Microtubule composition: Cryptography of dynamic polymers

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The posttranslational modification of microtubules reveals additional levels of diversity within the cytoarchitecture. Modified forms of microtubules are differentially distributed in cells and harbor distinct sets of microtubule-associated proteins. It has been difficult to ascertain whether these posttranslational modifications dictate microtubule dynamics, or whether particular modifications follow changes in microtubule dynamics. A study in budding yeast published in a recent issue of PNAS (1) enabled genetic modifications of the primary transcript and analysis of microtubules assembled from modified tubulin.

Microtubule Structure

Microtubules are composed of tubulin dimers (α and β) that exhibit stochastic growth and shortening, termed dynamic instability (2). Dynamic instability is regulated by a stabilizing GTP "cap," found at the exchangeable GTP-binding site of β-tubulin exposed on the plus end of a microtubule. The GTP-bound nucleotide is hydrolyzed on incorporation of β-tubulin into the microtubule lattice. Upon hydrolysis of GTP at the plus end, microtubules become less stable, resulting in the release of energy stored within the microtubule lattice and polymer shortening (3). Shortly after the discovery of dynamic instability, it was found that posttranslationally modified microtubules can be very stable and turn over with half times on the order of hours, rather than minutes (4, 5). A growing repertoire of plus-end-binding proteins has been shown to regulate microtubule dynamics and interactions between microtubules and nonmicrotubule cytoskeletal elements. Key posttranslational modifications may dictate both microtubule dynamics and the binding of specific plus-end-binding proteins. That posttranslational acetylation of tubulin can be removed by the same deacetylases that modify histones (6, 7) raises the specter that these modifications contribute to the transfer of critical information at the chromatin/microtubule boundary.

Both α- and β-tubulin subunits are subject to posttranslational modifications (8). One of these modifications is the removal of the C-terminal amino acid from α-tubulin. The C terminus of α-tubulin is aromatic (tyrosine in mammals and phenylalanine in yeast) and is preceded by two glutamate (EE) residues. A carboxypeptidase catalyzes the cyclic removal of tyrosine from tubulin and tubulin-tyrosine ligase can reattach tyrosine in a tRNA-independent reaction. The early studies in this area indicated that detyrosination itself does not confer stability unto the microtubule polymer (9). However, it was difficult to distinguish whether the modification dictated stability, or whether stabilized microtubules become modified. For the first time, a genetic system has been used to study the incorporation of detyrosinated tubulin into microtubules (1).

Microtubule Stability in Cells Expressing Detyrosinated Tubulin (Glu Tubulin)

Badin-Larçon *et al.* (1) introduced the truncated version of α-tubulin (lacking the terminal phenylalanine, thus exposing glutamate; Glu tubulin) into the yeast genome in place of endogenous *TUB1*. Yeast microtubules comprised of this truncated form of α-tubulin (Glu tubulin), and wild-type β-tubulin were able to support cell growth. However, the cells are sensitive to microtubule poisons such as benomyl and are slightly cold-sensitive (at 10°C). Phenylalanine was not added posttranslationally to the Glu tubulin, demonstrating that there is no tubulin-ligase activity in yeast, despite the presence of a gene, YBR094w, that exhibits significant homology (6e-53) to tubulin-tyrosine ligase in other organisms. In addition, overexpression of this gene (YBR094w) was unable to convert Glu tubulin to wild-type tubulin *in vivo*.

The phenotypic analysis of cells expressing Glu tubulin revealed significant defects in several microtubule-based processes. In budding yeast, nuclei migrate to the site of cell division, which is chosen at the time of bud emergence. In the course of nuclear migration, the nucleus traverses the cell and may oscillate between the mother and daughter cell before cell division. These oscillations are severely dampened in cells expressing Glu tubulin (1). Additional features of spindle dynamics, including the velocity and amplitude of spindle movement (μm/min) were decreased >2-fold in cells expressing Glu tubulin. In previous studies (4, 10, 11), Glu tubulin-containing microtubules have been reported to be more stable than their tyrosinated counterparts, indicating that a decrease in microtubule dynamics may contribute to the observed phenotypes. Yeast cells expressing altered forms of β-tubulin that attenuate microtubule dynamics *in vitro* also result in decreased spindle dynamics *in vivo* (12). Thus, it is likely that microtubules containing Glu tubulin are less dynamic than wild-type tubulin. The key advance by the Badin-Larçon *et al.* (1) study is that Glu tubulin can be incorporated in the lattice. In mammalian cells, the tubulin-tyrosine carboxypeptidase preferentially acts on polymers versus tubulin dimers, which is indicative of detyrosination of microtubules. Moreover, incorporation of Glu tubulin directly into the microtubule indicates that this modification can dictate the behavior of the microtubule. In tissue cells, it has been difficult to establish cause and effect between Glu tubulin and microtubule stability. It has been inferred that stable microtubules are detyrosinated, because inhibiting the tubulin-tyrosine ligase did not alter the global dynamics of microtubules (9). The studies in yeast indicate that indeed incorporation of Glu tubulin into the lattice dictates microtubule stability.

Plus-End-Binding Proteins at Glu Tubulin Ends

How does Glu tubulin stabilize the microtubule polymer? Glu tubulin could directly dampen microtubule dynamics or alter the repertoire of microtubule-associated proteins that in turn attenuate dynamics. It has been shown that Glu tubulin preferentially accumulates kinesin in a mechanism that promotes Glu tubulin-containing microtubule interaction with intermediate filaments (13, 14). However in these studies, the Glu tubulin could be modified after recruitment of kinesin, or interaction with intermediate filaments. The expression of Glu tubulin-containing microtubules allows one to distinguish these possibilities. Badin-Larçon *et al.* (1) examined the recruitment of two plus-end-binding proteins in yeast, Bim1 and Bik1 (the homologs of mammalian EB1 and CLIP-170, respectively; ref. 15). EB1 is responsible for recruiting the adenoma polyposis coli protein, whereas CLIP170 is

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a key factor for dynein-dependent microtubule capture in tissue cells. Bim1-GFP was recruited to microtubules containing wild-type or Glu tubulin; however, there was reduced force production from microtubule-cortical interactions in cells with Glu tubulin. In contrast, Bik1-GFP binding at microtubule plus ends was significantly reduced (3× reduction) on Glu tubulin-containing microtubules. In addition to directly demonstrating that Glu tubulin dictates the repertoire of plus-end-binding proteins (and extending previous studies; see ref. 11), these results provide evidence that Bik1p and Bim1p recognize different features of microtubule plus ends, as discerned by the Glu tubulin mark.

Microtubule Acetylation

α-Tubulin is also acetylated at position lysine 40. This modification is predominantly associated with stable microtubules in structures like the axoneme. After microtubule assembly, acetylation occurs at a site predicted to be within the inner wall or lumen of the microtubule. Whereas the tubulin acetyltransferase activity has not been identified, several deacetylases have been uncovered. Histone deacetylase 6 and human sir2 ortholog deacetylate tubulin (6, 7), and moreover, exhibit preferential activation toward a tubulin peptide substrate in comparison with a histone substrate.

The function of tubulin acetylation remains unclear. Experiments in which the lysine was changed to nonacetylatable amino acids does not have any phenotypic consequences for *Tetrahymena* (16) or *Chlamydomonas* (17). Interesting, nature herself may reveal the function of these modifications. In *Caenorhabditis elegans*, a novel tubulin encoded by MEC-12 is the only identified tubulin in this organism that contains lysine at position 40 (18). This tubulin isotype results in the formation of an unusual 15-protofilament microtubule that is highly expressed in touch receptor neurons. Phenotypic rescue by using a MEC-12 variant lacking the lysine 40 showed that acetylation is not required for MEC-12 activity. Acetylation might effect microtubule stability, directly or indirectly through the binding of plus-end proteins (see above and ref. 19).

Implications of the Posttranslational Modification of Dynamic Polymers

A microtubule code that specifies whether there are rapidly growing microtubules, stable microtubules, or recruitment of signaling molecules for a specific cell state, or more critically for a specific subdomain within a cell adds another level of regulation to differentiate subcellular structures. How is the code read? The expression of exclusively Glu tubulin and the lack of Bik1p binding in budding yeast indicate that specific binding proteins are able to distinguish structural aspects of wild-type vs. truncated α-tubulin. Whether microtubules containing Glu tubulin are structurally distinct, or whether some other feature of this modification is recognized, it is clear that this modification will have functional consequences. Likewise, it may be that acetylated tubulin dictates differential plus-end-protein binding, although this remains to be demonstrated. If the acetylation is inside the lumen, the molecular basis of recognition is less clear.

If distinctly modified microtubule plus ends expand their diversity, then we may find specialized functions for these differentiated ends. Microtubules are essential for intracellular trafficking, cell polarity establishment and maintenance, and chromosome segregation. However, the microtubule cytoskeleton also constrains chromosome movement in interphase (20), promotes chromosome arm motility in mitosis [polar ejection forces (21) and chromokinesins (22, 23)], and promotes chromosome motility after DNA damage (24). Whereas the specificity of these interactions remain to be elucidated, it is clear that microtubule plus ends are intimately involved with the chromosome. A recent study (25) reveals that such interactions may provide a conduit for loading proteins such as histone variants. The histone variant macroH2A1 (macroH2A1) contains an N-terminal domain that is highly similar to core histone H2A and a larger C terminus of unknown function. This histone localizes to the inactive X chromosome of differentiated female embryonic stem cells. In the course of X-inactivation, Xist RNA is expressed and becomes localized to the inactivate X chromosome. Shortly after Xist RNA localization, macroH2A1 becomes deposited. The accumulation of macroH2A1 comes from a pool of this protein at the centrosome. For a microtubule to specifically promote loading of centrosomal proteins to the X chromosome, there must be strict interactions between the microtubule and this chromosome, which may involve differentiated plus ends. Furthermore, this work may reveal a pathway for histone deacetylases as well. Nuclear histone deacetylase 6 and human sir2 ortholog deacetylases may travel from chromatin to a microtubule, providing a chromatin-directed regulation of microtubule plus ends. Histone deacetylases are typically associated with inactive or regions of heterochromatin. Would the delivery of deacetylases from inactive chromatin to microtubule plus ends stabilize the microtubule and provide a positive feedback mechanism for keeping the chromatin condensed? Whether or not this is the mechanism, the notion of chromatin-microtubule-based transport and functional specialization of plus ends is indicative of lines of communication between these dynamic polymers.

The prospect of defining the raison d'etre for posttranslational modifications of tubulin function is an exciting one. Posttranslationally modified tubulin accumulates in cancer cells and cardiac tissue in model systems for heart failure (26, 27). The common denominator in these models may be the requirement for cellular and/or chromatin remodeling. Perhaps Glu tubulin or other posttranslational tubulin modifications accompany (or direct) changes in the mechanical properties of the cytoskeleton in these altered states.

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