Mitosis

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Data that describe both the structure and the physiology of the mitotic spindle are reviewed. Some of the molecules that have been shown to play a role in mitosis are tabulated, and how mitosis might work is considered.

MITOSIS IS THE PROCESS BY WHICH EUKARYOTIC CELLS assure the equipartition of their chromosomes at cell division. The key events of mitosis are (i) prophase—the condensation of the duplicate chromosomes from a dispersed and metabolically active state to a compacted condition, suitable for transport; (ii) prometaphase—the positioning of the condensed chromosomes, first by orientation so one copy of each chromosome addresses one end of the cell and then by the motion of each chromosome to the cell's midplane to form the "metaphase plate"; (iii) anaphase—the separation of each chromosome into two identical parts, followed by their movement toward the opposite ends of the cell; and (iv) telophase—the reformation of nuclei and the decondensation of the chromosomes to reestablish the interphase condition.

Each of these steps is contemporaneous with, and in some cases the result of, other cellular processes. During prophase, the microtubules and microfilaments of the cytoskeleton disassemble and rearrange, destabilizing the interphase organization of the cytoplasm. Microtubule (MT) rearrangement is due, in part, to changes in the "centrosome," the principal MT organizing center of the cell. This structure duplicates during interphase, and at about the time of mitosis it separates into two distinct parts, each of which increases the number of MTs it initiates (Fig. 1, A through C). The centrosomes serve as organizational foci for the "mitotic spindle," the array of MTs and other proteins that guides and moves the chromosomes during mitosis. Also during prophase, the Golgi apparatus disperses into a large number of small vesicles, which become distributed throughout the cytoplasm; pinocytosis and RNA synthesis stop; and protein synthesis slows to about 25% of its normal rate.

The start of prometaphase is defined by the onset of interactions between the chromosomes and the "spindle fibers," the bundles of MTs and associated proteins that emanate from the centrosomes. One locus on each chromosome, the "centromere," is specialized to bind a set of proteins to form "kinetochores," the domains on each copy of the duplicate chromosomes that interact with the spindle fibers (Fig. 1, D and G). As a result of these interactions, a force is exerted on the chromosomes that effects both orientation and registration at the metaphase plate (Fig. 1, E and H). In higher eukaryotes, the nuclear envelope breaks down to allow these interactions, but in some fungi and algae the spindle simply forms inside the nucleus. Other centrosome-initiated MTs interact with their counterparts from the opposite centrosome to form "interpolar fibers"; these are often arranged in a spindle-shaped cluster that gives the chromosome-moving apparatus its name.

The onset of anaphase is a global event, and in some cells the duplicate chromosomes will separate into two parts without a spindle. Chromosome segregation into two complete genomes, however, requires spindle action (Fig. 1, F and I). Late in anaphase, as the chromosomes near the poles, they contract to form two tight masses. The nuclear envelopes then re-form about each mass, reestablishing the nuclear compartments. The reconstruction of two synthetically active, interphase nuclei is accomplished during telophase, thus completing the process of "karyokinesis" (Fig. 1, J and K) [see (*1*)].

Nuclear division is generally followed by cell division, or "cytokinesis." In animal cells, protozoa, and many unicellular plants, cytokinesis is mediated by an actomyosin-dependent contractile ring which, after anaphase, divides the cytoplasm into two roughly equal parts, each containing about half of the cell's constituents (*2*). In higher plants, cytokinesis results from the action of the "phragmoplast," a specialized structure that forms at or near the plane where the metaphase plate used to be. The phragmoplast assembles small, Golgi-derived vesicles into one large, flat vesicle, which ultimately fuses with the plasma membrane and divides one cell into two.

Characterization of Chromosome Movement

Chromosome kinematics. Early in prometaphase, the chromosomes are often drawn toward one centrosome or the other, suggesting a mutual attraction. The resulting motions range in speed from 0.05 to 1 μm/s, approximately the same range observed for the motions of cytoplasmic particles. This motion slows to 0.01 to 0.05 μm/s as prometaphase progresses, suggesting that as the spindle forms, its structure imposes constraints on chromosome mobility (*3*). Soon, the chromosomes become engaged with MTs from both poles, whereupon they orient so that each of the two copies of one chromosome faces one centrosome. The chromosomes then move

Fig. 1. (**opposite page**) Mammalian PtK_1 cells stained to reveal components of the mitotic apparatus. The chromosomes are stained blue with DAPI (4′,6-diamidino-2-phenylindole dihydrochloride); the MTs are stained green with antiserum to tubulin and fluorescein-labeled secondary antibodies; and kinetochores are stained red with serum from a human patient with scleroderma and rhodamine-labeled secondary antibodies. (**A**) MTs, (**B**) kinetochores, and (**C**) chromatin of an early prophase cell. Centrosome activity is revealed in the two asters of cytoplasmic MTs, but the nuclear envelope blocks interaction with the kinetochores. (**D** through **F** and **G** through **I**) Prometaphase through anaphase cells stained to reveal chromosomes and spindles or kinetochores, respectively. (**J** and **K**) A pair of cells in late telophase-interphase, when the nuclei are essentially reestablished, but the two cells are still interconnected by a cytoplasmic bridge.

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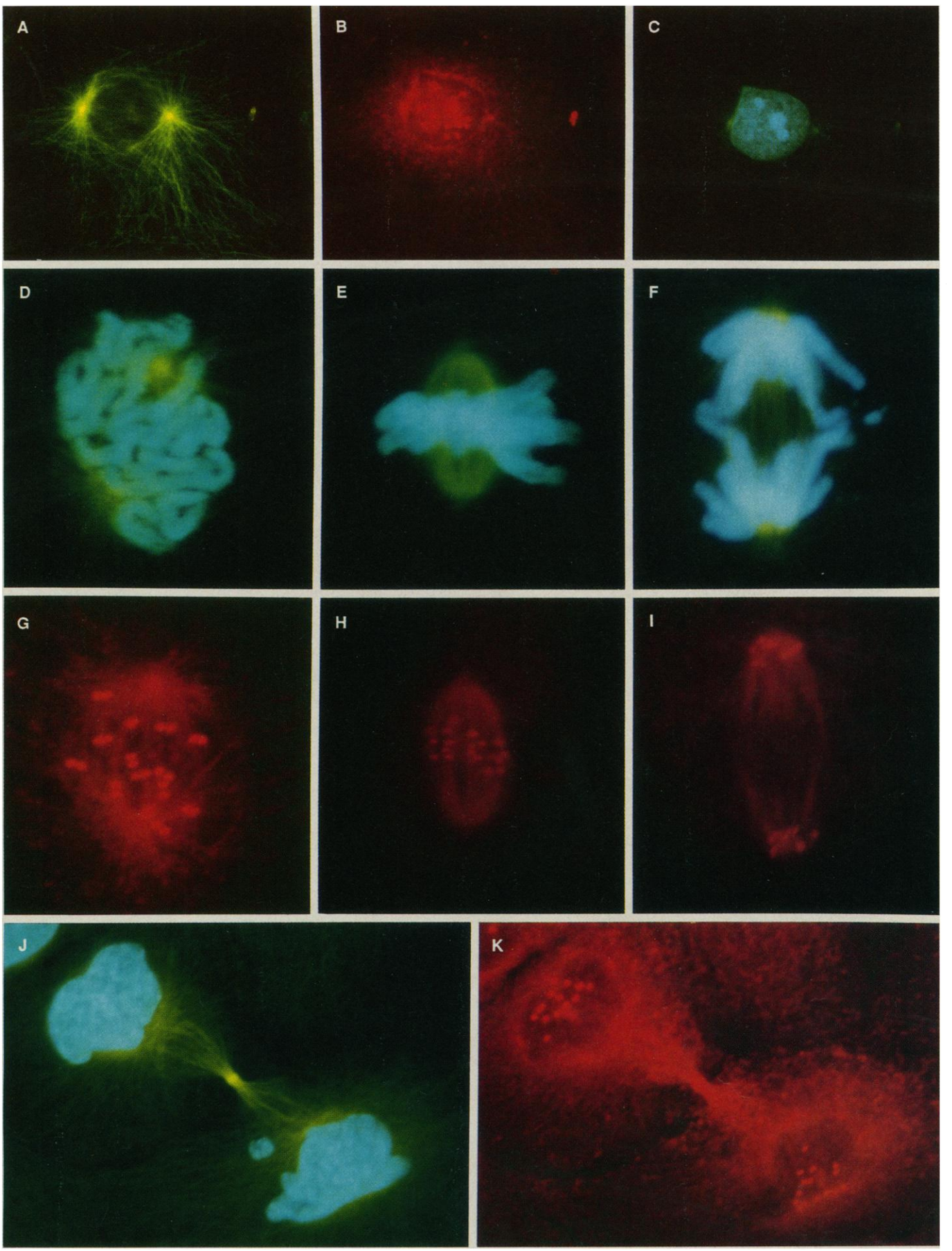
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back and forth, but over time they congregate at the metaphase plate.

During anaphase, chromosomes move toward the poles at an approximately constant speed, about as fast as the movements of late prometaphase. Chromosome speed is virtually independent of position, but in insects, chromosome speed increases about fourfold for an increase of temperature from 15° to 30°C (*4*). Anaphase motion toward the spindle pole (anaphase A) is first accompanied by, and then followed by, spindle elongation (anaphase B), which in large cells can increase the separation between the already segregated chromosomes by tens of micrometers.

Chromosome mechanics. The ablation of one kinetochore during prometaphase with either an ultraviolet (UV) or a laser microbeam results in a shortening of the distance between the nonirradiated kinetochore and the centrosome it faces (*5*), suggesting that during this time a chromosome is under tension from both ends of the forming spindle. When fine glass needles are used to reposition or pull on individual chromosomes, the resulting perturbations confirm the existence of centrosome-directed forces acting at each kinetochore (*6*).

Kinetochore–spindle fiber interactions are unstable until there is an opposing force that resists the tendency of a chromosome to move (*7*). Micromanipulation of chromosomes during prometaphase of meiosis I has shown that these opposing forces are usually supplied by the fibers that connect each centrosome to the kinetochores on one of the two parts of this "bivalent" chromosome. The stability of a chromosome's attachment to the spindle is therefore dependent on its proper orientation with respect to the spindle. Such behavior assures that improper chromosome associations are unstable, so only chromosome orientations that promote correct chromosome segregation will survive.

It has been proposed that chromosome motion during prometaphase is the result of kinetochore-centrosome attractions, the magnitudes of which are proportional to distance (*8*). This hypothesis accounts both for the orientation of the chromosomes and for their migration to the spindle equator. Anaphase would then be a natural consequence of parting the mechanical connection between the two chromosome halves, allowing each to respond to the pole-directed forces acting on it. There is some direct evidence for this idea. Abnormal associations of meiotic chromosomes can produce "trivalent" structures that have three functional kinetochores. Such chromosomes take a metaphase position nearer the centrosome that interacts with two kinetochores, and the sum of the distances from these kinetochores to the centrosome they face is about the same as the distance from the single kinetochore to its centrosome (*9*). Laser ablation of a portion of one kinetochore on a bivalent chromosome also leads to an asymmetric metaphase position; the tension generated by a half-sized kinetochore attached to a long spindle fiber balances that generated by a half-length fiber attached to a full-sized kinetochore (*10*). It appears, therefore, that during prometaphase, there is a tension on each kinetochore that is proportional to both the length and the number of the component MTs in the fiber that connects it with the spindle pole.

Chromosomes associated with a single centrosome for a prolonged period do not, however, gather at the pole. They move away from, as well as toward, the pole, showing that one centrosome can push as well as pull on a chromosome (*11*). Laser microsurgery has been used on such "monopolar spindles" to sever chromosome arms from their centromeres. The resulting fragments that lack kinetochores move slowly away from the centrosome, while the kinetochore-containing piece moves closer (*12*). This observation suggests that a single centrosome pulls on a kinetochore and pushes on the rest of the chromosome, a duality of forces that may be significant for prometaphase motions to the metaphase plate.

The forces acting on anaphase chromosomes have been measured by seeing how much counter force must be applied with a thin glass needle to slow an anaphase chromosome by a given amount (*13*). Anaphase speed is reduced to zero by the application of $\sim 7 \times 10^{-5}$ dynes. This force exceeds the viscous drag on a chromosome by a factor of $\sim 10^4$, suggesting that normal anaphase velocity is regulated. One significant component of this control is probably the resistance to movement imposed by the need to disassemble spindle MTs in order to get a kinetochore closer to the centrosome it faces. This resistance may also account for the slowing of chromosome movement during prometaphase.

The sum of the many kinetochore-centrosome attractions must pull inward on the centrosomes. Because the poles of the spindle do not collapse to the spindle equator, there must be forces acting outward. Some cells contain obvious bundles of fibers that run from one centrosome to the other. In diatoms, damaging of this bundle by microbeam irradiation results in a collapse of the intercentrosome distance, suggesting that the "interpolar spindle" is the support that keeps the poles apart (*14*). Microscopy has revealed the presence of interpolar MT bundles in many cell types (*1*). Thus the pattern of one group of spindle fibers pulling the chromosomes toward the poles, while another group holds the poles apart, may be a general feature of spindle design.

Sometime late in prometaphase, centrosomes become dispensable; they may be removed by micromanipulation without loss of mitotic progression (*15*). Apparently the interactions between kinetochore-associated and interpolar MTs that provide support for the chromosome-moving forces can occur away from the centrosomes themselves, presumably through interactions between MTs. These interactions continue during anaphase B, so elongation of the interpolar spindle contributes to the extent of chromosome separation.

The Polymerization Properties of Mitotic Microtubules

Microtubules are the most abundant spindle component and the only one whose presence is clearly essential for chromosome movement. We can now relate aspects of MT behavior to the properties of chromosome motion.

Most mitotic microtubules turn over rapidly. Spindle MTs are labile, as indicated by their ephemeral birefringence (*16*); their instability after cell lysis in many buffers; their sensitivity to cold, hydrostatic pressure, and several tubulin-binding drugs [see (*1*)]; and their rapid turnover, as visualized by both the rate of postinjection incorporation of labeled tubulin and the rate of fluorescent tubulin redistribution after photobleaching (*17*). Most mitotic MTs are more labile than their interphase counterparts (*18*). This increased turnover rate may be due in part to a reduction in the mean MT length, which results from the increased initiation activity at the centrosomes (*19*) and, in part, to changes in the interactions between MTs and their associated proteins (*20*); it is probably not due to the synthesis of a different tubulin, because interphase tubulin microinjected into mitotic cells shows rapid turnover in the spindle (*17, 18*) and because tubulin and the other essential spindle proteins are made before mitosis begins. At the time of spindle formation there is an increase in the phosphorylation of several proteins (*21*). Such posttranslational modifications may be important for the control of mitotic MT behavior.

Some metaphase microtubules are more stable than others. The MTs that interact with kinetochores are partially stabilized, as indicated by their relative insensitivity to cold and colchicine and by their slower turnover (*22*). Presumably the binding of a kinetochore to a free MT

end helps to stabilize that polymer against disassembly. Kinetochore MTs can both add and lose subunits at or near their kinetochores (*22*). This polymerization has also been investigated by injecting cells during prophase with a labeled tubulin that can be stimulated to fluorescence by a pulse of long wavelength UV light (*23*). If the spindle equator is exposed to UV light during late prometaphase, then some of the resulting fluorescent label migrates at ~0.01 μm/s from the equator toward both centrosomes, suggesting that there is a net addition of tubulin subunits near the kinetochores and a net loss near the centrosomes. This pattern of tubulin assembly and polymer flux may be important during prometaphase chromosome motions to the spindle equator, because the centrosome-directed forces that act at the kinetochores could be developed along the length of each kinetochore MT, pushing it slowly poleward. The resulting force acting on each kinetochore would then be proportional to both the length of the attached fiber and the number of component MTs, accounting for both prometaphase motions and the experimental data cited above (*9, 10, 23*).

Microtubule stabilities and position changes in anaphase. As a cell enters anaphase, the characteristics of MT polymerization change: the centrosomes lose their ability to initiate new MTs (*24*), and some spindle fibers shorten while others elongate. Both the previously stable kinetochore fibers and the majority of the other spindle MTs depolymerize during anaphase A (*25*). The major site of subunit loss from kinetochore MTs is at, or near, the kinetochores (*22, 26*). The transition from prometaphase behavior to anaphase behavior may result in part from the change in force balance that accompanies chromosome separation at the onset of anaphase. After the anaphase chromosomes have separated, there is no longer a force pulling each kinetochore away from the centrosome it faces. This change in tension may be an important variable in the tubulin assembly reaction (*27*).

While most spindle MTs shorten during anaphase, the interpolar MT framework elongates. In diatoms, this elongation is accompanied by a reduction in the extent of interdigitation by the two halves of the interpolar spindle (*28*). In many cells, there is also an elongation of the MTs that comprise the interpolar spindle (*28*). The pattern of incorporation of labeled tubulin injected into anaphase cells suggests that MT elongation is due to subunit addition at the centrosome-distal ends of the interpolar MTs (*29*), and direct studies of both spindle structure (*14, 28*) and of marks bleached on fluorescent spindles (*29*) suggest that the two interdigitating sets of interpolar spindle MTs slide apart as they increase their length by polymerization. These results imply that the zone where centrosomal MTs interdigitate promotes polymer assembly, even while the rest of the spindle is depolymerizing. The results also suggest that there are motors that make the interpolar MTs slide.

Mitotic Processes in Vitro

Isolated spindles. Given the complexity of spindle behavior in vivo, many investigators have tried to develop experimental models of chromosome motion in lysed cells or spindle isolates. Work in the 1970s demonstrated both centrosome-directed assembly of MTs and a labile, anaphase-like chromosome movement in gently lysed mammalian cells (*30*). Improvements in methods for achieving cell lysis have yielded models in which anaphase A appears to be independent of exogenous adenosine triphosphate (ATP), whereas anaphase B requires the addition of this energy source (*31*); as these lysed cells still contained many proteins, however, the result has been difficult to interpret. Spindles truly isolated from clam embryos can now be induced to elongate by the addition of ATP and exogenous tubulin, but this promising system has not been available for long enough to tell us much about mitotic mechanisms (*32*).

The most informative studies on isolated spindles to date have been on anaphase B in a diatom (*33*). These spindles are stable in a variety of isolation buffers and may be activated by the addition of the magnesium salt of ATP (Mg^{2+}ATP). The resulting MT motion is a sliding apart of the interdigitating, interpolar MTs. Addition of exogenous tubulin increases the extent of bundle elongation by extending the spindle microtubules at their centrosome-distal ends, permitting a longer path for the sliding process. Analogous results have been obtained with spindles isolated from a yeast, suggesting that the sliding-polymerization mechanism is not confined to diatoms (*34*).

Isolated chromosomes. Given the apparent importance of kinetochores for many aspects of chromosome motion, much recent work on mitosis in vitro has focused on isolated chromosomes and their interactions with MTs. Kinetochores will initiate MT assembly in vitro (*35*), but unlike the fibers that form in vivo, the resulting MTs are randomly oriented (*36*). The addition of Mg^{2+}ATP to this complex results in kinetochores associated only with the "plus" MT ends (the ends with which they associate in cells), suggesting that kinetochores can translocate toward the plus end of an MT (*37*). This result is puzzling, however, because the predominant MT-dependent movements of chromosomes in both prometaphase and anaphase are toward the "minus" MT end. One possibility is that kinetochores contain a motor that moves over MTs toward their plus ends. Another is that the dissociation rate of MT-kinetochore complexes depends on the part of the MT to which the kinetochore is bound. Dissociation is faster for kinetochores associated with either the minus MT ends or the MT walls than for those associated with the plus MT ends (*36*), raising the possibility that the in vitro motion of kinetochores toward MT plus ends is a result of association-dissociation and MT diffusion to a state from which the dissociation rate is low.

When kinetochore-MT complexes in vitro are diluted so as to reduce the concentration of soluble tubulin, MTs attached to kinetochores by their plus ends shorten, losing subunits from their kinetochore-associated ends (*38*). The resemblance between this movement and anaphase A is striking, but several important issues must still be explored. Chromosome movement during anaphase occurs at a constant speed (*4*), whereas the speed of kinetochore motion toward the MT minus ends in vitro decreases with time (*38*). Further, the anaphase spindle can exert a considerable force on the chromosomes it moves (*13*), but there are as yet no data on the forces developed by the in vitro system.

Analysis of Spindle Components

Structural and physiological studies have characterized many aspects of the mitotic mechanism, but an understanding of mitosis at a molecular level will require the identification and characterization of the proteins that function in spindle behavior.

Biochemical descriptions of spindle components. Biochemical analyses of isolated spindles have been rather uninformative because such isolates are complex and, until recently (*32, 33*), no bulk spindle preparation has been able to move chromosomes. Functional assays for the significance of a spindle-associated component have therefore been impossible, and indirect criteria for assessing the role of a protein in mitosis have been developed.

MTs from mitotic cells can be stabilized with the drug Taxol and used as an affinity matrix for the isolation of proteins that bind to them (*39*). Antibodies against these or other proteins, whose role in mitosis has been either inferred or discovered by chance, can then be used with immunofluorescence to probe antigen localization during

mitosis. Many putative spindle components have been identified this way, but it is not clear that localization in the spindle is good evidence for a role in mitosis. With monoclonal antibodies there is the additional problem that a shared epitope may cause confusion between a known antigen and a true spindle component. For a few molecules there is corroborating evidence from antibody microinjection, drug treatment, or other procedures that the spindle localization is significant (Table 1).

An alternative has been to isolate a spindle component, such as the chromosomes or centrosomes, and then to analyze it. These components are still biochemically complex and such isolations are not easy, so indirect analyses have still been important. Some human autoimmune disorders, such as scleroderma, induce antibodies that react with kinetochores or centrosomes (*40*). These antibodies and a few monoclonal antibodies have begun to help identify proteins in important regions of the spindle (Table 1).

Molecular biology of spindle components. Autoimmune antibodies have provided a route to the cloning of DNA sequences that code for some of the proteins in kinetochores. Kinetochore proteins of ~18, 80, and 140 kD have been identified immunologically in a variety of cell types. The ~18-kD component has some histone-like features (*41*), and its extraction from isolated chromosomes does not greatly affect their ability to initiate MT assembly in vitro (*42*), suggesting that this protein is associated more with chromatin than MTs. The gene for the 80-kD component has been cloned and sequenced (*43*). By immunocytochemistry, the antigen was found to be localized over the centromeric heterochromatin, whereas cross-linking studies suggest that it interacts with tubulin (*42*); its role in kinetochore function is unclear.

An alternate approach to understanding the kinetochore has come from the cloning of centromeric DNA, that is, sequences that confer on a piece of DNA the ability to form a normal spindle attachment and to segregate faithfully (*44*). Yeast centromere sequences are only 120 to 140 bp long. They are conformationally distinct and contain three functional sequence elements, one of which is a conserved 25-bp sequence with internal symmetry that appears to be crucial for binding of kinetochore proteins (*45*). Yeast centromere sequences are specific for yeast cells, but they may prove useful in identifying the components of a functional kinetochore; proteins that bind to them are just now beginning to be identified (*45–47*).

Genetics of mitosis. Genetic analysis has been used to try to unravel the complexities of mitosis (Table 2) (*48*). Although mitotic mutants of fruit flies have been known for years (*49*), only recently has there been progress in going from mutant phenotype to an understanding of some mitotic process (*50, 51*). A major effort on the isolation of cell division cycle mutants in yeast has turned up some strains with specific mitotic defects (*52*), but it appears that the genetic target offered by the whole cell cycle is so large that it is difficult to collect significant numbers of mitotic mutants with this general kind of search. Nonetheless, a gene essential for centrosome separation (*53*) and one important for the regulation of spindle action (*54*) have been identified this way. Studies on *Aspergillus* have taken advantage of the combination of genetic analysis and mitotic cytology that this organism provides to identify several genes in

Table 1. Spindle-associated components.

Spindle component	Functional information	Reference
	Regulatory	
MAP 4	Injection of antibodies to MAP 4 promotes spindle dissolution. *Drosophila* gene has been cloned and sequenced.	(*59*)
Calcium	Injection causes spindle dissolution and anaphase onset.	(*60*)
	EGTA injection prolongs metaphase.	(*61*)
	Injected antibodies against Ca^{2+}-transport proteins cause spindle dissolution.	(*62*)
Calmodulin	Chlorpromazine (non-specific inhibitor of calmodulin) inhibits mitosis.	(*63*)
	Decrease in cellular levels causes growth arrest at metaphase.	(*64*)
62-kD Ca^{2+}-binding protein	Spindle-associated. Phosphorylation by Ca^{2+} calmodulin-dependent protein kinase promotes MT-instability. Antibody injection causes anaphase arrest.	(*65*)
Calpain II (Ca^{2+}-activated protease)	Injection causes metaphase induction, anaphase onset, and spindle dissolution.	(*66*)
cAMP-dependent protein kinase*	Localized to spindle poles by immunofluorescence. Injection of antibodies to catalytic subunit delays anaphase.	(*67*) (*68*)
cdc-2 kinase	Localized at spindle poles; antibody injection arrests cells at G_2/M.	(*69*)
p13 SUC-1	Antibody injection causes mitotic abnormalities and micronuclei formation.	(*69*)
Phosphorylated epitopes	The MPM-2 antibody recognizes phosphoproteins of various molecular sizes. Antigens for this antibody are present in kinetochores, centrosomes, and spindles, but can only be detected during mitosis.	(*21,70*)
Dynein, kinesin	Localized immunocytochemically in some spindles, but not others.	(*71*)
	Structural	
Kinetochore proteins	Various sizes (14 to 140 kD). Identified with CREST antisera.	(*41,72*)
	CENP-A has histone-like features.	(*41*)
	CENP-B possibly binds to tubulin.	(*42,43*)
	CBP-1, a 16-kD centromere-specific DNA-binding protein from *Saccharomyces cerevisiase*.	(*46*)
	CP1, a 57- to 64-kD *S. cerevisiae* centromere-specific DNA-binding protein.	(*47*)
INCENP proteins	Proteins (155, 135, and 38 kD) that migrate from chromosomes to the interpolar spindle during anaphase.	(*73*)
"Matrix" proteins	105- and 95-kD antigens in Chinese hamster ovary cells. Antibody injection causes metaphase arrest.	(*74*) (*75*)
Centrosome	59-kD SPA-1 participates in yeast spindle pole body duplication.	(*56*)
	185- and 66-kD proteins localized immunocytochemically in *Drosophila*. Protein-coding sequences of the 185-kD protein have been isolated.	(*76*)
	Centrin (caltractin), a 20-kD Ca^{2+}-binding protein in *Chlamydomonas*. Cloned and sequenced; shows homology to calmodulin and yeast cdc-31.	(*77*)

*cAMP, adenosine 3′,5′-monophosphate.

addition to tubulin that are essential for mitotic progression (*55*). Several factors that regulate the entry of a cell into mitosis have also been identified, but it is clear that a more focused attack would facilitate the identification of genes important for mitosis itself. Two such approaches have recently been successful: the use of human autoimmune sera to look for cross-reacting components in yeasts (*56*), and the construction of yeast strains that will allow a selection for mitotic-defective mutants (*57, 58*).

Generalizations and Conclusions

Centrosomes are important in spindle formation, because they control MT initiation. They may serve thereafter as anchor points for one end of the polymers they initiate, including those that attach to kinetochores, but once the spindle is established at metaphase, the centrosomes become dispensable. Kinetochores are essential for the attachment of chromosomes to the spindle, and they are capable of both binding to and moving on MTs. They pull the chromosome toward the spindle pole through a connection that will permit both addition and loss of MT subunits. They are therefore the site of mechanochemical processes that relate the forces acting on the MTs to the polymerization reactions that define their length. The interpolar spindle fibers are coupled to the chromosomal fibers at or near the poles, and they provide a framework for chromosome movement by pushing away from the spindle equator with a combination of MT sliding and polymerization. The resulting tension in the kinetochore fibers may contribute to the organization of the chromosomes

Table 2. Genetic analysis of spindle components.

Organism	Gene	Location of gene product	Mutant phenotype and molecular information	Reference
Drosophila				
	mrg	Centrosome	Defective in centrosome replication or separation, resulting in monopolar spindles.	(*50*)
	polo	Centrosome	Defective in structure or function; causes multiple poles.	(*51*)
	asp	Spindle	Abnormal structure. Metaphase arrest. Possibly due to hyperstabilized MTs.	(*78*)
Saccharomyces cerevisiae				
	SPA-1	Spindle pole	56-kD gene product; overexpression correlated with multiple poles; underexpression correlated with growth defects.	(*56*)
	CDC-31	Spindle pole	Duplication defect. Protein has sequence homology to Ca^{2+}-binding domains.	(*53*)
	NDC-1	Unknown	Segregation defect. Possibly due to abnormal chromosomal attachment to spindle apparatus.	(*57*)
	KAR-1	Unknown	Defects seen in spindle pole body replication and chromosome disjunction. Gene encodes a 53-kD protein required for normal MT function.	(*54*)
	TOP-2	Chromosome	Topoisomerase II; necessary for chromosome disjunction.	(*79*)
Schizosaccharomyces pombe				
	nuc-2+	Nuclear scaffold	Defective in spindle elongation. 76-kD protein.	(*80*)
	dis	Unknown	Unequal chromosome distribution. Possibly affects disjunction, spindle function, or both. *dis 2* encodes a 37-kD protein with strong homology to protein phosphatase type 1.	(*81*)
	bws 1+	Unknown	Identified as a bypass of *wee* suppression. Gene sequence is identical to *dis 2* (see above). Encodes a putative protein phosphatase.	(*82*)
	cdc-2	Spindle, centrosomes	34-kD protein kinase necessary for mitosis.	(*69*)
Aspergillus				
	BENA33	Microtubules	β-tubulin mutation resulting in metaphase arrest. Possibly hyperstabilizes MTs. Spindle has normal appearance.	(*83*)
	BIM-G	Unknown	BIM-G11 mutation blocks anaphase spindle elongation and chromosome separation and causes hyperphosphorylation of nuclear structures. Gene product shows strong homology to protein phosphatase type 1.	(*84*)
	BIM-7	Unknown	UV and temperature-sensitive 706 mutant is anaphase defective.	(*85*)
Caenorhabditus elegans				
	lin 5	Unknown	Chromosomes fail to undergo anaphase movements, possibly due to lack of spindle attachment.	(*86*)
Mouse				
	OS	Unknown	Oligosyndactyly mutation causes metaphase arrest in blastula stage. Spindles are intact but possibly show reduced phosphoprotein staining with the MPM-2 antibody (see Table 1).	(*87*)

during prometaphase. When the chromosomes divide at anaphase onset, both the disassembly of kinetochore-associated MTs and the elongation of the interpolar spindle become harnessed to chromosome movement. The molecules that make all these connections and serve as motors for these movements are yet to be identified.

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