

## COMMENTARY

## Assembly and disassembly of plant microtubules: tubulin modifications and binding to MAPs

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Microtubules are dynamic heteropolymers of  $\alpha$ - and  $\beta$ -tubulin that assemble co-ordinately in response to a variety of intracellular and extracellular signals and participate in a number of different functions in eukaryotic cells, from cell division to organelle transport, from RNA positioning to flagellar beating. In plant cells, microtubules assemble and disassemble during the cell cycle to organize different microtubule arrays. Interphase cortical microtubules have a critical role in the construction of the cell wall by controlling the correct deposition of cell wall polymers (Lloyd and Chan, 2008). During cell division, microtubules are arranged into characteristic structures. The preprophase band (a circular array of microtubules) defines the future construction site of the cell plate, thus imposing asymmetry on the daughter cells. The mitotic spindle shares the function of the analogous structure of animal and fungal cells but its organization shows some critical differences, mainly caused by the absence of centrioles. The phragmoplast is a special microtubule array that substitutes the contractile ring of animal cells during cytokinesis, allowing the synthesis of a new cell wall that physically separates the two daughter cells. Since the four different microtubule arrays have distinct features and structures, use of different proteins (tubulin and non-tubulin) is a critical requisite for the assembly of each array. Understanding how individual proteins are used in the assembly of microtubules will allow a clearer picture of how microtubules perform their function and pass from interphase to mitotic arrays (and vice versa).

In this issue, Jovanovic and colleagues (Jovanovic *et al.*, 2010) report that the tyrosination/detyrosination cycle of tubulin could regulate the transition of plant cells from the elongation to the division stages. In their work, a specific compound (nitrotyrosine) is used irreversibly to incorporate tyrosine into detyrosinated  $\alpha$ -tubulin; the consequence of this post-translational modification is the inhibition of

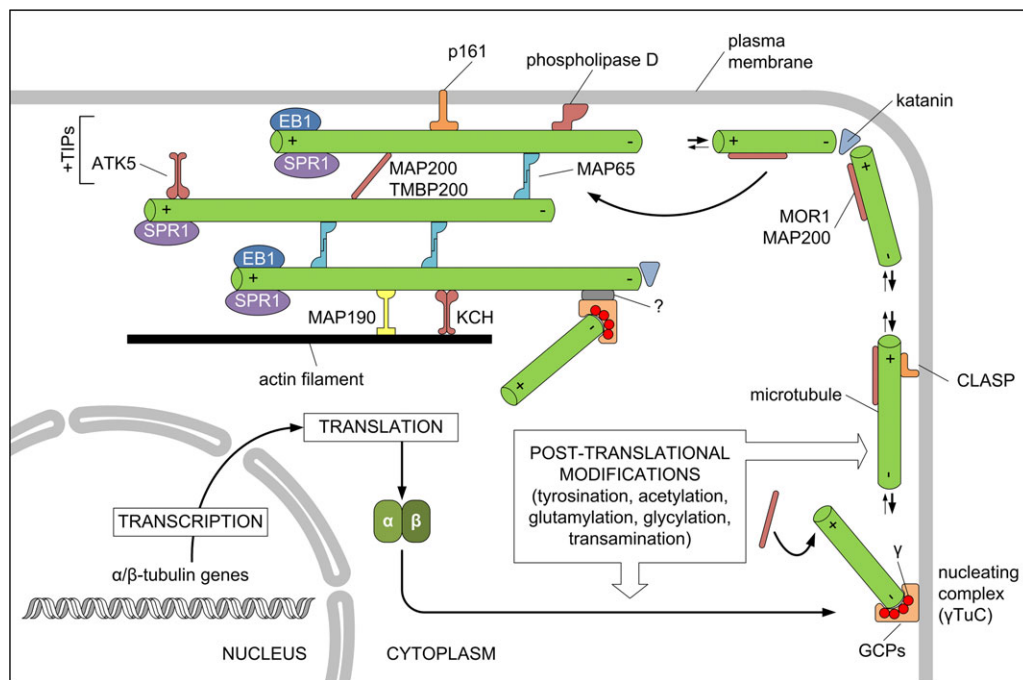
mitosis and the increase in cell elongation. The article points to the importance of post-translational modification of tubulin in the reorganization of the microtubule cytoskeleton during the life cycle of plant cells. Although microtubules within each array are apparently identical in structure, plants have distinct gene sets coding for both  $\alpha$  and  $\beta$ -tubulin (Guo *et al.*, 2009). Tubulin genes are not expressed uniformly during plant development; for example, a particular gene is expressed almost exclusively in reproductive organs (Yu *et al.*, 2009). At cellular levels, distinct  $\alpha$ -tubulin genes can be specifically expressed in cells that exit from mitosis when transverse microtubules determine the cell shape (Schröder *et al.*, 2001). Generally, the expression of tubulin isotypes seems to be tissue-specific, a model that is also supported by immunological approaches (Parrotta *et al.*, 2009). The use of different tubulin isoforms is further complicated by mechanisms of post-translational modifications which are used to label subpopulations of microtubules, and that work individually or in combination at the level of single cells to control specific microtubule functions in particular cell domains. The detyrosination/tyrosination of tubulin is probably involved in controlling the binding of plus-end tracking proteins and motor proteins with microtubule depolymerizing activity (Peris *et al.*, 2009); glutamylation and glycylation are hypothetically involved in the mechanism of microtubule severing by katanin (Sharma *et al.*, 2007). On the other hand, acetylation is a post-translational modification detected in stable microtubules of most cells, but is also likely to be involved in regulating kinesin-based motility (Gardiner *et al.*, 2007). Recently, the discovery of phosphorylated tobacco tubulin suggested that tyrosine phosphorylation is also involved in regulating the properties of plant microtubules (Blume *et al.*, 2008). A recent report suggests that addition of putrescine to tubulin by pollen transglutaminase can also regulate the binding and release of kinesin to/from microtubules (Del Duca *et al.*, 2009). Consequently, current data from genetic and biochemical approaches suggest a model in which development of specific plant cells and tissues is characterized by the expression of distinct tubulin genes and, consequently, by the use of distinct tubulin isotypes, which are post-translationally modified to control the binding of microtubule-associated proteins (MAPs).

MAPs are used to assemble different microtubule arrays according to the specific stage of the cell cycle and their interaction with microtubules is critical at almost every stage of microtubule life. After one microtubule is assembled by a  $\gamma$ TuC nucleating complex (containing  $\gamma$ -tubulin and several gamma complex proteins or GCPs), stabilization of the growing end is supported by binding to specific proteins, such as MOR1, and/or by putative association with the

plasma membrane through plus-end proteins like CLASP. Subsequent detachment of microtubules from the nucleating complex is probably supported by the activity of katanin. Net addition of  $\alpha/\beta$ -subunits to the plus-end and the concomitant removal of subunits at the minus-end generate a treadmilling process, which is responsible for the translocation of microtubules in the cell cortex (plant MAPs are reviewed by Hamada, 2007). This process brings microtubules in contact with other cortical (bundles of) microtubules. In this context, post-translational modifications like glutamylation and glycylation hypothetically constitute signals for the severing activity of katanin (Sharma *et al.*, 2007), which could cut microtubules in order to increase their number. Microtubules moving by treadmilling in the cell cortex can be captured by other microtubules and organized into larger bundles by, for example, proteins of the MAP65 family (Van Damme *et al.*, 2004). In interphase cells, microtubules are anchored along their length to the plasma membrane by proteins that stabilize microtubules, such as p161 (Cai *et al.*, 2005), and/or by proteins that mediate the intercellular communication, like phospholipase D (Gardiner *et al.*, 2001). Association of the plus-end of microtubules with the plasma membrane and with the endomembrane system is probably mediated by a class of proteins collectively known as +TIPs, so far including EB1, SPR1, and the kinesin-14A ATK5 (Pastuglia and Bouchez, 2007). Association of +TIPs with microtubules is potentially regulated by the C-terminal detyrosination/tyrosination of tubulin. Although previous studies showed that detyrosinated microtubules are less dynamic than those containing tyrosinated

tubulin, more recent investigations indicate that the detyrosinated form of tubulin interacts specifically with kinesin-1 (Liao and Gundersen, 1998), while tyrosinated microtubules interact preferentially with the plus-end protein CLIP170 (Hammond *et al.*, 2008). These findings suggest that the tyrosination/detyrosination of plus-end tubulin can regulate the binding of microtubules to MAPs and, consequently, their dynamics. Association of microtubules with motor MAPs could also be controlled by other post-translational modifications: microtubules containing acetylated tubulin interact preferentially with the motor proteins kinesin-1 and dynein (Fukushima *et al.*, 2009) while the addition of polyamines by transglutaminase changes the binding properties of kinesin (Del Duca *et al.*, 2009). Consequently, the interaction of microtubules with associated proteins regulating their function and dynamics is speculatively dependent on various types of local post-translational modifications. It is still unknown whether the lateral association of microtubules with actin filaments, which is required for the assembly and directionality imposed on transverse microtubules by the actomyosin-based cytoplasmic streaming (Sainsbury *et al.*, 2008), needs specific post-translational modification of tubulin. Plant kinesins with a calponin-homology domain (KCH) (Frey *et al.*, 2009) and structural MAPs, like MAP190 (Igarashi *et al.*, 2000), are candidates for this function and they could putatively be regulated by post-translational modifications of tubulin (Fig. 1).

The transition from interphase to mitotic microtubules is marked by the disassembly of microtubule bundles beneath



**Fig. 1.** Speculative activity of post-translational modifications and MAPs during the assembly of microtubule bundles in interphase cells. After transcription and translation, tubulins are modified by the addition/removal of specific groups. Such modifications, which are likely to occur at the level of both monomers and polymers, hypothetically regulate the binding of motor and non-motor MAPs to microtubules. See the text for further explanation. Abbreviations:  $\gamma$ , gamma tubulin; ?, hypothetical protein. The pair of diverging short arrows at the minus-end and plus-end of the microtubules indicates shortening and elongation. (This figure is available in colour at JXB online.)

the plasma membrane and by the co-ordinated assembly of new microtubules. Current literature provides few indications on the post-translational modifications of tubulin during the transition from interphase to mitosis in plant cells. Since the dephosphorylated form of tubulin interacts specifically with kinesin-1 while tyrosinated microtubules interact with plus-end proteins, the incorporation of tyrosine could enhance the binding to specific MAPs (such as +TIPs), which changes the equilibrium between cell elongation and mitosis. In this context, the work of Jovanovic *et al.* (2010) suggests that the post-translational modifications of tubulin could be part of the mechanism that regulates the transition from interphase to mitotic microtubules. Another 'controller' could be acetylated tubulin, which interacts preferentially with kinesin-1 and dynein (Fukushima *et al.*, 2009) and is localized at the poles of the plant mitotic spindle (Smertenko *et al.*, 1997). Speculatively, acetylation of tubulin could mark specific subsets of microtubules involved in the assembly of the mitotic spindle. The organization of the mitotic apparatus also requires the co-ordinated bundling of microtubules into parallel and anti-parallel orders. The MAP65 protein family performs a critical role because distinct members of MAP65 are localized to specific sites of the mitotic spindle (MAP65-1 and MAP65-3 localize to the spindle midzone during metaphase and anaphase while MAP65-4 is preferentially localized at the spindle poles) (Van Damme *et al.*, 2004). A progressively increasing literature suggests that a number of mitosis-specific kinesins plays a crucial role in the assembly of the mitotic spindle in vascular plants, such as AtKRP125c/kinesin-5 (Bannigan *et al.*, 2007) and the PAKRP1/Kinesin-12A and PAKRP1L/Kinesin-12B, which localize at the juxtaposing plus-ends of antiparallel microtubules in the phragmoplast (Lee *et al.*, 2007). Depicting the interplay between post-translational modifications of tubulin and the microtubule binding of both motor and structural MAPs in interphase and mitotic plant cells may be a critical priority in the future.

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## ERRATA

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