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Review

Microtubule dynamics

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Abstract. Microtubules are fibrous elements in the cytoplasm of eukaryotic cells, where they perform a wide variety of functions. Microtubules are major organizers of the cell interior and are vitally involved in motility events such as chromosome migration during cell division. To fulfill their physiological function, microtubule arrays have to undergo dramatic changes in their spatial arrangement, and this depends to a large extent on the complex and special dynamic properties of the individual polymers. In this review we first describe the intrinsic dynamic properties of microtubules assembled in vitro from purified tubulin and examine the relationships between these properties and microtubule functions. Subsequent sections concern microtubule dynamics in vivo, their similarity and differences with microtubule dynamics in vitro, and the nature of the cellular regulators which act on microtubule assemblies in physiological conditions.

Key words. Microtubules; microtubule associated proteins; dynamics; organization; regulation.

Introduction

Microtubules are hollow cylindrical aggregates, present in the cytoplasm of eukaryotic cells [1]. Microtubules are used by cells for many different purposes, being vitally involved in cell motility and division, in organelle transport, and in cell morphogenesis and organization (fig. 1). This amazing variety of microtubule functions is paralleled by an equally remarkable flexibility in microtubule organization. Microtubules are the main components of the complex and highly organized axonemal structures found in cilia and flagella. In most interphase cells, microtubule arrays are nucleated and organized by the centrosome from which the polymers irradiate into the cell cytoplasm. Interphase microtubules are thought to be major organizers of the cell's interior. They are essential determinants of cell shape and motility, required for the assembly of essential cell structures such as the endoplasmic reticulum and the Golgi apparatus, and used as highways for organelle transport. During mitosis, microtubules rearrange to form the so-called mitotic spindle. The mitotic spindle orients the plane of cell cleavage and functions as a supramolecular motor to segregate the chromosomes to the cell poles during anaphase.

The precise ways in which microtubules achieve their cellular functions is not fully understood yet. However, it is thought that the functional and structural flexibility of microtubule arrays relies to a large extent on their remarkable properties. Microtubule arrays in cells are generally dynamic, capable of assembly, disassembly and rearrangement on a time scale of seconds to minutes. Microtubule dynamics in vivo are based on intrinsic dynamic properties of the polymers themselves determined by the biochemical properties of the microtubule building block, the tubulin $\alpha\beta$ heterodimer. Tubulin is able to interact with a bewildering number of proteins and small molecules such as nucleotides and drugs. Furthermore, tubulin is a GTPase. GTP hydrolysis during tubulin assembly is apparently a phenomenon of central im-

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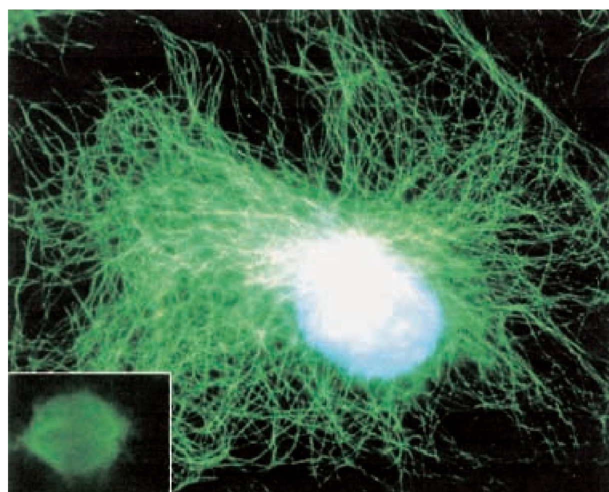

Figure 1. Microtubule arrays in cells. Analysis of microtubule arrays using immunofluorescence with primary antibody directed against tubulin (green). Interphasic cell: microtubules irradiate all the cytoplasm. Metaphasic cell (insert): microtubules are major components of the mitotic spindle. DNA staining using Hoescht (blue).

portance for microtubule physiology. The energy input from GTP hydrolysis serves to generate nonequilibrium dynamics, and this is probably a basic physicochemical mechanism underlying microtubule-dependent organization and motility.

The first sections of this review include a brief description of tubulin and microtubule structure. Further sections deal with microtubule dynamics in vitro and in vivo and their possible relationships with microtubule functions. Final sections concern microtubule regulation by associated proteins.

Tubulin and microtubule structures

α- and β-tubulin monomers are proteins of about 450 amino acids each and are about 50% identical at the amino acid level [2]. Each monomer has a molecular mass of about 50,000 Da. Tubulins are ubiquitous in eukaryotes and belong to a growing family that now includes seven different monomers (for a review, see [3]). Each monomer binds a GTP molecule, nonexchangeable in α tubulin and exchangeable in β tubulin. GTP from β tubulin is required for microtubule assembly, and its hydrolysis follows addition of a dimer to the microtubule end, upon which it becomes nonexchangeable within the microtubule. The structure of the tubulin dimer has been solved by electron crystallography of the two-dimensional (2D), crystalline sheets of tubulin that form in the presence of zinc ions [4]. This atomic model of tubulin provides structural information and gives a picture of the properties of tubulin and microtubules at the molecular level [5].

The origin of tubulin in evolution has long been enigmatic. In this regard, one of the most striking results that followed the resolution of the tubulin three-dimensional (3D) structure is that the 3D structure of a prokaryotic protein, FtsZ, shares a remarkable similarity with that of α and β tubulins [6], although the sequences of FtsZ and of tubulins have only a low degree of sequence identity. FtsZ has a relative molecular mass of 40,000 Da, is ubiquitous in eubacteria, archaebacteria, and was also identified in chloroplasts. During septation, FtsZ localizes early at the division site to form a ring-shaped septum. Purified bacterial FtsZ assembles in vitro into protofilament sheets, into minirings and within chloroplasts into tubules. Both eukaryocyte microtubules and FtsZ tubules are hollow and composed of protofilaments. However, in microtubules the arrangement of protofilaments is longitudinal, whereas in FtsZ tubes it is helical. Both proteins are GTPases, and tubulin is now thought to be an evolutionary derivative of FtsZ (for a review, see [7]).

Microtubules are arranged linearly into protofilaments that associate laterally to form a 25-nm-wide cylindrical structure (fig. 2 A). In vitro, the number of protofilaments of microtubules varies between 10 and 15 depending on the assembly conditions. Microtubules in vivo and microtubules nucleated in vitro from centrosomes and axonemes generally have 13 protofilaments. Ultrastructural analysis of motor-decorated microtubules has showed that each protofilament is shifted slightly lengthwise by about 0.9 nm with respect to its neighbour, so that tubulin dimers describe helices around the microtubule. This arrangement also leads to the observation of a seam, parallel to the long axis of the microtubule (for a detailed description, see [8]).

The organization of α- and β-tubulin heterodimers in the microtubule lattice is polarized, and this feature results in structural and kinetic differences at the microtubule ends. The faster growing end (named the plus end) has the β-tubulin subunit of each heterodimer exposed, whereas the slower growing end (named the minus end) has the α-tubulin subunit exposed. In vivo, the minus end of the microtubule is associated with the centrosome. It is localized near the center of the cell, whereas the plus end is peripheral.

Both the structure of the lattice and the polarity of the microtubule are central to the function of microtubule motor proteins of the kinesin and dynein families, which move unidirectionnally along the microtubule.

Electron microscopic studies have examined the pathway of early assembly of microtubules (reviewed in [9]). The earliest structures are small sheets of protofilaments. This is also the case when nucleation is seeded from stable microtubule portions. Later, the sheet closes to form a microtubule.

Once assembled, tubulin addition to and loss from existing microtubules occur only at microtubule ends. Cryoelectron microscopy reveals that the ends of growing mi-

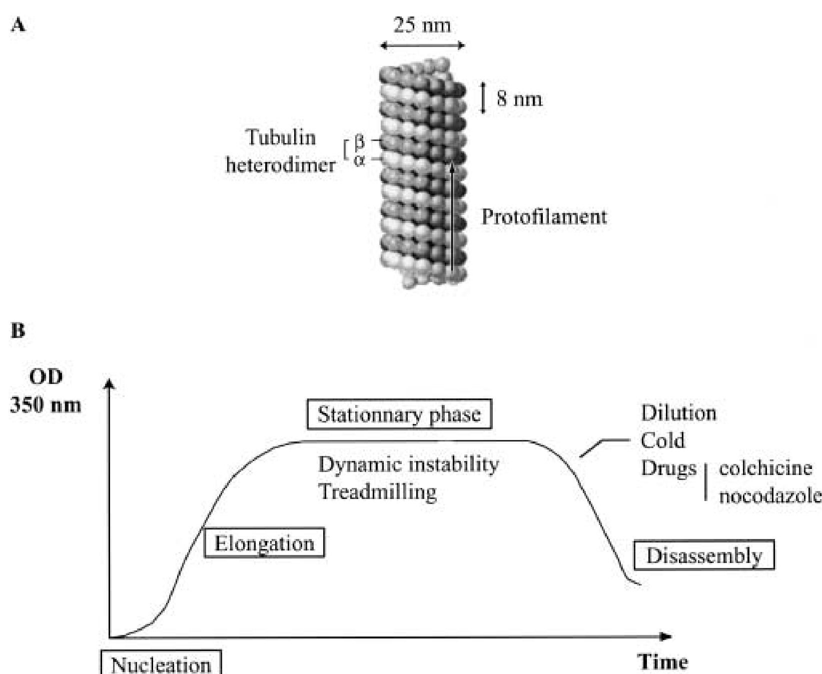

Figure 2. Microtubule structure and assembly. (*A*) Schematic representation of microtubule structure. (*B*) Various phases of microtubule assembly as seen in turbidimetry (see text).

crotubules from purified tubulin range from blunt to long, gently curved sheets [10]. It has been postulated that microtubules elongate by formation of an outwardly curved sheet which closes at a variable rate to form the microtubule cylinder. Ends of microtubules grown from *Xenopus* egg extracts were also observed using electron cryomicroscopy. It has been shown that, under physiological conditions, microtubules also grew by the extension of a 2D sheet of protofilaments, which later closes into a tube [11]. The ends of disassembling microtubules were blunt, or showed small oligomeric or coiled structures [12]. The curvature of the sheets at the end of growing microtubules and the small oligomeric structures observed at the end of disassembling microtubules indicate that tubulin molecules undergo conformational changes during both assembly and disassembly. As discussed in the next section, these conformational changes seem to be central to microtubule dynamics.

Microtubule dynamics in vitro

General features

Microtubule assembly proceeds in three phases: nucleation, elongation and steady state. These phases have been extensively studied both in vitro and in vivo. Microtubule dynamic properties were first observed in vitro for suspensions of microtubules from purified tubulin. In further studies, the intrinsic dynamic properties of microtubules have been found again in vivo in a cellular context where dynamics are regulated through the overall environment.

The aggregation of purified tubulin to form microtubules can be induced in a variety of conditions. Two factors play an important role. First, purified tubulin microtubules do not form at low temperature. Therefore, tubulin aggregation is generally induced by warming up tubulin solutions to 30–37 °C. Second, tubulin is incorporated into microtubules as a complex with GTP, as seen above. Following warming and GTP addition to soluble tubulin dimers, tubulin assembly proceeds in the three phases mentioned above. Two principal methods have been used to follow microtubule assembly: turbidity measurements and videomicroscopy. Microtubule suspensions are turbid and cause an apparent absorption at 350 nm. This property is used to measure the total mass of assembled microtubules and gives information about the behaviour of the whole microtubule population (fig. 2B). Videomicroscopy using dark field and DIC (differential interference contrast) follows the assembly and disassembly of individual microtubules.

As measured by turbidimetry, the first phase of assembly is usually referred to as the nucleation phase. During this phase, poorly defined microtubule seeds are formed. Microtubule seeds do not convert into microtubules below a tubulin threshold concentration defined as critical concentration. This critical concentration depends on experimental assembly conditions. The second phase of assembly is that of elongation. During this phase, tubulin dimers add at the end of microtubule.

The third phase of assembly is that of the steady-state. Analysis of steady-state microtubules has demonstrated that a fixed proportion of tubulin is in the assembled form, whereas another tubulin pool remains soluble. The concentration of free tubulin dimers at steady state varies with buffer conditions and corresponds to the so-called tubulin critical concentration for microtubule assembly.

Tubulin assembly has two main features: the microtubules formed are intrinsically labile and, at steady state, the microtubule system remains out of equilibrium. Microtubules are readily disassembled by temperature drop below 10–15 °C, removal of GTP, or dilution of microtubule suspensions and subsequent decrease of the free tubulin concentration. Microtubule disassembly can also be triggered by adding specific drugs to a microtubule solution, such as colchicine, vinblastine or nocodazole. Drugs such as colchicine or vinblastine poison tubulin assembly onto microtubule ends and thereby induce microtubule disassembly. Nocodazole acts by sequestering tubulin dimers, with which it forms an inactive complex. The result is that the free tubulin concentration decreases, and when it drops below the critical concentration, microtubules disassemble.

At steady state, microtubules remain out of equilibrium. Continuous GTP hydrolysis and thereby energy consumption is required for the maintenance of steady-state microtubules, which apparently use energy to generate active exchanges between their constitutive subunits and the tubulin molecules of the soluble pool. Two mechanisms are thought to account for such tubulin exchanges. The first has been called treadmilling. Treadmilling relies on a particular property of microtubules: at steady state, microtubule ends behave differently [13]. The plus end continuously incorporates new tubulin dimers, whereas the minus end continuously looses them (fig. 3A). Such asymmetric behavior clearly requires energy consumption. However, the precise mechanisms that couple GTP hydrolysis to the generation of the functional asymmetry in microtubules are so far unknown. When microtubule ends are not anchored at a fixed position, treadmilling results in an apparent microtubule migration that actually reflects the propagation of a wave of tubulin assembly-disassembly. When microtubule ends are kept stationary, treadmilling generates an apparent flux through the polymers of tubulin molecules travelling from the plus ends toward the minus ends [14].

The second mechanism through which steady-state microtubules exchange their constitutive tubulin with soluble tubulin relies on another property, known as dynamic instability (fig. 3B). This model is based on an analysis of the length distribution of fixed microtubules [15, 16]. According to this model, although a population of microtubules exhibits a bulk steady state, an individual microtubule never reaches an equilibrium length but persists in prolonged phases of assembly and rapid phases of disassembly that interconvert infrequently. Transition from slow assembly to fast disassembly is referred to as 'catastrophe', and transition from disassembly to assembly as 'rescue'. Moreover, microtubules sometimes pause for a period of time, during which their length remains constant [17]. Dynamic instability was indeed extensively studied using videomicroscopy and real-time analysis of individual microtubules [17, 18]. Four parameters have been proposed to describe dynamic instability: rates of assembly and disassembly, and frequencies of catastrophe and of rescue [17].

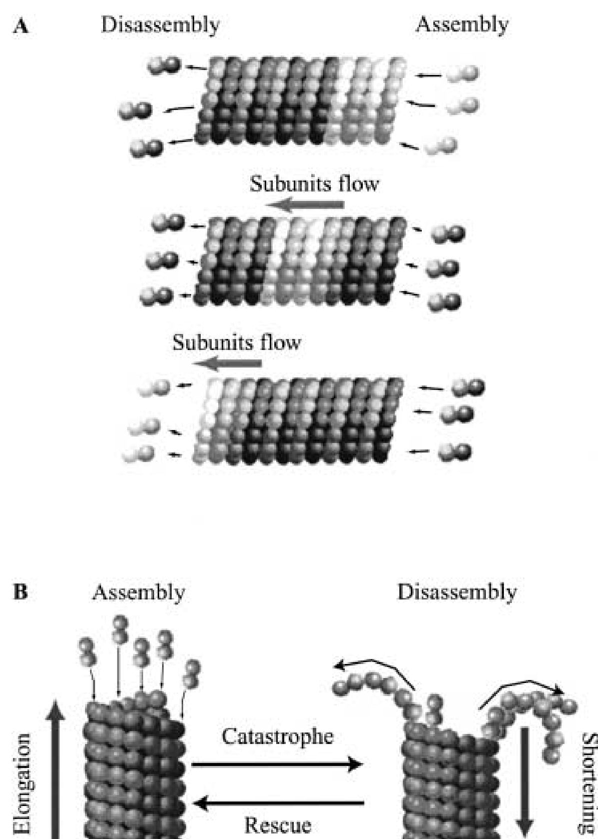

Figure 3. Schematic diagram of microtubule behaviour at steady state. (*A*) Treadmilling: the microtubule plus end is steadily growing, whereas the minus end is shrinking. The polymer length remains constant, but there is continuous exchange between the pool of tubulin molecules in the polymer and the free tubulin pool. (*B*) Dynamic instability individual polymers show extensive length fluctuations due to spontaneous phases of catastrophe, rescue and regrowth.

Kinetic analysis of microtubule dynamics

For many years, the kinetics of microtubule assembly have been interpreted according to simple law of mass action-based models [19–21]. Exchanges between the free tubulin and the assembled tubulin pools were supposed to be rapid, with a continuous and direct effect of the free tubulin concentration on microtubule behaviour. In this

view, the rates of microtubule nucleation and microtubule elongation are both supposed to be dependent at all time points on the free tubulin concentration. When the free tubulin concentration decreases during microtubule assembly, the rate of nucleation decreases as a function of a high power of this concentration, whereas the rate of microtubule elongation varies linearly with the free tubulin concentration [22–25]. Hence, microtubule assembly seems to proceed in two phases, a phase of microtubule nucleation followed by a phase of microtubule elongation at constant microtubule number. Microtubule disassembly, caused for instance by a decrease in the free tubulin concentration, is viewed as mechanically identical with microtubule elongation, with a negative net assembly rate.

The discovery of dynamic instability has modified these views, suggesting that microtubule dynamics are strongly influenced by structural transitions occurring at microtubule ends. It is still thought that microtubule nucleation and elongation are dependent at all time points on the free GTP-tubulin concentration. But a central hypothesis of all dynamic instability models is that assembly and disassembly are different processes. According to these models, disassembling and assembling microtubules react in different ways with their molecular environment, including free GTP-tubulin dimers [10, 12, 16, 26, 27].

The ability of tubulin molecules to form various types of oligomers has long been recognized, and these oligomers have been proposed as important regulators of microtubule dynamics [28–33].

Nucleation models have involved tubulin oligomers in the formation of microtubule nuclei [28, 29, 33]. These oligomers were thought to be at rapid equilibrium with tubulin dimers, whose concentration was thought to drive the nucleation reaction. Different mechanisms of nuclei formation from tubulin dimers have been postulated, and different values of the apparent nucleation exponent have been observed in different experimental conditions [22–25, 34] (fig. 4). These results indicated that the mean number of tubulin dimers necessary to obtain a microtubule nucleus was between 6 and 13 following the authors. We have recently reexamined the kinetics of microtubule nucleation. In agreement with previous work, we have found that the rate of nucleation is strongly dependent on the initial concentration of tubulin [35]. But, in contrast with models in which the rate of nucleation is supposed to be dependent at all time points on the free tubulin concentration, our study showed that the rate of nucleation remained constant during most of the assembly phase, being completely insensitive to wide drops in the free tubulin concentration during assembly. These results deviate from current models of assembly, in that the limiting factor for nucleation is not the free tubulin concentration. To account for the fact that the rate of microtubule nucleation is determined by the initial conditions and remains constant during assembly, we propose that microtubule nuclei form from precursor oligomers which are present at a concentration determined by the initial tubulin concentration. According to observed nucleation exponents, about six oligomers combine to form microtubule nuclei [35].

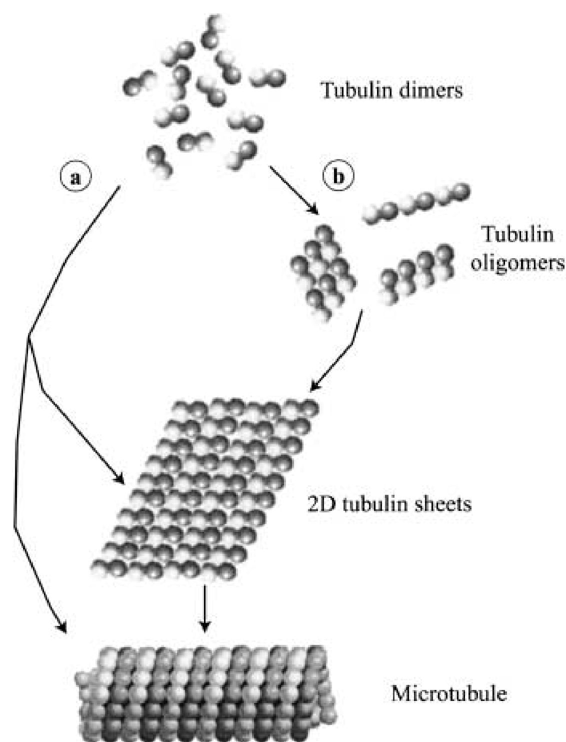

Figure 4. Nucleation models: from tubulin dimers to microtubules. Schematic representation of two models of microtubule nucleation. (*a*) Tubulin dimers associate to form sheets which subsequently close either into microtubules or microtubules. (*b*) Tubulin molecules associate to form oligomers that subsequently combine to form sheets and microtubules.

The rate of elongation far from steady state has recently been reinvestigated using different approaches. In contrast with the predictions of law-of-mass-action-based models, this rate was found to be constant, in a given buffer condition, insensitive to wide variations in the free tubulin concentration [24, 35]. Calculations showed that the rate of elongation is limited by the probability of tubulin dimers sticking to the microtubules, and not the rate at which they diffuse to the microtubule ends. Therefore, the rate of elongation is probably mainly determined by intrinsic properties of the microtubule system, independently of the free tubulin concentration. These data fit well with structural data, suggesting that assembly proceeds through the formation of 2D tubulin sheets that progressively close into tubes [10]. During microtubule elongation, sheet extensions are present at microtubule ends as long as the tubulin concentration is above its critical value. Under these conditions, the rate-limiting step for microtubule elongation probably is the intrinsic rate

of sheet closure. Close to the critical tubulin concentration, the rate of sheet closure into microtubules apparently becomes higher than the rate of formation of sheet extensions because the free tubulin concentration can become rate limiting.

As mentioned above, catastrophe rates and frequencies have been measured at steady state, under the critical tubulin concentration, in conditions where no spontaneous nucleation is observed. From these results, it has been concluded that catastrophe can be considered as a rare event, followed by rapid microtubule disassembly. These data were also observed far from steady state [35]. Moreover, a strong influence of the initial tubulin concentration on the rate of catastrophe has also been shown. To explain such an influence, the authors postulated that catastrophe factors could be present in tubulin solutions, and that the amount of these factors could be dependent on the initial tubulin concentration.

Taken together, these results show that a microtubule suspension obtained from purified tubulin has rich intrinsic capacities. It contains not only tubulin dimers and microtubules, but also several kinds of intermediate aggregates which are competent to drive both nucleation and disassembly. However, many questions remain to be solved with respect to the precise molecular mechanisms controlling these behaviours.

Microtubule oscillations and microtubule self-organization

Self-organization can occur in dissipative, nonlinear chemical systems. Dissipative systems continuously consume energy, are thermodynamically irreversible and are kept out of equilibrium. With this definition, microtubule solutions clearly represent dissipative systems. Microtubules continuously incorporate GTP-tubulin molecules, which results in energy consumption through GTP hydrolysis. The reaction is thermodynamically irreversible, since GDP-tubulin dissociation from microtubules does not generate GTP-tubulin. Finally, as discussed above, microtubules are out of equilibrium at steady state due to dynamic instability and treadmilling. A salient feature of nonlinear system is their capacity to undergo oscillatory behaviour, when placed in the proper conditions. Depending upon the conditions under which assembly is initiated, tubulin can assemble either following monotonic kinetics or adopt an oscillatory mode [31, 36–38]. When present, microtubule oscillations involve successive and spontaneous phases of extensive (sometimes complete) microtubule disassembly followed by reassembly, until a steady state is reached progressively. Microtubule oscillations can be triggered in different ways. A basic requirement for such oscillations to occur is that the rate of initial tubulin assembly should be rapid as compared with the rate of GTP-tubulin regeneration from GDP-tubulin. The molecular mechanisms underlying oscillations are still debated. But the existence of oscillations in microtubular suspensions is solid evidence for the fact that some of the chemical reactions involved in microtubule formation are nonlinear processes.

Clearly, microtubule assembly results from dissipative and nonlinear chemical reactions. Such systems can, in principle, generate patterns known as dissipative space structures or Turing structures. Pattern formation in microtubular solutions is easy to observe. Initial observations concerned pure tubulin assembled into microtubules in spectrophotometric cells. Following microtubule formation, macroscopic striped patterns arose [39, 40], consisting of a series of horizontal white and dark stripes encompassing the whole cell (fig. 5). The stripe pattern was shown to result from periodic changes in microtubule orientation throughout the cell and was not observed in stable, nondynamic, microtubule solutions. Microtubule organization has been tested in low gravity conditions produced during space flight. The samples prepared in microgravity showed almost no self-organization and were locally disordered [41].

Microtubules provide the first simple biochemical system with self-organizing capacity in acellular systems and are amenable to biochemical assays and tests of morphogenesis. For instance, microtubule self-organization has been investigated by assessing the assembly and positioning of microtubule asters in microfabricated chambers that mimic the geometry of living cells. Microtubules from purified tubulin were nucleated from centrosomes, and microtubule assembly alone was shown to be sufficient to position asters at the center of the chamber. It has been postulated that dynamic instability is responsible for this centering. This fundamental behaviour of microtubules could provide an effective way for the aster to explore intracellular space [42]. From a conceptual point of view, microtubule self-organization through dynamic instability is another example of self-organization through nonlinear dissipative systems.

In *Xenopus* egg extracts, microtubules self-organize to form different types of spindle assembly. This has allowed the development of biochemical approaches to study the molecular basis of microtubule self-organization in complex yet acellular systems. Self-organization of microtubules was demonstrated in this type of system such that magnetic beads, coupled with plasmid DNA, act as artificial chromosomes. Spindle assembly does not require centrosomes or centromeric DNA sequences. Mitotic chromatin itself is the driving force for microtubule organization into bipolar spindles [43]. The self-organizing processes involve both microtubules and associated molecular motors.

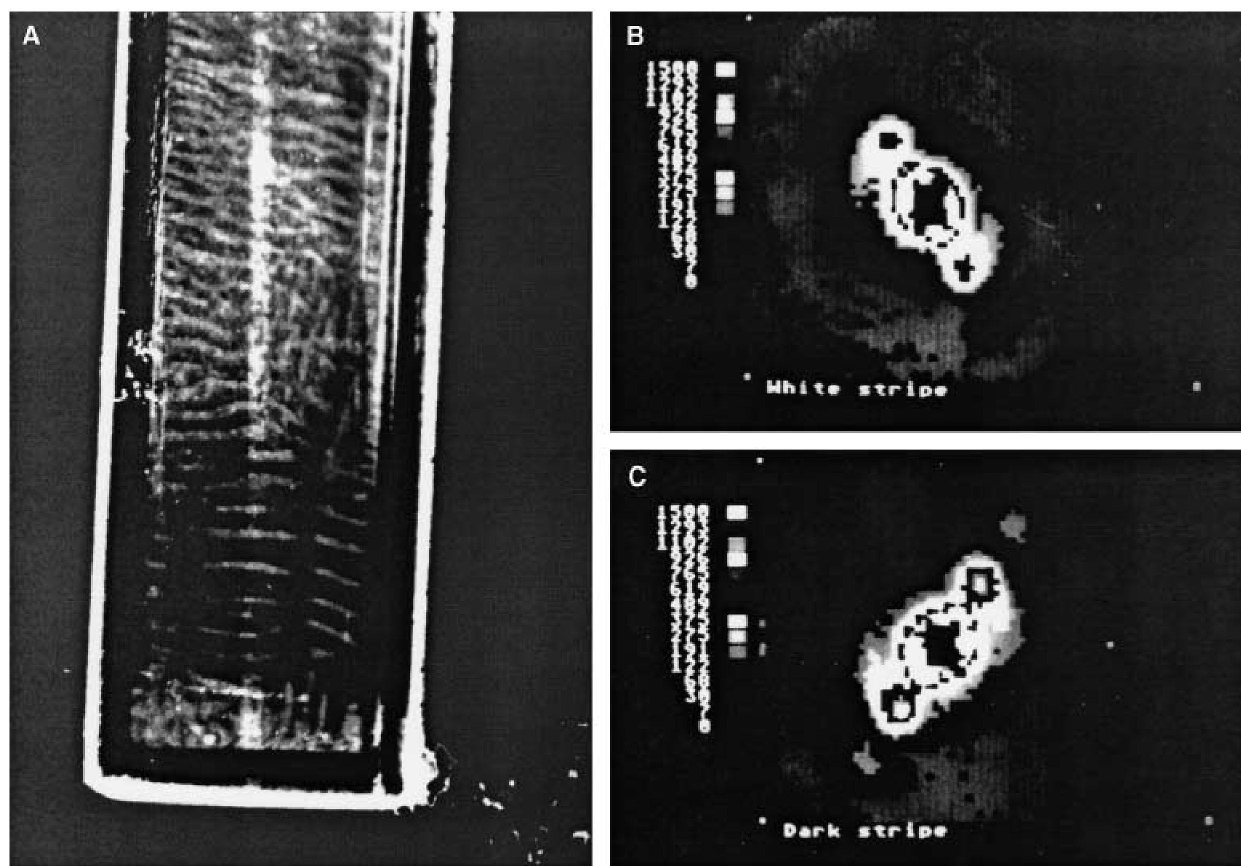

Figure 5. Microtubule dissipative space structures. Pure tubulin was assembled in a spectrophotometer cell (40 × 10 × 1 mm). (*A*) Observed through crossed polarized filter light, the microtubule solution showed a pattern formed of successive dark and white stripes. Each stripe was about 2 mm. (*B*) Neutron scattering spectra from (*B*) a white stripe and (*C*) a dark stripe. Stripes contain parallel microtubules, and microtubule orientation varies by 90° between adjacent stripes.

Microtubule dynamics in vivo

Relationships between microtubule dynamics in vitro and microtubular functions in vivo

Microtubule dynamics in vivo share fundamental features with microtubule dynamics in vitro. Cellular microtubules are highly dynamic, and both dynamic instability and treadmilling have been observed in vivo. Cellular microtubules exhibit a more rapid assembly rate and higher transition frequencies than in vitro microtubules formed from pure tubulin [44]. The assembly rate of tubulin in vivo is about 5–10-fold higher than that of pure tubulin at a similar concentration.

It is thought that the assembly-disassembly behaviour of microtubules may be the driving force originally used during evolution to move chromosomes during anaphase. One of the raisons d'être of microtubules is to participate in mitosis. Anaphase is the phase of mitosis during which chromosomes are segregated in the dividing cell, through migration from an equatorial position toward the two opposite cell poles. During anaphase, microtubules form two hemi-spindles, connecting the cell poles to the migrating chromosomes. A crucial observation is that poleward chromosome migration is accompanied by concomitant shortening of spindle microtubules. An obvious question thus concerns the existence of plausible physicochemical mechanisms which could couple microtubule disassembly and chromosome movement. Such coupling can be achieved through a mechanism known as polymer-guided [45] or polymer-biased diffusion [46]. Polymer-biased diffusion relies first on microtubule disassembly at the kinetochore. The microtubule becomes shorter and pulls the chromosome, which moves by diffusion in the direction of the pole. This behaviour has been demonstrated in reconstituted systems [46, 47]. Furthermore, polymer-biased diffusion is a general 'push-pull' mechanism which applies to different systems. For instance, it is now generally accepted that the bacterium *Listeria* moves inside cells through polymer-guided diffusion [48].

Treadmilling and dynamic instability have been observed in cellular microtubules. Dynamic instability seems to represent the main mechanism of microtubule turnover [49]. It is also thought to be involved in microtubule self-organization [50]. According to 'search-capture' models,

dynamic instability in cells allows microtubules to search 3D space more effectively than equilibrium assembly, and this enables microtubules to find specific cell target sites. Plus ends of mitotic microtubules were hypothesized to probe through the cytoplasm, searching for binding sites on kinetochores that could capture them. This process has been visualized in living newt lung cells [51].

Analysis of microtubule dynamics in another model system, the fish melanophore, revealed the existence of plus and minus pathways in microtubule turnover. Turnover occurs by instability dynamics at distal plus ends of microtubules. However, a subpopulation of microtubules was also found, shortening from their minus ends, presumably after constitutive release from the centrosome. It was concluded that turnover occurs by a combination of plus and minus end pathways, the plus end dominating at the cell periphery and the minus end dominating near the cell center [52]. Other processes can also account for dynamic instability, for instance local regulation of factors controlling microtubule dynamics.

Treadmilling has been observed in cytoplasmic fragments of fish melanophores. Microtubules were shown to detach from their nucleation sites and free microtubules moved toward the periphery by an apparent treadmilling, independent of molecular motors [53]. In other studies, the mechanism of cytoskeletal protein transport, especially in neurons, has been investigated. Using the FRAP (fluorescence recovery after photobleaching) method in living zebrafish embryos, neurons incorporating microinjected fluorescent tubulin were observed. The results showed that microtubules themselves remain stationary among neurons, but that the moving form of tubulin travelling down the axon could be small tubulin oligomers or heterodimers [54]. Recent studies confirm these findings, showing that tubulin molecules were transported in squid giant axons in a unique form of large transporting complex composed of oligomeric tubulin and kinesin. This complex is clearly distinct of stable microtubules [55]. The basic mechanism described in these two studies is in fact treadmilling and suggests that the cytoskeleton is exported in neurites as chemical waves (treadmilling microtubules) that are stabilized to generate persistent microtubules when reaching their target location.

Despite extensive similarity with intrinsic microtubule behaviour in vitro, microtubules in vivo have specificity linked to tight polymer control by cell effectors. Cell regulation affects both microtubule nucleation and assembly. Nucleation in vivo does not occur randomly in the cytoplasm; it is organized by the centrosome (also called microtubule-organizing center). In most cells, the minus ends of microtubules are usually tethered at the centrosome. Centrosomes are complex structures composed of two centrioles (which are themselves microtubular structures), and of the so-called pericentriolar material, consisting of complex and still poorly defined protein assemblies. Microtubule nucleation is thought to occur on γ-tubulin ring complexes, containing 13 molecules of γ-tubulin proteins (γ-TuRC) tethered at the centrosome [56, 57]. Recent work using electron microscopy shows that γ-TuRC could act as a helical template that constitutes the basis of the newly formed polymer [58–60]. In vitro, monomeric γ-tubulin has been demonstrated to have the capacity to nucleate microtubules [61]. However, it seems that microtubule nucleation can also occur independent of γ-tubulin, and more experiments are needed to understand the overall mechanisms of centrosomal nucleation.

Microtubule-regulating proteins

Microtubule dynamics in vivo are regulated by many microtubule-associated proteins, and these are usually classified into two main groups: proteins that stabilize microtubules and proteins that destabilize microtubules. Recent studies have allowed a better mechanistic dissection of how these factors regulate microtubule dynamics. They have also highlighted other functions of these proteins, especially with regard to the regulation of the spindle assembly. New groups of proteins have emerged. In one of them, proteins are localized at the tip of microtubules, and they regulate the overall microtubule dynamics and organization in vivo, depending on the cellular context [62–64]. Another group of proteins [65–67] can interact not only with microtubules but also with the other cytoskeletal networks, namely actin and intermediate filaments. Finally, recent work has shown that cells use motor proteins to modulate microtubule dynamics.

Microtubule-stabilizing proteins

Within the microtubule-associated proteins (MAPs), first described as structural proteins (for a review, see [68]), several mammalian MAPs promote tubulin assembly and stabilize microtubules, although they do not suppress disassembly. Members of this family include the neuronal proteins tau and MAP2, which localize to the axon and the dendrites, respectively, and MAP4, which is present in all nonneuronal vertebrate cells. In all three proteins, the microtubule binding domain is contained within the C-terminus, whereas the N-terminal domain protrudes from the microtubule surface. The effect of MAPs on microtubule stability depends on phosphorylation, and all structural MAPs have been shown to be in vitro substrates for several protein kinases. For instance, microtubule-affinity-regulating kinases have been described as mammalian serine/threonine kinases that phosphorylate the tubulin binding domain of MAPs, cause their detachment from microtubules and therefore increase microtubule dynamics [69]. At most of the sites phosphorylation tends to weaken microtubule binding, but the extent of the cor-

responding effect differs considerably. Up to now, the complicated relationships involving protein kinases, phosphorylation sites, their effect on MAP-microtubule binding and the resulting impact on microtubule dynamics have not been well established.

As judged from immunohistochemistry experiments, the nervous system of tau-deficient mice appears to be normal. In cultured neurons, axonal elongation is not affected. In some small-calibre axons, microtubule stability is decreased and microtubule organization is significantly changed. It has also been postulated that the observed increase in MAP1A could compensate for the functions of tau in large-calibre axons. Thus, tau does not seem to have a role in axonal elongation, but its expression is necessary for the stabilization and organization of axonal microtubules of certain axons [70].

Gene targeting has also been used to disrupt the murine MAP1B gene. Homozygous mice showed a slightly decreased brain weight and a delay in the development of the nervous system. Hence, MAP1B is not essential for survival, but it is essential for the normal time course of brain development [71].

More recently, another group of microtubule-associated proteins inducing a much higher microtubule stability has been described. This group includes Lis1 and doublecortin, BPAG1, and STOP proteins.

Mutations in either Lis1 or doublecortin are the most common cause for type I lissencephaly, a human brain malformation characterized by a smooth cerebral surface and a disordered organization of the cortical layers resulting in a defect in neuronal migration (for a review see [72]). The Lis1 protein is a subunit of the platelet-activating factor acetylhydrolase, and in vitro experiments showed that the protein interacts with tubulin and microtubules. It reduces microtubule catastrophe events, and this leads to an increase in the maximum length of the microtubules [73]. Lis1 is distributed along microtubules and is not restricted to neuronal cells. However, recent results show that Lis1 is enriched in neurons relative to other cell types, and that Lis1 interacts with the microtubule motor dynein. Production of more Lis1 protein in nonneuronal cells increases retrograde movement of cytoplasmic dynein and leads to peripheral accumulation of microtubules. These results suggest that the amount of Lis1 protein in neurons may stimulate specific dynein functions in neuronal migration and axon growth. [74]. Homologues for Lis1 have been described, especially in *Aspergillus nidulans* (homologue named *nudF*), *Saccharomyces cerevisiae* and *Drosophila*. Mutations of *nudF* caused a severe defect in the migration of nuclei into and within the hyphal processes, resulting in grossly diminished colony size [75].

Doublecortin is expressed in migrating and differentiating neurons [76]. It is also directly associated with microtubules in vitro and in vivo and stabilizes them. Its sequence does not contain any known microtubule binding domain, suggesting that it defines a new microtubule-binding structure [77]. The interaction of doublecortin with microtubules is dependent upon a novel repeated tubulin binding motif, and missense mutations in that region of the protein shown to be present in patients lead to impaired microtubule assembly both in vitro and in vivo, as well as impaired microtubule stabilization [78]. Lis1 and doublecortin proteins interact physically both in vitro and in vivo, and they have been shown to interact with tubulin and microtubules. The addition of both proteins to tubulin enhances microtubule assembly in an additive fashion [79]. Taken together, these results emphasize the central role of regulation of microtubule dynamics and stability during neuronal morphogenesis.

DCAMKL1, a protein kinase with homology to doublecortin, has recently been identified. It associates with microtubules and stimulates assembly of purified tubulin [80]. DCAMKL1 is expressed throughout the central nervous system in migrating neuronal populations, and its expression overlaps with doublecortin on microtubules. These data suggest that DCAMKL1 and doublecortin may act together to regulate microtubules in migrating neurons.

Proteins have recently been identified that have the capacity to physically link actin, intermediate filament and microtubule networks. The best characterized are plakins, an emerging family of sequence-related cross-linker proteins that include plectins, the bullous pemphigoid antigen-1 proteins (BPAG1s), AC7 (referred to as kakapo in *Drosophila*) [65, 66]. Microtubule binding sites have been identified in several plakins, including a form of BPAG1 (BPAG1n3) and several ACF7 isoforms [81, 82]. In transfected cells, BPAG1 binds and stabilizes microtubules, which become resistant to depolymerizing agents, including cold. In mice, deletions within the BPAG1 gene result in severe neuronal degeneration throughout the peripheral nervous system. Dorsal root ganglion axons exhibit dramatic disorganization of neuronal intermediate filaments and microtubules accompanied by abnormal axonal myelination and axonal swellings [66].

ACF7/MACF (microtubule actin cross-linking factor) contains a plakin-like domain highly homologous to BPAG1, and a putative actin binding domain. The carboxy-terminal domain of the protein interacts with and stabilizes microtubules. In transfected cells MACF associates with both actin and microtubules [82]. Recent studies show that MACF is predominantly expressed in neural, muscle and lung development [83]. The closest relative of MACF is the *Drosophila* protein kakapo, a plectin-homologue protein essential for neuronal growth and adhesion between and within cell layers. Consistent with the properties of MACF, kakapo mutants cause disorganization of microtubules in epidermal muscle attach-

ment cells and some sensory neurons. Remarkably, kakapo has lost the entire intermediate filament binding domain.

Plectin, a cytoskeletal linker protein of very large size (>500 kDa), has been shown to copurify with microtubules assembled from extracts of cultured rat glioma C6 cells, and to bind to MAP fraction from brain (MAP2, and MAP1 subsets). The coassembly of plectin with microtubules probably occurred indirectly via its binding to MAPs. However, a direct interaction of plectin with microtubules cannot be ruled out, especially in cells that do not express neuronal MAPs (for a review, see [67]).

In a number of cell types, cytoplasmic microtubules are resistant to prolonged cold exposure. Cold stability is due to microtubule association with different variants of a calmodulin-regulated protein called STOP protein (fig. 6) (for a review, see [84]). The dynamic and hence physiological consequences of STOP association with microtubules vary in different tissues (fig. 6). There are several STOP variants, generated from a single gene by the use of alternative start sites for transcription. These variants are differentially expressed in different tissues and at different stages of development. All known STOP variants share the ability to interact with calmodulin and microtubules. STOP proteins have a different effect on microtubule turnover in neuronal cells compared with cycling cells. In neuronal cells, STOP proteins seem to be almost permanently associated with microtubules and are major factors responsible for the slow turnover of neuronal microtubules. STOP function is apparently required for neuronal differentiation. In cycling cells, only minor amounts of STOP are associated with interphase microtubules, and STOP does not measurably affect microtubule dynamics. However, STOP is associated with mitotic microtubules in the spindle. Such an association could be vital for meiosis and for the long-term fidelity of the mitotic process. Finally, knockout of the STOP gene leads to major behavioral abnormalities in mouse, [A. Andrieux et al., unpublished].

Three other microtubule-associated proteins were purified from *Xenopus* eggs, and they have distinct effects on microtubule assembly in vitro. XMAP230 is a potent microtubule stabilizer, increasing microtubule elongation rate and lowering the rate of catastrophe in vitro.

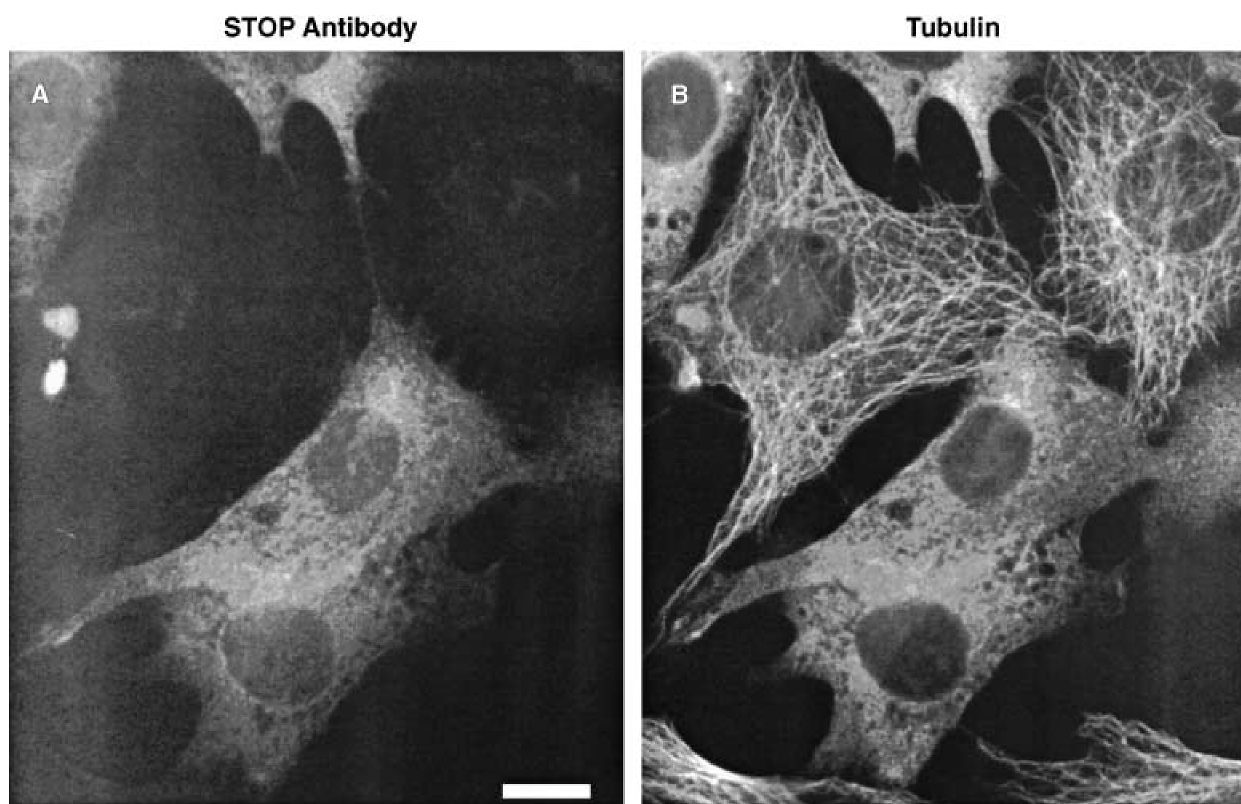

Figure 6. STOP proteins prevent microtubule disassembly in cells exposed to cold. NIH3T3 cells, constitutively expressing STOP proteins, were microinjected with STOP antibodies, incubated for 2 h, and subjected to cold (4 °C) during 30 min. Then, immunofluorescence was performed to detect STOP antibodies in microinjected cells (*A*) and tubulin in the whole cell population (*B*). Microinjected cells, visible in panel *A*, do not exhibit microtubule arrays in panel B, whereas non microinjected cells, invisible in panel A, still exhibit microtubule arrays in panel *B*, whereas nonmicroinjected cells, invisible in panel *A*, still exhibit microtubule arrays in panel *B*. STOP inactivation suppresses microtubule cold stability, showing that STOP proteins are major cell effectors responsible for such microtubule stabilization. Scale bar, 10 μm.

XMAP230 localizes to interphase microtubules, dissociates from microtubules at the onset of prophase and specifically binds to spindle microtubules during metaphase and anaphase [85]. XMAP310 promotes rescue in vitro. It localizes to the nucleus during interphase and is only associated with microtubules after nuclear envelope breakdown and entry in mitosis. It is phosphorylated during mitosis, but this observation has not yet been associated to a specific function [86]. XMAP215 promotes rapid microtubule assembly at plus ends [87]. XMAP215 speeds the microtubule plus-end growth rate by seven fold, mainly through an increase in the apparent on-rate constant, in contrast to other stabilizing MAPs, such as tau or MAP2 that increase growth rates about two-fold, primarily through a decrease of the rate of disassembly. Several homologues of XMAP215 have been identified in other organisms including proteins from yeasts, *Caenorhabditis elegans*, *Drosophila*. TOGp, a human homologue of XMAP215 has also been identified [88]. Mutations in several of the homologues result in mitotic effects, and it has been shown that XMAP215 and TOGp have a role in spindle or mitotic aster formation. Therefore, XMAP215/TOGp family proteins appear necessary for mitotic spindle assembly, probably through their microtubule-stabilizing activity. TOGp preferentially binds to microtubule ends, but is not limited to tips, in contrast to what is observed for CLIP-170 [89]. The microtubule binding domain of TOGp was found within the N-terminus, whereas the C-terminus contains a tubulin binding domain. The protein has also been shown to bind to sheetlike polymers and rings of protofilaments [90]. The mechanisms responsible for assembly promotion and catastrophe protection by XMAP215 are still not known. XMAP215 can counterbalance the activity of the destabilizing factor XKCM1, a member of the kinesin superfamily [91] (see below).

Microtubule-destabilizing proteins

Several types of proteins have been identified that destabilize microtubules through different mechanisms, when known.

Op18/stathmin is a small ubiquitous protein. It has been shown to destabilize microtubules in vitro by increasing the catastrophe frequency and to regulate microtubule levels both in *Xenopus* egg extracts and in tissue culture cells [92, 93].

Two mechanisms have been postulated by which Op18/stathmin destabilizes microtubules. Since Op18/stathmin exists in a ternary complex with two tubulin heterodimers [94], it has been proposed that Op18/stathmin acts as a sequestering protein, lowering the amount of tubulin available for assembly. The other possibility is that Op18/stathmin acts directly on microtubules as a catastrophe-promoting factor.

It is possible to dissociate in vitro the tubulin-sequestering and microtubule catastrophe-promoting activities by controlling the pH at which experiments are performed [95]. At pH 6.8, Op18/stathmin mainly acts through a mechanism of tubulin sequestration, but at pH 7.5 Op18/stathmin acts as a catastrophe promoter. The C-terminus of Op18/stathmin is required for tight binding to tubulin and the N-terminus is required for catastrophe activity.

Other studies in vivo have shown that mutations of a putative coiled-coil motif in Op18/stathmin poorly affect the ability of Op18/stathmin to interact with tubulin dimers, but significantly lowered the ability of Op18/stathmin to induce microtubule catastrophes [96]. In contrast, deletion of the C-terminal domain of Op18/stathmin causes an important decrease in tubulin binding, but the truncated protein remains nearly as effective as the wild-type protein in inducing microtubule disassembly [97]. Microinjections of anti-Op18/stathmin antibodies and antisense inhibition of Op18/stathmin result in a decrease of catastrophe frequency without affecting the growth rate of microtubules, suggesting that Op18/stathmin can induce catastrophes without affecting the amount of available tubulin [98]. All these results suggest that Op18/stathmin induces microtubule disassembly by a mechanism that mainly does not involve tubulin sequestration.

The molecular mechanism of microtubule destabilization in vivo may involve the alteration of the GTP state of the tips of microtubules [97]. Recent work in vitro shows that Op18/stathmin decreases GDP dissociation from tubulin depending on the phosphorylation state of Op/stathmin [99], and mutational analysis of Op18/stathmin involving regions interacting with tubulin showed that the binding cooperativity in the ternary complex controls tubulin GTP hydrolysis [100].

Op18/stathmin is multiply phosphorylated (for a review, see [101]). Phosphorylation site mutants of Op18/stathmin revealed that multiple phosphorylation events result in decreased microtubule-destabilizing activity. These multiple levels of phosphorylation of Op18/stathmin by different kinases might allow Op18/stathmin to play a central role in the rearrangement of the microtubule cytoskeleton in response to different signalling pathways. Phosphorylation of Op18/stathmin by mitotic cyclin-dependant kinases turns off the activity of this destabilizing protein, suggesting that Op18/stathmin activity decreases during the transition from interphase to mitosis. However, Op18/stathmin phosphorylation specifically in the vicinity of chromatin could be important for the stabilization of microtubules near chromosomes [102].

SCG10 is a neuron-specific protein highly enriched in growth cones. SCG10 has sequence homology with Op18/stathmin, but the protein is encoded by a different gene. SCG10 has been shown to bind to microtubules, to

inhibit their assembly and to induce their disassembly [103]. Non-phosphorylatable mutants had an increased microtubule-destabilizing effect, suggesting that the microtubule-destabilizing activity of SCG10 is regulated by phosphorylation just as in the case of Op18/stathmin [104].

A second class of proteins recently implicated in microtubule destabilization are certain members of the superfamily of kinesin-related microtubule motor proteins. XKCM1 is a kinesin-related motor protein isolated from *Xenopus* eggs. Loss of XKCM1 function in *Xenopus* extracts inhibited mitotic spindle formation [105], and purified XKCM1 protein was able to induce microtubule catastrophe in vitro [106]. XKIF2, a homologue of XKCM1, was also able to destabilize microtubules. These results suggest that several members of Kin I subfamily of kinesins might act as microtubule-destabilizing enzymes.

XKCM1 does not act as a conventional motor protein, but induces disassembly by a mechanism that does not involve direct motility [106]. XKCM1 was found to bind to both ends of the microtubule and to induce a conformational change in the microtubule lattice. This, resulted in a release of many tubulin dimers and a small number of tubulin dimer/XKCM1 complexes. Therefore, XKCM1 has the ability to interact with tubulin dimers as well as with assembled tubulin. ATP hydrolysis by XKCM1 is necessary to recycle the protein for a subsequent round of disassembly, and it has been shown that, in the presence of ATP, XKIF2 cannot bind to tubulin dimers.

Using *Xenopus* egg extracts, XMAP215 was shown to modulate the microtubule catastrophe frequency by opposing the microtubule-destabilizing activity of XKCM1, in interphase as well as in mitosis [91]. These results suggest that, at least in *Xenopus* egg extracts, microtubule dynamics could be regulated through a balance between stabilizing and destabilizing factors.

In the yeast *Saccharomyces cerevisiae*, two kinesin-related motors, Kar3p and Kip2p, have been shown to function antagonistically at spindle pole body by influencing the cytoplasmic microtubule number [107]. Kar3p is a minus-end-directed kinesin that can disassemble taxol-stabilized microtubules from their minus end in vitro [108]. In vivo loss of Kar3p motor stimulates cytoplasmic microtubule growth, whereas loss of Kip2p leads to a sharp reduction in cytoplasmic microtubule numbers [109].

Other proteins have also been shown to destabilize microtubules in in vitro experiments. Recent data revealed that the HIV-1 Rev protein can interact with microtubules and disassemble them to form stable bilayered rings [110]. The interaction involves the N-terminal domain of Rev and the face of tubulin exposed on the exterior of the microtubules. The N-terminal half of Rev exhibits sequence similarity to the tubulin-binding portion of the catalytic/motor domains of Kin I kinesin. These results may be consistent with the described disruption of microtubules after human immunodeficiency virus HIV-1 infection.

Heat-shock proteins HSP27, HSP70 and HSP90 were characterized as microtubule-associated proteins (for a review see [111]). The effect of HSP70 on microtubule dynamics remains controversial. HSP90 was shown to inhibit microtubule formation in vitro in the presence of taxol, although at high concentration only [112]. Several enzymes of glycolysis significantly bind to tubulin and to microtubules [113]. One of these enzymes, pyruvate kinase, inhibits taxol-induced tubulin assembly [114]. G protein α subunits were shown to activate the GTPase activity of tubulin, to inhibit microtubule assembly and to increase microtubule dynamic instability in vitro [115].

Finally, a small acidic polypeptide, MINUS, biochemically purified from cultured neural cells and bovine brain, was described to inhibit nucleation of tau and taxol-mediated microtubule formation in vitro, and to be inactivated by dephosphorylation [116]. Results indicate that MINUS blocks microtubule nucleation, but has no effect on microtubule growth and stability both in the presence and absence of centrosomes in vitro. Microinjection of MINUS in cells causes a transient disassembly of microtubules. Therefore, MINUS may function as a microtubule nucleation regulator, being regulated itself through its phosphorylation state.

Microtubule end-binding proteins

The EB1 family is a highly conserved group of proteins, localized to the centrosome, the mitotic spindle and the distal tips of cytoplasmic microtubules (for a review, see [62]). Recent work has shown in a cultured epithelial cell line that the distribution of EB1 may be variable. EB1 is present at the end of 75% of these microtubules containing predominantly tyrosinated tubulin, but only of 12% of microtubules containing a posttranslational modified form of tubulin known as detyrosinated tubulin [117]. The interaction of EB1 with the well-characterized tumor suppressor protein APC (adenomatous polyposis coli) has been demonstrated for the first time by yeast two-hybrid analysis. More recent findings show that the interaction of APC and EB1 targets APC to microtubule tips. The interaction between the two proteins is downregulated during mitosis, APC being phosphorylated and dissociated from microtubules [118, 119].

Indications for a potential role of EB1 on microtubule dynamics have been obtained from yeast studies. Budding yeasts possess a single EB1 sequence homologue, *BIM1*. In bim1p lacking yeast, microtubules were shorter, disassembled more slowly, showed less dynamic instability and longer pauses than in wild-type cells, resulting in globally less dynamic behaviour. These cells were also defective in mitotic spindle positioning. The first step of

the spindle orientation process consists of spindle movement to the yeast bud neck, resulting from cytoplasmic microtubule capture and end-on depolymerization at the bud tip. Consistent with its localization to the microtubule tip, Bim1p is a central component of this step. Bim1p both increases microtubule dynamicity and, interacting with the cortical protein Kar9p, forms a physical link between the microtubule end and the bud [120, 121]. Therefore, these results show that bim1p exerts a positive activity on the global dynamics of microtubules, and plays an essential role in mitosis in virtue of the correct positioning of microtubules within the cell.

CLIP-170 is a human protein which was initially described as linking microtubules to endosomes and was also found to associate with the desmosomal plaques in epithelial cells. CLIP-170 is localized in dots along microtubules and accumulates at the plus ends of growing microtubules, and not at the end of shrinking ones [122, 123]. The functional significance of CLIP-170 microtubule end localization remains unclear. However, it has been suggested that CLIP-170 might influence microtubular dynamics by promoting growth toward a microtubule target and serve as a capturing device once that target has been found [122]. Recently, similar data were obtained from tip1p, a CLIP170 protein family member [63] of the fission yeast *Schizosaccharomyces pombe*. Tip1p is located at the distal tip of microtubules, and is required to guide microtubules to their target zone located at the cell end [64]. In the absence of tip1p, microtubule catastrophe is no longer restricted to cell ends but occurs when microtubules reach any region of the cellular cortex. In addition, microtubules are shorter and no longer organized along the longitudinal axis. Thus, tip1p plays a crucial role in establishing overall microtubule organization within the cell [64].

Conclusion

The microtubule system has emerged over the years as a biological system of remarkably complex physical chemistry. Tubulin and microtubules have provided the first clear example of self-organization through reaction-diffusion mechanisms in a biological system involving only one protein and a few chemical reagents (principally GTP). It is intellectually satisfying that the special intrinsic properties of microtubules can be conceptually related to their main biological functions: generating organelle movement and organizing the cell's interior. It is also remarkable that microtubule dynamics in vitro and in vivo share extensive phenomenological similarity. Therefore, one may conclude that the intrinsic dynamic properties of microtubules are used by cells. However, such similarity should not obscure the apparent immense complexity of microtubule-regulating cascades in cells. It is now evident that cells control the microtubule system at all levels, including tubulin dimers, tubulin oligomers and microtubules themselves. Many cellular control mechanisms are mediated by regulatory proteins. The list of tubulin and/or microtubule-associated proteins has been rapidly growing over the past few years. Yet, most scientists in the field believe that we have seen only the tip of a huge iceberg.

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