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Tubulin Superfamily: Giving Birth to Triplets

Dispatch

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Two new studies show that e tubulin is required for centriole/basal body duplication in both *Chlamydomonas* and *Paramecium*, adding to the list of new tubulin family members specifically involved in forming the centriole triplet microtubules. The function of these triplets, and the precise role of e tubulin in triplet formation, remains unclear.

Centrioles are cylinders containing nine triplet microtubules. During mitosis, centrioles recruit microtubule-nucleating material to form microtubule organizing centers (MTOCs) called centrosomes, which eventually become the spindle poles. During interphase, centrioles can give rise to cilia and flagella, at which point the centrioles are referred to as basal bodies. Centrioles are absolutely essential for the assembly of cilia and flagella, but they are dispensable for spindle formation in certain cell types, for example in higher plants. It thus seems likely that centrioles first evolved to allow formation of cilia/flagella (we use the terms interchangeably here), with obvious adaptive benefits, and that *subsequently* they were co-opted to perform additional functions during cell division. As flagella are made of a nine-fold array of microtubule doublets, templated by the triplets of the basal body, this almost certainly explains why centrioles are microtubule-based structures. But how are the microtubule triplets formed?

The microtubule triplets are made of α and β tubulin, which in centrioles and flagellar axonemes are subject to extensive post-translational modification, particularly acetylation and polyglutamylation. Antibodies specific for polyglutamylated tubulin cause centrioles to disappear *in vivo*, suggesting a possible role for this modification in maintaining centriole structure [1]. Centriole microtubules are probably nucleated by γ tubulin, which is found within the centriole at the end containing the 'minus' ends of the microtubules [2], and is required for centriole duplication [3,4]. More recent work has revealed additional tubulin family members (reviewed in [5]), namely δ, η and ζ tubulin, all of which localize to centrioles. As one example, *Chlamydomonas* mutants lacking δ tubulin form centrioles that contain doublet, rather than triplet, microtubules [6].

A pair of recent papers [7,8] have now reported that another new tubulin, ε tubulin, is required for centriole duplication. ε tubulin was first identified molecularly by genome sequence analysis as a tubulin family member localizing to centrioles [9], but in fact the first ε tubulin mutant was obtained decades ago in a *Chlamydomonas* screen for cells that cannot mate because they lack flagella. The flagella-less phenotype of these '*bld2*' mutants was shown to be caused by their having defective centrioles which do not contain microtubule triplets, or even doublets, but which are composed rather of a ring of nine singlet microtubules [10]. A more severe *bld2* allele is lethal, possibly because centriole duplication is completely blocked [11]. It has now been shown that *BLD2* encodes ε tubulin [7]. High-resolution immunofluorescence indicated that ε tubulin localizes in a ring around the centrioles. Similar results were recently obtained using reverse genetics in *Paramecium*: when ε tubulin production was repressed, centriole duplication was blocked [8]. Immuno-electron microscopy in *Paramecium* showed that ε tubulin localizes within and around the centriole barrel at both the proximal and distal ends.

The centriole duplication defect in ε tubulin-deficient cells suggests a role for triplet microtubules in centriole duplication. New centriole assembly does not begin with microtubule polymerization, however, but rather with assembly of an amorphous disc-like structure containing no discernable microtubule structures [12]. This is most dramatically seen during differentiation of ciliated epithelial cells, in which a large spherical structure forms first and later gives rise to multiple microtubule-containing centrioles [13]. At least one component of the amorphous precursor is centrin, a protein that localizes to the site of future centriole assembly long before the microtubule structures begin to form [14,15]. Centrin plays an important role in centriole duplication [15–17] consistent with this early localization. Only after this precursor is formed, do microtubules begin to appear, first as a ring of nine short singlet microtubules, which are subsequently converted into doublets and then into triplets [12]. Presumably γ tubulin is recruited to the centrin-based precursor to nucleate the initial singlet microtubules, after which ε and δ tubulins are required sequentially to form doublets and then triplets.

But are these tubulins really required to build centriole microtubule triplets? A *Chlamydomonas bld2* null mutant that completely lacks ε tubulin could be rescued by an extragenic suppressor mutation *rgn1*, forming centrioles that contain a mixture of singlet, doublet and triplet microtubules [11], implying that the need for ε tubulin can be circumvented. Also, the basal bodies of *Drosophila* sperm contain triplet microtubules even though *Drosophila* does not contain δ or ε tubulin [18]. The function of ε tubulin in making triplets thus remains unclear.

Nor is it clear what function the microtubule triplets play in centriole duplication. *Chlamydomonas* mutants such as *bld2-1* (reduced ε tubulin) or *uni3* (missing δ tubulin) make centrioles containing singlets or doublets instead of triplets, and yet these defective centrioles duplicate at the wild-type rate. Centrioles in nematodes and *Drosophila* embryos contain only singlet microtubules [18,19], but nevertheless duplicate

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efficiently. Triplets are, however, important for making flagella. The singlet centrioles of worms and flies do not template flagella, and when flies need to make flagella during spermiogenesis, the centrioles are converted into a form that contains triplets. Similarly, the *bld2-1* and *uni3 Chlamydomonas* mutants, which have centrioles containing singlets or doublets, also show defects in flagellar assembly [6,10]. Evidently, centriole microtubule triplets are important for making flagella but are not essential for centriole duplication.

However, ε tubulin deficiency can in fact cause centriole duplication defects — as evidenced by the *bld2* null allele and by the *Paramecium* experiments — depending on the cell type and the extent of the ε tubulin deficiency, so these tubulins and the triplets they produce must play some secondary role in centriole assembly, perhaps by stabilizing the centriole. This could account for differences in effect depending on the severity of the mutation: a hypomorphic ε tubulin allele may stabilize centriole microtubules just enough to support centriole assembly, whereas complete loss of function may destabilize the nascent centriole microtubules to the point that centrioles cannot form.

Centriole microtubule triplets, like flagellar axoneme doublets, are extremely stable *in vitro* compared to singlet microtubules and do not fall apart spontaneously, for example after cold or colchicine treatment. *In vivo* pulse-label experiments indicated centriole microtubules turn over slowly, exchanging at most 10% of their tubulin per cell-cycle [20]. Injection of antibodies to glutamylated tubulin [1], as well as removal of γ tubulin [4], cause centrioles to disappear gradually over a period of time equivalent to many cell cycles. Triplet microtubule turnover is thus orders of magnitude slower than turnover in ordinary cytoplasmic microtubules or even in axonemes, implying triplets are significantly more stable than singlet or doublet microtubules.

The increased stability of triplets may explain an interesting correlation between centriole structure and length. Centrioles in the *bld2-1* ε tubulin mutation that has singlets instead of triplets, are much shorter than normal centrioles. Likewise, the singlet containing centrioles of Drosophila embryos and nematodes are much shorter than those seen in other animals whose centrioles contain triplets [18]. Drosophila sperm basal bodies, which have microtubule triplets, are much longer than the singlet-based embryonic centrioles, again suggesting triplets may be stabler than singlets or doublets.

The identification of centriole-specific tubulins is clearly just the starting point for understanding their function. At the same time, we should avoid concentrating exclusively on tubulins when the majority of centriole proteins remain to be discovered.

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