Microtubules and microtubule-associated proteins

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Microtubule research is becoming increasingly diverse, reflecting the many isoforms and modifications of tubulin and the many proteins with which microtubules interact. Recent advances are particularly visible in four areas: microtubule motor proteins (their structures, stepping modes, and forces); microtubule nucleation (the roles of centrosomes and γ-tubulin); tubulin folding (mediated by cytoplasmic chaperones); and the expanding list of microtubule-associated proteins, knowledge of their phosphorylation states, and information on their effects on microtubule dynamics.

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Introduction

Microtubules represent one of the fiber systems of the eukaryotic cytoskeleton. They are essential for a wide variety of cellular functions, notably cell motility, transport, cell shape and polarity, and mitosis. Microtubules consist of a core cylinder built from heterodimers of α- and β-tubulin motoromers. Three main classes of proteins interact with tubulin. The 'microtubule-associated proteins', or MAPs, are sometimes called more specifically 'structural' MAPs because they bind to, stabilize and promote the assembly of microtubules, and because they can be copurified with tubulin through several cycles of microtubule assembly and disassembly. Representatives are MAP1a and 1b, MAP2a, 2b, and 2c, MAP4, tau protein, 205 kDa MAP, and isoforms of these proteins that are often generated by alternative splicing.

Another broad class comprises the motor proteins, so called because they generate movement along microtubules using the chemical energy of ATP hydrolysis. Representatives are kinesin (a motor moving towards the distal, or plus, end of microtubules), dynein (a rearward motor, also involved in the bending of cilia and flagella), and their many relatives. The motors can bind tightly to microtubules, for example in the absence of nucleotides, but they do not copurify through cycles of assembly and disassembly.

A third and more heterogeneous class includes proteins that are not normally called MAPs but are often found associated with microtubules and may even copurify with them. Examples are glycolytic enzymes (e.g. GAPDH and aldolase), kinases (e.g. protein kinase A, GSK-3 and c-mos), proteins involved in biosynthesis (elongation factor EF-1α and even entire ribosomes), proteins linking up to membrane receptors (dynamin and G proteins), and ribonucleoproteins carrying mRNA.

These interactions are not always well defined, but they point to the cytoskeleton as a (transient) anchor for many cytoplasmic proteins.

The fields of microtubules, MAPs, and motors have expanded so much that they have become the subject of separate reviews. For example, microtubule structure, assembly and regulation have been reviewed recently [1-4], as have MAPs [5,6] and motors [7-9]. General surveys of microtubules and related proteins are given in [10,11].

This review covers selected articles concerning microtubules, MAPs and motor proteins published in the last eighteen months.

Structural aspects of microtubules revealed by motors

The information on microtubule structure we have today is derived mostly from electron microscopy combined with image reconstruction, X-ray scattering, and video microscopy; information on tubulin structure is indirect and derived mostly from biochemical experiments (binding sites of nucleotides or drugs, cross-linking studies, and so on). Novel insights on the microtubule lattice have recently come from studies of the interaction between microtubules and kinesin.

The head domain of kinesin binds to β-tubulin with a stoichiometry of one kinesin head per tubulin heterodimer, generating an axial periodicity of 8 nm (the height of the dimer) and a 'B'-lattice in which adjacent protofilaments are staggered by about 0.9 nm [12*-14*]. The dimer polarity is such that α-tubulin points to the plus (fast-growing) end of the microtubule and β-tubulin points towards the minus (slow-growing) end (see Fig. 1). This implies that the plus end has a crown of α-tubulin subunits and the minus end terminates with β-tubulin [14*]. This polarity enables the β-subunits to interact with γ-tubulin, which is a key component of microtubule-organizing centers (reviewed in [3]). As the exchangeable GTP-binding site is

Abbreviations

GAPDH—glyceraldehyde-3-phosphate dehydrogenase; GSK-3—glycogen synthase kinase-3; MAP—microtubule-associated protein.
on β-tubulin and the microtubule-bound GTP is at the plus end [15°], another consequence of this arrangement of the microtubule is that the 'GTP-cap' sits on the plus end in an inverted fashion so that the last β-tubulin–GTP is buried by a terminal layer of α-tubulin (see Fig. 1).

Kinesin moves along the microtubule in 8 nm intervals and produces forces in the pN range [16°-20°]. The direction of movement is parallel to the tracks of protofilaments [21], and the step size matches the separation of the β-tubulin 'stepping stones'. As kinesin works as a dimer in vivo and shows alternating head catalysis (using each head of the dimer alternately to hydrolyze ATP) [22°], one could integrate the structural and kinetic data by assuming that a kinesin dimer would 'walk' or 'hop' along one or two adjacent protofilaments. (Note that if the center of the kinesin molecule advances by 8 nm, this may imply 16 nm steps for each head.) It also seems that kinesin prefers rigid stepping stones over wobbly ones: microtubules are fairly stiff structures, with persistence lengths (a measure of their straightness) in the mm range [23°-25°], but it is possible to make them even stiffer using slowly hydrolyzable GTP analogues such as GMP-PCP, in which case the movement of kinesin becomes 30% faster [26].

The microtubule–motor interaction is surprisingly versatile: there are variants of kinesin such as Drosophila ncdr or Saccharomyces cerevisiae Kar3 that can walk in the opposite direction (towards the minus end), and this property is inherent to the head domains [27°,28°,29°]. The motor can twist its neck in order to attach to microtubules in the proper direction [30°], and may even cause rotation of microtubules instead of translation, as is suggested by the presence of kinesin-like proteins on the C2 central microtubule in flagella in Chlamydomonas [31°]. Finally, CENP-E, a kinesin-like protein that migrates from kinetochores to the midzone of mitotic spindles, cross-links the antiparallel microtubules and presumably controls their rigidity or gliding during anaphase [32°].

Tubulin structure and interactions

Drugs that target tubulin

Tubulin has many applications as a target of drugs (e.g. anti-cancer drugs) and therefore is a workhorse for certain drug screening tests. The different modes of action and binding sites on tubulin of various drugs continue to be explored (see reviews [33°,34°]). Colchicine and vinblastine are examples of drugs that destabilize microtubules and thus disrupt mitosis; however, their effect can be felt even at drug concentrations too low to cause net microtubule disassembly. This can be attributed to a pronounced effect of the drugs on microtubule dynamic instability parameters [35°-37°], which are closely linked to GTP binding and hydrolysis (see below). The binding site for colchicine has been mapped near the amino terminus of β-tubulin [38°]. By contrast, taxol stabilizes microtubules, thereby also disrupting mitosis. It was shown using photoactivatable taxol derivatives that taxol binds predominantly to the amino-terminal region of β-tubulin [39°,40°]. The stoichiometry of taxol binding is one molecule per tubulin dimer; dimers stabilized by taxol are capable of assembling with bound GDP, without the usual need for GTP hydrolysis [41°].

GTP binding to tubulin

Microtubules contain a non-exchangeable GTP on α-tubulin and an exchangeable one on β-tubulin (nucleotides on the latter site can be exchanged with nucleotides from the solution). Extending earlier work, two recent studies showed that the exchangeable GTP site is in the amino-terminal domain of β-tubulin; an additional ATP-binding site (not involved in microtubule assembly) was found on α-tubulin [42°,43°]. That microtubule stability is closely linked to GTP binding and hydrolysis has now been demonstrated directly by Davis et al. [44°], who made several mutant tubulins in which altered GTPase activity is paralleled by altered dynamic instability. Similarly, Reijo et al. [45°] made systematic mutations of clusters of charged amino acids for alanine in yeast β-tubulin and deduced that three regions are critical for β-tubulin function: near the carboxyl terminus, around residue 330, and around residue 150 (near the glycine cluster that is probably involved in GTP binding).

Modifications of tubulin

An enigmatic feature of tubulin is its heterogeneity. Not only are α- and β-tubulin each encoded by up to six different genes, but the protein can also be modified in several ways: phosphorylation, acetylation, detyrosination, and glutamylation. The complexity of neuronal tubulins is particularly great and appears to increase with differentiation. In other cells, such as avian erythrocytes, the degree of heterogeneity is conspicuously low [46°]. One function of neuronal tubulin modification may be the selective stabilization of certain microtubules [47°]. One of the modifying enzymes, the tubulin tyrosine ligase, has now been sequenced and characterized in detail [48°]. This enzyme restores a carboxy-terminal tyrosine to α-tubulin after it has been cleaved by a tubulin carboxypeptidase. However, α-tubulin can also lose two carboxy-terminal residues (glutamic acid and tyrosine). This Δ2-tubulin can no longer be retyrosinated by the ligase and is enriched in very stable neuronal microtubules [49°]. Another sign of neuronal differentiation is the increasing length of poly-glutamic acid chains attached to glutamic acid residues near the carboxyl terminus of α- and β-tubulin; this makes the already very acidic carboxyl termini even more negatively charged [50°]. The functions of these post-translational modifications have been difficult to ascertain, but Gurland and Gunderensen [51°] found that long-lived stable microtubules (known as 'Glu-microtubules' because they have lost the carboxy-terminal tyrosine from α-tubulin) selectively break down when phosphatases are inhibited by okadaic acid. One interpretation of this observation is that stabilization factors (caps) that are normally associated with stable microtubules can be lost as a result of unchecked phosphorylation.
Regulation of tubulin synthesis

The level of tubulin synthesis is regulated via the soluble tubulin pool, whose size is in turn coupled to the degree of microtubule assembly. In the case of β-tubulin this autoregulation involves the four amino-terminal residues (MRE1 in the single letter code for amino acids) [52]. In an extension of their earlier work, Cleveland and co-workers [52] showed that β-tubulin follows a different pathway (even though both subunits are downregulated by degrading their mRNAs).

Prokaryote homologs of tubulin?

Tubulin is thought to be restricted to eukaryotic cells, but there is a curious homology of the conserved glycine-rich cluster (GGGTGSG) to the bacterial FtsZ protein from Escherichia coli. Like tubulin, this protein is also involved in cell division and binds GTP, and Bramhill and Thompson [53] have now shown that it even polymerizes in a GTP-dependent fashion, suggesting that the similarity is more than accidental, even though the overall homology is very low.

Centrosomes and chaperones

Components of the centrosome

For microtubules to operate properly they must be nucleated at the right time and in the right place. This normally happens at the centrosome, which consist of a pair of centrioles surrounded by pericentriolar material; the latter is where microtubule nucleation actually occurs. The stages and components of centrosome assembly are beginning to be understood. One of the components is γ-tubulin, which exists in the cytoplasm as part of a multiprotein complex (‘gammasome’) with several other proteins. γ-Tubulin is recruited to the centrosome for nucleation of microtubules at their minus ends; nucleation is blocked when γ-tubulin is depleted or inactivated. Other components of the centrosome precursor include α- and β-tubulin, actin, and the molecular chaperone hsp70 [54–57]. Additional proteins are required to assemble the pericentriolar material, such as pericentrin, an elongated 220kDa protein with a coiled-coil stalk and two globular ends, identified using antisera from patients with scleroderma [58]. This general domain structure is shared with other proteins found in microtubule-organizing structures, such as the SP-H antigen/NuMa [59], and variants of tektin, an α-helical protein first found in flagellar axonemes and recently in centrosomes [60]. The microtubule-nucleating function of γ-tubulin in centrosomes appears to be highly conserved as human γ-tubulin functions even when introduced into yeast [61].

Chaperones

Before microtubules can be nucleated, the tubulin must first be folded; this is achieved by another oligomeric complex, the cytoplasmic chaperone complex TCP-1,
also known as TriC. This 800 kDa structure contains several homologs of the heat shock protein Hsp60 (termed CCTα, -β, and so on) arranged in the two-layered ring structure also found in bacterial and mitochondrial chaperones; this complex assists in the folding of relatively few proteins, among them the three tubulins and actin [62-64*]. TCP-1 subunits are the cytosolic equivalents of the bacterial chaperone GroEL and mitochondrial Hsp60. The folding takes place in several steps such that the initial association occurs with ADP bound to the complex, followed by a later release involving ATP hydrolysis [65•]. Yeast have equivalent chaperones (BIN2, BIN3 and so on); even though they are related, they appear to serve somewhat different functions and do not replace one another [66*].

Location of assembly and transport
A related and more specialized problem is the nucleation of microtubules in nerve cells, whose long processes are filled with microtubules that serve as tracks for intracellular transport. Are the microtubules assembled in the axon or in the cell body? The latter appears to be the case: nucleation sites containing γ-tubulin are found only in the cell body, and microtubules are then transported into the axons or dendrites (see below). Superimposed on this transport process is a length redistribution which allows microtubules to change their length, presumably by dynamic instability, in the axon. The plus ends of the transported microtubules are oriented distally, and this end is where incorporation of new subunits can occur [67].

Microtubule-associated proteins

The majority of studies have traditionally been done with brain MAPs, partly because brain tissue was the most abundant source of microtubules, and partly because the MAPs provide selective markers for brain development and cell type. The best known MAPs are the heat-stable proteins MAP2 and tau; they are localized (mainly) to the dendritic and axonal compartment, respectively, and they share homologous repeats in the carboxy-terminal region that contribute to microtubule binding (reviewed by Schoenfeld and Obar [5]).

Tau

Tau is thought to stabilize microtubules in axons and thus provide the basis for axonal transport. Consistent with this idea, it was found that when tau is transfected into culture cells microtubules become more stable and the degree of assembly increases [68,69]. This effect can be observed even more dramatically in insect SF9 cells transfected with tau using the baculovirus system: these normally round cells develop 'neurite-like' processes. In this system, tau also protects microtubules against drug-induced disassembly [70•,71•]. The simple picture does not always hold, however, as transfection of tau into Chinese hamster ovary cells did not result in an increase in microtubule stability or mass [72•]. Even more surprising is the fact that tau-null transgenic mice are viable; in this case, the stabilization of microtubules may be achieved by other MAPs [73•].

Functions of tau

Tau has several distinct effects on microtubules in vitro: it binds to them, promotes their nucleation and elongation, protects against disassembly, and induces them to form parallel arrays (bundles). These functions can be traced back to different domains of tau. For example, efficient assembly of microtubules requires not only the internal repeat domain, but also regions on either side, acting as 'jaws'; the flanking regions in turn tend to promote the formation of bundles [74-76]. Bundles of microtubules may be important not only in neurite outgrowth, but also in other functions such as in the erythrocyte marginal band, where tau is one of the MAPs responsible for cohesion [77]. Although the contribution of the repeat domain to microtubule binding is generally weak, there are certain 'hotspots' (e.g. the region between repeats 1 and 2) which could regulate the affinity, especially during the developmentally regulated alternative splicing of tau [78].

Distribution

Another characteristic feature of tau is its heterogeneous distribution, both within cells and between different cell populations. Whereas six tau isoforms are found in central nervous tissue (in humans), 'big' tau (distinguished mainly by the presence of an additional exon, 4a) occurs in peripheral nerves. Within neurons, tau is mainly axonal (in contrast to the somatodendritic MAP2), but there is also a nuclear variant of tau. This intracellular sorting may take place at the level of the transcripts, which come in sizes between 2.5 and 8 kilobases and may contain targeting information. Microtubules may play a role in distributing tau, because miRNA is transported along them [79-82].

Alzheimer's disease

Apart from its cell biological role, tau has generated considerable interest because of its relationship with Alzheimer's disease. Tau is the main component of the paired helical filaments that make up the neurofibrillary tangles in the brains of patients with Alzheimer's disease. In these tangles, tau is not only abnormally aggregated, but also modified in several other ways (reviewed in [83]). The most conspicuous modification is excess phosphorylation, frequently at Ser-Pro or Thr-Pro motifs (indicating the influence of proline-directed kinases). There are other sites (such as Ser262) that strongly influence the affinity of tau for microtubules [84•] and are phosphorylated in the tau protein found in Alzheimer's disease [85]; phosphorylation might explain why this tau does not bind to microtubules and ceases to stabilize them [86]. This effect contrasts with that resulting from 'fetal' phosphorylation of tau, which shares some of the phosphorylation sites of Alzheimer's disease.
but which still binds well to microtubules [87]. Several phosphorylation sites in fetal and adult rat tau have now been determined directly by mass spectrometry and sequencing [88]. From a practical point of view, most studies of phosphorylation rely on phosphorylation-dependent antibodies, which can also be used with cell models (epitopes are summarized in [83]). All of the sites found so far can be dephosphorylated by phosphatases PP-2A and PP-2B (calcineurin), both of which are abundant in brain [89]. Other modifications include ubiquitination at several lysines [90] and glycation [91].

**Structure**

How the normal and pathological properties of tau are related to its structure remains enigmatic. In solution, tau lacks secondary structure or even a defined shape and is best described as a random coil; the lack of secondary structure is observed even in paired helical filaments, suggesting that these may not assemble by way of interacting β-strands, in contrast to other fibers found in pathological states [92]. Nevertheless, tau tends to associate into antiparallel dimers, and in this form it is prone to assembly into paired helical filaments [93].

**MAP2, MAP1a, MAP1b and MAP4**

**MAP2**

MAP2 is a relative of tau in that it has a homologous repeat domain near the carboxyl terminus but has a much larger extension towards the amino terminus. The middle part can be spliced out to give MAP2c, a ‘juvenile’ isoform [94]. Whereas tau can have either three or four repeats, MAP2 used to be found with three repeats only; however, this gap has now been closed by the discovery of a four-repeat MAP2c. Unlike the neuronal MAP2, this isoform occurs in glia cells [94]. The question of how MAP2 binds to microtubules, and how they interfere with motor proteins, continues to be a matter of study. Wallis et al. [95] showed that MAP2 binds in a positively cooperative manner, leading to the clustering of MAP2 on the microtubule surface (which makes it distinct from tau [76]). Echoing an earlier debate, Hagiwara et al. [96] reported that MAP2 and motor proteins compete for the same binding sites (in the carboxy-terminal domain of tubulin), whereas Marya et al. [97] found distinct sites and no competition. A compromise solution was offered by Lopez and Sheetz [98], who found distinct sites but steric hindrance, as might be expected with molecules as large as MAP2. Whatever the explanation of the results, in practice there seems to be no doubt that motors can work their way along microtubules quite efficiently, even when MAPs are present.

**MAP1a and MAP1b**

MAP1a and MAP1b are two other high molecular weight neuronal MAPs; they do not belong to the same class as MAP2 and tau because they have different microtubule interaction motifs. MAP1a and MAP1b share partial sequence homology, and both are synthesized as a polyprotein coupled to a light chain (LC; MAP1b–LC1, 34 kDa, and MAP1a–LC2, 28 kDa, respectively [99,100]). As MAP1b binds to microtubules through a series of basic repeats of the type KKE, the same mode of binding had also been assumed for MAP1a. This is not the case, however, as Cravchik et al. [101] have discovered that MAP1a has a novel binding motif which is unique, as it is acidic rather than basic. They proposed that MAP1a has a bipolar helical structure, with positive and negative charges separated on the two sides. The third light chain, LC3 (18 kDa), is encoded separately and has now been cloned [102]. It is a basic protein, abundant in neurons, that binds to both MAP1a and MAP1b and could modulate their interaction with microtubules.

**MAP4**

In contrast to the neuronal proteins discussed above, MAP4 has a ubiquitinous distribution. It resembles MAP2 and tau in having a homologous repeat domain that interacts with microtubules. Contrary to the view that MAPs are freely diffusible in cells, Chapin and Bulinski [103] have observed a surprising extent of microheterogeneity of MAP4-microtubule interactions in cell cultures, suggesting the selective stabilization of subsets of microtubules within cells. A related MAP4-like protein has been purified from *Xenopus* eggs [104]. This protein becomes phosphorylated during mitosis and thus is a candidate for MAP4-dependent regulation of microtubule dynamics.

**Plant MAPs**

Compared to the proteins mentioned so far, the study of plant microtubules has been lagging behind, partly because of the difficulty in preparing sufficient quantities of protein. Recent work shows that plant microtubules are quite comparable with those of animals; for example, plant tubulin can be assembled reversibly using taxol, hybrid systems of maize tubulin assembled with neuronal MAP2 show tight binding (dissociation constant in the micromolar range), and some plant MAPs appear to be related to tau [105–107].

**Other proteins associated with microtubules**

As mentioned in the Introduction, not all associated proteins can be classified as structural or motor MAPs; conversely, as microtubules are ‘sticky’, not all associations may be meaningful. As phosphorylation regulates many intracellular interactions, it is intriguing that microtubules associate with a number of kinases (such as the mitogen-activated protein kinase cdk5, and GSK-3; see [83]) that can cause the ‘pathological’ phosphorylation of tau protein. The kinases often bind to microtubules via MAPs. An interesting case is that of protein kinase A associated with MAP2: phosphorylation of the regulatory subunit of protein kinase A by the cell cycle kinase cdc2 releases the catalytic subunit and presumably allows it to phosphorylate other targets [108]. Dynamin is a GTPase involved in endocytosis; it has two microtubule-bind-
ing sites near the amino terminus and a proline-rich domain (resembling Src homology region 3 domains) near the carboxyl terminus whose affinity is regulated by phosphorylation [109].

Several proteins involved in signal transduction bind tightly to tubulin or microtubules. Recent examples include neurofibromin (a neuronal GTPase-activating protein) [110], the α-subunit of several G proteins (which can take up GTP from tubulin) [111], and gephyrin (a protein linked to the glycine receptor) [112]. Associations with microtubules are not restricted to proteins; the binding of mRNAs was mentioned above [80], and recently Surridge and Burns [113] reported that the middle part of MAP2 has a high affinity for phospholipids, a property not found in tau or MAP2c.

Of special interest in this context are proteins that are capable of microtubule severing, which could contribute to their rapid reorganization, for example during mitosis. Examples include katanin, a heterodimeric protein (60 and 81 kDa) that severs microtubules in an ATP-dependent fashion [114*], and elongation factor EF-1α, a GTP-binding protein involved in protein synthesis [115*]. On the other hand, a protein like EF-1α has also been found to be able to cause the formation of bundles of microtubules [116*] and has been found in the pro-centrosomal protein complex (see above; [54*]). It will be interesting to see how these seemingly contradictory activities are related to one another. Finally, flagellar microtubules can be excised near their base, and it turns out that this is achieved by contractile fibers consisting of centrin, a relative of the calcium-binding protein calmodulin ([117*]; see also Salisbury, this issue, pp 39–45).

**Microtubule dynamics**

One of the most fascinating aspects of microtubules is their rapid turnover and potential for reorganization, unexpected for a cyto-skeletal polymer. The half-lives of most microtubules are in the range of minutes, and the predominant mechanism of redistribution appears to be dynamic instability (reviewed by Cassimeris, [118]), perhaps in combination with the severing factors just mentioned [114*,115*]. Dynamic instability can also be observed in plant cells [119]. On the other hand, many cellular microtubules are intrinsically stable [120], suggesting that the observed dynamics are tightly regulated by factors controlling catastrophes and rescues, including kinases such as the cell cycle dependent kinase cdc2. Independent of these mechanisms is a steady flux (or treadmill) of subunits, such that labeled subunits migrate slowly from the plus to the minus end of the microtubule [121]. In spite of this activity, and contrary to intuition, the assembled microtubule mass does not change much during mitosis, indicating that reorganization does not require net disassembly [122].

A reconstruction of an entire spindle microtubule assembly shows that the nearest-neighbor interactions are mainly between antiparallel microtubules, supporting the potential for bidirectional gliding during anaphase [123]. Plant cells have the capacity for reorienting their entire microtubule network in response to extracellular factors and gravity [124]. Some of these features can be reproduced *in vitro*, where microtubules can be induced to display elaborate waves of assembly and orientation in time and space [125*].

**Conclusions**

In contrast to the actin-based cytoskeleton, where the structures of the main players (actin, myosin and their complex) have been solved by X-ray crystallography [126,127], the microtubule system does not yet offer such insights. A direct structural analysis may be possible by electron crystallography; because zinc induces the formation of tubulin sheets that diffraction to near-atomic resolution [128*]. For the time being, we have to learn by analogy. For example, the structure of the actin-binding domains of gelsolin [129] illustrates that repetitive sequences need not reflect repetitive binding; the interactions may in fact be based on the variable parts, whereas the repeats could simply provide a framework for protein folding (this argument might apply to models of microtubule–MAP interactions). Tubulin is a GTP-binding protein that shares weak homology with signal-transducing G proteins. The structure of the α-subunit of transducin has become available [130]; part of it folds in a manner similar to the small G protein Ras/p21 (reviewed in [131]). Finally, if one is interested in an example of an assembly of α- and β-subunits, with nucleotides bound in a non-exchangeable and exchangeable fashion, then the recent solution of the F1-ATPase structure may be worth studying [132]. Even though these proteins have different functions from tubulin, they provide intriguing food for thought.

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
** of outstanding interest


12. Song Y-H, Mandelkow E: Recombinant kinesin motor domain
   • binds to β-tubulin and decorates microtubules with a B-surface lattice. *Proc Natl Acad Sci USA* 1993, 90:1671-1675.


14. Mitchison TJ: Localization of an exchangeable GTP-binding site


19. A centrifuge microscope is used to measure force-velocity relationships for kinesin, yielding force values in the range of 0.1 pN per kinesin head. See also [16*] annotation.


21. Hackney DD: Evidence for alternating head catalysis by kinesin

A kinetic analysis of the interactions between microtubules, kinesin, and ATP hydrolysis.


The paper shows that the directionality of the movement is encoded in the head domain of a motor, independently of its arrangement in the protein.


29. Mitchellon TJ: Localization of an exchangeable GTP-binding site

A direct demonstration that the exchangeable GTP on β-tubulin is near the plus end of microtubules.


This paper and [45] provide examples of how yeast genetics can be used to answer questions about tubulin structure, functional domains, and GTPase activity.


A systematic mutation analysis of β-tubulin (converting charged residues to alanine) to reveal regions important for the functions of microtubules in yeast.


Protein complexes that represent centrosome precursors are isolated from Chinese hamster ovary cells. They contain cytoskeletal proteins α-, β-, γ-tubulin and actin as well as elongation factors and chaperones.


A 25 S complex containing γ-tubulin in Xenopus eggs can combine with the sperm centriole to form the centrosome: this assembly requires ATP but not microtubules. The complex confers the microtubule-nucleating activity upon the centrosome.


This study distinguishes between the nucleation of microtubules on other microtubules (template elongation) and on the pericentriolar material (as-tal nucleation) in Xenopus eggs, and describes the role of γ-tubulin in the nucleation activity.


Pericentrin is a newly discovered 220 kDa protein involved in organizing the pericentriolar material and other structures from which microtubules can be nucleated.


The actin homologue cactin and γ-tubulin, two centrosomal proteins, are folded through the cytoplasmic chaperonin complex TCP-1; the folding reaction requires ATP.


A pulse-labeling analysis of actin and tubulin biogenesis in Chinese hamster ovary cells showing that they must associate with the 900 kDa TCP-1 complex in order to fold properly and associate into functional tubulin heterodimers.


Analysis of the interactions between the TCP-1 complex, actin, tubulin, and other cofactors involved in the folding reaction.


This paper describes the sequence of steps in the folding of actin and tubulin by the cytoplasmic chaperonin complex. The initial interaction requires the chaperonin to be in the ADP-bound state, whereas release requires ATP, which thus acts as a switching device.


The folding of actin and tubulin in yeast requires chaperonin complexes similar to the TCP-1 complex.


A review of how insect Sf9 cells can be used as a model system to generate ”neurite-like” cell processes by transfecting them with microtubule-associated proteins.
Expression of tau in insect S9 cells leads to the formation of ‘neurite-like’ cell processes based on assembled microtubules. Probing their resistance to depolymerizing drugs or low temperature shows that tau makes these microtubules stable against drugs but not against cold. 


COS cells were transfected with Drosophila 250 kDa MAP, human MAP4 and human tau. The exogenous MAPs had no major effect on the levels of assembled microtubules or their drug sensitivity, suggesting that MAPs may have roles distinct from those in microtubule stabilization.


The first tau-null mouse appears to survive reasonably well, suggesting that neurons have a back-up system for tau.


This paper describes a critical residue in the repeat region of tau protein whose phosphorylation detaches tau from microtubules and destabilizes them.


