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Deciphering the Tubulin Code

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Enzymes of the tubulin tyrosine ligase-like (TTLL) family posttranslationally modify and thereby mark microtubules by glutamylation, generating specific recognition sites for microtubule-interacting proteins. Garnham et al. report the first structure of a TTLL protein alone and in complex with microtubules, elucidating their mechanism of action.

Markers, flags, and signs are commonly used in our daily lives. Rangers mark trees in the forest so that wood workers know which tree is left in place and which one to cut. Barcodes on supermarket products carry hidden messages like the price, the destination of the product, or its best-before date. Street signs show us which street we are driving on, where it is leading, and how fast we are allowed to drive. Knowing the meaning of these symbols, we decode hidden information and make use of it. Biology also utilizes codes, and in the case of microtubules, posttranslational modifications (PTM) mark subpopulations and modify the interactions with microtubule effectors (Janke, 2014). This so-called "tubulin code" (Verhey and Gaertig, 2007) is established by detyrosination, glutamylation, glycylation, acetylation, phosphorylation, palmitoylation, and generation of Δ2-tubulin (Westermann and Weber, 2003) (Figure 1A). With the exception of acylation and palmitoylation, these modifications occur at the flexible C-terminal tails of α- and β-tubulin protruding from the surface of microtubules. Microtubule PTMs alter the interaction with microtubule-associated proteins (MAPs), motor proteins such as kinesin and dynein, and plus-end tracking proteins (+TIPs) and are therefore essential for intracellular trafficking, assembly and motility of cilia, microtubule dynamics, and mitosis. Dysfunction of microtubule PTM enzymes has detrimental effects for the organism, leading to developmental disorders and neurodegenerative diseases. Bacteria also make use of the tubulin code and specifically modify microtubules of the host. Toxin A from *Clostridium difficile*, for example, decreases acetylation of tubulin and thereby causes acute inflammation (Nam et al., 2010).

Due to missing structural information on tubulin PTM enzymes in complex with microtubules, it has so far not been apparent how these enzymes specifically recognize and modify the microtubule—that is, how they establish the "tubulin code." In this issue of *Cell*, the teams around Antonina Roll-Mecak, Ron Milligan, and Gabe Lander present a structural explanation for the specific binding and modification of microtubules by tubulin tyrosine ligase-like enzyme 7 (TTLL7) (Garnham et al., 2015), which is responsible for the ATP-dependent initiation and elongation of polyglutamylation of microtubules (Mukai et al., 2009).

The crystal structure of TTLL7 shows that its active site has the same ATP-grasp ligase fold found in the homologous tubulin tyrosine ligase (TTL) (Janke et al., 2005; Szyk et al., 2011). However, c-MTBD, a major positively charged domain of the protein that is not found in TTL, is not resolved in the structure. Interestingly, this domain is ordered in TTLL7 when bound to the microtubule, interacting with a negatively charged patch on α-tubulin (Figure 1B). The authors show convincingly that the positive charge is essential for proper binding not only of TTLL7, but also of other members of the TTLL glutamylases, even if the fold of the domain is probably not conserved.

The cryo-EM structure of the TTLL7-microtubule complex, which represents the first structure of a microtubule PTM enzyme in complex with its substrate, also reveals additional densities corresponding to the α- and β-C-terminal tails

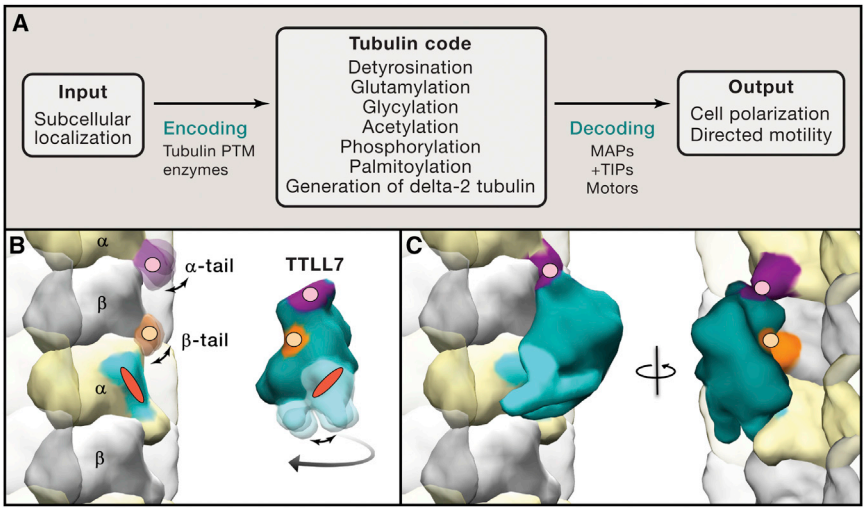

Figure 1. The Tubulin Code and the Interaction of TTLL7 with the Microtubule

(A) Schematic diagram of the tubulin code.

(B and C) Schematic drawing of the TTLL7-microtubule complex before (B) and after (C) binding of TTLL7. α-tubulin, β-tubulin, and TTLL7 are depicted in yellow, gray, and sea green, respectively. The regions involved in the interaction of the α and β tails with TTLL7 are shown in magenta and orange, respectively. The interface between α-tubulin and the c-MTBD domain of TTLL7 is depicted in cyan. In addition, all interfaces are highlighted with colored ovals. Note the flexibility (indicated by double arrows) of the α- and β-C-terminal tails of tubulin and the c-MTBD domain of TTLL7 prior to binding and the obvious surface complementarity between the microtubule and TTLL7.

of tubulin, which are normally flexible and not resolved in cryo-EM structures of microtubules (Figure 1C). The negatively charged β-C-terminal tail, which is the site for posttranslational glutamylation, clearly localizes to the active site of TTLL7 and interacts with positively charged residues (Arg106 and Arg352) at the entrance of the binding groove.

Different conserved domains of the enzyme interact with three different positions on the microtubule surface to allow for the proper localization of its active site (Figures 1B and 1C). TTLL7 fits like a 3D puzzle piece exactly onto the surface of the microtubule. Interestingly, besides this accurate fit, binding of TTLL7 also involves a bilateral induced fit mechanism, with the c-MTBD domain of TTLL7 and the α- and β-C-terminal tails of tubulin being ordered once the complex is formed. In addition, the active site of TTLL7 appears to clamp around the β tail of tubulin. Using single-molecule TIRF microscopy, the authors show that both tubulin tails contribute significantly to the binding energy and that a complete removal of the tails leads to loss of TTLL7 binding.

The addition of up to eight glutamates does not significantly change the interaction of TTLL7 with microtubules, and only longer polyglutamate chains result in "diffusion" of TTLL7 along the microtubule since the binding of TTLL7 is probably sterically hindered.

The next frontier is to reveal structural mechanisms of specifically recognizing and modifying microtubules by other tubulin PTM enzymes to better understand how the tubulin code is established. High-resolution structures of PTM enzymes in complex with the microtubule (especially with regard to the recent resolution revolution in electron cryomicroscopy [Kühlbrandt, 2014]) will help significantly to decipher the code. A final and likely most challenging step will be to shed light on how the code controls microtubule function.

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