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Meeting abstract

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Does tubulin phosphorylation correlate with cell death in plant cells?

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Background

Microtubules are necessary for a wide spectrum of cellular functions, which include cell division, intracellular transport, organelle positioning and generating of cell polarity. The major component of microtubules is tubulin heterodimeric protein which is consist of two subunits: α- and β-tubulin. Both tubulin subunits can be extensively altered by post-translational modifications, including detyrosination/tyrosination, acetylation/deacetylation, polyglutamylation, polyglycylation, and phosphorylation. As for the different tubulin isotypes, the functionality of the post-translational modifications is still a matter of debate. Although, it is known that some of them are associated with stable/dynamic populations of microtubules, while others seem to influence the binding of motor proteins [1]. One of post-translational modifications, tubulin phosphorylation, is not a widely observed and its precise function is unknown both in animal and plant cells.

It was shown recently that animal tubulin can be phosphorylated by different systems of cyclic nucleotide-dependent (cAMP- and cGMP-dependent) protein kinases, Ca^{2+}-dependent protein kinases (including Ca^{2+}-calmodulin-dependent and Ca^{2+}-dependent, phospholipid-stimulated types of enzymes), casein kinases and tyrosine kinases, too [2,3]. The combined data demonstrate that plant tubulin can also undergo extensive phosphorylation by different types of protein kinases and that the phosphorylation on serine\threonine as well as at tyrosine residues can participate in the generation of high level of polymorphism of plant tubulin [4]. It is interesting to establish a functional role of this tubulin modification as phosphorylation is a universal post-translational modification which is typical for most of the proteins. The effects of different activators and inhibitors of protein kinases on microtubule dynamics and cell cycle progression in plan cells are present in this report.

Materials and methods

Two plant lines, *Arabidopsis thaliana* [5] and tobacco BY-2 cell culture [6] (kindly handed over by Prof. J.-P. Verbelen, University of Antwerp, Belgium) both expressing GFP-tubulin as well as *A. thaliana* and *Nicotiana tabacum* wild types were used in this research. GFP-labeled microtubules in *A. thaliana* and BY-2 cells were analyzed by confocal laser scanning microscopy.

The root tips of 3-day old *Allium cepa* seedlings were also used in this study. The primary mouse monoclonal antibodies TU-01 (against α-tubulin) and TU-06 (against β-tubulin) (kindly provided by Drs. V. Viklicky and P. Draber, Institute of Molecular Genetics, Prague, Czech Republic) were used for visualisation of microtubules in onion meristematic root tip cells by immunofluorescence microscopy. FITC-conjugated anti-mouse antibody (Sigma, USA) was used as a secondary one. The fixation and staining of microtubules by antibodies were performed as described by us early [7].

As regulators of protein kinases, dibutyryl-cAMP (Serva, Germany) in combination with ATP, polymyxin B (Serva, Germany), trifluoperazine (Serva, Germany) and okadaic acid (Sigma, USA) were used.

Results

For more detailed analysis of the functional role of tubulin phosphorylation in plant cells several specific inhibi-

tors and activators of different types of protein kinases were used in our research. Dibutyryl-cAMP (10 μM in combination with 100 μM ATP) as an activator of cAMP-dependent phosphorylation, polymyxin B (5 mM) as an inhibitors of the protein kinase C, trifluoperazine (5 mM), as an inhibitor of the Ca^{2+}-calmodulin-dependent protein kinase, and okadaic acid (inhibitor of protein phosphatase type 2A, PP2A), in concentration 1–30 nM, were investigated with regard to their ability to affect microtubule dynamics and to induce structural changes of microtubules. The root tips of seedlings were treated with each of these compounds. The effects of these regulators of protein kinases on the structural reorganisation of interphase and mitotic microtubules were studied after exposure of plant material in the presence of activator or inhibitor during 6, 12 and 24 h.

Immunofluorescence analysis of microtubules showed that treatment by cAMP causes the disruption of both interphase and mitotic microtubules and accumulation of depolymerised tubulin around the nuclei in the cells. The treatment of onion cells by trifluoperazine caused the reorganization of microtubules and change of their spatial organization from a transverse to a longitudinal orientation and formation of thick longitudinal arrays. The treatment of *A. cepa* cells with polymyxin B caused the same effects on microtubular organisation as trifluoperazine.

Confocal laser scanning and light microscopy of *A. thaliana* and *N. tabacum* cells revealed that okadaic acid arrested cell growth, alter cell morphology, and affected the organization of microtubules.

Conclusion

It was reviewed by us that plant tubulin can undergo extensive phosphorylation by different types of protein kinases, and that tubulin phosphorylation participates in regulation of the plant cell cycle [4]. Many studies shown that different protein phosphatase inhibitors effect microtubules in animal and plant cells. For instance, it was shown that the treatment of *Tradescantia* stamen hair cells with okadaic acid and other protein phosphatase inhibitors caused changes of the metaphase transit times and the pattern of sister chromatid separation [8]. The treatment of *Arabidopsis* shoots with inhibitors of serine/threonine protein phosphatases (okadaic acid or calyculin A) provoked the destruction of root morphology, that can be explained by the influence of these compounds on cortical microtubules function [9]. The same authors later proved that phosphatase inhibitors as well as protein kinase inhibitors destroy not only root morphology but that cortical microtubules also become disorganized after exposure to some types of inhibitors [10]. In particular, these effects were characteristic of protein phosphatases such as calyculin A and cantaridin. The protein kinase inhibitor staurosporine also had similar effect in plant cells [11-13]. The disruption of microtubules was found recently after calyculin A and okadaic acid treatment in *Lilium* [14]. Thus, literature indicates that phosphorylation and dephosphorylation represent a part of the molecular mechanism responsible for both the organization of the cortical microtubular networks and of mitotic function.

Studies on animal cells clearly demonstrated that okadaic acid and other protein phosphatase inhibitors induce mitotic arrest [15,16], premature chromosome condensation [17,18], microtubule disassembly [18,19], DNA fragmentation [20,21] and apoptosis [16,17,20,21].

Summarizing our data obtained we can conclude that the changes in the spatial organisation of microtubules after treatment by cAMP and the protein kinase inhibitors lead to disturbances of cell cycle progression and it is most likely to launch of the cell death program in plant cells.

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References

1. Westermann S, Weber K: **Post-translational modifications regulate microtubule function.** *Nature Rev Mol Cell Biol* 2003, **4:**938-947.
2. Luduena RF: **Multiple forms of tubulin: different gene products and covalent modifications.** *Int Rev Cytol* 1998, **178:**207-275.
3. MacRae TH: **Tubulin post-translational modifications. Enzymes and their mechanisms of action.** *Eur J Biochem* 1997, **244:**265-278.
4. Blume YaB, Yemets AI, Lloyd CW: **Plant tubulin phosphorylation and its role in cell cycle progression.** *NATO Series* in press.
5. Ueda K, Matsuyama T, Hashimoto T: **Visualization of microtubules in living cells of transgenic *Arabidopsis thaliana*.** *Protoplasma* 1999, **206:**201-206.
6. Kumagai F, Yoneda A, Tomida T, Sano T, Nagata T, Hasezawa S: **Fate of nascent microtubules organized at the M/G1 interface, as visualized by synchronized tobacco BY-2 cells stably expressing GFP-tubulin: time-sequence observations of the reorganization of cortical microtubules in living plant cells.** *Plant Cell Physiol* 2001, **42:**723-32.
7. Yemets AI, Kundel'chuk OP, Smertenko AP, Solodushko VA, Rudas VA, Gleba YY, Blume YB: **Transfer of amiprophosmethyl-resistance from a *Nicotiana plumbaginifolia* mutant by somatic hybridisation.** *Theor Appl Genet* 2000, **100:**847-857.
8. Wolniak SM, Larsen PM: **Changes in the metaphase transit times and the pattern of sister chromatid separation in stamen hair cells of *Tradescantia* after treatment with protein phosphatase inhibitors.** *J Cell Sci* 1992, **102:**691-6715.
9. Smith RD, Wilson JE, Walker JC, Baskin TI: **Protein phosphatase inhibitors block root hair development and alter cell shape in *Arabidopsis* roots.** *Planta* 1994, **194:**516-524.
10. Baskin TI, Wilson JE: **Inhibitors of protein kinases and phosphatases alter root morphology and disorganize cortical microtubules.** *Plant Physiol* 1997, **113:**493-502.
11. Hasezawa S, Nagata T: **Okadaic acid as a probe to analyse the cell cycle progression in plant cells.** *Bot Acta* 1992, **105:**63-69.
12. Mizuno K: **Inhibition of gibberelin-induced elongation, reorientation of cortical microtubules and change of isoform of tubulin in epicotyl segments of azuki bean by protein kinase inhibitors.** *Plant Cell Physiol* 1994, **35:**1149-1157.
13. Zhang K, Tsukitani Y, John PCL: **Mitotic arrest in tobacco caused by the phosphoprotein phosphatase inhibitor okadaic acid.** *Plant Cell Physiol* 1992, **33:**677-688.

14. Foissner I, Grolig F, Obermeyer G: **Reversible protein phosphorylation regulates the dynamic organization of the pollen tube cytoskeleton: effects of calyculin A and okadaic acid.** *Protoplasma* 2002, **220:**1-5.
15. Wera S, Hemmings BA: **Serine/threonine protein phosphatases.** *Biochem J* 1995, **311:**17-29.
16. Lerga A, Richard C, Delgado MD, Caneles M, Frade P, Cuadrado MA, Leon J: **Apoptosis and mitotic arrest are two independent effects of the protein phosphatase inhibitor okadaic acid in K562 leukemia cells.** *Biochem Biophys Res Comun* 1999, **260:**256-264.
17. Ishida Y, Furukawa Y, Decaprio JA, Saito M, Griffin JD: **Treatment of myeloid leukemic cells with phosphatase inhibitor okadaic acid induces cell cycle arrest at either G1/S or G2/M depending on dose.** *J Cell Physiol* 1992, **150:**484-492.
18. Sun Q-Y, Wu G-M, Lai L, Bonk A, Cabot R, Park K-W, Day BN, Prather RS, Schatten H: **Regulation of mitogen-activated protein kinase phosphorylation, microtubule organization, chromatin behavior, and cell cycle progression by protein phosphatases during pig oocyte maturation and fertilization in vitro.** *Biol Reprod* 2002, **66:**580-588.
19. Zhang L, da Costa SR, Yarber FA, Runnegar M, Hamm-Alvarez SF: **Protein phosphatase inhibitors alter cellular microtubules and reduce carbachol-dependent protein secretion in lacrimal acini.** *Curr Eye Res* 2000, **20:**373-383.
20. Inomata M, Saijo N, Kawashima K, Kaneko A, Fujiwara Y, Kunikana H, Tanaka Y: **Induction of apoptosis in cultured retinoblastoma cells by the protein phosphatase inhibitor, okadaic acid.** *J Cancer Res Clin Oncol* 1995, **121:**729-738.
21. Nuydens R, Dispersyn G, van den Keiboom G, de Jong M, Connors R, Ramaekers F, Borgers M, Geerts H: **Bcl-2 protects against apoptosis-related microtubule alterations in neuronal cells.** *Apoptosis* 2000, **5:**43-51.