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Morphogenetic Properties of Microtubules and Mitotic Spindle Assembly

Review

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Introduction

One of the major problems in modern cell biology remains the description of cellular morphologies in terms of molecular interactions. How is the linear information stored in a genome translated into three-dimensional structures? In some cases, such as phage assembly, morphogenesis is defined by a multitude of stereo-specific interactions that combine to create one intricate structure. In other cases, polymeric systems such as the cytoskeleton are endowed with intrinsic dynamic and structural properties, allowing their organization into a variety of three-dimensional arrays. A classic example of polymeric self-organization is the assembly of a mitotic spindle by polymerization of microtubules from tubulin subunits. In a typical animal cell, an assembled spindle consists of microtubules originating from two centrosomes and extending to the chromosomes at the center of the spindle. Some microtubules attach to chromosomes via kinetochores, while others overlap with each other in the middle of the spindle, creating the typical fusiform shape. This complex structure assembles and dissassembles with remarkable speed and efficiency as cells proceed through mitosis.

The morphogenetic problem in the creation of a mitotic spindle is illustrated in Figure 1. Dynamic populations of microtubules, growing radially from a nucleating center (Figure 1B), are converted into assymetric arrays in which some microtubules are stabilized by association with chromosomes in mitosis (Figure 1C). Therefore, selective stabilization of microtubules by chromosomes lies at the heart of spindle assembly, and Kirschner and Mitchison (1986) proposed that this stabilization was driven by interaction of microtubules with chromosomes. This hypothesis had its roots in the then-recent discovery of the dynamic instability of microtubules, making microtubules "searching devices" capable of being "captured" by specific targets in the cell. A number of experiments 10 years later have shown that the principle of random growth of labile microtubules followed by capture is indeed one of the principles underlying assembly of the mitotic spindle (Holy and Leibler, 1994). However, analysis of spindle assembly in a number of different systems has shown that capture and stabilization cannot be the only principle driving spindle assembly (Sawin and Endow, 1993; Vernos and Karsenti, 1995).

In this review we discuss how two different properties of the microtubule polymer are exploited into two morphogenetic principles underlying spindle assembly. One property is the dynamic behavior of microtubules: we discuss the dynamic properties of microtubules and how their modulation is required for spindle assembly. The second property is the polarity of microtubules: we discuss how interactions between polar microtubules and microtubule-based motors organize randomly polymerized microtubules into ordered arrays. Spindle assembly today is extensively studied in a number of different systems, and we make no attempt here to review the whole field. In particular, we do not address the problem of chromosome segregation, concentrating rather on the principles underlying spindle assembly.

Modulation of Microtubule Dynamics: A Morphogenetic Principle in Spindle Assembly

Microtubules exist in dynamic equilibrium with tubulin subunits, growing and shrinking by addition or loss of tubulin dimers from the ends of the microtubules (Kirschner and Mitchison, 1986). Individual microtubules switch stochastically between phases of slow growth and fast shrinkage so that in a microtubule population some will be growing and some shrinking, a property known as dynamic instability (Mitchison and Kirschner, 1984; Walker et al., 1988). Microtubule dynamics is therefore defined by the rate of growth, the rate of shrinkage, and the transition frequencies between growing and shrinking (a catastrophe) and shrinking to growing (a rescue). It has been appreciated for some time that microtubules in a mitotic spindle must be selectively stabilized by modulating one or more of these parameters (e.g., Cassimeris et al., 1988; Kirschner and Mitchison, 1986). In this section we describe recent advances in our understanding of the molecular basis of dynamic instability and then describe how dynamic instability of microtubules is modulated during spindle formation.

Because microtubule dynamics are an intrinsic property of the polymer, it has been possible to investigate its basic mechanism by studying pure tubulin in the absence of other cellular factors. Microtubules polymerizing from centrosomes consist of 13 protofilaments that form the microtubule wall (Figure 2; Amos and Klug, 1974; Evans et al., 1985). They elongate by addition of tubulin subunits to the ends of the protofilaments, and polymerization appears to be a diffusion-limited reaction (Erickson and O'Brien, 1992). The energy allowing sudden catastrophe and fast depolymerization comes from GTP hydrolysis, with each tubulin dimer bringing two molecules of GTP into the lattice, one of which is rapidly hydrolyzed (Carlier, 1989; Purich and Kristoffersen, 1984). Experiments using nonhydrolyzeable analogs of GTP have shown that GTP hydrolysis is not required for assembly but instead is essential to produce unstable microtubules (Erickson and O'Brien, 1992; Hyman et al., 1992).

The question that has dogged investigators of microtubule dynamics is the following: why do microtubules turn over stochastically? It is clear that the energy from GTP hydrolysis is used to destabilize the lattice. However, GTP is hydrolyzed as soon as tubulin is incorporated in the polymer, and thus energy released on hydrolysis must be stored in the lattice until such time as it is

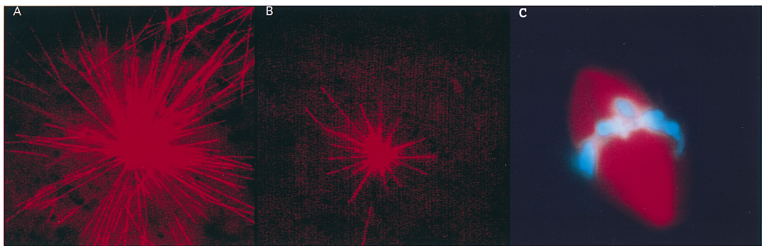

Figure 1. Changes in Microtubule Dynamics during the Interphase to Mitosis Transition

This figure shows microtubules at different times in a Xenopus extract cycling between interphase and mitosis. (A) Interphase. (B) Mitosis. (C) Mitotic dynamics in the presence of chromatin. Dynamics are measured in Xenopus extracts by addition of rhodamine tubulin followed by time-lapse microscopy. In this experiment, the growth rate was 14 μm/min in (A) and 12 μm/min in (B), while the catastrophe rate was 0.35 per minute for (A) and 2.8 per minute for (B). This translates into a turnover rate of 3 min in interphase and 20 s in mitosis. Turnover rates have been measured in Xenopus spindles by photoactivation of fluorescence (Sawin and Mitchison, 1991), with ~2 min half-life. Similar dynamic changes can be measured in tissue culture cells, by injection of biotin-labeled tubulin (Mitchison et al., 1986; Schulze and Kirschner, 1986). Biotin tubulin is injected into tissue culture cells, and then, after varying periods, the cells are fixed. Biotin tubulin can then be visualized either with a fluorescent-labeled anti-biotin antibody by light microscopy or with a gold-labeled anti-biotin antibody by electron microscopy. The amount of biotin tubulin incorporated in the network is then a measure of the turnover rate of microtubules. In published experiments, the half-life in interphase is a 10 min turnover and in mitosis is 15 s for astral microtubules turnover and in spindles is 2 min for nonkinetochore spindle microtubules.

released, when a microtubule undergoes a catastrophe. For many years, this problem was analyzed at a biochemical level, leading to the most popular hypothesis, that of the GTP cap. This model proposes that unhydrolyzed GTP subunits at the end of the microtubule protect it from shrinking (Carlier, 1989). Although there is little biochemical evidence for unhydrolyzed GTP in the lattice, it has recently been shown that a few GTP-like subunits at the end of the microtubule will stabilize a microtubule against depolymerization (Drechsel and Kirschner, 1994).

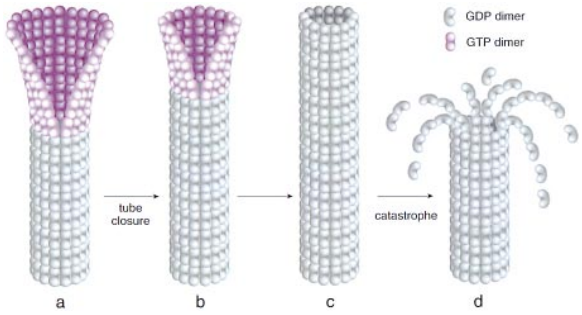

Figure 2. Microtubule Dynamic Instability: A Structural Cap Model

GTP dimers are in purple; dimers with hydrolyzed GTP are in gray. The GTP dimer is straight, while the GDP dimer has curled into a bean shape.

(a) The microtubule is growing with a sheet at its tip, and the open sheet contains GTP tubulin.

(b) The tube begins a stochastic tube closure event, forcing the GTP to hydrolyze.

(c and d) The tube has closed all the way to the end so that no GTP tubulin remains in the microtubule. The mechanical stress in the GDP lattice is no longer constrained by the GTP lattice, and the GDP tubulin subunits release the strain by curling out, causing catastrophic depolymerization. Close examination of the helical structure of a microtubule suggests a way in which tube closure could stimulate hydrolysis. A typical microtubule contains a three start helix determined by the small stagger between subunits in adjacent protofilaments. This suggests that as the tube closes, a GDP subunit will contact a GTP subunit during closure. If formation of lateral bonds between a GDP and a GTP subunit pushed the conformation of the GTP subunit into one in which GTP is hydrolyze, hydrolysis would propagate around the helix toward the extremity of the microtubule. If the tube closure is faster than the rate of subunit addition, the tube would close all the way to the end. A race between hydolysis and subunit addition has been called a vectorial hydrolysis model (Carlier, 1989).

Recent analysis has lead to a deeper understanding of the structural basis of dynamic instability. Cryoelectron microscopy has shown that the structure of the protofilament lattice at microtubule ends is different during growing and shrinking phases (Figure 2). During growth, the ends of microtubules are sheets of slightly curved protofilaments closing into tubes further down the lattice, while during shrinkage the ends of the protofilaments curl over as they “peel” off from the lattice (Chretien et al., 1995; Mandelkow et al., 1991; Simon and Salmon, 1990). Therefore, a large structural transition lies at the heart of the interconversion between growing and shrinking phases. Since the curled protofilaments associated with depolymerizing microtubule ends are GDP liganded and since the only energy input into the system is GTP hydrolysis, it is a strong working assumption that the shallow curvature of the sheets present at the tip of growing microtubules is due to the presence of GTP subunits (Figure 2). The simplest explanation for this is that GTP hydrolysis changes the conformation of the tubulin subunit from straight to curled (Arnal and Wade, 1995; Hyman et al., 1995; Melki et al., 1989; Mickey and Howard, 1995), and indeed a structural change has been measured between GTP-like and GDP subunits (Hyman et al., 1995). Because the bulk of the GDP tubulin subunits are constrained within the wall of the microtubule, the curvature of the protofilaments must be constrained within the straight wall of the microtubule, putting mechanical strain on the lattice. Interestingly, Mickey and Howard (1995) have calculated the amount of energy stored in the form of mechanical strain in the GDP microtubule lattice from the measurements of flexural rigidity of GDP and GTP-like microtubules. They found that the mechanical energy stored within the straight wall of a GDP microtubule is $\sim 10^{-19}$ J per dimer, comparable to the free energy available from GTP hydrolysis. Therefore, most of the energy from GTP hydrolysis can be stored as mechanical strain in the lattice, which is released when microtubules shrink. The conversion of chemical energy into mechanical strain is analogous to the energy stored in a compressed spring. Storage of energy in this way is analogous to the conformational change that takes place during movement of myosin in

muscle following the hydrolysis of ATP. What is remarkable about microtubules is how much energy is stored in a single polymer and for how long. A microtubule 50 μm long stores the free energy released from 80,000 GTP molecules for many minutes.

What triggers the sudden release of mechanical strain during catastrophe? Recent structural and thermodynamic analyses have suggested an exciting possibility: closure of the tube to the end of the microtubule could trigger catastrophe (Chretien et al., 1995; Mickey and Howard, 1995). The argument is as follows. Studies of microtubule dynamics have shown that catastrophe is suppressed by increasing growth rate (Mitchison and Kirschner, 1984; Walker et al., 1988). Structural studies of microtubules have shown that the length of the sheets at the end of the microtubule increases with increasing growth rate (Chretien et al., 1995). Thus, the length of the sheet appears to correlate with the stability of the microtubule: the longer the sheet, the more stable the microtubule. Furthermore, the lengths of the sheets at any particular tubulin concentration are variable from microtubule to microtubule, suggesting a stochastic rate of tube closure. Thus, if the sheet were, on average, shorter, there would be more chance that during a stochastic period of closure, the tube would close all the way to the end. If closure of the tube to the end of the microtubule stimulated catastrophe, a stochastic rate of tube closure would then determine the stochastic rate of catastrophes. We call this a structural cap model because it predicts that the dynamic properties of microtubules are regulated by the structure of the microtubule end and not simply by the rate constants of addition of GTP tubulin and GDP tubulin. Why would tube closure trigger microtubule depolymerization? A possible mechanism is that tube closure pushes the tubulin subunits into a conformation that triggers hydrolysis (Chretien et al., 1995; Mickey and Howard, 1995), as shown in Figure 2. Therefore, when the tube closes all the way to the growing end, this results in full GTP hydrolysis, and the lattice would begin to fall apart as mechanical stress stored in the lattice is released. However, another possibility is that GTP hydrolyzes randomly after polymerization, but that the sheet has an inherently stable conformation while the tube is inherently unstable. Therefore, sometimes when the tube closes, sufficient GTP is hydrolyzed in the sheet to trigger depolymerization. At other times, it is not, and the microtubule continues to grow.

Spatial Control of Microtubule Dynamics and the Generation of Polarized Microtubule Arrays

How are the basic dynamic parameters of microtubules modified to construct a mitotic spindle? A simple examination of microtubule growth in an interphase array (see Figure 1A) shows that the cell can modulate the relationship between the rates of growth, shrinkage, and catastrophe. Microtubules in vivo grow about five times faster than in vitro for an equivalent concentration of tubulin (Belmont et al., 1990; Sammak and Borisy, 1988; Simon et al., 1992; Verde et al., 1992). This stimulation of growth rate is due to microtubule-associated proteins (MAPs), which bind to the wall of the microtubule and can be mimicked in vitro by the addition of MAPs to pure tubulin (Andersen et al., 1994; Drechsel et al., 1992; Pryer et al., 1992; Vasquez et al., 1994). However, high growth rate in vivo is accompanied by high catastrophe frequency, a behavior never observed in pure tubulin solutions. Clearly, the cell has developed mechanisms that allow fast growth rate without concomitantly lowering the rate of turnover. We can model potential mechanisms for in vivo dynamics using the structural cap model. If the rate of tube closure was enhanced in vivo by specialized molecules in the cell, this could explain why fast-growing microtubules still interconvert between growing and shrinking. Alternatively, some factors in the cytoplasm may increase the rate of GTP hydrolysis in the tubulin sheets, thus destabilizing microtubules. Examination of the structure of growing ends in vivo should help to distinguish between these ideas.

The first step in modulation of microtubule dynamics prior to spindle assembly is a global increase in the catastrophe rate, under direct control of the cell cycle machinery, without a concomitant change in growth or shrinkage rate (see Figure 1B). Addition of the master cell cycle control enzyme cdc2 to an interphase Xenopus cytoplasmic extract drives the extract into mitosis, resulting in a rapid increase in microtubule catastrophe (Belmont et al., 1990; Verde et al., 1992). However, if chromosomes are present in Xenopus extracts, the dynamic microtubules associated with chromosomes are rapidly stabilized while those not associated remain highly dynamic (Sawin and Mitchison, 1991; see Figure 1C). Micromanipulation experiments in spermatocytes elegantly demonstrate the principle of microtubule stabilization by chromosome (Figure 3A; Zhang and Nicklas, 1995). If chromosomes and centrosomes are removed from a spindle, they will form a new spindle while the old one vanishes. If only one centrosome is removed with the chromosomes, microtubules grow from the centrosome toward the chromosome while the centrosome left behind nucleates only short microtubules (Figure 3A). Therefore the chromosomes have a strong effect on microtubule assembly by inducing growth of more and longer microtubules in their vicinity.

Examination of interactions between chromosomes and microtubules has shown that preferential growth of microtubules toward chromosomes is determined by at least two nonexclusive mechanisms. Chromosomes can capture and stabilize microtubules undergoing dynamic instability. This “search-and-capture” mechanism relies on the continual turnover of microtubules to ensure that some contact specific capture sites on chromosomes (Figure 3B). Chromosomes can also locally stabilize microtubule in the absence of direct contact by changing the local environment (Figure 3C). Because this effect would occur in the absence of direct contact between microtubules and chromosomes, this has been termed an à distance effect. By modulating the dynamic instability parameters of microtubules, this effect would ensure that microtubules grow preferentially toward chromosomes.

Mitotic chromosomes in their simplest form consist of kinetochores, specialized substructures that ensure that one sister chromatid of each pair is inherited by each daughter cell, and the chromosome arms, the nonkinetochore chromatin. The interaction between microtubules nucleated from centrosomes and kinetochores

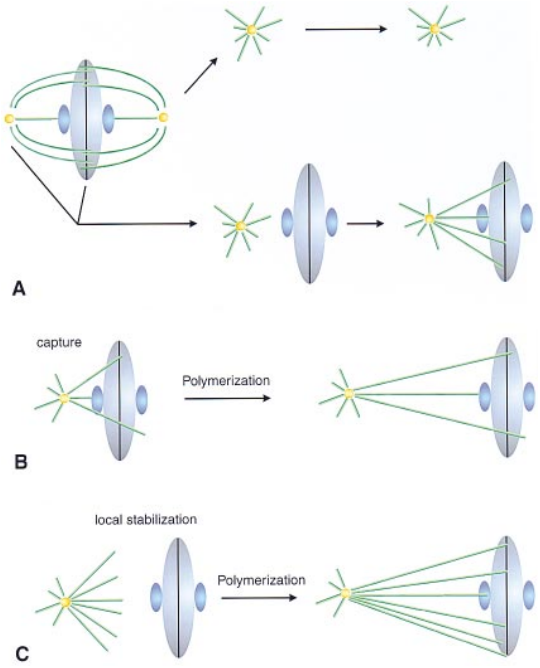

Figure 3. Local Modulation of Microtubule Dynamics by Chromosomes

(A) After removal by micromanipulation of one centrosome and the single chromosome from an insect spermatocyte in mitosis, the spindle disassembles, and the centrosome left behind nucleates an aster of very short microtubules. A half-spindle is assembled with longer and more microtubules between the other centrosome and the chromosome. This experiment demonstrates the effect of chromosomes on the local stabilization of microtubules.

(B) A model for microtubule stabilization by capture: if a centrosome happens to be close enough to a chromosome so that the short mitotic microtubules can contact it, they become captured by kinetochores or chromosome arm motors. These motors can then modulate microtubule dynamics, as described in Figure 4, and promote microtubule elongation.

(C) A model for microtubule stabilization by an à distance effect: even if a centrosome is too far from the chromosome for the short mitotic microtubules to contact it, microtubules are stabilized and grow longer toward the chromosomes until some become captured and further stabilized. This local stabilization could be achieved through a gradient of phosphporylation of MAPs around chromosomes; MAPs required to stabilize microtubules in interphase are probably inactivated by phosphorylation during mitosis, leading to the highly dynamic and therefore short mitotic microtubules. If a phosphatase is localized on chromosomes, it could preferentially dephosphorylate and activate these MAPs, leading to the preferential elongation of microtubules toward chromosomes.

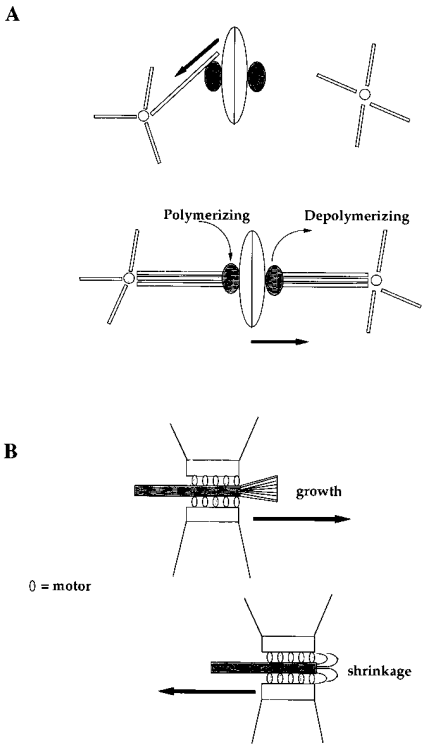

Figure 4. Modulation of Microtubule Dynamics by Kinetochores

(A) Kinetochore captures a single unstable microtubule (closed elipse), moving the microtubule toward the minus end. More microtubules are captured and stabilized by the kinetochores. Movement of kinetochores is then coupled to polymerization and depolymerization.

(B) Model for the structure of microtubule ends during growing and shrinking of kinetochore-associated microtubules.

provides the best example of a search-and-capture mechanism. Microtubules grow and shrink from centrosomes by dynamic instability, but occasionally individual kinetochores attach to one of the microtubules (Figure 4A; Merdes and DeMey, 1990; Rieder and Alexander, 1990). Gradually, more microtubules polymerizing from the centrosome associate with the kinetochore to form the kinetochore fiber, containing the most stable microtubules in the mitotic spindle (Gorbsky et al., 1987; Margolis et al., 1990; Mitchison et al., 1986). Thus, dynamic microtubules growing randomly from a centrosome have been captured and stabilized.

What aspects of microtubule dynamics are modulated during this stabilization process? Capture of microtubules does not prevent catastrophe. Microtubules depolymerize at kinetochores when chromosomes move toward poles and polymerize at kinetochores when chromosomes move away from poles (Figure 4A; Geuens et al., 1989; Mitchison et al., 1986). This ability of kinetochores to maintain attachment to dynamic microtubules has been confirmed by reconstitution in vitro. Preformed microtubules bind to the kinetochores in a way that mimics microtubule capture by kinetochores in vivo (Mitchison and Kirschner, 1985). When ATP and tubulin are added to the system, kinetochores use motors to slide along microtubules, where they follow the growing end (Hyman and Mitchison, 1991; Mitchison and Kirschner, 1985). In the same system, when microtubules interconvert between growing and shrinking, kinetochores maintain attachment and follow the shrinking end of the microtubule, probably by harnessing the energy released from GTP hydrolysis in the microtubule (Hyman and Mitchison, 1990; Koshland et al., 1988; Lombillo et al., 1995). From these in vivo and in vitro studies, it is clear that microtubules still undergo catastrophes when attached to kinetochores. This may seem paradoxical given the stability of kinetochore microtubules in vivo. However, the paradox can be resolved if we distinguish between catastrophe transitions of ends that

remain attached to the kinetochore from catastrophe transitions of ends that detach. Thus, a captured microtubule is stabilized at a kinetochore not because it cannot undergo catastrophe, but because it cannot detach. At a structural level, we can imagine that a kinetochore remains at the end of a microtubule by remaining at the furthermost extension of the microtubule tube (Figure 4B). Thus, when attached to a growing microtubule, the sheet is free to elongate, and the kinetochore motors follow the closing tube. When the microtubule shrinks, protofilaments peel off while the kinetochore remains attached to the tube (Coue et al., 1991; Koshland et al., 1988). Coupling microtubule dynamics to chromosome movement raises a further problem of synchronizing the dynamics of all microtubules at a kinetochore. Why do all microtubules at kinetochores depolymerize at the same time? Perhaps motors present in kinetochores regulate microtubule dynamics by affecting the structure of microtubule ends, preventing closure of the tube while a kinetochore moves away from the pole and forcing tube closure when it moves toward the pole. More detailed studies on the structure of microtubule ends in kinetochores should help to understand how kinetochore movement is coupled to dynamics.

While kinetochore–microtubule interactions are essential for segregation of chromosomes, interactions between chromosome arms and microtubules play the dominant role in formation of the fusiform shape of a spindle. In Xenopus eggs or extracts, spindles form in the apparent absence of kinetochores (Karsenti et al., 1984; Sawin and Mitchison, 1990). Removal of chromosomes from spindles in tissue culture cells results in a reduction in microtubule mass, dependent on the size of the chromosome removed, and moving one arm without kinetochore toward one pole in the experiment shown in Figure 3A results in an increase in microtubule number at that pole (Zhang and Nicklas, 1995). When the action of motors associated with chromosome arms is inhibited, the fusiform shape of the spindle is never established, and most microtubules are seen as two large asters growing from the poles, demonstrating the role of specific molecules on chromosome arms in stabilization of microtubules (Vernos et al., 1995). Because one cannot visualize specific capture sites on chromosome arms, the detailed mechanisms by which microtubule dynamics are modified by association with chromosome arms require more analysis in different systems.

Therefore, both kinetochores and chromosome arms can orient microtubules in the spindle by capturing them. In this mechanism, chromosomes first capture microtubules, and subsequent movement is coupled to further polymerization of captured microtubules (see Figure 3B). What is the evidence for modulation of the parameters of microtubules by chromosomes using the à distance effect (see Figure 3C)? If λ phage DNA is injected into an egg, it assembles into chromatin, locally stabilizing microtubules (Karsenti et al., 1984). This effect has been examined in an artificial system in which DNA is attached to a glass coverslip and incubated with Xenopus mitotic extract and centrosomes, demonstrating that microtubules growing from centrosomes toward the DNA have fewer catastrophes than those growing away from DNA (Dogterom, 1994). The à distance hypothesis proposes that chromatin modulates these growth parameters by creating a local "interphase" state due to immobilization of an enzyme on the chromatin that acts on a diffusible substrate that can modulate microtubule dynamics. Since the catastrophe frequency is increased by phosphorylation of some proteins by the cyclin B–dependent cdc2 kinase (Verde et al., 1990), it has been proposed that the à distance effect is due to the presence of a phosphatase on chromosomes that would dephosphorylate proteins, generating a gradient of protein phosphorylation around the chromosomes (Karsenti, 1991). The diffusible substrate could be MAPs that suppress catastrophes when they bind to microtubules in a phosphorylation-dependent manner (Andersen et al., 1994; Drechsel et al., 1992; Shiina et al., 1992). Although more work remains to be done on the à distance effect, these studies suggest that the cell does not rely only on a simple search-and-capture mechanism to assemble a spindle, but may also bias microtubule growth toward chromosomes by modulation of the dynamic instability parameters of microtubules before they are captured by chromosomes.

In this section, we have summarized the changing dynamics of microtubules during formation of a mitotic spindle. Modulation of the parameters of microtubule dynamic instability by MAPs and cell cycle enzymes results in the production of highly dynamic microtubules in the mitotic cytoplasm. The short, highly dynamic microtubules nucleated by the centrosomes become preferentially stabilized around chromosome arms, forming the fusiform shape of the spindle. Within this fusiform shape, kinetochores attach to microtubules, at least in part by a search-and-capture mechanism. Kinetochores thus do not play a privileged role in spindle assembly, but form a subset of microtubules with their own dynamic properties that ensure correct segregation at mitosis.

Microtubule Polarity and Morphogenesis of a Mitotic Spindle

So far, we have examined how temporal and spatial modulation of the dynamics of microtubules growing from centrosomes contributes to the assembly of mitotic spindles. However, local modulation of dynamics by chromosomes is not in itself sufficient to explain formation and stabilization of a bipolar spindle. We now know that motors are essential not only for chromosome movement but also for spindle assembly. For example, motors are instrumental in centrosome separation prior to spindle assembly, in the capture of microtubules by chromosomes, and in the stabilization of bipolarity (McIntosh, 1994; Vernos and Karsenti, 1995). The key to the operation of microtubule-based motors is the intrinsic polarity of microtubules. Some motors move toward minus ends while others move toward plus ends. Therefore, for motors to fulfill their biological function, microtubules in a spindle must have uniform polarity with their minus ends at centrosomes and their plus ends at chromosomes. For example, because of the uniform polarity, kinetochores move toward minus ends with minus end–directed motors (Rieder and Alexander,

1990). In mitotic spindles, this polarity is created by uniform growth of microtubules from centrosomes with plus ends leading. However, in many systems, such as meiotic spindle formation in Xenopus or Drosophila (Gard, 1992; Theurkauf and Hawley, 1992) or mitotic spindles in plants (Bajer and Mole Bajer, 1982; Lambert and Lloyd, 1994), there are no preexisting centrosomes. In these systems, microtubules grow randomly around the chromosome mass and are later organized into a bipolar array by de novo formation of poles. How is uniform polarity created in meiotic spindles in which initial growth appears random? De novo creation of such poles with defined polarity appears to be a mechanism in which motors play a sophisticated role, to organize and sort microtubules by reading their polarity, and is another essential morphogenetic principle in spindle assembly.

Self-Assembly of Mitotic Poles

The self-organization of microtubules into polar arrays in the absence of preformed centrosomes has been most clearly seen in several systems in interphase. Following removal of the centrosome from BSC-1 cells, initially disorganized microtubules take up an astral configuration by the movement of prepolymerized microtubules (Maniotis and Schliwa, 1991). In a classic experiment, McNiven and Porter (1988) isolated pieces of melanophore cells without centrosomes. The microtubules in these pieces, initially randomly distributed, gradually reorganized into astral arrays. Experiments in mitotic Xenopus egg extracts showed that microtubules stabilized by taxol progressively reorganized from an initial random distribution into small asters containing centrosomal material in their center. This experiment provided an early clue that motors organize microtubules into asters: removal of the minus end–directed motor dynein from the extracts blocked the assembly of asters, which was restored by addition of exogenous dynein (Verde et al., 1991).

A model for motor-dependent aster formation is shown in Figure 5A and depends on the principle that microtubules are polar polymers and that motors recognize this inherent polarity. If a multisubunit minus-end motor binds to two microtubules, it will move to the minus end of both microtubules (Figure 5A). If more microtubules are caught in the process, an aster composed of microtubules with uniform polarity will be created. While minus ends of microtubules are always clustered together in vivo, the general property that unidirectional motors form asters of uniform polarity has been demonstrated in pure systems composed of tubulin and the plus end–directed motor kinesin, which form asters with their plus ends at the center (Figure 5C; Urrutia et al., 1991). This model assumes two properties of the motors involved. The first is that motors have multiple subunits. Motors have been isolated with as many as four heads, but cellular particles with multiple motors attached could fulfil the same role. The second property is that when it reaches the minus end, the motor will not run off the end of the microtubule. The relationship between microtubule attachment and plus ends has been discussed in the section on kinetochore attachment to microtubules: in that case, it is clear that motors do not march off the plus ends (Mitchison and Kirschner, 1985). We know less about motors and minus ends, but it seems likely that mechanisms exist either to prevent minus-end motors running off the ends of microtubules or to keep minus ends attached once motors have clustered them.

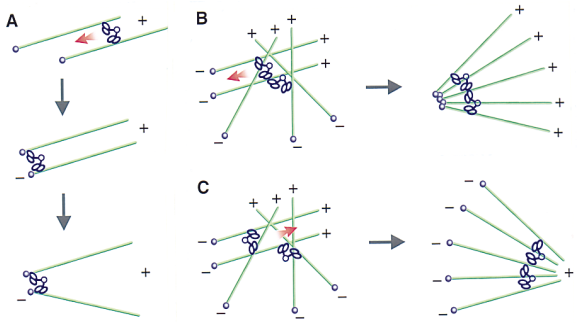

Figure 5. The Generation of Polar Arrays of Microtubules by Motors

(A) Multimeric minus end–directed motors can cross-link two microtubules and move toward microtubule minus ends, where they accumulate.

(B) If more than two microtubules are caught in the process, an aster of microtubules is generated with minus ends at the pole. That this can actually occur has been demonstrated in mitotic Xenopus egg extracts in which microtubules were stabilized by taxol. In these extracts, inactivation of a minus end–directed motor blocks aster formation.

(C) A plus end–directed motor could in principle also produce asters with plus ends at the pole. That this can occur has been shown by addition of pure kinesin to microtubules in vitro. These experiments show that astral microtubule arrays with uniform polarity can be produced by a motor-dependent reorganization of microtubules. Nucleation of microtubules by centrosomes is therefore not the only way of generating astral arrays of microtubules with uniform polarity in the cell.

The coalescence of initially randomly oriented microtubules into poles during spindle formation appears to use similar mechanisms. Microtubules, randomly nucleated around chromatin, are organized into arrays with uniform polarity (Figure 6B). A number of minus end–directed motors have been implicated in meiotic spindle pole formation. Ncd, a Drosophila minus end–directed motor, is required for pole formation, and dynein has been found localized at spindle poles (Endow et al., 1994; Pfarr et al., 1990). Although direct evidence showing a requirement for dynein in spindle pole assembly is lacking, it is quite possible that both dynein and Ncd play a major role in spindle pole assembly in the absence of centrosomes. There is also evidence that microtubules in mitotic spindles continuously move toward poles, as predicted by the presence of minus end motors. When caged fluorescein tubulin is injected into cells and a bar of fluorescence is activated across the spindle, the bar moves toward the pole (Mitchison, 1989). This continuous movement of microtubules poleward has been termed poleward flux, but its mechanism and function in metaphase are unclear (Mitchison, 1989). In tissue culture cells, part of the flux takes place in kinetochore microtubules and is probably involved in chromosome movement (Mitchison and Salmon, 1992). In spindles assembled in Xenopus extracts, microtubule flux appears to be mediated by nonkinetochore microtubules (Sawin and Mitchison, 1991). In these spindles, it

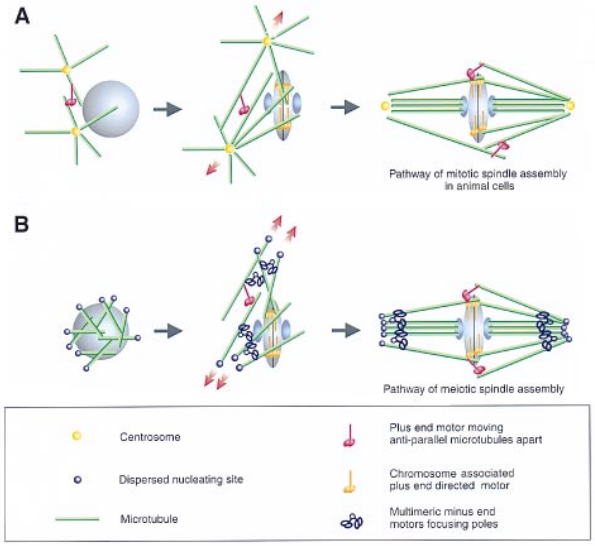

Figure 6. Establishment of Bipolarity in Mitotic and Meiotic Spindles Is Based upon Local Modulation of Microtubule Dynamics and Sorting of Microtubules by Motors

(A) In the mitotic spindle of animal cells, centrosomes are separated by motors in prophase, when microtubules start to shrink in response to the activation of cdc2 kinase. After nuclear envelope breakdown, microtubules start to grow preferentially toward chromosomes, where they become captured by chromosome arms and kinetochores. Finally, bipolarity is stabilized by motors that move apart and cross-link antiparallel microtubules originating from each centrosome.

(B and C) In meiotic spindles or plant cells, there are no centrosomes. Those microtubules are captured by kinetochores and chromosome arms, and their minus end moved away from the chromosomes by motors associated with the arms and motors that move antiparallel microtubules apart. The poles are focused by minus end–directed motors. The role of chromatin in stabilization of nucleated microtubules can be most clearly seen by injection of plasmid DNA, which contains no centromere sequences, into Xenopus eggs (Karsenti et al., 1984). When DNA is injected into the eggs, it assembles into chromatin, which promotes microtubule elongation in its vicinity. Spontaneous nucleation of microtubules requires a relatively high concentration of tubulin, and nucleation in the cell occurs where the critical concentration for nucleation is lowered. In most cell types, nucleation occurs only from centrosomes, but in some cells, such as Xenopus eggs, nucleation occurs throughout the cytoplasm. Thus, the current view of microtubule nucleation suggests that nucleation material can be either dispersed throughout the cytoplasm, leading to dispersed nucleation; concentrated at centrosomes, leading to focused nucleation; or both, leading to both dispersed and focused nucleation. It therefore seems likely that systems in which microtubules are initially randomly dispersed around chromatin are taking advantage of dispersed nucleation of microtubules. What molecules are responsible for nucleation? For many years the centrosomes was a source of mystery, but recent molecular analysis has begun to define some of the components involved. The most striking discovery is γ-tubulin, a third type of tubulin located at centrosomes of most species. A number of different experiments suggest that it is central to nucleation from centrosomes. For example, injection of antibodies to γ-tubulin abolishes microtubule nucleation in cells. It is also dispersed in systems such as Xenopus eggs that have dispersed nucleation. Furthermore, overexpression of γ-tubulin in mammalian cells leads to ectopic microtubule polymerization (for a recent review on nucleation, see Mandelkow and Mandelkow, 1995).

is possible that flux is driven in part by minus end motors involved in pole formation, predicting that inhibition of motors required for poleward flux will block pole formation.

Self-organization of microtubules into poles in the absence of centrosomes cannot on its own explain a fundamental aspect of spindle assembly, the generation of a bipolar spindle. Why are microtubules self-organized by motors into two poles and not four, for instance? In animal cells, bipolarity comes from the presence of two centrosomes that form the two poles (Figure 6A; Wilson, 1925). In meiotic systems, bipolarity appears to come from interactions between antiparallel microtubules (Figure 6B; Sawin and Endow, 1993). Analysis of the ultrastructure of spindles shows that microtubules from each pole make extensive antiparallel contacts with each other in the spindle midzone (Mastronarde et al., 1993). Thus, it seems likely that once motors have organized microtubules into arrays with uniform polarity, plus-end motors begin to cross-link antiparallel microtubules, eventually driving microtubules into two poles (Figure 6B; Vernos and Karsenti, 1995).

Asymmetric Dynamics and Self-Organization of Microtubules as Morphogenetic Principles in Bipolar Spindle Assembly

Although there are myriad different types of spindles in the plant and animal kingdoms, we believe that the morphogenetic principles that we have described can explain the basis of spindle assembly in most systems. Any spindle assembly mechanism must explain the generation of a bipolar spindle with uniform microtubule polarity in each half-spindle since this allows distribution of chromosomes to the two daughter cells. In animal mitotic cells, uniform polarity in each half-spindle derives from the uniform polarity of growth from the centrosome (Euteneur and McIntosh, 1981; Bergen et al., 1980; Evans et al., 1985). Therefore, animal cells must ensure that the spindle has two centrosomes that lie opposite each other on the spindle. In this pathway, the centrosome-nucleated microtubules become highly dynamic at the onset of mitosis, mostly through an increase in the catastrophe frequency (see Figure 1). Concomitantly, the duplicated centrosomes migrate around the nucleus through the activity of motors (Figure 6A). The local reduction of the catastrophe frequency produced by chromosomes makes microtubules elongate preferentially toward them (Figure 6A) and become captured by kinetochores and chromosome arms. As the microtubules from the two half-spindles mingle, they bind together through antiparallel interactions, further stabilizing the spindle in a bipolar configuration with the poles 180° apart.

In plants and meiotic cells, microtubules are nucleated randomly around chromosomes, probably from dispersed nucleation sites not organized in a centrosome (Figure 6B). They elongate only around chromosomes owing to the local stabilization effect, are captured by chromosomes, and are finally organized into poles by minus-end motors. Interactions between the plus ends of antiparallel microtubules then generate bipolarity.

In this picture, both meiotic and mitotic spindles assemble according to similar principles: local modulation

of microtubule dynamics by chromosomes and sorting of microtubules into a bipolar array by motors that read their polarity. The presence of organized centrosomes is not essential for bipolarity. On the contrary, their positioning on each side of the spindle relies on microtubule polarity and the action of motors.

Conclusion

Beyond the apparent diversity of spindle assembly pathways, it seems likely that only a few principles dictate the essential features of spindle assembly and that these principles derive from the structure and dynamic properties of microtubules. We believe that the steady-state size of the spindle in complex eukaryotes is largely determined by the dynamics of microtubules, which is itself determined by the opposing forces of the intrinsic dynamics of microtubules in the mitotic cytoplasm and the stabilization effect of chromosomes. Uniform microtubule polarity is essential in conjunction with the activity of motors to position the poles and thus generate a bipolar spindle. It is fascinating to see how much information can be stored in a single molecule: the tubulin dimer contains the essence of dynamic instability and polarity buried in its structure. Understanding how binding molecules and enzymes read this information is the key to understanding the mechanisms of spindle assembly.

In this review, we have attempted a difficult exercise: to explain the overall morphogenesis of a complex assembly of molecules from the intrinsic characteristics of its parts. Clearly, we have much to learn about spindle assembly. More detailed analysis of the modulation of microtubule dynamics is essential to tie down the principles generating stable arrays of microtubules in the spindle. For instance, one of the principles that emerges from the study of spindle assembly is that there is a continuous interplay between microtubule dynamics and motors. Microtubules are stabilized around chromatin and then organized by motors; during centrosome separation, microtubules elongate while motors push centrosomes apart; kinetochore motility is coupled to growth and shrinkage of microtubules. We know little about the coupling of motility to dynamics that lies at the heart of spindle assembly. Much work is needed to define the molecules that interact with microtubules during spindle formation, whether motors, MAPs, or the enzymes that control their activity. A combination of more sophisticated assays and greater understanding of the molecules involved should lead to an understanding of the mechanisms of spindle formation.

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