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How Kinesin Motor Proteins Drive Mitotic Spindle Function: Lessons from Molecular Assays

Linda Wordeman
Department of Physiology & Biophysics, University of Washington School of Medicine, 1705 NE Pacific St., Seattle, WA 98195-7290

Linda Wordeman: worde@u.washington.edu

Abstract

Kinesins are enzymes that use the energy of ATP to perform mechanical work. There are approximately 14 families of kinesins within the kinesin superfamily. Family classification is derived primarily from alignments of the sequences of the core motor domain. For this reason, the enzymatic behavior and motility of each motor generally reflects its family. At the cellular level, kinesin motors perform a variety of functions during cell division and within the mitotic spindle to ensure that chromosomes are segregated with the highest fidelity possible. The cellular functions of these motors are intimately related to their mechanical and enzymatic properties at the single molecule level. For this reason, motility studies designed to evaluate the activity of purified molecular motors are a requirement in order to understand, mechanistically, how these motors make the mitotic spindle work and what can cause the spindle to fail. This review will focus on a selection of illustrative kinesins, which have been studied at the molecular level in order to inform our understanding of their function in cells. In addition, the review will endeavor to point out some kinesins that have been studied extensively but which still lack sufficient molecular underpinnings to fully predict their contribution to spindle function.

Keywords

Mitosis; Kinesin; Motor; Microtubule; Mitotic Spindle

1. Introduction: The discovery of motors in the mitotic spindle

> "The venerable and provocative speculation that all forms of movement in biological systems have a common molecular basis can, at present, be subjected to few kinds of experimental test, for all of which muscle contraction provides the paradigms. One test is the search for proteins corresponding to actin and myosin in their physical properties, a second is the preparation of working "models,"' and a third is the demonstration that the molecules composing the working structure not only interact with but split ATP."(1)

Ever since the surprising discovery that myosin(2) was capable of hydrolyzing ATP(3), the energy source for muscle contraction, students of mitosis have exhibited a keen interest in

Correspondence to: Linda Wordeman, worde@u.washington.edu.

molecular motors. This might derive from the appearance of mitotic chromosomes which, even in fixed preparations, seemed to be experiencing motive force. It did not escape the eye of David Mottier (1903)(4) that chromosomes attached to the mitotic spindle appeared to be experiencing a pulling force exerted by the spindle fibers (Figure 1). The isolation of an ATPase from *Tetrahymena* cilia in 1965(5) only served to intensify the resolve among cell biologists to link these motors to mitotic spindle function. Especially compelling was the fact that this ciliar enzyme, dynein, resembled an ATPase isolated from mitotic spindles(1) more closely than it resembled myosin. Two decades later another microtubule motor called kinesin was purified from squid axoplasm and implicated as the motor powering fast axonal transport in neurons(6). Kinesin, which ironically, bears considerable structural similarity to myosin (6, 7), seemed like a valuable molecule for use within the spindle if it could only be identified in non-neuronal cells. Kinesin and dynein suggested a palette of force generators potentially available to the spindle. Dynein, which travels toward microtubule minus ends in combination with kinesin, whose founding member travels unidirectionally toward microtubule plus-ends seemed like a perfect complement of motors that could be employed for all the mechanical requirements of cell division. Unfortunately, neither of these molecules could be identified, unambiguously, with the mitotic spindle for a number of years using the biochemical techniques available at the time. The 1990's, however, were a comparatively rich decade for motors in the spindle in that several genetic studies simultaneously galvanized and revolutionized the mitosis field by identifying mitotic kinesins that strongly resembled but were not identical to previously identified neuronal kinesin(8–12). At the same time and after much hard work, cytoplasmic dynein was also unambiguously identified in the mitotic spindle (13,14). The mitotic spindle can now flex its muscles.

Kinesins, which now number over 45 unique genes in mammals, fall into approximately 14 subfamilies based on sequence homology within the catalytic core motor domain(15). It would not be an overstatement to say that almost all identified kinesin families participate to some extent in cell division. The purpose of this review will be to examine a subset of kinesins, which have been carefully evaluated for activity in isolation and then attempt to place this activity in the context of spindle function. Space constraints prevent me from a comprehensive review of all mitotic motors, which would include kinesins, dyneins and myosins. Finally, I hope to emphasize that there are a number of interesting, heavily investigated, mitotic kinesins which still lack thorough evaluation of enzymatic function *in vitro* and that without these data a complete understanding of cellular function is impossible.

2. Kinesin: The Movement Protein

2.1 Introduction

For the majority of enzymes it is difficult to "see" them work. One can measure the accumulation of product or the decrease in substrate. Detailed structural studies can reveal mechanistic snapshots of the protein in action. But generally speaking it is often difficult to watch an enzyme working in real time. In contrast, many details of motor protein mechanism can be viewed in the light microscope in real time. The early biochemical purification of neuronal kinesin made use of this property in the form of a "motility assay"(6). Motors can be purified, affixed to a substrate, a bead, or a fluorescent tag and fed with a little ATP. Then their directionality, processivity, on-rate and off-rate can be measured directly as they travel along the surface of the microtubule lattice.

To date approximately 45 unique kinesin superfamily genes have been identified in mammals falling into 14 families(15,16). These motors participate in a wide variety of often cell- and tissue-specific functions and the majority possess one or more members that assist during mitosis or meiosis. The 14 families of kinesin genes are classified based on a combination of sequence (and therefore structural) similarity and functional grouping(16,17). The functional

grouping of kinesins must be, by necessity, relatively broad because kinesins exhibit tissue-specific functions. An example is the microtubule-depolymerizing kinesin, MCAK, a member of the kinesin-13 family of which there are 3 unique members in mammals. MCAK depolymerizes microtubules(18). In mitotic cells, MCAK promotes the turnover of kinetochore fiber microtubules during cell division, which leads to improvements in error-correction, congression and spindle assembly(19–22). MCAK is not, thus far, found in post-mitotic or terminally differentiated cells(23). However, postmitotic neurons do express a kinesin-13 family member, Kif2A, which limits collateral branch extension in developing neurons(24). What is the functional link between these two jobs? In both cases they are accomplished by disassembling microtubules. Thus, the functional classification of kinesins ends up being dependent on how they move on, and what they do to, microtubules.

2.2 Kinesin Families

Kinesin families fall into three broad functional categories based on what they do in conjunction with microtubules: (i) kinesins that translocate to the plus ends of microtubules (kinesins-1 through 7, kinesin-12), (ii) kinesins that translocate to the minus ends of microtubules (kinesin-14), (iii) kinesins that depolymerize microtubules (kinesin-13, kinesin-8). As always, there are interesting exceptions to these classifications (kinesin-10) and a few not rigorously tested (kinesin-9, kinesin-11). Within these broad functional classifications detailed analyses of motility reveal themes consistent or likely to be consistent within each family. As a cautionary note, it is usually the case that individuals within a subfamily have not been tested for motile properties relative to each other. Perhaps this is because researchers are afraid such an analysis will be boring or repetitive. I suspect, however, that comparative studies within subfamilies will discover interesting, functionally relevant, specializations. Nevertheless, for most kinesin families there is at least one member who has been run through its paces (Table 1).

2.3 Kinesin Motility

Initial studies of representative family members in vitro revealed basic features that can easily be related to function. Using the energy of ATP, motile motors will translocate unidirectionally along the surface of a microtubule relative to the microtubule's inherent structural polarity (minus ends anchored at the spindle pole, plus ends extending distally to the cell membrane, kinetochores and spindle midzone). The direction a motor will "walk" will then define its suitability for certain tasks during cell division. The majority of kinesins walk toward the plus end of the microtubule putting them in an excellent position to transport chromosomes to the end of kinetochore fibers during congression, or to slide two half spindles apart during spindle elongation. Some kinesins (and all dyneins) walk toward the minus ends of microtubules. This predicts a potential utility in anaphase chromosome movement, focusing anastral spindle microtubules and positioning the spindle within the cell via minus-end directed motors anchored to the submembranous cytoskeleton. Two families of kinesins (kinesin-8 and kinesin-13) have been implicated in modulating the kinetics of microtubule assembly and disassembly from free tubulin dimers. These kinesins are suspiciously enriched near the centrosomes and centromeres, areas rich with microtubule ends, where assembly and disassembly occur. It continues to be useful for researchers to apply the characteristics that individual motors display on microtubules *in vitro* into the context of the mitotic spindle in order to evaluate the role a particular motor will play during cell division (Figure 2). In addition to motility and directionality, it is important to consider carefully the potential "attachment points", the structures, either identified or speculative, against which the motor will exert force during movement. These "attachment points" constitute one of the most poorly understood aspects of motor function in the spindle. For example, if a motor is proposed to use its plus end directed motor activity to slide a microtubule relative to another microtubule is the motor anchored statically to one microtubule and sliding the other? Or, is the motor anchored to a

"matrix" of structural proteins surrounding the microtubules? Is a motor anchored to a chromosome arm able to exert force on the entire chromosome given the inherent flexibility of the chromatin to which it is attached? These are the sorts of questions that still flourish, generally unanswered, in the mitotic motor field.

2.3.1 Eg5—Kinesin families have broad structural divisions, which, in turn, reflect their particular motility style. Kinesin-5 family members (Eg5/BimC, for example, are bipolar tetramers with two plus-end directed motors at each end(25). Such a molecule is admirably suited to slide two parallel microtubules relative to each other, minus end leading if it has a separate attachment site (such as a spindle matrix) against which to exert force(26). Alternatively, the molecule may push two anti-parallel microtubules relative to each other without attachment ot other structures. Not surprisingly, *in vitro* the molecule exhibits a clear preference to slide antiparallel microtubules relative to each other(27,28).

What does it mean for Eg5 to exhibit a "preference" for anti-parallel microtubules? This is where the fine details of motility *in vitro* become potentially important. Fine details include: processivity, the number of cycles the enzyme undergoes before detaching from the microtubule substrate; and diffusion, a non-energy requiring, gliding that motors do for short distances along the microtubule which is generally unbiased in directionality. Processivity, in the case of walking kinesins, refers to the number of steps that the motor will take before detaching. A motor that is highly processive can do a lot of work all on its own without the assistance of other motors. A non-processive motor, in contrast, may function most effectively in groups. Diffusion tends to be an extremely rapid method of scanning short distances along the microtubule but falls off in importance when efforts over a long distance are required. The bipolar tetramer, Eg5, is a rather poor motor at physiological salt conditions, dithering back and forth on the microtubule in ATP-independent diffusive mode. However, once the motor encounters another microtubule that it can crosslink and, presumably, push against, then it becomes an active, ATP-hydrolyzing directional motor(29). Furthermore, the motor domains appear to be oriented in a manner that favors the second bound microtubule to be in an antiparallel orientation(28). There would be no way to predict this without careful, detailed *in vitro* assays of purified motor and microtubules in isolation. Yet, the information fits, mechanistically, very well with the proposed functions of kinesin-5s in driving spindle elongation and establishing bipolarity(30–32). However, motors by virtue of their propensity to cycle through strong and weakly microtubule-bound states during ATP hydrolysis, have the potential capability to crosslink microtubules and oppose movement as well as promote it. Eg5 is no exception and has also been reported to limit spindle elongation in some systems(33, 34). Many excellent studies and essential tools, such as Eg5-specific small molecule inhibitors, have made Eg5 one of the mechanistically better understood motors contributing to mitotic spindle function. The motor has a clear role in "establishing spindle bipolarity". Yet, the complete manner in which it does so is still maddeningly elusive.

2.3.2 NCD—NCD (Non-Claret Disjunctional) is a minus-end directed kinesin originally identified as essential for proper chromosome segregation in Drosophila female meiosis and early embryos(8). The motor domain has been crystallized and shows striking similarity to plus-end directed kinesins (35). In fact, electron microscopy in conjunction with 3D reconstruction shows that motors kinesin motors strongly bound to microtubules will always bind in one orientation(36). Thus, directionality, as has been shown in numerous studies, relies on domains outside of the motor domain. NCD can be released from minus-end directionality by a single point mutation in either of two, highly conserved, interacting residues, one in the "neck" outside the motor domain and one in the core motor domain(37). The resultant motors can translocate in either direction in an ATP-dependent manner. This would be an excellent point at which to regulate directionality, the control of which has yet to be demonstrated, in any motor in a cellular setting.

Motility studies of NCD have long shown that it is relatively non-processive, suggesting that it is a motor that is most effective in groups(38). Kinesin-14 family members have long been known to be prodigious bundlers of microtubules in addition to their minus-end directed motility. Far from being a nuisance for motility assays, recent *in vitro* studies of full-length NCD and its orthologue from *S. pombe* have demonstrated that these molecules preferentially slide anti-parallel microtubules relative to each other in various bound motor orientations. In contrast, microtubules with uniform parallel orientation become locked in place by inter-microtubule-bound motors working against each other(39,40). HSET, the kinesin-14 orthologue in human cells, is non-essential for cell division in normal cells yet is helpful at rescuing multipolar spindles via centrosome clustering in cells with multiple centrosomes (41). Thus, its force-locked microtubule bundling activity may be more functionally important than sliding and may be especially important in female meiosis in Drosophila where the spindles lack the focusing activites of centrosomes or in situations in which too many centrosomes are present and need to be coalesced into one pole-focusing entity.

Minus-end directed directional sliding should implicate kinesin-14s in promoting spindle collapse and opposing the activity of Eg5(42). Yet recent studies have shown that overexpression of HSET, lengthens spindles while depletion modestly shortens them without interfering with cell division. Mutational analysis in which sliding ability was eliminated indicated that both sliding and crosslinking contribute to spindle elongation(43). How sliding of parallel microtubules in the half-spindle accomplishes this task when kinesin-14 molecules tend to become force-locked in these conditions is mysterious. It may be that minus-end directed motility and crosslinking are not fully separable activities. For example, in ATP, a microtubule may be immovable because the collective motors operating on it are equivalently balanced in opposition to each other. They may be able to progress partially through the cross-bridge cycle until a strongly bound or force-locked state is reached. This would result in strong crosslinking without a lot of overt microtubule sliding. Regardless, it is necessary to know the orientation and anchor points (both static and dynamic) of the motors to the half-spindle microtubules are essential in order to understand how HSET functions in the spindle. This is best understood by extension from *in vitro* studies of purified protein supplemented by computational modeling(44).

Kar3p is a kinesin-14 important for mitosis and karyogamy in *S. cerevesiae*. Up to this point we have been discussing kinesin-14 motors that are two-headed homodimers connected by a long coiled-coil stalk. The orthologous protein in yeast, Kar3, preferentially heterodimerizes with either Vik1p or Cik1p, although it can homodimerize as well(45). Structural studies have revealed these proteins to be kinesin motor domains that lack an ATP-binding site yet still bind microtubules(46). Dimerization with these “dead heads” significantly enhances Kar3p minus-end directed motility(45) and confers the ability to destabilize the microtubule plus-end as well (45,47). Nothing analogous to Vik1p or Cik1p has been identified in mammalian cells, but it is well within the realm of possibility that similar non-motor motors will be identified as a means to structurally and functionally diversify kinesin activity. More precise motility assays of these two heterodimers in order to mechanistically evaluate how they operate in promoting spindle positioning, spindle integrity, chromosome maintenance, and synaptogenesis(48,49). A key unanswered question is whether Kar3p and its partners behave similarly to other kinesin-14s with respect to microtubule crosslinking.

2.3.3 NOD—NOD is a kinesin-10 family member, which includes the mitotic chromokinesin, KID. Both KID and NOD are found on chromosome arms and both are implicated in chromosome positioning during congression. NOD is required for the proper positioning of achiasmate chromosomes during meiosis in *Drosophila*. KID appears to be monomeric(50) and exhibited plus-end directed motility only when surveyed using a bead assay (motors affixed to a bead rather than a glass coverslip)(51). NOD is widely considered to be non-motile(52)

and has been reported to bind preferentially to microtubule ends and stimulate microtubule polymerization(53). Structural studies suggest that NOD binds and releases microtubules concurrent with ATP hydrolysis but that ATP turnover is inhibited when the motor binds the microtubule end until the end elongates(54). This would be an excellent means by which to help coax chromosomes to the plus-ends of microtubules without relying on hand-over-hand motility.

It must be said, however, that the disparity between the motility described for NOD and for KID feels incomplete for two motors in the same general family. There is still much work to be done with these motors. Furthermore, there is another family of chromokinesins (kinesin-4), which is characterized by Kif4/XKLP1. An extensive study of XKLP1 shows that it is a plus-end directed motor that also modulates microtubule ends by inhibiting both microtubule growth and shrinkage(55). This activity and also those ascribed to the kinesin-10s, KID and NOD, are all compatible with their proposed role in promoting the congression of chromosomes to the metaphase plate. Previously, it was though that microtubules polymerizing from the spindle pole produced a “polar ejection force” that tended to assist with the job of moving chromosomes away from the pole and toward the spindle midzone(56). This force promotes the ejection of chromosomes from microtubule-dense areas near the spindle pole, toward the plus-ends of microtubules at the spindle midzone even when the chromosome arm is physically detached from the kinetochore(57). Now, with the discovery of chromokinesins (plus-end directed motors on chromosome arms), it is widely thought that motor-dependent plus-end directed motility associated with chromosome arms corresponds to the polar ejection force. Ejection force arising from polymerizing microtubules and microtubule motor-dependent polar ejection force are not mutually exclusive and it is not presently known which of these activities is acting on chromosome arms to produce the polar ejection force. Nevertheless, chromosome arm-associated microtubule-stabilizing activity would be a useful way of promoting congression either by facilitating the maintenance of a track for plus-end directed motility, or by promoting a preferential association with polymerizing microtubule ends (as is proposed for NOD), or by facilitating microtubule polymerization, an activity which is likely to be capable of producing a polar ejection force in isolation. These activities are extremely difficult to isolate within the context of the spindle so further assays of the chromokinesins in vitro are likely to be informative with respect to the motor activities possessed by chromosome arms.

2.3.4 MCAK—MCAK was originally identified as a kinesin-related protein that was enriched in the inner centromere region of mitotic chromosomes(58). Rapid depletion of this kinesin from cellular extracts led to explosive microtubule growth(59) and a classic study subsequently confirmed that the motor was capable of completely disassembling microtubules stabilized against disassembly by either paclitaxel or non-hydrolyzable GTP analogs(18). Subsequent studies confirmed that MCAK and other members of its kinesin-13 family are capable of disassembling microtubules(18,60,61) in a catalytic manner and that it is most likely that one motor can remove approximately twenty tubulin dimers prior to dissociating from the microtubule(62). The process involves the motor preferentially stabilizing a curved (rather than straight) protofilament conformation that weakens the association of tubulin with the microtubule(63,64). Tubulin dimer removal from the microtubule is tightly coupled to ATP hydrolysis and can be isolated from tubulin dimer release by the bound motor by introducing a point mutation into the switch II region motor domain(65). Such mutations have been commonly used to isolate the pre-and post-power stroke structures of myosin and other motile kinesins(66–68). In the case of MCAK, the process of ATP-binding and hydrolysis triggers the motor to sequentially bend, remove and release a tubulin dimer during one ATP hydrolysis cycle(65). Thus, MCAK and its family members are potent, catalytic microtubule depolymerizers and it is likely that this is the activity that they contribute to the centromeric, centrosomal, and spindle midzone regions of the mitotic spindle.

Evaluating the role of these depolymerizers in cells is challenging because the activity of MCAK in the cell appears to be intimately associated with the ratio of tubulin dimer to polymer. This is not surprising as it has been known for quite some time that cells adjust their level of tubulin expression to the level of free tubulin dimer in the cell(69,70). However, slow (12–36 hour) depletion of MCAK such as occurs during siRNA treatment, gives the cell ample time to adjust microtubule polymer dynamics and results in a rather subtle mitotic phenotype. Cells experience a modest increase in lagging chromosomes and longer astral microtubules(20,71). Defects in congression due to improper kinetochore microtubule attachment(72) and antagonism of bipolar spindle assembly have also been reported(20,72–75). MCAK's activity and centromere localization are regulated by Aurora B kinase, further implicating the motor in kinetochore function and error correction(76–79). Depletion of centromere-associated MCAK leads to decreased speed of chromosome movement and also to decreased kinetochore fiber microtubule turnover(21). In contrast, addition of more MCAK to the centromere resulted in fewer lagging chromosomes (suggesting increased error-correction activity), increased microtubule turnover in the kinetochore fiber and greater overall speed of chromosome movement and fewer erroneous microtubule connections(21). This suggests, commensurate with its potent microtubule depolymerizing activity, that MCAK facilitates kinetochore fiber microtubule turnover and error correction by gently antagonizing microtubule attachment at the kinetochore.

2.3.5 Kif18A—The kinesin-8 family possesses unique attributes that significantly inform our perception of their role during cell division. Members of this family were described in *Drosophila* as plus-end directed motors whose elimination led to unexpectedly long microtubules. This conundrum was partially solved with the discovery that the kinesin-8, Kip3p, from *S. cerevesiae* is a highly processive plus-end directed motor that disassembles microtubules from the plus-end in a length dependent manner(80,81). This length dependence is related to the high processivity of the motor in that, once it is translocating along the microtubule, it is unlikely to detach. This leads to an accumulation of the motors at the plus end of the microtubule if the motors do not walk off the end of the microtubule. This ability of kinesin-8s to stay attached at the end of the microtubule is an interesting phenomenon in and of itself. Not all motile kinesins do this. Once the concentration of Kip3p is high enough the microtubule begins to shorten in a length dependent manner. This is because the high processivity of the motor results in longer microtubules accumulating more motor over time, thus leading to a positive relationship between the length of the microtubule and the rate of disassembly. The importance of a high concentration of motor is underscored by the observation that one molecule of Kip3 will not remove a tubulin dimer until another motor kicks it off(82). Thus, the motor will only remove tubulin dimers when the lattice near the plus-end is essentially saturated.

The mammalian orthologue of Kip3p, Kif18A, is also capable of disassembling microtubules (80) and distributes in a gradient along kinetochore fibers suggesting it may provide positional information to congressing chromosomes(83). However the activity that Kif18A supplies to the gradient may not be as simple to interpret as the depolymerase activity of MCAK. Based on the length-dependent disassembly of microtubules by Kip3p(81), the molecule has been implicated in using its depolymerase activity to limit microtubule length within the spindle. Furthermore, loss of Kif18A leads to a modest lengthening in spindles and longer microtubules. So far so good. However, there is more than one way to shorten a microtubule. Suppressing microtubule dynamics can shift the steady state length of microtubules to a narrow distribution around a shorter length. One can accomplish this by either simultaneously suppressing rate of assembly and disassembly or by simultaneously increasing both catastrophe and rescue (the transitions between disassembly and assembly). Kip3p in living yeast appears to have its principal effect *in vivo* by increasing the transitions between growth and shortening(84). Thus a microtubule has less time to grow and less time to shorten in the presence of Kip3p. Overall

this is going to shift the distribution of microtubule lengths in the cell to a narrow distribution of shorter lengths. Interestingly, the expression of excess Kif18A protein in mitotic cells has similar effects on chromosome oscillations(83). Bioriented chromosomes exhibit oscillatory movement during metaphase. This movement is related to the growth and shrinkage of microtubule ends bound by kinetochores in that it can be suppressed by microtubule drugs that suppress microtubule dynamics. Similarly, expression of Kif18A suppresses chromosome movement without appreciably affecting their ability to congress. Conversely, depletion of Kif18A increases the speed at which chromosomes travel and decreases the frequency with which they switch directions, leading to an inability to congress because they sail past the metaphase plate without stopping(83). By extension, this resembles the situation in yeast whereby microtubule transition frequencies decrease with the loss of Kip3p(84). This suggests that further evaluation of the effect of purified Kif18A protein on dynamic microtubule *in vitro* is definitely warranted in order to reconcile the microtubule shortening effect of kinesin-8s with their cellular role in suppressing microtubule dynamics.

2.3.6 MKLP1—Both MKLP1 and MKLP2 are kinesin-6 family members. Previous evidence indicates that they are dimers in metazoans(85) but recent crosslinking data suggests that klp9p, the kinesin-6 family member in *S. pombe*, may form a tetramer(86). This is consistent with the recent discovery that klp9p may slide bipolar mitotic spindles apart in *S. pombe* during anaphase B (spindle elongation)(86). Klp9p accomplishes this in conjunction with a microtubule bundling protein Ase1 (Prc1 in metazoans). In a perplexing twist, prc1 in mammals also bundles microtubules and interacts with three plus-end directed kinesins, the chromokinesin, Kif4A (kinesin-4 family), the kinesin-6, MKLP1, and the kinesin-7, CENP-E (87).

In *Drosophila*, which possesses all three representatives implicated in spindle midzone function: Kif4A (Klp3A; kinesin-4), MKLP1 (Pavarotti; kinesin-6) and MKLP2 (Subito; kinesin-6), the kinesin-4 Klp3A, is proposed to operate in spindle elongation by promoting midzone bundling and suppressing flux(88). As expected for a chromokinesin there is also a mild congression defect associated with Klp3A loss (89). In mammalian cells, depletion of Kif4A lead to long anaphase spindles implying a role in opposing spindle elongation(90). This is interesting as both kinesin-6s and kinesin-4s are plus-end directed. Most studies implicating a balance of forces involved antagonism between motors of opposite directionality. A detailed *in vitro* study of the Xenopus kinesin-4, Xklp1, may provide some answers. Xklp1 was shown to be a rapid plus-end directed motor with the ability to suppress both depolymerization and polymerization in an ATP-independent manner once it reached the end of the microtubule (55). This suggests how kinesin-4s might antagonize the sliding activities of kinesin-6s, by limiting the polymerization of interzonal microtubules and thus limiting the extent of the zone of anti-parallel microtubule overlap.

Kinesin-6s such as MKLP1/2 have not been as clearly implicated in pole separation by sliding of antiparallel microtubules in cells other than *S. pombe*. This may be because anaphase B spindle elongation is less pronounced in other systems. Furthermore, the role of kinesin-6s in “organizing” the central spindle can lead to mechanistically difficult to interpret phenotypes such as broken, splayed or collapsed spindles. Instead, a much larger body of work from *C. elegans*, *Drosophila* and mammalian cells (reviewed in (91)) demonstrates that a dimer of the kinein-6 family member, MKLP1, forms a complex with a dimer of RhoGAP to form the centralspindlin complex, which is required for Rho-dependent cleavage furrow ingression (85). MKLP2 (found in *Drosophila* and mammals but not *C. elegans*) relocalizes from the inner centromere at mitosis to the spindle midzone, interacts with polo kinase, INCENP and Aurora kinase and is required to localize Aurora kinase to the midzone, which then phosphorylates MKLP1(92). Phosphorylation of MKLP1 is, in turn, required for specification of the cleavage furrow (93). Less well understood is the more phylogenetically restricted

kinesin-6, MPP1, although it is also implicated in the completion of cytokinesis in human cultured cells(94). Regardless, the take-home message is that, in contrast to *S. pombe*, the kinesin-6 family in other organisms seems to be primarily involved in positioning and facilitating the function of the cleavage furrow, rather than physically pushing the spindle poles apart. This is curious in that pushing the spindle poles apart might be an excellent way to locally reduce the number of cortically associated astral microtubules and to redundantly localize furrowing(95). Perhaps the redundancy is not as evolutionarily robust when imparted by the same motor?

In organisms that possess more than one kinesin-6, they appear to interact with different signaling molecule complexes. For example, in contrast to MKLP2 which complexes with midzone passenger proteins, MKLP1 forms a complex with RhoGAP and RhoGEF and is required for Rho-dependent cleavage furrow ingression(96). Both of these kinesin-6s are strongly associated with anti-parallel microtubules in the spindle midzone. MKLP1 is a plus-end directed motile kinesin that can crosslink and appears to be able to slide anti-parallel microtubules relative to each other(97). The activity of MKLP2 *in vitro* is likely to be similar but has not been tested. It would be interesting to understand whether kinesin-6s can elongate mitotic spindles by exerting plus-end directed force on each half spindle and whether this contributes to their role in cleavage furrow specification or represents a separate function.

Our understanding of the role of MKLP1 in directing the establishment of the cleavage furrow has benefited from informative biochemical and genetic studies. However, from the perspective of MKLP1, the motor, the ability of the kinesin-6 family proteins to slide anti-parallel microtubules relative to each other is consistent with their localization to the interdigitating anti-parallel microtubules of the midzone but superficially inconsistent with their role as transporters of signaling complexes. Such tasks could be accomplished by any plus-end directed motor. Thus, a true mechanistic evaluation of their function remains to be determined. How, precisely does MKLP1 direct the accumulation of RhoA to the forming cleavage furrow and is the ability to slide antiparallel microtubules required for this process? How is its actin-binding activity useful for this process? Does MKLP1 transport RhoA regulators to the cortex or does it localize them at the midzone and rely on diffusion to signal cortical actin? More detailed analysis of the activity kinesin-6 family motors on single and bundled microtubules coupled with live imaging of the dynamic behavior of these motors during cell division would help answer some of these questions.

3. Conclusions

A comprehensive survey of kinesins in cell division is not possible within the constraints of this review. However, I have endeavored to touch on a few kinesins for which detailed analysis *in vitro* has been or is likely to be informative with respect to cellular function. This is especially the case for mitosis, as compared to other microtubule-based processes, because the organization of microtubules during mitosis is complex and dynamic. Mitotic spindle microtubules form parallel arrays, anti-parallel arrays, asters and bundles. They undergo dynamic instability, flux, sliding and exhibit high rates of nucleation and turnover during cell division. Future studies *in vitro* should include experiments designed to mimic these substrates.

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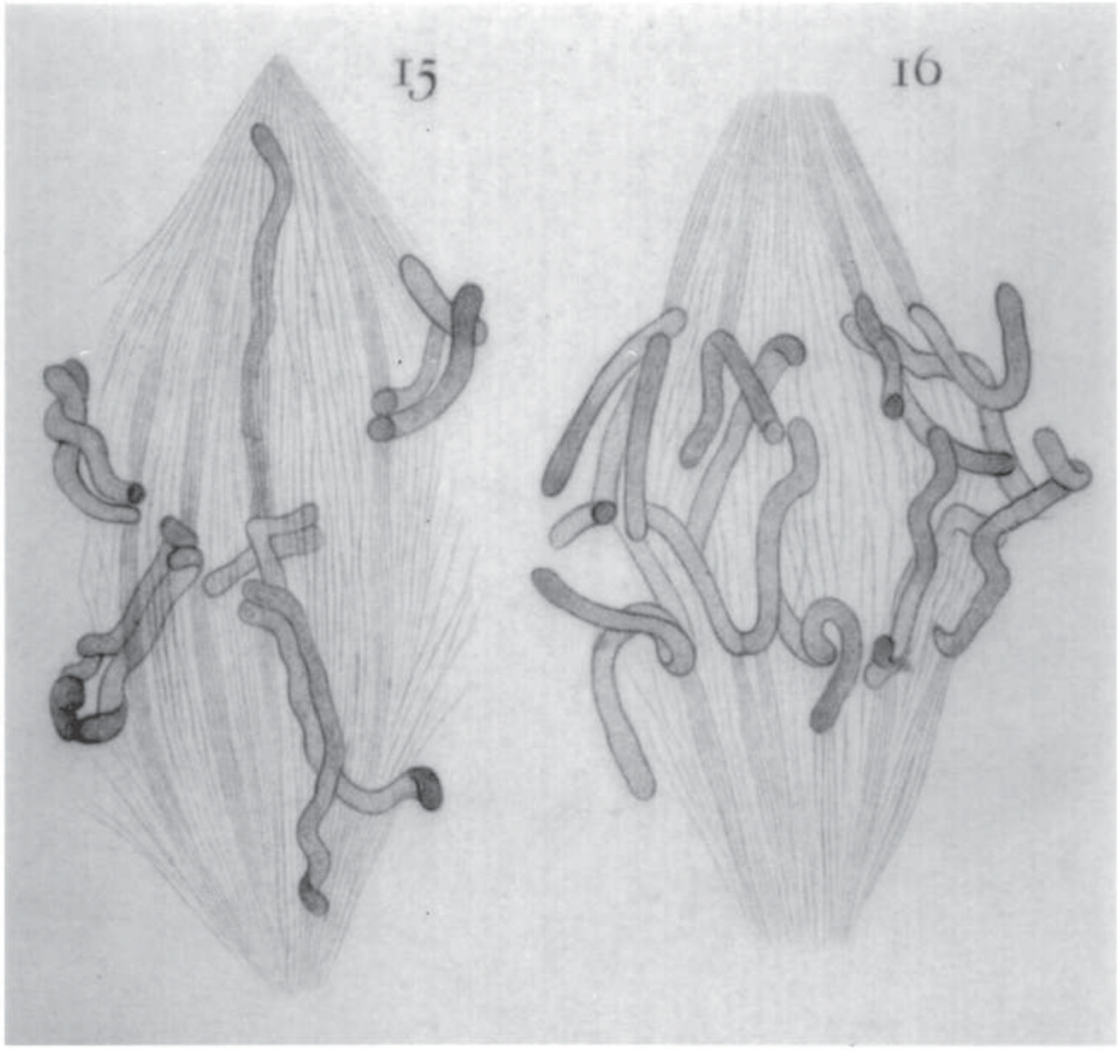

Figure 1.
Chromosome segregation in the *Lillium* pollen shore mother cell from Mottier (1903)(4).

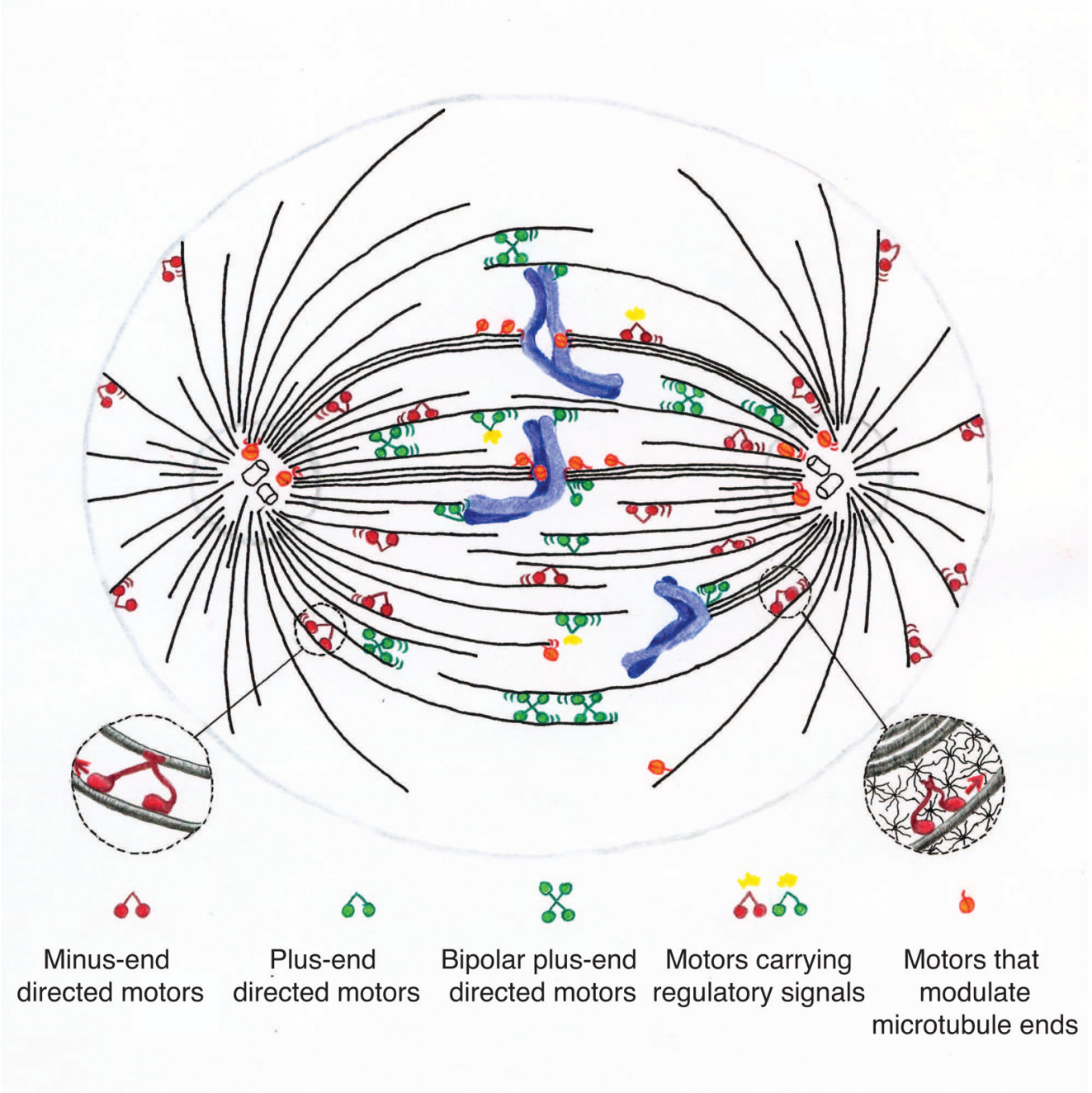

Figure 2.
Motors contribute diverse mechanical activities to the mitotic spindle. Minus-end directed motility (examples are NCD and dynein), plus-end directed motility (Kid, MKLP1, MKLP2, and others); bipolar plus-end directed motility (Eg5, presently no bipolar minus-end directed motors have been reported); plus- and minus-end directed motility transporting signaling molecules (MKLP1, dynein); depolymerizing and end modulating activity (MCAK, Kif18A, Xklp1, Nod). Motors may couple to microtubules (left inset), spindle matrix components such as NuMa(140,141) (right inset) or other cargo.

Table 1

Motility and Function of Kinesin Families

Family	Common Names	Structure	Motility *in vitro*	Mitotic Function
Kinesin-1	UKHC, Kif5, UNC-116	Heterotetramer: 2 HC and 2 LC	Plus-end directed, processive, hand-over-hand motility (68).	No known function in mitotic spindles but mediates translocation of meiotic spindle to the oocyte cortex in *C. elegans* meiosis (98).
Kinesin-2	Kif3A/B/C	Hetero- and Homodimer	Plus-end directed, fast, processive, variable based on composition of heterodimer(99).	Dominant-negative mutants result in aneuploidy and multipolar spindles(98, 100).
Kinesin-3	Kif14; Kif13B/Gakin	Dimer	Plus-end directed rapid motility (101).	Interacts with PRC1, implicated in late stage cytokinesis (102–104).
Kinesin-4	Kif4, Xklp1, Klp38B	Not confirmed, Dimer?	Plus-end directed motility, inhibits dynamics(55,105).	Congression, spindle assembly, cytokinesis.(87,106)
Kinesin-5	Eg5, BimC, Cin8	Bipolar tetramer	Bundling, parallel and antiparallel microtubule sliding (29,107).	Spindle elongation(108), spindle assembly(109), congression(110).
Kinesin-6	MKLP1, MKLP2, Pavarotti, Subito, Klp9p, Cho1, Kif12, Rab6Kinesin, Kif20, Kif23	Dimer or Tetramer	Plus-end directed, antiparallel microtubule sliding (97).	Spindle assembly(111), spindle elongation(86), cleavage furrow positioning(96), regulation of midzone assembly(112), cytokinesis (113).
Kinesin-7	Cenp-E	Dimer	Plus-end directed processive motility (114–116).	Congression(117,118)
Kinesin-8	Kip3, Kif18A, Klp5/6, Klp67A	Not confirmed, Dimer(119)	Length-dependent depolymerization(81), increase catastrophe and rescue, decreased dynamicity (84).	Congression(80,83), kinetochore fiber dynamics(83,120), central spindle dynamics(121).
Kinesin-9	Kif6, Kif9, Klp1	Unknown	Unknown	Tumor suppressor(122), flagellar(123).
Kinesin-10	Kif22, Kid, Nod	Monomer(50)	Weak plus-end directed motility(51) or no motility (52,54).	Congression(124–126). chromosome compaction (127), meiotic chromosome positioning (128).
Kinesin-11	Smy1, Kif26A, Vab-8	Unknown	Unknown	None identified
Kinesin-12	Krp180, Klp-10, Xklp2, Hklp2, Kif12, Kif15	Unknown	Slow plus-end directed	Centrosome separation(129). Spindle positioning(130), Ki-67 interaction (131).
Kinesin-13	Kif2A,B,C, MCAK, Klp10A, Klp57C, XKCM1, Dsk1	Homodimer	Depolymerizer(18,62), promote catastrophes (132).	Congression(22), error correction(76, 78), increase K-fiber turnover(21).
Kinesin-14	Ncd, CHO2, Xctk2, Kar3,KlpA, KifC2, KifC2, Kata	Dimer	Nonprocessive minus-end directed motility (10,133), sliding of anti-parallel microtubules, bundling (134), depolymerizer (47,135).	Bipolar spindle assembly (43,136,137), pole focusing(138), regulate microtubule length and number(139).