Mitotic Spindle Form and Function

Mark Winey* and Kerry Bloom[†,1]
*Molecular Cell and Developmental Biology, University of Colorado, Boulder, Colorado 80309 and [†]Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599-3280

ABSTRACT The *Saccharomyces cerevisiae* mitotic spindle in budding yeast is exemplified by its simplicity and elegance. Microtubules are nucleated from a crystalline array of proteins organized in the nuclear envelope, known as the spindle pole body in yeast (analogous to the centrosome in larger eukaryotes). The spindle has two classes of nuclear microtubules: kinetochore microtubules and interpolar microtubules. One kinetochore microtubule attaches to a single centromere on each chromosome, while approximately four interpolar microtubules emanate from each pole and interdigitate with interpolar microtubules from the opposite spindle to provide stability to the bipolar spindle. On the cytoplasmic face, two to three microtubules extend from the spindle pole toward the cell cortex. Processes requiring microtubule function are limited to spindles in mitosis and to spindle orientation and nuclear positioning in the cytoplasm. Microtubule function is regulated in large part via products of the 6 kinesin gene family and the 1 cytoplasmic dynein gene. A single bipolar kinesin (Cin8, class Kin-5), together with a depolymerase (Kip3, class Kin-8) or minus-end-directed kinesin (Kar3, class Kin-14), can support spindle function and cell viability. The remarkable feature of yeast cells is that they can survive with microtubules and genes for just two motor proteins, thus providing an unparalleled system to dissect microtubule and motor function within the spindle machine.

TABLE OF CONTENTS

Continued

doi: 10.1534/genetics.111.128710
Manuscript received March 15, 2011; accepted for publication September 6, 2011
[1]Corresponding author: CB #3280, Coker Hall, University of North Carolina, Chapel Hill, NC 27599-3280. E-mail: kerry_bloom@unc.edu

IN the budding yeast *Saccharomyces cerevisiae*, the mitotic spindle is exemplified by its simplicity and elegance. Microtubules (MTs) are nucleated by the highly organized array of proteins inserted in a fenestra in the nuclear envelope known as the spindle pole body (SPB, analogous to the centrosome in larger eukaryotes). The SPB organizes two classes of nuclear spindle microtubules: kinetochore (KT) and interpolar microtubules. One kinetochore microtubule attaches to a single centromere on each chromosome, while approximately four interpolar microtubules emanate from each pole and interdigitate with interpolar microtubules from the opposite spindle to provide stability to the bipolar spindle. On the cytoplasmic face, two to three microtubules extend from the SPB toward the cell cortex. Processes requiring microtubule function are limited to spindles in mitosis and spindle orientation and nuclear positioning in the cytoplasm. Microtubule function is regulated in large part via products of the 6 kinesin genes and the 1 cytoplasmic dynein gene. A single bipolar kinesin (Cin8, class Kin-5), together with a depolymerase (Kip3, class Kin-8) or minus-end-directed kinesin (Kar3, class Kin -14), can support spindle function and cell viability. The remarkable feature that both budding and fission yeast cells can survive with microtubules and genes for just a few motor proteins provides an unparalleled system to dissect microtubule and motor function within the spindle machine.

Spindle Structure

Three-dimensional ultrastructural analysis of yeast mitotic spindle microtubules has been accomplished by reconstruction from serial thin sections of cells (Winey *et al.* 1995) and by electron tomography of thick sections of cells (O'Toole *et al.* 1999) (Figure 1). The work confirmed that kinetochores are attached by a single microtubule as suggested in early high-voltage electron microscopy (EM) studies of isolated yeast spindles (Peterson and Ris 1976). Reconstruction of yeast mitotic spindle microtubules from serial thin sections or electron tomograms revealed that the spindle is a highly stereotypic microtubule array in these cells. In metaphase, it is 1.4–1.5 μm long, and haploid cells contain ~20 microtubules from each SPB. These microtubules can be divided into shorter microtubules that do not interact with antiparallel microtubules from the other SPB, suggesting that they are kinetochore microtubules, particularly since there are very nearly 16 per haploid SPB (Winey *et al.* 1995). Once formed, the 1.5- 2.0-μm spindle length is constant for ~15–20 min. At this stage the spindle appears fairly rigid and can rotate up to 90° obliquely to the mother/bud axis. Anaphase onset is characterized by rapid linear elongation of the spindle with a velocity of ~1 μm/min to a length of 6 μm. These rates are consistent with earlier measurements taken from low-level DAPI-stained cells (Palmer *et al.* 1989). In anaphase the kinetochore microtubules shorten from their plus-end (anaphase A). As spindle elongation ensues (anaphase B), the number of interpolar microtubules decreases to one or two interpolar microtubules from each pole prior to spindle disassembly.

The lack of detectable kinetochore structures required that kinetochore microtubules be detected by various computational techniques. Two key findings about the kinetochore microtubules came of these analyses. First, the plus ends of the kinetochore microtubules from opposing SPBs were never found to be near each other as one might expect at metaphase when the paired sister chromatids achieve bipolar spindle attachment. What was observed was a gap of ~250 nm between the ends of the kinetochore microtubules from each SPB, leading to the suggestion that the sister centromeres were separated from each other at metaphase in yeast (Winey *et al.* 1995). This proposal was confirmed by fluorescent microscopy studies of live cells with marked tubulin (Tub-GFP, Figure 1) centromeric DNA or kinetochores (Goshima and Yanagida 2000; Tanaka *et al.* 2000; He *et al.* 2001; Pearson *et al.* 2001). The position of lacO arrays relative to the centromere is critical to visualizing separated sister centromeres. The most proximal centromere marked DNA spots [within 1 kilobase (kb) pairs from the centromere] are spatially separated for the duration of mitosis (Goshima and Yanagida 2001; Pearson *et al.* 2001). Second, the appearance of clusters of sister kinetochore proteins provide evidence for metaphase in budding yeast (Pearson *et al.* 2001). Third, the electron tomography confirmed that anaphase A (kinetochores moving toward the poles) occurred in budding yeast (O'Toole *et al.* 1999). Chromosome movement in anaphase proceeds at a rate of ~1 μm/min. As a point of reference, if you read down this page at the rate a yeast chromosome moves, it would take you ~150 days to get to the foot of the page. Anaphase B (increasing pole-to-pole distance) is so significant in yeast (the ~1.5-μm metaphase spindle will lengthen to nearly 10 μm) that this movement alone may be sufficient to separate sister chromatids. However, the reconstructions revealed that the kinetochore MTs of ~0.5 μm long at metaphase (Figure 1) are shorter in anaphase cells. In fact, they shrink all the way to 30–50 nm in late anaphase. These remnants

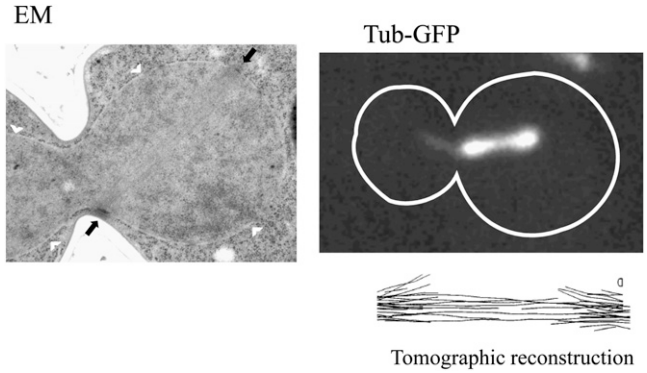

Figure 1 Yeast mitotic spindle structure. Sixteen kinetochore microtubules and four interpolar microtubules emanate from each spindle pole in a haploid cell: 40 MTs/1.5 μm spindle. (Left) The yeast mitotic spindle as seen in the electron microscope (EM) using thin sections of high-pressure frozen and freeze-substituted cells (*e.g.*, Winey *et al.* 1995). The budded cell contains two SPBs (black arrows) that form a bipolar spindle (microtubules are the filaments between the SPBs in the nucleus). The nuclear envelope (white carets) extends through the bud neck, a typical configuration at this point in the cell cycle. (Right) The yeast mitotic spindle as seen in the light microscope using α-tubulin fused to green fluorescent protein (Tub-GFP, shown in Figure as white protein on black background). The outline of the cell is illustrated by the outline in white. The spindle can be seen at the bud neck and extending into the mother (larger) cell. Astral microtubules that emanate from the spindle pole body can be seen penetrating the daughter (budded cell). (Bottom right) A tomographic reconstruction of a spindle from serial thin sections.

are detectable only by electron tomography and hold the kinetochores very close to the SPB at the end of mitosis (O'Toole *et al.* 1999).

The remaining few nonkinetochore microtubules from each SPB interact (defined as being close to each other in 3D space) with nonkinetochore microtubules from the other SPB (O'Toole *et al.* 1999). These microtubules are thought to form a core bundle or the central spindle that lengthens during anaphase B to maintain spindle integrity and contributes to driving the SPBs apart. In shorter spindles, these microtubules can be difficult to distinguish from the kinetochore microtubules, but as the spindle lengthens this group of six to eight microtubules (three to four from each SPB) become very apparent as they are tightly packed (~40 nm from each other) and even twist around each other (Winey *et al.* 1995). Crosslinking structures can be detected between these microtubules that could be various motors or microtubule-associated proteins (MAPs), but those molecular identifications have not been made (Winey *et al.* 1995).

Finally, the long microtubules of the anaphase B central spindle seem to persist into G1 after the spindle is severed during karyokinesis. The fact that the budding yeast SPB is embedded in the nuclear envelope throughout the entire life cycle of the organism is consistent with the observation that microtubules are continually present in the nucleus. The G1 nuclear microtubule array have a median length of 150 nm (O'Toole *et al.* 1999).

The Parts List

Spindle pole bodies

The *S. cerevisiae* SPB was first observed in the electron microscope by Robinow and Marak (1966) (Figure 2). As the sole microtubule-organizing center (MTOC) in these cells that have a closed mitosis (the nuclear envelope stays intact throughout the cell cycle), the SPB must have access to the nucleoplasm to form the microtubules of the mitotic spindle and to the cytoplasm to form the astral microtubules that will position the nucleus. This is accomplished by positioning the SPB in the nuclear envelope where it faces forming microtubules in each cellular compartment, the nucleus, and the cytoplasm. When observed under the electron microscope, a longitudinal section of SPB reveals a layered structure made up of a series of plaques (flat disks, ~80 nm in diameter in a haploid and 160 nm in a diploid) apparently stacked on each other~150 nm tall (Byers and Goetsch 1974, 1975). By definition, the inner plaque of the SPB is in the nucleus, the central plaque is in the plane of the nuclear envelope, and the outer plaque is the cytoplasm. In addition to the plaques of the SPB, there is an associated region of the nuclear envelope called the "half-bridge" that is modified with the addition of membrane proteins and a layer of proteins on the cytoplasmic face of this small region of the envelope.

Importantly, it is likely that genetic analysis, proteomic screens, and genome-wide GFP tagging of yeast ORFs has led to the identification of all of the SPB components (Huh *et al.* 2003; Keck *et al.* 2011). There are 18 core SPB components, those being components found in the structure throughout the mitotic cell cycle (Table 1). Many additional

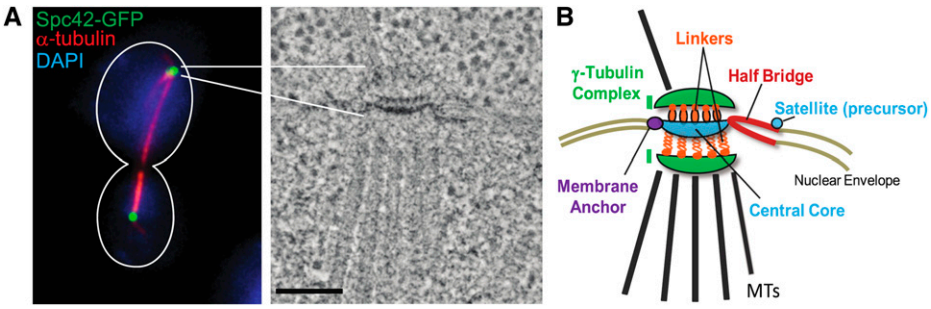

Figure 2 The mitotic spindle in yeast (A, left) is formed from spindle pole bodies (A, right) that are composed of five subcomplexes (B). (A, left) Immunofluoresence of a large-budded mitotic yeast cell showing SPBs marked by Spc42-GFP (green), microtubules (red), and DNA (blue) and electron micrograph (A, right) showing trilaminar ultrastructure. Bar, 100 nm. (Eileen O'Toole, University of Colorado, Boulder). (B) Schematic of the five major functional centrosome subcomplexes. This figure is from Keck *et al.* (2011) and is reprinted with permission.

Table 1 SPB components

Protein	Gene	Structural motif/function	Biological process
γ-Tubulin complex			
γ-Tubulin	*TUB4*		Microtubule nucleation
TUBGCP2	*SPC97*		Microtubule nucleation
TUBGCP3	*SPC98*		Microtubule nucleation
γ-Tubulin complex linkers			
Kendrin	*SPC110*	Coiled coil	Links γ-tubulin complex to SPB
Calmodulin	*CMD1*	Calcium binding	Binds to Spc110
TACC	*SPC72*	Coiled coil	Links γ-tubulin complex to SPB
Central plaque and satellite components			
	SPC29		Satellite/central plaque structure
	SPC42	Coiled coil	Satellite/central plaque structure
	CNM67	Coiled coil	Satellite/central plaque structure
Centriolin	*NUD1*		Satellite/central plaque structure
Half-bridge			
HSfi1	*SFI1*	Cdc31 bindng	Cytoplasmic side of half-bridge
Centrin	*CDC311*	Calcium binding	Cytoplasmic side of half-bridge
SUN protein	*MPS3*	Membrane protein	Half-bridge structure
	KAR1	Membrane protein	Half-bridge structure
Membrane anchors			
hNDC1	*NDC1*	Membrane protein	Nascent SPB insertion
	NBP1	Binds Ndc1	Nascent SPB insertion
	MPS2	Membrane protein	Nascent SPB insertion
	BBP1	Binds Mps2	Nascent SPB insertion

proteins are transiently associated with the SPB during the cell cycle in its functions as a signaling platform or as a MTOC (Caydasi *et al.* 2010a,b). As befits an essential cellular structure, 16 of the 18 core SPB components are encoded by essential genes. The two nonessential genes, *SPC72* and *CNM67*, are essential in some genetic backgrounds, and cells containing the null alleles of these genes are slow growing (Brachat *et al.* 1998; Soues and Adams 1998; Hoepfner *et al.* 2000, 2002; Schaerer *et al.* 2001). Conditional mutations in any of the 16 essential genes encoding core SPB components cause SPB structural defects in mutant cells incubated at the restrictive condition. These defects range from the loss of microtubules to failed assembly of new SPBs to disintegration of parts of the SPB. Despite the clear structural differences between SPBs and the centrosome of vertebrate cells with its centrioles, 11 of the 18 core SPB components have vertebrate orthologs, most of which are centrosomal proteins (Table 1).

In addition to our knowledge of its composition, the SPB has been subjected to extensive structural analyses including electron tomography (O'Toole *et al.* 1999), cryo-EM (Bullitt *et al.* 1997), and mapping fluorescence resonance energy transfer (FRET) interactions *in vivo* (Muller *et al.* 2005) (Figure 3). A stunning finding from this work is that the central plaque is organized around a paracrystalline array of the Spc42 protein (Bullitt *et al.* 1997). This protein is nearly all coiled coil for which there is a crystal structure (Zizlsperger *et al.* 2008), and it packs in a hexagonal array as seen in cryo-EM of isolated SPBs (Bullitt *et al.* 1997). This structural feature of the SPB was also observed *in situ* using electron tomography (Geiser *et al.* 1993; Kilmartin *et al.* 1993; O'Toole *et al.* 1999). Spc42 interacts with the other major central plaque proteins, Spc29 and the C terminus of Spc110 bound to calmodulin (Cmd1). Spc42 spans the gap from the central plaque to part of the outer plaque where it interacts with Cnm67. These interactions have been verified *in vivo* by FRET analysis, and a model of their organization has been developed (Muller *et al.* 2005) (Figure 3).

The central plaque is attached to the inner (nuclear side) and outer (cytoplasmic side) plaques, respectively, by "strut" proteins that span the gap between these layers of the SPB. The inner and outer plaques are critically important because they are the sites from which microtubules are nucleated. The sole "strut" protein connecting the central plaque to the inner plaque is Spc110 (Geiser *et al.* 1993; Kilmartin *et al.* 1993). The protein has a central coiled-coil domain that forms homodimers, and the C-terminal end binds calmodulin (Cmd1) and is where it interacts with Spc42 in the central plaque. Interestingly, Spc110 is an essential target of calmodulin in budding yeast, and this function of calmodulin does not require calcium binding (Geiser *et al.* 1991, 1993; Stirling *et al.* 1994). In an elegant set of experiments altering the coiled-coil domain within Spc110, John Kilmartin showed that Spc110 is the sole determinant of central plaque to inner-plaque distance (Kilmartin *et al.* 1993). At the inner plaque, the N terminus of Spc110 binds the γ-tubulin complex and may be integral to its function in nucleating microtubules (Knop and Schiebel 1997; Nguyen *et al.* 1998; Vinh *et al.* 2002; Kollman *et al.* 2010).

Outer plaque construction is more complex, involving Cnm67, Nud1, and Spc72. Cnm67 spans the gap from central to outer plaque. It is a dimeric, coiled-coil protein with globular N- and C-terminal domains. The C-terminal domain of Cnm67 binds Spc42 and is sufficient for SPB localization, and an X-ray crystallographic structure was recently reported (Klenchin *et al.* 2011). The structure reveals a novel dimeric,

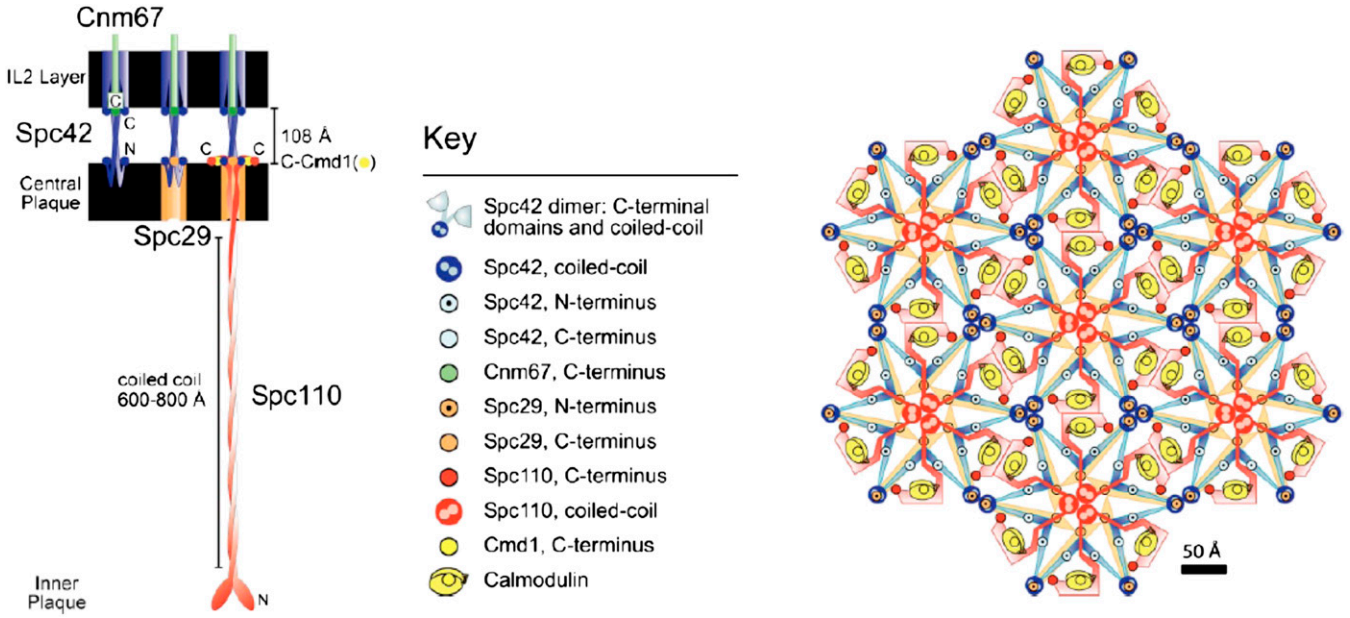

Figure 3 A model of the SPB central-plaque structure in cross section (left) and *en face* (right) based on FRET mapping of interactions and incorporating various structural data about the components in this substructure of the SPB. This figure is from Muller *et al.* (2005) and is reprinted with permission.

interdigitated, all α-helical fold with the very C-terminal eight residues being disordered. Nonetheless, these residues are critical for protein folding and structural stability. The N-terminal region of Cnm67 binds Spc72, which is the protein that directly contacts the γ-tubulin complex (Knop and Schiebel 1998). Interestingly, the N-terminal γ-tubulin-complex-binding domain of Spc72 can replace the N terminus of Spc110, suggesting that this domain of these two proteins serves largely the same purpose (Knop and Schiebel 1998). Spc72 is also found on the half-bridge attached to Kar1 during a period in SPB duplication in which cytoplasmic microtubules originate from the half-bridge (Pereira *et al.* 1999), and Spc72 interacts with the microtubule dynamicity factor Stu2 (Chen *et al.* 1998; Gruneberg *et al.* 2000). Finally, Nud1 is also found at the outer plaque, where it connects Cnm67 to Spc72 (Gruneberg *et al.* 2000). Nud1 is also the SPB component that binds several signaling molecules of the mitotic exit network (MEN) (*e.g.*, Gruneberg *et al.* 2000; Yoshida *et al.* 2002).

In the end, the function of the SPB is to nucleate microtubule formation. This SPB function is carried out by the γ-tubulin complex that is composed of the conserved components Tub4 (γ-tubulin), Spc97, and Spc98 (Schiebel 2000; Vinh *et al.* 2002; Kollman *et al.* 2010) (Figure 4). Together, these proteins form the γ-tubulin small complex with a stoichiometry of two γ-tubulin molecules for each Spc97 and Spc98 molecule (Kollman *et al.* 2008, 2010). This complex is assembled in the cytoplasm and can bind the cytoplasmic face of the SPB. The complex is transported into the nucleus by virtue of a nuclear localization signal (NLS) on Spc98 (Pereira *et al.* 1998). The complex does not appear to nucleate microtubules when not at the SPB and that may be the result of microtubule nucleation requiring a higher-order structure (Kollman *et al.* 2010).

At the SPB, the γ-tubulin complex likely forms the cap seen on the minus ends of the microtubules that were first detected in isolated SPBs (Byers *et al.* 1978) and also were observed in electron tomograms of SPBs (O'Toole *et al.* 1999). It is not really known if microtubule formation is constitutive, meaning that the addition of γ-tubulin complexes to the SPB immediately leads to microtubule formation. Alternatively, there may be γ-tubulin complexes at the SPB that are not active in nucleation and lack an attached microtubule. A domain of Spc110, in addition to the three-protein γ-tubulin complex, is necessary *in vitro* to enable the formation of cap-like structures that can nucleate microtubule formation (Vinh *et al.* 2002; Kollman *et al.* 2010). However, this *in vitro* nucleation is inefficient, raising questions about whether all of the proteins and their appropriately modified forms have been incorporated in the assay. The N-terminal domain of Spc110 interacts primarily with Spc98, contributing to the formation of the higher-order complex. This domain of the protein is known to be phosphorylated by both Cdc28 (CDK) and Mps1, suggesting that this modification may control assembly or nucleation activity (Friedman *et al.* 2001; Huisman *et al.* 2007).

An unusual feature of the SPB when compared to other spindle pole structures is that it is embedded in a membrane, namely the nuclear envelope. This location in the cell gives the SPB access to both the nucleoplasm and the cytoplasm, and it forms microtubules in both compartments. The two membranes of the nuclear envelope join to form a fenestra in which the SPB sits. The edge of the SPB forms an interface with the joined membranes that is similar to the interface between a nuclear pore complex and the nuclear envelope. The comparison is apropos because one of the four membrane proteins associated with SPBs, Ndc1, is associated with both

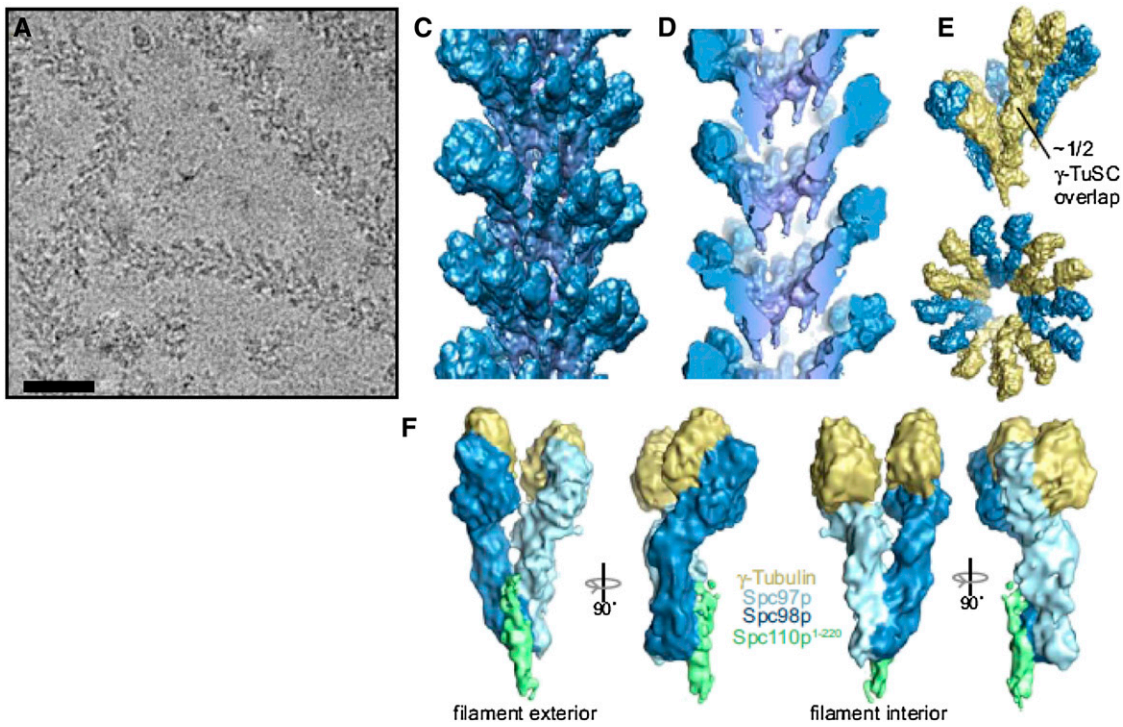

Figure 4 The γ-tubulin complex (Tub4 γ-tubulin, Spc97, and Spc98 as indicated) structure in complex with the N terminus of Spc110 based on cryo-EM from Kollman *et al.* (2010). A micrograph (A) of the complex in its 13-fold polymeric form. Various views (C–E) of the γ-tubulin complex with the N terminus of Spc110 in the context of the higher-order structure. Various views (F) of a single γ-tubulin complex with the N terminus of Spc110. Note: Panel B from the original figure was not included in the figure reproduction because the panel was not relevant to this article. Reprinted by permission from Macmillan Publishers Ltd: Nature. Kollman *et al.*, copyright 2010.

SPBs and nuclear pore complexes (NPCs) (Chial *et al.* 1998) and is required to insert both structures into the envelope during assembly (Lau *et al.* 2004; Onischenko *et al.* 2009). Mps2 is also found at the SPB/nuclear envelope interface (Munoz-Centeno *et al.* 1999). Both of these proteins are in complex with a second protein—Ndc1 with Nbp1 (Araki *et al.* 2006) and Mps2 with Bbp1 (Schramm *et al.* 2000)—which are thought to serve as linkers from their membrane protein partner to the core SPB structure.

The remaining two SPB membrane proteins, Kar1 and Mps3, are found in the special modified region of the nuclear envelope adjacent to the SPB, the half-bridge (Vallen *et al.* 1992; Spang *et al.* 1995; Jaspersen *et al.* 2002). The half-bridge is critically important to SPB assembly yet is poorly understood. It is seen in the electron microscope as an ~90-nm electron-dense region of the nuclear envelope on one side of the SPB (oddly, it does not surround the SPB) (Byers and Goetsch 1975). The membrane likely stains dark because of the presence of the membrane proteins. Electron microscopic studies revealed a structure layered over the cytoplasmic face of the half-bridge (O'Toole *et al.* 1999). This layer is likely composed of Sfi1 and its bound centrin (Cdc31) molecules (Kilmartin 2003; Li *et al.* 2006). Interestingly, both Mps3 and Kar1 have been reported to bind Cdc31 (Biggins and Rose 1994; Spang *et al.* 1995; Jaspersen *et al.* 2002). Perhaps this binding can occur when Cdc31 is in complex with Sfi1, allowing for the retention of the Sfi1/centrin complex at the half-bridge and for the overall organization of the half-bridge. Indeed, mutations in any of these four genes lead to losing the half-bridge structure (Byers 1981; Rose and Fink 1987; Jaspersen *et al.* 2002).

The budding yeast SPB is the best-characterized spindle pole MTOC. From the molecular architecture, one can begin to estimate the environment surrounding the microtubules, such as packaging and crowdedness. The spindle pole is ~160 nm in diameter in metaphase for diploid cells, yielding an area of 20,106 sq nm (area = πr^2). Simply dividing by the cross-sectional area of a microtubule (490 nm^2) gives an estimate of the carrying capacity for microtubules of ~40. This is important in understanding whether the ratio of microtubule capacity to chromosome number might be a contributing factor to aneuploidy. It is possible that, as chromosome number approaches the microtubule-carrying capacity, the error rate in chromosome segregation increases. This could provide a selective pressure for MTOC size and/or chromosome number in different organisms. The electron tomography of the mitotic spindle reveals that microtubules are packed nearly wall-to-wall. If we assume that each microtubule occupies a space of 25 × 25 nm (they are circular but pack more like squares), then the capacity is only 32. The packing pattern is likely to reflect the solution that both maximizes the number of microtubules and provides adequate spacing for active and/or thermal fluctuations of the

Table 2 Spindle parts list

Protein	Gene	Biochemical function	Biological process
α-Tubulin	*TUB1*, *TUB3*	Heterodimer with tub2	Microtubule cytoskeleton
β-Tubulin	*TUB2*	Heterodimer with tub1 or tub3	Microtubule cytoskeleton
Motors			
Kinesin-5	*CIN8*, *KIP1*	Plus-end homotetramer	Mitotic spindle, chromosome segregation
Kinesin-14	*KAR3*	Minus-end heterdimer with *CIK1* or *VIK1*	Mitotic spindle, spindle orientation, mating
	CIK1	Noncatalytic subunit of Kar3	Targets Kar3 to MT plus end, promoting depolymerization, functions in mating and spindle orientation
	VIK1	Noncatalytic subunit of Kar3	Targets Kar3 to mitotic spindle, cross-links spindle microtubules
Kinesin-8	*KIP3*	MT depolymerase	Spindle orientation, chromosome congression
Orphan kinesin	*KIP2*	Plus-end motor	Targets Bik1, dynein to MT plus end
Orphan kinesin	*SMY1*	Myosin processivity factor	Interacts electrostatically with actin
Dynein	*DHC1*	Minus-end, multisubunit complex	Spindle orientation MAPs
MAPS			
Ase1	*ASE1*	Cross-linker	Spindle stability, anaphase spindle elongation
CLIP-170	*BIK1*	Plus-end tracking	Spindle orientation
CLASP	*STU1*	Microtubule binding	Spindle stability
XMAP-215	*STU2*	Microtubule binding	Dynamicity factor
EB1	*BIM1*	Plus-end tracking	Spindle orientation
Orphans	*IRC15*	MT-binding protein	Chromosome segregation, checkpoint

polymer as microtubules encounter changes in force from chromosome attachment and bi-orientation in mitosis. In diploid cells, each spindle pole body nucleates at least 36 microtubules (32 kinetochore microtubules plus 4 interpolar microtubules). The spindle pole is operating at, or close to, its microtubule-carrying capacity. Nonetheless, major questions remain as to what limits microtubule nucleation. In addition, it is not known how close to the edge of the SPB microtubules can be nucleated. There may be a region at the periphery of the SPBs that cannot form microtubules. There could be a dynamic interplay between kinetochore microtubule capture and SPB size/microtubule nucleation capacity. This idea stems from the observation that SPBs grow and microtubule nucleation increases in *cdc20* mutants held at their restrictive growth temperature (O'Toole *et al.* 1997). These spindles contain approximately fourfold the number of microtubules (81 *vs.* 23) relative to a wild-type haploid spindle pole. This observation raises the notion that checkpoint activation is able to drive SPB assembly and more microtubule formation, giving the cell a better chance of capturing the unattached kinetochores, and raises the question of whether there are other limiting factors for the number of microtubules nucleated from each pole (Yoder *et al.* 2003). In addition, MTs exhibit different angles of exit from the spindle pole body, and their minus ends can be slightly displaced from the pole. The area occupied by the γ-tubulin complexes is larger than the central plaque and provides a greater nucleation capacity than strictly defined by the SPB.

Microtubules

Microtubules are large polymeric filaments composed of α- and β-tubulin heterodimers. The polymeric form is composed of 13 protofilaments arranged cylindrically to form the 25-nm diameter microtubule. There are $\sim 3 \times 10^5$ molecules/cell (Abruzzi *et al.* 2002) and 3×10^4 free tubulin molecules (Sprague *et al.* 2003), or 30–35 μM tubulin is polymerized into microtubules, and 3 μM tubulin is free in solution (see Tables 2 and 3). *TUB1* and *TUB3* encode α-tubulin and *TUB2* encodes β-tubulin. *TUB1* accounts for ~90% of α-tubulin protein in the cell, with *TUB3* contributing the remaining 10%. These genes are constitutively expressed, as microtubules are present throughout the cell cycle. Nuclear and cytoplasmic (or astral) microtubules are evident throughout the cell cycle. Cytoplasmic astral microtubules are needed for the orientation and positioning of the nucleus during the mitotic cell cycle of budding yeast. This subset of microtubules is thought to help in orienting the mitotic spindle by a combination of pushing and pulling forces exerted on the cell cortex, coupled with microtubule assembly and disassembly. Upon SPB duplication and the onset of mitosis, the new SPB must gain competence to nucleate nuclear microtubules that, together with nuclear microtubules from the old SPB and microtubule-based motors, develop into the mitotic spindle.

Microtubules are inherently polar polymers with a "plus" and "minus" end that have distinct structures and dynamic properties. The minus end is nucleated at the SPB and the plus end extends away from the spindle pole. The plus end is considerably more dynamic and undergoes frequent changes from growth to shortening. Switching from growth to shortening (catastrophe) or from shortening to growth (rescue) is stochastic. This behavior is known as dynamic instability and is believed to be responsible for kinetochore attachment and chromosome segregation. Fluorescence recovery after photobleaching (FRAP) and fluorescent speckle microscopy shows that minus ends contribute little or nothing to the assembly dynamics of cytoplasmic or nuclear microtubules (Maddox *et al.* 2000b). Unlike microtubule dynamics in several other systems, polymer flux through

Table 3 Rates and numbers

Tubulin concentration
3×10^5 molecules/cell as polymer: ~30–35 μM
3×10^4 molecules/cell as dimer
Spindle dynamics: tubulin half-life (t 1/2) ~1 min in metaphase, plus-end dynamics, no minus-end disassembly
Young's modulus microtubule: 2 GPa
Persistence length: 6 mm
Motors
Cin8, Kip1: 100 molecules/cell
Dynein: 10 molecules/cell
Key rates
Microtubule growth velocity: Vg 1.2 μm/min
Microtubule-shortening velocity: Vs 1.2 μm/min
Catastrophe frequency: Kc 0.25–30 min^{-1}(depends on spindle position of KT)
Rescue frequency: Kr 9–24 min^{-1} (depends on distance between KT, tension-based rescue)
Motors
Unloaded motor velocity 50 nm/sec (3 μm/min)
Motor stall force: 6pN

the minus end is negligible or nonexistent in budding yeast (Table 3).

More recently, microtubule assembly dynamics have been examined at the nanoscale (Kerssemakers *et al.* 2006; Schek *et al.* 2007). These studies reveal extensive variability in growth rate. The plus end of the microtubule undergoes frequent excursions of shortening (up to five layers of tubulin) throughout net growth. Thus net changes of state at the macro scale (observable in the light microscope) hide the mechanics of tubulin subunit addition and subtraction occurring at the nano level. These hidden fluctuations are likely to be critical for the sensitivity of the switch between growth and shortening and regulation of the switch through tension or compression (Howard and Hyman 2009).

Microtubules are stiff (Young's modulus of 1.2 GPa) (Gittes *et al.* 1993), meaning that they are structurally rigid like plastics such as polycarbonate centrifuge tubes. One of the challenges in understanding the behavior of polymers such as microtubules in living cells is the realization that, at the size scale of the molecules in question, there is essentially no inertia. Instead, thermal fluctuations and viscous forces dominate reactions, and the force required to drive a given reaction may be only slightly greater than that of thermal motion. We must turn to physical definitions that define a material's properties. Young's modulus is a measure of the stiffness of a material and is the ratio of stress (pressure) to strain (change in length). Persistence length describes a filament's resistance to thermal force and is the distance over which the correlation of the direction of the two ends of a polymer is lost. The persistence length of a microtubule is ~6 mm. This is three orders of magnitude longer than a typical yeast cell; thus, microtubule bending observed in live cells reflects active chemical processes. Spindle microtubules self-assemble from a pool of tubulin subunits *in vivo*. The process is endothermic and driven by the loss of ordered water surrounding the tubulin dimer (Inoue *et al.* 1975; Salmon 1975). The free energy difference between dimer and polymeric states is small; therefore, the dimer *vs.* polymer concentrations are comparable (since neither state is energetically favored over the other). In fact, in interphase mammalian cells, the experimental measurements bear this out (Schliwa *et al.* 1979; Morris and Lasek 1984).

Microtubule motor proteins

Microtubule motor proteins convert chemical energy to mechanical energy to generate forces that can slide antiparallel microtubules apart, couple cargo (*e.g.* chromosomes) to growing or shortening microtubule plus ends, regulate chromosome position in metaphase, and cross-link microtubules into bundles with specific polarity patterns to fortify spindle stability. In budding yeast, the repertoire of molecular motors is restricted to six kinesin-like proteins and one dynein (Table 2). Through pioneering experiments from Saunders and Hoyt to dissect kinesin function, it was found that Cin8 and Kip1 provide a pushing force for spindle elongation that is antagonized by Kar3 (Roof *et al.* 1992; Saunders and Hoyt 1992). In addition, yeast can survive with only two motors: Cin8 and either Kar3 or Kip3 (Cottingham *et al.* 1999) The Cin8 motor is able to support cell viability in the presence of small quantities of benomyl to dampen microtubule dynamics. The major spindle motors are thus the class 5 bipolar kinesins, Cin8 and Kip1. Their dominant function is to slide antiparallel microtubules apart (Roof *et al.* 1991, 1992). These kinesins are regulated directly through phosphorylation (Chee and Haase 2010) and contribute to spindle formation, stability, and anaphase spindle elongation. In addition, kinesin-5 motors have been found to be responsible for chromosome congression via spatial gradients in microtubule catastrophe rates (Gardner *et al.* 2008a).

Kar3, originally isolated in a screen for karyogamy mutants (hence Kar) (Meluh and Rose 1990), is antagonistic to the plus-end-directed Cin8 and Kip1 (Saunders and Hoyt 1992; Saunders *et al.* 1997b). Kar3 is a minus-end-directed kinesin-14 family member (Endow *et al.* 1994). It was proposed that Kar3 provides an inward-directed force, opposing the outward-directed pushing force of the kinesin-5 family members (Saunders and Hoyt 1992). This antagonistic motor model for mitotic spindle function was revolutionary for the field in the late 1990s and provided an important framework for thinking about motor function and dynamic microtubules in spindle function (Saunders *et al.* 1997b). Kar3 also provides structural support to the spindle by cross-linking antiparallel microtubules in anaphase (Gardner *et al.* 2008b). The inward force opposing Cin8 and Kip1 could be due to minus-end-directed walking of Kar3 on antiparallel microtubules or to its ability to cross-link antiparallel microtubules. Defects in Kar3 function will effectively antagonize Cin8 and Kip1 via two distinct mechanisms. One mechanism counteracts directional force. Forces are exerted in opposite directions—an outward extension due to Cin8 and Kip1 *vs.*

an inner contraction from Kar3. A second, not mutually exclusive, mechanism is the loss of structural stability of the spindle. The loss of Kar3 cross-linking antiparallel microtubules leads to reduced spindle extension since the effectiveness of the outward motors is reduced. The latter model is an indirect mechanism but one that satisfies the genetic findings of shorter spindles in *kar3Δ* mutants (Saunders *et al.* 1997b).

Analysis of Kar3 function during mating revealed that Kar3 binds to microtubule plus ends and also functions as a microtubule depolymerase, thereby bringing two nuclei together in the mating projection for efficient fusion (Molk and Bloom 2006). The ability to visualize microtubules during mating provided an alternative hypothesis to the original sliding model of Meluh and Rose (1990). Kar3 is complexed with either of two noncatalytic subunits, Cik1 or Vik1 (Page *et al.* 1994; Manning *et al.* 1999). Kar3 is complexed with Cik1 in mating cells and along the spindle in vegetative cells. At spindle poles, Kar3 is complexed with Vik1 in vegetative cells. Kar3's function can be tuned depending on the identity of the associated proteins Vik1 or Cik1 (Manning *et al.* 1999). In elegant biophysical experiments, it has been shown how the nucleotide-insensitive subunit (Vik1) coordinates the interaction of the Kar3 motor with microtubules (Allingham *et al.* 2007).

Kip3 is a kinesin-8 family member and exhibits *bona fide* microtubule depolymerase activity (Gupta *et al.* 2006; Varga *et al.* 2006). In the cytoplasm, Kip3 regulates astral cytoplasmic microtubule length and, together with microtubule plus-end-binding proteins Bim1, Kar9, Bni1, and Bud6, plays a critical role in directing the mitotic spindle to the bud (reviewed in Pearson and Bloom 2004). In the nucleus, Kip3 contributes to kinetochore clustering in metaphase (Wargacki *et al.* 2010). It has been found that longer microtubules accumulate higher concentration of MAPs and that, remarkably, Kip3 depolymerizes longer microtubules faster than shorter ones (Varga *et al.* 2006). How does a motor know how long the microtubule is? If the rate of motor movement is faster than microtubule dynamics, then motors accumulate at MT plus ends. Once there, various motors, such as Kip3, might enhance the catastrophe frequency.

Kip2 is another predominantly cytoplasmic kinesin and is antagonistic to Kip3. Astral microtubules are extremely short in *kip2* mutants, indicative of potential microtubule polymerization activity of Kip2. Kip2 targets proteins such as Bik1 and cytoplasmic dynein to microtubule plus ends (Carvalho *et al.* 2003, 2004), where they interact with cytoplasmic cues (Kar9, Num1) to orient the spindle toward the bud and ensure chromosome and nuclear segregation into mother and daughter cells.

The sixth kinesin protein in yeast, Smy1, was found in a genetic screen as synthetic lethal with myosin (Lillie and Brown 1992, 1994). It has since been found to be a processivity factor *in vitro* (Ali *et al.* 2008; Hodges *et al.* 2009). Smy1 enhances myosin processivity and intracellular transport by electrostatic interactions with actin. This passive electrostatic tether is likely to be an important new mechanism that contributes to the effective processivity of individual motor proteins for a given track.

Dynein is a minus-end microtubule motor whose exclusive function is in spindle orientation (Eshel *et al.* 1993; Li *et al.* 1993). The heavy chain itself (Dyn1 or Dhc1) is a processive motor *in vitro* (Reck-Peterson *et al.* 2006). The heavy chain is complexed with several intermediate and light chains (IC/Pac11, LIC/Dyn3, LC8/Dyn2) (Geiser *et al.* 1997) as well as with the dynactin complex (p150glued/Nip100, p24/YLL049w, dynamitin/Jnm1, Arp1, Arp11/Arp10), Bik1 (a CLIP-170 ortholog), and Kip2 (Carvalho *et al.* 2004). Bik1 is carried out to microtubule plus ends via Kip2 where they target dynein to its site of function. Dynein exerts its function by force generation at the microtubule-binding site on the cortex (Lee *et al.* 2005; Moore *et al.* 2009b).

The motor field has exploded from the early days when antagonistic motor function was the reigning paradigm. It is now clear that, in addition to plus-end- and minus-end-directed motility, motors can function as microtubule polymerases or depolymerases by biasing fluctuations in dimer addition or subtraction at the plus end and by cross-linking of antiparallel or parallel microtubules, electrostatic couplers to enhance motor processivity and in organelle transport (*e.g.*, mitochondria). While some of these functions are segregated to one class of kinesin (Kin-5 sliding motors, Kip3 depolymerase), in other kinesin classes the functions may be dictated by interacting proteins (Kar3-Vik1 cross-linking *vs.* Kar3-Cik1 depolymerase) (Sproul *et al.* 2005) or yet additional mechanisms that remain for future investigations (*e.g.*, Cin8 function as a depolymerase at plus ends) (Gardner *et al.* 2008a).

Microtubule-associated proteins

MAPs regulate motor protein function, kinetochore microtubule dynamics, spindle orientation, and stability in metaphase and anaphase. Historically, MAPs have been defined as any microtubule-binding protein. As such, the list is large and diverse. Recent biophysical experiments reconstituting microtubule plus-end-tracking activity and the structural determination of microtubule plus-end-tracking proteins have revealed mechanistic insights into MAP function (Figure 5) (Slep 2010). The major plus-end-tracking nonmotor proteins in yeast are Bim1 (EB1), Bik1 (CLIP-170), Stu1 (CLASP), Stu2 (XMAP215), and Nip100 ($p150^{glued}$). The major binding motifs are a calponin homology domain (CH), protein glycine-rich domains (CAP-GLY), and a TOG domain (tumor overexpressing gene, a motif of 200-amino-acid residues repeated two to five times depending on the organism). Calponin homology domains are found in Bim1, CAP-GLY domains in Bik1, and TOG domains in Stu2. Each TOG domain contains up to five HEAT motifs (Figure 5). HEAT motifs form rod-like helical structures and are found in a number of proteins, including the regulatory subunit of the type 2A protein phosphatase and the auxiliary subunits

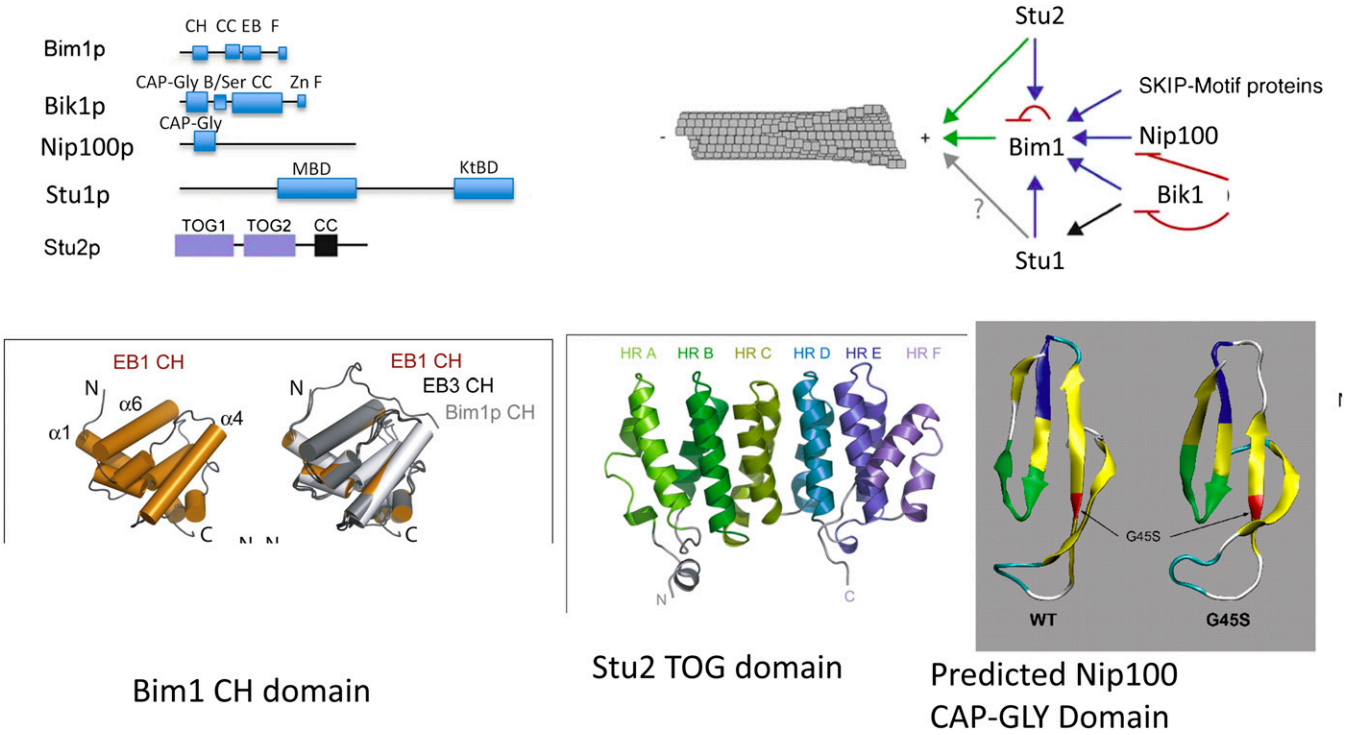

Figure 5 Domain structure and hierarchy of microtubule plus-end-tracking proteins Bim1 (EB1), Bik1 (CLIP-170), Nip100 ($p150^{glued}$), Stu1 (CLASP), and Stu2 (XMAP215). (Top left) Bim1 (EB1 family) is delineated by an N-terminal calponin homology domain (CH), an EB1 domain that confers dimerization and a C-terminal EEY motif that binds CAP-Gly domains. Bik1, of the CLIP-170 family, contains an N-terminal CAP-Gly domain, a central coiled-coil (CC) dimerization domain, a C-terminal Zn^{2+} knuckle domain that can inhibit the ability of CAP-Gly domains to bind tubulin, and a C-terminal ETF motif akin to the EEY motif found in EB1. Nip100 (of the $p150^{glued}$ family) contains a CAP-Gly domain. In the CLASP ortholog, Stu1 contains a microtubule-binding domain (MBD) as well as a kinetochore-binding domain (KtBD). Stu2 contains two N-terminal TOG domains followed by a C-terminal CC dimerization domain. (Top right) Bim1 and Stu2 bind to and regulate plus-end microtubule dynamics (green arrows). SKIP-motif proteins and the CAP-Gly-containing proteins $Nip100^{p150}$ and Bik1 show a Bim1-dependent microtubule plus-end-tracking activity (blue arrows). Whether Stu1 can plus-end-track autonomously or is Bim1-dependent remains to be elucidated. Regulatory elements are depicted in red. Adapted with permission from K. Slep (2010). (Bottom left) EB1 structure and microtubule binding. Structure of the EB1 N-terminal calponin homology domain (left, orange) reveals seven peripheral helices packed around the central conserved α3 helix. The structures of human EB1 (orange), EB3 (dark gray), and *S. cerevisiae* Bim1p (light gray) superimposed (bottom) reveal a high degree of structural identity across the EB1 family. The conserved C-terminal EB1 dimerization domain is formed through a coiled coil that folds back at its C-terminal region to form a four-helix bundle. The interface between the coiled coil and the four-helix bundle contains a signature FYF motif involved in SKIP-motif binding. (Bottom middle) Structure and mechanism of the TOG domain-containing Stu2. A cartoon representation of the *Drosophila* XMAP215 TOG2 domain, delineating the six HEAT Repeats (HR), A–F, forms the elongated domain. Each HEAT repeat is formed by two antiparallel helices bridged by an intra-HEAT loop, positioned here at the top of the domain. (Bottom right) The G59S mutation of human $p150^{glued}$ constructed in yeast. Predicted structures of residues 25–83 for wild-type Nip100 and the Nip100-G45S mutant. The sequence of Nip100 was threaded onto the structure of $p150^{glued}$. Moore *et al.* (2009a) have demonstrated that the CAP-Gly domain has a critical role in the initiation and persistence of dynein-dependent movement of the mitotic spindle and nucleus. A single amino acid change, G59S, in the conserved cytoskeletal-associated protein glycine-rich (CAP-Gly) domain of the p150 (glued) subunit of dynactin can cause motor neuron degeneration in humans and mice, which resembles ALS.

of condensin and cohesin and are proposed to promote protein–protein interactions [HEAT: **H**untingtin, elongation factor 3 (**E**F3), protein phosphatase 2A (PP2**A**), and the yeast PI3-kinase **T**OR1] (Neuwald and Hirano 2000). These repeats undergo force-induced conformational changes and may be critical in linking mechanical responses with microtubule-based force generation (Grinthal *et al.* 2010).

Bim1 requires a GTP hydrolysis-competent cap at the microtubule plus end and recognizes unique features of the plus end. Bim1 promotes lateral α/β-tubulin contacts. Bim1 in turn recruits CAP-GLY domains. The Tog domains such as found in Stu2 fold into intra-HEAT loops that form a facade delineating the tubulin-binding site and catalyze the incorporation of a tubulin dimer (Widlund *et al.* 2011). The biological function of the major MAPs in yeast bears out the diversity gleaned from the structural information. Bim1 tracks microtubule plus ends in yeast and is involved in spindle orientation (Miller *et al.*, 2000; Hwang *et al.* 2003). In the nucleus it is found on kinetochore microtubule plus ends. Its spindle function is not a critical determinant in mitosis. In contrast, it is critical at astral microtubule plus ends to orient relative to asymmetric protein cues in the bud (Hwang *et al.* 2003). Bik1 (Clip-170, CAP-GLY domains) is transported to plus ends via Kip2 (Carvalho *et al.* 2004), where it has been proposed to stimulate dynein function. More recently, Bik1 has been shown to interact with Bim1. Bim1 and Bik1 form

homodimers that interact to create a tetramer that in turn binds microtubule plus ends (Blake-Hodek *et al.* 2010). Combinatorial interactions among the different MAPS provide a mechanism to increase the number of distinct activities from a limited number of proteins. Different complexes may generate novel functions, provide modes of inhibition or activation (Blake-Hodek *et al.* 2010), or modulate the strength of microtubule-binding interactions at distinct sites.

The homolog of Stu2, XMAP215, is a microtubule polymerase, which accelerates the growth of microtubules 10-fold after fertilization in frog eggs. In yeast, Stu2 seems to be a dynamicity factor. *In vivo*, Stu2 acts to increase the numbers of cytoplasmic microtubules, consistent with a stabilizing activity. At kinetochores, Stu2 appears to promote turnover of attached microtubule ends, suggesting a destabilizing activity (Pearson *et al.* 2003). Pure Stu2 has been reported to destabilize microtubules *in vitro* as well, but a *Xenopus* homolog, XMAP215, has been found to promote both assembly and disassembly, depending on conditions (Shirasu-Hiza *et al.* 2003). Given this variety of reported activities, Stu2 seems to act as a dynamicity factor, stabilizing or destabilizing microtubule dynamics, depending on the local environment and protein interaction (Pearson *et al.* 2003; Brittle and Ohkura 2005). Most recently, the TOG domains and a basic region have been engineered to recapitulate almost full microtubule polymerase activity (Widlund *et al.* 2011). Stu2 is therefore a significant catalyst of tubulin polymerization at microtubule plus ends.

The CLASP (**CL**IP-**as**sociated **p**rotein) homolog is Stu1 (Akhmanova and Hoogenraad 2005; Ortiz *et al.* 2009). CLASPs are plus-end-tracking proteins that stabilize MT plus ends by facilitating the incorporation of tubulin subunits (Maiato *et al.* 2005). In addition, by localizing at specialized MT regions distinct from plus ends, at least one CLASP member contributes to the stability of MT bundles in interphase (Bratman and Chang 2007). Metazoan CLASPs bind to KTs, where they regulate the dynamic behavior of KT MTs (kMTs) and, consequently, chromosome congression (Maiato *et al.* 2002, 2003; Cheeseman *et al.* 2005; Mimori-Kiyosue *et al.* 2006). Stu1 is required for spindle formation (Yin *et al.* 2002) and is found on the spindle. More recently, it has been shown that Stu1 binds unattached kinetochores in budding yeast (Ortiz *et al.* 2009). Unattached kinetochores sequester Stu1 to prevent premature spindle stabilization, thereby keeping the two spindle poles together to facilitate sister kinetochore bi-orientation.

The spindle midzone is the region of overlapping antiparallel microtubules, which, on average, constitutes about eight microtubles (three to four from each spindle pole) (Winey *et al.* 1995). The interpolar microtubules vary in length and rarely span the length of the spindle. The region of overlap is ~3/4 μm in length. As the spindle elongates in anaphase, polar microtubules increase in length and decrease in number, generating an elongated late-anaphase spindle with a pair of overlapping microtubules. This structure provides physical support until spindle disassembly. Proteins binding to the region of overlap (known as the midzone) provide a critical function in spindle stability. One of the major midzone proteins is Ase1 (**a**naphase **s**pindle **e**longation) (Pellman *et al.* 1995). Ase1 is a member of a conserved family of cross-linking proteins (PRC1 in humans, fascetto and sofe in Drosophila, MAP65 in plants). Ase1 forms a dimer and has a microtubule-binding domain. It diffuses randomly on single microtubules and plays a key role in cross-linking antiparallel microtubules. In mammals, a kinesin 4 moves the Ase1 homolog PRC1 to the plus ends of antiparallel microtubules. Ase1 phosphorylation plays an important role in regulating spindle assembly as well (Kotwaliwale *et al.* 2007). Thus, while these MAPs have primary functions in specific spatial or temporal domains of spindle morphogenesis, such as the spindle midzone, their functions are not restricted to these domains. More subtle phenotypes and contribution to a variety of spindle features will undoubtedly be discovered as we continue to tease apart the molecular nuts and bolts of the spindle.

With the advent of large-scale genomic screens applied to spindle morphogenesis (Hwang *et al.* 2003; Vizeacoumar *et al.* 2010) and spindle disassembly (Woodruff *et al.* 2010), it is certain that MAPs will continue to be identified. Of note is Irc15 (Keyes and Burke 2009), which is found to bind microtubules *in vitro* and localize to spindles *in vivo*. Cells mutant in Irc15 have a prolonged duration of mitosis.

DNA, cohesion, and condensin springs in the spindle

The 16 kinetochore microtubules (on average 0.35 μm in length in the half-spindle) and 4 interpolar microtubules (~1 μm in length from each spindle pole) yield ~20 μm of microtubule polymer in the spindle. At 8 nm per tubulin dimer, 13 protofilaments per microtubule, and 110 kDa per dimer, $\sim 3.25 \times 10^6$ kDa of tubulin is polymerized in the spindle (Figure 1). The number of Cin8 and Kip1 microtubule motor proteins (~100 kDa) in the spindle is on the order of just a few hundred (A. P. Joglekar and K. Bloom, unpublished results), and their mass is dwarfed by that of tubulin. Likewise, each kinetochore (see below) is $\sim 5 \times 10^3$ kDa (De Wulf *et al.* 2003), and the 32 per spindle constitute $\sim 0.16 \times 10^6$ kDa, or <5% the mass of tubulin. The mass of chromatin in a cell is on the order of 1.4×10^7 bp × 660 Da = 10×10^6 kDa. Thus the spindle is just 1/3 the mass of the DNA (and ~1/6 the mass of the chromatin) that needs to be faithfully segregated.

It has recently been found that chromatin not only is the site of kinetochore formation, but also provides an elastic component to spindle function (Bouck and Bloom 2007). This elastic component is built from loops of pericentric chromatin and cohesin (Yeh *et al.* 2008) (Figure 6). Each loop is ~20 kb; thus, for 16 chromosomes at 20 kb per sister chromatid × 2 sister chromatids = 640 kb DNA. This is ~4.5% of the genome ($6.4 \times 10^5 / 1.4 \times 10^7 = 4.5\%$ of the genome) or 640 kb × 660 Da/bp = 0.4×10^6 kDa. Assuming an equal weight of histone yields a chromatin mass of 0.8×10^6 kDa. This structural perspective reveals that pericentric

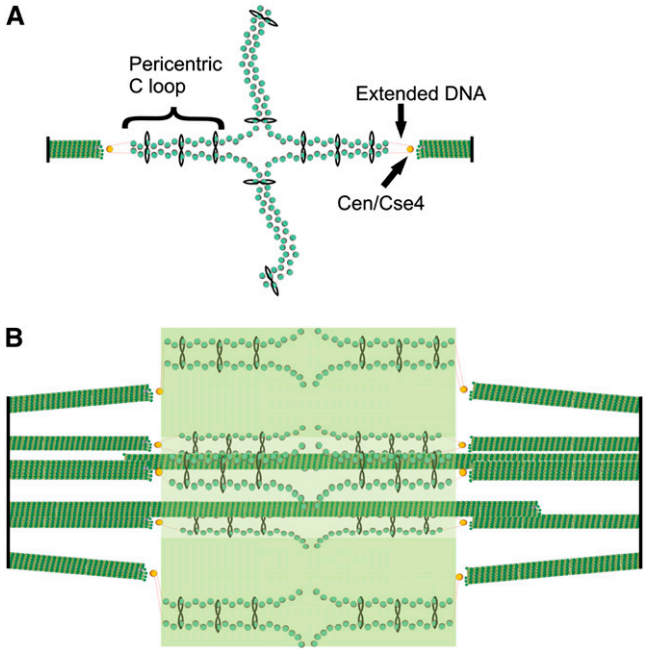

Figure 6 DNA springs in the spindle: model of the organization of cohesin and pericentric chromatin in metaphase (A) DNA of each sister chromatid is held together via intramolecular bridges that extend ~11.5 kb on either side of the centromere. A transition from intra- to intermolecular linkages results in a cruciform structure. (B) Five (of 16) bioriented sister chromatids are shown with two (of eight) interpolar microtubules. We have proposed that the transition between intramolecular looping and intermolecular cohesion is mobile and, on average, 11.5 kb from the centromere core. DNA adjacent to the centromere may extend to its form length *in vivo* (depicted as thin orange lines), thereby linking the centromere at kinetochore–microtubule plus ends to strands of intramolecularly paired pericentric chromatin and cohesin that are displaced radially from spindle microtubules. Microtubules and spindle pole bodies are represented by green and black rods, respectively. The 125-bp centromere is wrapped around the Cse4-containing histone in yellow. Nucleosomal chromatin is depicted as green histone cores wrapped around DNA in thin red line. Cohesin is depicted as black ovals linking two strands of nucleosomal DNA. The fluorescence distribution of cohesin is depicted in transparent green. Pericentric chromatin from each of the 16 chromosomes is displaced 70–90 nm radially from the central spindle microtubules. The entire spindle is composed of 32 kinetochore microtubules and eight pole–pole microtubules. From Yeh *et al.* (2008).

chromatin constitutes roughly one-fifth the mass of the segregation apparatus (0.8×10^6 kDa/$0.8 + 3.25 \times 10^6$ kDa), a significant fraction relative to spindle microtubules. Together with microtubules, the pericentric chromatin provides a structural component that contributes to mitotic spindle function. The pericentric chromatin is organized into a molecular spring that opposes the outward-directed microtubule-based pushing forces. The spring is bound to the spindle at the kinetochore/microtubule interface and linked to its sister chromatid via cohesin. Thus, motors bound to antiparallel microtubules provide struts in the spindle, while DNA springs are elastic elements in the spindle. These loops provide an inward force to counterbalance outward-pulling forces generated by kinetochore and spindle microtubules. The loops are compliant and contribute to mechanical strain in response to tension generated in mitosis.

It is unlikely that the elastic spring is dictated by the properties of DNA itself. In addition to histones, the major constituents of chromatin in the pericentric chromosomal region include topoisomerase 2, cohesion, and condensin. Cohesin and condensin are members of the structural maintenance of chromosome (SMC) proteins that assemble into complexes that adopt a ring-like conformation. The backbone of the ring is formed by the SMC proteins themselves (MukB in bacteria, Smc2 and Smc4 in *S. cerevisiae* condensin, and Smc1 and Smc3 in *S. cerevisiae* cohesion) (reviewed in Melby *et al.* 1998). In eukaryotes, the SMC monomer is folded in an antiparallel coiled coil. At one end, the two monomers associate to form a hinge, and at the other end is an ATP-binding head domain. Closure of the ring at the head domain is carried out by proteins known as kleisins, including Scc1 (also known as Mcd1) and Brn1. Each dimer is associated with additional proteins [*e.g.*, Ycs4, Ycg1, Scc3 (also known as Irr1), Rad61, and Pds5] at the head domain to form a functional complex *in vivo*. In bacteria, the SMC coiled coils are bound by ScpA and ScpB. The primary biochemical function of cohesin is to hold together sister-chromatid strands while condensin functions in chromatin compaction (Hirano 2006; Nasmyth and Haering 2009). Cohesin and condensin are enriched threefold in the pericentromeric chromatin where they contribute to the spring-like elastic properties of this region of the chromosome (Gerlich *et al.* 2006; Bachellier-Bassi *et al.* 2008; D'Ambrosio *et al.* 2008; Ribeiro *et al.* 2009; Samoshkin *et al.* 2009). Cohesin and condensin exhibit distinct patterns of localization within the spindle (Stephens *et al.* 2011). Cohesin (Smc3) exhibits a bi-lobed structure when viewed from the side and a cylindrical array when viewed on end (Yeh *et al.* 2008) (Figure 6). In contrast, condensin (Smc4) is enriched along the spindle axis (Bachellier-Bassi *et al.* 2008). Pericentric cohesin is radially displaced from the spindle microtubules, while condensin lies proximal to the spindle axis where it is primarily responsible for axial compaction of pericentric chromatin. Together with the intramolecular centromere loop, these SMC complexes constitute a molecular spring that balances spindle microtubule forces in metaphase (Stephens *et al.* 2011). The segregation apparatus is therefore a composite material of microtubules and chromatin (Bouck and Bloom 2007; Bouck *et al.* 2008).

The kinetochore

The kinetochore is a large multiprotein complex that links the sister chromatids to the mitotic spindle during chromosome segregation. The physical organization of the kinetochore into a trilaminar structure (Brinkley and Stubblefield 1966; Jokelainen 1967) is visible in electron microscopy images of vertebrate kinetochores, and the molecular composition in yeast follows this three-layer organization (Joglekar *et al.* 2006, 2008, 2009; Cheeseman and Desai 2008; Anderson *et al.* 2009; Santaguida and Musacchio 2009) despite the inability to see yeast kinetochores in the electron microscope (Peterson and Ris 1976). The kinetochore forms on the microtubule plus end as

a "basket" of elongated molecules (namely Ndc80) that recruit the outer-kinetochore components of the KMN network (which comprises the Knl1 complex, the Mis12 complex, and the Ndc80 complex). These outer-kinetochore proteins dangle from the expanded basket surface generated by Ndc80 and can interact with the chromatin and proteins of the constitutively centromere-associated network. The purpose of the basket is to allow the outer-kinetochore components to move over a greater distance and increase the likelihood of encountering an unattached centromere. In addition, this basket-like structure is likely to be part of the solution that contributes the mechanism in which a dynamic microtubule remains attached to the kinetochore. Namely, tubulin subunits must be free to diffuse into and away from the microtubule plus end while it is embedded in the kinetochore. The geometry of the pericentric chromatin loops predisposes the centromere DNA (CEN) to be at the surface of the chromosome, where CEN is able to recruit a centromere histone H3 variant (CENPA) as well as the centromere DNA-binding proteins (CBF3). The outer and inner halves of the kinetochore can then interact and form a stable attachment, connecting the chromosome to the microtubule. The kinetochore serves several important roles during chromosome segregation: it links chromosome movement to microtubule dynamics, monitors chromosome biorientation, and serves as a site of catalysis for synchronizing chromosome segregation with cell cycle events.

We propose that the whole budding yeast mitotic spindle might serve as a model for a single regional centromere with multiple microtubule attachments per chromosome (Zinkowski *et al.* 1991) and that the cruciform structure found at budding yeast centromeres is analogous to the looping model for more complex centromeres (Figure 6). The cruciform structure of the pericentromere places the centromeres at the apex of the intramolecular loop loaded with cohesin, maximizing the distance between sister centromeres and thus reconciling increased cohesin and maximal spot separation during mitosis (Yeh *et al.* 2008). Further work has revealed that the formation of this structure is promoted by the DNA-binding components of the kinetochore (Anderson *et al.* 2009). This function is likely to be inherent in the structure of the proteins. For example, the *S. cerevisiae* protein Ndc10 (also known as Cbf3a) is needed to form the looping cruciform structure; it is thought to bind as a dimer, and it is possible that these dimers serve to bring two regions of chromatin together to form a loop. Given the high level of conservation in composition between yeast and higher eukaryotic kinetochores (Joglekar *et al.* 2006, 2008, 2009), one view is that multiple binding-site kinetochores of regional centromeres are repeats of the basic kinetochore of budding yeast, as proposed by the repeated subunit hypothesis (Zinkowski *et al.* 1991). However, electron microscopy work has suggested that the mammalian kinetochore is disorganized and lacks the recurring subunits proposed by the repeated subunit hypothesis (Dong *et al.* 2007; McEwen and Dong 2010). A view that reconciles these perspectives is that the inner-kinetochore–centromere interface resembles a woven fabric, rather than two separate fixed structures.

Building the Spindle

Spindle pole duplication and separation

SPB duplication is the cell cycle process by which the two SPBs responsible for mitotic spindle assembly are formed. The first morphologically recognizable steps of the process begin early in the cell cycle during G1. However, the first regulatory events may occur at the end of the previous cell cycle in the form of a licensing event conceptually similar to the licensing of chromosomal DNA replication (Hasse *et al.* 2001). The classic work of Breck Byers and Loretta Goetsch revealed the morphological pathway of SPB duplication (Byers and Goetsch 1975). They also demonstrated that SPB duplication is coordinated with the cell cycle, such that the numerous *cdc*$^-$ mutants identified in the neighboring laboratory of Lee Hartwell not only gave uniform arrests by cell morphology and DNA content, but also arrested with uniform SPB and spindle morphologies (Byers and Goetsch 1974). This profound and fundamental observation is now taken for granted. Also fundamentally important in this early work was the discovery of a single *cdc*$^-$ mutant that violated the coordinated arrest finding. Strains mutant in *CDC31* failed to duplicate their SPBs, yet went onto a mitotic arrest that should have included a bipolar spindle (Byers 1981). *CDC31* was the first gene identified in yeast, or in any organism, to have a specific function in spindle pole duplication. It is not surprising that this cellular process in yeast, like most others, has been dissected genetically in great detail.

The first recognizable intermediate in SPB duplication is the satellite-bearing stage (Figure 7). This stage is most easily observed in mating factor-arrested cells or in cells at the *cdc28* arrest (budding yeast CDK) (Byers and Goetsch 1974). This stage is also observed in cycling cells, indicating that it is a normal intermediate in SPB duplication. At this early point in duplication, the half-bridge has lengthened from ~90 nm to nearly twice its length, and a tuft of dark staining material (called the "satellite") is observed at the SPB-distal end of the bridge on the cytoplasmic side. It is interesting that both the lengthening of the half-bridge and the deposition of the satellite occur in G1, but how these events are regulated is not completely known.

Presumably, lengthening of the half-bridge occurs prior to the addition of the satellite. John Kilmartin's discovery of Sfi1 as a centrin (Cdc31)-binding partner at the half-bridge produced an intriguing model for the dynamic behavior of this structure (Li *et al.* 2006). Sfi1 contains 15 repeats of a motif that is distantly related to the IQ-gap domain that binds calmodulin (Kilmartin 2003; Li *et al.* 2006). In this case, the Sfi1 repeat (pfam designation: Sfi1, PF08457) binds centrin. In co-crystals of centrin and the Sfi repeat, the Sfi moiety is an α-helix that binds centrins in a head-

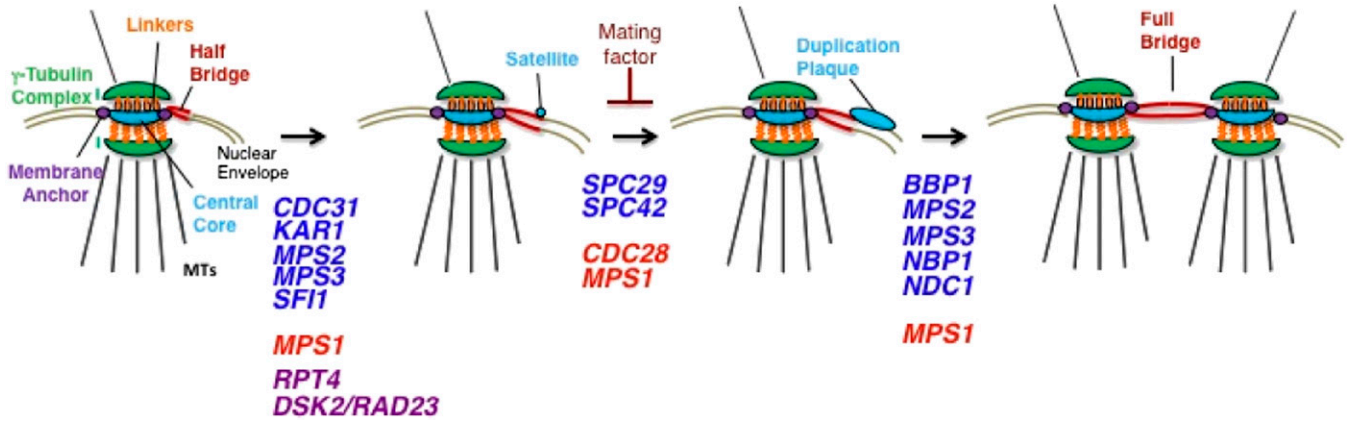

Figure 7 The SPB duplication pathway. The various steps and the genes required at each step of the pathway are indicated (see *Spindle pole duplication and separation*). Those in blue encode SPB components, those in red encode protein kinases that regulate SPB duplication, and those in purple are other regulators. In brief, the first step is elongation of the half-bridge and the formation of the satellite. The second step is the expansion of the satellite into the duplication plaque and the beginning of the fenestra in the nuclear envelope. The third step is the insertion of the new SPB into the nuclear envelope and the maturation of its nuclear face, creating duplicated side-by-side SPBs. The point of arrest of the duplication pathway by mating factor, in preparation for cellular fusion and karyogamy, is indicated.

to-tail arrangement that is not significantly altered by calcium binding (Kilmartin 2003; Li *et al.* 2006). Kilmartin estimated the length of Sfi1 bound to centrin as ~90 nm, or the length of the half-bridge throughout most of the cell cycle. Using epitope-tagged alleles of Sfi1, Kilmartin showed that the N terminus of Sfi1 was proximal to the SPB and that the C terminus was distal from the SPB. This result suggests that Sfi1 determines the length of the half-bridge and that its orientation in the bridge offers a molecular basis for asymmetry in the bridge that is likely important in SPB duplication (Li *et al.* 2006). Importantly, the elongated bridge seen in mating factor-arrested cells or seen between two SPBs (called the full bridge) has two apparent end-to-end populations of Sfi1 along its length. In these cases, the N termini are at the ends of the long half-bridge and the C-terminal ends are in the middle, potentially interacting with each other (Li *et al.* 2006; Anderson *et al.* 2007). It has been suggested that the N terminus of Sfi1 not associated with the existing SPB defines the site of SPB assembly, potentially by recruiting satellite components (Li *et al.* 2006).

Adams and Kilmartin (1999) showed that the satellite is composed of the core SPB components Spc42, Spc29, Nud1, and Cnm67. After the satellite is formed, it will expand while still on the cytoplasmic face of the nuclear envelope, forming what the authors termed a "duplication plaque" that appears similar to the central plaque of the SPB (Adams and Kilmartin 1999) (Figure 7). Expansion of the duplication plaque is concurrent with its insertion into the nuclear envelope. This is accomplished by the creation of a fenestra at the distal end of the half-bridge into which the growing duplication plaque is inserted, giving it access to the nucleoplasm. How the membrane fenestration is created is unknown, but it appears to require the functions of the SPB membrane proteins Ndc1 and Mps2 and their respective binding partners Nbp1 and Bbp1 (Winey *et al.* 1991, 1993; Schramm *et al.* 2000; Araki *et al.* 2006). Mps2 also interacts with the half-bridge membrane and SUN-domain protein Mps3, but this interaction is involved with bridge assembly or maintenance (Jaspersen *et al.* 2006). Nonetheless, Mps3 is also required for insertion of the duplication plaque into the nuclear envelope (Sue Jaspersen, personal communication).

The still mysterious relationship between SPBs and NPCs was first hinted at by the discovery that Ndc1 is found at both structures (Chial *et al.* 1998). Ndc1 was known to be required for SPB assembly (Winey *et al.* 1993) and has been subsequently shown to be required for the assembly of NPCs into the intact nuclear envelope of yeast (Lau *et al.* 2004; Onischenko *et al.* 2009). However, the relationship goes beyond a shared component. EM studies have shown that NPCs are found clustered near SPBs in mitotic cells, possibly to ensure the segregation of a sufficient number of NPCs to the daughter cells with each SPB (Winey *et al.* 1997). Furthermore, Adams and Kilmartin (1999) reported observing an NPC-like structure at the site of insertion of the SPB into the nuclear envelope, raising a potential role for NPCs in SPB duplication. Various genetic interactions have been detected between alleles of genes encoding SPB components and NPC components. For example, the deletion of genes coding for the nonessential membrane nucleoporins Pom152 or Pom34 suppressed the SPB insertion defects caused by certain mutant alleles of *NDC1*, *MPS2*, or *BBP1* (Chial *et al.* 1998; Sezen *et al.* 2009). This includes the intriguing finding that the null allele of the essential *MPS2* gene can be rescued by deletion of Pom152 or Pom34 (Sezen *et al.* 2009). Similarly, the deletion of Pom152 or the nucleoporin Nup157 will rescue the deletion of the *MPS3* gene, another essential SPB membrane protein (Witkin *et al.* 2010). Finally, a screen for SPB-remodeling factors, described below, identified a role for Pom152 in controlling

SPB size (Greenland *et al.* 2010). These interactions between SPBs and NPCs are complex and poorly understood, but involve shared components (Chial *et al.* 1998), shared environment in the nuclear envelope (Witkin *et al.* 2010), and coordinated translational control of component synthesis (Sezen *et al.* 2009).

Upon insertion into the nuclear envelope, the duplication plaque matures into a SPB by its acquisition of microtubule nucleation capacity. The resulting structure is called the duplicated side-by-side SPB. As noted above, this activity is dependent on γ-tubulin complexes, the outermost components of the outer and inner plaques. Outer plaque development is complicated because of MT nucleation from the bridge of duplicated side-by-side SPBs on the basis of the interaction of Spc72 with the bridge component Kar1 (Pereira *et al.* 1999). The fact that the duplicated SPBs at this stage are different from each other with respect to age leads to the older "mother" SPB being able to nucleate microtubules, whereas the younger "daughter" SPB lags in the cell cycle in gaining this activity, which is controlled by Cdc28/Clb5 (Segal *et al.* 2000; Pereira *et al.* 2001). Furthermore, the phosphorylation of γ-tubulin (Tub4) also influences the behavior of the cytoplasmic microtubules (Vogel *et al.* 2001).

Inner-plaque formation involves the localization of both the γ-tubulin complex and its inner-plaque linker, Spc110, into the nucleus. This is accomplished by NLS on Spc110 (Adams and Kilmartin 1999) and on Spc98, which is sufficient to transport the entire γ-tubulin complex into the nucleus (Pereira *et al.* 1998). Mutation of the NLS in Spc110 relocalizes it to the cytoplasm, and overexpression of this mutant form of the protein will interfere with SPB duplication (Adams and Kilmartin 1999). Furthermore, proper interactions between Spc110 and calmodulin (Cmd1) are required for normal assembly (Sundberg *et al.* 1996). Similar to Spc110, mutation of an NLS in Spc98 blocks localization of the γ-tubulin complex to the nucleus and is lethal (Pereira *et al.* 1998). This mutant allele enabled Schiebel and co-workers to show that an Mps1-phosphorylated form of Spc98 is found only in the nucleus, but the function of this phosphorylation event is not known (Pereira *et al.* 1998).

Conditional mutations in the various genes encoding SPB components block every step of the duplication pathway. The generic phenotype of these types of mutants is mitotically arrested cells (large-budded cells with replicated chromosomes) displaying aberrant microtubule organization arising from the single SPB instead of two SPBs forming a bipolar spindle. The single SPB is the "mother" SPB that the cells possessed prior to attempting duplication, and it forms a monopolar spindle (*e.g.*, Winey *et al.* 1991). These monopolar spindles arising from SPB duplication defects cause a mitotic arrest by triggering the spindle assembly checkpoint (Weiss and Winey 1996). Examination of the defective SPBs by electron microscopy has proven instructive about the nature of the failed assembly process. As mentioned above, mutations in the genes encoding components of the half-bridge lose this structure at the restrictive temperature and block duplication by eliminating the site of satellite assembly (Byers 1981; Rose and Fink 1987; Jaspersen *et al.* 2002; Kilmartin 2003).

Failure to insert the duplication plaque into the nuclear envelope is observed in cells mutated in the membrane anchor proteins Ndc1 and Mps2 or in their respective binding partners, Nbp1 and Bbp1 (Winey *et al.* 1991, 1993; Schramm *et al.* 2000; Araki *et al.* 2006). Specific alleles of the SUN-domain membrane protein Mps3 also cause this phenotype (Sue Jaspersen, personal communication). Mutant cells include not only the monopolar spindle formed from the mother SPB, but also a second defective SPB derived from the duplication plaque. This defective SPB is attached to the cytoplasmic face of the nuclear envelope and is assembled to the point where it has cytoplasmic microtubules (Winey *et al.* 1991, 1993). Oddly, these cytoplasmic microtubules can exert force on the defective SPB, presumably via cortical attachments, and pull it away from the mother SPB and into the bud on a thin extension of the nuclear envelope that lacks chromatin.

Finally, inner-plaque defects can be observed in Spc110, Cmd1, and some alleles of the genes encoding components of the γ-tubulin complex. In one such allele (tub4-34), the inner plaque appears to be missing although the SPB has been inserted into the envelope (Marschall *et al.* 1996). *CMD1* alleles exhibit pleiotropic defects because of the numerous functions of calmodulin, but SPB defects are evident (*e.g.*, Sun *et al.* 1992). More exotic are alleles of Spc110 defective in calmodulin binding that form aberrant structures in the nucleus that act as MTOCs (Sundberg *et al.* 1996). In addition, the *spc110-226* allele causes a loss of integrity of SPB structure at the restrictive temperature (Yoder *et al.* 2005). In these mutants, the inner-plaque γ-tubulin complexes along with the mutant Spc110 can "delaminate" from the SPB and form foci in the nucleoplasm that are associated with the minus end of microtubules. This observation of SPB failure in mitosis suggests that significant forces are applied to the structures at this point in the cell cycle when the chromosomes are achieving biorientation.

Duplicated side-by-side SPBs are observed upon the completion of SPB duplication. In this case, a complete bridge tethers the two SPBs, mother and daughter, together. SPB duplication can go to this late step in the process during G1 as evidenced by the finding that the *cdc*$^-$ mutant alleles of SCF components (*cdc4*, *cdc34*, or *cdc53*) each arrest with duplicated side-by-side SPBs but fail to enter S phase (see Jaspersen and Winey 2004). Interestingly, there are mutants that arrest with duplicated side-by-side SPBs, but which have also completed S phase and have grown a bud. This arrest may be equivalent to G2, being a very brief part of the cell cycle in cycling cells. The separation event involves an enigmatic severing of the bridge, leaving a half-bridge on each of the SPBs as they are separated to form the mitotic spindle. The genes involved in separation of duplicated side-by-side SPBs are the bridge component Sfi1 (Anderson *et al.* 2007); the major yeast CDK, Cdc28 (Fitch *et al.* 1992); and

the kinesin-like motor proteins, Cin8 and Kip1 (Roof *et al.* 1992; Saunders and Hoyt 1992). Mutations in the C-terminal domain of Sfi1 block separation (Anderson *et al.* 2007). This is the domain of the protein that is at the center of the bridge that may be exposed once the bridge is severed, producing the site for bridge elongation during the next round of duplication. How the C-terminal ends of the Sfi1 function to create a dissolvable junction in the middle of the bridge, whether it be via direct interactions or via interacting with an another bridge component, is not known. Separation of the SPBs does require force to be applied to the SPBs such that they move in the nuclear envelope. The kinesin-like motor proteins Cin8 and Kip1 are required for SPB separation and presumably work by cross-linking the microtubules from each of the SPBs. Upon moving toward the plus ends of the microtubules during spindle assembly, the motors participate in driving the SPBs apart. Cytoplasmic microtubules can exert forces on SPBs (Tolic-Norrelykke 2010), but SPB separation will occur without these microtubules (Huffaker *et al.* 1988; Jacobs *et al.* 1988; Li *et al.* 1993) or without dynein function (Li *et al.* 1993). Finally, the bridge is not severed in cyclin B (*CLB1-4*)-depleted cells (Fitch *et al.* 1992) or in cells overexpressing Swe1, which inhibits CDK activity (Lim *et al.* 1996). These results reveal that the increasing CDK/CyclinB activity in mitotic cells is required to separate SPBs.

Regulation of SPB duplication

Most SPB components are phosphorylated (Wigge *et al.* 1998), and this modification is required for the proper regulation and assembly of several components (*e.g.*, Jaspersen *et al.* 2004). Recent phospho-proteomics studies of overexpressed γ-tubulin complexes (Tub4, Spc97, Spc98) (Lin *et al.* 2011) and of entire intact SPBs (Keck *et al.* 2011) revealed extensive phosphorylation of SPB components. For example, Keck *et al.* (2011) report 297 phosphorylation sites over the 17 of 18 core SPB components; only Mps3 lacked mapped sites, likely due to poor coverage of this membrane protein. Furthermore, these authors showed that there are substantial changes in phosphorylation-site usage at different points in the cell cycle. Both groups showed that phosphorylation of γ-tubulin (Tub4) is functionally important, as previously shown for Y445 (Vogel *et al.* 2001; position Y445 verified in Keck *et al.* 2011). Lin *et al.* (2011) found that phospho-mimetic mutations in *TUB4* at S74, S100, and S360 are lethal. Keck *et al.* (2011) showed that the highly conserved Tub4-S360 was phosphorylated *in vivo* and is a CDK site *in vitro* as predicted by its sequence. In these authors' research, the *tub4-S360D* allele displayed a temperature-sensitive lethal phenotype (Keck *et al.* 2011). At the restrictive temperature, the cells displayed significant defects in the mitotic spindle similar to what was reported by Lin *et al.* (2011). At the permissive temperature, the *tub4-S360D* strains exhibit abberations in spindle elongation during anaphase, revealing an intriguing link between γ-tubulin complex phosphorylation and spindle dynamics.

A few protein kinases have been implicated in SPB assembly and function, but unique among these is Mps1 protein kinase (Winey and Huneycutt 2002). This is the only yeast protein kinase-encoding gene for which SPB duplication defective alleles have been identified. At the restrictive condition, the original allele, *mps1-1*, produced monopolar spindles formed by SPBs with a long half-bridge. This result indicated that Mps1 is required for satellite formation, but not for bridge elongation (Winey *et al.* 1991). Further analysis of an allelic series and an analog-sensitive allele revealed that Mps1 is involved in bridge elongation, in satellite formation and utilization, and in membrane insertion of the duplication plaque (Pereira *et al.* 1998; Schutz and Winey 1998; Friedman *et al.* 2001; Castillo *et al.* 2002; Jones *et al.* 2005; Holinger *et al.* 2009; Araki *et al.* 2010). This may not be surprising in retrospect now that Cdc31, Spc29, Spc42, Spc98, and Spc110 are known substrates (Pereira *et al.* 1998; Friedman *et al.* 2001; Castillo *et al.* 2002; Holinger *et al.* 2009; Araki *et al.* 2010). The relevant substrate(s) for SPB nuclear envelope insertion is not known. In addition to the multiple SPB phenotypes observed in *mps1*$^{-}$ mutant strains, it was also observed that these cells failed to arrest in mitosis similar to other SPB duplication-defective mutant strains (Winey *et al.* 1991). This was found to result from failure of the spindle assembly checkpoint in which Mps1 also functions (Weiss and Winey 1996). Like other mitotic protein kinases, Mps1 has multiple functions during mitosis, including important functions at the kinetochore (*e.g.*, Maure *et al.* 2007; Dobra *et al.* 2011).

As with chromosomal DNA replication, the precise duplication of the SPBs to produce a single new SPB per cell cycle is ultimately under the control of Cdc28, the CDK that regulates the budding yeast cell cycle. As mentioned above, Cdc28 activity in mitotic cells is required to separate duplicated side-by-side SPBs from the mitotic spindle. Similar to the chromosome cycle, CDK activity must be inactivated during anaphase to allow for SPB duplication (Hasse *et al.* 2001). This finding hints at a licensing event, but molecular basis of that event is unknown. Finally, CDK activity in late G1 is required to proceed past the satellite-bearing stage. Cdc28 is known or thought to phosphorylate several SPB components, including Spc29, Spc42, Spc110, Nbp1, Bbp1, and Nud1 (Friedman *et al.* 2001; Ubersax *et al.* 2003; Jaspersen *et al.* 2004; Park *et al.* 2004, 2008; Holinger *et al.* 2009). The function of most of these phosphorylation events is unknown, but Cdc28 phosphorylation of Spc42 is required for proper assembly (Jaspersen and Winey 2004; Keck *et al.* 2011). Other potential regulators of the SPB duplication pathway are the yeast polo kinase Cdc5 (Park *et al.* 2004, 2008; Maekawa *et al.* 2007) and the proteosome. A double-mutant strain lacking the polyubiquitin-binding proteins Dsk2 and Rad23 exhibits a SPB duplication defect whose basis is unknown (Biggins *et al.* 1996). Also, mutations in the proteosome cap subunit Rpt4 fail in SPB duplication (McDonald and Byers 1997). These results offer the possibility that some protein must be degraded to specifically allow for SPB duplication.

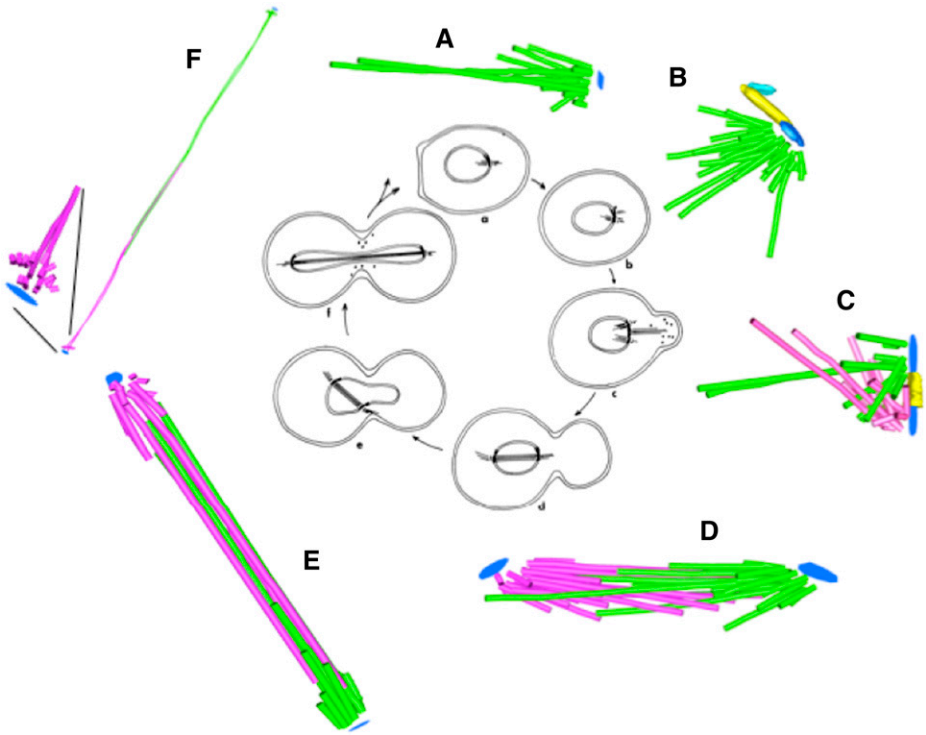

Figure 8 Breck Byer's classic drawing of the yeast cell cycle indicating the state of the SPBs (Byers and Goetsch 1975) to which are added representative models of SPBs and microtubules from electron microscopy and electron tomography studies (Winey *et al.* 1995; O'Toole *et al.* 1999). A single SPB in G1 phase (A) has nuclear and cytoplasmic microtubules. A duplicating SPB (B, blue) has a satellite or early duplication plaque (green) on the half-bridge (yellow). (C) Duplicated side-by-side SPBs. Each SPB has nuclear microtubules, and there are a few cytoplasmic microtubules. A short bipolar spindle (D, ~600 nm) is not well organized. A medial length, likely metaphase, spindle (E, ~1.5 um) is clearly organized with shorter kinetochore microtubules and the fewer longer microtubules that will form the central spindle. A late-anaphase spindle (F, ~8 um) and a SPB in such a spindle (enlarged) as seen by electron tomography. The predominant feature of the anaphase spindle are the few tightly packed and twisted microtubules that interdigitate at the spindle midzone. However, the SPB has numerous (nearly 16) very short (30–50 nm) microtubules that are presumably still attached to kinetochores.

The step-wise assembly and separation of SPBs tells only part of the story of SPB assembly (Figure 8). The exchange of transiently expressed GFP-tagged SPB components into SPBs (Jaspersen and Winey 2004), FRAP of GFP-tagged SPB components (Yoder *et al.* 2003), and the continued growth of SPBs in mitotically arrested cells (O'Toole *et al.* 1997) all reveal dynamic behavior of SPB components. Not known are the mechanism of the exchange, the extent to which any given SPB component can be exchanged, and the biological significance of the exchange. It possible that the exchange of components allows for the repair of damaged SPBs because there is no known instance of *de novo* SPB formation. Hence, cells must maintain their SPBs because they cannot be replaced. Some studies have begun to address this exchange of components. The exchange of transiently expressed Spc42 into SPBs fails in cells mutated for its binding partner Spc29 and fails in cells lacking Cdc28 or Mps1 activities, suggesting that the exchange can share requirements with assembly during SPB duplication (Jaspersen *et al.* 2004). A system to specifically damage SPBs was engineered by inserting TEV protease sites into the Spc110 protein (Greenland *et al.* 2010). Cleavage of Spc110 leads to a spindle checkpoint-mediated cell cycle delay. This system was used for a screen for genes required for SPB remodeling and identified mutations in genes with various functions in the nuclear envelope and in protein turnover (Greenland *et al.* 2010). Maintenance of MTOC structures is an emerging and important area of study (Pearson *et al.* 2009) that is accessible to genetic analysis in budding yeast.

Spindle Dynamics

Regulation of microtubule dynamics, kinetochore and interpolar microtubules

The half-life of a tubulin dimer in the microtubule lattice is ~50 sec (Maddox *et al.* 2000a). The microtubules do not shorten completely prior to rescue. Rather, there is a gradient in dynamics, with the most rapid turnover exhibited at the plus ends (near the spindle midzone) and the least turnover at the minus ends (proximal to the spindle pole) (Figure 9). Thus kinetochore microtubules make many short excursions into catastrophe followed by frequent rescue. It has been proposed that these robust short excursions contribute to mechanisms responsible for the fidelity of chromosome segregation (Pearson *et al.* 2006).

The study of the regulatory gradients of microtubule dynamics in budding yeast requires methods to obtain spatial information below the resolution of the light microscope. The resolution limit is defined as the smallest distance between two points in an object that can be distinguished as two points in the image. The resolution limit of the light microscope when illuminating with green light is ~1/4 micrometer. In addition, since the interpolar and kinetochore microtubules are confined to a spindle 1/4 μm in diameter, it is not possible to study single microtubules, and as a consequence one is always looking at a population average. To circumvent the limitation of light microscopy and to deduce the behavior of individual microtubules in the budding yeast spindle, a method known as

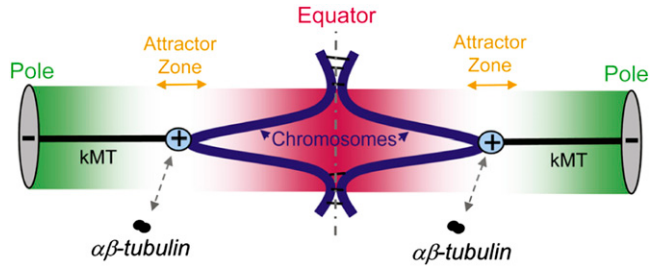

Figure 9 Proposed spatial gradient in net kMT plus-end assembly mediates kinetochore congression in yeast. Kinetochores (cyan) congress to attractor zones (yellow arrows) on either side of the spindle equator (dashed-and-dotted line) during yeast metaphase via the plus-end assembly dynamics of kMTs (black). The kMT plus-end assembly dynamics are spatially regulated such that plus-end assembly is favored near the poles (gray) when kMTs are relatively short (favorable assembly zone shown as green gradient) and suppressed near the spindle equator (dashed line) when kMTs are relatively long (assembly suppression zone shown as red gradient). Adapted from Gardner *et al.* (2008c)

model convolution has been developed (Sprague *et al.* 2003; Gardner *et al.* 2005, 2007, 2010). In this method, spatial distributions of cellular objects generated from computer models or other structural information (from, *e.g.*, electron micrographs or tomograms) are convolved with the three-dimensional distribution of the objective point spread function. The resolution of the computer model is theoretically very small (sub-nanometer), but as it is convolved to simulate an experimental image (including background and noise), resolution is reduced and nanometer differences in different models cannot be distinguished.

A stochastic model of kinetochore MT plus-end dynamics in the metaphase spindle was developed and then evaluated by simulating images of kinetochore-associated fluorescent probes (Sprague *et al.* 2003). Computer simulations of kinetochore MT dynamics combined with statistical measures of how well the simulation data predict experimental fluorescence kinetochore distributions recorded by live-cell imaging have been used to build an understanding of budding-yeast mitotic spindle kMT dynamics (Sprague *et al.* 2003; Gardner *et al.* 2005, 2007, 2010). It was found that models based on any set of constant dynamic instability parameters were insufficient to explain how kinetochores tend to cluster midway between the poles and the equator in yeast metaphase spindles (Sprague *et al.* 2003). The best fit to the experimental data was achieved by kinetochores sensing a stable gradient between the poles to spatially control kMT plus-end catastrophe frequency and by sensing tension generated via chromatin stretching between sister kinetochores to control kMT plus-end rescue frequency. This finding raised the question as to the source of the putative catastrophe gradient. Gardner *et al.* (2008a) proposed that the source of the catastrophe gradient is the Cin8 plus-end motor protein. The notion that a microtubule motor might generate any kind of gradient was introduced in the landmark study from Varga *et al.* (2006) showing that plus-end depolymerases promote a length-dependent disassembly of microtubules. Gardner *et al.* (2008a) observed Cin8 on the plus ends of cytoplasmic microtubules and, upon shifting the concentration from the nucleus to the cytoplasm (deletion of the nuclear localization sequence of Cin8) (Hildebrandt and Hoyt 2001), showed that Cin8 promotes microtubule shortening. Extrapolating to the spindle reveals a length-dependent mechanism to promote disassembly of kinetochore microtubules, *i.e.*, a catastrophe gradient. It has recently been found that the depolymerizing kinesin-8 motor Kip3p also contributes to the spatial regulation of yeast kMT assembly (Wargacki *et al.* 2010). Methods to resolve microtubule dynamics below the limit of resolution are pioneering new views and hypotheses toward our understanding of how individual microtubules are regulated and participate in spindle function.

Regulation of spindle length and stability

Microtubule-based motors are the major regulators of spindle length (Saunders and Hoyt 1992; Saunders *et al.* 1997b). Upon loss of either of the plus-end motors, Cin8 or Kip1, spindle length decreases. The loss of outward-directed motors reduces the outward force vector, and a new balance between outward and inward forces is found at a shorter spindle length. Following this logic, loss of an inward-directed motor such as Kar3 was expected to result in longer spindles. Unexpectedly, *kar3* mutants have shorter spindles (Saunders *et al.* 1997a). Using model convolution (described above) Gardner *et al.* (2008b) found that Kar3 functions in bundling interpolar microtubules. Upon loss of bundling activity, microtubules in the spindle midzone may be farther apart, resulting in the reduction of the number of kinesin-5 motors bound to antiparallel microtubules. This would result in reduced outwardly directed spindle forces and provides an explanation for the long-standing enigma of how spindle lengths could be shorter in *kar3* Δ mutants even though Kar3 acts to resist outwardly directed spindle forces when ipMTs are properly bundled.

A second regulator of spindle length is pericentric chromatin. Loops of pericentric chromatin occupy the space between separated sister chromatids in metaphase. These loops compose ~20% of the mass of the segregation apparatus and are enriched in cohesin and condensin relative to the remainder of the genome (Megee *et al.* 1999; Weber *et al.* 2004; Bachellier-Bassi *et al.* 2008). Since chromatin loops cannot be removed from the genome, the strategy to test their contribution to spindle length was to change their state of compaction. By reducing the concentration of histone to half its wild-type concentration, it was found that the metaphase spindle increased ~50% in length (Bouck and Bloom 2007). Deletion of the outward-force generators kinesins Cin8 and Kip1 shortens the long spindles observed in histone-repressed cells. The increase in spindle length upon histone repression and restoration of wild-type spindle length by the loss of plus-end-directed motors suggests that during metaphase spindle length is governed by the stretching of pericentric chromatin. Chromatin is an elastic molecule that is stretched in direct opposition to the outward-force

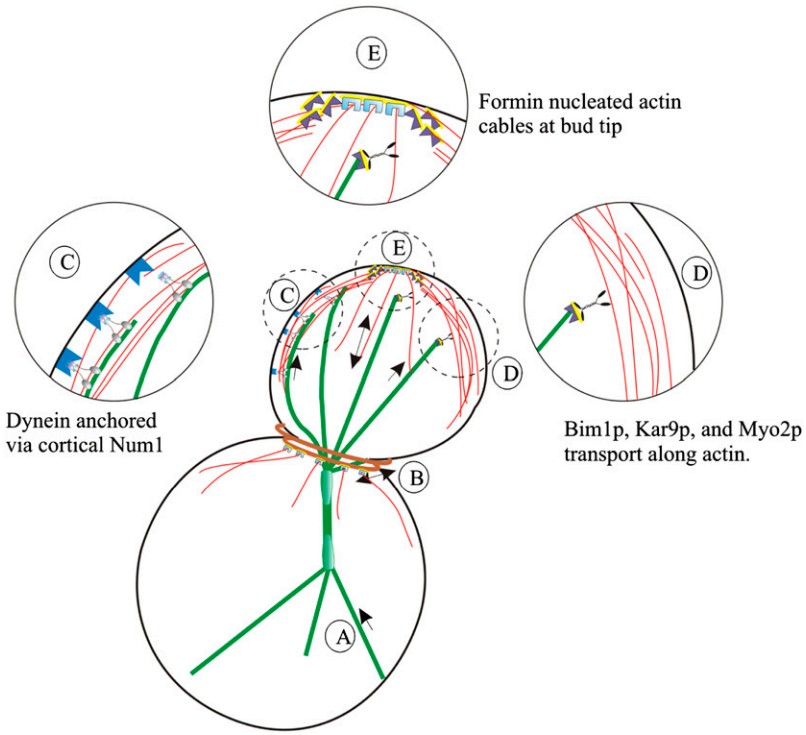

Figure 10 Balance of dynamic pushing and pulling forces in *S. cerevisiae*.To properly position the pre-anaphase spindle at the bud neck without moving the spindle into the bud, *S. cerevisiae* provides a balance of pushing and pulling forces (arrows). (A) Growing and shortening microtubules in the mother cell facilitate searching of the cytoplasmic space and establish pushing forces against the cortex to orient the spindle to the bud neck. (B) Stable attachment at the neck could provide a stabilizing force to limit pulling forces from the bud; it could also provide a loading site for actin-based transport of microtubules and dynein-dependent sliding, and/or it could maintain proper positioning at the bud neck. (C) Minus-end-directed movement of cortically anchored dynein provides a strong pulling force to bring the spindle to the bud neck. Dynein is off-loaded to cortical anchors (Num1) where the minus-end activity is stimulated to result in a pulling force that brings the nucleus into the bud (Lee *et al.* 2005; Markus and Lee 2011). (D) In a redundant pathway, microtubule plus ends are linked through Bim1 and Kar9 to class-V myosin (Myo2) that is moving along polarized actin arrays, facilitating plus-end transport to the bud site. In addition, transport might generate pulling forces to pull the spindle to the bud neck. (E) Finally, end-on attachment at the bud tip where formin nucleates actin growth may also generate force by maintaining attachment to both growing and shortening microtubule plus ends. Adapted from Pearson and Bloom (2004).

generators Cin8 and Kip1, and its packaging contributes to the mechanisms that maintain spindle length.

Spindle Orientation and Translocation

A consequence of cell division by budding necessitates a mechanism that orients the mitotic spindle relative to the mother cell and daughter bud. The bud site is selected well before bipolar spindle formation, and astral microtubules emanating from the SPB (old pole) provide the conduit to translate spatial information from the bud into a motive force for spindle translocation (Figure 10). The force for spindle translocation involves spindle elongation via bipolar Kin5 motors (Cin8 and Kip1) that drive antiparallel microtubules apart and microtubule cortical interactions via cytoplasmic dynein or microtubule actin interactions via type V myosin (Myo2). Defects in spindle translocation were observed by the increase in binucleated cells (two nuclei in one cell) in dynein mutants (Eshel *et al.* 1993; Li *et al.* 1993). The low fraction of binucleated cells (5–20%) is indicative of the redundancy between the two major spindle translocation mechanisms, as well as a powerful spindle positioning checkpoint that prevents exit from mitosis until the spindle aligns along the mother–daughter axis (Caydasi *et al.* 2010a). Spindle elongation and nuclear division can occur completely in the mother cell, giving rise to binucleated cells.

The analysis of spindle dynamics and orientation using video-enhanced differential interference microscopy and fluorescence microscopy of dynein-GFP provided critical insights into the mechanism of spindle translocation (Yeh *et al.* 1995). Prior to anaphase one or both SPBs can migrate through the nuclear envelope. One SPB remains proximal to the neck, while the other SPB traverses the nuclear envelope. Microtubules labeled with cytoplasmic dynein in G1 cells are destined for the bud (Shaw *et al.* 1997). Upon SPB duplication, one SPB retained the existing cytoplasmic microtubules well before the second pole was competent to acquire dynein and nucleate astral microtubules (Shaw *et al.* 1997; Segal *et al.* 2000). Using slow-folding red fluorescent proteins, Pereira *et al.* (2001) found that the old pole was destined for the bud. Thus the new pole is delayed in its ability to nucleate astral microtubules, providing the mechanism for partitioning the poles according to their age to mother or bud.

Cytoplasmic dynein is required both for pre-anaphase nuclear movements and spindle alignment. In the absence of dynein, the nucleus will migrate proximally to the neck, but the spindle does not align along the mother–bud axis. In addition, nuclear (but not spindle) movements into the isthmus of the neck were not observed in the absence of dynein. These early nuclear movements are mediated by interactions between dynein and components of the nuclear envelope and are indicative of dynein's role in docking the nucleus into a position at the neck where simultaneous spindle translocation and elongation can occur to move chromosomes into both the mother and the bud. This result is reminiscent of the phenotype observed when astral microtubule assembly is selectively disrupted (Sullivan and Huffaker 1992). Thus forces from the central spindle, in the absence of astral microtubules, are insufficient for nuclear movement into the bud. The temporal deficiency in the absence of dynein revealed that dynein is not functionally unique, as other forces acting

on cytoplasmic microtubules are able to compensate for dynein's absence. We understand now that there is a hierarchical array of force producing proteins in the cytoplasm.

A critical aspect of spindle translocation into the daughter cell is the interaction of cytoplasmic microtubules with either the polarized actin cytoskeleton or cortical cues in the newly formed bud (Figure 10). The cytoplasmic microtubules provide the beacon for distinguishing the bud from the mother cell. The microtubule plus ends are decorated with a plethora of MAPs and motors (see *Microtubule motor proteins* and *Microtubule-associated proteins* above). At the plus end, dynein interacts with its cortical receptor, Num1. In ways that remain to be determined, dynein docks to Num1, is activated by plus-end-localized dynactin, and begins to walk to the microtubule minus end. Num1 provides the anchor, and thus the spindle moves toward the bud. This is referred to as the offloading model, indicated by loading dynein off the free ends to the cortex where it then walks along the lattice toward the spindle pole (Figure 10) (Lee *et al.* 2000, 2005; Moore *et al.* 2008, 2009b; Moore and Cooper 2010). Dynactin is necessary for dynein function as evidenced by the binucleated phenotype in mutants lacking various subunits (McMillan and Tatchell 1994; Kahana *et al.* 1998). The mechanism of dynactin action in budding yeast was extensively investigated (Moore *et al.* 2008). They find that dynein is responsible for recruiting dynactin to plus ends and for a codependency for off-loading. The results indicate that the major role for dynactin is in the transfer of dynein from microtubule plus end to the Num1 anchor.

A second pathway utilizes the linkage between the microtubule plus end and the actin cytoskeleton. Actin is required for delivering vesicles to sites of new growth and is highly polarized toward the bud early in the division process. Kar9 is instrumental in mediating the actin/microtubule interaction. Kar9 was isolated as a bilateral karyogamy mutant and subsequently found to be required for spindle positioning in mitosis (Miller and Rose 1998). Kar9 contains a coiled-coil domain at its N-terminal half and a conserved Bim1 binding domain. The conserved domain is also found in the adenomatous polyposis coli protein (APC). That Kar9 mediates the actin/microtubule interaction was exquisitely demonstrated in the bypass of Kar9 by fusion between the plus-end-binding proteins Bim1 and Myo2 (Yin *et al.* 2000). A key role of Kar9 is revealed in its asymmetric localization to microtubules and the SPB destined for the bud. Kar9 is found on both SPBs early in spindle assembly and becomes concentrated on the SPB destined for the bud as mitosis proceeds. Kar9 is also found on daughter-bound cytoplasmic microtubules. Interestingly, Kar9 is stably associated with microtubule plus ends (Liakopoulos *et al.* 2003), unlike a variety of other MAPs that exhibit on-and-off rates consistent with tubulin assembly and disassembly dynamics at plus ends. Kar9's ability to oligomerize (R. K. Miller, S.-C. Cheng, and M. D. Rose, unpublished results) may contribute to its ability to maintain persistent attachment to a dynamic substrate. Kar9 is a substrate of cyclin-dependent kinase (Liakopoulos *et al.* 2003; Maekawa *et al.* 2003), sumoylation (Leisner *et al.* 2008; Meednu *et al.* 2008), and ubiquitylation (Kammerer *et al.* 2010). These modifications contribute to various extents to the asymmetric positioning of Kar9 on bud-directed cytoplasmic microtubules. A recent finding involving the polarization driven by actin cables proposes an interesting mechanism wherein myosin transport of cytoplasmic microtubules along actin cables results in a feedback loop for the build-up of Kar9 at the daughter spindle pole (Cepeda-Garcia *et al.* 2010). The idea is that Myo2 transport delivers cytoplasmic microtubules to sites of polarized growth, and upon microtubule disassembly, Kar9 at plus ends returns to the spindle pole. Upon repeated cycles of growth, orientation, and shortening, Kar9 asymmetry is sustained throughout mitosis. In some ways, this may be analogous to the accumulation of plus-end microtubule-based motors at microtubule plus ends (see *Microtubule motor proteins*). The plus-end-binding protein Kar9 accumulates at the spindle pole due to cycles of growth and shortening, thereby predisposing microtubules nucleated from the daughter spindle pole body to bind Kar9 and thus be transported to the daughter cell. There are non-intuitive consequences of the behavior of long polymers like actin and microtubules, such as differential behavior of short or long polymers due to the differential accumulation of microtubule-associated proteins or motors or changes in stiffness depending on whether the total polymer is longer or shorter than its persistence length (see *Microtubules*). The physical properties of these polymers provide the framework for spindle and nuclear dynamics.

Regulation of spindle orientation and translocation

The coordination of microtubule plus-end dynamics with force generation and transport (such as Kar9 and dynein) is critical for networking with the actin cytoskeleton or other cortical and/or polarity determinants relative to cell cycle progression (Schuyler and Pellman 2001; Carvalho *et al.* 2003; Howard and Hyman 2003). Spindle positioning is therefore dependent not only on the function of microtubule-binding proteins, but also on the accurate timing and placement of these proteins on specific microtubules (Figure 10).

Regulation of motor protein and MAP targeting occurs by different modes of delivery and maintenance, which generates a temporally and spatially regulated landscape of MAP localization. The plus-end-directed microtubule-based motors transport cargoes such as signaling components and other motor proteins to plus ends. For example, Kip2 transports the minus-end-directed motor cytoplasmic dynein to microtubule plus ends (Carvalho *et al.* 2004), where it is delivered to the cortex (Sheeman *et al.* 2003). Once at the plus ends, dynein provides a vector for spindle orientation via cortical anchors to generate pulling forces for spindle movements. The plus-end-directed kinesin Kip2 is also necessary for the transport of Kar9 (Maekawa *et al.* 2003) to microtubule plus ends, to which Kar9 binds through its interaction with Bim1 (Korinek *et al.* 2000; Lee *et al.* 2000). In yeast with mutations in both the Cdk Cdc28 and its cyclin partner Clb5, Kar9 spreads along the entire length of cytoplasmic

microtubules (Maekawa *et al.* 2003),which results in premature migration of the entire spindle into the bud/daughter cell (Segal *et al.* 2000; Maekawa *et al.* 2003). Clb5–Cdc28 promotes Kar9 transport to cytoplasmic microtubule plus ends that are destined for the bud/daughter cell (Maekawa *et al.* 2003), thereby regulating the selective attachment of microtubule plus ends to the bud/daughter-cell cortex. In contrast, Clb4–Cdc28 confers tight spatial control of Kar9 to the bud/daughter-cell-bound spindle pole body (Liakopoulos *et al.* 2003). This spatial and cell-cycle-mediated control of Kar9 localization highlights the role of its transport mechanism in limiting the number and position of directed microtubules and possibly in limiting the force that is necessary for proper spindle placement. The minus-end-directed motor protein Kar3, like Kar9, is spatially restricted to a subset of the plus ends of microtubules in mating *S. cerevisiae* cells (Maddox *et al.* 1999, 2003). Approximately three microtubules form a bundle that is directed toward the "mating projection" (Byers and Goetsch 1974), where they function to bring together the nuclei of mating cells (Meluh and Rose 1990). Kar3 is preferentially localized to the plus ends of shortening microtubules within this bundle and is excluded from the ends of other microtubules in these cells (Meluh and Rose 1990; Maddox *et al.* 2003). The regulatory factors that allow Kar3 to discriminate between specific plus ends by virtue of their subcellular localization remain to be elucidated.

Spindle Disassembly: Mitotic Exit and Preparation for the Next Cycle

Breaking down the spindle requires a regulatory cascade that coordinates the timing of sister-chromatid separation upon and during anaphase, inactivation of mitotic CDKs, dissolution of the spindle midzone, and microtubule depolymerization. The regulatory cascade revolves around Cdc14, a protein phosphatase that antagonizes the mitotic cyclin-dependent kinases. This phosphatase is compartmentalized in the nucleolus for much of the cell cycle. The FEAR complex (Cdc 14 early anaphase release) regulates its release, and the MEN promotes additional release and maintains its release during anaphase and telophase (Stegmeier and Amon 2004). How Cdc14 controls spindle breakdown requires identification of substrates and linking of these substrates to the mechanical processes such as microtubule cross-linking and dynamic instability that regulate spindle assembly and disassembly.

One of the early consequences of Cdc14 release is the dephosphorylation of the yeast inner centromere protein Sli15 and localization of Sli15, Ipl1, and the kinetochore protein Slk19 to the midzone (Pereira and Schiebel 2003). The budding-yeast midzone consists of overlapping antiparallel microtubule plus ends (Winey *et al.* 1995b; Maddox *et al.* 1999). Slk19 and Sli15 have been proposed to contribute to anaphase spindle stability, whereas Ipl1 has been proposed to regulate the timing of spindle disassembly (Zeng *et al.* 1999; Buvelot *et al.* 2003; Pereira and Schiebel 2003). To appreciate the subtlety required in regulation, it is important to point out that, as spindle elongation ensues, the number of interpolar microtubules diminishes from approximately eight to two. From the microtubule perspective, the spindle is a fragile machine. Aurora kinase (Ipl1) plays a critical role in spindle disassembly. This was first reported from live-cell studies showing Ipl1-GFP accumulation in the midzone and the stabilization of anaphase spindle in *ipl1* mutants (Buvelot *et al.* 2003). One of the key kinetochore proteins, Ndc10 is also required for spindle stability. Ndc10 is released in a Cdc14-dependent fashion from the kinetochore to microtubule plus ends where it contributes to spindle stability specifically in anaphase (Bouck and Bloom 2005). Ndc10 is critical for spindle elongation to the full 10–12 μm prior to spindle disassembly and cytokinesis (Widlund *et al.* 2006).

Additional substrates have recently been identified in studies directed at exploring this critical aspect of spindle morphogenesis (Vizeacoumar *et al.* 2010; Woodruff *et al.* 2010). Vizeacoumar *et al.* (2010) use a high-throughput approach to demonstrate how the kinetochore is dismantled and components recycled to the midzone [*e.g.*, Ndc10, Bouck and Bloom 2005)]. Ipl1 is directed to the midzone following the sumoylation of a kinetochore component (mcm21), as well as via the release of Cdc14 (Vizeacoumar *et al.* 2010). Interestingly, the spindle localization of Ndc10, like that of Ipl1, is dependent on its sumoylation (Montpetit *et al.* 2006). Thus, in an elegant feat of cellular recycling, the sumoylation of several kinetochore proteins results in the dismantling of the kinetochore and the relocalization of key proteins to a locale where they function to stabilize the anaphase spindle.

In a classical genetic screen, Woodruff *et al.* (2010) demonstrate that spindle disassembly can be genetically dissected into (1) spindle splitting and (2) microtubule depolymerization. Spindle splitting is dependent upon the activation of the APC that leads to the degradation of microtubule cross-linking proteins in the spindle midzone [ubiquitination of Ase1 and Cin8 (Hildebrandt and Hoyt 2001)]. In a separate pathway, Ipl1 promotes microtubule depolymerization by phosphorylating the microtubule plus-end-binding protein Bim1. Bim1 functions in G1 cells to promote microtubule dynamics (Tirnauer *et al.* 1999). In anaphase, Ipl1-dependent loss of Bim1 must reflect the inability of shortening microtubules to switch to rescue in the absence (or reduction) of Bim1. In this regard, Woodruff *et al.* (2010) found that the microtubule depolymerase Kip3 (see *Microtubule motor proteins*) promotes microtubule depolymerization independently of Ipl1. Thus the switch to depolymerization reflects independent mechanisms that remove key plus-end-binding proteins while promoting depolymerases to shorten spindle microtubules.

Prospective

The first iteration of the molecular biology of the yeast *S. cerevisiae* brought us Breck Byers' classic electron micrographs

(Byers and Goetsch 1975) that focused on the morphology of spindles and spindle poles through the mitotic and meiotic cycle. Twenty-two years later the yeast cytoskeleton chapter provided the first interaction maps for actin (that predated the current explosion in "omics"), spindle dynamics in live cells, and a glimpse of what would be forthcoming from green fluorescent protein fusions (through tubulin-GFP) (Botstein *et al.* 1997). In the ensuing 15 years every yeast protein has been fused with GFP, and the dynamics of actin and microtubule cytoskeletons have been extensively studied. Large repertoires of binding proteins and their structures have been determined. Their dynamics in living cells reveals a level of complexity that makes the genetic interaction maps look remarkably simple. With added complexity comes greater reliance on methods to integrate large data sets and computational models. Network modeling has become a cottage industry in genome research. The application of physical models to understand chromosome and spindle function has already provided important insights, predictions, and solutions to spatial gradients regulating chromosome congression and spindle oscillation (Visintin *et al.* 1997; Pearson *et al.* 2006; Gardner *et al.* 2008a). Computational models, together with optical convolution methods to overcome the Abbe limit that restricts the resolution to ~1/4 wavelength of green light, represent a powerful strategy to "see" at the nanometer scale in live cells. An emerging frontier will see structural biology being performed inside living cells. It is possible through technologies such as total internal reflection microscopy, super-resolution (STORM, PALM, SIM), and SHREC (single molecule high-resolution co-localization) to break the Abbe limit and map sites of protein interaction and dynamic changes in live cells (Joglekar *et al.* 2009; Wan *et al.* 2009). Further development of these methods will revolutionize light microscopy and present new challenges to the structural biologists. The challenge for young scientists in the field is to become versed in molecular, genetic, structural, and computational biology.

Acknowledgment

We thank Elaine Yeh for critical reading of this article; Shelly Jones, Jamie Keck, Tom Giddings, and Eileen O'Toole for comments and for help with the figures; and Sue Jaspersen for communicating results prior to publication. Our work on yeast mitotic spindles has been supported by the National Institutes of Health (GM051312 to M.W., GM32238 to K.B.).

Literature Cited

Abruzzi, K. C., A. Smith, W. Chen, and F. Solomon, 2002 Protection from free beta-tubulin by the beta-tubulin binding protein Rbl2p. Mol. Cell. Biol. 22: 138–147.

Adams, I. R., and J. V. Kilmartin, 1999 Localization of core spindle pole body (SPB) components during SPB duplication in Saccharomyces cerevisiae. J. Cell Biol. 145: 809–823.

Akhmanova, A., and C. C. Hoogenraad, 2005 Microtubule plus-end-tracking proteins: mechanisms and functions. Curr. Opin. Cell Biol. 17: 47–54.

Ali, M. Y., H. Lu, C. S. Bookwalter, D. M. Warshaw, and K. M. Trybus, 2008 Myosin V and kinesin act as tethers to enhance each other's processivity. Proc. Natl. Acad. Sci. USA 105: 4691–4696.

Allingham, J. S., L. R. Sproul, I. Rayment, and S. P. Gilbert, 2007 Vik1 modulates microtubule-Kar3 interactions through a motor domain that lacks an active site. Cell 128: 1161–1172.

Anderson, M., J. Haase, E. Yeh, and K. Bloom, 2009 Function and assembly of DNA looping, clustering, and microtubule attachment complexes within a eukaryotic kinetochore. Mol. Biol. Cell 20: 4131–4139.

Anderson, V. E., J. Prudden, S. Prochnik, T. H. Giddings Jr., and K. G. Hardwick, 2007 Novel sfi1 alleles uncover additional functions for Sfi1p in bipolar spindle assembly and function. Mol. Biol. Cell 18: 2047–2056.

Araki, Y., C. K. Lau, H. Maekawa, S. L. Jaspersen, T. H. Giddings Jr. *et al.*, 2006 The Saccharomyces cerevisiae spindle pole body (SPB) component Nbp1p is required for SPB membrane insertion and interacts with the integral membrane proteins Ndc1p and Mps2p. Mol. Biol. Cell 17: 1959–1970.

Araki, Y., L. Gombos, S. P. Migueleti, L. Sivashanmugam, C. Antony *et al.*, 2010 N-terminal regions of Mps1 kinase determine functional bifurcation. J. Cell Biol. 189: 41–56.

Bachellier-Bassi, S., O. Gadal, G. Bourout, and U. Nehrbass, 2008 Cell cycle-dependent kinetochore localization of condensin complex in Saccharomyces cerevisiae. J. Struct. Biol. 162: 248–259.

Biggins, S., and M. Rose, 1994 Direct interaction between yeast spindle pole body components: Kar1p is required for Cdc31p localization to the spindle pole body. J. Cell Biol. 125: 843–852.

Biggins, S., I. Ivanowska, and M. Rose, 1996 Yeast ubiquitin-like genes are involved in duplication of the microtubule organizing center. J. Cell Biol. 133: 1331–1346.

Blake-Hodek, K. A., L. Cassimeris, and T. C. Huffaker, 2010 Regulation of microtubule dynamics by Bim1 and Bik1, the budding yeast members of the EB1 and CLIP-170 families of plus-end tracking proteins. Mol. Biol. Cell 21: 2013–2023.

Botstein, D., D. Amberg, J. Mulholland, T. Huffaker, A. Adams *et al.* (Editors), 1997 *The Yeast Cytoskeleton*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Bouck, D. C., and K. S. Bloom, 2005 The kinetochore protein Ndc10p is required for spindle stability and cytokinesis in yeast. Proc. Natl. Acad. Sci. USA 102: 5408–5413.

Bouck, D. C., and K. Bloom, 2007 Pericentric chromatin is an elastic component of the mitotic spindle. Curr. Biol. 17: 741–748.

Bouck, D. C., A. P. Joglekar, and K. S. Bloom, 2008 Design features of a mitotic spindle: balancing tension and compression at a single microtubule kinetochore interface in budding yeast. Annu. Rev. Genet. 42: 335–359.

Brachat, A., J. V. Kilmartin, A. Wach, and P. Philippsen, 1998 Saccharomyces cerevisiae cells with defective spindle pole body outer plaques accomplish nuclear migration via half-bridge-organized microtubules. Mol. Biol. Cell 9: 977–991.

Bratman, S. V., and F. Chang, 2007 Stabilization of overlapping microtubules by fission yeast CLASP. Dev. Cell 13: 812–827.

Brinkley, B. R., and E. Stubblefield, 1966 The fine structure of the kinetochore of a mammalian cell in vitro. Chromosoma 19: 28–43.

Brittle, A. L., and H. Ohkura, 2005 Mini spindles, the XMAP215 homologue, suppresses pausing of interphase microtubules in Drosophila. EMBO J. 24: 1387–1396.

Bullitt, E., M. Rout, J. Kilmartin, and C. Akey, 1997 The yeast spindle pole boby is assembled around a central crystal of Spc42p. Cell 89: 1077–1086.

Buvelot, S., S. Y. Tatsutani, D. Vermaak, and S. Biggins, 2003 The budding yeast Ipl1/Aurora protein kinase regulates mitotic spindle disassembly. J. Cell Biol. 160: 329–339.

Byers, B., 1981 Mulitple roles of the spindle pole body in the life cycle of Saccharomyces cerevisiae, pp. 119–133 in *Molecular Genetics in Yeast*, edited by D. V. Wettstein, A. Stenderup, M. Kielland-Brandt, and J. Friis. Munksgarrd, Copenhagen.

Byers, B., and L. Goetsch, 1974 Duplication of spindle plaques and integration of the yeast cell cycle. Cold Spring Harb. Symp. Quant. Biol. 38: 123–131.

Byers, B., and L. Goetsch, 1975 Behavior of spindles and spindle plaques in the cell cycle and conjugation of *Saccharomyces cerevisiae*. J. Bacteriol. 124: 511–523.

Byers, B., K. Shriver, and L. Goetsch, 1978 The role of spindle pole bodies and modified microtubule ends in the initiation of microtubule assembly in Saccharomyces cerevisiae. J. Cell Sci. 30: 331–352.

Carvalho, P., J. S. Tirnauer, and D. Pellman, 2003 Surfing on microtubule ends. Trends Cell Biol. 13: 229–237.

Carvalho, P., M. L. Gupta Jr., M. A. Hoyt, and D. Pellman, 2004 Cell cycle control of kinesin-mediated transport of Bik1 (CLIP-170) regulates microtubule stability and dynein activation. Dev. Cell 6: 815–829.

Castillo, A. R., J. B. Meehl, G. Morgan, A. Schutz-Geschwender, and M. Winey, 2002 The yeast protein kinase Mps1p is required for assembly of the integral spindle pole body component Spc42p. J. Cell Biol. 156: 453–465.

Caydasi, A. K., B. Ibrahim, and G. Pereira, 2010a Monitoring spindle orientation: spindle position checkpoint in charge. Cell Div. 5: 28.

Caydasi, A. K., B. Kurtulmus, M. I. Orrico, A. Hofmann, B. Ibrahim *et al.*, 2010b Elm1 kinase activates the spindle position checkpoint kinase Kin4. J. Cell Biol. 190: 975–989.

Cepeda-Garcia, C., N. Delgehyr, M. A. Ortiz, R. ten Hoopen, A. Zhiteneva *et al.*, 2010 Actin-mediated delivery of astral microtubules instructs Kar9p asymmetric loading to the bud-ward spindle pole. Mol. Biol. Cell 21: 2685–2695.

Chee, M. K., and S. B. Haase, 2010 B-cyclin/CDKs regulate mitotic spindle assembly by phosphorylating kinesins-5 in budding yeast. PLoS Genet. 6: e1000935.

Cheeseman, I. M., and A. Desai, 2008 Molecular architecture of the kinetochore-microtubule interface. Nat. Rev. Mol. Cell Biol. 9: 33–46.

Cheeseman, I. M., I. MacLeod, J. R. Yates III., K. Oegema, and A. Desai, 2005 The CENP-F-like proteins HCP-1 and HCP-2 target CLASP to kinetochores to mediate chromosome segregation. Curr. Biol. 15: 771–777.

Chen, X. P., H. Yin, and T. C. Huffaker, 1998 The yeast spindle pole body component Spc72p interacts with Stu2p and is required for proper microtubule assembly. J. Cell Biol. 141: 1169–1179.

Chial, H. J., M. P. Rout, T. H. Giddings, and M. Winey, 1998 Saccharomyces cerevisiae Ndc1p is a shared component of nuclear pore complexes and spindle pole bodies. J. Cell Biol. 143: 1789–1800.

Cottingham, F. R., L. Gheber, D. L. Miller, and M. A. Hoyt, 1999 Novel roles for Saccharomyces cerevisiae mitotic spindle motors. J. Cell Biol. 147: 335–350.

D'Ambrosio, C., C. K. Schmidt, Y. Katou, G. Kelly, T. Itoh *et al.*, 2008 Identification of cis-acting sites for condensin loading onto budding yeast chromosomes. Genes Dev. 22: 2215–2227.

De Wulf, P., A. D. McAinsh, and P. K. Sorger, 2003 Hierarchical assembly of the budding yeast kinetochore from multiple subcomplexes. Genes Dev. 17: 2902–2921.

Dobra, J., R. Vankova, M. Havlova, A. J. Burman, J. Libus *et al.*, 2011 Tobacco leaves and roots differ in the expression of proline metabolism-related genes in the course of drought stress and subsequent recovery. J. Plant Physiol. 168: 1588–1597.

Dong, Y., K. J. Vanden Beldt, X. Meng, A. Khodjakov, and B. F. McEwen, 2007 The outer plate in vertebrate kinetochores is a flexible network with multiple microtubule interactions. Nat. Cell Biol. 9: 516–522.

Endow, S. A., S. J. Kang, L. L. Satterwhite, M. D. Rose, V. P. Skeen *et al.*, 1994 Yeast Kar3 is a minus-end microtubule motor protein that destabilizes microtubules preferentially at the minus ends. EMBO J. 13: 2708–2713.

Eshel, D., L. A. Urrestarazu, S. Vissers, J. C. Jauniaux, J. C. van Vliet-Reedijk *et al.*, 1993 Cytoplasmic dynein is required for normal nuclear segregation in yeast. Proc. Natl. Acad. Sci. USA 90: 11172–11176.

Fitch, I., C. Dahmann, U. Surana, A. Amon, K. Nasmyth *et al.*, 1992 Characterization of four B-type cyclin genes of the budding yeast Saccharomyces cerevisiae. Mol. Biol. Cell 3: 805–818.

Friedman, D. B., J. W. Kern, B. J. Huneycutt, D. B. Vinh, D. K. Crawford *et al.*, 2001 Yeast Mps1p phosphorylates the spindle pole component Spc110p in the N-terminal domain. J. Biol. Chem. 276: 17958–17967.

Gardner, M. K., C. G. Pearson, B. L. Sprague, T. R. Zarzar, K. Bloom *et al.*, 2005 Tension-dependent regulation of microtubule dynamics at kinetochores can explain metaphase congression in yeast. Mol. Biol. Cell 16: 3764–3775.

Gardner, M. K., D. J. Odde, and K. Bloom, 2007 Hypothesis testing via integrated computer modeling and digital fluorescence microscopy. Methods 41: 232–237.

Gardner, M. K., D. C. Bouck, L. V. Paliulis, J. B. Meehl, E. T. O'Toole *et al.*, 2008a Chromosome congression by kinesin-5 motor-mediated disassembly of longer kinetochore microtubules. Cell 135: 894–906.

Gardner, M. K., J. Haase, K. Mythreye, J. N. Molk, M. Anderson *et al.*, 2008b The microtubule-based motor Kar3 and plus end-binding protein Bim1 provide structural support for the anaphase spindle. J. Cell Biol. 180: 91–100.

Gardner, M. K., D. J. Odde, and K. Bloom, 2008c Kinesin-8 molecular motors: putting the brakes on chromosome oscillations. Trends Cell Biol. 18: 307–310.

Gardner, M. K., B. L. Sprague, C. G. Pearson, B. D. Cosgrove, A. D. Bicek *et al.*, 2010 Model convolution: a computational approach to digital image interpretation. Cell. Mol. Bioeng. 3: 163–170.

Geiser, J. R., D. van Tuinen, S. E. Brockerhoff, M. M. Neff, and T. N. Davis, 1991 Can calmodulin function without binding calcium? Cell 65: 949–959.

Geiser, J., H. Sundberg, B. Chang, E. Muller, and T. Davis, 1993 The essential mitotic target of calmodulin is the 110-kilodalton component of the spindle pore body in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 13: 7913–7924.

Geiser, J. R., E. J. Schott, T. J. Kingsbury, N. B. Cole, L. J. Totis *et al.*, 1997 Saccharomyces cerevisiae genes required in the absence of the CIN8-encoded spindle motor act in functionally diverse mitotic pathways. Mol. Biol. Cell 8: 1035–1050.

Gerlich, D., T. Hirota, B. Koch, J. M. Peters, and J. Ellenberg, 2006 Condensin I stabilizes chromosomes mechanically through a dynamic interaction in live cells. Curr. Biol. 16: 333–344.

Gittes, F., B. Mickey, J. Nettleton, and J. Howard, 1993 Flexural rigidity of microtubules and actin filaments measured from thermal fluctuations in shape. J. Cell Biol. 120: 923–934.

Goshima, G., and M. Yanagida, 2000 Establishing biorientation occurs with precocious separation of the sister kinetochores, but not the arms, in the early spindle of budding yeast. Cell 100: 619–633.

Goshima, G., and M. Yanagida, 2001 Time course analysis of precocious separation of sister centromeres in budding yeast: Continuously separated or frequently reassociated? Genes Cells 6: 765–773.

Greenland, K. B., H. Ding, M. Costanzo, C. Boone, and T. N. Davis, 2010 Identification of Saccharomyces cerevisiae spindle pole body remodeling factors. PLoS ONE 5: e15426.

Grinthal, A., I. Adamovic, B. Weiner, M. Karplus, and N. Kleckner, 2010 PR65, the HEAT-repeat scaffold of phosphatase PP2A, is an elastic connector that links force and catalysis. Proc. Natl. Acad. Sci. USA 107: 2467–2472.

Gruneberg, U., K. Campbell, C. Simpson, J. Grindlay, and E. Schiebel, 2000 Nud1p links astral microtubule organization and the control of exit from mitosis. EMBO J. 19: 6475–6488.

Gupta, M. L. Jr., P. Carvalho, D. M. Roof, and D. Pellman, 2006 Plus end-specific depolymerase activity of Kip3, a kinesin-8 protein, explains its role in positioning the yeast mitotic spindle. Nat. Cell Biol. 8: 913–923.

Hasse, S. B., M. Winey, and S. I. Reed, 2001 Multi-step control of spindle pole body duplication by cyclin-dependent kinase. Nat. Cell Biol. 3: 38–42.

He, X., D. R. Rines, C. W. Espelin, and P. K. Sorger, 2001 Molecular analysis of kinetochore-microtubule attachment in budding yeast. Cell 106: 195–206.

Hildebrandt, E. R., and M. A. Hoyt, 2001 Cell cycle-dependent degradation of the Saccharomyces cerevisiae spindle motor Cin8p requires APC(Cdh1) and a bipartite destruction sequence. Mol. Biol. Cell 12: 3402–3416.

Hirano, T., 2006 At the heart of the chromosome: SMC proteins in action. Nat. Rev. Mol. Cell Biol. 7: 311–322.

Hodges, A. R., C. S. Bookwalter, E. B. Krementsova, and K. M. Trybus, 2009 A nonprocessive class V myosin drives cargo processively when a kinesin-related protein is a passenger. Curr. Biol. 19: 2121–2125.

Hoepfner, D., A. Brachat, and P. Philippsen, 2000 Time-lapse video microscopy analysis reveals astral microtubule detachment in the yeast spindle pole mutant cnm67. Mol. Biol. Cell 11: 1197–1211.

Hoepfner, D., F. Schaerer, A. Brachat, A. Wach, and P. Philippsen, 2002 Reorientation of mispositioned spindles in short astral microtubule mutant spc72Delta is dependent on spindle pole body outer plaque and Kar3 motor protein. Mol. Biol. Cell 13: 1366–1380.

Holinger, E. P., W. M. Old, T. H. Giddings Jr., C. Wong, J. R. Yates III. *et al.*, 2009 Budding yeast centrosome duplication requires stabilization of SPC29 via Mps1-mediated phosphorylation. J. Biol. Chem. 284: 12949–12955.

Howard, J., and A. A. Hyman, 2003 Dynamics and mechanics of the microtubule plus end. Nature 422: 753–758.

Howard, J., and A. A. Hyman, 2009 Growth, fluctuation and switching at microtubule plus ends. Nat. Rev. Mol. Cell Biol. 10: 569–574.

Huffaker, T. C., J. H. Thomas, and B. Botstein, 1988 Diverse effects of beta-tubulin mutations on microtubule formation and function. J. Cell Biol. 106: 1997–2010.

Huh, W. K., J. V. Falvo, L. C. Gerke, A. S. Carroll, R. W. Howson *et al.*, 2003 Global analysis of protein localization in budding yeast. Nature 425: 686–691.

Huisman, S. M., M. F. Smeets, and M. Segal, 2007 Phosphorylation of Spc110p by Cdc28p-Clb5p kinase contributes to correct spindle morphogenesis in S. cerevisiae. J. Cell Sci. 120: 435–446.

Hwang, E., J. Kusch, Y. Barral, and T. C. Huffaker, 2003 Spindle orientation in Saccharomyces cerevisiae depends on the transport of microtubule ends along polarized actin cables. J. Cell Biol. 161: 483–488.

Inoue, S., J. Fuseler, E. D. Salmon, and G. W. Ellis, 1975 Functional organization of mitotic microtubules. Physical chemistry of the in vivo equilibrium system. Biophys. J. 15: 725–744.

Jacobs, C. W., A. E. Adams, P. J. Szaniszlo, and J. R. Pringle, 1988 Functions of microtubules in the Saccharomyces cerevisiae cell cycle. J. Cell Biol. 107: 1409–1426.

Jaspersen, S. L., and M. Winey, 2004 The budding yeast spindle pole body: structure, duplication, and function. Annu. Rev. Cell Dev. Biol. 20: 1–28.

Jaspersen, S. L., T. H. Giddings Jr., and M. Winey, 2002 Mps3p is a novel component of the yeast spindle pole body that interacts with the yeast centrin homologue Cdc31p. J. Cell Biol. 159: 945–956.

Jaspersen, S. L., B. J. Huneycutt, T. H. Giddings Jr., K. A. Resing, N. G. Ahn *et al.*, 2004 Cdc28/Cdk1 regulates spindle pole body duplication through phosphorylation of Spc42 and Mps1. Dev. Cell 7: 263–274.

Jaspersen, S. L., A. E. Martin, G. Glazko, T. H. Giddings Jr., G. Morgan *et al.*, 2006 The Sad1-UNC-84 homology domain in Mps3 interacts with Mps2 to connect the spindle pole body with the nuclear envelope. J. Cell Biol. 174: 665–675.

Joglekar, A. P., D. C. Bouck, J. N. Molk, K. S. Bloom, and E. D. Salmon, 2006 Molecular architecture of a kinetochore-microtubule attachment site. Nat. Cell Biol. 8: 581–585.

Joglekar, A. P., D. Bouck, K. Finley, X. Liu, Y. Wan *et al.*, 2008 Molecular architecture of the kinetochore-microtubule attachment site is conserved between point and regional centromeres. J. Cell Biol. 181: 587–594.

Joglekar, A. P., K. Bloom, and E. D. Salmon, 2009 In vivo protein architecture of the eukaryotic kinetochore with nanometer scale accuracy. Curr. Biol. 19: 694–699.

Jokelainen, P. T., 1967 The ultrastructure and spatial organization of the metaphase kinetochore in mitotic rat cells. J. Ultrastruct. Res. 19: 19–44.

Jones, M. H., B. J. Huneycutt, C. G. Pearson, C. Zhang, G. Morgan *et al.*, 2005 Chemical genetics reveals a role for Mps1 kinase in kinetochore attachment during mitosis. Curr. Biol. 15: 160–165.

Kahana, J. A., G. Schlenstedt, D. M. Evanchuk, J. R. Geiser, M. A. Hoyt *et al.*, 1998 The yeast dynactin complex is involved in partitioning the mitotic spindle between mother and daughter cells during anaphase B. Mol. Biol. Cell 9: 1741–1756.

Kammerer, D., L. Stevermann, and D. Liakopoulos, 2010 Ubiquitylation regulates interactions of astral microtubules with the cleavage apparatus. Curr. Biol. 20: 1233–1243.

Keck, J. M., M. H. Jones, C. C. L. Wong, J. Binkley, D. Chen *et al.*, 2011 A cell cycle phosphoproteome of the yeast centrosome. Science 332: 1557–1561.

Kerssemakers, J. W., E. L. Munteanu, L. Laan, T. L. Noetzel, M. E. Janson *et al.*, 2006 Assembly dynamics of microtubules at molecular resolution. Nature 442: 709–712.

Keyes, B. E., and D. J. Burke, 2009 Irc15 Is a microtubule-associated protein that regulates microtubule dynamics in Saccharomyces cerevisiae. Curr. Biol. 19: 472–478.

Kilmartin, J. V., 2003 Sfi1p has conserved centrin-binding sites and an essential function in budding yeast spindle pole body duplication. J. Cell Biol. 162: 1211–1221.

Kilmartin, J. V., S. L. Dyos, D. Kershaw, and J. T. Finch, 1993 A spacer protein in the Saccharomyces cerevisiae spindle poly body whose transcript is cell cycle-regulated. J. Cell Biol. 123: 1175–1184.

Klenchin, V. A., J. J. Frye, M. H. Jones, M. Winey, and I. Rayment, 2011 Structure-function analysis of the C-terminal domain of CNM67, a core component of the Saccharomyces cerevisiae spindle pole body. J. Biol. Chem. 286: 18240–18250.

Knop, M., and E. Schiebel, 1997 Spc98p and Spc97p of the yeast γ-tubulin complex mediate binding to the spinde pole body via their interaction with Spc110p. EMBO J. 18: 6985–6995.

Knop, M., and E. Schiebel, 1998 Receptors determine the cellular localization of a γ-tubulin complex and thereby the site of microtubule formation. EMBO J. 17: 3952–3967.

Kollman, J. M., A. Zelter, E. G. Muller, B. Fox, L. M. Rice *et al.*, 2008 The structure of the gamma-tubulin small complex: im-

plications of its architecture and flexibility for microtubule nucleation. Mol. Biol. Cell 19: 207–215.
Kollman, J. M., J. K. Polka, A. Zelter, and T. N. Davis, and D. A. Agard, 2010 Microtubule nucleating γTuSC assembles structures with 13-fold microtubule-like symmetry. Nature 466: 879–882.
Korinek, W. S., M. J. Copeland, A. Chaudhuri, and J. Chant, 2000 Molecular linkage underlying microtubule orientation toward cortical sites in yeast. Science 287: 2257–2259.
Kotwaliwale, C. V., S. B. Frei, B. M. Stern, and S. Biggins, 2007 A pathway containing the Ipl1/aurora protein kinase and the spindle midzone protein Ase1 regulates yeast spindle assembly. Dev. Cell 13: 433–445.
Lau, C. K., T. H. Giddings Jr., and M. Winey, 2004 A novel allele of Saccharomyces cerevisiae NDC1 reveals a potential role for the spindle pole body component Ndc1p in nuclear pore assembly. Eukaryot. Cell 3: 447–458.
Lee, L., J. S. Tirnauer, J. Li, S. C. Schuyler, J. Y. Liu *et al.*, 2000 Positioning of the mitotic spindle by a cortical-microtubule capture mechanism. Science 287: 2260–2262.
Lee, W. L., M. A. Kaiser, and J. A. Cooper, 2005 The offloading model for dynein function: differential function of motor subunits. J. Cell Biol. 168: 201–207.
Leisner, C., D. Kammerer, A. Denoth, M. Britschi, Y. Barral *et al.*, 2008 Regulation of mitotic spindle asymmetry by SUMO and the spindle-assembly checkpoint in yeast. Curr. Biol. 18: 1249–1255.
Li, S., A. M. Sandercock, P. Conduit, C. V. Robinson, R. L. Williams *et al.*, 2006 Structural role of Sfi1p-centrin filaments in budding yeast spindle pole body duplication. J. Cell Biol. 173: 867–877.
Li, Y. Y., E. Yeh, T. Hays, and K. Bloom, 1993 Disruption of mitotic spindle orientation in a yeast dynein mutant. Proc. Natl. Acad. Sci. USA 90: 10096–10100.
Liakopoulos, D., J. Kusch, S. Grava, J. Vogel, and Y. Barral, 2003 Asymmetric loading of Kar9 onto spindle poles and microtubules ensures proper spindle alignment. Cell 112: 561–574.
Lillie, S. H., and S. S. Brown, 1992 Suppression of a myosin defect by a kinesin-related gene. Nature 356: 358–361.
Lillie, S. H., and S. S. Brown, 1994 Immunofluorescence localization of the unconventional myosin, Myo2p, and the putative kinesin-related protein, Smy1p, to the same regions of polarized growth in Saccharomyces cerevisiae. J. Cell Biol. 125: 825–842.
Lim, H. H., P. Y. Goh, and U. Surana, 1996 Spindle pole body separation in Saccharomyces cerevisiae requires dephosphorylation of the tyrosine 19 residue of Cdc28. Mol. Cell. Biol. 16: 6385–6397.
Lin, T. C., L. Gombos, A. Neuner, D. Sebastian, J. V. Olsen *et al.*, 2011 Phosphorylation of the yeast gamma-tubulin Tub4 regulates microtubule function. PLoS ONE 6: e19700.
Maddox, P., E. Chin, A. Mallavarapu, E. Yeh, E. D. Salmon *et al.*, 1999 Microtubule dynamics from mating through the first zygotic division in the budding yeast Saccharomyces cerevisiae. J. Cell Biol. 144: 977–987.
Maddox, P., K. Bloom, and E. D. Salmon, 2000a Polarity and dynamics of microtubule assembly in the budding yeast *Saccharomyces cerevisiae*. Nat. Cell Biol. 2: 36–41.
Maddox, P. S., K. S. Bloom, and E. D. Salmon, 2000b The polarity and dynamics of microtubule assembly in the budding yeast Saccharomyces cerevisiae. Nat. Cell Biol. 2: 36–41.
Maddox, P. S., J. K. Stemple, L. Satterwhite, E. D. Salmon, and K. Bloom, 2003 The minus end-directed motor Kar3 is required for coupling dynamic microtubule plus ends to the cortical shmoo tip in budding yeast. Curr. Biol. 13: 1423–1428.
Maekawa, H., T. Usui, M. Knop, and E. Schiebel, 2003 Yeast Cdk1 translocates to the plus end of cytoplasmic microtubules to regulate bud cortex interactions. EMBO J. 22: 438–449.
Maekawa, H., C. Priest, J. Lechner, G. Pereira, and E. Schiebel, 2007 The yeast centrosome translates the positional information of the anaphase spindle into a cell cycle signal. J. Cell Biol. 179: 423–436.
Maiato, H., P. Sampaio, C. L. Lemos, J. Findlay, M. Carmena *et al.*, 2002 MAST/Orbit has a role in microtubule-kinetochore attachment and is essential for chromosome alignment and maintenance of spindle bipolarity. J. Cell Biol. 157: 749–760.
Maiato, H., E. A. Fairley, C. L. Rieder, J. R. Swedlow, C. E. Sunkel *et al.*, 2003 Human CLASP1 is an outer kinetochore component that regulates spindle microtubule dynamics. Cell 113: 891–904.
Maiato, H., A. Khodjakov, and C. L. Rieder, 2005 Drosophila CLASP is required for the incorporation of microtubule subunits into fluxing kinetochore fibres. Nat. Cell Biol. 7: 42–47.
Manning, B. D., J. G. Barrett, J. A. Wallace, H. Granok, and M. Snyder, 1999 Differential regulation of the Kar3p kinesin-related protein by two associated proteins, Cik1p and Vik1p. J. Cell Biol. 144: 1219–1233.
Markus, S. M., and W. L. Lee, 2011 Regulated offloading of cytoplasmic Dynein from microtubule plus ends to the cortex. Dev. Cell 20: 639–651.
Marschall, L. G., R. L. Jeng, J. Mulholland, and T. Stearns, 1996 Analysis of Tub4p, a yeast γ-tubulin-like protein: implications for microtubule-organizing center function. J. Cell Biol. 134: 443–454.
Maure, J. F., E. Kitamura, and T. U. Tanaka, 2007 Mps1 kinase promotes sister-kinetochore bi-orientation by a tension-dependent mechanism. Curr. Biol. 17: 2175–2182.
McDonald, H. B., and B. Byers, 1997 A proteasome cap subunit required for spindle pole body duplication in yeast. J. Cell Biol. 137: 539–553.
McEwen, B. F., and Y. Dong, 2010 Contrasting models for kinetochore microtubule attachment in mammalian cells. Cell. Mol. Life Sci. 67: 2163–2172.
McMillan, J. N., and K. Tatchell, 1994 The JNM1 gene in the yeast Saccharomyces cerevisiae is required for nuclear migration and spindle orientation during the mitotic cell cycle. J. Cell Biol. 125: 143–158.
Meednu, N., H. Hoops, S. D'Silva, L. Pogorzala, S. Wood *et al.*, 2008 The spindle positioning protein Kar9p interacts with the sumoylation machinery in *Saccharomyces cerevisiae*. Genetics 180: 2033–2055.
Megee, P. C., C. Mistrot, V. Guacci, and D. Koshland, 1999 The centromeric sister chromatid cohesion site directs Mcd1p binding to adjacent sequences. Mol. Cell 4: 445–450.
Melby, T. E., C. N. Ciampaglio, G. Briscoe, and H. P. Erickson, 1998 The symmetrical structure of structural maintenance of chromosomes (SMC) and MukB proteins: long, antiparallel coiled coils, folded at a flexible hinge. J. Cell Biol. 142: 1595–1604.
Meluh, P. B., and M. D. Rose, 1990 KAR3, a kinesin-related gene required for yeast nuclear fusion. Cell 60: 1029–1041.
Miller, R. K., and M. D. Rose, 1998 Kar9p is a novel cortical protein required for cytoplasmic microtubule orientation in yeast. J. Cell Biol. 140: 377–390.
Miller, R. K., S.-C. Cheng, and M. D. Rose, 2000 Bim1p/Yeb1p mediates the Kar9p-dependent cortical attachment of cytoplasmic microtubules. Mol. Biol. Cell 11: 2949–2959.
Mimori-Kiyosue, Y., I. Grigoriev, H. Sasaki, C. Matsui, A. Akhmanova *et al.*, 2006 Mammalian CLASPs are required for mitotic spindle organization and kinetochore alignment. Genes Cells 11: 845–857.
Molk, J. N., and K. Bloom, 2006 Microtubule dynamics in the budding yeast mating pathway. J. Cell Sci. 119: 3485–3490.

Montpetit, B., T. R. Hazbun, S. Fields, and P. Hieter, 2006 Sumoylation of the budding yeast kinetochore protein Ndc10 is required for Ndc10 spindle localization and regulation of anaphase spindle elongation. J. Cell Biol. 174: 653–663.

Moore, J. K., and J. A. Cooper, 2010 Coordinating mitosis with cell polarity: molecular motors at the cell cortex. Semin. Cell Dev. Biol. 21: 283–289.

Moore, J. K., J. Li, and J. A. Cooper, 2008 Dynactin function in mitotic spindle positioning. Traffic 9: 510–527.

Moore, J. K., D. Sept, and J. A. Cooper, 2009a Neurodegeneration mutations in dynactin impair dynein-dependent nuclear migration. Proc. Natl. Acad. Sci. USA 106: 5147–5152.

Moore, J. K., M. D. Stuchell-Brereton, and J. A. Cooper, 2009b Function of dynein in budding yeast: mitotic spindle positioning in a polarized cell. Cell Motil. Cytoskeleton 66: 546–555.

Morris, J. R., and R. J. Lasek, 1984 Monomer-polymer equilibria in the axon: direct measurement of tubulin and actin as polymer and monomer in axoplasm. J. Cell Biol. 98: 2064–2076.

Muller, E. G., B. E. Snydsman, I. Novik, D. W. Hailey, D. R. Gestaut *et al.*, 2005 The organization of the core proteins of the yeast spindle pole body. Mol. Biol. Cell 16: 3341–3352.

Munoz-Centeno, M. C., S. McBratney, A. Monterrosa, B. Byers, C. Mann *et al.*, 1999 Saccharomyces cerevisiae MPS2 encodes a membrane protein localized at the spindle pole body and the nuclear envelope. Mol. Biol. Cell 10: 2393–2406.

Nasmyth, K., and C. H. Haering, 2009 Cohesin: its roles and mechanisms. Annu. Rev. Genet. 43: 525–558.

Neuwald, A. F., and T. Hirano, 2000 HEAT repeats associated with condensins, cohesins, and other complexes involved in chromosome-related functions. Genome Res. 10: 1445–1452.

Nguyen, T., D. B. Vinh, D. K. Crawford, and T. N. Davis, 1998 A genetic analysis of interactions with Spc110p reveals distinct functions of Spc97p and Spc98p, components of the yeast γ-tubulin complex. Mol. Biol. Cell 9: 2201–2216.

Onischenko, E., L. H. Stanton, A. S. Madrid, T. Kieselbach, and K. Weis, 2009 Role of the Ndc1 interaction network in yeast nuclear pore complex assembly and maintenance. J. Cell Biol. 185: 475–491.

Ortiz, J., C. Funk, A. Schafer, and J. Lechner, 2009 Stu1 inversely regulates kinetochore capture and spindle stability. Genes Dev. 23: 2778–2791.

O'Toole, E. T., D. N. Mastronarde, T. H. Giddings Jr., M. Winey, D. J. Burke *et al.*, 1997 Three-dimensional analysis and ultrastructural design of mitotic spindles from the cdc20 mutant of Saccharomyces cerevisiae. Mol. Biol. Cell 8: 1–11.

O'Toole, E. T., M. Winey, and J. R. McIntosh, 1999 High-voltage electron tomography of spindle pole bodies and early mitotic spindles in the yeast Saccharomyces cerevisiae. Mol. Biol. Cell 10: 2017–2031.

Page, B. D., L. L. Satterwhite, M. D. Rose, and M. Snyder, 1994 Localization of the Kar3 kinesin heavy chain-related protein requires the Cik1 interacting protein. J. Cell Biol. 124: 507–519.

Palmer, R. E., M. Koval, and D. Koshland, 1989 The dynamics of chromosome movement in the budding yeast *Saccharomyces cerevisiae.* J. Cell Biol. 109: 3355–3366.

Park, C. J., S. Song, T. H. Giddings Jr., H. S. Ro, K. Sakchaisri *et al.*, 2004 Requirement for Bbp1p in the proper mitotic functions of Cdc5p in Saccharomyces cerevisiae. Mol. Biol. Cell 15: 1711–1723.

Park, C. J., J. E. Park, T. S. Karpova, N. K. Soung, L. R. Yu *et al.*, 2008 Requirement for the budding yeast polo kinase Cdc5 in proper microtubule growth and dynamics. Eukaryot. Cell 7: 444–453.

Pearson, C. G., and K. Bloom, 2004 Dynamic microtubules lead the way for spindle positioning. Nat. Rev. Mol. Cell Biol. 5: 481–492.

Pearson, C. G., P. S. Maddox, E. D. Salmon, and K. Bloom, 2001 Budding yeast chromosome structure and dynamics during mitosis. J. Cell Biol. 152: 1255–1266.

Pearson, C. G., P. S. Maddox, T. R. Zarzar, E. D. Salmon, and K. Bloom, 2003 Yeast kinetochores do not stabilize Stu2p-dependent spindle microtubule dynamics. Mol. Biol. Cell 14: 4181–4195.

Pearson, C. G., M. K. Gardner, L. V. Paliulis, E. D. Salmon, D. J. Odde *et al.*, 2006 Measuring nanometer scale gradients in spindle microtubule dynamics using model convolution microscopy. Mol. Biol. Cell 17: 4069–4079.

Pearson, C. G., D. P. Osborn, T. H. Giddings Jr., P. L. Beales, and M. Winey, 2009 Basal body stability and ciliogenesis requires the conserved component Poc1. J. Cell Biol. 187: 905–920.

Pellman, D., M. Bagget, Y. H. Tu, G. R. Fink, and H. Tu, 1995 Two microtubule-associated proteins required for anaphase spindle movement in Saccharomyces cerevisiae. J. Cell Biol. 130: 1373–1385.

Pereira, G., and E. Schiebel, 2003 Separase regulates INCENP-Aurora B anaphase spindle function through Cdc14. Science 302: 2120–2124.

Pereira, G., M. Knop, and E. Schiebel, 1998 Spc98p directs the yeast gamma-tubulin complex into the nucleus and is subject to cell cycle-dependent phosphorylation on the nuclear side of the spindle pole body. Mol. Biol. Cell 9: 775–793.

Pereira, G., U. Gruenberg, M. Knop, and E. Schiebel, 1999 Interaction of the yeast γ-tubulin complex-binding protein Spc72p with Kar1p is essential for microtubule function during karyogamy. EMBO J. 18(15): 4180–4195.

Pereira, G., T. U. Tanaka, K. Nasmyth, and E. Schiebel, 2001 Modes of spindle pole body inheritance and segregation of the Bfa1p-Bub2p checkpoint protein complex. EMBO J. 20: 6359–6370.

Peterson, J. B., and H. Ris, 1976 Electron-microscopic study of the spindle and chromosome movement in the yeast *Saccharomyces cerevisiae*. J. Cell Sci. 22: 219–242.

Reck-Peterson, S. L., A. Yildiz, A. P. Carter, A. Gennerich, N. Zhang *et al.*, 2006 Single-molecule analysis of dynein processivity and stepping behavior. Cell 126: 335–348.

Ribeiro, S. A., J. C. Gatlin, Y. Dong, A. Joglekar, L. Cameron *et al.*, 2009 Condensin regulates the stiffness of vertebrate centromeres. Mol. Biol. Cell 20: 2371–2380.

Robinow, C. F., and J. Marak, 1966 A fiber apparatus in the nucleus of the yeast cell. J. Cell Biol. 29: 129–151.

Roof, D. M., P. B. Meluh, and M. D. Rose, 1991 Multiple kinesin-related proteins in yeast mitosis. Cold Spring Harb. Symp. Quant. Biol. 56: 693–703.

Roof, D. M., P. B. Meluh, and M. D. Rose, 1992 Kinesin-related proteins required for assembly of the mitotic spindle. J. Cell Biol. 118: 95–108.

Rose, M., and G. Fink, 1987 *KAR1*, a gene required for function of both intranuclear and extranuclear microtubules in yeast. Cell 48: 1047–1060.

Salmon, E. D., 1975 Pressure-induced depolymerization of spindle microtubules. II. Thermodynamics of in vivo spindle assembly. J. Cell Biol. 66: 114–127.

Samoshkin, A., A. Arnaoutov, L. E. Jansen, I. Ouspenski, L. Dye *et al.*, 2009 Human condensin function is essential for centromeric chromatin assembly and proper sister kinetochore orientation. PLoS ONE 4: e6831.

Santaguida, S., and A. Musacchio, 2009 The life and miracles of kinetochores. EMBO J. 28: 2511–2531.

Saunders, W. S., and M. A. Hoyt, 1992 Kinesin-related proteins required for structural integrity of the mitotic spindle. Cell 70: 451–458.

Saunders, W., D. Hornack, V. Lengyel, and C. Deng, 1997a The Saccharomyces cerevisiae kinesin-related motor Kar3p acts at

preanaphase spindle poles to limit the number and length of cytoplasmic microtubules. J. Cell Biol. 137: 417–432.
Saunders, W., V. Lengyel, and M. A. Hoyt, 1997b Mitotic spindle function in Saccharomyces cerevisiae requires a balance between different types of kinesin-related motors. Mol. Biol. Cell 8: 1025–1033.
Schaerer, F., G. Morgan, M. Winey, and P. Philippsen, 2001 Cnm67p is a spacer protein of the Saccharomyces cerevisiae spindle pole body outer plaque. Mol. Biol. Cell 12: 2519–2533.
Schek, H. T. III., M. K. Gardner, J. Cheng, D. J. Odde, and A. J. Hunt, 2007 Microtubule assembly dynamics at the nanoscale. Curr. Biol. 17: 1445–1455.
Schiebel, E., 2000 Gamma-tubulin complexes: binding to the centrosome, regulation and microtubule nucleation. Curr. Opin. Cell Biol. 12: 113–118.
Schliwa, M., U. Euteneuer, W. Herzog, and K. Weber, 1979 Evidence for rapid structural and functional changes of the melanophore microtubule-organizing center upon pigment movements. J. Cell Biol. 83: 623–632.
Schramm, C., S. Elliot, A. Shevchenko, A. Shevchenko, and E. Schiebel, 2000 The Bbp1p/Mps2p complex connects the SPB to the nuclear envelope and is essential for SPB duplication. EMBO J. 19(3): 421–433.
Schutz, A. R., and M. Winey, 1998 New alleles of the yeast MPS1 gene reveal multiple requirements in spindle pole body duplication. Mol. Biol. Cell 9: 759–774.
Schuyler, S. C., and D. Pellman, 2001 Microtubule "plus-end-tracking proteins": the end is just the beginning. Cell 105: 421–424.
Segal, M., D. J. Clarke, P. Maddox, E. D. Salmon, K. Bloom *et al.*, 2000 Coordinated spindle assembly and orientation requires Clb5p-dependent kinase in budding yeast. J. Cell Biol. 148: 441–452.
Sezen, B., M. Seedorf, and E. Schiebel, 2009 The SESA network links duplication of the yeast centrosome with the protein translation machinery. Genes Dev. 23: 1559–1570.
Shaw, S. L., E. Yeh, P. Maddox, E. D. Salmon, and K. Bloom, 1997 Astral microtubule dynamics in yeast: a microtubule-based searching mechanism for spindle orientation and nuclear migration into the bud. J. Cell Biol. 139: 985–994.
Sheeman, B., P. Carvalho, I. Sagot, J. Geiser, D. Kho *et al.*, 2003 Determinants of S. cerevisiae dynein localization and activation: implications for the mechanism of spindle positioning. Curr. Biol. 13: 364–372.
Shirasu-Hiza, M., P. Coughlin, and T. Mitchison, 2003 Identification of XMAP215 as a microtubule-destabilizing factor in Xenopus egg extract by biochemical purification. J. Cell Biol. 161: 349–358.
Slep, K. C., 2010 Structural and mechanistic insights into microtubule end-binding proteins. Curr. Opin. Cell Biol. 22: 88–95.
Soues, S., and I. R. Adams, 1998 SPC72: a spindle plie component required for spindle orientation in the yeast Saccharomyces cerevisiae. J. Cell Sci. 111: 2809–2818.
Spang, A., I. Courtney, K. Grein, M. Matzner, and E. Schiebel, 1995 The Cdc31p-binding protein Kar1p is a component of the half bridge of the yeast spindle pole body. J. Cell Biol. 128: 863–877.
Sprague, B. L., C. G. Pearson, P. S. Maddox, K. S. Bloom, E. D. Salmon *et al.*, 2003 Mechanisms of microtubule-based kinetochore positioning in the yeast metaphase spindle. Biophys. J. 84: 3529–3546.
Sproul, L. R., D. J. Anderson, A. T. Mackey, W. S. Saunders, and S. P. Gilbert, 2005 Cik1 targets the minus-end kinesin depolymerase kar3 to microtubule plus ends. Curr. Biol. 15: 1420–1427.
Stegmeier, F., and A. Amon, 2004 Closing mitosis: the functions of the Cdc14 phosphatase and its regulation. Annu. Rev. Genet. 38: 203–232.
Stephens, A. D., J. Haase, L. Vicci, R. M. I. Taylor, and K. Bloom, 2011 Cohesin, condensin, and the intramolecular centromere loop together generate the mitotic chromatin spring. J. Cell Biol. 193: 1–14.
Stirling, D., K. Welch,, and M. Stark, 1994 Interaction with calmodulin is required for the function of Spc110p, an essential component of the yeast spindle pole body. EMBO J. 13: 4329–4342.
Sullivan, D. S., and T. C. Huffaker, 1992 Astral microtubules are not required for anaphase B in Saccharomyces cerevisiae. J. Cell Biol. 119: 379–388.
Sun, G. H., A. Hirata, Y. Ohya, and Y. Anraku, 1992 Mutations in yeast calmodulin cause defects in spindle pole body functions and nuclear integrity. J. Cell Biol. 119: 1625–1639.
Sundberg, H. A., L. Goetsch, B. Byers, and T. N. Davis, 1996 Role of calmodulin and Spc110p interaction in the proper assembly of spindle pole body compenents. J. Cell Biol. 133: 111–124.
Tanaka, T., J. Fuchs, J. Loidl, and K. Nasmyth, 2000 Cohesin ensures bipolar attachment of microtubules to sister centromeres and resists their precocious separation. Nat. Cell Biol. 2: 492–499.
Tirnauer, J. S., E. O'Toole, L. Berrueta, B. E. Bierer, and D. Pellman, 1999 Yeast Bim1p promotes the G1-specific dynamics of microtubules. J. Cell Biol. 145: 993–1007.
Tolic-Norrelykke, I. M., 2010 Force and length regulation in the microtubule cytoskeleton: lessons from fission yeast. Curr. Opin. Cell Biol. 22: 21–28.
Ubersax, J. A., E. L. Woodbury, P. N. Quang, M. Paraz, J. D. Blethrow *et al.*, 2003 Targets of the cyclin-dependent kinase Cdk1. Nature 425: 859–864.
Vallen, E., M. Hiller, T. Scherson, and M. Rose, 1992 Separate domains of KAR1 mediate distinct functions in mitosis and nuclear fusion. J. Cell Biol. 117: 1277–1287.
Varga, V., J. Helenius, K. Tanaka, A. A. Hyman, T. U. Tanaka *et al.*, 2006 Yeast kinesin-8 depolymerizes microtubules in a length-dependent manner. Nat. Cell Biol. 8: 957–962.
Vinh, D. B., J. W. Kern, W. O. Hancock, J. Howard, and T. N. Davis, 2002 Reconstitution and characterization of budding yeast gamma-tubulin complex. Mol. Biol. Cell 13: 1144–1157.
Visintin, R., S. Prinz, and A. Amon, 1997 CDC20 and CDH1: a family of substrate-specific activators of APC-dependent proteolysis. Science 278: 460–463.
Vizeacoumar, F. J., N. van Dyk, F. S. Vizeacoumar, V. Cheung, J. Li *et al.* 2010 Integrating high-throughput genetic interaction mapping and high-content screening to explore yeast spindle morphogenesis. J. Cell Biol. 188: 69–81.
Vogel, J., B. Drapkin, J. Oomen, D. Beach, K. Bloom *et al.*, 2001 Phosphorylation of gamma-tubulin regulates microtubule organization in budding yeast. Dev. Cell 1: 621–631.
Wan, X., R. P. O'Quinn, H. L. Pierce, A. P. Joglekar, W. E. Gall *et al.*, 2009 Protein architecture of the human kinetochore microtubule attachment site. Cell 137: 672–684.
Wargacki, M. M., J. C. Tay, E. G. Muller, C. L. Asbury, and T. N. Davis, 2010 Kip3, the yeast kinesin-8, is required for clustering of kinetochores at metaphase. Cell Cycle 9: 2581–2588.
Weber, S. A., J. L. Gerton, J. E. Polancic, J. L. DeRisi, D. Koshland *et al.*, 2004 The kinetochore is an enhancer of pericentric cohesin binding. PLoS Biol. 2: E260.
Weiss, E., and M. Winey, 1996 The Saccharomyces cerevisiae spindle pole body duplication gene MPS1 is part of a mitotic checkpoint. J. Cell Biol. 132: 111–123.
Widlund, P. O., J. S. Lyssand, S. Anderson, S. Niessen, J. R. Yates III. *et al.*, 2006 Phosphorylation of the chromosomal passenger protein Bir1 is required for localization of Ndc10 to the spindle during anaphase and full spindle elongation. Mol. Biol. Cell 17: 1065–1074.
Widlund, P. O., J. H. Stear, A. Pozniakovsky, M. Zanic, S. Reber *et al.*, 2011 XMAP215 polymerase activity is built by combin-

ing multiple tubulin-binding TOG domains and a basic lattice-binding region. Proc. Natl. Acad. Sci. USA 108: 2741–2746.
Wigge, P. A., O. N. Jensen, S. Holmes, S. Soues, M. Mann *et al.*, 1998 Analysis of the Saccharomyces spindle pole by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. J. Cell Biol. 141: 967–977.
Winey, M., and B. J. Huneycutt, 2002 Centrosomes and checkpoints: the MPS1 family of kinases. Oncogene 21: 6161–6169.
Winey, M., L. Goetsch, P. Baum, and B. Byers, 1991 MPS1 and MPS2: novel yeast genes defining distinct steps of spindle pole body duplication. J. Cell Biol. 114: 745–754.
Winey, M., M. A. Hoyt, C. Chan, L. Goetsch, D. Botstein *et al.*, 1993 NDC1: a nuclear periphery component required for yeast spindle pole body duplication. J. Cell Biol. 122: 743–751.
Winey, M., C. L. Mamay, E. T. O. Toole, D. N. Mastronarde, T. H. Giddings Jr. *et al.*, 1995 Three-dimensional ultrastructural analysis of the Saccharomyces cerevisiae mitotic spindle. J. Cell Biol. 129: 1601–1615.
Winey, M., D. Yarar, T. H. Giddings Jr., and D. N. Mastronarde, 1997 Nuclear pore complex number and distribution throughout the Saccharomyces cerevisiae cell cycle by three-dimensional reconstruction from electron micrographs of nuclear envelopes. Mol. Biol. Cell 8: 2119–2132.
Witkin, K. L., J. M. Friederichs, O. Cohen-Fix, and S. L. Jaspersen, 2010 Changes in the nuclear envelope environment affect spindle pole body duplication in *Saccharomyces cerevisiae*. Genetics 186: 867–883.
Woodruff, J. B., D. G. Drubin, and G. Barnes, 2010 Mitotic spindle disassembly occurs via distinct subprocesses driven by the anaphase-promoting complex, Aurora B kinase, and kinesin-8. J. Cell Biol. 191: 795–808.
Yeh, E., R. V. Skibbens, J. W. Cheng, E. D. Salmon, and K. Bloom, 1995 Spindle dynamics and cell cycle regulation of dynein in the budding yeast, Saccharomyces cerevisiae. J. Cell Biol. 130: 687–700.
Yeh, E., J. Haase, L. V. Paliulis, A. Joglekar, L. Bond *et al.*, 2008 Pericentric chromatin is organized into an intramolecular loop in mitosis. Curr. Biol. 18: 81–90.
Yin, H., D. Pruyne, T. C. Huffaker, and A. Bretscher, 2000 Myosin V orientates the mitotic spindle in yeast. Nature 406: 1013–1015.
Yin, H., L. You, D. Pasqualone, K. M. Kopski, and T. C. Huffaker, 2002 Stu1p is physically associated with beta-tubulin and is required for structural integrity of the mitotic spindle. Mol. Biol. Cell 13: 1881–1892.
Yoder, T. J., C. G. Pearson, K. Bloom, and T. N. Davis, 2003 The Saccharomyces cerevisiae spindle pole body is a dynamic structure. Mol. Biol. Cell 14: 3494–3505.
Yoder, T. J., M. A. McElwain, S. E. Francis, J. Bagley, E. G. Muller *et al.*, 2005 Analysis of a spindle pole body mutant reveals a defect in biorientation and illuminates spindle forces. Mol. Biol. Cell 16: 141–152.
Yoshida, S., K. Asakawa, and A. Toh-e, 2002 Mitotic exit network controls the localization of Cdc14 to the spindle pole body in Saccharomyces cerevisiae. Curr. Biol. 12: 944–950.
Zeng, X., J. A. Kahana, P. A. Silver, M. K. Morphew, J. R. McIntosh *et al.*, 1999 Slk19p is a centromere protein that functions to stabilize mitotic spindles. J. Cell Biol. 146: 415–425.
Zinkowski, R. P., J. Meyne, and B. R. Brinkley, 1991 The centromere-kinetochore complex: a repeat subunit model. J. Cell Biol. 113: 1091–1110.
Zizlsperger, N., V. N. Malashkevich, S. Pillay, and A. E. Keating, 2008 Analysis of coiled-coil interactions between core proteins of the spindle pole body. Biochemistry 47: 11858–11868.

Communicating editor: T. N. Davis