Cytoskeleton: **Functions for tubulin modifications at last**

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New studies show that three types of evolutionarily conserved post translational tubulin modification, polyglutamylation, polyglycylation and detyrosination, play important roles *in vivo*. These modifications appear to act by modulating the binding of molecular motors to the external surface of microtubules.

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In most eukaryotes, tubulin proteins, the building blocks of microtubules, are subjected to several types of evolutionarily conserved post-translational modification, including detyrosination [1], acetylation [2], phosphorylation [3], palmitoylation [4], polyglutamylation [5] and polyglycylation [6]. Despite systematic characterization of these tubulin post-translational modifications in diverse organisms, until recently the biological significance of these cellular mechanisms remained unknown. A recent paper by Gaertig and colleagues [7] reports that one of the post-translational modifications, tubulin polyglycylation, is essential *in vivo* and its presence is required for normal ciliary motility in the ciliate *Tetrahymena*.

For most of the past twenty years, the study of tubulin post-translational modifications has been a cottage industry based on antibodies that easily enabled the determination of which microtubules in a cell were modified. It was shown that all the α-tubulin of the ciliary axoneme — with its characteristic arrangement of '9 + 2' microtubule protofilaments — is acetylated on a single lysine residue, and many labs showed that the more stable cytoplasmic microtubules of a variety of cells are the ones that are acetylated. It became clear, however, that this microtubule stability is not caused by the acetylation [8]. Rather, microtubules are acetylated after assembly, and acetylation appears to be a marker for how long the microtubules have been available to serve as a substrate for the tubulin acetyltransferase.

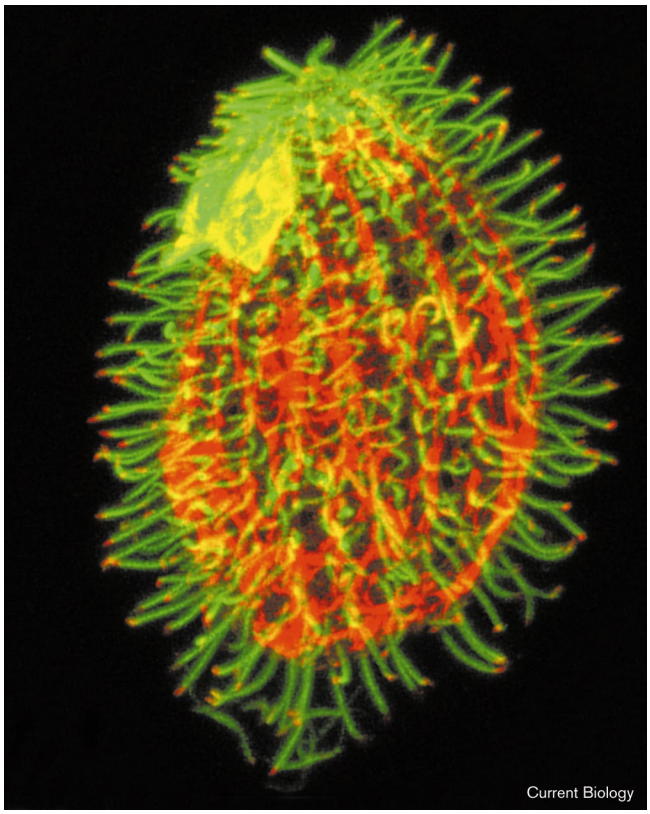
In many eukaryotes, a conserved carboxy-terminal tyrosine of α-tubulin is removed post-translationally. This detyrosination, like acetylation, is found on more stable microtubules and also appears to be the result, rather than the cause, of the increased longevity of the detyrosinated microtubules. For example, Borisy and colleagues [9] showed that conversion of microtubules of mammalian cells into their detyrosinated form by injection of antibodies directed against the enzyme tubulin tyrosine ligase, which converts detyrosinated α-tubulin back to its unmodified form, did not change the stability of microtubules. In an especially clear demonstration of the relationship between tubulin detyrosination and microtubule age, Gull and collaborators [10] used gold labeled antibodies and electron microscopy to show that newly assembled plus ends of trypanosome microtubules were unmodified, whereas segments of the same microtubules that had been assembled earlier were detyrosinated.

Like most cottage industries, the use of antibodies to characterize the modification state of microtubules has been largely superceded by new (molecular) technologies. In two studies, DNA-mediated transformation was used to show that ciliated or flagellated protists can use a non-acetylatable α-tubulin with no observable ill effects on the microtubules or the organisms [11,12]. In one of these studies, α-tubulin was completely replaced by a non-acetylatable isoform in all microtubules of the ciliate *Tetrahymena*. Although *Tetrahymena* maintains high levels of acetylation in ciliary and cell body microtubules, mutants which had no acetylated tubulin exhibited no detectable abnormalities [11]. Clearly, either there are alternative pathways to supplant the function of tubulin acetylation or it is not very important.

Recent studies, however, have provided the first direct evidence that at least some of the tubulin post-translational modifications have important functions. Xia *et al.* [7] have now shown that, in the ciliate *Tetrahymena*, there is an essential function for tubulin polyglycylation. *Tetrahymena* is a superb tool for such studies because it has a complex microtubule architecture comparable to that of the diverse cells of a multicellular organism, but its highly modified microtubules (Figure 1) are assembled from a single, major α/β-tubulin dimer that can be easily mutated by gene replacement [13]. It had been shown earlier by Eddé *et al.* [5] and by Redeker *et al.* [6] that microtubules can be modified by polyglutamylation and polyglycylation of glutamic acid residues in the carboxy termini of both α-tubulin and β-tubulin.

By extensive analysis of site-directed mutations of tubulin genes expressed *in vivo*, Xia *et al.* [7] showed that the carboxyl termini of both α-tubulin and β-tubulin of *Tetrahymena* have multiple sites of polyglycylation. Interestingly, multiple substitutions could completely eliminate α-tubulin glycylation with no observable phenotypic effect. Not so with the higher level of β-tubulin glycylation,

Figure 1



A *Tetrahymena* cell doubly labeled by general anti-tubulin antibodies C140 (red) [23] and anti-polyglycylated tubulin antibodies AXO 49 (green) [24]. The polyglycylated tubulins are seen to be restricted to oral and locomotory cilia, with the exception of the distal tips of cilia, where the addition of new ciliary subunits is known to take place (image courtesy Jacek Gaertig and Marie-Helene Bré).

which was found to be required for cells to survive. Xia *et al.* [7] also showed that all sites of polyglycylation on β-tubulin can be eliminated if, at the same time, the carboxyl terminus of α-tubulin is replaced by the carboxyl terminus of β-tubulin carrying a normal set of polyglycylation sites. This surprising result suggests that a threshold level of polyglycylation on microtubules, and not specific sites of modifications on each tubulin subunit, is required for viability. By modifying some, but not all, of the β-tubulin glycylation sites, these workers were able to make 'hypomorphs' with just enough tubulin glycylation to permit cells to survive so that phenotypic observations could be made. The cilia of these cells moved poorly, suggesting that the β-tubulin glycylation was affecting the function of the ciliary dynein motor.

The slow motility of polyglycylation mutants observed by Xia *et al.* [7] is especially intriguing in the light of reports suggesting that detyrosination also affects the interaction of microtubules with the motor protein, kinesin. Gurland and Gundersen [14] showed in cultured mammalian cells, that vimentin intermediate filaments preferentially co-align with the detyrosinated microtubules. Recently, Gundersen and colleagues [15] were able to disrupt this co-localization of microtubules and intermediate filaments by injecting detyrosinated α-tubulin molecules that had been chemically altered to prevent their polymerization. Moreover, kinesin motors appear to be responsible for distributing intermediate filaments along detyrosinated microtubules, as injection of either anti-kinesin [16] or anti-detyrosinated tubulin antibodies [14] led to a collapse of the intermediate filament network. Consistent with these *in vivo* results, a non-polymerizable detyrosinated α-tubulin strongly inhibited binding of kinesin to microtubules *in vitro* [15].

In studies on polyglutamylation, Bornens and colleagues [17] reported the spectacular result that injecting monoclonal antibodies specific to the polyglutamylated isoforms of tubulin into HeLa cells caused the disappearance of centrioles, an organelle known to have the most stable of any of the cell's microtubules. Gagnon *et al.* [18] had shown earlier that this same antibody interfered with the motility of ATP-reactivated flagellar axonemes, probably by affecting the ability of dynein to bind to the B-subfiber of the outer doublet microtubule.

Taken together, these results suggest that the post-translational tubulin modifications, detyrosination, glycylation and glutamylation, affect binding of motors to the microtubule wall. All these modifications occur on the negatively charged tubulin carboxyl terminus, which has recently been shown to be important for the processive movement of single-headed kinesin along microtubules [19]. It will be interesting to see if molecular motor interactions with microtubules are also affected by acetylation, which occurs near the amino terminus of α-tubulin. It also should soon be possible to determine the structural consequences of post-translational modifications on microtubule–motor interactions, as a great deal has been learned recently about the high-resolution structure of both motor proteins and microtubules [20–22]. It is here that the approaches developed by Gaertig, Gorovsky and their colleagues should add a great deal, as it is possible to construct all manner of microtubules assembled entirely from mutated α-tubulins and β-tubulins expressed by genes that have been transformed into *Tetrahymena*.

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