

Mitotic Motors

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THE complex but well-controlled motions of chromosomes during mitosis have long evoked the view that the mitotic spindle contains enzymes capable of transducing chemical energy into mechanical work, but the nature of these "motor" molecules has been elusive. During the last 40 years, three ideas have dominated the field: (a) microtubules (MT)¹ attach to chromosomes and move them by the addition and removal of tubulin; (b) mitotic forces are produced by mechanochemical enzymes, probably ATPases, that interact with MTs; and (c) mitotic forces are generated by muscle proteins. Possibility *c* now seems remote, but recent data from studies on the dynamics of spindle MTs, on the genes and gene products that are required for normal chromosome motion, and on the time-dependent changes in spindle structure reveal that both possibilities *a* and *b* are pertinent for explaining mitotic movements. While it is too soon to say just how chromosomes are moved during cell division, there is now enough information to identify key phenomena and the kinds of proteins involved. This paper is a sketch of the motile events that occur during mitosis and a discussion of the ways that MTs and their associated motor enzymes may cause these motions.

Mitotic Motions and Forces

Motions

The essential features of chromosome motion are well known (Fig. 1). The interactions between MTs and chromosomes begin at the transition from prophase to prometaphase; in higher eukaryotes this occurs when the nuclear envelope disperses, while in many lower eukaryotes spindle proteins assemble in the nucleus. MTs grow out from the centrosomes (spindle poles) and interact with the chromosomes and with each other. Initial chromosome movements are usually directed toward the nearby pole and are often comparatively fast (ca. 25 $\mu\text{m}/\text{min}$). One of the two sister kinetochores is the principal site at which the force for this motion is generated (71). Eventually sister kinetochores attach to MTs growing from opposite poles, and each chromosome shows a net motion toward the spindle equator. During this "congression" to the metaphase plate, which is usually the sum of many movements toward and away from each pole, the rate of chromosome motion decreases. Anaphase begins with the splitting of the centromeres, and sister chromatids move apart at 0.5–2 $\mu\text{m}/\text{min}$. The decrease in distance be-

tween chromosomes and poles is called anaphase A, while the increase in separation between the poles is called anaphase B. For fuller descriptions of mitosis see references 6, 25, 28, 44, 47, 50, 57, and 69.

In addition to these widely recognized events, there are several less well-known movements that reflect motile properties of the spindle and may therefore yield insights into its motile mechanism. Chromosomes without kinetochores (6) and cytoplasmic objects placed experimentally into the metaphase spindle (59) are usually ejected from it. The speed of ejection is ca. 1 $\mu\text{m}/\text{min}$, about the same as the velocity of an anaphase chromosome. The trajectories vary, but the most common is radially outward from the pole-to-pole axis. Ejection forces also appear to act on the arms of long chromosomes, which often point out from the spindle axis. Indeed, large chromosomes are usually confined to the periphery of the metaphase plate, and only the smaller ones lie near the spindle axis. The "ejection forces" responsible for these effects are also seen in the interaction of chromosomes with a single centrosome. Such "mono-oriented" chromosomes usually take up a stable position some micrometers from the centrosome, and if they are broken by micro-irradiation, the kinetochore-containing fragment moves in toward the centrosome, while the rest of the chromosome moves out (70). Apparently, the position of a mono-oriented chromosome reflects, at least in part, a balance of pole-directed forces acting on the kinetochore and the aster-dependent ejection forces, which act on the chromosome as a whole (72). Spindles occasionally induce comparatively rapid motions in non-chromosomal objects. Particles which encounter astral MTs often move toward the centrosome at speeds up to 5 $\mu\text{m}/\text{s}$ (68). Aster-associated particles can also move out from the centrosome, but these motions are less frequent and more saltatory than the poleward movements. In higher plants, vesicles are transported to the spindle midplane during late anaphase and telophase, where their fusion contributes to the formation of the cell plate (5).

Spindle MTs themselves move during mitosis. Lesions made on chromosomal spindle fibers of insect spermatocytes by UV microbeam irradiation sometimes migrate poleward at ca. 1 $\mu\text{m}/\text{min}$ (94). Direct evidence for migration of MTs attached to kinetochores has been obtained by photo activation of a "caged" fluorescent analogue of tubulin; spots so made on metaphase MTs migrate poleward at 0.6 $\mu\text{m}/\text{min}$ (51). The same technology has now revealed a 3 $\mu\text{m}/\text{min}$ flux in non-kinetochore MTs growing from centrosomes in extracts of *Xenopus* oocytes (74, 75). The latter flux is blocked by a non-hydrolyzable analogue of ATP. Together these data

1. Abbreviations used in this paper: MT, microtubule.

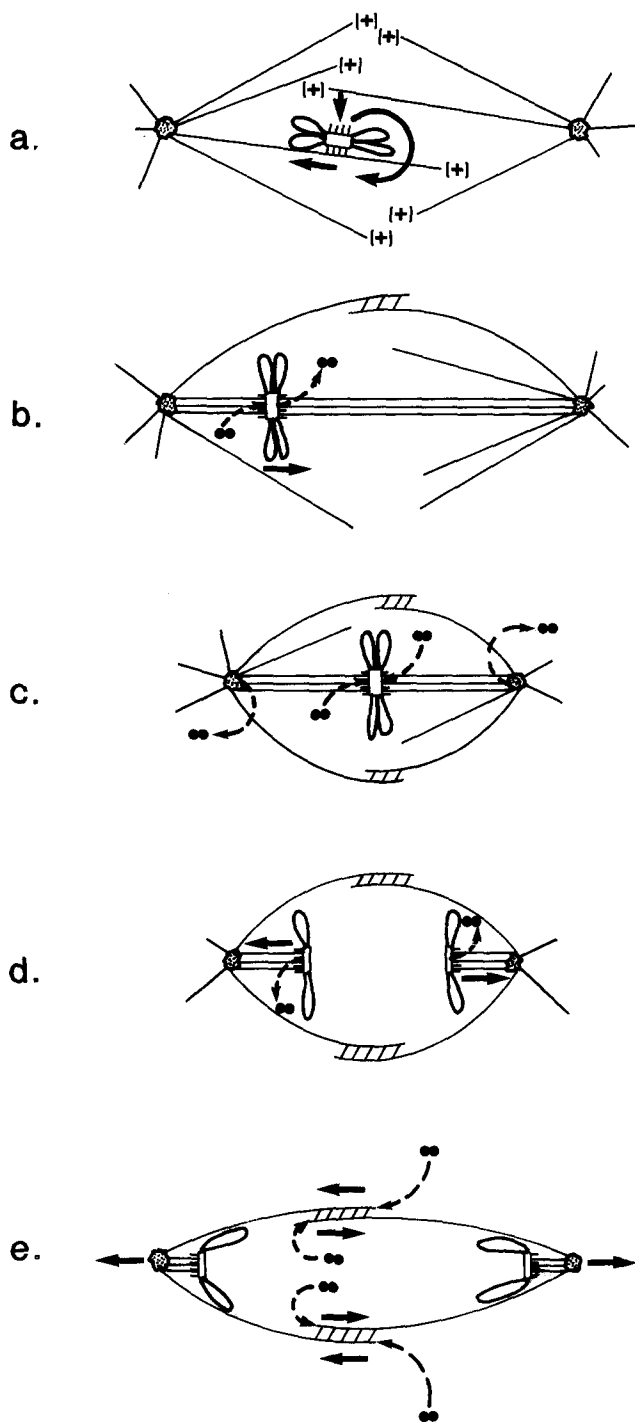


Figure 1. The principal events of spindle action. The centrosomes of a forming spindle initiate numerous MTs that are oriented with their plus ends (the faster growing ends) pointing outward. Chromosomes that interact with the lateral surfaces of MTs from one centrosome undergo a rapid ($5\text{--}30\ \mu\text{m}/\text{min}$) movement toward that pole of the spindle (a). Eventually each chromosome interacts with MTs from both poles, which induces a rotation that orients sister kinetochores toward opposite poles (a and b). The chromosomes then oscillate slowly toward and away from the poles. As the kinetochore MTs lengthen and shorten, tubulin subunits are added and lost predominantly at the MT ends attached to the kinetochores (b). The net effect of these motions is to position all of the chromosomes near the spindle equator, though the oscillations often continue thereafter. Sister kinetochores are pulled toward opposite poles

suggest that there is net tubulin addition at the plus ends of spindle MTs and an ATP-dependent net depolymerization from their centrosome-associated minus ends. Such concerted movements are distinct from the rapid fluctuations of individual MTs between polymerization and depolymerization characteristic of the “dynamic instability” (32) that leads to the lability of most metaphase MTs (73).

Forces

The forces acting on chromosomes have been studied in living cells by watching the details of chromosome movement, by characterizing the deformations that mitotic motions cause in chromosome structure, and by measuring the forces that must be applied to impede normal chromosome movements (reviewed in reference 57). Throughout prometaphase and metaphase, kinetochores are pulled poleward by their interactions with the fibers to which they bind (48, 70). The initial interactions, which promote fast chromosome movements, appear to be between the kinetochore and the lateral surface of one or more MTs (71, 85). The magnitude of these forces is ca. $1\ \mu\text{d}/\text{MT}$ (1, 57), but the velocities vary rapidly, so the forces themselves may be erratic.

If the chromosomes are being pulled toward the poles, then one would expect to find an equal and opposite reaction pulling the poles toward the chromosome. Microirradiation studies suggest that during prometaphase the spindle poles are pulled inward by a mechanism that depends on spindle fibers. If the fibers in one half of a metaphase newt spindle are cut with a UV microbeam, the side of the cut near the chromosomes is stable, while the side defined by the free plus ends recedes toward the pole, widening the cut. New astral MTs then grow out from the pole near the cut, and when they reach the rest of the spindle, the pole moves quickly in toward the metaphase plate. There is also a shortening of the half spindle that was not irradiated (83). Apparently the metaphase spindle pulls each pole in toward the equator. The stability of a normal spindle’s length therefore implies a mechanism to hold the poles apart. Such support is probably based, at least in part, on the MTs that emanate from each pole and interdigitate near the spindle equator (see Fig. 1, b and c) (46, and reviewed in reference 43). Certainly the destruction of this framework in diatom spindles by UV microbeam irradiation leads to the collapse of the interpolar distance (34). The ejection forces that push acentric chromosome fragments out of the spindle (70) may also help to push the poles apart, but the magnitude of these forces has not yet been measured, and it may be very small. An alternative possibility is that the spindle MTs that project into the cytoplasm (astral rays) interact with material outside the spindle and pull each centrosome outward from the spindle equator. Indeed, a single centrosome associated with an asymmetric array of MTs can move through the cytoplasm (4, 17). Such

throughout this time. Spindle MTs move poleward during metaphase, with tubulin subunits added at their plus ends and lost near the centrosomes (c). At the onset of anaphase, sister chromatids separate and move toward their respective poles (d). The kinetochore MTs disassemble largely at their kinetochore ends. The spindle then elongates. Some of the MTs emanating from each pole interdigitate near the spindle midplane; these lengthen by tubulin addition at their plus ends and slide apart as the poles separate (e).

motility could contribute to spindle length stability, to the increase in spindle length during anaphase B, and to the translation and rotation of spindles seen in some embryos (26, 36).

Prometaphase congression to the spindle equator poses one of the most interesting puzzles of the whole mitotic process. Once a bipolar attachment has been achieved, the pole-directed forces described above should pull each chromosome toward both poles at once. Net movement to the spindle equator suggests either that the strength of the pull on the kinetochore is proportional to the length of the chromosome fiber (62), or that there is some mechanism for telling a chromosome where it lies in the spindle and thus how hard its kinetochore-associated fibers should pull (50). Alternatively, the strength of a kinetochore fiber's pulling may be independent of position, and some other force (e.g., the ejection force) acts on chromosomes in a position-dependent manner to effect congression (70, 72).

Force Balance and Spindle Structure

The lengths of spindle MTs depend in part on forces that act on their ends. When a force parallel to the pole-to-pole axis is experimentally applied to a prometaphase chromosome, the chromosome moves in the direction of the applied force (reviewed in reference 57). The kinetochore MTs linking that chromosome to each pole shorten or lengthen to accommodate this movement. Likewise, if microneedles are used to apply force to both spindle poles, either in toward the equator or outward from it, the spindle shortens or elongates in response (18). The rate of chromosome fiber shortening at anaphase can be retarded by applying a force that opposes chromosome motion (56). On the other hand, the rates of spindle-associated movements can be increased by treatments that promote MT depolymerization (reviewed in reference 28). These observations all suggest that the assembly of tubulin is modulated by the mechanical loads placed on tubulin polymers (21, 22); thus the polymerization of tubulin can be a factor in MT-dependent movement (30).

The Generation of Mitotic Forces

The development of optical and video methods for visualizing individual MTs in cell-free systems (2, 29) has led to the discovery and characterization of several motor enzymes that move over a MT surface: kinesin, which generally moves over MTs in vitro toward their plus ends (86–89), and cytoplasmic dynein, which usually moves in the opposite direction (37, 64, and reviewed in reference 45). More recently, however, members of the kinesin family have been found that move in the same direction as dynein (42, 93, and reviewed in reference 11), and a dynein-like protein seems capable of moving in either direction (81). One distinction between a kinesin and a dynein family has therefore become blurred. Additional complexity comes from the fact that the polymerization of MTs (here meaning either the addition or the loss of tubulin subunits) can do mechanical work (10, 33, 55, and modeled in references 21, 40). Thus, several distinct molecular mechanisms may contribute to spindle-associated motions. Below we describe some properties of MT-dependent motor molecules and explore the roles that they might play in mitotic movements.

The Kinesin Family

Initial studies with antibodies to kinesin purified from sea urchin eggs localized this motor in the spindle of early embryos, suggesting that it might contribute to chromosome movement (35, 80), but subsequent work has shown that this kinesin is associated with membranes of the endoplasmic reticulum whose spindle-specific localization is limited to early blastomeres (96). Immunolocalization with several affinity purified polyclonal antibodies raised against mammalian brain kinesin localized these epitopes at the poles of spindles in cultured cells (60), but other workers failed to confirm this finding (66, 77). Data to help understand this discrepancy are now coming from molecular biology. Antibodies against *Drosophila* kinesin heavy chain (78) have led to the cloning and sequencing of the corresponding gene (97). A combination of deletion analysis (98) and biochemistry (reviewed in reference 45) has identified a ca. 43-kD domain near kinesin's NH₂ terminus that binds MTs and ATP and is sufficient for the enzyme's motor activity. Several previously identified genes, whose products are required for normal mitosis and/or meiosis, have now been characterized as "kinesin-like" by comparisons of their primary sequences with the motor domain of *Drosophila* kinesin. For example, the "Blocked in Mitosis" gene of *Aspergillus nidulans* (*bim-C*) encodes a protein in which a 419 amino acid segment near the NH₂ terminus shows 42% sequence identity with *Drosophila* kinesin (14). This domain contains both putative ATP and MT binding regions with even greater similarity to those of the *Drosophila* motor enzyme. The rest of the *bim-C* protein, however, shows no similarity to kinesin, leading to its description as "kinesin-like." *Bim-C* cells are unable to separate their spindle poles, suggesting that this protein is part of the mitotic machinery, but neither the localization nor the biochemical and motile properties of the protein have yet been determined.

A gene encoding a different kinesin-like protein contributes to spindle function in the fission yeast *Schizosaccharomyces pombe* (16). A temperature-sensitive mutation of the *cut7⁺* gene blocks spindle formation at restrictive temperatures. The *cut7⁺* gene product contains an NH₂-terminal 415 amino acid domain that has 57% sequence identity with the product of *bim-C*⁺. The phenotype of *cut7⁺* cells at restrictive temperatures is similar to that of *bim-C*⁺ cells; duplicated spindle poles initiate MTs but fail to separate and form the normal mitotic array. Outside the motor domain, however, the *cut7⁺* protein shows no homology with either BIM-C or kinesin. Neither the *bim-C*⁺ nor the *cut7⁺* proteins contain the heptad repeat motif that is found in kinesin outside its motor domain and supports its alpha-helical, coiled-coil structure. It remains to be seen whether the *bim-C* and *cut-7* gene products perform analogous functions in different cells, or whether both *A. nidulans* and *S. pombe* have representatives of each gene type.

The *KAR3* gene of *Saccharomyces cerevisiae* encodes a protein that is essential for karyogamy, i.e., the coalescence of gamete nuclei after zygote formation (49). The sequence of this gene predicts a protein containing a 328 amino acid region with 38% identity to the motor domain of *Drosophila* kinesin. Within this region there are ATP and MT binding regions with greater sequence similarity, but in the *KAR3* protein this domain is near the COOH terminus, not the NH₂ terminus. The middle region of the *KAR3* protein con-

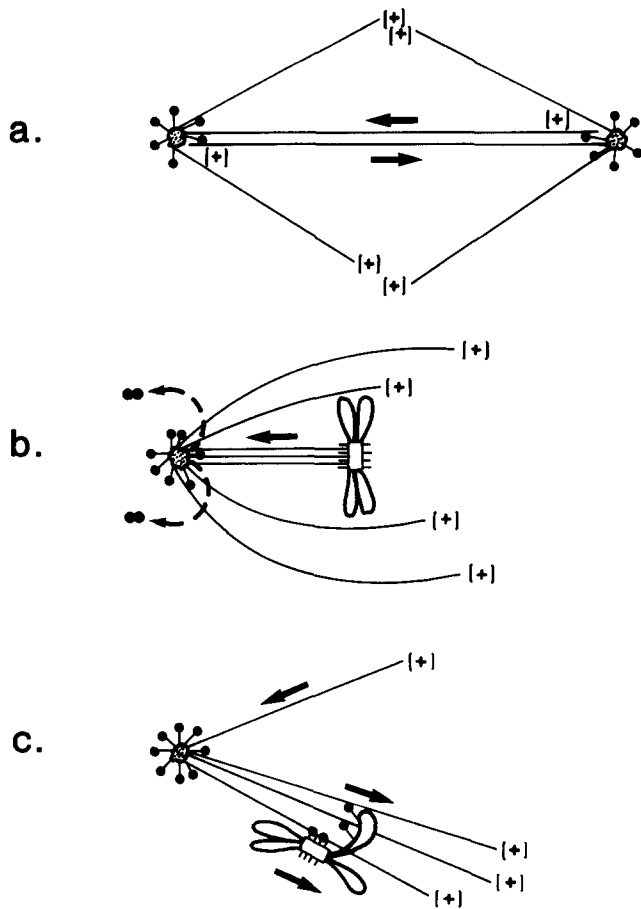


Figure 2. Possible roles for plus end-directed motor enzymes in spindle movements. Motors bound to the centrosomal region of the spindle could interact with the plus ends of MTs emanating from the opposite pole to generate forces that would push the poles apart (a). They could also act to pull poleward the MTs emanating from that pole. This action would contribute to the poleward flux of both kinetochore MTs (b) and other spindle MTs (c). Plus end-directed motors associated with a chromosome could promote two types of movement: (a) distributed along a chromosome arm and interacting with non-kinetochore MTs, they could contribute to the ejection forces that push objects slowly away from the poles; (b) located at the kinetochore, they could contribute to the movements of prometaphase (c).

tains sequences that predict a coiled-coil structure, analogous to those of kinesin's heavy chain, but the NH₂ terminus is similar to MT-binding domains of certain MT-associated proteins from mammalian brain, suggesting that this protein might cross-link MTs as well as effect movements over their surfaces. Indeed, a fusion protein expressing this portion of the molecule associates with MTs in cells. Strains that are mutant in *KAR3* grow more slowly than wild type cells, because ca. 40% of the cells fail in mitosis, but the disruption of vegetative growth is less pronounced than that seen in either *bim-C*⁻ or *cut7*⁻ cells.

While true kinesin does not appear to play a role in mitosis (77, 96), mutations in two kinesin-like genes in *Drosophila*, *ncd* (13, 41), and *nod* (101), reduce the fidelity of meiotic chromosome segregation. Both these genes contain sequences that resemble kinesin's motor domain. Some alleles of *ncd* (also called *ca^{nd+}*) result in a high frequency of non-

disjunction during both meiosis in females and mitosis of the early zygotic divisions. These abnormalities affect both chiasmatic and non-chiasmatic chromosomes, whereas most alleles of *nod* lead to a loss of only the chromosomes that have failed to cross-over (reviewed in reference 9). The motor domain of NOD is near its NH₂ terminus (101), while the molecular organization of NCD resembles that of the *KAR3* protein (13, 41).

The NCD coding sequence has been expressed in *Escherichia coli* to produce an ATP-dependent motor activity that is sensitive to vanadate ions and moves over MTs toward their minus ends (42, 93). Thus, the motor properties of *ncd* protein resemble those of a dynein, not kinesin. Perhaps NCD is a "mini-dynein" in the sense that myosin I is a "mini-myosin". Sequence data on the dyneins and other kinesin-like proteins will certainly help to determine whether both kinds of MT-dependent motor enzymes belong to the same superfamily (see Note Added in Proof).

Since three *Drosophila* kinesin-like proteins have been identified in so short a time, one might expect that the whole kinesin family is rather large. DNA probes have been made by the polymerase chain reaction, using a *Drosophila* cDNA library and degenerate primers from the kinesin motor domain; these identify more than 30 sites of hybridization to polytene chromosomes (12). While some of these loci may not encode functional genes, the result suggests that there are enough kinesins to allow different motors to be specialized for specific groups of tasks.

The fertility of *Drosophila* with mutations at the *nod* or *ncd* loci suggests that meiosis involves motor molecules in addition to the products of these two genes, yet the genetic interactions between some alleles at these loci (reviewed in reference 9) suggests that the functions of their products overlap. Even in a single celled eukaryote, there appears to be a family of kinesin-like proteins involved in mitosis. Molecular and genetic techniques have identified at least four kinesin-like proteins in *S. cerevisiae*. Mitosis can continue in the absence of any one of them, but certain pairwise deletions are lethal (Mark Rose, Princeton University, Princeton, NJ; and Andrew Hoyte, Johns Hopkins University, Baltimore, MD, personal communication). It appears that there are several genes encoding kinesin-like motors whose products cooperate in generating mitotic movements.

Mitotic Roles for Plus End-Directed Motor Enzymes

The role for plus end-directed motor enzymes in mitosis might be indirect, e.g., helping to position vesicles whose ability to sequester ions is essential for spindle function. In our view, however, plus end-directed motors are likely to cause some of the MT motions described above, as diagrammed in Fig. 2. A plus end-directed motor bound at the spindle pole could push away on the MTs projecting from the opposite pole, contributing to the prophase separation of the centrosomes (vis. BIM-C and CUT7) (Fig. 2 a). It could also promote the poleward flux of kinetochore (51) and non-kinetochore (75) MTs (Fig. 2 b and c). Bound to kinetochores, it could contribute to the motion of chromosomes away from nearby poles (85) and account for the in vitro movement of kinetochores toward MT plus ends (27, 53). Distributed over vesicles or bound to a chromosome's surface, a plus end-directed motor could contribute to both the ejection forces seen in normal bipolar spindles and the posi-

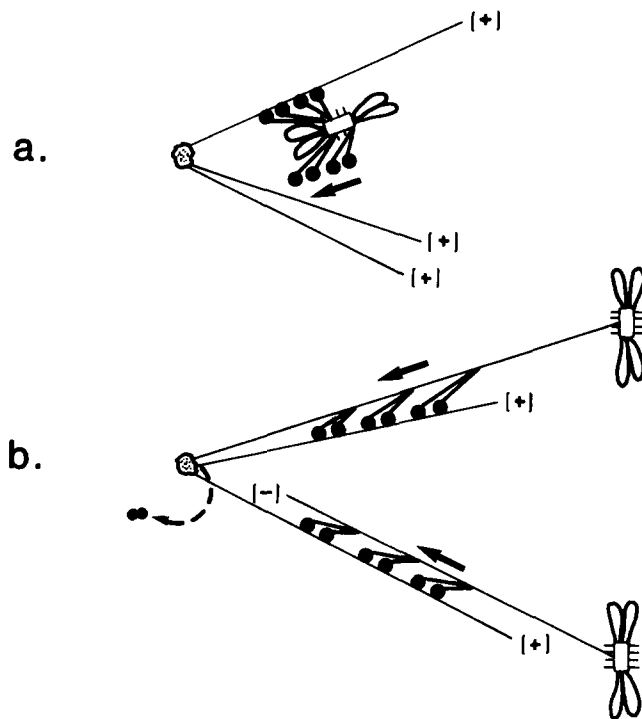


Figure 3. Possible roles for minus end-directed motor enzymes in spindle movements. Bound to the kinetochores, they could provide the force for the rapid, early prometaphase movements toward the pole (a). After kinetochores bind to MT ends, such movements would necessarily be coupled with MT subunit loss, imposing constraints on the rates of movement, as in anaphase. A minus end-directed motor enzyme with the properties of dynein, which is distributed along kinetochore MTs, could induce MT-MT sliding interactions if ATP insensitive bonds were formed predominantly with kinetochore MTs (b). Dynein "walking" along the surface of non-kinetochore MTs would then pull a kinetochore MT and its associated chromosome poleward. If the kinetochore MT extended to the pole, subunit loss at the minus end would allow shortening to occur.

tional stability of chromosomes next to a single centrosome (70). Note, however, that these spindle motions are slow in comparison to the movements induced by true kinesin *in vitro*, so either some members of the kinesin family move more slowly than kinesin or a regulatory process, such as tubulin polymerization, is involved.

Kinesin-like proteins could also serve to promote the relative sliding of antiparallel MTs, like that which occurs in the interzone regions of the anaphase and telophase spindle (3, 43, 76) (Fig. 1, c and d). In diatoms, however, the nucleotide specificity and the inhibitor sensitivity of spindle elongation *in vitro* (7) suggest that the relevant motor differs from the kinesin-like proteins that have been characterized so far. Further, the motor for anaphase spindle elongation is likely to possess either a second MT-binding domain that is static, like that postulated for KAR3, or a matrix-binding domain that would allow it to attach to the osmiophilic material of the midbody. Two motor molecules mechanically coupled by matrix could interact with two antiparallel MTs to effect their relative movement. The localization of kinesin-like proteins in mitotic cells should help to tell whether these enzymes serve as MT cross-linkers and/or function as the pole-associated motors, depicted in Fig. 2.

Cytoplasmic Dynein

Biochemical and immunological work on dynein suggests that this MT motor, in addition to kinesin-like proteins, may be important for mitosis. Cytoplasmic dyneins that do not participate in axoneme formation have recently been identified in mammalian brain, nematode and fruit fly embryos, cultured cells, and a variety of vertebrate tissues (reviewed in references 45, 91). These enzymes share with kinesin the ability to promote ATP-dependent MT gliding *in vitro*. Most of the dyneins so far examined share a distinct set of structural and biochemical similarities and move toward the minus MT end (31, 38, 63), though one dynein appears to be able to promote motion over MTs in either direction (81).

The possibility that dynein contributes to mitotic movements has been considered for years, but the evidence for spindle dynein has been controversial. Some antibodies to flagellar dynein have shown antigen concentrated in spindles (23, 67, 100), while others have not (102). Recently, however, antibodies to cytoplasmic dynein from *Drosophila* embryos, HeLa cells (65), and chicken brains (84) have been used to localize these antigens in cultured cells. During mitosis, cytoplasmic dynein concentrates at the spindle poles, at the kinetochore regions of the chromosomes, and along the fibers that connect them. Dynein antibodies also bind to the kinetochore regions of isolated chromosomes. In immunoblots of chromosomal proteins these antibodies recognize polypeptides of molecular weights that correspond to dynein's heavy chain and one of its intermediate chains (65, 84). Dynein localization is cell cycle dependent; during interphase, the antigen is confined to the cytoplasm where it localizes to small spherical objects, probably vesicles, with little apparent concentration on centrosomes, MTs, or inside the nucleus. These results indicate that cytoplasmic dynein is recruited into spindle structures at the onset of mitosis. We therefore think it likely that dynein participates in the generation of spindle movements.

Mitotic Roles for Minus End-Directed Motor Enzymes

Cytoplasmic dynein and/or kinesin-like, minus end-directed motors (e.g., NCD) could contribute to mitotic movements in several ways. While there is not yet information available on the localization of NCD in mitotic cells, the localization of dynein on kinetochores, beginning at prophase, suggests its involvement in the early poleward motions of chromosomes during their initial attachment to the spindle (71) (Figs. 1 and 3). Dynein's concentration at the centrosomes throughout mitosis may result from its being bound to vesicles that aggregate around the pole, but motor protein so arranged may play a direct role in the formation or stability of the astral array. Dynein participates in the formation of aster-like structures in extracts of *Xenopus* ooplasm, presumably because it can cross-link MTs and mediate their relative movement into a minus end-associated configuration (92). An analogous action might contribute to aspects of normal spindle formation.

Recent work has identified a MT- and ATP-dependent motility at the kinetochores of isolated chromosomes (27). Both minus end-directed, and plus end-directed activities have been demonstrated. Which activity predominates appears to depend on the state of phosphorylation of not yet identified kinetochore components. While *ncd* protein might be the

relevant minus end-directed motor, the fact that preparations of isolated mammalian chromosomes contain polypeptides that bind antibodies to both a heavy and an intermediate chain of cytoplasmic dynein suggests that the minus end-directed movement is due to cytoplasmic dynein. The identity of the plus end-directed kinetochore motor is not yet known, but its response to inhibitors is distinct from that of the minus end-directed activity, suggesting that this movement is the result of a different enzyme (27).

Dynein's localization on spindle MTs could lead to the generation of additional forces that would pull the kinetochores towards the poles and vice versa. Cytoplasmic dynein, like its flagellar counterpart, may possess two distinct MT binding domains: one that is "active", coupling ATP hydrolysis with MT binding and force generation, and one that is ATP-insensitive, coupling dynein to a MT that will be pulled (reviewed in reference 31). If cytoplasmic dynein were to form ATP-insensitive bonds with only kinetochore MTs (because, for example, these MTs are more stable than other spindle MTs and can therefore accumulate a slowly binding species), the minus end-directed forces generated by the motor domain's binding to non-kinetochore MTs would lead to pole-directed forces acting on kinetochore MTs and equator-directed forces acting on the poles. The latter could account for the inward motion of a pole cut from a spindle and then allowed to reattach (83). The former might contribute to prometaphase chromosome congression to the spindle equator. Since kinetochore MTs can lose subunits at the pole (51, 54), concomitant MT sliding would result in forces acting on the kinetochores themselves (Fig. 3 b). The magnitude of this force at each kinetochore should be proportional to the number of dynein-mediated interactions between kinetochore and non-kinetochore MTs, and thus depend on both the number of kinetochore MTs and their lengths. Experiments with meiotic chromosomes in grasshopper spermatocytes provide direct evidence for such behavior (19, 20), but it must be noted that current evidence suggests that the majority of subunit loss from kinetochore MT shortening during prometaphase occurs at the kinetochores (95). If this were the only pathway for kinetochore MT shortening, then forces generated along their length could play no role in the forces acting at kinetochores. Given the poleward flux of kinetochore MTs, however (Fig. 1 c), a dynein-based, MT-MT force generating mechanism might be important for the refinement of metaphase chromosome position.

After anaphase onset, minus end-directed motor enzymes could contribute to chromosome motion both by directly promoting the movement of each kinetochore over the MTs to which it is bound (Fig. 1 d) and by generating forces like those described above for prometaphase. Several lines of evidence show that chromosome to pole motion is accomplished largely by the movement of kinetochores relative to the fibers to which they bind (15, 54, 58), so the kinetochore-associated activity is likely to be the more important. Minus end-directed motors might also contribute to the interactions between astral MTs and material outside the spindle, helping to pull the spindle poles apart or draw the spindle to a particular region of the cell cortex (26, 36). An analysis of these possibilities by genetics has just begun. The gene for one dynein-associated protein has been cloned, and its sequence shows strong homology with that of the glued gene of *Drosophila* (24). The phenotype of a dominant mutant al-

lele at this locus includes pleiotropic developmental defects, so it is not yet possible to say whether a perturbation of mitosis is involved.

Additional Mitotic Motors?

A study of MT-activated ATPases has recently led to the identification of a third, MT-dependent motor activity called dynamin (82). This protein is distinct from kinesin and dynein in polypeptide composition and in its manner of binding to MTs. The 100-kD polypeptide of dynamin cross-links MTs into bundles, and in the presence of one or more protein co-factors, it mediates an ATP-dependent sliding between MTs. Brain dynamin is, however, largely associated with vesicles (79). Molecular cloning of the principal dynamin polypeptide has revealed a strong sequence similarity with known GTP binding proteins, but little homology with any of the kinesin-like proteins or myosin (61). While there is no evidence to show a concentration of dynamin in the spindle, the primary structure of dynamin shows 45% sequence identity with the product of *SPO15*, a gene of *S. cerevisiae* that is necessary for the proper separation of duplicated spindle poles at the onset of meiosis I (99). The *SPO15* gene is also identical with *VSP1*, a previously identified yeast gene known to be involved in the sorting of vacuolar proteins. Recent data suggest that dynamin is a better GTPase than ATPase (61), but the ability of GTP to support MT sliding in dynamin cross-linked bundles has not yet been reported. The assignment of a mitotic role to dynamin must await further clarification of its enzymology and mechanochemistry.

Polymer Assembly and Disassembly

The idea that MT assembly and disassembly contribute to chromosome movement has a long history (reviewed in reference 30). Experiments on spindles in situ have demonstrated that MT assembly can lead to an increase or decrease in the distance between chromosomes and poles (reviewed in reference 28). However, the complexity of the mitotic apparatus in a living cell has made it impossible to know whether MT assembly actually produces force for these spindle-associated movements or merely regulates their rates and extents. It is now clear, however, that MT assembly can do work; MTs polymerized in lipid vesicles grow longer than the vesicle diameter and deform the membrane that surrounds them (55). Polymer disassembly can also do work: MTs attached to kinetochores by their plus ends will disassemble in a low concentration of tubulin by loss of subunits from their kinetochore-associated ends. As these MTs shorten, their minus ends move in toward the kinetochores (33). This process does not require added nucleotide triphosphate, suggesting that MT subunit loss provides the driving force for movement.

A recently developed assay system has allowed the real time visualization of chromosomes and particles moving at ca. 20 $\mu\text{m}/\text{min}$ together with the ends of disassembling MTs (10). Such movements will occur when the concentration of ATP is less than 10^{-12} M and/or in the presence of 100 μM NaVO_4 , an inhibitor of both dynein's and NCD's mechanochemistry, suggesting that ATP hydrolysis is not required for these chromosome movements. The movements will even occur against a rapid flow of buffer. The force that disassembling MTs can exert exceeds 0.1 μdyne , as calculated from

the speed of buffer flow and the geometry of the chromosomes and particles being moved. Given the tendency for GDP-tubulin in MTs to disassemble (8, 52), there is sufficient free energy available from tubulin depolymerization to do considerable work on any object that can remain bound to the disassembling MT. Several models for the molecular mechanism of such a process have been suggested (21, 22, 33, 50). They share the concept of a loosely attached adaptor that binds the moving object to the disassembling MT. There is evidence that both a dynein (90) and the STOP protein (39) possess this property. In such a mechanism, MT disassembly is probably better thought of as the fuel for motion than the motor itself.

While MT assembly is clearly essential for normal mitosis, it is difficult to measure the energy contributed by polymerization to any particular spindle-associated movement. For example, MTs polymerize at the kinetochore as a prometaphase chromosome moves away from a nearby pole (Fig. 1 b), but assessing the contribution of this assembly to the forces that cause chromosome congression requires a yet to be achieved depth of analysis. There are, however, several experimental approaches to understanding what MT assembly can do. One is to produce mitosis-like motions in a well defined system that permits MT assembly but lacks functional motor enzymes; the *in vitro* motions of chromosomes relative to disassembling MTs in the absence of ATP are examples (10, 33). A second approach is to study spindle isolates that manifest mitosis-like movements under well-controlled conditions. For example, diatom spindles will elongate *in vitro*, but this movement requires ATP as well as tubulin assembly, implying that ATPases are required in addition to tubulin polymerization to effect the motion (7). A third approach is to inactivate motor enzymes *in vivo*, by either mutational, immunological, or chemical inhibition, and ask what movements remain. Results from these approaches to mitotic movements are now forthcoming.

The coupling between MT length and experimentally applied forces, cited above, suggests that the thermodynamic drive for tubulin polymerization is linked directly to forces acting at MT ends. Motor enzymes should therefore influence spindle fiber assembly reactions and vice versa. The lengths of spindle MTs, the distance pole-to-pole, and the positions of chromosomes on the spindle are probably regulated, at least in part, by forces generated by motor enzymes. For example, when sister centromeres split and chromatids part at anaphase onset, the force balance on each kinetochore fiber changes. The ensuing disassembly of the kinetochore fiber may be a direct result of this change in force balance. On the other hand, the rate of motor enzyme action is probably governed in part by MT assembly. Chromosome motion is fast during early prometaphase but slows as the spindle forms, as if the velocity of chromosome movement becomes limited by the rates at which MTs can assemble (46, 57). Thus, spindle structure links MT motor activity and MT assembly into a single system of reactions that produces a well-regulated and concerted set of motions. This linkage may be at the heart of spindle function, because it contributes to the fidelity with which chromosomes are oriented, positioned, and segregated.

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Note Added in Proof. DNA sequences for β -dynein heavy chain have now been established for two species of sea urchins (Gibbons, I. R., B. H. Gibbons, G. Mocz, D. J. Asai. 1991. *Nature (Lond.)*. 342:640-643; Ogawa. 1991. *Nature (Lond.)*. 352:643-645). They show no extensive regions of similarity to the kinesin-like proteins now known, so these two classes of MT-dependent motors appear to be distinct. A recent analysis of the kinesin-like genes suggests, however, that the variation in this set alone is sufficiently great to regard the enzymes they encode as a super-family (Goldstein, L. S. B. 1991. *Trends Cell Biol.* In press).

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