Microtubules and Microtubules Associated Proteins

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SUMMARY/ABSTRACT

Microtubules act as railways for motor-driven intracellular transport, interact with accessory proteins to assemble into larger structures like the mitotic spindle, and provide an organizational framework to the rest of the cell. Key to these functions is the fact that microtubules are *dynamic*. As with actin, the polymer dynamics are driven by nucleotide hydrolysis and influenced by a host of specialized regulatory proteins. However, microtubule turnover involves a surprising behavior termed *dynamic instability*, where individual polymers switch stochastically between growth and depolymerization. Dynamic instability allows microtubules to explore intracellular space and remodel in response to intra- and extracellular cues. It is central to the assembly of many microtubule-based structures and to the robust functioning of the microtubule cytoskeleton.

INTRODUCTION

Along with actin (Chapter 2) and intermediate filaments (Chapters 4 and 9-12), microtubules (**Figures 1-2**) constitute one of the three main classes of cytoskeletal filaments in eukaryotic cells. Microtubules are found in all characterized eukaryotic organisms. Thus the last common ancestor of eukaryotes had microtubules; this ancestor also had the dynein and kinesin motors that operate on the microtubule cytoskeleton (Chapter 5). Many prokaryotes have at least one gene homologous to tubulin, the most common of which is FtsZ, a protein that forms polymers involved in cytokinesis. These observations suggest that the tubulin gene family appeared very early, perhaps in the last common ancestor of all forms of life on earth (Chapter 1). Even without knowing anything else about microtubules, the maintenance of these structures and their constituent proteins across such a large span of time and in highly divergent organisms indicates that they have a fundamental role in eukaryotic cell biology.

Indeed, microtubules and their accessory proteins form the *mitotic spindle* – the dynamic self-organized machine that separates the chromosomes during mitosis, arguably the most important of all eukaryotic cell processes (Chapter 23). In addition, complexes of microtubules and motors form the core of *cilia* and *flagella* (Chapter 21), making

microtubules essential for motility of many organisms, including numerous protists and most metazoan sperm. Microtubules also provide tracks for motors that catalyze the movement of organelles, transport vesicles, and other structures (Chapter 16). This microtubule-based intracellular transport contributes to the efficient function of many organisms and cell types, but it is crucial for the dramatically elongated neurons of animals. Microtubules also play fundamental roles in cell organization by localizing organelles and establishing the polarity of a wide variety of cells in both animals and plants (Chapter 16).

How do microtubules contribute to these diverse cellular activities? As described below and elsewhere in this book, there are many answers to this question, only some of which are well understood. One central theme is that the dynamic behavior of microtubule polymers is essential to many microtubule-based processes. Briefly, structures assembled from microtubules and actin filaments usually have longer lifetimes than the individual polymers from which they are assembled. In both cases hydrolysis of nucleotides bound to the polymer subunits drives their turnover. However, the patterns of turnover differ. Actin filaments *in vivo* typically grow at their barbed-end and disassemble at their pointed-end as a result of multiple reactions including severing by accessory proteins[1]. In contrast, microtubule polymers usually (but not exclusively) display a surprising behavior termed *dynamic instability*, where individual polymers switch stochastically (i.e., randomly) between growth and shortening. As explained below, this dynamic instability behavior is fundamental to many of the functions and properties of the microtubule cytoskeleton.

Because microtubule function and behavior derive ultimately from the structure and biochemistry of the microtubule filaments, this chapter starts with a description of their structure and biochemistry. The chapter then discusses the subcellular structures that form from microtubules, and returns to examine in more depth dynamic instability and its mechanism. The chapter concludes with a discussion of the proteins that regulate microtubule dynamics and interact with microtubules to interface with the rest of the cell.

[1] *In vitro,* purified actin can by itself slowly treadmill (undergo assembly at one end and disassembly at the other). In vivo, treadmilling of the actin network is driven by proteins that regulate assembly, capping, severing and depolymerization (see Chapter 2).

MAIN TEXT

PHYSICAL ATTRIBUTES OF MICROTUBLES AND TUBULIN

Microtubule Structure

Microtubules are assembled from heterodimers of alpha and beta tubulin into long hollow polymers that are ~25 nm wide and range in length from less than 1 μm to more than 100μm (**Figure 2**). These heterodimeric tubulin subunits are referred to as αβ-*tubulin*, *tubulin dimers*, or simply *tubulin*.

Microtubule structure is most straightforwardly described as being composed of ~13 linear *protofilaments* (PFs) that are associated laterally and closed into a hollow tube. The resulting polymer is polar, with a fast growing *plus-end* that has exposed β tubulin, and a slowly growing *minus-end* with exposed α tubulin (**Figure 2A**).

In typical 13-PF microtubules, the boundary where the tube closes is unlike the other interfaces between protofilaments, creating a *seam* where the lateral interactions between protofilaments differ from those elsewhere in the microtubule (**Figure 2A**). This seam is generally believed to be a weak point of the microtubule structure, although there is some evidence to the contrary (Sui and Downing, 2010). While most microtubules *in vivo* have 13-PF, there are some exceptions, and microtubules assembled *in vitro* can have a wide range of protofilament number. One striking difference between the typical 13-PF microtubules and those with 15- or 16-PFs is that the 15- and 16-PF microtubules do not have a recognizable seam (**Figure 2C**).

The number of protofilaments and presence/absence of a seam might seem like arcane details, but the idea that PF number can have physiological relevance is supported by the observation that microtubules with consistently different numbers of PFs do appear in nature (reviewed by Sui and Downing, 2010). In addition, the issue of protofilament number has practical significance for researchers because the presence of a seam interferes with structure determination by helical reconstruction-based methods. In contrast, the

seamless body of 15- and 16-PF microtubules is fully symmetric (**Figure 2C**). Thus, *in vitro* methods to produce (limited numbers of) 15- and 16-PF microtubules can be quite useful for determination of the structure of microtubules and their binding proteins, such as motors (e.g. Arnal et al., 1996).

Although protfilament-based descriptions of microtubule structure are easiest to visualize, microtubules are sometimes described in terms of their lattice structure and/or helical structure (**Box 1**).

Box 1. Description of microtubule structure in terms of lattices and helices. The main body of a typical 13-protofilament microtubule can be described as being composed of a so-called *B lattice*, in which alpha subunits are next to alpha subunits (α-α) and beta next to beta (β-β). However, at the seam, the alpha subunits associate laterally with beta subunits in the adjacent protofilaments (α-β) in an *A lattice* (**Figure 2A-B**). The lattice structure changes at the seam because each protofilament in the main body of a microtubule is shifted slightly relative to its neighbor, resulting in an offset at the seam of 1.5 dimers for a 13-PF microtubule (**Figure 2C**). Changing the number of protofilaments changes the offset, so that the offset for microtubules with 15- or 16-PFs is two full subunits, resulting in a situation where there is no discernable seam (these microtubules are composed entirely *B lattice:* α-α and β- β).

In addition, microtubules are sometimes described as helices, although the presence of a seam means that a 13-PF microtubule is not a true helix: it just appears to be one in low resolution electron microcopy images where α and β tubulin are indistinguishable. Given this ambiguity, a 13-PF microtubule can be described as a left handed *3-start helix* because each of the three monomers in the 1.5 tubulin dimer offset at the seam can be viewed as starting a new helix (**Figure 2C**). As noted above, changing the number of protofilaments changes the offset, so that the offset for microtubules with 15- or 16-PFs is two full subunits. This situation results in a *4-start helix*, where as noted above *B lattice* is found uniformly through the microtubule (**Figure 2C**, see also Amos, 2004).

Although considering microtubules as (pseudo) helices can be useful in structural studies, it is usually more informative to view microtubules as protofilament-based structures because a consensus is building that microtubules grow by adding subunits to these linear protofilaments, not by extending the helixes. This idea is based in part on evidence that the longitudinal interactions between subunits within a protofilament are stronger and more extensive than the lateral bonds between subunits in different protofilaments (Sept et al., 2003; Zhang et al., 2015). The predominance of these longitudinal bonds means that interactions within a given protofilament are more significant than those within a given helix.

Several additional aspects of microtubule structure are functionally significant. First, the surface of microtubules is negatively charged, because the *C-terminal tails* of α and β tubulins contain several acidic residues and are located on the outer surface. These C-terminal tails (also called *E-hooks* because they are glutamate-rich) are key sites of interaction for many microtubule binding proteins (summarized by Roll-Mecak, 2015). Second, while the walls of microtubules are often represented as being solid, high resolution structural analysis shows that microtubule walls contain holes large enough to allow diffusion of water and small molecules like Taxol (Li et al., 2002). The inside of a microtubule is an intriguing space that is as yet relatively unexplored in terms of its significance and potential interactions. Some EM images of microtubule cross-sections have visualized densities of unknown composition inside microtubules (Garvalov et al., 2006); one explanation of these luminal structures is that they are enzyme complexes involved in post-translational modification of tubulin subunits, such as acetylation (e.g. Soppina et al., 2012).

Microtubules are Structurally Rigid

Microtubules and actin filaments both have physical properties similar to the stiff plastic plexiglass, but microtubules are much more rigid owing to their larger diameter and tubular construction. To make this comparison more quantitative, the persistence length of microtubules is ~5000μm compared with ~20μm for actin filaments (Gittes et al., 1993; Hawkins et al., 2010). The rigid nature of microtubules is hard to reconcile with the observation that microtubules are often highly curved *in vivo*. One explanation is the curvature results from forces exerted by motors and connections to other cytoskeletal elements. Alternative explanations include the idea that curvature is induced by lattice defects (i.e., missing and/or mis-incorporated subunits) and/or binding of microtubule binding proteins. Mechanical aspects of microtubules are discussed in more depth in (Hawkins et al., 2010).

Isoforms of Tubulin are Specialized for Specific Functions

Microtubules are composed of subunits consisting of heterodimers of αβ-tubulin, an

arrangement that appears to be ubiquitous in eukaryotes (see Chapter 1 for a discussion of microtubule related proteins in prokaryotes). However, most eukaryotic cells also contain multiple tubulin isoforms, many of which are both ancient in origin and fundamental to aspects of microtubule function as explained briefly below. For more detailed discussion of the tubulin superfamily (see Findeisen et al., 2014; McKean et al., 2001).

- α **and** β **tubulin** assemble into obligate heterodimers that form the body of cytoplasmic microtubules (**Figure 2A**) in all eukaryotic organisms characterized thus far. Both bind the nucleotide GTP, but only the β subunit hydrolyzes its GTP in the course of normal microtubule polymerization. Folding of α and β tubulin requires the assistance of a set of dedicated chaperones (Tian and Cowan, 2013).
- γ **tubulin** is a key part of the machinery that nucleates the growth of new microtubule structures (**Figure 2D;** more about this below). Like α and β tubulin, γ tubulin has been found in all free-living (i.e., non-parasitic) eukaryotic organisms examined, and the combination of these three proteins appears to form the minimal set of eukaryotic tubulins (Findeisen et al., 2014; Gull, 2001).
- δ, ε, and ζ **tubulin** isoforms are found in cilia, flagella and/or basal bodies, and in general they are specific to organisms with these structures. These ancient proteins have been identified in some of the most divergent known eukaryotic organisms, but they have also been lost from many lineages (Findeisen et al., 2014; McKean et al., 2001).
- Other tubulin isoforms (e.g. η) have been described, but appear to be limited to specific lineages (Findeisen et al., 2014).

New Microtubules Appear Through the Process of Nucleation

Given the complex structure of a microtubule, one might imagine that generation of new microtubules is a difficult process. Indeed, spontaneous appearance of new microtubules is rare in solutions of pure αβ-tubulin heterodimers unless tubulin concentrations are high. Nucleation of pure tubulin is an unfavorable and highly cooperative process, with the nucleation rate depending on the some large power (6-12) of the tubulin concentration (Caudron et al., 2002). This naturally suppresses spontaneous nucleation and allows cells to regulate when and where new microtubules will be assembled. Careful control of the

localization and activity of microtubule nucleators plays an important role in generating different types of microtubule arrays (e.g., **Figure 1**).

Generation of new microtubules *in vivo* involves the action of specialized nucleation machinery, generally associated with γ-tubulin. The most well-known and intuitively understandable nucleator is the *gamma tubulin ring complex* (γ-TuRC), a lock-washer shaped structure that appears to act as both a template for microtubule assembly and a cap for the minus-end (**Figure 2D**). γ-TuRC is well-characterized biochemically in vertebrates, but components are found in many organisms including fungi and plants, so involvement of γ-TuRC in nucleation appears to be ancient and widespread (Kollman et al., 2011).

While most well-characterized examples of microtubule nucleation *in vivo* involve γ-tubulin, additional mechanisms for increasing microtubule numbers exist. Many microtubule-stabilizing proteins have nucleation activity *in vitro*. However, it is not clear whether these proteins nucleate microtubules *in vivo* or simply stabilize new microtubules nucleated by other mechanisms. More significantly, augmin can promote nucleation of new microtubules from the sides of pre-existing microtubules (Petry et al., 2013). The significance of this side nucleation is still being debated, but it has been implicated in the functioning of the mitotic spindle and beyond (Sanchez-Huertas and Luders, 2015). Finally, microtubule severing proteins, such as katanin, can potentially increase microtubule number by breaking existing microtubules into multiple pieces (discussed in Ehrhardt and Shaw, 2006). The contributions of these mechanisms to the function of the microtubule cytoskeleton are still being elucidated.

Microtubules Undergo Post-translational Modifications

One curious and as-yet poorly understood aspect of microtubule structure is that tubulin subunits undergo a series of post-translational modifications. These include common modifications like phosphorylation, acetylation, and sumoylation, as well more unusual and/or tubulin-specific modifications such as detyrosination (removal of the C-terminal

tyrosine) and polyglutamylation (addition of free glutamates to the side chain of a glutamate in the polypeptide; reviewed by Garnham and Roll-Mecak, 2012; Janke and Bulinski, 2011). It is interesting to note that most of these modifications occur on the residues of the C-terminal E-hook; an exception is acetylation, which occurs in the microtubule lumen (Soppina et al., 2012). Experiments in a wide range of organisms and cell types have shown that in many cases the modifications are more common in older (stabilized) polymers. It is tempting to think on the basis of this observation that the modification(s) cause the stabilization. However, tests of this hypothesis have generally been inconclusive, so at present it is safest to conclude only that many modifications correlate with polymer stabilization.

In contrast, it is clear that the modifications can alter the affinity of binding proteins for microtubules, so it is possible that the partial correlation between modification and stability is mediated by microtubule binding proteins. For example, detyrosination inhibits binding of both the plus-end tracking protein EB1 and depolymerizing motor MCAK, while increasing binding of the transport motor kinesin-1 (reviewed by Garnham and Roll-Mecak, 2012). A number of studies have suggested that other post-translational modifications influence the affinities of motors for microtubules, although interpreting this work is complicated (reviewed by Janke and Bulinski, 2011). Though much work remains to be done, an emerging notion is that post-translational modifications create chemical marks along stabilized microtubules that specialize them for specific functions. Determining the functional significance and spatiotemporal regulation of microtubule modifications is an important focus for future study.

STRUCTURES FORMED FROM MICROTUBULES

The Interphase Microtubule Array

The interphase array of microtubules (usually dynamic, sometimes stabilized) helps to determine cell shape and organization and acts as a substrate for motor driven intracellular transport. The organization of the interphase microtubule array varies by cell

type and organism (**Figure 1**). In many cell types ranging from vertebrate fibroblasts to *Dictyostelium* amoebas, the interphase microtubule cytoskeleton is a radially organized structure that emanates from a centrally located *microtubule organizing center* (MTOC, see below) (**Figure 1A**). In radially organized cells, the plus-ends of the microtubules are oriented towards the cell boundary, with the minus-ends embedded in the MTOC.

While radial organization of the microtubule cytoskeleton is common and is sometimes presented as canonical, it is by no means universal. As one example of a different arrangement, the microtubule array in vertebrate polarized epithelial cells has a more parallel organization, with the microtubule minus-ends towards the apical membrane and the plus ends towards the base of the cell (**Figure 1B**) (Bartolini and Gundersen, 2006). In higher plant cells (*e.g., Arabidopsis*), microtubules are found in cortically associated parallel arrays oriented transverse to the axis of cell elongation (Ehrhardt and Shaw, 2006) (**Figure 1D**).

The organization of the microtubule array is important in most cells because it plays a central role in determining the organization of the rest of the cell. For example, when microtubules are oriented radially as in fibroblasts, the membranes of the Golgi apparatus are generally located near the MTOC, with most other membranes distributed more peripherally. In contrast, the parallel microtubule arrays endow polarized epithelial cells with a more linear internal organization with the Golgi membranes at the apical face, and other membranes located more basolaterally (Bartolini and Gundersen, 2006). In these cells and others, loss of microtubules leads to loss of normal internal organization as well other aspects of cell polarity (de Forges et al., 2012). For example, many animal cells can move without microtubules, but lose directionality (Ganguly et al., 2012), and plant cells with depolymerized microtubules grow aberrantly (Ehrhardt and Shaw, 2006).

How is microtubule organization established and maintained? These processes are not yet well-understood, but the microtubule array as observed in a particular cell emerges from interactions between dynamic microtubules, their nucleators, their regulators, microtubule

motors, and the cell boundary (**Box 2**).

Box 2. How is the organization of the microtubule network established and maintained?

The obvious answer is that microtubule organization depends on localization of the microtubule organizing center, but functional bipolar spindles can form in cells even after the centrosomes have been removed by microsurgery (Khodjakov and Rieder, 2001), and a number of cell types (most obviously plant cells) lack centrosomes (Ehrhardt and Shaw, 2006). Moreover, spindle-like bipolar structures can form around artificial chromosomes *in vitro* in the absence of centrosomes (Heald et al., 1996). Examination of gamma tubulin localization in these and other non-centrosomal systems leads to a related proposal: perhaps microtubule organization depends on the localization of the microtubule nucleators. Centralized nucleation machinery correlates with radial organization, and distributed nucleation machinery correlates with distributed networks (**Figure 1**). While compelling, this explanation raises the next question: what determines the localization of the nucleators?

A hint to resolving this conundrum is provided by the observation that mixtures of stabilized microtubules and purified active motors can spontaneously self-organize into a range of different structures; the details of these structures depend on the specific activities and ratios of the proteins involved (Surrey et al., 2001). Motor-driven microtubule organization is also involved in the formation of dynamic radial microtubule arrays in melanophore cell fragments (Vorobjev et al., 2001).

However, it is important to remember that other aspects of the cellular environment can also influence the microtubule cytoskeleton. The pigment granules in melanophores appear to participate in microtubule organization (Vorobjev et al., 2001). The Golgi apparatus can nucleate microtubules and/or act as an MTOC (de Forges et al., 2012). Moreover, the signal transduction pathways that were originally hypothesized by Mitchison and Kirschner to allow selective stabilization of microtubules near particular regions of the cell boundary (Kirschner and Mitchison, 1986) are becoming elucidated (Akhmanova et al., 2009). Finally, it is important to understand that the physical barrier presented by the cell boundary can itself influence microtubule organization and dynamics (Maly and Borisy, 2002; Gregoretti et al., 2006; Dogterom and Surrey, 2013).

In summary, it is becoming apparent that the interphase microtubule array emerges from dynamic interactions between microtubules, motors, their regulators, and their physical environment. Gaining a deeper understanding of how particular large-scale structures emerge from local interactions will likely require computational modeling and other approaches used to study complex systems.

Other Subcellular Microtubule-based Structures

The mitotic spindle (**Figure 1 G-H**) is the complex and beautiful self-assembled machine

that separates the chromosomes in all eukaryotic cells. The mitotic spindle is composed of dynamic microtubules, a wide array of motors, and a series of other microtubule associated proteins. The mitotic spindle forms when the interphase microtubule array undergoes a dramatic reorganization upon entry into mitosis. The assembly, activities, and properties of the mitotic spindle have been and continue to be the subjects of intense study, and they are discussed in more detail in Chapter 23.

MTOCs, centrosomes, and spindle pole bodies are various names given to localized foci of microtubule nucleating machinery (see Chapter 8). Microtubule Organizing Center (MTOC) is a term that applies to all of these structures, while centrosome usually applies more specifically to the perinuclear MTOC of radially organized cells, and spindle pole body applies to the nuclear-membrane embedded MTOC of fungi such as *S. cerevisiae* and *S. pombe*. While centrosomes and spindle pole bodies have similar functions in terms of microtubule nucleation and contain many similar proteins, their ultrastructure is quite different (Adams and Kilmartin, 2000). MTOCs contain gamma tubulin and the gamma tubulin ring complex (γ-TURC, **Figure 2D**), but may contain a complex array of other proteins such as motors and +TIPs and can include *centrioles* (see below). In the past, centrioles were thought to be fundamental to the function of MTOCs, but many organisms (e.g., most higher plants) lack centrioles, and fly mutants lacking centrioles develop in a largely normal way (Basto et al., 2006). One explanation for the frequent association between centrioles and centrosomes is that the co-localization of these structures keeps the microtubule nucleating activities organized in a single focus.

Flagella and cilia (Chapter 21) are complex organelles that are composed of highly organized arrangements of microtubules, motors, and other proteins. Structurally they are similar, but they can differ in terms of attributes such as their function, motion, length, and details of their protein composition. Flagella and cilia are highly conserved and ancient organelles, existing in similar form in organisms ranging from humans to some of the most divergent protists (Carvalho-Santos et al., 2010).

Centrioles and Basal bodies are complex structures typically composed of nine sets of triplet microtubules and a set of well-conserved associated proteins (Chapter 8), although with some variation (Carvalho-Santos et al., 2011). They are found at the base of flagella and cilia (where they are called basal bodies) and in centrosomes (where they are called centrioles). Centrioles and basal bodies can interconvert as cells pass through the cell cycle. Centrioles undergo a mysterious form of replication in which new centrioles appear at 90° angles from the parent centrioles, and this replication is typically tightly coordinated with cell replication and assembly of the mitotic spindle (Nigg and Stearns, 2011).

The midbody is an enigmatic structure composed of bundled microtubules and associated proteins derived from the mitotic spindle. The midbody forms during cytokinesis at the point of abscission (separation) of the two daughter cells. Midbodies have often been viewed as waste depots for the cell division process, but increasing evidence suggests that these structures are transient organelles with still-mysterious functions of their own (Chen et al., 2013).

Organism-specific structures: Protists contain a wide variety of complex microtubule based structures that are important for their viability and/or pathogenicity. Striking examples include the *Toxoplasma* conoid (Morrissette, 2015), the *Giardia* ventral disk (i.e., the suction plate) (Schwartz et al., 2012) (**Figure 1F**), and the cilia array of ciliates (Winey et al., 2012). The diversity of cellular architecture in protists is remarkable, and mechanisms leading to generation of these structures are only beginning to be defined (Slabodnick and Marshall, 2014).

MICROTUBULE ASSEMBLY AND DYNAMICS

Introduction to Microtubule Dynamics

The term *cytoskeleton* brings to mind a static structure, an idea that is reinforced by immunofluorescence images such as those in **Figure 1**. Nothing could be farther from the truth: as with actin (Chapter 2), the microtubule cytoskeleton in eukaryotic cells is constantly turning over in a process that is driven by nucleotide hydrolysis (GTP

hydrolysis in the case of microtubules). However, the energy of the nucleotides is not used to build actin filaments and microtubules, but rather to destroy them. This concept can be demonstrated by replacing the GTP in the tubulin subunits with the slowly hydrolyzable GTP analog GMPCPP: the microtubules grow, but fail to disassemble normally (Hyman et al., 1992). Where does the energy for building the filament come from? Assembly of GTP tubulin into microtubules is a spontaneous process that is driven primarily by the hydrophobic effect (Vulevic and Correia, 1997), like many other forms of macromolecular assembly.

Microtubules in most cell types display a behavior known as *dynamic instability*, where the ends of individual polymers transition randomly between periods of growth and shortening (**Figure 3**). In many animal cells, the minus-ends of most microtubules are embedded in the MTOC (see discussion above), and so dynamic instability occurs primarily at the plus-end, but uncapped minus end can also exhibit dynamic instability, at least *in vitro* (**Figure 3A**). Some microtubules can also treadmill, a behavior that is particularly important in the cortical microtubule array of plants (Ehrhardt and Shaw, 2006) but is also seen in more limited cases in animal cells (e.g., Rodionov and Borisy, 1997; Vorobjev et al., 2001).

The constant turnover of cytoskeletal structures might seem wasteful – just consider the amount of GTP that is "burned" by a single microtubule undergoing dynamic instability. Why would cells expend so much energy constantly destroying structures that they have just built? It turns out that microtubule turnover is necessary for many aspects of cell physiology and is an essential aspect of the microtubule cytoskeleton. Some examples include:

- The dynamic nature of the microtubule cytoskeleton allows cells to adapt to changes in cell shape and environment.
- The random probing generated by dynamic instability allows individual microtubules to explore cellular space and bring the microtubule "train tracks" into contact with cargo like vesicles, organelles, and chromosomes, which are too large to diffuse effectively

in the highly crowded environment of the cytoplasm.

- Spatially localized ("selective") stabilization of dynamic microtubules provides a mechanism for generating morphological change in response to internal or external signals (Kirschner and Mitchison, 1986)
- The combination of spatial exploration and selective stabilization (sometimes summarized by the phrase "search-capture") plays a fundamental role in the self-assembly of structures like the mitotic spindle (reviewed by Mogilner et al., 2006).

The process of building and maintaining such a dynamic microtubule cytoskeleton might be energy-intensive, but it is profoundly *robust* – in other words it is unlikely to fail even if significant perturbations (e.g. changes in cell shape, number of microtubules) occur to the system. This realization is important for understanding how the cytoskeleton works, but it also has implications for understanding the process by which the cytoskeleton evolved (Kirschner and Gerhart, 2005).

Microtubule Assembly can be Altered by Changes to the Environment and by Drugs

Microtubules assembled from pure GTP tubulin are remarkably unstable polymers – reduce the concentration of GTP-tubulin subunits for a few seconds, and they disappear. Rapid depolymerization can also be induced by reducing the temperature or shifting other aspects of the environment (e.g. an increase in calcium). The transient and unstable nature of microtubules is all the more striking when considered alongside the ability of these structures to withstand physical perturbations (bending, tension) as outlined above.

Researchers can manipulate the assembly state of microtubules through the use of small molecules. The natural product Taxol and its relatives induce microtubule assembly and can stabilize microtubules against dilution-induced depolymerization and (to a lesser degree) cold temperatures, while molecules such as nocodazole, colchicine, vinblastine, and vincristine destabilize microtubules. The mechanism of Taxol-mediated stabilization has been unclear (Amos, 2011), but recent high resolution structural data might be

resolving this issue (Alushin et al., 2014).

Microtubule-altering drugs are very important in agriculture and medicine, especially cancer chemotherapy, at least partly because of the role microtubules play in spindle assembly. Microtubule drugs can be somewhat organism-specific, and some commercially significant compounds target microtubules of fungi or plants (e.g. Benomyl). Hence, work continues on developing new compounds that target microtubules (Amos, 2011). Researchers are also developing drugs that target microtubule binding proteins, for example motors (as possible cancer fighting agents) and Tau (as possible treatments for neurodegenerative disease) (Rath and Kozielski, 2012).

Mechanism of Microtubule Dynamic Instability

As discussed above, the apparently random[2] transitions between growth and depolymerization that characterize microtubule dynamic instability are functionally significant, but they are also intriguing. Transitions from growth to depolymerization are termed *catastrophes,* while those from depolymerization to growth are called *rescues* (**Figure 3B**). What could cause such abrupt switching? In other words, what is the *mechanism* of dynamic instability? The answers to these questions have become clearer in the thirty years that have passed since dynamic instability was first recognized (Mitchison and Kirschner, 1984), but some important questions still remain.

Some generally accepted experimental observations relevant to the mechanism of dynamic instability include:

- As discussed above, soluble tubulin binds GTP. The alpha subunit binds without hydrolyzing or exchanging. The exchangeable GTP on the beta subunit hydrolyzes and releases its phosphate quickly after polymerization, but slowly in the absence of polymer (Melki et al., 1998).

- Assembly promotes hydrolysis of the GTP bound to the beta subunits. This occurs because the incoming alpha subunit functions as a GAP (GTPase activating protein) for the beta subunit by completing its nucleotide active site (Nogales et al., 1998).

[2] the transitions are often described as random, but this is not completely accurate: microtubules that have been growing longer are more likely to undergo catastrophe (Coombes et al., 2013; Odde et al., 1995).

- GTP tubulin will assemble into microtubules if the free tubulin concentration is sufficiently high (i.e., above the critical concentration). GDP tubulin will not assemble into more than oligomers (its critical concentration is impractically high; Howard, 2001).

- Growing microtubules have slightly curved protofilaments and/or sheet-like extensions at their tips (Chretien et al., 1995), but depolymerizing microtubules have at their tips tightly curled "ram's horns" consisting of curved protofilaments that appear to be peeling off the microtubules. Isolated GDP tubulin can form rings similar to these rams' horns.

- These observations led early on to the idea that GTP and GDP tubulin have different preferred conformations. Initially it was proposed that GTP tubulin is straight and GDP tubulin is curved. Later structural data suggests that both are curved, but that GTP tubulin can become straight in the context of a microtubule (Nogales et al., 1998). The bending appears to occur both within and between subunits (reviewed by Brouhard and Rice, 2014).

- Microtubules assembled from the slowly hydrolyzing GTP-like analog GMPCPP polymerize as normal, but depolymerize much more slowly (Hyman et al., 1992). This is a key point: it means that the energy of GTP is used to destroy the microtubule, not to build it.

- Microtubule growth and dynamic instability can occur at either end, although the kinetics of the two ends differ (e.g., the plus end grows faster than the minus end).

For the many citations not given above see (Desai and Mitchison, 1997; Howard, 2001; Howard and Hyman, 2003).

These and other experiments have led to the standard textbook model of dynamic instability, which typically includes the following ideas:

- After a new subunit adds to a growing microtubule tip, a short delay in hydrolysis and phosphate release result in a GTP-rich region (the *GTP cap*[3]) at the growing tip, while older parts of the microtubule are primarily composed of GDP tubulin (this region is sometimes referred to at the "GDP lattice") (**Figure 3C**).
- GTP hydrolysis promotes depolymerization in at least two ways:
  - First, the GDP tubulin in the microtubule lattice is under strain because it is forced to be

[3] It is not clear whether it is actually the GTP form, the GDP-Pi form, or both that have the stabilizing activity. For simplicity, most publications refer this stabilizing GTP- and/or GDP-Pi- rich structure as the GTP cap.

in an unfavorable straight conformation. This strain effectively weakens the already weak lateral bonds between protofilaments (Zhang et al., 2015).

- In addition, conformational changes associated with hydrolysis and phosphate loss weaken the longitudinal bond (Rice et al., 2008; Zhang et al., 2015).
  Note: it used to be thought that the lateral bonds of the GTP and GDP lattices are significantly different, but as has been seen from recent cryo-EM work, the lateral contacts of the GTP and GDP lattices are similar ; GTP hydrolysis further weakens the lateral bonds by increasing the strain on these bonds (Zhang et al., 2015);

- When the GTP cap is present (i.e., as long as new GTP tubulin is added to the tip faster than it is lost through dissociation and/or hydrolysis), its relatively strong lateral bonds maintain the tubular structure and allowing continued polymerization. However, if the cap is lost, a *catastrophe* occurs: the GDP tubulin is exposed, the protofilaments splay apart as the GDP subunits adopt their preferred bent conformation, and the microtubule rapidly depolymerizes.
- According to this model, the GDP lattice below the GTP cap stores the energy of GTP hydrolysis, allowing the depolymerizing microtubule to do work (Grishchuk, 2005; Mogilner and Oster, 2003).

While this conceptual model is attractive, it leaves many questions unresolved. For example, what are the size and shape of the GTP cap? Are GTP and GDP the only important conformations, or do intermediates (i.e., GDP-Pi) play an important role? Why do the minus- and plus-ends exhibit quantitatively different dynamic instability behaviors?[4] What are the detailed molecular mechanisms of catastrophe and rescue? One can imagine fluctuations in tubulin addition and first-order GTP hydrolysis leading to catastrophe, but what series of events could cause a rapidly depolymerizing microtubule to start polymerizing again?

The sudden and apparently unpredictable nature of catastrophe and rescue have been so

[4] One reasonable (but not necessarily complete) explanation for the asymmetry is that a subunit attaching at the plus end does not hydrolyze its GTP until after a new subunit attaches to it, while one attaching at the minus end will start the process of first-order hydrolysis as soon as it attaches.

puzzling that some researchers have proposed that they result entirely from "outside" influences such as lattice defects, thermal fluctuations, and/or require the action of microtubule binding proteins. Alternatively, it has been proposed that microtubules normally grow as open sheets, and that catastrophe may be caused by tube closure, which could be related in an unspecified way to GTP hydrolysis (Wieczorek et al., 2015; Chretien et al., 1995). It is important to note that some of these ideas are not mutually exclusive with the standard model presented above and that multiple mechanisms are likely at work (e.g., catastrophe can be both a spontaneous event and one caused by microtubule binding proteins).

Current Research into the Mechanism of Dynamic Instability

Recently an array of new experiments such as nanoscale assembly measurements, super-resolution microscopy, and localization of conformation-specific binding proteins have provided new insight into the microtubule assembly processes (e.g., Coombes et al., 2013; Dimitrov et al., 2008; Gardner et al., 2011; Seetapun et al., 2012). These approaches have been complimented by determination of microtubule structure at higher resolution (Alushin et al., 2014; Zhang et al., 2015) and improved computer simulations (e.g. Margolin et al., 2012; VanBuren et al., 2005, Bowne-Anderson et al., 2005). The picture that is emerging is broadly consistent with the classical model of dynamic instability outlined above, but it contains additional molecular detail, and it is beginning to address some of the open questions.

More specifically, the model that emerges from multiple experimental and theoretical studies is one where rapidly exchanging GTP subunits add to protofilament tips (with only a small fraction being incorporated), and the non-terminal subunits undergo first-order (not vectorial[5]) GTP hydrolysis. The resulting microtubule structure has an unstable GDP lattice capped by a stabilizing region rich in GTP (or GDP-Pi). This GTP cap has a size that depends on the elongation rate, as well as an approximate exponential

[5] Early conceptual models of microtubule dynamic instability assumed the GTP hydrolysis occurred vectorially, i.e., in a wave that travels up from the base towards the tip. Later mathematical modeling

shape with a poorly defined lower boundary, and it can extend ~10 or more subunits into the microtubule (Bowne-Anderson et al., 2015; Coombes et al., 2013; Margolin et al., 2012, Seetapun et al., 2012). In these models, open sheets are not observed, but instead flat extensions protruding from a closed tube are seen; these sheets may explain the age-dependent catastrophe that has been experimentally observed (Coombes et al., 2013).

Dimer-based computational simulations based on this conceptual model recapitulate many aspects of experimentally observed dynamic instability, supporting its broad structure, but they also make new predictions. For example, one of these models suggests that the rapid subunit exchange mentioned above occurs because cracks (laterally unbonded regions) exist between adjacent protofilaments in the region close to the tip, allowing subunits to exchange until they form lateral bonds and become incorporated into the lattice (Li et al., 2014; Margolin et al., 2012). One appealing aspect of this and related ideas is that the healing of these cracks by microtubule binding proteins and the resulting suppression of loss of recently added subunits could potentially account for the otherwise surprising ability of some microtubule binding proteins to increase the rate of microtubule growth (Howard and Hyman, 2009; Gardner, 2011).

These computer simulations are also providing specific hypotheses about the molecular-scale mechanisms of catastrophe and rescue. One idea is that catastrophe results from stochastic fluctuations in the extent to which interprotofilament cracks extend into the GDP-rich region (Li et al., 2014; Margolin et al., 2012) and/or the lengths of the protofilament extensions (Coombes et al., 2013). Rescue (a rare event in the absence of microtubule binding proteins) might be promoted by stochastic blunting of the microtubule tip followed by reestablishment regions of laterally bonded GTP tubulin (Li et al., 2014; Margolin et al., 2012). Regulatory proteins could potentially promote or suppress transitions by altering the stability of lateral bonding between subunits or the tip extensions (e.g. Gupta et al., 2013) or by altering delivery of tubulin subunits to the tip (Ayaz et al., 2014). In addition, the ability of computer simulations to simultaneously

(Flyvbjerg et al., 1994) showed that this mechanism was not consistent with the data from sudden dilution experiments (Walker et al., 1991). See Bowne-Anderson et al., 2015 for more discussion.

follow a system at different scales means that these models have the potential to provide insight into long-standing questions about how the properties of the tubulin dimers relate to the observed dynamic instability parameters and to bulk-scale properties such as the critical concentration.

MICROTUBULE BINDING PROTEINS

Introduction to Microtubule Binding Proteins

Naked microtubules assembled from pure tubulin are unstable structures that are poised, quite literally, at the edge of catastrophe. This situation enables microtubule assembly to respond quickly to changes in environment and the influence of regulatory proteins. Some microtubule regulatory proteins are widespread in eukaryotes but others vary significantly by organism and cell type. Even among the organism-specific proteins there are some common motifs and themes as discussed below.

The term *Microtubule Binding Protein* (often abbreviated MTBP) applies broadly to any protein that can be shown experimentally to bind to microtubules. Another term, *Microtubule Associated Protein* (MAP), is often used to describe the subset of MTBPs that cosediment with microtubules through multiple rounds of polymerization and depolymerization, a group that includes proteins such as MAP2 and Tau.

Broadly speaking, the MTBPs that regulate assembly can be categorized functionally as *stabilizers*, *destabilizers* (including *severing proteins)*, *capping proteins*, and *bundlers/crosslinkers* (**Figure 4**). Other MTBPs include *motors* that use microtubules as tracks for intracellular transport (Chapter 5) and *cytoplasmic linker proteins* (CLIPs), which anchor organelles to microtubules to promote cell organization. Some MTBPs are *cytoskeletal integrators* (i.e., proteins that connect to other components of the cytoskeleton). In addition, some proteins involved in signal transduction, translation and metabolism bind microtubules or other components of the cytoskeleton. Many MTBPs have multiple activities. Determining the functions of MTBPs can be challenging because the activity observed can depend on the details of the assay or on other aspects of the

experimental conditions.

Microtubule regulatory proteins can also be categorized as to where they localize on dynamic microtubules (**Figure 4**). *Lattice binding proteins* associate with microtubules along their length, while *end-binding proteins* localize more specifically to one or both of the microtubule extremities. *Microtubule plus-end tracking proteins* (+TIPs) are a subset of end-binding proteins that dynamically track growing microtubule ends, which *in vivo* are typically the plus-ends (Akhmanova and Steinmetz, 2015). The recently recognized microtubule *minus-end targeting proteins* are specifically recruited to minus-ends (Akhmanova and Hoogenraad, 2015).

Specific Classes of Microtubule Binding Proteins

For the purposes of this discussion, **major classes** of MTBPs are in **bold**, *subclasses* are in *italics*, and individual proteins or protein families are underlined. **Figure 4** lists some of the major MTBPs along with their predominant activities (plus and minus signs indicate positive and negative regulation, respectively).

Microtubule binding proteins categorized by activity

Stabilizers are proteins that promote polymerization and/or slow depolymerization. While these two activities are similar, they are not necessarily identical: a protein could potentially stabilize a microtubule by inducing pause (inhibiting shortening but also inhibiting growth) without promoting polymerization. In practice, it can be difficult to distinguish between these two activities.

Many microtubule stabilizers can be categorized into one or another broad groups based on shared sequences or behaviors. Microtubule stabilizers are often less conserved than analogous actin stabilizers, but relatedness can still be recognized across organisms by the presence of conserved domains, which are frequently found repeated. For example, Mutli-TOG-domain proteins such as XMAP215/DIS1 and CLASP are found in a wide range of organisms (Al-Bassam and Chang, 2011), as are CH-domain_containing proteins like the +TIP EB1 (Komarova et al., 2009) and the kinetochore-microtubule linker NDC80

(Varma and Salmon, 2012). CAP-GLY containing proteins are found in most if not all eukaryotes, although the CAP-GLY proteins with stabilizing activity (e.g., the +TIP CLIP-170) may be restricted to the animal-fungi lineage (Steinmetz and Akhmanova, 2008 and Goodson, unpublished). Animal cells also contain a host of more lineage-restricted protein families with stabilizing activities. This group includes the classical MAPs (Tau, MAP2, and MAP4), which are poorly conserved but do contain common repeat structures (Dehmelt and Halpain, 2005), the STOP proteins, which are particularly effective in stabilizing microtubules against cold (Bosc et al., 2003), and the doublecortin and EMAP families, which have roles in neuronal development (Fourniol et al., 2013) and cancer (Bayliss et al., 2016), respectively.

Exactly how most stabilizers work is unsettled, but the common presence of multiple microtubule binding domains suggests that they work at least in part by cross-linking protofilaments laterally or longitudinally. Such cross-linking could stabilize the structure of the microtubule and thus prevent catastrophe, promote rescue, or both. Some proteins might stabilize microtubules by suppressing GTP hydrolysis, but none with this activity have (as yet) been clearly identified. Some proteins (e.g. XMAP-215) promote polymerization in part by increasing the microtubule growth rate, but the mechanism is still debated. One possibility is these proteins bind free tubulin dimers and help deposit them at the tip (Ayaz et al., 2014). Alternatively, a tip-localized subunit cross-linker could potentially increase the growth rate by increasing the fraction of incoming tubulin subunits that incorporate into the lattice (Gardner et al., 2011). Stabilizers might work synergistically to promote microtubule polymerization (Gupta et al., 2014; Zanic et al., 2013). Such cooperation is just starting to be investigated. This issue is discussed more in the section on +TIPs below.

Destabilizers shift a pool of dynamic microtubules towards free subunits by one or more mechanisms:

- *Sequestering proteins* depolymerize microtubules indirectly, by binding free tubulin subunits and preventing them from polymerizing. The best-characterized sequestering protein is the animal protein stathmin, which works in part by binding two dimers in a

curved conformation that cannot incorporate into a microtubule (Cassimeris, 2002).

- *Tip destabilizers* act by directly attacking the sensitive microtubule tip. The best-characterized examples of these are the depolymerizing kinesins (e.g., kinesin-13), which use cycles of ATP hydrolysis to actively remove subunits. This activity causes net depolymerization in part by promoting catastrophe, but these proteins can even depolymerize Taxol and GMPCPP-stabilized microtubules (Walczak et al., 2013). Stathmin can also destabilize microtubule tips, acting at least in part by interfering with lateral bonding between subunits (Gupta et al., 2013).
- *Microtubule severing proteins* use the energy of ATP to cut microtubules into pieces. Katanin, Spastin, Fidgetin and related proteins are AAA ATPases that sever microtubules and are found in a wide range of organisms (reviewed by Roll-Mecak and McNally, 2010). Katanin and at least some members of this family appear to work by utilizing ATP hydrolysis to extract tubulin dimers from the lattice and destabilize the polymer. The new ends created by severing lack GTP caps, so they typically depolymerize rapidly (Sharp and Ross, 2012).
- Other possible mechanisms for promoting depolymerization including increasing the tubulin GTPase (proposed for stathmin Cassimeris, 2002) and capping PF ends (see below).

Capping proteins adhere to the microtubule plus- or minus-end, and thus have the potential to stop both dimer association and dissociation. While actin filament capping proteins are well-characterized (see Chapter 2), less is known about microtubule capping proteins, perhaps because of the greater size and complexity of the microtubule tip. For example, the only known minus-end capping proteins are large complexes rather than individual proteins. The best-characterized examples are the related complexes Gamma TuRC and Gamma TuSC, which not only cap minus-ends but also nucleate microtubules (Kollman et al., 2011). Some evidence suggests that stathmin can cap protofilaments and suppress subunit addition without stabilizing the polymer (Gupta et al., 2013). Patronin and other proteins known as CAMSAPs are sometimes considered capping proteins, although they appear to associate laterally with the minus-end (see also minus-end targeting proteins below).

Bundlers and cross-linkers associate microtubules laterally. Proteins variously called MAP65/Ase1/PRC1 preferentially bundle antiparallel microtubules (Walczak and Shaw, 2010), an activity important in the mitotic spindle. Most stabilizers have some bundling activity, but whether this activity is physiologically relevant is unclear. Simply coating negatively charged microtubules with Tau peptides can result in bundling (Melki et al., 1991), as can crowding agents such as polyethylene glycol (Sanchez et al., 2012).

Cytoskeletal integrators bind to and/or modulate microtubules and at least one other cytoskeletal element. This diverse category contains large scaffolding molecules such as the cancer-associated protein APC and the "plakin" family (Suozzi et al., 2012). It also includes the actin-nucleating formin family (Bartolini et al., 2008; Gaillard et al., 2011), myosin 10 (Weber et al., 2004), and even classic MAPs such as Tau (Gallo, 2007). Communication between the actin and microtubule cytoskeletons is essential for proper functioning of processes such as cytokinesis and generation and maintenance of cell polarity, and these proteins play fundamental yet still poorly understood roles in these processes (Rodriguez et al., 2003).

Other microtubule-associated activities:

- *Microtubule motors* kinesin, dynein, and their accessories such as dynactin complex are covered in Chapters 5 and 16.
- *Motor modulators* are proteins such as Tau (Dixit et al., 2008) and ensconcin (Barlan et al., 2013) that bind the microtubule lattice and alter the behavior of motors acting on those microtubules.
- *Membrane-microtubule linkers* such as CLIMP63 (Vedrenne et al., 2005) provide organelles with alternative (non-motor) connections to microtubules (Gurel et al., 2014).
- *Metabolic proteins* including most enzymes of the glycolytic pathway often bind microtubules. One possible physiological function of this interaction is to increase the local concentration of proteins in the same biochemical pathway, but some of these proteins are capable of altering microtubule assembly (at least *in vitro* or upon

overexpression). These and related observations suggest that the metabolic state of the cell can influence microtubules, and *vice versa* (reviewed by Cassimeris et al., 2012).

Microtubule binding proteins as categorized by localization

Lattice Binding Proteins bind along the body of the microtubule, and include all proteins that do not target either the plus- or minus-ends. As a result, this category includes proteins with a range of activities. Well-recognized examples of lattice binding proteins include the classical MAPs Tau, Map2, and Map4 (Dehmelt and Halpain, 2005). Tau and Map2 are neuronal microtubule stabilizers that are localized to axons and dendrites respectively, while Map4 is expressed in most tissues. Tau is the focus of much research, because of its involvement in Alzheimer's disease (Iqubal et al., 2016). Tau and MAP2 also seem to play a role in spacing of microtubules in tightly-packed neuronal extensions, as well as regulating motor activity (Dixit et al., 2008).

Microtubule Plus-End Trafficking Proteins (+Tips) dynamically track growing microtubule ends. Since the configuration of the microtubule tip determines whether a microtubule grows or shrinks, +TIP behavior allows a polymerization regulator to be dynamically localized where it needs to act. While the abbreviation +TIP is attractive, it can be misleading: the canonical +TIP EB1 is specific not to plus-ends, but to growing ends, as it will track growing minus-ends if presented with the opportunity, at least *in vitro* (Akhmanova and Steinmetz, 2008). The set of +TIPs includes some of the most conserved and significant polymerization-promoting microtubule binding proteins known, including EB1, XMAP-215, CLASP, CLIP-170, and their relatives. It is important to note that not all +TIPs promote polymerization – some +TIPs including kinesin-13 depolymerize microtubules (for overview of +TIPs, see Akhmanova and Steinmetz, 2008, 2015).

A small number of +TIPs (most notably EB1) track growing ends by binding preferentially to a short-lived tip-specific conformation (Maurer et al., 2014). Most other +TIPs localize to ends by binding to EB1, so EB1 is often considered to be “the master +TIP" (reviewed by Akhmanova and Steinmetz, 2015). Consistent with the idea that EB1 has a central and

ancient role in microtubule dynamics, EB1 is found across the spectrum of eukaryotic organisms, including *Giardia* (Dawson, 2010). In this wide diversity of organisms, EB1 has a canonical homodimeric structure consisting of a CH-domain followed by an apparently unstructured region, a short and highly conserved coiled-coil by which the protein dimerizes, and a tail that folds back to make a four-helix bundle. This four helix bundle contains the hydrophobic pocket into which inserts the SxIP motifs of most EB1-binding +TIPs. In many organisms, EB1 terminates with an EEY motif that mimics the tubulin C-terminal tail; this appears to be involved in autoinhibition (EB1 structure and function are reviewed by Akhmanova and Steinmetz, 2008, 2015).

Most if not all +TIPs bind other +TIPs, and so they are said to form the *+TIP network* – a loose web of interacting proteins that work together to regulate microtubule dynamics and integrate microtubules with the rest of the cell (Akhmanova and Steinmetz, 2015). These interactions localize many of the proteins indirectly to growing microtubule tips and often release autoinhibition, allowing the +TIP network to integrate signals from many pathways (Akhmanova and Steinmetz, 2015). The +TIP network may also act as a superstructure that promotes microtubule growth by stabilizing the structure of the microtubule tip and thus helping to promote microtubule polymerization (Gupta et al., 2014).

Minus-end targeting proteins: two types of proteins localize to microtubule minus-ends, the gamma tubulin ring complex (gamma TuRC) (Kollman et al., 2011), and a more recently discovered set of proteins known collectively as CAMSAPs (which includes the *Drosophila* protein Patronin) (Akhmanova and Hoogenraad, 2015). Gamma TuRC blocks both subunit association and dissociation by interacting with all of the protofilaments (**Figure 2D**). CAMSAPs bind at the minus-end and effectively cap it, stabilizing it against depolymerization and stopping or slowing tubulin addition; some CAMSAPs can even track growing minus ends (Akhmanova and Hoogenraad, 2015). CAMSAPs consist of an N-terminal CH domain, some regions of coiled-coil, and a conserved C-terminal CKK motif, and they seem to accomplish their stabilization of minus ends by assembling laterally at the minus-end instead of by creating a classical cap. CAMSAPs are found in most metazoans

and appear to be absent from plants and fungi, but they may be more widely distributed since some sequence motifs are present in the genomes of organisms such as ciliates (Akhmanova and Hoogenraad, 2015)(H. Goodson, unpublished). As another example of a minus-end binding protein, some evidence suggests that stathmin binding may be biased to the minus-end (cited by Gupta et al., 2013).

CONCLUDING REMARKS

The microtubule cytoskeleton is one of the most remarkable components of eukaryotic cells: during interphase, the microtubule array explores the cytoplasm, finds cargo, provides a substrate for transport, adjusts to internal and external signals, and directs the organization of the rest of the cell. Then, upon transitioning into mitosis, it disassembles and reassembles into the strikingly different mitotic spindle, a structure with the profoundly important purpose of precisely and consistently segregating to each daughter cell the correct set of chromosomes. Central to all of these processes are microtubule dynamics.

In reflecting on the role of dynamics in the function of the microtubules, it is interesting to realize that the combination of random probing and selective stabilization as seen in microtubule cytoskeleton is a theme that occurs throughout biology: random exploration of space followed by reinforcement/selection of the optimized variants is found in processes as diverse as acquired immunity, insect forging behavior, and even Darwinian evolution itself. This strategy produces highly robust systems (i.e., systems that are adaptable and hard to break), and its recurring appearance in biology provides an explanation for the robustness of life itself (Karsenti, 2008; Kirschner and Gerhart, 2005).

Much has been learned in the ~30 years since dynamic instability was discovered, but a great deal remains to be understood about microtubules, how their assembly is regulated, how they are dynamically organized, and how their organization drives the organization of the rest of the cell. At the molecular scale, key problems include establishing the mechanisms of the catastrophe and rescue transitions, determining how microtubule binding proteins alter these transitions, and understanding how groups of microtubule

binding proteins work together to create particular behaviors. At the cell scale, major challenges include understanding the assembly and dynamics of large scale structures such as the mitotic spindle and flagellum, and elucidating the organizational cross-talk between microtubules and the rest of the cell. In both cases, an important goal will be to obtain a quantitative and predictive understanding of these processes. Gaining this knowledge will be important for cell biology, but the resulting information about self assembling systems also has the potential to impact fields as distant as nanotechnology and synthetic biology.

To make these advances will require a multidisciplinary strategy that is founded on the classical approaches of biochemistry, cell biology, and genetics, but includes new types of thinking and approaches imported from the study of physics, chemistry, and complex systems. Already, input from these fields has made significant contributions, and promises to make many more (e.g., Karsenti, 2008; Sanchez et al., 2011). Now that efforts over the last ~50 years have identified most of the cellular components and made progress in determining their activities, it is time to start understanding how these components work together to create a dynamic and functional cell. The microtubule cytoskeleton will be a central focus for this endeavor.

Acknowledgements

We apologize to those authors whose work we were not able to cite for reasons of space. The authors thank members of the Goodson lab for critically reading the manuscript. This work was supported by a grant from the NSF (MCB-1244593) to HVG.

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Figure Legends

Figure 1. The microtubule cytoskeleton in varied cell types. Each pair of panels contains a fluorescence microscopy image of a specific cell/group of cells (left) with a cartoon depicting the generalized microtubule organization in that cell type (right). The color schemes for the microscope images are described below. In the cartoons, microtubules are shown in green, the DNA in blue, and centrosome in red. Non-centrosomal microtubule nucleation machinery exists in many cell types (see text) but is not depicted.
A: Radial microtubule array in interphase cells. Microtubules (green), DNA (blue), MTOC (red). Image from Gundersen lab website (http://www.columbia.edu/~wc2383/pictures.html).
B: Columnar microtubule array in polarized epithelial cells (GFP-tubulin expressed in MDCK cells). Image from Figure 1 of (Reilein et al., 2005).
C: Microtubules in a neuronal growth cone. Microtubules (green) and actin filaments (red). Image from Figure 1 of (Kalil et al., 2011).
D: Cortical microtubule array in plant cells (GFP-tubulin expressed in *Arabidopsis* cells). Image from Figure 1 of (Ehrhardt and Shaw, 2006).
E: Fission yeast interphase microtubules (GFP-tubulin). Image from Figure 1 of (Chang and Martin, 2009)
F: Microtubule cytoskeleton in *Giardia.* Microtubules (red), DNA (blue). Image from Figure 1 of (Dawson, 2010).
G: Animal cell mitotic spindle. Microtubules (green) and DNA (blue). Image from Figure 1 of (O'Connell and Khodjakov, 2007).
H: Metaphase plant mitotic spindle. Microtubules (green) and DNA (blue). Image from Figure 4 of (Yu et al., 1999).

Figure 2. Microtubule structure
A, B: Key aspects of microtubule structure as indicated. Figures modified from Figure 2 of (Kollman et al., 2011)**.**
C: The relationship between protofilament number and microtubule structure. Figures modified from Figure 2 of (Amos, 2004).
D: Model of γ-TuRC (colors) associated with the minus end of a microtubule (grey). Figured modified from (Kollman et al., 2011).

Figure 3. Microtubule dynamics and assembly
A: Kymograph (length/time plot derived from a movie) of a microtubule undergoing dynamic instability in vitro, with dynamics at both the minus (left) and plus (right) ends. Green represents Alexa488-labelled tubulin and red tetrarhodamine-labelled tubulin GMPCPP-stabilized microtubule seeds. Image from (Zanic et al., 2013).
B: Cartoon of a length-history plot (also called a life-history plot) of a microtubule undergoing dynamic instability. The key processes of microtubule dynamics are indicated.
C: Standard model of dynamic instability. As long as the microtubule has a GTP cap, it can grow, but it transitions to rapid depolymerization (catastrophe) upon loss of the GTP cap.
D, E: Cartoons depicting different approaches to measuring the critical concentration (Cc). **D** shows the dependence of the rate of plus end elongation on the concentration of GTP-tubulin dimers. **E** shows the dependence of the concentrations of polymer and soluble GTP-tubulin dimers on the total concentration of GTP-tubulin dimers.

Figure 4. Microtubule binding proteins
Model summarizing some of the major microtubule binding proteins according to their localization

on the microtubule and their activities. Green plus symbol (+) means positive regulation and Red minus symbol (-) means negative regulation.

- At the plus end (fast-growing), the members of the +TIP network (EB1, XMAP215, CLASP, CLIP170, doublecortin, and others not shown) associate with the stabilizing (GTP or GDP-P_i) cap of the growing microtubule, and stabilize this dynamic structure to promote growth. Conversely, proteins such as the depolymerizing kinesins and stathmin facilitate microtubule disassembly.
- At the minus end (slow-growing), proteins such as γ-TURC and Patronin/ CAMSAP associate with the α-tubulin subunit to cap the end of the filament to prevent depolymerization, which is promoted by stathmin.
- In the central part of the microtubule, the GDP microtubule lattice can be stabilized by the activities of classical MAPs (Tau, Map2, Map4, stop proteins), or destabilized by severing proteins (e.g. Katanin). MTBPs that regulate the activity of microtubule motors also bind along the GDP lattice. Microtubules can form large networks through the activities of bundlers/cross linkers, such as MAP65/ASE1/PRC1.
- Tubulin dimer binding proteins include stathmin (which promotes depolymerization by sequestering tubulin), as well as CLIP-170, Tau, and XMAP-215 (which promote polymerization).

Please see the main text for further discussion and references.

Figure 1

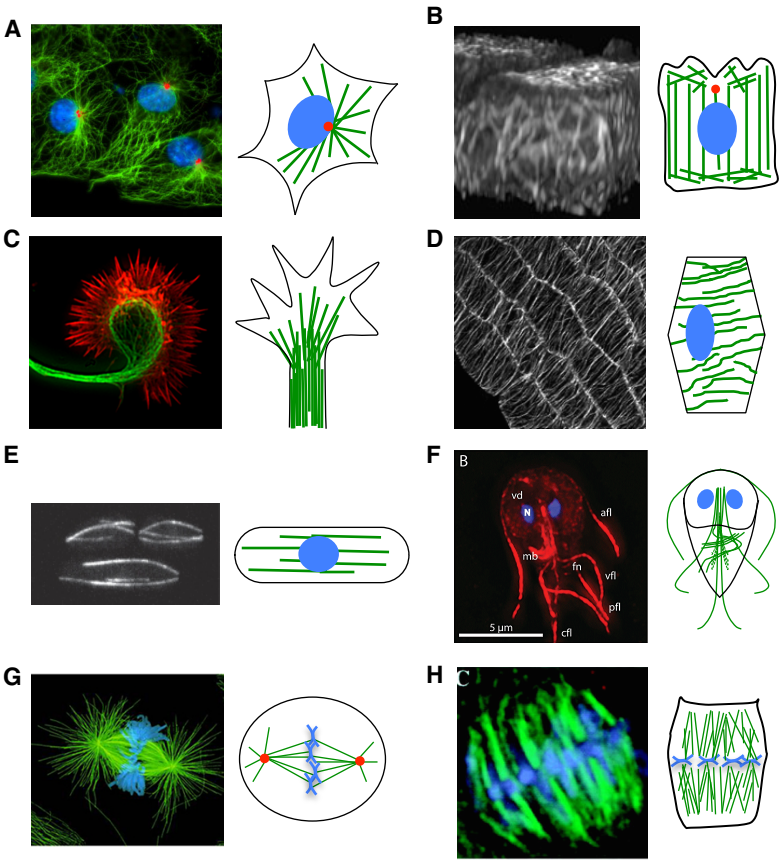
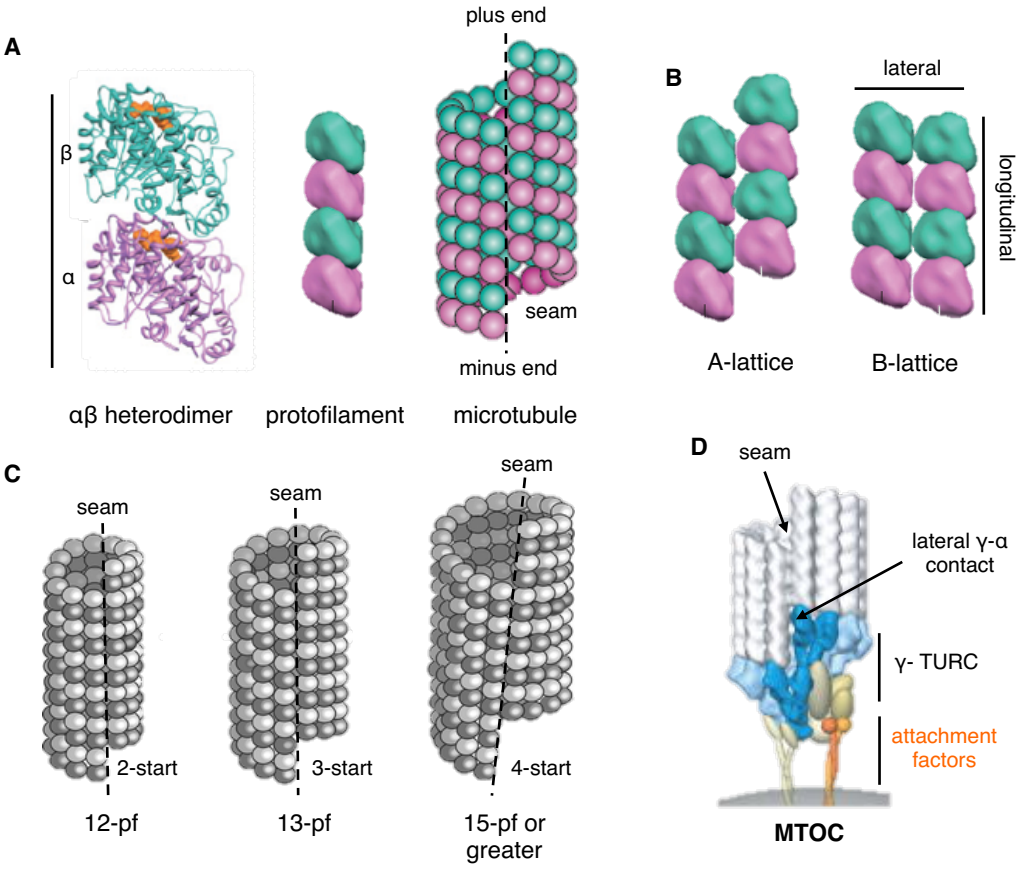


Figure 2


A
β
α
plus end
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B
lateral
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B-lattice
C
seam
2-start
12-pf
seam
3-start
13-pf
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4-start
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contact
γ- TURC
attachment
factors
MTOC

Figure 3

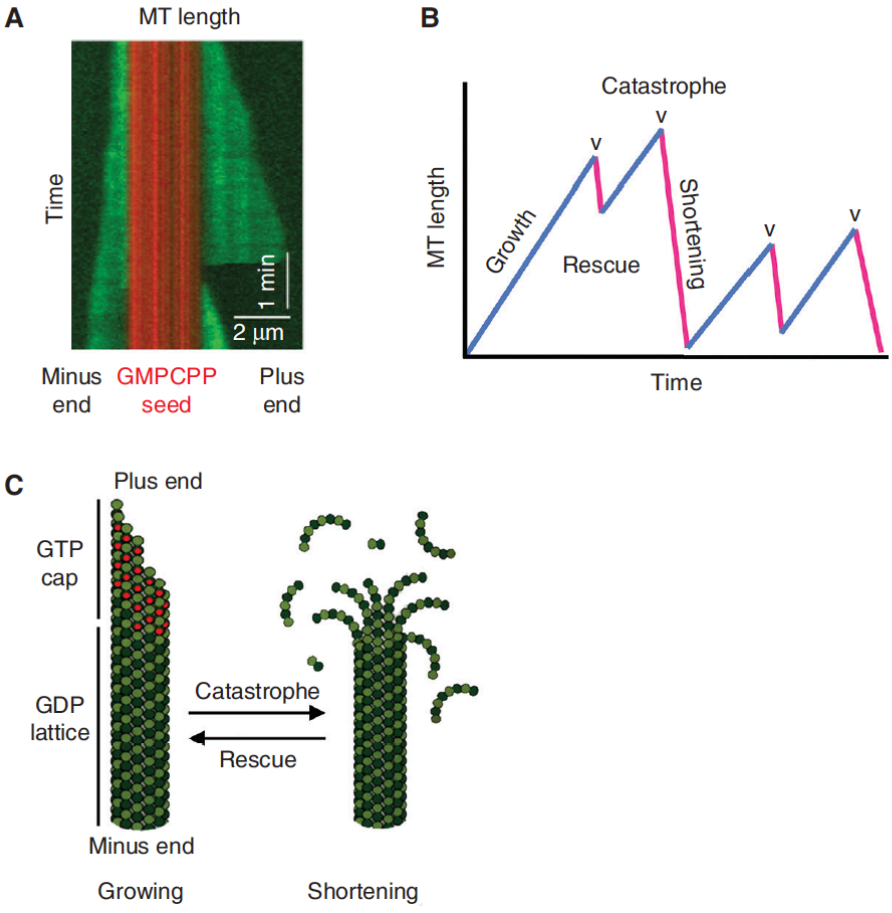
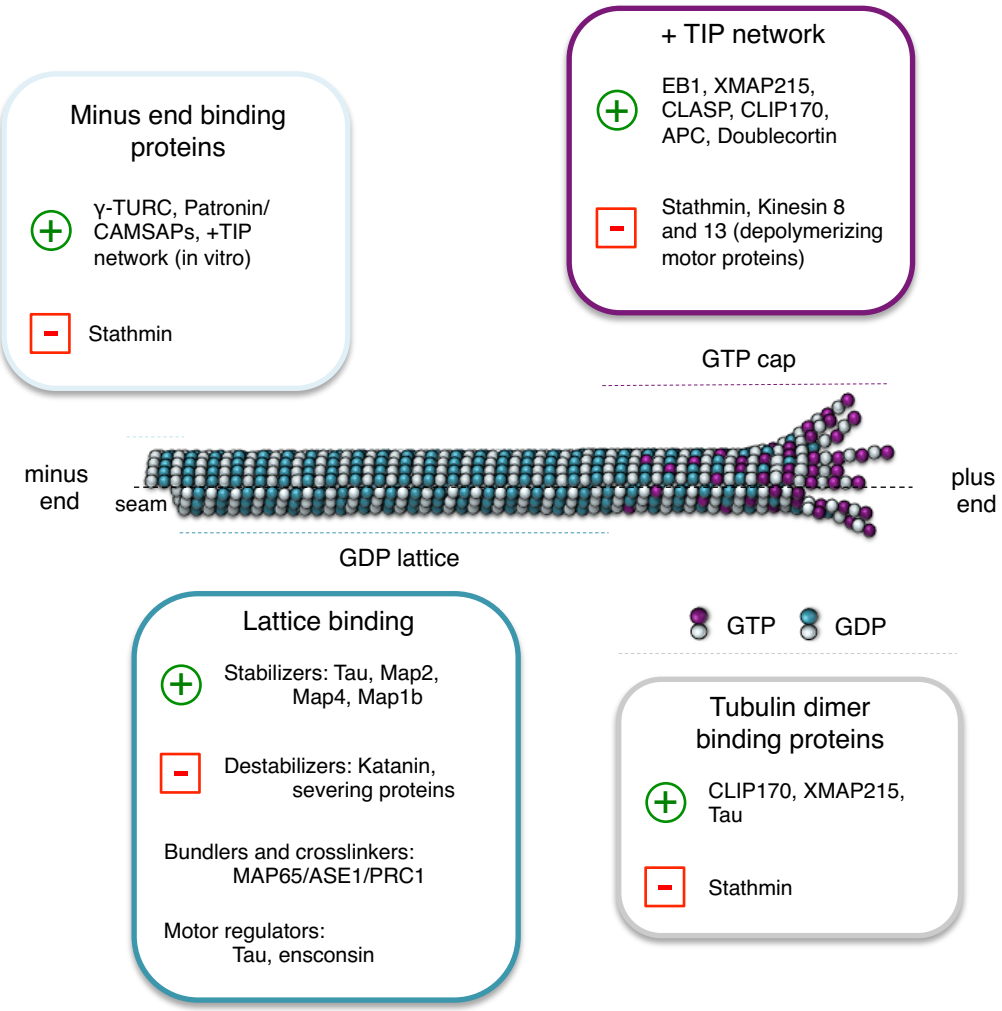

A
MT length
Time
1 min
2 µm
Minus end
GMPCPP seed
Plus end
B
Catastrophe
MT length
Growth
Rescue
Shortening
Time
C
Plus end
GTP cap
GDP lattice
Catastrophe
Rescue
Minus end
Growing
Shortening

Figure 4


+ TIP network
EB1, XMAP215, CLASP, CLIP170, APC, Doublecortin
Stathmin, Kinesin 8 and 13 (depolymerizing motor proteins)
Minus end binding proteins
γ-TURC, Patronin/ CAMSAPs, +TIP network (in vitro)
Stathmin
GTP cap
minus end
seam
plus end
GDP lattice
Lattice binding
Stabilizers: Tau, Map2, Map4, Map1b
Destabilizers: Katanin, severing proteins
Bundlers and crosslinkers: MAP65/ASE1/PRC1
Motor regulators: Tau, ensconsin
GTP
GDP
Tubulin dimer binding proteins
CLIP170, XMAP215, Tau
Stathmin