Spindle microtubules in flux

Gregory C. Rogers[1,2,*], Stephen L. Rogers[2] and David J. Sharp[1]

[1]Department of Physiology and Biophysics, 223 Ullmann Building, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[2]Department of Biology, CB#3280, 241 Wilson Hall, University of North Carolina, Chapel Hill, NC 27599, USA
*Author for correspondence (e-mail: gcrogers@email.unc.edu)

Journal of Cell Science 118, 1105-1116 Published by The Company of Biologists 2005
doi:10.1242/jcs.02284

Summary

Accurate and timely chromosome segregation is a task performed within meiotic and mitotic cells by a specialized force-generating structure – the spindle. This micro-machine is constructed from numerous proteins, most notably the filamentous microtubules that form a structural framework for the spindle and also transmit forces through it. Poleward flux is an evolutionarily conserved mechanism used by spindle microtubules both to move chromosomes and to regulate spindle length. Recent studies have identified a microtubule-depolymerizing kinesin as a key force-generating component required for flux. On the basis of these findings, we propose a new model for flux powered by a microtubule-disassembly mechanism positioned at the spindle pole. In addition, we use the flux model to explain the results of spindle manipulation experiments to illustrate the importance of flux for proper chromosome positioning.

Key words: Mitosis, Spindle, Microtubule, Poleward flux, Kinesin

Introduction

The eukaryotic spindle is a fusiform-shaped, microtubule (MT)-based machine that primarily serves a single purpose: the proper separation of chromosomes in meiotic and mitotic cells (Wittmann et al., 2001). Different pathways can be used by cells to assemble the array of filamentous MTs to form the characteristic bipolar spindle (Budde and Heald, 2003; Wadsworth and Khodjakov, 2004), in which MT plus-ends are directed towards the spindle center and MT minus-ends are focused into poles at either end of the spindle. Within the spindle, MTs are distinguished further by their positions, and these differences are relevant to their respective functions. Kinetochore MTs (kMTs) dock with kinetochores, which are multiprotein complexes assembled onto centromeric DNA (Maiato et al., 2004), and bundle together to form kinetochore fibers (K-fibers). Interpolar MTs (ipMTs) extend towards the spindle equator and may interact with MTs from the opposite pole. Finally, astral MTs project from poles towards the cell cortex (Fig. 1). Successful chromosome segregation requires proper assembly and function of the spindle MT array and, although numerous studies have identified MT-dependent forces underlying chromosome movement and spindle assembly (Mitchison and Salmon, 2001), we possess an incomplete understanding of the force-generating components. Here, we discuss the impact of an important force-generating engine in the spindle – poleward MT flux – whose function, until recently, was mostly speculation.

The flux phenomenon: a brief history

The proposal that spindles and their constituent fibers (later identified as MTs) are dynamic rather than static structures predates the discoveries of tubulin as the molecular subunit of MTs and the dynamic behavior of MT assembly in vitro (Desai and Mitchison, 1997). In 1965, Arthur Forer in Shinya Inoue's lab irradiated localized regions within meiotic spindles of living crane fly spermatocytes, using an ultraviolet microbeam, and observed the movement of the resulting marks of reduced birefringence. Surprisingly, the irradiated areas moved towards the nearest spindle pole and disappeared. Forer concluded that spindle fibers "move to the pole, are broken down at the pole, and are re-cycled, and that the area of reduced birefringence is just a marker in this continuously moving system" (Forer, 1965).

The subsequent discovery of MT treadmilling (Margolis and Wilson, 1978) led to a theoretical model of spindle function based on the poleward flow of the MT array. Margolis et al. proposed that the parallel MTs in each half-spindle treadmill while their plus-ends (at kinetochores and in the spindle equator) assemble by tubulin subunit addition and their minus-ends (at spindle poles) simultaneously disassemble by tubulin loss (Margolis et al., 1978). Where the two half-spindles overlap at the spindle equator, the ATP-dependent sliding apart of the anti-parallel ipMTs would result in poleward translocation of MTs. If kMTs were linked to the translocating ipMTs by crossbridges, then chromosomes attached to the kMTs would experience a poleward force. During metaphase, the coordinated poleward sliding of crosslinked, treadmilling MTs would create an isometric tension in the spindle. During anaphase, features of this system could be modulated to precipitate anaphase events; for example, the cessation of kMT plus-end assembly would lead to the segregation of disjoined sister chromatids to their respective poles. Notably, MTs assembled from pure tubulin do not treadmill at steady-state as initially thought by Margolis and Wilson (Margolis and Wilson, 1978); instead their plus- and minus-ends exhibit dynamic instability (Grego et al., 2001).

The development of novel tubulin derivates allowed a more direct examination of spindle MT dynamics (Mitchison et al., 1986; Gorbsky et al., 1988; Mitchison, 1989). For instance,

Journal of Cell Science

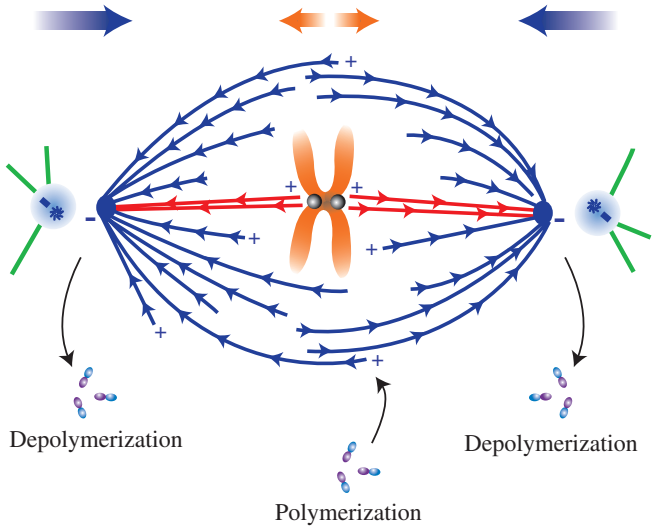

Fig. 1. Poleward MT flux in a metaphase-stage mitotic spindle. Flux occurs on both kMTs (red lines) and non-kMTs (blue lines) in the spindle. Tubulin subunits are incorporated into polymer at MT plus-ends and removed at their minus-ends focused at the spindle poles. Arrows within the red and blue lines indicate the direction of continuous ATP-dependent polymer movement. kMT plus-end assembly stops at the transition to anaphase (although there are exceptions to this rule) (Chen and Zhang, 2004; LaFountain et al., 2004) (see text). Astral MTs (green lines), whose minus-ends are embedded in the centrosomes, do not flux. Orange arrows above the spindle indicate the poleward direction of force exerted by flux on each sister kinetochore. Likewise, opposing blue arrows indicate the metaphase plateward direction of force exerted by flux on each spindle pole. Importantly, the major source of MT assembly dynamics in the spindle is plus-end dynamic instability, which is not shown here for simplicity.

Mitchison et al. microinjected live metaphase monkey fibroblasts (BSC1 cells) with biotin-labeled tubulin and showed that the tubulin incorporates at kMT plus-ends embedded in kinetochores and subsequently extends towards the pole, which indicated a poleward 'flux' of subunits through the lattice of kMTs (Mitchison et al., 1986). Mitchison later microinjected an ultraviolet (UV)-photoactivatable, caged fluorophore-tubulin into cultured mitotic porcine kidney epithelial (LLC-PK1) and rat kangaroo (PtK_2) cells and allowed it to incorporate into MTs and eventually distribute throughout the spindle (Mitchison, 1989). Irradiation of a metaphase spindle with a focused UV microbeam generated a narrow, fluorescent bar on the spindle that traveled towards and eventually disappeared at a pole. Mitchison concluded that metaphase kMTs move poleward and must assemble at kinetochores at the same rate as disassembly at poles in order to maintain a constant spindle length (Mitchison, 1989). Furthermore, he proposed that kMTs are made to slide poleward by a plus-end-directed force produced by kinesin-like motors anchored to an unidentified, non-MT 'spindle matrix' (Scholey et al., 2001).

Interestingly, subsequent photoactivation studies demonstrated that this flux is unique to spindles and does not occur in the interphase MT array (Zhai et al., 1995). In addition, spindle MT flux rates change between mitotic stages in cultured mammalian tissue cells (Table 1) (Mitchison and Salmon, 1992; Zhai et al., 1995). During anaphase, MT flux continues but slows to a rate approximately half the metaphase rate. Comparisons of the rates of flux and chromosome movement during a particular mitotic stage indicate the flux rate is only approximately a third of the rate of chromosome movement, irrespective of the mitotic stage (Mitchison and Salmon, 1992; Skibbens et al., 1993; Zhai et al., 1995). Therefore, kMT plus-ends attached to moving chromosomes must disassemble faster than the kMTs flux, utilizing a 'Pacman'-based mechanism that couples kinetochore motility to plus-end disassembly (McIntosh et al., 2002).

Flux has also been studied in vitro, which increases the scope of possible experimental manipulations. For instance, Sawin and Mitchison found that robust flux occurs in both kMTs and ipMTs assembled in vitro in *Xenopus* egg extracts (Sawin and Mitchison, 1991). Importantly, the non-hydrolyzable ATP analog AMP-PNP completely inhibits flux when added to preformed extract spindles, demonstrating the ATP dependence of the process.

The development of fluorescent speckle microscopy (FSM) dramatically enhanced the resolution and ease of imaging flux (Waterman-Storer et al., 1998). In this technique, small amounts of fluorescently labeled tubulin are randomly incorporated into polymerizing MTs, creating fluorescent 'speckles' that allow observation of spindle dynamics in living cells, including invertebrate systems (Brust-Mascher and Scholey, 2002; Maddox et al., 2002). It has also revealed that flux does not occur in astral MTs but is restricted to kMTs and ipMTs (Waterman-Storer et al., 1998).

Flux versus treadmilling: who are the players?

While analyzing the MT lattice motion of metaphase spindles, Mitchison coined the descriptive name 'polewards MT flux' to distinguish this process from 'treadmilling' (Mitchison, 1989). As with actin filaments, treadmilling describes a specific class of steady-state polymer dynamics where differences in subunit affinities at the two ends drive the constant addition of subunits from one end and the balanced subunit loss at the opposite end (Margolis and Wilson, 1981). Because the mechanism driving the poleward movement of tubulin subunits was unknown and potentially distinct from treadmilling, Mitchison chose the term 'flux'. Accordingly, 'flux' constitutes two activities: (1) the poleward movement of kMTs and (2) balanced rates of kMT plus-end assembly and minus-end disassembly. This description is satisfactory for the metaphase spindle but is not adequate at anaphase, when kMTs continue to move polewards while disassembling at both ends (Mitchison and Salmon, 1992). Therefore, we support the more encompassing definition of flux, proposed by Maddox et al., which can be applied regardless of the mitotic stage: flux is the poleward movement of MTs that is coupled to minus-end disassembly at the spindle pole (Maddox et al., 2003). During metaphase, fluxing kMTs display an additional treadmilling-like activity in which plus-ends grow at kinetochores while minus-ends disassemble at poles, achieving a balance that maintains a constant spindle length.

Central to solving flux is the understanding of how MTs move poleward. Although not mutually exclusive, two popular models describe how this movement is accomplished utilizing either a MT depolymerization or sliding mechanism

Table 1. Rates of poleward microtubule flux and anaphase A chromosome segregation

Organism/cell type	Visual method	Average metaphase flux rate (μm/sec)×10^{-3}	Average anaphase flux rate (μm/sec)×10^{-3}	Average anaphase A rate (μm/sec)×10^{-3}	Flux contribution to anaphase A (%)	Reference
Mitosis						
Porcine kidney epithelial	Photoactivation	8.3†	–	–	–	Mitchison, 1989
(LLC-PK1)	Photoactivation	6.5†; 7.5‡	3.3†	10.2§	32	Zhai et al., 1995
Rat kangaroo epithelial						
(PtK_1)	Photoactivation	7.7†	3.5†	–	–	Zhai et al., 1995
(PtK_2)	Photoactivation	10.0†	–	–	–	Mitchison, 1989
Newt lung	Photoactivation	9.0	7.3; 3.0¶	28.3; 9.0¶	37	Mitchison and Salmon, 1991
	Photoactivation	9.0	3.3	–	–	Waters et al., 1996
	FSM	12.5; 27.2**	–	–	–	Waterman-Storer et al., 1998
Drosophila embryo	FSM	31.7; 31.7‡‡	31.7	106.7	30	Brust-Mascher and Scholey, 2002
	FSM	–	53.3; 86.7††	60.0; 110.0††	89; 79††	Maddox et al., 2002
	FSM	36.7	36.7	93.3	39	Rogers et al., 2004
Meiosis						
Xenopus oocyte extract	Photoactivation	48.3	–	–	–	Sawin and Mitchison, 1991
	Photoactivation	–	33.3	36.7	91§§	Desai et al., 1998
	FSM	33.3	–	–	–	Waterman-Storer et al., 1998
	FSM	18.3	–	–	–	Kapoor and Mitchison, 2001
	FSM	33.3; 38.3‡‡	26.7	36.7	73	Maddox et al., 2003
	FSM	40.0	–	–	–	Gaetz and Kapoor, 2004
	FSM	43.8	–	–	–	Shirasu-Hiza et al., 2004
	FSM	36.8; 32.8¶¶	–	–	–	Miyamoto et al., 2004
Crane fly spermatocyte	FSM	11.7; 13.3‡‡	15; 15‡‡	8.8	100	LaFountain et al., 2004

†Cells maintained at 30°C.
‡Cells maintained at 23°C.
§Anaphase chromosome segregation displayed a biphasic (early fast – late slow) movement with the indicated overall average rate.
¶Anaphase chromosome segregation and flux displayed a biphasic movement with rates of the early and late phases indicated, respectively.
**Metaphase flux rates were measured in the kinetochore fiber and in the spindle equator, respectively.
††Syncytial blastoderm-stage *Drosophila* embryos were maintained at 18°C and 24°C, respectively.
‡‡Flux was measured in kinetochore and interpolar microtubules, respectively.
§§No significant difference was found between the average rates of anaphase A and anaphase flux in this study.
¶¶Speckle movement was measured using kymography and a cross-correlation algorithm, respectively.

(Miyamoto et al., 2004). Importantly, experiments by Waters et al. on mitotic newt lung cells identified the spindle pole as the origin of force production for flux (Waters et al., 1996). This group applied taxol to cells to suppress kMT plus-end dynamics and found that metaphase spindles collapsed at near flux velocities. Photoactivation of fluorescent tubulin bars on collapsing spindles confirmed the loss of plus-end dynamics but, significantly, showed that minus-end disassembly continued at poles. Also, tension exerted across centromeres (perhaps driven by flux) produces a measurable gap between sister kinetochores and was identical in taxol-treated and control cells. The authors drew several important conclusions about the nature of the flux mechanism: (1) it is not dependent on plus-end dynamics; (2) it must hold onto depolymerizing MT minus-ends while linking them to the centrosome; and (3) it must promote MT minus-end depolymerization. Additionally, these data support a role for flux in regulating spindle length and generating a poleward force on kinetochores.

These findings also have implications for the traction fiber model (Ostergren, 1950) proposed as a mechanism for chromosome congression, which has been attributed to flux (Kapoor and Compton, 2002). The model has two notable features: (1) bi-oriented chromosomes are moved to the spindle equator through a tug-of-war of opposing traction forces that are delivered through kMTs to sister kinetochores; and (2) the poleward force accrues from force-generators distributed uniformly along kMTs and, therefore, has a magnitude proportional to kMT length. Plus-end-directed motors, such as Eg5 (a kinesin-5 family member) (Lawrence et al., 2004), have been proposed to be positioned along a non-MT spindle matrix and to produce flux (and traction force) by interacting with nearby kMTs to slide them poleward (Mitchison and Sawin, 1990). Although the Waters et al. result does not exclude a MT sliding component for flux, the results argue against this model as the flux mechanism (Waters et al., 1996). Taxol-treated spindles collapse as poles move towards the metaphase plate by the persistent disassembly of spindle MT minus-ends. This is consistent with a flux model in which the force-generating activity is located at the poles.

By contrast, recent work on the Eg5 motor suggests that a MT sliding component for flux does exist in spindles assembled in *Xenopus* egg extracts. Initially, neither immunodepletion of Eg5 nor addition of the Eg5 inhibitor monastrol blocked flux in these in vitro spindles (Sawin and Mitchison, 1994; Kapoor and Mitchison, 2001). However, a repeat of these experiments revealed a dramatic reduction in flux after using a more-thorough immunodepletion of Eg5 or by again adding small-molecule Eg5 inhibitors (Miyamoto et al., 2004). Kinesin-5 family members are a functionally

conserved class of kinesins essential for maintaining spindle bipolarity (Sharp et al., 1999). Similar to the results by Waters et al. (Waters et al., 1996), loss of kinesin-5 activity results in collapse of the prometaphase spindle into a monaster. Therefore, to measure flux after Eg5 inhibition, Miyamoto et al. prevented spindle collapse by either pinning spindles by using a coverslip squash method or by co-inhibiting the dynein-dynactin complex by addition of p50/dynamitin protein to the extracts (Miyamoto et al., 2004; Wittmann and Hyman, 1999). Interestingly, the simultaneous inhibition of Eg5 and dynein-dynactin stopped flux and did not result in spindle shortening as observed by Waters et al. (Waters et al., 1996), suggesting that flux is driven by Eg5-dependent MT sliding. However, it is not clear how perturbing both Eg5 and dynein-dynactin rescues spindle bipolarity in vitro, and further study is needed to clarify the basis for the divergent results of the in vitro and live cell studies. It is clear that adding p50 to extract spindles disrupts the spindle pole and may mis-localize the MT depolymerization factors that reside there (Wittmann and Hyman, 1999; Gaetz and Kapoor, 2004). It would be important and compelling if future studies could demonstrate a contribution to flux by Eg5 in live cells.

Additional insight into the flux mechanism has come from the study of meiotic spindle mechanics of insect spermatocytes. Grasshopper and crane fly spermatocytes offer exceptional systems for the study of flux: during anaphase, their kMT plus-ends do not disassemble and flux appears to be the sole means to move chromosomes poleward (Wilson et al., 1994; Chen and Zhang, 2004). In fact, their kMT plus-ends polymerize during anaphase A at a rate identical to the chromosome-to-pole velocity (Chen and Zhang, 2004). Although this should hinder anaphase chromosome-to-pole movement, crane fly spermatocytes accomplish chromosome segregation by fluxing kMTs at rates almost twice that of chromosome velocity (LaFountain et al., 2004). Spermatocytes are also amenable to micromanipulation. Chen and Zhang have exploited this feature, maneuvering single anaphase chromosomes away from their spindles with a micromanipulation needle and then severing the K-fibers of the chromosomes with a laser microbeam (Chen and Zhang, 2004). New MT plus-ends created at the severing site quickly depolymerized to their pole, leaving behind an immobile chromosome with an attached K-fiber 'stub' whose length did not change. Microinjection of labeled tubulin revealed that MTs within the K-fiber stub treadmill (by assembly at plus-ends and disassembly at minus-ends), but this activity alone could not move the chromosome. Chromosome-to-pole movement resumed only after the K-fiber stub regained its connection to the pole through MTs growing from the pole. These results establish that the poleward chromosome force does not require non-kinetochore MTs and probably does not require a spindle matrix (assuming that attachment to a spindle matrix is lost when the chromosome is repositioned), but does require kMTs to be attached to a spindle pole.

Another approach used to identify the source of flux is examination of monopolar spindles assembled in cytostatic factor (CSF) *Xenopus* egg extracts (Sawin and Mitchison, 1994). These can be obtained by adding sperm nuclei (each with a single adherent centrosome) to egg extracts or by adding dimethyl sulfoxide (DMSO) to egg extracts that lack chromosomes and centrosomes. In both cases, photoactivation assays revealed MT flux (by disassembly of the focused minus-ends) at rates similar to those of bipolar spindles (Sawin and Mitchison, 1994). However, FSM studies, which allow more-precise measurement of MT lattice motion, have since found that MTs in these mono-arrays do not flux (Mitchison et al., 2004). Evidently, the preparation of a sample for microscopy can cause the centrosome of a monopolar spindle to spontaneously split and slide apart, setting the stage for some bipolarity to arise in these spindles. It was these bipolarizing structures that were examined in the earlier *Xenopus* extract studies. This indicates that flux initiates only after anti-parallel ipMTs, which are not present in monopolar spindles, form and begin to slide polewards. Presumably, this is driven by plus-end-directed Eg5 motors (Mitchison and Sawin, 1990; Miyamoto et al., 2004) positioned within the zone of overlapped ipMTs (Mitchison et al., 2004).

By contrast, the results of Waters et al. and Chen and Zhang described above (Waters et al., 1996; Chen and Zhang, 2004), pinpoint the spindle pole as the origin of force production for flux. The results of grasshopper spermatocyte microsurgery reinforce this view. Early anaphase spermatocytes were cut in half through their spindles to create two cells, each containing a half-spindle with a single pole (Alsop and Zhang, 2003). Although the authors did not directly assay flux or determine the polarity of the MTs within the severed half-spindles, they observed that chromosomes complete their poleward movement in these cells (in which flux is the sole means for anaphase chromosome movement), suggesting that flux continues even on monopolar half-spindles.

If flux arises at the poles and generates a pole-directed force on spindle MTs, then two predictions follow. First, the flux engine should be positioned at poles; second, its inhibition should stop flux and slow chromosome segregation. Recently, we and others identified the first molecular component of the flux-generation apparatus, a Kin I (also known as kinesin-13 family) kinesin in *Drosophila* named KLP10A that localizes to centrosomes and spindle poles throughout mitosis (Rogers et al., 2004). Members of this kinesin subfamily possess a central kinesin-like ATPase domain (Hunter and Wordeman, 2000). Unlike most kinesins, which move unidirectionally along the surface of a MT, these bind to MT ends (both plus and minus) and catalyze depolymerization through class-specific neck/motor elements that target and are predicted to bend terminal αβ tubulin subunits at their intradimer region (Desai et al., 1999; Moores et al., 2002; Ogawa et al., 2004; Shipley et al., 2004). Increased intradimer curvature is thought to destabilize MT ends by 'peeling' protofilaments apart from each other, thereby disrupting their stabilizing lateral interactions. Furthermore, these kinesins localize to mitotic spindle poles, centrosomes and centromeres, and are required for spindle assembly as well as chromosome movements (Wordeman and Mitchison, 1995; Walczak et al., 1996; Maney et al., 1998; Kline-Smith and Walczak, 2002; Moore and Wordeman, 2004; Rogers et al., 2004). KLP10A displays ATP-dependent MT-depolymerization activity in vitro that is inhibited by AMP-PNP. In addition, FSM of spindles in live early embryos microinjected with a function-blocking anti-KLP10A antibody reveals that this inhibits flux: >90% of speckled spindle MTs display little or no movement (Rogers et al., 2004).

Prior to anaphase, sustained MT plus-end assembly is needed to balance minus-end disassembly and to maintain

spindles at constant length. We propose that specific factors promote such plus-end assembly, reside at kinetochores prior to anaphase and are tightly regulated throughout mitosis. Excellent candidates for these are the conserved CLASP family of non-motor MT-associated proteins (MAPs) (Carvalho et al., 2003). CLASP homologs in humans (CLASP1) and *Drosophila* (MAST/Orbit) localize to spindle MTs, centrosomes and kinetochores, and are required to maintain spindle bipolarity (Lemos et al., 2000; Inoue et al., 2000; Maiato et al., 2003). Specifically, CLASP1 in HeLa cells localizes to growing MT plus-ends and promotes kMT growth at the kinetochore; inhibition results in paused kMT plus-ends and halts the chromosome oscillations commonly found in vertebrate cultured cells (Maiato et al., 2003; Skibbens et al., 1993). Furthermore, live cell analysis of green fluorescent protein (GFP)-labeled centrosomes in *mast* mutant *Drosophila* embryos revealed a metaphase collapse of the spindle strikingly reminiscent of the taxol-induced spindle collapse observed in newt lung cells (Maiato et al., 2002). Although we do not know how purified CLASPs influence the dynamic instability of pure MTs in vitro, the mitotic phenotype of CLASP inhibition indicates a role for this protein in treadmilling, specifically in promoting MT growth at kinetochores.

A model for flux

To integrate the recent findings, we propose a model for the mechanism of flux that is dependent on a spindle-pole-associated Kin I kinesin as the primary force generator. Initially, γ-tubulin ring complexes (γ-TuRCs) embedded in the centrosome nucleate MT growth and cap minus-ends, preventing their disassembly (astral MTs constitute this non-fluxing class of MTs) (Wiese and Zheng, 2000; Keating and Borisy, 2000). During prometaphase, free MT minus-ends could arise near the centrosome by either the activity of the centrosome-associated MT-severing enzyme katanin (Buster et al., 2002) or the regulated release of γ-TuRC caps from MT ends (Fig. 2A). MT release is important not only for spindle pole formation but also, in our model, to allow Kin I kinesins to associate with minus-ends at the pole and modulate disassembly. However, it is not known whether a γ-TuRC-capped MT end is protected from Kin I activity nor exactly when flux begins prior to metaphase.

Next, the spindle pole formed as MT minus-ends are zipped together into a tapered bundle and are anchored to the centrosome by an insoluble 'spindle pole matrix' (distinct from the 'spindle matrix' mentioned above) (reviewed by Scholey et al., 2001). Proteins implicated in this process include Ncd, dynein/dynactin, NuMA and *Drosophila* Asp (Matthies et al., 1996; Merdes and Cleveland, 1997; Dionne et al., 1999; Zeng, 2000; Wakefield et al., 2001). In our model, the Kin I kinesin is first recruited to the pole matrix (Fig. 2b) and, second, begins continuous depolymerization of minus-ends, creating the polymer-free 'gap' commonly observed between the centrosome and spindle pole in living and fixed mitotic animal spindles (Fig. 2c). Third, the pole-tethered Kin I kinesins depolymerize MT minus-ends processively (as probably occurs in vitro) (Hunter et al., 2003), generating a force that 'reels in' MTs (and attached chromosomes) to the pole (Fig. 2d). However, these kinesins are not required for anchoring spindle MTs to the centrosome, because MTs remain focused at poles following Kin I kinesin inhibition (Rogers et al., 2004). Instead, pole matrix proteins fulfill this role by transient dynamic binding of fluxing MTs, akin to the 'sleeve-like' MT minus-end-capping complex proposed by Waters et al. (Waters et al., 1996). Finally, CLASP proteins promote the polymerization of kMTs and ipMTs to promote a treadmilling behavior that is downregulated at the kinetochore at anaphase onset (Fig. 2d) (Sharp, 2002).

Central to this model is the proposal that flux and force are generated by an AMP-PNP-sensitive Kin I kinesin positioned at the pole. Therefore, the total poleward force exerted by Kin-I-powered flux on an attached kinetochore should be directly proportional to the number of fluxing kMTs in a K-fiber. But the velocity of a poleward-moving chromosome should be independent of the number of kMTs, assuming that Kin-I-induced kMT depolymerization at the pole is rate limiting.

Our model predicts that flux should cease after loss of the pole. This prediction is contradicted by a study that found anaphase chromosomes move poleward even after the surgical removal of a pole from meiotic spindles. Working with demembranated grasshopper spermatocytes, Nicklas used a micromanipulation needle to cut and sweep away one pole of anaphase spindles (Nicklas, 1989). Chromosomes continued to segregate properly, even moving towards the spindle end lacking a pole. The traction fiber model predicts that kMTs should be extruded away from the spindle through the cut region, provided that their minus-ends do not disassemble. Since this was not observed, Nicklas concluded that the activity responsible for chromosome segregation and kMT depolymerization is positioned at or near kinetochores and is not distributed along kMTs. However, we now know that kMT plus-ends assemble during anaphase and that kinetochores do not contribute to poleward movement in this cell type (Chen and Zhang, 2004). Nevertheless, the continued movement of anaphase chromosomes in the absence of a spindle pole conflicts with our flux model. After examination of the images of cut spindles in the study by Nicklas, it is clear that centrosomes were successfully removed; however, the extent of spindle pole amputation is not obvious. Focused bundles of MTs can still be seen at the cutting sites in several images, suggesting that these pole-like remnant structures could support proper targeting of the Kin I kinesin to drive flux and chromosome movement. This issue could be resolved if the localization of Kin I kinesins and other pole proteins such as NuMA/Asp were examined in cut meiotic spindles.

The relevance to spindle function

The identification of a molecular component responsible for generation of flux allows us to test its role in spindle function. We and others therefore inhibited the *Drosophila* Kin I KLP10A by microinjecting function-blocking reagents into living embryos; these nearly eliminated flux (Rogers et al., 2004). Quantitative analysis of mitotic movements in microinjected embryos suggests that flux plays a crucial dual role in exerting a poleward force on chromosomes and regulating metaphase spindle length. KLP10A inhibition affects chromosome positioning by perturbing prometaphase congression and retarding anaphase chromatid-to-pole motion.

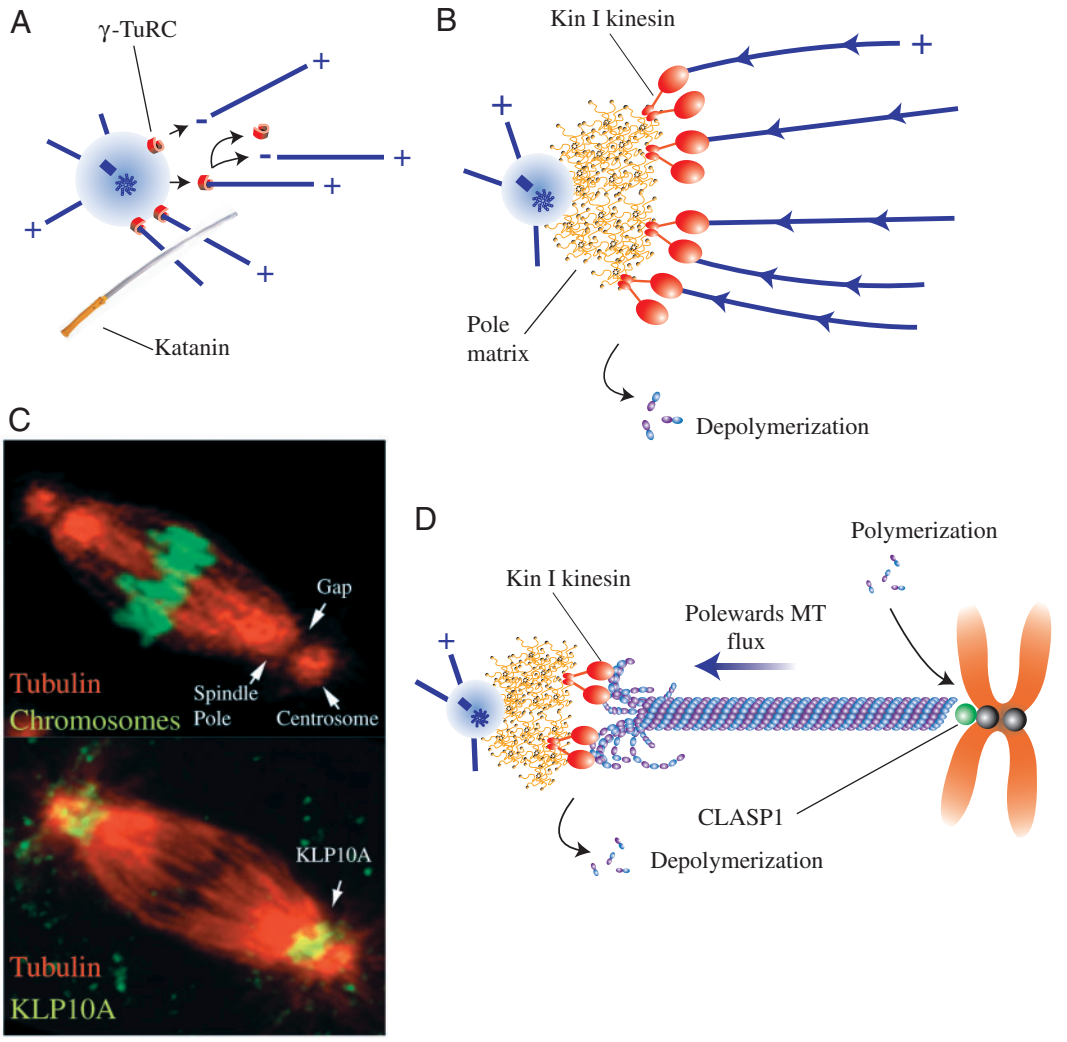

Fig. 2. A model for flux. (A) MT minus-end release from centrosomes could occur either by separation from the γ-tubulin ring complex (γ-TuRC) at or near the centrosome or from the MT-severing activity of centrosome-associated katanin (McNally et al., 1996). (B) A Kin I kinesin is targeted and tethered to an insoluble spindle pole matrix that anchors the spindle pole to the centrosome. Kin I actively drives flux (depicted as blue lines with arrows) by disassembling MT minus-ends. (C) This activity produces a polymer-free gap between the centrosome and the spindle pole that is observed in both live (top panel) and fixed (bottom panel) *Drosophila* syncytial blastoderm-stage embryos. The top panel shows rhodamine-labeled MTs (red) and GFP-histones (green). Indirect immunofluorescence in the bottom panel shows MTs (red) and KLP10A (green), which localizes within the gap and on centrosomes. (D) Poleward MT flux is driven in the metaphase half-spindle by a spindle-pole-associated Kin I kinesin. kMT length is maintained by the activity of the kinetochore-associated CLASP protein that induces plus-end polymerization.

Likewise, loss of KLP10A function (and flux) should lead to a loss of tension between sister kinetochores; however, measurements of tension were not performed in this study. During prometaphase, the contribution of flux to chromosome alignment and tension is not clear, because KLP10A is also found on prometaphase centromeres and could promote kinetochore motility by depolymerizing kMT plus-ends. More revealing, however, is the role of flux during anaphase A[†], when KLP10A is primarily restricted to the poles. The wild-type anaphase flux velocity in *Drosophila* embryos is approximately 40% of the rate of chromatid-to-pole movement (similar to spindles in vertebrate cultured cells; Table 1). Notably, blocking flux by inhibiting KLP10A results in a 40% decrease in the anaphase A chromosome velocity and consequently produces defects that included severe chromatin bridges and lagging chromosomes. Nonetheless, without flux, anaphase chromosomes moved poleward, albeit slowly, because of the Pacman mechanism that operates at the kinetochore and supplies the remaining poleward force for this movement. Although the role of flux during congression is uncertain, it probably exerts a constant poleward force on attached kinetochores throughout mitosis and produces tension between sister kinetochores.

An equally important task for flux is spindle length regulation. In the absence of KLP10A activity, bipolar spindles do not display the series of transient steady-state pauses characteristic of spindle lengthening in *Drosophila* embryos (Rogers et al., 2004; Sharp et al., 2000a). Instead, spindles elongate continuously from prometaphase through anaphase, lengthening at a constant average rate almost identical to anaphase B rates and approximately twice that of flux, presumably due to the continued polymerization of spindle MT plus-ends (i.e. elongation of each half-spindle occurs at a near-flux rate). By metaphase, spindles are twice their normal length. These results are consistent with the hypothesis that flux exerts a major inward force on spindle poles (Waters et al., 1996), producing tension across bi-oriented sister centromeres and counterbalancing outward force generators (such as the plus-end-directed Eg5 kinesin that bridges and slides apart ipMTs) (Sharp et al., 2000b). Furthermore, the premature anaphase-B-like spindle elongation that occurs in the absence of minus-end disassembly suggests that cells normally downregulate KLP10A to initiate anaphase B. In fact, flux within ipMT bundles ceases at the onset of anaphase B (by which time chromosomes have reached the poles) in wild-type

[†]Anaphase is composed of two stages: anaphase A, the movement of sister chromatids towards opposite spindle poles, and anaphase B, elongation of the spindle.

Drosophila embryonic spindles, which switch to a sliding mechanism to accomplish elongation (Brust-Mascher and Scholey, 2002). A quantitative model that can describe the dynamics of anaphase B in terms of this specific molecular mechanism has been formulated (Brust-Mascher et al., 2004).

Looking back to see forward

Can our new understanding of flux explain results from previous studies of chromosome positioning? Chromosomes in mitotic vertebrate spindles display characteristic abrupt oscillations as kinetochores switch between three states: movement towards (P) or away (AP) from the pole or relatively no movement (the 'neutral state', in which plus-end assembly balances minus-end disassembly) (Skibbens et al., 1993). The most compelling model for switching (also termed directional instability) includes a role for plus-end-directed, chromosome-arm-binding kinesin-4 and kinesin-10 family members (also called chromokinesins) that power a polar ejection force to move chromosomes away from the poles along MTs (Brunet and Vernos, 2001; Levesque and Compton, 2001; Powers et al., 2004). This ejection force, in turn, is relayed along chromosome arms and can produce tension at kinetochores (of either mono- or bi-oriented chromosomes) (Rieder and Salmon, 1994). Tension induces kMT plus-end assembly (AP motion), whereas a lack of tension results in active disassembly (P motion) probably driven by a Pacman-type mechanism (Skibbens et al., 1993; Rieder and Salmon, 1998). Thus, tension exerted on kinetochores regulates the assembly state of kMT plus-ends and therefore the directional switching. Directional instability provides an explanation for chromosome congression, but such models should be modified to recognize a contribution from flux.

Forces governing chromosome motion in the meiotic spindles of grasshopper and crane fly spermatocytes appear less complicated. As mentioned above, kinetochores do not produce a poleward force because kMT plus-ends polymerize during anaphase A, and instead poleward motion arises entirely from flux (Chen and Zhang, 2004). By contrast, most animal mitotic spindles studied to date appear to use a combination of Pacman and flux mechanisms to accomplish anaphase A (Mitchison and Salmon, 1992; Zhai et al., 1995; Rogers et al., 2004). In addition, although chromosome arms can bind MTs in these spindles (Fuge, 1990; LaFountain et al., 2001), they do not display the extensive oscillations observed in vertebrate mitotic cells (D. Zhang, personal communication), presumably owing to the lack of a polar ejection force (LaFountain et al., 2001). Likewise, extensive metaphase chromosome oscillations do not occur in early *Drosophila* embryonic and *Xenopus* egg extract spindles (Maddox et al., 2002; Brust-Masher and Scholey, 2002; Maddox et al., 2003), even though chromokinesins are present and play an important role in aligning chromosomes at the metaphase plate (Alphey et al., 1997; Vernos et al., 1995; Antonio et al., 2000; Funabiki and Murray, 2000). The extremely fast flux rates in these spindles probably produce a sufficiently high tension at kinetochores that the switch to the depolymerization state is prevented (Maddox et al., 2003) (Table 1).

In a provocative study, Hays and Salmon examined the effects of altering kMT number by partially damaging one kinetochore on bi-oriented meiosis I bivalents aligned at the metaphase plate in grassphopper spermatocytes, using a laser microbeam (Hays and Salmon, 1990). This produces an imbalance in the number of kMTs attached to the two homologous kinetochores, resulting in movement towards the pole of the unirradiated kinetochore. Bivalents did not travel all the way to the pole, but instead established new equilibrium positions at some distance from the pole. Greater doses of irradiation further reduced kMT number, leading to increased poleward movement. In each case, poleward chromosome velocities were unaffected. The authors concluded that a balance of forces determines the equilibrium position of bi-oriented chromosomes and that net poleward force is dependent on the difference in kMT length and number between the homologous kinetochores.

Although the traction fiber model does predict that equilibrium chromosome position should be a function of kMT length and number, these results do not conform to its prediction of a simple linear relationship. Therefore, this model does not adequately explain the available data. We propose that equilibrium chromosome position is determined by both the poleward force of flux produced by flux generators (Kin I kinesins) at the pole and the kMT plus-end polymerization that opposes flux. These two factors account for the observation that the net force on a kinetochore depends on kMT number and length. Force contributed by flux arises from the fixed number of flux generators associated with each kMT minus-end; so, the force of flux should be directly proportional to kMT number. However, plus-end polymerization is increased by tension on a kinetochore, which could result, for instance, from a chromosome approaching a pole and experiencing an increasing resistance from the increasingly dense MT array. Therefore, tension and plus-end assembly have a non-linear relationship with chromosome position (and kMT length).

In this scenario, the metaphase position of meiotic chromosomes is maintained by a balance of rates between plus-end assembly at kinetochores (Skibbens et al., 1993) and Kin-I-driven flux (Fig. 3a). This model predicts that loss of kMTs from an irradiated kinetochore decreases the force of flux on that kinetochore, causing an imbalanced bivalent to shift towards the pole that has more kMTs (and, therefore, more flux-derived force). Hays and Salmon (1990) postulated that an increase in stress induces a greater rate of polymerization at the remaining kMT plus-ends of the damaged kinetochore and, consequently, exerts a compressive force on the kMTs attached to the opposing unirradiated kinetochore. This compressive force either blocks kMT plus-end assembly, producing stable, paused ends [the 'parked state' described by Skibbens et al. (Skibbens et al., 1993)], or slows the rate of assembly such that disassembly at the poles is faster (as occurs during anaphase A). Flux continues in both half-spindles, and the poleward march of the bivalent continues until its arms make contact with an increasing density of MTs at the pole. Eventually, this resistance creates sufficient tension on the leading kinetochore to induce kMT plus-end polymerization and the establishment of a new equilibrium position. The proximity of this new position to the pole is thus dependent on the difference in the number of kMTs attached to the homologous kinetochores.

At first glance, such a model appears to fail to explain chromosome movements during prometaphase. For instance, work by McEwen et al. suggested that kMT number does not determine direction of movement because a congressing

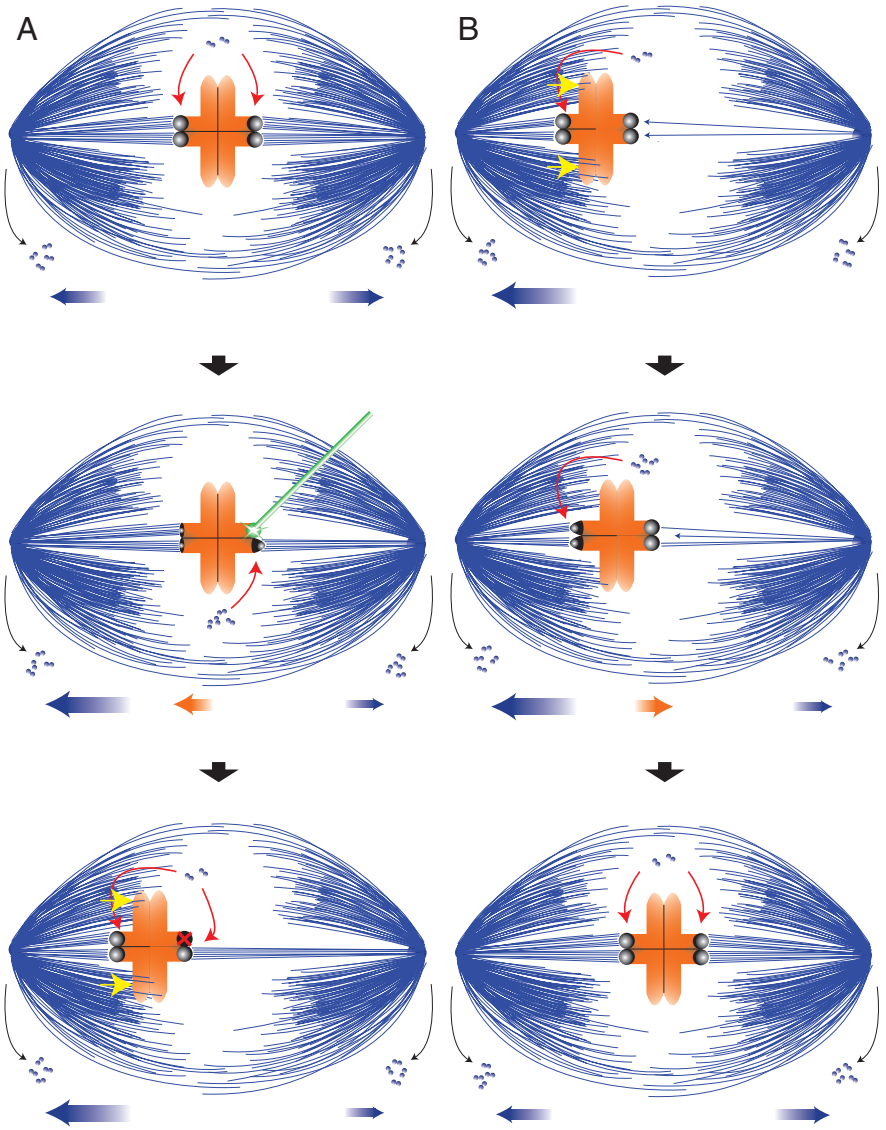

Fig. 3. Mechanistic models for bivalent positioning and congression in meiotic spindles of grasshopper spermatocytes, using the force from flux and plus-end kMT assembly. (A) Bivalent positioning. A bivalent maintains an equilibrium position at the spindle equator owing to an equivalent amount of flux (blue arrows) on homologous kinetochores attached to an equal number of kMTs. Moderate tension on the kinetochores induces kMT plus-end assembly (red arrows). Laser irradiation (green line) partially destroys a kinetochore, which reduces the number of kMTs to which it can bind. This sudden imbalance in kMT number increases the stress on the remaining kMTs of the irradiated kinetochore, inducing a greater rate of kMT plus-end assembly (red arrow). Compressive force on the opposing kMTs inhibits or decreases the rate of plus-end assembly, and, consequently, the bivalent moves (orange arrow) towards the pole that has the greater number of kMT attachments and the larger flux-generated force (larger blue arrow). As chromosome arms bind to an increasing density of spindle MTs that resist their poleward movement (yellow arrows), the increased tension on the unirradiated kinetochore induces plus-end polymerization. A new equilibrium position is reached when tension-induced plus-end assembly on the unirradiated kinetochore equals the rate of disassembly at the pole. (B) Congression. Initially, a bivalent close to one spindle pole becomes mono-oriented and is pulled poleward by flux (blue arrow). Chromosome arms bind to an increasing density of spindle MTs and the resulting resistance (yellow arrows) increases kinetochore tension to induce plus-end polymerization (red arrows). Poleward movement stops, facilitating the capture of the homologous kinetochore by the opposite pole (blue arrows in the spindle). Capture of the unattached kinetochore produces an even greater amount of tension and polymerization (red arrow) at the opposite kinetochore, allowing the bi-oriented bivalent to move to the spindle equator (orange arrow), even though kMT number and flux-generated force (blue arrows) are greater at the lagging homologous kinetochore. Finally, an equilibrium position is established at the spindle equator when the leading kinetochore is captured by an equal number of kMTs. Poleward force from flux (blue arrows) is equivalent in each half-spindle and is balanced by an equal rate of kMT plus-end assembly (red arrows) induced by moderate tension.

chromosome in a mitotic rat kangaroo epithelial (PtK_1) cell has more kMTs attached to the lagging sister kinetochore (closer to its pole) than the leading sister kinetochore (closer to the spindle equator) (McEwen et al., 1997). At the start of mitosis, the kinetochore closest to a pole is more likely to have captured more kMTs than its sister, and yet this chromosome can eventually move to the spindle equator. We believe our model can explain congression under these circumstances even in the absence of a polar ejection force. In fact, congression of bi-oriented chromosomes occurs efficiently after elimination of the polar ejection force in mitotic human cells by microinjection of antibodies directed against the chromokinesin Kid (Levesque and Compton, 2001). Although Kid inhibition blocks the characteristic chromosome oscillations, congressing chromosomes move at normal P and AP velocities. Interestingly, these velocities are much greater than that of flux measured in vertebrate mitotic cells, which suggests that forces at the kinetochore (presumably Pacman mechanisms) contribute to the faster-than-flux rates (Mitchison, 1989; Zhai et al., 1995; Levesque and Compton, 2001). Similarly to a polar ejection force, flux can thus probably also alter tension across centromeres and regulate kMT plus-end behavior to mediate congression. But, unlike the constant poleward force exerted by flux on stable kMTs, polar ejection forces arise from the stochastic encounters between chromokinesins and dynamic spindle MTs, stimulating abrupt kinetochore switching and chromosome oscillations.

At the start of meiosis, a mono-oriented bivalent is pulled poleward by its fluxing kMTs. Owing to the low tension on the

attached kinetochore, kMT plus-end assembly does not occur or is slower than flux. When chromosome arms bind the high density of MTs at the pole, tension on the attached kinetochore increases and induces kMT plus-end polymerization. Consequently, the bivalent pauses some distance from the pole, thereby facilitating capture of the homologous unattached kinetochore by MTs emanating from the opposite pole (Fig. 3b). As the unattached kinetochore becomes captured, increasing tension and polymerization at the opposite kinetochore allows the now bi-oriented bivalent to move towards the spindle equator, even though the kMT number and poleward force from flux is greater at the lagging kinetochore. A new equilibrium position is established when the bivalent reaches the spindle equator and the leading kinetochore is captured by an equal number of kMTs. At this point, the poleward force from flux is equivalent in each half-spindle and is balanced by an equal rate of kMT plus-end assembly as both kinetochores transit to the neutral state. This mechanism for bivalent congression is very similar to a congression model described by Rieder and Salmon (Rieder and Salmon, 1994), especially with regard to the link between kinetochore tension and chromosome motion. Both models describe a 'force' preventing chromosomes from reaching the pole whose magnitude is dependent on the distance and density of MTs relative to the center of the pole. However, in the model by Rieder and Salmon, this force has two components: (1) 'steric' resistance to chromosome penetration into regions of high MT density; and (2) an active, away-from-the-pole, pushing force (also called the 'polar winds').

Conservation of flux and its components

Given its importance in regulating spindle length and chromosome positioning, flux in the spindle appears to be the rule and not the exception in higher eukaryotes. Flux is observed in metazoan mitotic and meiotic spindles and, although flux velocities can vary greatly between cell types (and even within individual spindles themselves) (Rogers et al., 2004), a comparison of average flux and anaphase chromosome segregation rates reveals an important trend. The contribution of flux to anaphase A differs between mitotic and meiotic systems (Table 1). Meiotic spindles appear to have a greater dependence on flux to drive chromosome movement. By contrast, mitotic spindles presumably rely more on kinetochore-based Pacman mechanisms to power anaphase A (an average 60-70% contribution). This is not to say that meiotic systems lack a poleward-force-generating mechanism at the kinetochore. Indeed, Pacman-type kinetochore motility is observed in *Xenopus* extract spindles (Maddox et al., 2003), and mutations that prevent kinetochore targeting of cytoplasmic dynein (a putative component of the Pacman mechanism) (Sharp et al., 2000c) decrease the rate of anaphase A in *Drosophila* spermatocytes (Savoian et al., 2000). Table 1 reveals an additional unique feature of vertebrate mitotic spindles: the rate of flux decreases at the metaphase-anaphase transition. The purpose of this is not understood, nor are the factors that inactivate flux at the completion of anaphase A in *Drosophila* embryonic spindles (Brust-Mascher and Scholey, 2002), although it is possible that these events have common regulatory components. Interestingly, in most mammalian tissue cells, anaphase B starts at the same time as anaphase A and may also be related to a decrease in depolymerization at the poles.

It is not known whether flux occurs in plant spindles, which lack centrosomes. Flux is thought not to occur in yeast spindles (both *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*) (Mallavarapu et al., 1999; Maddox et al., 2000); however, conclusive evidence is lacking since the methods required to visualize flux are technically challenging (if not impossible) to apply. In common with metazoans, budding yeast encodes kinesins (Kip2, Kip3 and Kar3) whose mutation indicates a role in the regulation of MT dynamics (Hildebrandt and Hoyt, 2000). In fact, one would predict flux in the budding yeast spindle given that Kar3 localizes to spindle pole bodies (SPBs) and has a MT minus-end-destabilizing activity in vitro (Zeng et al., 1999; Endow et al., 1994). However, Kar3-null mutants display a short spindle phenotype instead of the longer spindles that would be expected for a flux component involved in MT minus-end disassembly (Saunders et al., 1997). Furthermore, the minus-ends of nuclear MTs anchored to the SPBs of *S. cerevisiae* appear capped with electron-dense material and lack the flared morphology often seen at the dynamic plus-ends (O'Toole et al., 1999). Assuming the morphology of MT ends accurately reflects their assembly state, then it appears that the presence of a cap at nuclear MT minus-ends precludes flux.

Recent evidence indicates that evolution has preserved the role of Kin I kinesins as essential MT minus-end-destabilizing factors for flux in vertebrate cells, as in *Drosophila*. There are three Kin I kinesins in humans and mice (KIF2A, KIF2B and KIF2C) (Miki et al., 2003) and an additional mouse isoform of KIF2A (KIF2Aβ) (Santama et al., 1998). Although a comparison of their primary sequences does not identify clear homology between *Drosophila* and vertebrate Kin I kinesins, KIF2A appears to be the functional KLP10A ortholog. KIF2A can destabilize MTs in vitro in an AMP-PNP sensitive manner and concentrates on spindle poles in both mitotic human cells and *Xenopus* extract spindles (Desai et al., 1999; Ganem and Compton, 2004; Gaetz and Kapoor, 2004). Significantly, when dominant-negative dynactin or NuMA proteins are added to metaphase *Xenopus* extract spindles, KIF2A is displaced from poles and flux is blocked (Gaetz and Kapoor, 2004). Also, the loss of flux in the metaphase extract spindles results in abnormally long spindles that elongate at twice the flux rate – a result of the continued polymerization and sliding of spindle MT plus-ends. This phenotype is strikingly similar to that generated by KLP10A inhibition in *Drosophila* (Rogers et al., 2004). Therefore, the Kin I kinesins in these diverse systems could be orthologous, and a pole-targeted Kin I kinesin is probably required by all metazoans to drive flux.

Concluding remarks

Poleward MT flux is an important aspect of higher eukaryotic spindle function – both in mitotic and in meiotic spindles. It is a dominant regulator of spindle length as well as a constant poleward pulling force for chromosomes. From its initial description to the recent identification of one of its key components, our knowledge of this unique dynamic behavior of spindle MTs has been almost forty years in the making, but much work still remains for mitosis researchers. A comprehensive understanding of the flux engine first requires

identification of the molecular components that drive it. For instance, what are the polymerization factors that promote the treadmilling behavior of metaphase spindle MTs? Identifying factors that target and regulate Kin I kinesin function at spindle poles will probably be particularly important, since these should provide insight into the integration of flux with mitotic events, such as the slowing and then cessation of flux observed in late-anaphase elongating spindles. In addition, analyzing the biophysical properties of the flux-producing motor should provide one means to test and refine flux models. For example, could a Kin I kinesin, tethered to an immobilized bead, 'reel in' free MTs in solution by binding and depolymerizing their ends? If so, what amount of force is generated on the MT and does the flux velocity vary with load? Does flux increase if MT ends are driven towards a Kin-I-coated bead, which might occur in the spindle when Eg5 slides apart anti-parallel ipMTs?

The cause and function of the variable flux rates within an individual spindle are unknown. Why do kMTs and ipMTs flux, whereas astral MTs do not? This might be due to a selective MT minus-end capping at centrosomes by γ-TuRCs that interferes with the MT-destabilizing activity of KinI kinesins. Interestingly, ipMTs appear to flux faster than kMTs in both mitotic and meiotic spindles (Waterman-Storer et al., 1998; Maddox et al., 2003; LaFountain et al., 2004), although the reason for this is not clear. The difficulty in resolving flux between these two populations of spindle MTs might explain the large variation in flux rates within individual spindles. However, improved imaging and resolution will certainly provide important information. In sum, a deeper understanding of the function and molecular mechanism of flux is an important goal not only because flux serves crucial functions during mitosis and meiosis but also because this specific phenomenon could one day be the target of anti-cancer therapy.

We thank D. Buster, J. Scholey and D. Zhang for enjoyable, insightful discussions and for critically reading the manuscript.

References

Alphey, L., Parker, L., Hawcroft, G., Guo, Y., Kaiser, K. and Morgan G. (1997). KLP38B: a kinesin-related protein that binds PP1. *J. Cell Biol.* **138**, 395-409.

Alsop, G. B. and Zhang, D. (2003). Microtubules continuously dictate distribution of actin filaments and positioning of cell cleavage in grasshopper spermatocytes. *J. Cell Sci.* **117**, 1591-1602.

Antonio, C., Ferby, I., Wilhelm, H., Jones, M., Karsenti, E., Nebreda, A. R. and Vernos, I. (2000). Xkid, a chromokinesin required for chromosome alignment on the metaphase plate. *Cell* **102**, 399-402.

Brunet, S. and Vernos, I. (2001). Chromosome motors on the move. From motion to spindle checkpoint activity. *EMBO Rep.* **2**, 669-673.

Brust-Mascher, I. and Scholey, J. M. (2002). Microtubule flux and sliding in mitotic spindles of *Drosophila* embryos. *Mol. Biol. Cell* **13**, 3967-3975.

Brust-Mascher, I., Civelekoglu-Scholey, G., Kwon, M., Mogilner, A. and Scholey, J. M. (2004). Model for anaphase B: role of three mitotic motors in a switch from poleward flux to spindle elongation. *Proc. Natl. Acad. Sci. USA* **101**, 15938-15943.

Budde, P. P. and Heald, R. (2003). Centrosomes and kinetochores, who needs 'em? The role of noncentromeric chromatin in spindle assembly. *Curr. Top. Dev. Biol.* **56**, 85-113.

Buster, D., McNally, K. and McNally, F. J. (2002). Katanin inhibition prevents the redistribution of γ-tubulin at mitosis. *J. Cell Sci.* **115**, 1083-1092.

Carvalho, P., Tirnauer, J. S. and Pellman, D. (2003). Surfing on microtubule ends. *Trends Cell Biol.* **13**, 229-237.

Chen, W. and Zhang, D. (2004). Kinetochore fibre dynamics outside the context of the spindle during anaphase. *Nat. Cell Biol.* **6**, 227-231.

Desai, A. and Mitchison, T. J. (1997). Microtubule polymerization dynamics. *Annu. Rev. Cell Dev. Biol.* **13**, 83-117.

Desai, A., Maddox, P. S., Mitchison, T. J. and Salmon, E. D. (1998). Anaphase A chromosome movement and poleward spindle microtubule flux occur at similar rates in *Xenopus* extract spindles. *J. Cell Biol.* **141**, 703-713.

Desai, A., Verma, S., Mitchison, T. J. and Walczak, C. E. (1999). Kin I kinesins are microtubule-destabilizing enzymes. *Cell* **96**, 69-78.

Dionne, M. A., Howard, L. and Compton, D. A. (1999). NuMA is a component of an insoluble matrix at mitotic spindle poles. *Cell Motil. Cytoskeleton* **42**, 189-203.

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

Forer, A. (1965). Local reduction of spindle fiber birefringence in living *Nephrotoma suturalis* (Loew) spermatocytes induced by ultraviolet microbeam irradiation. *J. Cell Biol.* **25**, 95-117.

Fuge, H. (1990). Non-kinetochore transport phenomena, microtubule-chromosome associations, and force transmission in nuclear division. *Protoplasma* **158**, 1-9.

Funabiki, H. and Murray, A. W. (2000). The *Xenopus* chromokinesin Xkid is essential for metaphase chromosome alignment and must be degraded to allow anaphase chromosome movement. *Cell* **102**, 411-424.

Gaetz, J. and Kapoor, T. M. (2004). Dynein/dynactin regulate metaphase spindle length by targeting depolymerizing activities to spindle poles. *J. Cell Biol.* **166**, 465-471.

Ganem, N. J. and Compton, D. A. (2004). The Kin I kinesin Kif2a is required for bipolar spindle assembly through a functional relationship with MCAK. *J. Cell Biol.* **166**, 473-478.

Gorbsky, G. J., Sammak, P. J. and Borisy, G. G. (1988). Microtubule dynamics and chromosome motion visualized in living anaphase cells. *J. Cell Biol.* **106**, 1185-1192.

Grego, S., Cantillana, V. and Salmon, E. D. (2001). Microtubule treadmilling in vitro investigated by fluorescence speckle and confocal microscopy. *Biophys. J.* **81**, 66-78.

Hays, T. S. and Salmon, E. D. (1990). Poleward force at the kinetochore in metaphase depends on the number of kinetochore microtubules. *J. Cell Biol.* **110**, 391-404.

Hildebrandt, E. R. and Hoyt, M. A. (2000). Mitotic motors in *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* **1496**, 99-116.

Hunter, A. W. and Wordeman, L. (2000). How motor proteins influence microtubule polymerization dynamics. *J. Cell Sci.* **113**, 4379-4389.

Hunter, A. W., Caplow, M., Coy, D. L., Hancock, W. O., Diez, S., Wordeman, L. and Howard, J. (2003). The kinesin-related protein MCAK is a microtubule depolymerase that forms an ATP-hydrolyzing complex at microtubule ends. *Mol. Cell* **11**, 445-457.

Inoue, Y. H., do Carmo Avides, M., Shiraki, M., Deak, P., Yamaguchi, M., Nishimoto, Y., Matsukage, A. and Glover, D. M. (2000). Orbit, a novel microtubule-associated protein essential for mitosis in *Drosophila melanogaster*. *J. Cell Biol.* **149**, 153-165.

Kapoor, T. M. and Mitchison, T. J. (2001). Eg5 is static in bipolar spindles relative to tubulin: evidence for a static spindle matrix. *J. Cell Biol.* **154**, 1125-1133.

Kapoor, T. M. and Compton, D. A. (2002). Searching for the middle ground: mechanisms of chromosome alignment during mitosis. *J. Cell Biol.* **157**, 551-556.

Keating, T. J. and Borisy, G. G. (2000). Immunostructural evidence for the template mechanism of microtubule nucleation. *Nat. Cell Biol.* **2**, 352-357.

Kline-Smith, S. L. and Walczak, C. E. (2002). The microtubule-destabilizing kinesin XKCM1 regulates microtubule dynamic instability in cells. *Mol. Biol. Cell* **13**, 2718-2731.

LaFountain, J. R., Jr, Oldenbourg, R., Cole, R. W. and Rieder, C. L. (2001). Microtubule flux mediates poleward motion of acentric chromosome fragments during meiosis in insect spermatocytes. *Mol. Biol. Cell* **12**, 4054-4065.

LaFountain, J. R., Jr, Cohan, C. S., Siegel, A. J. and LaFountain, D. J. (2004). Direct visualization of microtubule flux during metaphase and anaphase in crane-fly spermatocytes. *Mol. Biol. Cell* **15**, 5724-5732.

Lawrence, C. J., Dawe, R. K., Christie, K. R., Cleveland, D. W., Dawson, S. C., Endow, S. A., Goldstein, L. S. B., Goodson, H. V., Hirokawa, N., Howard, J. et al. (2004). A standardized kinesin nomenclature. *J. Cell Biol.* **167**, 19-22.

Lemos, C. L., Sampaio, P., Maiato, H., Costa, M., Omel'yanchuk, L. V.,

Liberal, V. and Sunkel, C. E. (2000). Mast, a conserved microtubule-associated protein required for bipolar mitotic spindle organization. *EMBO J.* **19**, 3668-3682.
Levesque, A. A. and Compton, D. A. (2001). The chromokinesin Kid is necessary for chromosome arm orientation and oscillation, but not congression, on mitotic spindles. *J. Cell Biol.* **154**, 1135-1146.
Maddox, P. S., Bloom, K. S. and Salmon, E. D. (2000). The polarity and dynamics of microtubule assembly in the budding yeast *Saccharomyces cerevisiae*. *Nat. Cell Biol.* **2**, 36-41.
Maddox, P., Desai, A., Oegema, K., Mitchison, T. J. and Salmon, E. D. (2002). Poleward microtubule flux is a major component of spindle dynamics and anaphase A in mitotic *Drosophila* embryos. *Curr. Biol.* **12**, 1670-1674.
Maddox, P., Straight, A., Coughlin, P., Mitchison, T. J. and Salmon, E. D. (2003). Direct observation of microtubule dynamics at kinetochores in *Xenopus* extract spindles: implications for spindle mechanics. *J. Cell Biol.* **162**, 377-382.
Maiato, H., Sampaio, P., Lemos, C. L., Findlay, J., Carmena, M., Earnshaw, W. C. and Sunkel, C. E. (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., Fairley, E. A., Rieder, C. L., Swedlow, J. R., Sunkel, C. E. and Earnshaw, W. C. (2003). Human CLASP1 is an outer kinetochore component that regulates spindle microtubule dynamics. *Cell* **113**, 891-904.
Maiato, H., DeLuca, J., Salmon, E. D. and Earnshaw, W. C. (2004). The dynamic kinetochore-microtubule interface. *J. Cell Sci.* **117**, 5461-5477.
Mallavarapu, A., Sawin, K. and Mitchison, T. (1999). A switch in microtubule dynamics at the onset of anaphase B in the mitotic spindle of *Schizosaccharomyces pombe*. *Curr. Biol.* **9**, 1423-1426.
Maney, T., Hunter, A. W., Wagenbach, M. and Wordeman, L. (1998). Mitotic centromere-associated kinesin is important for anaphase chromosome segregation. *J. Cell Biol.* **142**, 787-801.
Margolis, R. L. and Wilson, L. (1978). Opposite end assembly and disassembly of microtubules at steady state in vitro. *Cell* **13**, 1-8.
Margolis, R. L. and Wilson, L. (1981). Microtubule treadmills: possible molecular machinery. *Nature* **293**, 705-711.
Margolis, R. L., Wilson, L. and Kiefer, B. I. (1978). Mitotic mechanism based on intristic microtubule behaviour. *Nature* **272**, 450-452.
Matthies, H. J., McDonald, H. B., Goldstein, L. S. and Theurkauf, W. E. (1996). Anastral meiotic spindle morphogenesis: role of the non-claret disjunctional kinesin-like protein. *J. Cell Biol.* **134**, 455-464.
McEwen, B. F., Heagle, A. B., Cassels, G. O., Buttle, K. F. and Rieder, C. L. (1997). Kinetochore fiber maturation in PtK_1 cells and its implications for the mechanisms of chromosome congression and anaphase onset. *J. Cell Biol.* **137**, 1567-1580.
McIntosh, J. R., Grishchuk, E. L. and West, R. R. (2002). Chromosome-microtubule interactions during mitosis. *Annu. Rev. Cell Dev. Biol.* **18**, 193-219.
McNally, F. J., Okawa, K., Iwamatsu, A. and Vale, R. D. (1996). Katanin, the microtubule-severing ATPase, is concentrated at centrosomes. *J. Cell Sci.* **109**, 561-567.
Merdes, A. and Cleveland, D. W. (1997). Pathways of spindle pole formation: different mechanisms; conserved components. *J. Cell Biol.* **138**, 953-956.
Miki, H., Setou, M., Hirokawa, N.; RIKEN GER Group; GSL Members (2003). Kinesin superfamily proteins (KIFs) in the mouse transcriptome. *Genome Res.* **13**, 1455-1465.
Mitchison, T. J. (1989). Polewards microtubule flux in the mitotic spindle: evidence from photoactivation of fluorescence. *J. Cell Biol.* **109**, 637-652.
Mitchison, T. J. and Sawin, K. E. (1990). Tubulin flux in the mitotic spindle: where does it come from, where is it going? *Cell Motil. Cytoskeleton* **16**, 93-98.
Mitchison, T. J. and Salmon, E. D. (1992). Poleward kinetochore fiber movement occurs during both metaphase and anaphase-A in newt lung cell mitosis. *J. Cell Biol.* **119**, 569-582.
Mitchison, T. J. and Salmon, E. D. (2001). Mitosis: a history of division. *Nat. Cell Biol.* **3**, E17-E21.
Mitchison, T. J., Evans, L., Schulze, E. and Kirschner, M. (1986). Sites of microtubule assembly and disassembly in the mitotic spindle. *Cell* **45**, 515-527.
Mitchison, T. J., Maddox, P., Groen, A., Cameron. L., Perlman, Z., Ohi, R., Desai, A., Salmon, E. D. and Kapoor, T. M. (2004). Bipolarization and poleward flux correlate during *Xenopus* extract spindle assembly. *Mol. Biol. Cell* **15**, 5603-5615.
Miyamoto, D. T., Perlman, Z. E., Burbank, K. S., Groen, A. C. and Mitchison, T. J. (2004). The kinesin Eg5 drives poleward microtubule flux in *Xenopus laevis* egg extract spindles. *J. Cell Biol.* **167**, 813-818.
Moore, A. and Wordeman, L. (2004). The mechanism, function and regulation of depolymerizing kinesins during mitosis. *Trends Cell Biol.* **14**, 537-546.
Moores, C. A., Yu, M., Guo, J., Beraud, C., Sakowicz, R. and Milligan, R. A. (2002). A mechanism for microtubule depolymerization by Kin I kinesins. *Mol. Cell* **9**, 903-909.
Nicklas, R. B. (1989). The motor for poleward chromosome movement in anaphase is in or near the kinetochore. *J. Cell Biol.* **109**, 2245-2255.
O'Toole, E. T., Winey, M. and McIntosh, J. R. (1999). High-voltage electron tomography of spindle pole bodies and early mitotic spindles in the yeast *Saccharomyces cerevisiae*. *Mol. Biol. Cell* **10**, 2017-2031.
Ogawa, T., Nitta, R., Okada, Y. and Hirokawa, N. (2004). A common mechanism for microtubule destabilizers-M-type kinesins stabilize curling of the protofilament using the class-specific neck and loops. *Cell* **116**, 591-602.
Ostergren, G. (1950). Consideration of some elementary features of mitosis. *Hereditas* **36**, 1-19.
Powers, J., Rose, D. J., Saunders, A., Dunkelbarger, S., Strome, S. and Saxton, W. M. (2004). Loss of KLP-19 polar ejection force causes misorientation and missegregation of holocentric chromosomes. *J. Cell Biol.* **166**, 991-1001.
Rieder, C. L. and Salmon, E. D. (1994). Motile kinetochores and polar ejection forces dictate chromosome position on the vertebrate mitotic spindle. *J. Cell Biol.* **124**, 223-233.
Rieder, C. L. and Salmon, E. D. (1998). The vertebrate cell kinetochore and its roles during mitosis. *Trends Cell Biol.* **8**, 310-318.
Rogers, G. C., Rogers, S. L., Schwimmer, T. A., Ems-McClung, S. C., Walczak, C. E., Vale, R. D., Scholey, J. M. and Sharp, D. J. (2004). Two mitotic kinesins cooperate to drive sister chromatid separation during anaphase. *Nature* **427**, 364-370.
Santama, N., Krijnse-Locker, J., Griffiths, G., Noda, Y., Hirokawa, N. and Dotti, C. G. (1998). KIF2beta, a new kinesin superfamily protein in non-neuronal cells, is associated with lysosomes and may be implicated in their centrifugal translocation. *EMBO J.* **17**, 5855-5867.
Saunders, W., Hornack, D., Lengyel, V. and Deng, C. (1997). 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**, 417-431.
Savoian, M. S., Goldberg, M. L. and Rieder, C. L. (2000). The rate of poleward chromosome motion is attenuated in *Drosophila* zw10 and rod mutants. *Nat. Cell Biol.* **2**, 948-952.
Sawin, K. E. and Mitchison, T. J. (1991). Poleward microtubule flux in mitotic spindles assembled in vitro. *J. Cell Biol.* **112**, 941-954.
Sawin, K. E. and Mitchison, T. J. (1994). Microtubule flux in mitosis is independent of chromosomes, centrosomes, and antiparallel microtubules. *Mol. Biol. Cell* **5**, 217-226.
Scholey, J. M., Rogers, G. C. and Sharp, D. J. (2001). Mitosis, microtubules, and the matrix. *J. Cell Biol.* **154**, 261-266.
Sharp, D. J. (2002). Cell division: MAST sails through mitosis. *Curr. Biol.* **12**, R585-R587.
Sharp, D. J., Yu, K. R., Sisson, J. C., Sullivan, W. and Scholey, J. M. (1999). Antagonistic microtubule-sliding motors position mitotic centrosomes in *Drosophila* early embryos. *Nat. Cell Biol.* **1**, 51-54.
Sharp, D. J., Brown, H. M., Kwon, M., Rogers, G. C., Holland, G. and Scholey, J. M. (2000a). Functional coordination of three mitotic motors in *Drosophila* embryos. *Mol. Biol. Cell* **11**, 241-253.
Sharp, D. J., Rogers, G. C. and Scholey, J. M. (2000b). Microtubule motors in mitosis. *Nature* **407**, 41-47.
Sharp, D. J., Rogers, G. C. and Scholey, J. M. (2000c). Cytoplasmic dynein is required for poleward chromosome movement during mitosis in *Drosophila* embryos. *Nat. Cell Biol.* **2**, 922-930.
Shipley, K., Hekmat-Nejad, M., Turner, J., Moores, C., Anderson, R., Milligan, R., Sakowicz, R. and Fletterick, R. (2004). Structure of a kinesin microtubule depolymerization machine. *EMBO J.* **23**, 1422-1432.
Skibbens, R. V., Skeen, V. P. and Salmon, E. D. (1993). Directional instability of kinetochore motility during chromosome congression and segregation in mitotic newt lung cells: a push-pull mechanism. *J. Cell Biol.* **122**, 859-875.
Vernos, I., Raats, J., Hirano, T., Heasman, J., Karsenti, E. and Wylie, C.

(1995). Xklp1, a chromosomal *Xenopus* kinesin-like protein essential for spindle organization and chromosome positioning. *Cell* **81**, 117-127.

Wadsworth, P. and Khodjakov, A. (2004). *E pluribus unum*: towards a universal mechanism for spindle assembly. *Trends Cell Biol.* **14**, 413-419.

Wakefield, J. G., Bonaccorsi, S. and Gatti, M. (2001). The *Drosophila* protein asp is involved in microtubule organization during spindle formation and cytokinesis. *J. Cell Biol.* **153**, 637-648.

Walczak, C. E., Mitchison, T. J. and Desai, A. (1996). XKCM1: a *Xenopus* kinesin-related protein that regulates microtubule dynamics during mitotic spindle assembly. *Cell* **84**, 37-47.

Waterman-Storer, C. M., Desai, A., Bulinski, J. C. and Salmon, E. D. (1998). Fluorescent speckle microscopy, a method to visualize the dynamics of protein assemblies in living cells. *Curr. Biol.* **8**, 1227-1230.

Waters, J. C., Mitchison, T. J., Rieder, C. L. and Salmon, E. D. (1996). The kinetochore microtubule minus-end disassembly associated with poleward flux produces a force that can do work. *Mol. Biol. Cell* **7**, 1547-1558.

Wiese, C. and Zheng, Y. (2000). A new function for the γ-tubulin ring complex as a microtubule minus-end cap. *Nat. Cell Biol.* **2**, 358-364.

Wilson, P. J., Forer, A. and Leggiadro, C. (1994). Evidence that kinetochore microtubules in crane-fly spermatocytes disassemble during anaphase primarily at the poleward end. *J. Cell Sci.* **107**, 3015-3027.

Wittmann, T. and Hyman, T. (1999). Recombinant p50/dynamitin as a tool to examine the role of dynactin in intracellular processes. *Methods Cell Biol.* **61**, 137-143.

Wittmann, T., Hyman, A. and Desai, A. (2001). The spindle: a dynamic assembly of microtubules and motors. *Nat. Cell Biol.* **3**, E28-E34.

Wordeman, L. and Mitchison, T. J. (1995). Identification and partial characterization of mitotic centromere-associated kinesin, a kinesin-related protein that associates with centromeres during mitosis. *J. Cell Biol.* **128**, 95-104.

Zeng, C. (2000). NuMA: a nuclear protein involved in mitotic centrosome function. *Microsc. Res. Tech.* **49**, 467-477.

Zeng, X., Kahana, J. A., Silver, P. A., Morphew, M. K., McIntosh, J. R., Fitch, I. T., Carbon, J. and Saunders, W. S. (1999). Slk19 is a centromere protein that functions to stabilize mitotic spindles. *J. Cell Biol.* **146**, 415-425.

Zhai, Y., Kronebusch, P. J. and Borisy, G. G. (1995). Kinetochore microtubule dynamics and the metaphase-anaphase transition. *J. Cell Biol.* **131**, 721-734.