INTRODUCTION

The cytoplasm of most eukaryotic cells is continuously remodeled by hundreds of growing or shrinking microtubules. These polymers not only provide structural architecture to the cytoplasm, but also contribute to several forms of intracellular motility including chromosome segregation during meiosis and mitosis and the distribution and transport of various organelles such as the Golgi and endoplasmic reticulum. In addition, microtubules have specific structural and motile roles in specialized cells including flagellar-mediated locomotion, the specification and maintenance of the asymmetric morphology of neurons, and the transport of organelles and vesicles into the axonal processes of such cells.

One of the intriguing questions is what specifies which microtubules are used for each of these roles? An obvious place to look for such information is within the structure of the basic subunit, the tubulin heterodimer of one α and one β tubulin polypeptide. It is now clear that (with the exception of the simplest eukaryotes) tubulin exists in all cells as a mixture of similar, but not identical, polypeptides. As might be expected, within an individual cell this diversity is generated at two levels: first is the selective transcriptional activation of one or more genes encoding either subunit. Second, there are a variety of post-translational modifications of the primary gene products. We shall focus this review on the extent to which this tubulin diversity contributes to overall microtubule function.

TUBULIN ISOTYPES: REDUNDANCY OR SPECIFICITY OF FUNCTION?

Comparative sequence analysis of vertebrate tubulin genes gave the first look at the heterogeneity of tubulin subunits encoded in vertebrate genomes. This revealed the persistence during evolution of a very highly conserved set of ~6 α and ~6 β tubulin polypeptides. Within either family, individual subunits diverge from each other (both within and across species) at less than 10% of the amino acid positions [for a detailed review, see Sullivan, 1988]. The greatest divergence among the tubulin molecules is localized to a variable domain comprised of the carboxy-terminal 15 residues (see for an example, Fig. 1a). For β tubulin, the observation that these variable domains are nearly absolutely conserved in the subunits utilized in the same cell types of different species led to the identification of five evolutionarily conserved isotypic classes [Sullivan and Cleveland, 1986]. The only exception to this rule is a hematopoetic β tubulin [Murphy et al., 1987; Wang et al., 1986], which is very highly divergent in sequence and which is not conserved between species. The definition of unique tubulin β isotypes and their conserved programs of expression during development provided the initial experimental support for the hypothesis (initially advanced by Stephens [1975] and Fulton and Simpson [1976]) that different tubulin subunits contribute unique functional properties.

For α tubulin, distinct isotypes are also present, although the sequence differences are less striking than for β tubulin [Villasante et al., 1986; Pratt and Cleveland, 1988].
VERSATILITY OF ISOTYPES

Although interspecies conservation of isotypes is consistent with a unique, essential function for one or more isotypes, an alternative hypothesis (advanced most clearly by Raff [1984]) that interspecies conservation of specific isotypes may simply reflect a slow sequence drift after the time of gene duplication could not be discounted. Indeed, in several systems where genetics are available (Drosophila, yeast, Aspergillus), multiple lines of evidence have suggested functional equivalence of different tubulin polypeptides. The first of these demonstrated that in Drosophila one β tubulin is expressed only in the testis where it is multifunctional and used for constructing most classes of microtubules required during spermatogenesis [Kemphues et al., 1982].

In the fission yeast Schizosaccharomyces pombe, as well as in the budding yeast Saccharomyces cerevisiae, two genes encode α tubulin. The encoded polypeptides share only 80% sequence identity and gene disruption analyses have shown that only one of the two genes is essential for viability [Adachi et al., 1986; Shatz et al., 1986]. However, mutations in the essential gene are complemented by increased expression of the non-essential gene. Thus, while it is not clear how many different classes of microtubules yeast really do have, the two α isotypes appear redundant. The odd thing is why then each of these two yeasts (which are only very distantly related) have retained two different α tubulin genes?

A similar situation has been documented in Aspergillus: there are two β tubulin genes whose encoded proteins are only 83% identical [May et al., 1987]. One isoform is used for normal mitotic growth, while the other is expressed exclusively during the events of asexual sporulation (conidiation). But mutants that fail to produce this second β tubulin have no observable phenotype [Weatherbee et al., 1985] and mutations in the essential gene are complemented by forcing expression of the essential gene by fusion cloning [May, 1989]. The conclusion that the subunits are interchangeable and that the conidiation-specific product is redundant seems inescapable, although the nagging doubt remains that the laboratory definition of "essentialness" may obscure some real world selective constraint.

In cells from vertebrates, the preparation of isotype-specific antibodies for α tubulins [Gu et al., 1988] or β tubulins [Lewis et al., 1987; Lopata and Cleveland, 1987] has localized each isoform within cells. At least at the resolution allowed by light microscopy, interphase and mitotic microtubules are copolymers of the available isotypes, a finding consistent with interchangeability of isotypes.

Evidence for Functional Selectivity

Despite the evidence that isotypes may be interchangeable in some instances, there are at least four examples of selective utilization of specific isotypes in specialized cells:

First, in all vertebrate red blood cells (except mammals), the mature cell contains a circumferential ring of microtubules, known as the marginal band. Not only are differences in in vitro assembly properties apparent for the erythroid cell-specific β tubulin that comprises ~80% of marginal band tubulin [Rothwell et al., 1986], but microtubules enriched in this erythroid isotype are more stable to temperature-induced depolymerization [Joshi et al., 1987]. However, the physiological significance of such a distinction remains in question, a doubt encouraged by the finding that, alone among the vertebrate β tubulins, the sequence of erythroid-specific subunits has not been conserved during evolution [Murphy et al., 1987; Wang et al., 1986].

Second, null mutations in the mec7 β tubulin gene of Caenorhabditis elegans not only prevent assembly of the unusual 15 protofilament microtubules found only in touch receptor neurons, but also result in assembly of 11 protofilament microtubules. These 11 protofilament microtubules are not normally found in touch cells and are presumably assembled from the product of another β tubulin gene that by itself cannot assemble 15 protofilament microtubules [Savage et al., 1989].

Third, in rat PC12 cells the five β tubulins expressed during NGF-induced neurite extension are not used randomly; rather, three isotypes (classes I, II, and IV) are preferentially assembled into neurite microtubules, while one (class V) is partially excluded from neurites [Joshi and Cleveland, 1989].
Fourth, using DNA transfection to produce hamster cell lines that stably express a chicken class IV β tubulin polypeptide at a rate two to three times that of endogenous β tubulins, Sisodia et al. [1989] found that the c-IV polypeptides accumulated to <10% of the total β tubulin. Furthermore, when cIV transcription was further elevated transiently, there was a compensatory loss in the cell lines and that the stability of individual isotypes is endogenous class IV isotype so that the level of class IV polypeptides accumulated to <10% of the total nouseous other cellular factors.

The difficulty with each of these observations is that it is not known whether the documented biochemical differences reflect physiologically significant distinctions between isotypes. However, at long last this question has been unequivocally settled through experiments of Hoyle and Raff (1990). These authors have focused on β-tubulin utilization in Drosophila testes. Normally, the β2 gene product (one of 4 β-tubulins in this organism) is the major β-tubulin utilized for construction of most classes of microtubules during spermiogenesis. By introducing into the Drosophila genome an additional gene whose transcriptional control elements were derived from the β2 gene, but whose encoded polypeptide is the wild-type product of one of the other Drosophila genes (β3), these authors have determined that when β3 is co-expressed in the male germ line with β2, at any level, spindles and all classes of cytoplasmic microtubules are assembled and function normally. However, when β3 tubulin exceeds 20% of the total testes β-tubulin, it acts in a dominant way to disrupt normally axoneme assembly. Moreover, when β3 is expressed in testes derived from mutant strains that do not accumulate significant levels of β2, only one cytoplasmic microtubule based process continues (mitochondrial derivative elongation), but meiosis and nuclear reshaping do not occur. These results insist that β2 and β3 have different intrinsic functional capabilities. What remains untested is whether the multi-functional β2 is competent to perform all of the functions of β3, whose functional properties appear more limited.

**USUAL AND UNUSUAL POSTTRANSLATIONAL MODIFICATIONS OF TUBULIN ISOTYPES**

Both α and β tubulins are known to be modified subsequent to their translation. α Tubulins undergo removal, then enzymatic re-addition of a single tyrosine residue at the extreme carboxy-terminus of the molecule [Barra et al., 1973]. Another modification, a reversible addition of an acetyl group to lysine 40 of α tubulin [LeDizet and Piperno, 1987] (initially discovered by L'Hernault and Rosenbaum [1983] during flagellar growth in the Chlamydomonas), has been widely found in various differentiated cell types [Gundersen and Bulinski, 1986]. The precise functional significance of these modifications still remains elusive. Both deetyrosination and acetylation are enriched in the subpopulation of microtubules that is more stable, but it is also clear that neither modification directly causes stabilization [Khawaja et al., 1988]. In fact, the only current evidence that either modification alters an important microtubule property is that the modification takes place!

Modification by phosphorylation is known only for a single β-tubulin isotype [Edde et al., 1981; Gard and Kirschner, 1985]. Indeed, this subunit is the neuron-specific, class III isotype that during neurite extension is modified at a carboxy-terminal serine present only in the class III variable domain [Luduena et al., 1988; see Fig. 1a,b].

A final, unprecedented post-translational modification has only just been discovered [Edde et al., 1990; (D.F. Hunt, J.E. Alexander, J. Shabanowitz, H. Michel, M.K. Lee, S.C. Berlin, T.L. Macdonald, R.J. Sundberg, and A. Frankfurter, personal communication): using mass spectrometry to sequence proteolytic fragments of α or β tubulins, this alteration was determined to be addition of one to five glutamic acid residues to the γ carboxyl group of a unique glutamic acid residue that lies in the carboxy-terminal variable region of α or β tubulin. For α, this polyglutamylation occurs at residue 445 and presumably occurs on most (or all) neuronal α tubulins. For β, only the neuron-specific class III isotype has been shown to be glutamylated (on glu residue 438—see Fig. 1b). This is also the only tubulin isotype that is a known substrate for phosphorylation (see above). Whether other β tubulins are also glutamylated is not settled, but as much as 40–50% of the total α tubulin is glutamylated in chick embryonic neurons. Discovery of this modification (which is found only on brain tubulin [Edde et al., 1990]) accounts for the long-known heterogeneity in brain tubulins and requires a change in the way we envision neuronal microtubules. These polyglutamyl residues must extend out from the surface of each modified microtubule resulting in highly negatively charged "bottle-brush" structures. It is not yet known whether poly-glutamylated microtubules are present in axons, dendrites, and/or neuronal cell somas. Unknown too is what influence this modification has on microtubule assembly/disassembly or on binding of microtubule-associated proteins.

**THE CASE FOR COEXISTENCE OF BIOCHEMICALLY DIFFERENTIATED MICRO TUBULES WITHIN THE SAME CELL**

How might tubulin heterogeneity yield important in vivo differences between microtubules? In certain differ-
entiated cell types (e.g., neurons), there is clear evidence to indicate coexistence of microtubules that differ with respect to their biochemical properties. Two-dimensional reconstruction of serial thin sections has revealed that discrete segments of long neuritic microtubules are refractile to cold-induced depolymerization [Joshi et al., 1986]. Another example of the presence of a differentiated subset of microtubules in the squid giant axons is provided by Arai and Matsumoto [1988] who showed that immunologically distinguishable β tubulin isoforms were differentially distributed between the peripheral and central axoplasm. Finally, there is ultrastructural evidence that in lobster axons only a subpopulation (25% of the axonal microtubules) of central microtubules provide the substrate for vesicle transport [Miller et al., 1986], indicating some biochemical differences among axonal microtubules. The mechanism that generates such biochemically differentiated microtubules in the same intracellular milieu is unknown, but an obvious possibility is through microtubules enriched in one or more isoforms and/or post-translational modifications.

A NEW MEMBER OF THE TUBULIN FAMILY—GAMMA TUBULIN

A genetic approach has identified a further complication in tubulin heterogeneity. Beginning with a temperature sensitive β tubulin mutation in Aspergillus nidulans, the mipA gene was isolated by its ability (when expressed at increased levels) to suppress growth arrest at the non-permissive temperature. Sequence of this gene revealed not the anticipated microtubule associated protein, but rather a new tubulin that is neither α or β, but about equally similar to both [Oakley and Oakley, 1989]. This tubulin, named γ, is an essential gene and disruption of it leads to microtubule-less mitotic arrest [Oakley et al., 1990]. Antibody localization reveals a striking enrichment at the spindle poles, suggesting that the major role of γ tubulin is in microtubule nucleation. γ Tubulin is not restricted to Aspergillus and a Drosophila homologue has already been isolated [Zheng and Oakley, 1989].

CONCLUSIONS

It is now clear that the original multi-tubulin hypothesis that individual tubulin isoforms would be utilized to assemble microtubules of different functions is incorrect. This is most clearly demonstrated in lower eukaryotes where a single isotype is sufficient for all essential microtubules and in Drosophila where a single gene product is capable of constructing multiple classes of microtubules. On the other hand, experiments with molecular genetics in Drosophila have proven that β-tubulin isoforms are not functionally equivalent in that organism. In vertebrates, biochemical distinctions between isoforms have also been documented and the potential that a specific isotype or post-translationally modified form (such as the neuron specific-glutamylation of class III β tubulin) has a unique in vivo function remains a real possibility.

REFERENCES


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