Microtubule MAPping

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Fifteen years have passed since microtubule-associated proteins (MAPs) were first identified as proteins that copurify with tubulin during repeated cycles of assembly. Initial interest focused on the ability of neuronal MAPs to stimulate microtubule nucleation and elongation from purified tubulin in vitro. These properties were always unsatisfying, not only because an alarming list of other agents could supplant the assembly-inducing properties, but also because the restricted focus on assembly ignored other potential MAP functions. Recently, a flurry of reports, primarily using molecular genetics, has uncovered unexpected properties of MAPs.

The original MAPs were isolated from mammalian neurons and named according to the three major size classes of polypeptides: MAP1 (>250 kd), MAP2 (~200 kd), and tau (35–65 kd). MAP1 is a bit of a misnomer in that the size class to which it refers contains at least three polypeptides (MAP1A, MAP1B, and MAP1C) that are probably unrelated. Ironically, although the MAP1 components are the least studied, MAP1C is the only neuronal MAP with an accepted function—it is the long-anticipated cytoplasmic dynein (Paschal and Vallee, 1987) that powers transport along microtubules of components in a direction from the nerve tip back toward the cell body. MAP1C is not restricted to neurons, however, and probably serves similar (but as yet undocumented) transport functions in other cell events, possibly during mitosis.

Interest in tau was renewed when it was recognized by several groups to be a major component of the abnormal, intracellular tangles of filaments that accumulate in the brains of patients with Alzheimer's disease. Distressingly heterogeneous in size, the various tases were initially feared to be proteolytic products of the larger MAPs. This is not the case; rather, a single tau gene produces multiple polypeptides through alternative RNA splicing. Five of the 14 exons of the >63 kb bovine tau gene may be included or deleted during splicing, and three different carboxyl termini utilized (Himmler, 1989), resulting in a minimum of 64 potential tau polypeptide products.

The determination of the primary structures of both tau (Lee et al., 1988) and MAP2 (Lewis et al., 1988) revealed a common feature: both proteins carry a similar set of three (sometimes four) imperfect, 18 amino acid repeats that comprise a portion, but probably not the entirety, of the tubulin binding domain. (The schematic illustrates the salient features of these two MAPs.) An adrenal MAP (~190 kd) also carries a similar microtubule binding domain (Aizawa et al., 1989). Although these findings raised the possibility that this repeat is a general feature of microtubule binding proteins, no such domain is present on kinesin (Yang et al., 1989), a more recently discovered MAP that moves particles along microtubules in the direction opposite to that of MAP1C. The sequence of the 2464 amino acid MAP1B polypeptide (also known as MAP1.2, MAP1(X), and MAP5) has revealed a basic domain containing multiple copies of the short motif KKEE or KKEE (Nobel et al., 1989). Assembly experiments revealed that this repeat element represents the tubulin binding domain of MAP1B, but it too is unrelated to that of tau and MAP2.

Immunolocalization of MAP2 and tau gave the first clue as to what properties they might contribute. Although they are coexpressed within most neurons, they localize to separate subcellular compartments. Tau is restricted to axons, the thin tubes that constitute the conducting unit of the neuron. MAP2 is found largely in dendrites, the arborized extensions of the cell body that serve as the neuron's chief signal receptor apparatus. How this segregation is achieved is unsettled, particularly because these MAPs share a common tubulin binding domain. Microinjection of biotinylated MAP2 into primary cultures of neurons has revealed that MAP2 can be transported into axons as well as dendrites. The odd thing is that in axons MAP2 barely binds to microtubules and is rapidly degraded (Hirokawa and Okabe, 1989).

What do tau and MAP2 do? This has been addressed in two ways: first, microinjection of tau into fibroblasts (which do not express their own tau gene) clearly demonstrated that tau both induced additional tubulin assembly and stabilized microtubules against depolymerization without an obvious change in filament organization (Drubin and Kirschner, 1986). Furthermore, in transfected fibroblast lines that express a single tau cDNA either transiently (Kanai et al., 1989; Lewis et al., 1989) or stably (Kanai et al., 1989), tau accumulation led to a dramatic reorganization of microtubules into bundles and an increase in total tubulin content. Equally striking bundling was observed when MAP2 was expressed by transfection (Lewis et al., 1989).

Insight into the mechanism of bundling came from the analysis of a series of truncated MAP2 and tau products (Lewis et al., 1989). While MAP2 has long been known to project from the surface of the microtubule, it is not this long amino-terminal domain that is responsible for cross-bridging. Rather, the important feature is a short, hydrophobic a-helical domain that resides at the carboxyl terminus of the short arm that lies beyond the tubulin binding repeats (see figure). MAP2 dimerizes through this domain...
and thus cross-links adjacent microtubules. This interaction is homologous to, and can be functionally replaced by, an authentic leucine zipper from the yeast transcription factor GCN4. Even MAP2C, an embryonic form of MAP2 that lacks 1324 amino acids through an alternative splice (Papandrikopoulou et al., 1989), retains cross-bridging activity (Lewis et al., 1989).

Of the dozen or so tau isoforms now cloned, all but one terminate with a hydrophobic zipper domain similar to that of MAP2. Expression of these isoforms induces bundling, whereas the one exception, which contains an additional 25 amino acids as a consequence of an alternative 5' splice site in exon 13, does not cross-link microtubules. Presumably this extension disrupts the ability of the zipper domains to dimerize. While there is no truly satisfying explanation of why bundling is not observed using micro-injection, it could be due to too little (or too much) tau injected or the specific isoforms present in the purified tau. In any event, it seems certain that some tau and MAP2 isoforms can cross-link microtubules.

The in vivo implications for tau and MAP2 cross-linking are far-reaching. First, it would be astonishing (although as yet untested) if the well-documented microtubule instability (reviewed in Kirschner and Mitchison, 1986) were not suppressed in cells expressing tau or MAP2 at reasonable levels. Second, both MAP2 and tau yield intermicrotubule cross-bridges about 20 nm in length, similar to the closest-spaced microtubules in neurons. The average microtubule spacing in both dendrites and axons is greater than this, but this could be a consequence of high levels of expression during transfection and a consequent tightening of microtubules within the bundle. Further, because microtubules in axons are all oriented with their plus ends distal to the cell body whereas filament polarity is random in dendrites, major features of neuronal morphogenesis may be determined by the ability of (axonal) tau to bundle only microtubules oriented with the same polarity, while (dendritic) MAP2 causes antiparallel filament bundling.

Bundling of a qualitatively different sort has been found for a newly identified MAP named dynamin. This 100 kd polypeptide was identified by Shpetner and Vallee (1989) as one of three polypeptides that bind to microtubules in a nucleotide-dependent manner, the other two being the translocators MAP1C and kinesin. Released from microtubules in the presence of GTP and AMPPNP, purified dynamin was shown to induce the formation of hexagonally packed bundles of microtubules spaced 13 nm apart. The binding is extraordinarily cooperative, yielding portions of a microtubule covered with dynamin, while adjacent domains are completely unbound. In addition, in the presence of ATP, the bundies fragment and elongate, indicating dynamin-induced sliding between microtubules. Dynamin thus joins MAP1C and kinesin as known mechanical enzymes capable of exerting force on a microtubule. The in vivo role of dynamin is still obscure, particularly because the close-packed bundling seen in vitro is not observed in neurons. However, as in the case of MAP1C and kinesin, dynamin is probably not restricted to neurons, and it is plausible that it (or a relative) contributes to mitotic spindle organization and movement, particularly during microtubule sliding at anaphase B (when the pole-to-pole distance increases).

References


