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Polyglutamylation: The Glue that makes Microtubules Sticky

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Abstract

The beating of cilia and flagella depends on microtubule sliding generated by dynein motors, but the interaction of these motors with their tracks is still under investigation. New evidence suggests that some dynein motors won't work unless their track has been modified by a specific post-translational modification.

Discussion

Tubulins can undergo a dizzying number of post-translational modifications, including phosphorylation, acetylation, glutamylation, glycylation, and tyrosination, but determining their significance has not been a simple task. For example, all of these modifications have been seen in cilia, but some appear non-essential while others are thought to play poorly-understood roles in ciliary assembly [1]. A recent key to finding out what they really do has come from discovery of the tubulin tyrosine ligase-like (TTLL) enzyme family, whose members are responsible for addition of tyrosine, polyglycine, or polyglutamine to residues near the tubulin C-terminus. Among these TTLL homologs are polyglutamylases that modify ciliary tubulins [2], and two papers in this issue of CB by Kubo et al. [3] and Surayavanti et al. [4] describe the effects of mutations that knock out these cilia-specific polyglutamylases.

Tubulin polyglutamylation adds strings of glutamines onto the gamma carboxyl group of any of several glutamine residues near the C-terminus of either alpha or beta tubulin [2]. This generates multiple negative charges in regions of the tubulin dimer that face the microtubule surface, and therefore could regulate the interaction of other proteins with microtubules, including both microtubule-associated proteins (MAPs) that alter microtubule stability and function, and molecular motors that use microtubules for tracks. In the nervous system, polyglutamylation has been linked to differential binding of MAPs such as MAP2, which may in turn modulate neurite outgrowth [5,6], and a mutation that alters patterns of alpha tubulin polyglutamylation in mice, ROSA22, selectively blocks neuronal vesicle transport by KIF1A kinesin [7]. In vitro, removing tubulin C-termini by subtilisin digestion reduces the processivity of both cytoplasmic dynein and kinesin motors [8], forging another potential link between polyglutamylation and motor function.

Early evidence on the function of tubulin polyglycylation and polyglutamylation in cilia came from two approaches, mutations to the tubulin residues that get modified, and use of modification-specific antibodies [1]. Motility of ciliary axonemes reactivated in vitro can be inhibited by antibodies that bind to polyglutamylated tubulins, but not by antibodies that bind polyglycylated tubulins or unmodified tubulins [9], suggesting that polyglutamylation plays an important role in motility, and additional studies showed that long glutamyl side chains occur predominantly on outer doublet B-tubules [10]. However, in the ciliate *Tetrahymena*, mutation of residues near the C-terminus of alpha or beta tubulin that can be polyglycylated or polyglutamylated indicated that modification of beta tubulin is essential not simply for motility, but for normal ciliary assembly [11]. A second link between

polyglutamylation and ciliary assembly came from mutations in homologs of a TPR protein, Dyf1, which appears to regulate the activity of some polyglutamylases. These mutations disrupt normal levels of tubulin glutamylation and cause ciliary assembly defects in fish [12], ciliates [13], trypanosomes [14] and worms [12,15]. One common feature of these Dyf1 defects is formation of defective cilia with incomplete B-tubules, which suggests a possible link between the motility defects caused by antibodies to polyglutamylated tubulin and the assembly defects seen with tubulin or *dyf1* mutations. If B-tubules are highly modified by polyglutamylation, then this modification might participate in the dual function of stabilizing doublet microtubules and providing a surface for processive dynein motor activity.

New evidence for the significance of polyglycylation and polyglutamylation in axonemal assembly and motility now comes from studies of the TTLL enzymes themselves. Knockdown of TTLL3 in *Tetrahymena* or zebrafish disrupts polyglycylation and specifically alters ciliary assembly [16], whereas disruption of TTLL6 homologs in *Tetrahymena* or TTLL9 homologs in *Chlamydomonas*, as reported in this issue of CB, specifically disrupt axonemal polyglutamylation, and alter motility without affecting overall microtubule structure or ciliary assembly. In both organisms, cells defective for polyglutamylation swim slowly, due in large part to reductions in ciliary beat frequency.

The question then becomes one of exactly why a change in tubulin modification changes the motility of these organelles. One obvious answer would be that axonemal dyneins depend on modified tubulin C-terminal sequences to use microtubules as tracks and act as effective motors. Alternatively, lack of polyglutamylation could block assembly of a specific dynein or a dynein-associated regulatory protein. In fruit flies, mutations near the beta tubulin N-terminus can block attachment of outer dynein arms [17]. In these new studies, however, EM analysis shows a general conservation of all normal structural components; loss of polyglutamylation does not result in disruption of B-tubules or central pair tubules, loss of outer dynein arms or radial spokes, or a change in overall axonemal length. Because there are so many *inner* row dyneins in axonemes it can be quite challenging to tell if any are missing by EM alone, so Kubo et al. used high resolution biochemical separation and SDS-PAGE to show that all of the major classes of inner row dyneins are retained at normal levels in their *tpg1* mutant axonemes.

A further clue to the role of polyglutamylation in motility comes from the location of tubulin subunits that sport this modification. Both papers show by immunofluorescence and immuno-EM that elongated polyglutamine chains are concentrated on outer doublet microtubules, not central pair microtubules, and more precisely on B-tubules, not Atubules. Axonemal B-tubules have far fewer MAPs than do central pair or doublet Atubules, and B-tubule MAPs appear to have extensive interactions with the inside of the microtubule [18], and therefore are not likely to interact with the C-terminal regions of tubulin that are modified by TTLLs. What does interact with this B-tubule surface? The microtubule-binding stalk of the dynein motor domain! The hypothesis is therefore that one or more dyneins cannot use the B-tubule as a motility track unless either beta tubulin (*Tetrahymena*) or alpha tubulin (*Chlamydomonas*) has been polyglutamylated.

Which dyneins need polyglutamylated tubulin for their normal force generation? Both studies show that the ciliary motility phenotype becomes more severe when outer row dyneins are missing, indicating that the remaining inner row dyneins are the ones particularly sensitive to this modification. The last eukaryotic common ancestor had already evolved a two-headed outer row dynein, a two-headed inner row dynein, and three single-headed inner row dyneins, and the single-headed dyneins have since undergone great diversification in each branch of eukaryotes [19]. Here the power of the extensive set of

dynein assembly mutations in *Chlamydomonas* provides at least a partial answer, and suggests that it is one or more of these single-headed inner row dyneins that needs polyglutamylated tubulin for normal force generation.

Remarkably, by measuring doublet microtubule sliding rates in protease-treated axonemes, both papers show that microtubule sliding velocities actually *increase* in these polyglutamylation mutants, when outer row dyneins are also absent. How can sliding rates increase, when ciliary beating is reduced by the same mutations? Sliding measures the activity of dyneins as linear motors, under no-load conditions. In contrast, bending requires dyneins to work against the combined loads of intrinsic sliding (shear) resistance and bending resistance of the microtubules, which together constitute axoneme stiffness, and external hydrodynamic (viscous) resistance of the medium. The interpretation here is that one or more dynein cannot get a good grip on the non-polyglutamylated B-tubule surface, and therefore cannot generate its normal level of force under load. Monomeric inner row dynein c, for example, appears to be particularly important to maintain force generation under increased viscous load [20]. However, when sliding occurs under no-load conditions, then these same dyneins may act very differently. In wild type axonemes, where they can hold onto the MT surface, they may actually slow down the sliding that can be generated by other dyneins (such as outer row dynein) which may be intrinsically faster motors. In mutants that can’t make polyglutamylated tubulin, these dyneins no longer get in the way because they cannot hold onto the slippery non-polyglutamylated MT surface, and sliding is faster than expected. Use of this tubulin post-translational modification in axonemes therefore most likely co-evolved with axonemal dyneins as a mechanism to fine-tune motility, improving the swimming skill of our single-celled ancestors as they motored through thick and thin.

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