2006). Salt stress affects cortical microtubule organization and helical growth in Arabidopsis. Plant Cell Physiol. *47*, 1158–1168.
16. Wang, S., Kurepa, J., Hashimoto, T., and Smalle, J.A. (2011). Salt stress-induced disassembly of Arabidopsis cortical microtubule arrays involves 26S proteasome-dependent degradation of SPIRAL1. Plant Cell *23*, 3412–3427.

Department of Plant Biology, University of California, Davis, CA 95616, USA.
E-mail: bliu@ucdavis.edu

http://dx.doi.org/10.1016/j.cub.2013.08.019