Minireview

Molecular mechanisms of spindle function

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Abstract

The key molecules involved in regulating the assembly and function of the mitotic spindle are shared by evolutionarily divergent species. Studies in different model systems are leading to convergent conclusions about the central role of microtubule nucleation and dynamics and of kinesin-related motor proteins in spindle function.

The faithful segregation of genetic material to daughter cells is essential for the survival of an organism. The cell has an elegant macromolecular machine, the mitotic spindle, made up of microtubules and associated proteins, to segregate its chromosomes. Microtubules are dynamic, polar polymers composed of α-β tubulin heterodimers that are nucleated from the centrosomes and extend to the spindle equator where they interact with the chromosomes. The chromosomes are attached to the spindle via kinetochores, specialized proteinaceous structures at the centromere that interact with microtubules.

The study of spindle function has evolved dramatically during the last 50 years. Work in the 1950s-1980s focused primarily on cytological observation of spindle function through ever-improving microscopy techniques. These studies provided an observational account of the precise movements achieved by the chromosomes and other partners in the spindle. The 1990s were the molecular years, when many of the key players in spindle assembly were identified and their precise roles began to be elucidated. The next decade should offer great additional insights into the mechanism of mitosis as many key components are identified in each of the model systems used to analyse spindle function and the precise roles of these proteins are being elucidated, in combination with improvements in high-resolution imaging. The wealth of information provided by genomics and proteomics should help identify additional components that were not previously identified by traditional genetic or biochemical approaches. The picture emerging is one in which we hope to assign a specific molecule or group of molecules to each key event in mitosis.

Several themes emerge from studies of spindle assembly and chromosome segregation (see Figure 1). First, microtubule nucleation is necessary, either from centrosomes or in the region of chromatin. Second, microtubule dynamics must be regulated for a spindle to be assembled. Third, motor proteins are key players, both in the assembly of the spindle and in the segregation of chromosomes. Finally, accurate movement of chromosomes requires a complex integration of motor-protein action and microtubule dynamics. The 'Spindles and Spindle Poles' minisymposium, as well as many other talks and posters, at the 39th annual meeting of the American Society for Cell Biology (December 11-15 1999, Washington DC) addressed these issues.

Microtubule nucleation

Microtubule nucleation is one of the key early events during spindle assembly. Nucleation is thought to require the activity of γtubulin and its associated proteins, often referred to as the γ-tubulin ring complex [1]. The current model is that γtubulin is required to nucleate microtubules from the centrosome that are assembled into the spindle. Recent data suggest that γtubulin may have additional functions in the spindle. Work in the fungi *Schizosaccharomyces pombe* and *Aspergillus nidulans* as well as the nematode *Caenorhabditis elegans* leads to the idea that alteration of γtubulin function by mutation or by RNA-mediated interference (RNAi)

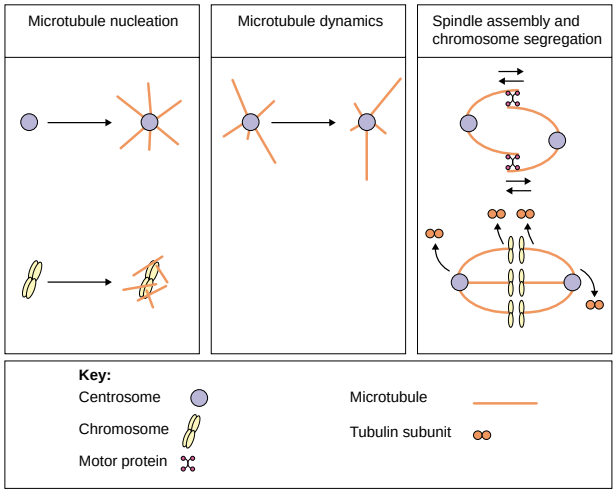

Figure 1
Key aspects of spindle function.

results in spindles with altered microtubule dynamics and mis-segregated chromosomes due to improper attachment of the microtubules to the spindle.

In *S. pombe*, a novel γtubulin mutation was isolated in a screen looking for genes that were synthetically lethal with the kinesin-related protein (KRP) Pkl1. Paluh and colleagues described a unique mutation in γ tubulin that requires *pkl1* for viability [2]. Several phenotypes were observed in this γtubulin mutant. At 19°C bipolar spindles formed and elongated, but chromosome segregation was impaired. Paluh and colleagues also examined the microtubule cytoskeleton in these cells and found that microtubules were hyper-elongated, resulting in long microtubule bundles that wrapped around the cell. The work in *S. pombe* is consistent with reports from *A. nidulans*: Oakley's group isolated point mutations in the γtubulin gene and then looked for genetic interactions with other spindle components [3]. They found that a null mutation in the KRP gene *klpA* caused a dramatic increase in the cold-sensitivity of two γtubulin alleles, which could be suppressed by the anti-microtubule drug benomyl. Interestingly, klpA is the *A. nidulans* homolog of the *S. pombe pkl1*, suggesting conservation of a functional pathway.

In *C. elegans*, a slightly different approach resulted in similar conclusions about the same process [4]. RNAi was used to deplete γ tubulin in early embryos. Embryos that had been treated with γ-tubulin RNAi contained bipolar spindles with numerous long astral microtubules but very few microtubules between the poles. These embryos had chromosomes that did not align properly on the spindle and failed to segregate during anaphase. Taken together, all of the results described above suggest that γtubulin must play an additional role in the spindle other than nucleation of microtubules from the centrosomes. It appears that this additional role may involve controlling microtubule dynamics, and that the combined effects of a defect in nucleation and a defect in dynamics result in defective chromosome segregation.

Microtubule dynamics

In addition to microtubule nucleation, microtubule dynamics must be precisely controlled to allow formation of a spindle. In the self-assembly pathway of spindle formation, microtubule dynamics must be regulated in the region around chromatin to allow for the local nucleation of microtubules that will be sorted and arranged into a bipolar spindle. An *in vitro* reconstitution of spindle assembly that recapitulates these events can be achieved using cytoplasmic extracts from *Xenopus* eggs. This system has been very useful in examining protein function during spindle assembly and chromosome segregation. In egg extracts, stathmin/Op18 may be a key component in regulating microtubule dynamics in the region of chromatin [5]. Op18 is a small heat-stable protein that destabilizes microtubules *in vitro*, in cell extracts and in intact cells (reviewed in [6]). It is regulated by phosphorylation; hyperphosphorylation of Op18 inactivates the protein, making microtubule polymerization more favorable. It has been shown previously that Op18 becomes hyperphosphorylated in the presence of chromatin, suggesting the existence of a chromatin-associated Op18 kinase.

Heald and coworkers described the development of a kinase assay to examine the effects of Op18 phosphorylation and to identify this kinase [7,8]. *Xenopus* Op18 is normally phosphorylated on three residues: Ser16, Ser25 and Ser39. Two triple mutants were created and then analyzed. Mutation of all three serines to alanine (AAA mutant) should generate a constitutively active version of Op18 because phosphorylation cannot occur, whereas mutation of all three serines to glutamate (EEE mutant) should generate a constitutively inactive protein, because the glutamates should mimic phosphorylated residues. Addition of wild-type Op18 to *Xenopus* egg extracts resulted in small spindles whereas addition of AAA Op18 generated virtually no spindles, and extract with EEE Op18 looked very similar to controls with no Op18. The effects of the different proteins on microtubule polymer levels in extracts when examined by a microtubule sedimentation assay were similar to their respective effects on extracts. By contrast, addition of wild-type Op18 or any of the mutated Op18 derivatives to purified microtubules without the cytoplasmic extract results in microtubule destabilization. These results suggest that extracts contain other factors that can modulate the activity of Op18. The identification of these additional factors, as well as the identification of the chromatin-associated kinase that regulates Op18 activity, will be important areas of future study.

Motor proteins

The proper assembly and function of the mitotic spindle would not be possible without the presence of motor proteins,

which couple the energy of ATP hydrolysis to force production. Studies in multiple systems, such as the yeast *Saccharomyces cerevisiae* and HeLa cell extracts have established the concept that antagonistic forces by multiple motor proteins are essential for spindle assembly and function (reviewed in [9]). Studies presented by Sharp and colleagues provide a beautiful illustration of how one can examine the antagonistic forces among different motor proteins in mitosis *in vivo* using early embryos of the fruitfly *Drosophila melanogaster* [10]. They focused their studies on three motor proteins known to be important for spindle assembly in flies: KLP61F, Ncd, and cytoplasmic dynein. KLP61F is a bipolar KRP proposed to cross-link anti-parallel microtubules to generate sliding forces in the mitotic spindle [11]. Sharp and colleagues examined the function of KLP61F by microinjection of antibodies into early *D. melanogaster* embryos that had previously been injected with fluorescently labeled tubulin so that mitosis could be followed with time-lapse imaging. Inhibition of KLP61F resulted in initial separation of centrosomes that then collapsed back together after nuclear envelope breakdown. These results suggest the presence of an antagonistic force acting against KLP61F and resulting in centrosome collapse. Sharp and coworkers suggested that this opposing force probably comes from the KRP Ncd, which also cross-links microtubules and plays a role in spindle assembly and chromosome segregation [12]. Injection of KLP61F antibodies into *ncd* null *D. melanogaster* embryos resulted in abnormal spindles, but centrosome collapse was never observed, suggesting that KLP61F and Ncd provide antagonistic forces for centrosome positioning. Another motor important in spindle function is cytoplasmic dynein. In *D. melanogaster*, cytoplasmic dynein is found localized at the cortex. Injection of inhibitory dynein antibodies or of p50 dynamitin, which disrupts the dynein-dynactin complex, resulted in inhibition of bipolar spindle formation. Surprisingly, inhibition of both dynein and Ncd resulted in restoration of bipolar spindles. The working model is that dynein acts at the cortex to pull on astral microtubules, resulting in forces that keep centrosomes apart. Taken together, these antibody inhibition experiments reveal the complex integration of motor function that acts to maintain spindle bipolarity in living embryos.

The question of what drives spindle assembly and generates spindle bipolarity remains a mystery. One of the most amazing recent findings is that bipolar spindles can be assembled in the absence of centrosomes, kinetochores or DNA. Activation of the small GTPase Ran induces microtubule aster formation and bipolar spindle assembly (reviewed in [13]). Wilde [14] presented work analyzing the mechanism by which this could occur. Wilde rationalized that Ran must act by inducing microtubule formation, or by inducing microtubule organization, or both. One argument against the idea that Ran acts solely by inducing microtubule formation is that agents that stabilize microtubules, such as dimethylsulfoxide (DMSO) or taxol, induce aster formation but not bipolar spindle formation when added to *Xenopus* egg extracts. But addition of a dominant-active version of Ran to taxol- or DMSO-induced asters assembled in extracts stimulates bipolar spindle formation, favoring the idea that microtubule organization is affected. An analysis of the movements of stabilized microtubule seeds on asters revealed that Ran stimulated plus-end-directed seed motility (microtubule 'plus' ends are at the periphery of the aster), suggesting that a plus-end-directed motor might be activated. Wilde hypothesized that Eg5, a bipolar KRP shown previously to be important in establishing spindle bipolarity, might be a downstream target of Ran. Indeed, inhibition of Eg5 reduced the proportion of seeds moving toward the plus ends of microtubules, strengthening the idea that Eg5 might be stimulated after the addition of Ran. The idea that a motor required for spindle bipolarity is stimulated by Ran makes sense in understanding how Ran could induce bipolar spindle formation. Understanding the mechanism of this stimulation and identifying other players in this process will clearly be important areas of future research.

Once a bipolar spindle is assembled, the chromosomes must be aligned at the metaphase plate and then segregated during anaphase to the two daughter cells. Anaphase is functionally subdivided into anaphase A, when the chromosomes separate but the spindle does not change length, followed by anaphase B in which the spindle poles separate from each other. There have been two primary mechanisms proposed for how chromosomes can be moved poleward in anaphase A. In vertebrate somatic cells, microtubule depolymerization at kinetochores accounts for approximately 70-90% of poleward movement, with depolymerization at the poles via poleward microtubule flux accounting for the rest. Recent experiments in spindles assembled in egg extracts had suggested that the majority of anaphase A movement occurs via depolymerization from poles by poleward flux [15]. Is this a unique property of extract-assembled spindles or could it be a general mechanism for chromosome segregation in embryonic systems? Using fluorescent-speckle microscopy [16], Desai described finding that chromosome-to-pole movement in *D. melanogaster* embryos occurred at 2 μm/min, the same rate at which tubulin subunits fluxed toward the poles [17]. This suggests that depolymerization at poles due to poleward flux may be the main driving force for chromosome segregation in embryonic systems. A re-analysis of chromosome movement and flux in spindles assembled in *Xenopus* extracts showed that flux occurred at 75% of the rate of chromosome-to-pole movement, suggesting that some depolymerization at kinetochores must occur in the extract-assembled spindles. Desai and coworkers also began to explore the molecular mechanisms associated with flux. Inhibition of the microtubule-depolymerizing KRP, XKCM1, did not inhibit poleward microtubule flux but did result in an inhibition of microtubule depolymerization at poles, such that microtubules at poles curved around. When XKCM1 was inhibited in combination with the inhibition of the KRP Eg5, microtubule flux

virtually stopped, suggesting that these two proteins might be important constituents of the flux machinery. Eg5 is the *Xenopus* homolog of the bipolar kinesin KLP61F; thus it would be very interesting to perform similar experiments in *D. melanogaster* embryos and establish whether the two motors behave in the same way in both organisms.

In summary, the field of mitosis is making great progress in establishing the molecular mechanism of mitotic spindle assembly and chromosome segregation. The importance of these processes to the survival of each cell and thus of the organism itself has allowed for the evolution of an intricate network of overlapping players and mechanisms to ensure fidelity. One striking outcome of the studies presented at the meeting is that there are redundant processes in place for each aspect of mitosis and that multiple related proteins are involved at each step. There must be a complete integration of function of the proteins that are involved in mitosis, including those that act directly on the spindle, such as motor proteins, those that control microtubule polymerization dynamics and those that coordinate the function of the spindle with the rest of the cell. Clearly the regulation of this complex machinery will be an important area of future research, as we move beyond the identification of additional protein components and into an era of more mechanistic analysis.

References

1. Wiese C, Zheng Y: **Gamma-tubulin complexes and their interaction with microtubule- organizing centers.** *Curr Opin Struct Biol* 1999, **9:**250-259.
2. Paluh JL, Nogales E, Oakley BR, McDonald K, Pidoux AL, Cande WZ: **A novel role for γ tubulin in microtubule dynamics and organization for chromosome segregation involves the kinesin-like protein pk11p and dynein.** *Mol Biol Cell* 1999, **10:**123a.
3. Oakley CE, Ma Q, Prigozhina N, Jung MK, Oakley BR: **Genetic interactions between γ-tubulin mutations and a kinesin-like protein null mutation.** *Mol Biol Cell* 1999, **10:**18a.
4. Strome S, Powers J, Saxton W: **A novel role for gamma tubulin revealed by analysis of GFP-tagged microtubules and chromosomes in living, gamma-depleted *C. elegans* embryos.** *Mol Biol Cell* 1999, **10:**127a.
5. Andersen SSL, Ashford AJ, Tournebize R, Gavet O, Sobel A, Hyman AA, Karsenti E: **Mitotic chromatin regulates phosphorylation of Stathmin/Op18.** *Nature* 1997, **389:**640-643.
6. Walczak CE: **Microtubule dynamics and tubulin interacting proteins.** *Curr Opin Cell Biol* 2000, **12:**52-56.
7. Budde PP, Rao N, Heald R: **A study of stathmin/Op18 phosphorylation in mitosis.** *Mol Biol Cell* 1999, **10:**128a.
8. Heald R, Merlie JJ, Wignall SM, Budde P, Gadde S: **Studying mitotic spindle assembly and function using DNA beads.** *Mol Biol Cell* 1999, **10:**123a.
9. Heald R, Walczak CE: **Microtubule-based motor function in mitosis.** *Curr Opin Struct Biol* 1999, **9:**268-274.
10. Sharp DJ, Brown HM, Kwon M, Rogers GC, Holland G, Scholey JM: **Cooperation between three mitotic motors revealed by real-time analysis of *Drosophila* embryos.** *Mol Biol Cell* 1999, **10:**123a.
11. Sharp DJ, McDonald KL, Brown HM, Matthies HJ, Walczak C, Vale RD, Mitchison TJ, Scholey JM: **The bipolar kinesin, KLP61F, cross-links microtubules within interpolar microtubule bundles of *Drosophila* embryonic mitotic spindles.** *J Cell Biol* 1999, **144:**125-138.
12. Sharp DJ, Yu KR, Sisson JC, Sullivan W, Scholey JM: **Antagonistic microtubule-sliding motors position mitotic centrosomes in *Drosophila* early embryos.** *Nat Cell Biol* 1999, **1:**51-54.
13. Desai A, Hyman A: **No longer an also Ran.** *Curr Biol* 1999, **9:**R704-R707.
14. Wilde A, Walczak CE, Zheng Y, Wiese C: **Ran activates microtubule polymerization and organization in mitosis.** *Mol Biol Cell* 1999, **10:**123a.
15. Desai A, Maddox PS, Mitchison TJ, Salmon ED: **Anaphase A chromosome movement and poleward spindle microtubule flux occur at similar rates in *Xenopus* extract spindles.** *J Cell Biol* 1998, **141:**703-713.
16. Waterman-Storer C, Desai A, Salmon ED: **Fluorescent speckle microscopy of spindle microtubule assembly and motility in living cells.** *Methods Cell Biol* 1999, **61:**155-173.
17. Desai A, Oegema K, Maddox P, Kapoor TM, Walczak C, Field C, Inoue S, Mitchison T, Salmon ED: **Probing mechanisms of chromosome movement using fluorescent speckle microscopy.** *Mol Biol Cell* 1999, **10:**123a.