14
Mitotic Spindle Motors

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14.1
Microtubules, Motors and Mitosis

Mitosis, the process by which identical copies of the replicated genome are distributed to the daughter products of each nuclear division, depends upon the action of the mitotic spindle, a protein machine that uses microtubules (MTs) and MT-based motor proteins to assemble itself and to segregate sister chromatids (Karsenti and Vernos, 2001, Mitchison and Salmon, 2001, Wittman et al., 2001). Spindle morphogenesis begins during prophase and pro-metaphase when MTs, motors, chromosomes and centrosomes interact and self-organize into a bipolar structure (Fig. 14.1) which by metaphase consists of pairs of sister chromatids aligned on the spindle equator facing opposite spindle poles. During the subsequent anaphase, sister chromatids are moved to opposite poles while the poles themselves move further apart and finally, during telophase, the nuclear envelope re-assembles around the segregated sisters. The movement of chromosomes and the positioning of spindle poles throughout mitosis depend upon mitotic motors, proteins that use nucleotide hydrolysis to generate force and directed motion. Mitotic motors include polymerizing and depolymerizing MTs that exert pushing and pulling forces, respectively as well as some members of the dynein and kinesin families, which generate force in the spindle by stepping along the MT polymer lattice. Here we discuss general principles of force generation by dynamic MTs and motor proteins and their deployment in the spindle. We do not present a comprehensive review of the recent literature on mitotic motors which is covered in other reviews (Banks and Heald, 2001, Brunet and Vernos, 2001, Heald, 2000, Hidebrandt and Hoyt, 2000, Sharp et al., 2000b).

MTs are the major cytoskeletal filaments of the spindle and the structural organization of spindle MTs has been elucidated using careful electron microscopic analysis which reveals that spindle MTs comprise two overlapping radial arrays emanating from spindle poles with their plus ends distal, forming the astral, kinetochore and interpolar MT bundles (McIntosh and McDonald, 1989; Fig. 14.1). Spindle MTs use GTP hydrolysis to facilitate two types of dynamic behavior, dy-

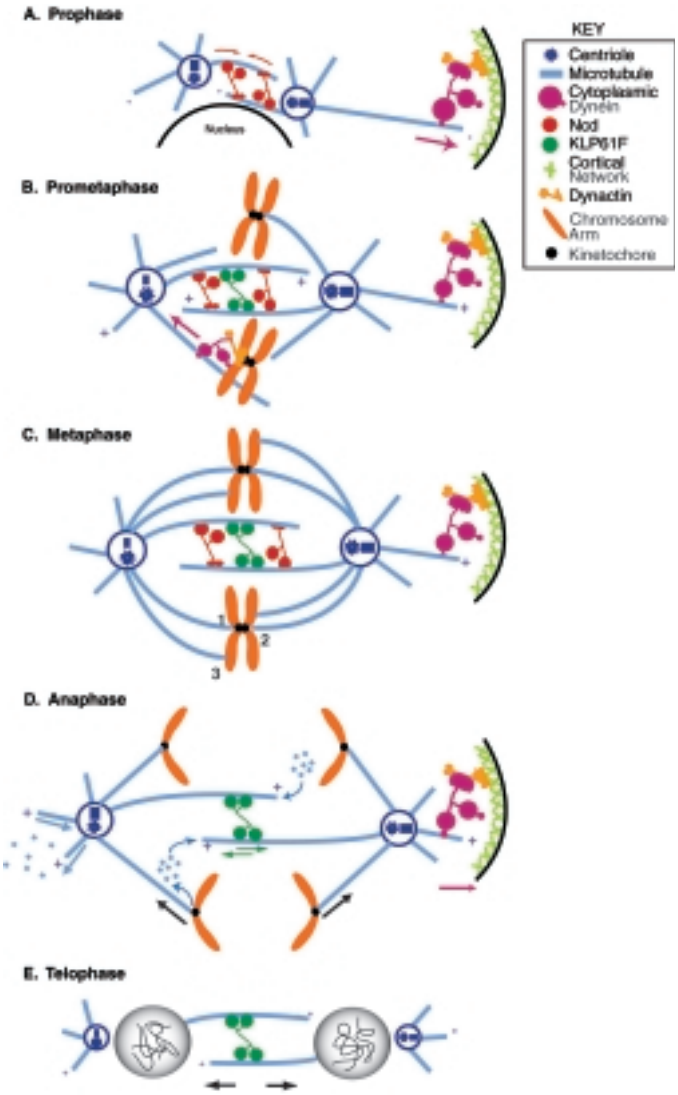

Figure 14.1. Events of mitosis. (A) Prophase. Dynein motors pull on astral MT generating outward force. Ncd motors cross-link interpolar MTs and develop inward force. The sum of the forces drives the centrosomes apart. (B) Prometaphase. Both Ncd, and bipolar kinesin motors cross-link the interpolar MTs. The chromosome is captured by the MT polymer and transported poleward (possibly, by dynein motors). (C) Metaphase. The chromosomes are aligned at the 'equator'. This alignment is the result of dynamically coupled forces generated at the sister kinetochores (1 and 2) and of the polar ejection forces developed at the MT plus end/chromokinesin complexes at the chromosome arms (3). (D) Anaphase. Segregated chromosomes are pulled poleward by a force generated at the kinetochores and coupled to MT's plus ends disassembly. At the same time, interpolar MTs undergo poleward flux disassembling at the minus ends and polymerizing at the plus ends. (E) Telophase. Chromosomes are separated, and nuclear envelopes form.

namic instability in which MTs grow and shrink by polymerizing and depolymerizing at their plus ends, and poleward flux in which MT plus-ends facing the spindle equator polymerize while their minus ends located at the poles depolymerize. It is clear that the dynamic properties of MTs are critical for spindle morphogenesis and also for generating forces for mitotic movements (Inoue and Salmon, 1995).

Spindle formation and function also depends upon the action of multiple MT-based motor proteins, enzymes that couple ATP hydrolysis to the generation of force and motion relative to MT tracks (Sharp et al., 2000a). It is clear that these motors act by a variety of mechanisms to coordinate chromosome movements, acting for example by a 'sliding filament mechanism' and sliding adjacent MTs in relation to one another, driving the intracellular transport of chromosomes or vesicles along MTs, modulating the dynamic properties of spindle MTs, or regulating progression through mitosis by acting as components of the spindle assembly checkpoint.

Given this rich repertoire of MT and motor functions in the spindle, a current challenge is to understand how the activities of the individual components are coordinated to produce a precision machine capable of segregating chromatids with the fidelity observed in cells. In this review we discuss current ideas about the physical nature of mitotic movements, mechanisms of force generation by MT dynamics and motor proteins in the spindle, and how these force-generating elements are deployed in the spindle to produce this impressive protein machine.

14.2
The Physical Nature of Mitotic Movements

Mitotic movements occur at the microscopic scale under conditions, where viscosity (Purcell, 1977) and thermal fluctuations (Berg, 1983, Mogilner et al., 2002) play dominant roles. Given the stochastic character of physical processes occurring at this scale, the reliability and precision with which the mitotic spindle operates is quite remarkable.

The spindle machinery operates in an aqueous environment. A water molecule is about 0.1 nm in radius, while globular proteins are two orders of magnitude larger. This size difference suggests that the fluid can be treated as a continuum. When an object of size l is moving through the fluid with velocity u, the fluid acceleration around the object is characterized by the term $\rho(du/dt)$, where ρ is density, and t is time, and the viscous drag on the object has the magnitude $\eta(d^2u/dx^2)$, where η is the dynamic viscosity, and x is the spatial coordinate. In this situation, l and $t = l/u$ are the characteristic spatial and temporal scales, respectively, and the orders of magnitude of the inertial and viscous forces on the fluid are $(\rho u^2/l)$ and $\eta(u/l^2)$, respectively. The ratio of these forces is the dimensionless Reynolds number: $\mathrm{Re} = ul/\nu$, where $\nu = \eta/\rho$ is the fluid *specific* viscosity (in water, $\nu \sim 10^6\ \mu m^2\ s^{-1}$). The characteristic size and rate of movement in mitosis are $l \sim 1\ \mu m$ and $u \sim 1\ \mu m\ s^{-1}$, so mitosis is characterized by very low Reynolds numbers: $\mathrm{Re} \sim 10^{-6}$ (see Tab. 14.1). In this limit, the viscous force is dominant, while the inertial force is negligible. In a sense, spindle movements are governed by Aristo-

Table 14.1 Physical parameters of the spindle environment.

Symbol	*Definition*	*Value and unit*
Re	Reynolds number	$\sim 10^{-6}$
η_c	Effective viscosity of the cytoplasm	100-300 cP ($0.1–0.3$ pN·s/μm^2)
m	Characteristic size of a molecular motor protein	~ 10–100 nm
r_{ch}	Radius of the chromosome	~ 0.2 μm
l_{ch}	Length of the chromosome	~ 6 μm
ζ_{ch}	Viscous drag coefficient of the chromosome	~ 10 pN·s/μm
D_{ch}	Effective diffusion coefficient of the chromosome	$\sim 10^{-4}$ μm^2/s
δ	Size of tubulin dimer	~ 8 nm
d_m	MT diameter	~ 25 nm
l_m	Characteristic average length of MTs	~ 10 μm
N_s	Characteristic number of spindle MTs	~ 100
N_f	Characteristic number of MTs in kinetochore and interpolar fiber	10–20
τ	MT turnover time in mitosis	20–60 s
V_{mt}	Characteristic rates of MT growth	10–50 μm/min
ΔG	Strain energy stored in the MT lattice from the GTP hydrolysis	~ 26 pN·nm/dimer
F_m	Characteristic force that can be generated by a single MT or motor	~ 1–10 pN
F_{pr}	Characteristic outward (dynein) and inward (Ncd) forces in prophase	~ 10–100 pN
T	Characteristic duration of various events in mitosis	Minutes
N_{ch}	Number of chromosomes	~ 10
F_{ej}	Polar ejection force per MT polymer	~ 1 pN
F_k	Poleward kinetochore force	0.1 pN (min), 100s pN (max)
V_{pol}	Characteristic rate of poleward movement in anaphase	1–4 μm/min
V_{mot}	Characteristic rates of motor transport	10–50 μm/min
λ_{mt}	MT persistence length	1–5 mm
λ_{ch}	Chromosome persistence length	10–100 μm
C	Tubulin concentration	10–20 μM
k_BT	Thermal energy	~ 4 pN·nm

telian mechanics ($F \propto u$), rather than by Newtonian physics ($F \propto du/dt$): the velocity (not the acceleration) of motion is proportional to the applied force.

At low Reynolds numbers, the viscous force F_v resisting the object's motion is proportional to the velocity according to Stokes's formula: $F_v = -\zeta u$. Here the viscous drag coefficient is $\zeta =$ (geometric factor)$\cdot \eta l$ in the case of a spherical object with radius l, $\zeta = 6\pi \cdot \eta l$, whereas for a cylinder of length l and radius r moving sidewise, $\zeta = 4\pi \cdot \eta l/(\ln(l/r) + 0.5)$. For small proteins, the cytoplasm appears aqueous, and its effective *microscopic* viscosity is close to that of water, $\eta \sim 1$ cP $= 10^{-3}$ pN s^{-1} μm^{-2} and thus for a motor protein of characteristic size $l \sim 10$ nm, $\zeta_{pr} \sim 6\pi \cdot \eta l \sim 10^{-4}$ pN s^{-1} μm^{-1}. For objects the size of a *Drosophila* chromosome (radius of arm, $r_{ch} \sim 0.2$ μm and length $l_{ch} \sim 6$ μm (Marshall et al., 2002)), the effective cytoplasmic viscosity arises mainly from cytoskeletal deformation rather than aqueous shearing, and the corresponding *macroscopic* viscosity is two orders of magnitude greater, than that of water, $\eta_c \sim 200$ cP $= 0.2$ pN s^{-1} μm^{-2} (Alexander and Rieder, 1991, Marshall et al., 2002). Consequently, if the chromosome is pulled sidewise, its effective drag coefficient is $\zeta_{ch} \sim 10$ pN s^{-1} μm^{-1}.

A protein moving through the fluid is acted on by frequent and uncorrelated momentum impulses arising from the thermal motions of the fluid. This leads to a 'random walk', when the protein makes extremely frequent and short steps (of length ~ 0.01 nm and duration $\sim 10^{-13}$ s (Mogilner et al., 2002)) in a random direction. The resulting *Brownian movement* is equivalent to the diffusion of the protein. The corresponding diffusion coefficient is given by the *Einstein Relation*: $D = k_BT/\zeta$. Here k_BT is the so-called thermal energy that serves as a gauge of energy in the microscopic world. At the temperature of a spindle, $k_BT \approx 4$ pN nm, the diffusion coefficient of a globular protein is $D_p \sim 10$ μm^2 s^{-1}, and that of a chromosome is $D_{ch} \sim 10^{-4}$ μm^2 s^{-1}.

In the intracellular world, diffusion is very effective over small time intervals and short distances, while forced drift is more effective at greater times and longer distances. The reason for this effect is that the distance traveled by diffusion grows as a square root of time, $d \sim \sqrt{Dt}$, unlike the drift increasing proportionally with time, $d \sim ut$. Thus for a globular protein it would take ~ 10 s to move randomly over 10 μm – faster than if it were moved the same distance by a unidirectional molecular motor. However, for larger objects, this is not so. A chromosome would need a few days to move the same distance ($t \sim (10\ \mu m)^2/10^{-4}$ μm^2 s^{-1})! Clearly, the chromosomes cannot move effectively by diffusion in the course of mitosis (whose characteristic time is minutes), and instead the biased walk of mitotic motors underlies the movement of chromosomes and spindle poles.

14.3
MT Polymerization and Depolymerization as Mitotic Motors

Spindle MTs are built from αb-tubulin heterodimers each containing two molecules of GTP, one of which is concealed in the α-subunit while the other is bound to the β-subunit and exposed to water. Within the MT polymer lattice,

these subunits are organized into a helical B-lattice of usually 13 protofilaments (pf) that form a hollow tube of diameter 25 nm with a discontinuity, or seam, between two of the 13 pfs (Nogales et al., 1999). Along a pf, all subunits point in the same direction (αβ-αβ-αβ) which gives the MT a structural polarity and by convention the β-tubulin end is called the plus end while the α-tubulin end is called the minus end. This structural polarity is crucial for MT function because the two ends have different polymerization kinetics with the plus ends polymerizing and depolymerizing faster than the minus ends, and because it constrains the directionality of kinesin and dynein motors which move unidirectionally along the polymer lattice.

The polymerization and depolymerization of spindle MTs coupled to GTP hydrolysis underlie dynamic instability and flux (Desai and Mitchison, 1997, Mitchison, 1989, Mitchison and Kirshner, 1984a,b). Flux is the term used to describe the movement of tubulin subunits from MT plus-ends facing the spindle equator to the MT minus-ends facing the poles and is thought to depend upon MT polymerization at the plus ends and MT depolymerization at the minus ends being coupled to poleward translocation of the polymer lattice. Dynamic instability describes the behavior of the ends of individual MTs which alternate between phases of polymerization (growth) and depolymerization (shrinkage) with the transitions from growth to shrinkage being termed *catastrophes*, and the converse transitions being termed *rescues*. Thus, dynamic instability is characterized by four parameters: rates of growth and shrinking and frequencies of catastrophes and rescues. While individual MTs are engaged in this stochastic behavior, a population of MTs can be described by its length distribution and average number as determined using these parameters together with the effective nucleation rate (Dogterom and Leibler, 1993). In the spindle, MTs are nucleated on γ-tubulin ring complexes located in the centrosome of amphiastral spindles (Schiebel, 2000). The α-ends of tubulin heterodimers bind to the γ-tubulin ring complexes and consequently the plus ends grow radially outward and display dynamic instability.

The end of a growing MT can act as a motor that generates a pushing force. For example, experiments with MTs growing inside liposomes showed that polymerization of MTs can generate enough force to deform the membrane (Fygenson, 1995, Hotani and Miyamoto, 1990). More recently, Dogterom and Yurke (1997) showed that MTs polymerizing against the wall of a chamber could generate a pushing force of several pN. Thus in the spindle, a centrosome-nucleated MT could, in principle, polymerize at its distal plus end and exert a force that pushes a chromosome away from the pole, during pro-metaphase congression, for example. Theoretical modeling has demonstrated that such pushing forces can be explained by a thermal ratchet mechanism (Mogilner and Oster, 1999, van Doorn et al., 2000).

According to the elastic polymerization ratchet model, a MT growing against an obstacle is involved in Brownian motion and consequently it undulates and bends very frequently. When the MT is bent, a gap appears between its tip and the obstacle. If a large enough gap persists for a sufficiently long time interval, a tubulin dimer can intercalate into the gap and assemble onto the tip of the growing poly-

mer. This increases the MT's length so when the longer polymer's tip contacts the obstacle again, the MT remains bent and the corresponding elastic force pushes the obstacle forward. The energy for this pushing force is supplied by the binding free energy of GTP dimers that associate at the growing tip of the MT and is used to rectify the Brownian motion of the tip. Strictly speaking, the force is generated by thermal fluctuations of the MT, and the binding free energy is used to rectify its thermal bending.

If a MT assembles against no resistance, then its elongation rate is simply $V_{mt} = \delta(k_{on}C - k_{off})$, where $\delta = 8$ nm/13 $\sim$ 0.6 nm is the MT length increment associated with the addition of a dimer to the tip of one of 13 pfs, C is GTP tubulin concentration, and k_{on} and k_{off} are the subunit association/dissociation rates, respectively. Detailed statistical physical analysis (Mogilner and Oster, 1999) shows that the thermal bending undulations of the fiber are much faster than the process of dimer assembly. When a MT polymerizes against a load force F_L which resists MT growth, the effective association rate $k_{on}C$ is modified by a probability $p(F_L, \delta)$ of there being a gap of width δ or greater between the MT tip and the obstacle: $V_{mt} = \delta(k_{on}C\ p(F_L, \delta) - k_{off})$. This equation with the specified function $p(F_L, \delta)$ gives the force-velocity relation for a polymerizing MT pushing against a load. At low loads the MT can polymerize rapidly, but as the load force increases it will slow down the rate of growth, until polymerization stalls. The stall force corresponds to the maximal pushing force achieved when growth is stopped. Near the stall, the probability function is given by the Boltzmann factor: $p(F_L, \delta) \sim \exp[-F_L\ \delta/k_BT]$. Thus the elongation rate decreases exponentially with increasing load, and the stall force can be found from the balance of the effective association and dissociation rates: $F_s \sim (k_BT/\delta)\cdot\ln[k_{on}C/k_{off}]$. The order of magnitude of the pushing force is common for all thermal ratchet mechanisms and is equal to the thermal energy divided by the step of polymerization, $F_s \sim k_BT/\delta \sim 6.5$ pN. The logarithmic factor is of the order of unity in a wide range of the system's parameters, so one MT fiber can generate the pushing force from a few to tens of pN.

While polymerizing MTs can generate a pushing force, depolymerizing MTs can develop pulling forces. For example, using *in vitro* assays, Coue et al. (1991) observed that the depolymerizing ends of MTs could pull particles at rates of almost 1 μm s^{-1} against estimated viscous forces of $\sim$ 10 pN, and subsequently Lombillo et al. (1995a) found that plastic beads coated with plus end-directed MT motors remain attached to the plus ends of depolymerizing MTs and are carried towards the MT minus ends as the polymer shortens. These results suggest that, in the spindle, it is possible that plus end-directed MT motors on the kinetochore could attach to the plus end of a depolymerizing centrosome-bound MT during anaphase in a way that allows the depolymerizing MT to pull the chromosome to the pole.

The nature of the pulling force associated with MT depolymerization remains elusive. The earliest theory by Hill (1985) suggested that the tip of the depolymerizing MT is associated with a docking protein having the form of a sliding collar that allows tubulin dimers to dissociate freely from the MT tip. The interior of this 'collar' has high affinity for the MT lattice and consequently when subunits dissociate from the tip of the MT, the binding free energy gradient drives the dock-

ing protein toward the MT minus end producing a 'pulling' force. Another possibility is the 'conformation wave' model proposed by Mitchison (1988), in which the elastic force from the pfs curving outward at the disassembling plus end can drive the sliding collar toward the minus end. Finally, Peskin and Oster (1995) developed a quantitative model in which a bead coated with high affinity tubulin-binding proteins undergoes diffusion along the MT polymer lattice. The binding energy gradient prevents the bead from detaching from the plus end of the MT and as it rolls it weakens the bonds between neighboring tubulin dimers and facilitates depolymerization.

All such models allow estimates of the order of magnitude of the pulling force developed by MT depolymerization. The origin of this force is very likely a combination of the thermal ratchet mechanism (Brownian motion of a docking protein ratcheted by disassembly at the end of the MT) and the protein elasticity associated with a conformational change in tubulin. The former component can be estimated as $k_BT/\delta \sim 6.5$ pN modified by the logarithmic factor of order 1, and the latter can be estimated from the above by the strain energy stored in the MT lattice from the GTP hydrolysis, $\Delta G \sim 26$ pN nm/dimer (Inoue and Salmon, 1995), divided by δ: 26 pN nm/(8 nm/13) $\sim$ 45 pN. So, both pushing polymerization and pulling depolymerization forces can range from a few pN to a few tens of pN per MT fiber. These forces are comparable in magnitude to those generated by kinesin and dynein motors, and they are likely to play significant roles in driving mitotic movements.

14.4
Kinesins and Dyneins as Mitotic Motors

Natural selection has created motor proteins that have specialized motor domains capable of converting chemical energy into the generation of force and movement (Howard, 2001, Mogilner et al., 2002). Many of these molecular motors walk vectorially along MT tracks using nucleotide hydrolysis as a fuel and thereby generate forces for mitotic movements (Brunet and Vernos, 2001, Hildebrandt and Hoyt, 2000, Sharp et al., 2000b,c).

The generation of force and motion by molecular motors is thought to depend on a mechanical cycle consisting of the *power stroke* in which the bound motor domain changes its conformation and generates force, alternating with the *recovery stroke* when the motor domain detaches from the MT and undergoes a diffusive search for the next binding site on the track. This produces a biased random walk whose directionality is determined by the polarity of the MTs and stereospecificity of the motor's binding to the MT. Tight coupling of these events to a cycle of hydrolysis make the resulting mechanochemical cycle irreversible and unidirectional, so that motors move either towards the plus or the minus ends of MT tracks, corresponding to movement towards or away from the spindle poles. A striking property of molecular motors that distinguishes them from macroscopic motors is the overwhelming importance of thermal fluctuations. For this reason,

all motor proteins must be regarded as 'Brownian machines' in which force generation and movement depend on Brownian motion as well as the elastic (and other physical) forces associated with the power stroke.

The forces developed by individual molecular motors near stall can be estimated using thermodynamic arguments similar to those used above to estimate the magnitude of forces generated by MT polymerization and depolymerization. The ratchet part of the force is of the order of $k_BT/\delta \sim 1$ pN, where $\delta \sim 8$ nm is now the size of the motor's 'step'. The active power stroke force is limited from the above by the energy of ATP hydrolysis, $\Delta G_h \sim 80$ pN nm, divided by the step size: $\Delta G_h/\delta \sim 10$ pN. Thus, molecular motors can develop forces in the range of a few pN. The rate of free movement of the molecular motors is limited by the rates of the associated ATP hydrolysis cycle and the time of the recovery stroke. Normally, ten to hundreds of cycles/steps take place every second, so the motors advance at $\sim 0.1-1$ μm s^{-1}.

The motor's displacement along the MT track is not steady, because stochastic processes of chemical reactions and searches for binding sites govern the steps of the mechanochemical cycle. One quantity that can be monitored as the motor advances is the variance of its displacement, $\mathrm{Var}[x(t)]$ about the mean, $\langle x(t)\rangle = Vt$. Normally, the variance grows linearly with time: $\mathrm{Var}[x(t)] = 2D_{\mathrm{eff}}\, t$, where D_{eff} is the effective diffusion coefficient of the motor. The greater the number of reaction processes associated with each mechanochemical cycle, the less random the motor's 'walk' becomes. In addition to the force–velocity relations and statistical properties, motor proteins are characterized by the duty ratio, which is the fraction of time when the motor domains are bound to MTs and developing force (or, roughly speaking, ratio of the time of the power stroke to the time of the cycle). Most of these relations and parameters have not been determined for mitotic motors.

Several members of the kinesin and dynein families function as mitotic motors (Brunet and Vernos, 2001, Dujardin and Vallee, 2002, Goldstein, 2001, Hildebrandt and Hoyt, 2000, Hirokawa et al., 1998, Holzbaur and Vallee, 1994, Karki and Holzbaur, 1999, Sharp et al., 2000a, Vale and Fletterick, 1997). The founding member of the kinesin family, conventional kinesin (Howard, 1996), is an intracellular transport motor capable of traveling long distances along a microtubule without dissociating (Howard et al., 1989) and which may drive transport along spindle fibers. It has two heads – motor domains – at the N-terminus of the heavy chain and moves in a fascinating head-over-head fashion toward the plus end: while one head is attached, another is 'searching' for the next binding site in the plus-end direction. Kinesin-related proteins share the same highly conserved motor domain but outside the motor domain they differ. Motors that have N-terminal motor domains (KIN-N) move towards the plus end of MTs, those with C-terminal motor domains (KIN-C) move towards the minus end, whereas motors from the KIN-I subfamily which contain internally located motor domains seem to destabilize MT ends (Desai et al., 1999, Schroer, 2001). Dyneins are structurally unrelated to kinesins, but they also use ATP hydrolysis to move (in a minus-end direction) and generate force. The dynein motor domain consists of six AAA (ATPases) domains only one of which hydrolyzes ATP (King, 2000), and a short but structurally

complex stalk that contains MT-binding sites (Gee et al., 1997). Dynein can produce force by a conformational change in the head applying tension to the rigid stalk and pulling on the microtubule. Alternatively, the stalk might rotate about a fulcrum located within the head, like a windshield wiper (Gee et al., 1997).

Mitotic motors act by a variety of mechanisms (Brunet and Vernos, 2001, Heald, 2000, Sharp et al., 2000a). For example, it has long been proposed that force is generated in the spindle by motor-driven MT sliding (McIntosh et al., 1969, Scholey et al., 2001, Sharp et al., 2000a), and accordingly, members of the plus-end-directed bipolar (BimC) family of kinesins oligomerize to form bipolar homotetramers with motor domains positioned at opposite ends of a central rod, that are thought to be capable of cross-linking MTs throughout the spindle and sliding apart anti-parallel MTs within interpolar MT bundles (Sharp et al., 1999). On the other hand, members of the minus-end-directed C-terminal kinesin family form homodimers, containing C-terminal motor domains, linked by a coiled-coil rod to a tail that contains nucleotide-insensitive MT binding sites. Thus, C-terminal kinesins are also thought to be capable of cross-linking and sliding adjacent MTs (Karabay and Walker, 1999), possibly acting on interpolar MTs to draw the poles together (Sharp et al., 2000a). Similarly dynein appears to be capable of sliding MTs relative to adjacent MTs or relative to cortical actin filaments, exerting pulling forces on spindle poles (Dujardin and Vallee, 2002).

There also exist mitotic motors that transport chromosomes as cargo along the surface lattice of spindle MTs. For example, the plus-end-directed motor, CENP-E and the minus-end-directed motor, dynein, both localize to kinetochores, and are likely to participate in chromosome congression and segregation (Sharp et al., 2000b, Savoian et al., 2000, Yucel et al., 2000). Several presumptively plus-end-directed motors, collectively referred to as chromokinesins, bind to chromosome arms as cargo, where they are thought to provide forces that push chromosomes towards the metaphase plate (Brunet and Vernos, 2001) and some intracellular transport proteins appear to move vesicles and protein complexes along spindle MTs (Section 14.8).

Finally it is clear that mitotic motors can regulate spindle MT assembly dynamics. For example, the KIN-I motor, XKCM1/MCAK, localizes to kinetochores where it is thought to induce disassembly of kinetochore MTs (Desai et al., 1999) whereas the orphan motor, CENP-E is thought to be able to use its plus-end-directed motor activity to anchor kinetochores to the shortening plus ends of MTs during anaphase (Lombillo et al., 1995b). Thus XKCM1/MCAK motors could induce the shortening of kinetochore-to-pole MTs during anaphase, and this could work in concert with the plus-end anchoring activity of CENP-E to transduce MT shortening into poleward forces on chromosomes.

The diversity of mitotic motors can be appreciated by considering a single organism, *Drosophila melanogaster* which has 36 MT-based motors and of these, 11 are strong candidates for being mitotic motors (Table 14.2). Cytoplasmic dynein, which is localized to cortical structures and kinetochores in syncytial blastoderms, has been implicated in spindle pole positioning, poleward chromosome movements and the transport of checkpoint proteins (Savoian et al., 2000, Sharp et al.,

Table 14.2 Mitotic motors in *Drosophila* embryos.

Motor Protein	*Cytogenetic position*	*Structure*	*Function*
1. Cytoplasmic dynein	64C		Pole–pole separation; poleward chromosome motion; checkpoint
2. Bipolar kinesin, KLP61f	61F		Cross-linking spindle MTs; pole–pole separation
3. C-terminal kinesin, Ncd	99C		Cross-linking spindle MTs; pulling poles together; pole organization
4. Pav KLP (MKLP1)	64B	?	Mid-zone organization; cytokinesis
5. KLP 3A	3A	?	Pole–pole separation; nuclear positioning; mid-zone organization
6. CENP-meta 7. CENP-ana	32E		Checkpoint; coupling to depolymerizing MTs; congression
8. KIN-I: KLP59C (XKCM1) 9. KIN-I: KLP10A	59C 10A		• Depolymerizing kinetochore's fibers at the kinetochores • Depolymerizing astral MTs at spindle poles
10. Chromokinesin: KLP54D 11. Chromokinesin: KLP38B	54D 38B	?	• Antipolar chromosome movement • Pole–pole separation

2000a,d, Wojcik et al., 2001). The bipolar kinesin, KLP61F and the C-terminal kinesin, Ncd are thought to cross-link MTs within interpolar MT bundles in embryonic spindles, where they generate antagonistic outward and inward forces on spindle poles (Sharp et al., 1999, 2000a). Two other kinesins, KLP3A and PavKLP localize to the spindle inter-zone (Adams et al., 1998, Williams et al., 1995), where they may also associate with interpolar MT bundles and contribute to the generation of forces that position spindle poles. Like dynein, the kinesins, KLP59D, KLP10A (both KIN-I motors), CENP-meta and CENP-ana, are candidates for being kinetochore motors (Desai et al., 1999, Yucel et al., 2000). There are three chromokinesins that are candidates for driving the transport of chromosome arms towards the spindle equator (Brunet and Vernos, 2001) namely KLP38B, KLP54D and Nod. Of these, Nod appears to function specifically in the female oocyte meiotic spindle, and there is no evidence for a mitotic role.

14.5 Functional Coordination of Mitotic Motors

An important issue facing mitosis researchers is why the mitotic spindle uses so many motors. Our current view is that cells use multiple mitotic motors in parallel to generate a delicate balance of complementary and antagonistic forces (Hoyt and Gieser, 1996, Sharp et al., 2000a). These ideas emerged initially from elegant genetic studies carried out in yeast, but they have recently been extended and refined by exploiting the *Drosophila* embryo, where it is possible to visualize and quantify specific mitotic movements at high temporal and spatial resolution in the presence and absence of specific motor inhibitors (Hildebrandt and Hoyt, 2000, Sharp et al., 2000a). The results argue that specific mitotic movements are not driven by individual mitotic motors acting alone, but instead depend on shifts in the balance of forces generated by multiple motors. For example, in *Drosophila* embryos, when spindle pole positioning is measured as a function of time, pole separation proceeds in a complex fashion, with stops, starts and rate changes that are thought to reflect changes in the net force acting on the poles, and these net forces in turn reflect the action of multiple MT-motors that serve to position the poles (Sharp et al., 2000a). When these forces balance one another, spindle pole spacing is maintained under isometric tension in a quasi-stable steady state structure. During mitosis, the spindle appears to pass through a series of these steady-state structures, at which points multiple complementary and antagonistic motors precisely balance one another. Transitions from one steady state to the next are thought to reflect the up- or downregulation of subsets of motors, which alters the net force acting on the spindle poles, allowing a specific mitotic movement that is visible as a change in the spacing of the spindle poles. We refer to this model as the multiple motor-dependent transient steady state model for spindle pole positioning (Sharp et al., 2000a).

Transient motor-generated steady-state structures may also serve as a mechanism for chromosome positioning during chromosome capture, congression and segregation. Progression through this pathway could plausibly be signaled by the density and polarity patterns of MTs surrounding chromosome arms and kinetochores. The response of a chromosome to its position would, in turn, be determined by the relative strength of the poleward versus plateward forces generated by motors positioned on these structures together with MT polymerization–depolymerization dependent forces. Thus, chromosomes would always tend to move toward a specific steady state or balance position, which could be altered at specific stages of the cell cycle by alterations in the activity of specific motors as well as by subtle changes in spindle and chromosome structure. For example, it is easy to imagine how a balance of kinetochore motor-, chromokinesin- and MT polymerization–depolymerization-generated forces could position chromosomes on the equator during the metaphase steady state, but testing this hypothesis and discerning the details require more work (Kapoor and Compton, 2002; Section 14.6.2).

14.6. Motor Action and Force-Generation during Mitosis

Spindle formation and chromosome segregation (Fig. 14.1) involves a complex interplay between dynamic MTs and motor proteins associated at spindle poles, kinetochores and chromosome arms, and is likely to be dependent on forces generated by both MT polymerization–depolymerization and by molecular motor action (Banks and Heald 2001, Brunet and Vernos, 2001, Heald, 2000, Hunter and Wordeman, 2000, Inoue and Salmon, 1995, Scholey et al., 2001, Sharp et al., 2000a). In what follows, we discuss the motor and MT-related mechanisms in mitosis.

14.6.1 Mitotic Motors and Spindle Formation at Early Stages of Mitosis

During interphase, MTs are nucleated from γ-tubulin ring complexes associated with the centrosome, forming a single radial array (Schiebel, 2000). A subset of these MTs have their minus ends anchored on the centrosome, while others appear to be released by katanin-dependent severing, but maintain a physical association with centrosomes via MT cross linking motors (Heald 2000, Merdes et al., 1996).

The formation of a bipolar spindle is associated with an increase in the dynamic instability properties of MTs (Salmon et al., 1984), the migration of the two centrosomes around the nuclear envelope (Sharp et al., 2000a) and the organization of centrosome-associated MTs into a bipolar array (Karsenti and Vernos, 2001). Motor proteins play important roles in many aspects of this self-organization process, although the details appear to vary between systems (Heald et al., 1997, Karsenti and Vernos, 2001, Sharp et al., 2000a). For example, in *Drosophila* embryos during interphase–prophase, centrosomes separate around the surface of the nuclear envelope at an initial fast rate that slows down as the poles separate until the centrosomes come to lie a few microns apart on opposite sides of the nucleus where they are maintained for 2–3 min in the 'prophase steady state' (Sharp et al., 2000a). Function inhibition experiments suggest that these events depend upon antagonistic outward and inward forces generated by cortical dynein and interpolar Ncd, respectively (Fig. 14.1A).

The following model can explain these experiments quantitatively (Cytrynbaum et al., unpublished data). In this model the outward force responsible for the initial fast rate of pole separation is due to cortical dynein generating a few tens of pN pulling force on a subset of astral MTs together with a pushing force due to polymerization of interpolar MTs. As the centrosomes separate towards opposite sides of the nucleus, this outward force becomes directed almost perpendicular to the surface of the nuclear envelope, and its projection onto this surface decreases. The decreasing outward force is opposed by an increasing inward force generated by Ncd motors that cross-link MTs in the interpolar MT bundle between the poles. Assuming there are about a dozen Ncd motors per micron, ~ 10 pN inward force would be generated per micron of interpolar bundle, and the total inward force would grow in proportion to the overlap between the interpolar MTs which in

turn increases as the poles separate. At a few microns separation, the outward and inward forces equilibrate, explaining the stable steady state.

One important question concerns the precision and robustness of pole separation. During the prophase steady state (as well as those occurring at other stages of mitosis), the fluctuations in the interpolar distance are of the order of only a few percent. According to probabilistic arguments, if, on the average, N MTs reach the cell cortex, then the average fluctuations in this number of MTs is of the order of $\sqrt{N}$. Therefore the relative fluctuations of the outward force and consequently the relative fluctuations of the separation distance between the poles, would be $\sim 1/\sqrt{N}$. For example, a 3 % fluctuation corresponds to $N \sim 1000$ MTs which is far greater than the number of MTs observed. This could indicate that additional control mechanisms maintain the spindle dimensions. Further quantitative research is needed to address the problem of the precision of spindle morphogenesis.

14.6.2
Mitotic Motors and Force Generation in Prometaphase–Metaphase

Following nuclear membrane fenestration there is another pause, the pro-metaphase steady state, followed by an episode of pole separation that increases pole–pole spacing to distances characteristic of the metaphase–anaphase A steady state. These changes have been explained qualitatively in terms of changes in the balance of bipolar kinesin, Ncd and dynein motor-generated forces, although further work is required to learn the roles of other motors and MT dynamics in these events, as well as to elucidate the details (Sharp et al., 2000a). Furthermore, following the fenestration, spindle formation and function is more complex as new players, the chromosomes and kinetochores, emerge and begin to play dominant roles.

Kinetochores are specialized sites on condensed chromosomes which form a localized, high-affinity site for the capture of spindle MTs. They ensure a high fidelity of segregation and act as central players in chromosome motility by monitoring chromosome attachment to MTs and regulating the metaphase–anaphase transition.

During pro-metaphase, dynamically unstable MTs associated with centrosomes probe the cytoplasm in an exploratory fashion, and chromosome capture depends upon a chance attachment of the side of a kinetochore to the wall of a growing MT. The dependence of this attachment process on the stochastic phenomenon of dynamic instability seems unreliable, but the quantitative model of Holy and Leibler (1994) argues that, in fact, spindle MT dynamics seemed to be tuned to optimize this 'search and capture process'. For example, the distance between the spindle poles and the chromosomes is $d \sim 10$ μm, similar to the average length of growing MTs in metaphase. If MTs were much longer than this, those that 'miss' a kinetochore would wastefully grow too long but if they were too short, most of them would fail to reach the kinetochores. When ~ 100 MTs (which is the order of magnitude of the number of MTs radiating from each pole) grow to ~ 10 μm, there is

one MT fiber per few square microns of the surface on which the chromosomes are distributed. The area of a kinetochore is $\sim 1\ \mu m^2$, so a MT 'finds' a kinetochore after just a few tries. Each trial takes less than a minute, because the plus ends polymerize at rates of tens of microns per minute, so over a few minutes each kinetochore is 'captured' by a MT fiber. This estimate is in good qualitative agreement with observations.

Note, that dynamic instability provides a much more effective mechanism of 'search and capture', than equilibrium polymerization kinetics could. The latter would result in an effective random walk of the polymer's tips with the steps equal to the dimer's size $\delta \sim 8$ nm. The corresponding average time for such polymers to reach $d \sim 10\ \mu m$ length is greater than that for the unstable growing MT by the factor $(d/\delta) \sim 1000$, which would make the 'search and capture' time equal to many hours.

Once attached, the kinetochore translocates polewards along the wall of the MT at rates approaching $1\ \mu m\ s^{-1}$, close to the velocity of MT gliding induced by cytoplasmic dynein (Rieder and Alexander, 1990) and within a minute the MT develops a stable plus end-contact with the kinetochore (Fig. 14.1B). Subsequently, 15–30 MTs rapidly establish end-on connections with each kinetochore and become cross-linked into a tight bundle (MT–MT spacing = 50–100 nm) referred to as a kinetochore (kt) fiber. MTs do not randomly 'search for' the kinetochore after the first attachment, but grow and connect to it in a cross-linked state, which reduces the time of kt fiber formation. The establishment of bipolar attachment involves the formation of such kinetochore fibers on each member of a pair of sister chromatids.

The fascinating process of congression follows, in which position-dependent forces align the chromosomes on the metaphase plate (Kapoor and Compton, 2002). Current evidence suggests that these forces are generated locally at the kinetochores and chromosomes rather than by 'traction fibers' acting along the length of the kt fiber, with pulling forces on the kinetochore being antagonized by pushing forces exerted on the chromosome arms from astral ejection forces (Hays et al., 1982, Kapoor and Compton, 2002, Rieder and Salmon, 1998, Rieder et al., 1986; Fig. 14.1C). By measuring the bending of chromosome arms, Marshall et al. (2001) estimated that the magnitude of the astral ejection force exerted by one MT fiber is ~ 1 pN.

During congression, mono- and bi-oriented chromosomes undergo a series of oscillatory movements at rates $\sim 2–3\ \mu m\ min^{-1}$ and amplitudes of $\sim 2–3\ \mu m$. These oscillations are coupled to MT polymerization at the lagging kinetochore and depolymerization at the leading one. Some observations indicate that poleward motion is driven by an action at the leading kinetochore, while the lagging one is passive and does not push. It is likely that some bi-stability based on a positive feedback between sister kinetochores underlies these oscillations, superficially similar to the cooperative bi-stability of dynein and kinesin motors observed in *in vitro* motility assays (Vale et al., 1992).

It is likely that multiple mitotic motors cooperate to position chromosomes during chromosome capture, congression and alignment. MT polymerization can, in

principle, exert pushing forces on kinetochores and chromosome arms, whereas depolymerization can generate pulling forces on these structures. Cytoplasmic dynein is associated with kinetochores in many systems, and may contribute to chromosome positioning by transporting kinetochores polewards. Indeed, the inhibition of cytoplasmic dynein function in *Drosophila* embryos interferes with congression and prevents the proper alignment of chromosomes on the metaphase equator (Sharp et al., 2000d), although the significance and generality of this observation is a matter of debate (Kapoor and Compton, 2002). MT-destabilizing KIN-I motors could depolymerize MTs at the kinetochore and they have been implicated in many aspects of kinetochore function (Maney et al., 2000). The plus-end-directed orphan kinesin motor, CENP-E may be associated with kinetochore movements towards and away from the spindle equator, being capable of both translocating kinetochores along the polymer lattice towards the plus ends of MTs and also coupling plus-end motility to the depolymerization of kt MTs (Lombillo et al., 1995b, Schaar et al., 1997, Yucel et al., 2000).

The polar ejection force acting to generate antipolar forces on chromosome arms appears to depend upon a combination of MT polymerization and plus-end transport driven by chromokinesins (Brunet and Vernos, 2001). Thus both polymerization and plus end-directed motors can contribute to powering anti-poleward movement of chromosomes while depolymerization and minus-end-directed motors can drive poleward movement, while kinetochore motors can couple the translocation of kinetochores to assembly/disassembly of the MT plus-ends. Understanding exactly how these force-generating elements cooperate to position chromosomes and achieve the balance that aligns chromosomes on the spindle equator at the metaphase steady state presents a fascinating technical challenge (Section 14.4). It has been proposed that chemical gradients originating at the poles could contribute to the positioning of the chromosomes in spindles (Karsenti and Vernos, 2001), but the large fluctuations characteristic of these gradients deem this suggestion unlikely.

An even greater puzzle than the mechanism of force generation by mitotic motors, is how the kinetochore machinery detects and responds to tension. During congression, high tension promotes switching to anti-poleward movement, whilst low tension tends to lead to poleward movement (Rieder and Salmon, 1994). Also, high tension stabilizes attachment of MT fibers to the kinetochore (Nicklas and Ward, 1994). Whether mitotic motors contribute to tension detection and the response at the kinetochore is another fascinating question.

Finally, mitotic motors may also contribute to the poleward flux of MTs in half spindles, which is superimposed on kinetochore-localized movements (Desai et al., 1998, Mitchison, 1989, Mitchison and Sawin, 1990). During metaphase, when the spindle length is constant, there is continuous concerted depolymerization of minus ends near the spindle poles, possibly mediated by the MT severing factor, katanin, and polymerization of plus ends near the cell's equator. These dynamic events are likely to be coupled to the motor-driven poleward translocation of the MT polymer lattice itself, but the identity of the motor(s) responsible, the factors that regulate this process, as well as its function, remain mysterious.

14.6.3
Mitotic Motors and Force Generation in Anaphase

Segregation of chromosomes at the onset of anaphase begins when all chromosomes are properly aligned on the spindle equator and the anaphase-promoting machinery exerts its effects (Section 14.9) including the degradation of chromokinesins with the consequent decrease in the plateward forces acting on chromosome arms which tips the balance of forces in favor of poleward motion (Funabiki and Murray, 2000). Multiple motors located on kinetochores could contribute to poleward chromosome movement (Fig. 14.2).

How much force is required to move a chromatid at the observed rate of poleward chromosome movement (microns per minute)? The viscous drag coefficient of a chromosome in a fluid with the viscosity of cytoplasm (Alexander and Rieder, 1991, Marshall et al. 2000, Nicklas, 1983) is $\sim$ 10 pN s μm^{-1}, which means that a small force $\sim$ (10 pN s μm^{-1})·(0.1 μm s^{-1}) $\sim$ 1 pN would be sufficient to drag the chromosome poleward at the velocities observed. Nicklas (1983: 0.1 pN), Houchmandzadeh et al. (1997: 1 pN) and Alexander and Rieder (1991: 10 pN) made similar estimates. This force is comparable to the force generated by a single depolymerizing MT, or by a single molecular motor. However, the kinetochores seem to generate much greater poleward forces.

The force the generated on the kinetochore in anaphase was measured using a calibrated flexible glass needle (Nicklas, 1983). Nicklas discovered that chromosome velocity was not affected until the opposing force reached approximately 100 pN, and then fell rapidly with increasing force. The opposing force that caused chromosome velocity to fall to zero – the force that matched the maximum force the spindle could exert on the chromosome – was of the order of 700 pN, which is several orders of magnitude greater than the calculated value of 0.1–10 pN required to overcome viscous drag. Similarly, Houchmandzadeh et al. (1997) mea-

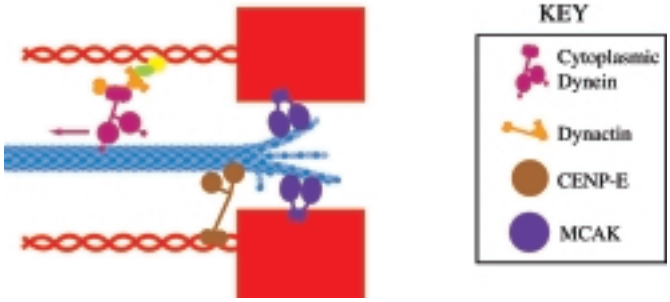

Figure 14.2. Generation of poleward force at the kinetochore. The kinetochore-associated motor (CENP-E) walks to the plus end of depolymerizing MTs (blue) and couples MT disassembly to polar transport of the kinetochore. KIN-I motors, such as MCAK, facilitate MT disassembly as indicated by the formation of curved protofilaments. In addition, the minus-end-directed kinetochore-associated dynein motor pulls the chromosome poleward. Its movement is governed by the MT plus end disassembly. The dynein is shown attached via dynactin to the fibrous 'corona' that emanates from the kinetochore (red) itself.

sured the force exerted by the spindle on a newt chromosome at anaphase as being hundreds to thousands of pN, again supporting the conclusion that kinetochores generate far larger forces than necessary for poleward movement. Moreover, poleward chromosome movements are accompanied by depolymerization of kt MTs (Inoue and Salmon, 1995), but kinetochore motility is much slower (microns per minute) than the free depolymerization rate (tens of microns per minute). These findings suggest that some *velocity governors* have to exist at the kinetochores.

Why are the poleward movement rates an order of magnitude slower than the free depolymerization velocity and how is the maximal force of few hundreds pN per kinetochore generated? What is the nature of the velocity governors? Finally, what is the reason for generating this excessive force, when a much smaller one would be sufficient?

First of all we note that 15–20 depolymerizing kt MTs acting together could generate more than 100 pN pulling force on the kinetochore. Similarly, a few tens of cytoplasmic dynein motors associated with the kinetochore could generate $\sim$ 100 pN force so in principle such forces can be developed by either motor proteins or MT dynamics. One of the simplest possibilities for the mechanism of the kinetochore force is that dynein motors associated to the kinetochore pull it poleward (Savoian et al., 2000, Sharp et al., 2000d; Fig. 14.2). One could speculate that the release of disassembling tubulin dimers from the plus ends of the kinetochore fiber MTs is hindered sterically at the kinetochore. This would explain the slow rate of the poleward movement. This slow depolymerization would stall the action of the dynein motors, which would explain the large measured maximal force developed at the kinetochore: this force would be equal to the sum of the stall forces for all motors. Such a mechanism could provide more faithful and precise poleward movement. Indeed, the randomness (rate of growth of displacement variance) of a number of motors (including kinesin (Vischer et al., 1999) and a depolymerizing MT (Peskin and Oster, 1995)) is large at the free movement of the motor and decreases dramatically when a load force opposes this movement, reaching minimum near stall. So, it could be that the force-generating elements at the kinetochore are dynein motors. The role of slow MT depolymerization is to stall the dyneins, which makes the effective poleward movement very steady. Further experiments are necessary to put more stringent constraints on models of kinetochore movement and force generation.

Separation of chromosomes consists of anaphase B spindle elongation in addition to chromatid-to-pole motion. In many systems anaphase B involves anti-parallel sliding of interpolar MTs coupled to the polymerization of overlapping MTs at their plus ends, which effectively adds to the separation distance between the chromosomes. Bipolar kinesins and dynein have been implicated in driving MT–MT sliding for anaphase B in both yeast and *Drosophila* (Hildebrandt and Hoyt, 2000, Sharp et al., 2000a) but the detailed mechanism and its method of regulation is unclear. Recent work carried out in *Drosophila* suggests that the spindle poles are maintained at a constant spacing throughout the metaphase–anaphase A steady state by a balance of forces involving MT flux, inward forces generated by interpolar C-terminal kinesin, Ncd, and outward forces exerted by cortical dynain and interpolar bipolar

kinesin, KLP61F. This balance appears to be tipped by a downregulation of Ncd activity and a suppression of MT depolymerization at the poles, which tips the balance of forces in favor of outward forces which drive pole–pole separation and spindle elongation (I. Brust-Mascher and J. M. Scholey, unpulished data).

14.7 Does a Spindle Matrix Facilitate the Function of Mitotic Motors?

Microtubules and motors are obviously critical components of the spindle machinery, but recent findings have revived interest in the long-standing and important problem of whether the spindle contains another, unidentified mechanical component, a spindle matrix, that could serve as a stationary substrate against which MTs and motors function (Bloom, 2002, Scholey et al., 2001, Wells, 2001). One such finding is the observation made using fluorescence speckle microscopy that the bipolar kinesin, Eg5 remains relatively static in the spindle while its underlying MT tracks flux poleward (Kapoor and Mitchison, 2001). The explanation favored by the authors of this provocative study is that Eg5 is immobilized on a stationary matrix against which the MTs are translocated poleward as they polymerize at the equator and depolymerize at the poles, although other explanations for the slow dynamics of Eg5 are also possible (Wells, 2001). Additional findings that draw attention to the matrix hypothesis concern the recent discovery of two novel filamentous nuclear proteins, Skeletor in fruitfly and Fin1 in yeast, that are proposed to assemble into spindle-shaped structures independent of spindle MTs. It is proposed that these proteins could function as components of a spindle matrix that serves as a stationary platform on which MTs and motors can perform their function (van Hemert et al., 2002, Walker et al., 2000), but unfortunately, the evidence obtained so far is merely guilt by association, as no clear functional evidence is available in support of any mitotic functions for Skeletor or Fin1. Thus the reality of the spindle matrix remains an unproven but fascinating issue.

If the spindle matrix is real, one of its possible functions could be to strengthen the spindle machine. The effects of MT elasticity on spindle mechanics have not been investigated thoroughly, but here we make some rough estimates. The MT's persistence length is $\lambda_{mt} \sim 10^3$ μm (Bray, 2001). An $l = 10$ μm long polymer with this persistence length would buckle if a force of the order $F \sim 10\ k_B T\ \lambda_{mt}/l^2 \sim 1$ pN was applied to the end of the polymer. In fact, defects in MT lattice would reduce the MT persistence length, and thus the buckling force even more. It is very likely that forces of the order of tens of pN are applied to the interpolar MTs by bipolar kinesin and Ncd motors, and that forces of hundreds of pN are developed at the kinetochores, more than enough to buckle the MT. Cross-linking of a few MTs would strengthen the bundle of MTs dramatically, and this may be a primary function of motors like bipolar kinesins that cross-link MTs throughout the spindle in a way that allows the underlying MT tracks to remain dynamic. A dense bundle of N MTs would have rigidity and buckling force roughly N^2 times greater than a single MT so that for example, 20 MTs cross-linked into a kinetochore fiber would buckle at ~ 400 pN, which is the same

order of magnitude, as the maximal kinetochore force. Similarly, an interpolar bundle of ~ 10 cross-linked MTs during prophase would buckle at ~ 100 pN, which is the same order of magnitude as the force that multiple bipolar kinesin and Ncd motors could generate. It is possible that something in addition to MT cross-linkers is required to strengthen kt and interpolar MT fibers, and a spindle matrix could provide this. Indeed, if the proposed matrix turned out to be an effective elastic medium with a Young's modulus of $Y \sim 10^4$ Pa, similar to that of a dense actin meshwork, then a MT associated with it would be buckled by only very large forces of magnitude ~ 100 pN. A bundle of such MTs would be impossible to buckle by the forces characteristic of the spindle. Moreover, this buckling force would be independent of the length of the fiber (Landau and Lifshitz, 1995), unlike an equivalent fiber in an aqueous medium, so even very long MTs would be stable. Such arguments underscore the importance of resolving the fascinating question of whether the spindle matrix does indeed exist (Bloom, 2002, Scholey et al., 2001).

14.8 Mitotic Motors and Intracellular Transport Systems

The notion that vesicle transport in spindles could be significant for the mechanism of mitosis (Sawin and Scholey, 1991) has recently gained renewed interest, based on recent work suggesting that some MT-based motor proteins function during late stages of mitosis to transport both vesicles and signaling molecules along spindle MTs and that these activities are required for the completion of cytokinesis (Finger and White, 2002, Shuster and Burgess, 2002, Skop et al., 2001). This aspect of mitotic motor function in some ways resembles the actions of neuronal and intraflagellar transport motors, including for example kinesin-I, which appears to attach to transmembrane receptor proteins on its vesicular cargo via the Jun N-terminal kinase signal scaffolding proteins, JIP1/2 and JIP3/syd (Goldstein, 2001).

In animal cells, cytokinesis is biphasic, involving the determination and ingression of the cleavage furrow, followed by the scission of the mid-body remnant resulting in the final separation of daughter cells (Finger and White, 2002). MT-based vesicle transport is thought to be required to provide additional membrane and thus to increase the surface area for the ingression of the cleavage furrow (at least in some systems) and to seal off the plasma membrane of the two daughter cells as they separate (Fig. 14.3). The scission events associated with vesicle transport along anti-parallel mid-body MTs resemble those involved in cell plate formation associated with the phragmoplast of dividing plant cells, which has long been understood to depend upon the MT-based transport of Golgi-derived vesicles (Lee et al., 2001). The precise roles of the signaling molecules that are associated with MT-based transport systems during these events are not well understood, but they could be involved in controlling MT dynamics in the mid-body or phragmoplast, in regulating the activity of the motors themselves, in signaling cleavage furrow positioning, ingression and scission, or they could be precursors of signaling complexes that assemble in association with new plasma membrane.

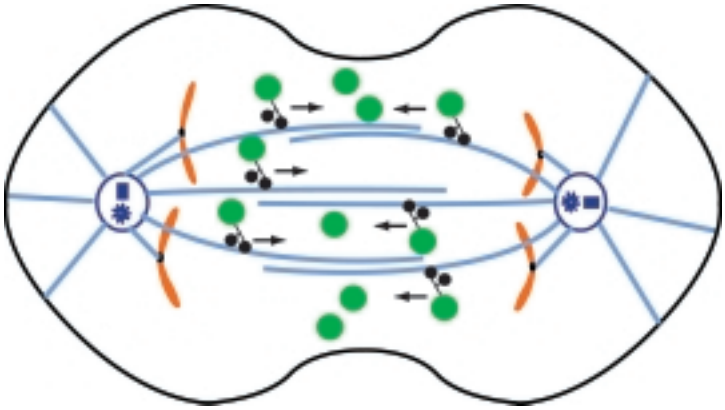

Figure 14.3. Intracellular transport by mitotic motors. New work indicates that some molecular motors in the spindle transport vesicles (green) and signaling complexes, delivering new surface membrane for the completion of cytokinesis (see text).

Which mitotic motors are involved in these aspects of cell division? The best candidates are those that associate with the anti-parallel MT arrays that constitute the animal cell mid-zone and the plant cell phragmoplast. For example, the two phragmoplast kinesins, AtPAKRP1 and AtPAKRP2 bind to MTs in a nucleotide-sensitive fashion and, by immunofluorescence microscopy, display different distributions within the phragmoplast (Lee and Liu, 2000, Lee et al., 2001). Thus, AtPAKRP1 forms a relatively tight band that may correspond to the plus ends of overlapping MTs, and is proposed to cooperate with bipolar and C-terminal kinesins to control the organization of mid-zonal MT bundles, whereas AtKRP2 localizes to a relatively broad band of punctate structures, presumably Golgi-derived vesicles, and it is proposed to deliver these vesicles to the developing cell plate (Lee et al., 2001). An additional phragmoplast kinesin, NACK1, is proposed to bind and activate the signaling molecule, NPK1, a Map kinase, and to transport the kinase to the equator of the phragmoplast where the complex is required for the outward expansion of the cell plate and the completion of cytokinesis (Nishihama et al., 2002). The precise mechanism of action of these phragmoplast motors is currently unclear, but the work shows how multiple motors can cooperate to facilitate different aspects of phragmoplast function during cytokinesis, including organizing MTs into ordered arrays that can serve as tracks for the efficient transport of vesicles to the site of abscission.

A similar functional cooperation is seen among the motors associated with mid-zonal MTs during animal cell cytokinesis, most notably members of the MKLP1 family. For example, the *C. elegans* mid-zonal kinesin, ZEN-4/MKLP1, is required for the tight bundling of mid-zonal MTs into a normal mid-body, and the loss of its function gives rise to a failure of the completion of cytokinesis (Powers et al., 1998, Raich et al., 1998). This motor appears to interact functionally with components of G-protein signaling pathways because ZEN-4/MKLP1 appears to interact with a

Rho-family GAP protein, CYK-4, forming a tight complex that cross-links MTs into bundles (Mishima et al., 2002) potentially forming organized tracks in the mid-zone for efficient vesicle delivery to the site of scission. Since CYK-4 binds to the neck region of ZEN-4/MKLP1 it may regulate motility and MT−MT bundling by the motor complex. The transport of Golgi-derived vesicles along the bundled MTs may be mediated by another mid-zonal MKLP1-related kinesin, Rab6-KIFL, which binds the small ras-related GTPase, Rab6, and is implicated in membrane traffic associated with the Golgi apparatus. Rab6−KIFL localizes to the spindle mid-zone of mitotic vertebrate cells much like ZEN-4/MKLP1, and the perturbation of its activity disrupts cytokinesis, suggesting that Rab6−KIFL may transport Golgi-derived vesicles along MTs that have been bundled into organized tracks by ZEN-4/MKLP1-related proteins, thus providing membrane for the final stages of cell−cell scission (Hill et al., 2000).

This is by no means the whole story for intracellular transport motors in the spindle mid-zone however, as ZEN-4 and its *Drosophila* homolog, PavKLP display functional interactions with the Polo and Aurora kinase regulatory systems, respectively (Carmena et al., 1998, Severson et al., 2000). The finding that the MKLP1-related kinesin, CHO-1 has an extra actin-binding domain that is missing in other MKLP1s, and which may allow this motor to connect mid-zonal MTs to the cell cortex (Kuriyama et al., 2002), introduces additional complexity. Moreover, the kinesin KLP3A from *Drosophila* is not a member of the MKLP1 family yet it is also required for the proper organization of MTs in the spindle mid-zone, and loss of its function leads to failures in cytokinesis (Williams et al., 1995), further complicating the issue of how these mid-zonal motors may cooperate to ensure the successful completion of cytokinesis.

There are some indications that the participation of the aforementioned mid-zone and phragmoplast motors in cytokinesis represents only the tip of the iceberg. For example, there exist motors with well-characterized intracellular transport functions in non-mitotic cells that might also be deployed to perform cell division-related functions in the spindle, although the evidence is currently less compelling. One of these is conventional kinesin-I itself, a protein that associates with vesicles in sea urchin embryonic mitotic spindles (Wright et al., 1991) and is proposed to deliver exocytotic vesicles out along astral MTs to the cell surface for resealing damaged membranes (Bi et al., 1997). The resealing of wounded membranes by Ca^{2+}-regulated exocytosis resembles the membrane fusion events that are involved in the scission of daughter cells during cytokinesis (Finger and White, 2002) and it is plausible to think that kinesin-I-dependent vesicle transport along astral MTs could be responsible for the new membrane addition that occurs in the late telophase cleavage furrows of sea urchin embryos (Shuster and Burgess, 2002), although antibody and dominant negative inhibition experiments did not reveal a requirement for kinesin-I in cell division (Bi et al., 1997, Wright et al., 1993). Heterotrimeric kinesin-II is another candidate (Cole et al., 1993). This motor is best known for its role in intraflagellar transport and ciliogenesis (Goldstein, 2001) but it localizes to punctate detergent-sensitive structures in some mitotic spindles (Henson et al., 1995) and, like Rab6−KIFL it is implicated in G-protein signaling and Golgi-associated membrane

trafficking (Le Bot et al., 1998, Shimizu et al., 1996). This suggests that kinesin-II may participate in spindle MT-based targeted secretion in association with cytokinesis, although no functional data support its mitotic role. Finally, the monomeric kinesin, UNC-104 is a well-characterized axonal pre-synaptic vesicle transport motor that has also been implicated in Golgi-associated membrane traffic (Dorner et al., 1998) raising the possibility that it might also be involved in targeted secretion during cell division, although in this case we are not aware of any evidence localizing the protein to mitotic spindles. Finally, it is possible that cytoplasmic dynein also drives vesicle transport in spindles, as it has been shown to play an important role in breaking down the nuclear envelope and transporting fragments of membrane along centrosomal MTs (Beaudouin et al., 2002).

In summary, abundant evidence suggests that some intracellular transport motors are deployed in spindles where they play critical roles in cytokinesis by transporting vesicle and signaling molecules along spindle MTs. It is also clear that deciphering the precise roles and interactions of the complex network of motors that participate in these events will remain an active and fascinating research topic for some time.

14.9 Mitotic Motors and the Spindle Assembly Checkpoint

It has become clear that the force-generating and intracellular transport properties of mitotic motors are also used to regulate chromatid segregation during anaphase, by acting as components of the spindle assembly checkpoint (Shah and Cleveland, 2000). This checkpoint ensures faithful chromatid segregation by inhibiting anaphase onset until all the chromosomes in a mitotic cell are properly aligned in a bipolar configuration on the metaphase spindle equator. Prior to bipolar attachment, unattached kinetochores release a diffusible inhibitor of the cell cycle proteolysis machinery that initiates anaphase onset, but MT attachment to kinetochores and/or the development of tension across kinetochore pairs prevents the release of the soluble inhibitor, allowing the proteolysis machinery to degrade key substrates including the cohesins that 'glue' together sister chromatids, a chromokinesin which pushes chromosomes towards the spindle equator, and some bipolar kinesins which cross-link MTs throughout the spindle (Funabiki and Murray, 2000, Gordon and Roof, 2001, Hildebrandt and Hoyt, 2001, Shah and Cleveland, 2000). The degradation and inactivation of these substrates removes constraints on chromatid-to-pole motion allowing progression through anaphase A, and removes restraining cross-linkers thus permitting spindle elongation, disassembly and reassembly for subsequent mitosis.

What is the nature of the soluble inhibitor and how is it inactivated? The outer kinetochore region contains binding sites for a complex set of proteins that are thought to participate in the spindle assembly checkpoint, including the kinesin, CENP-E, the dynein/dynactin complex, and several checkpoint proteins including MAD2, ZW10 and Rod (Shah et al., 2000). It is hypothesized that the soluble protease inhibitor is a fraction of MAD2 that binds the kinetochore where it is induced to

oligomerize and is then released as an active diffusible oligomer capable of inhibiting the cell cycle proteolysis machinery. Mitotic motors are thought to contribute to the inactivation of this checkpoint in at least two ways. First, CENP-E is essential for the stable bi-oriented attachment of kinetochores to spindle MTs and, together with dynein (Howell et al., 2001), is involved in the development of tension across chromosomes once they are properly aligned; this CENP-E-dependent MT attachment and tension generation is proposed to block the formation of active, oligomeric MAD2 (Yao et al., 2000). Secondly, once kinetochores are attached to MTs and held under tension, the dynein/dynactin complex is proposed to deplete the checkpoint machinery from the kinetochores and thus to inactivate the checkpoint by actively transporting checkpoint proteins such as MAD2, ZW10 and Rod towards the minus ends of kinetochore MTs, from the kinetochores to the spindle poles where these proteins are observed to accumulate subsequent to metaphase (Howell et al., 2001, Wojcik et al., 2001). Thus these mitotic motors not only contribute directly to the physical alignment and segregation of chromosomes, but they also contribute to the regulation of these processes, thus ensuring the fidelity of mitosis.

14.10 Conclusions and Future Studies

Despite great progress in the field, there remain many outstanding questions about the roles of motor proteins and MT dynamics in mitosis. How are the force-generating properties of dynamic MTs and motor proteins coordinated? What is the role of the multiple redundant force-generating and velocity-governing systems? How are they integrated and regulated? What mechanisms insure the precise temporal and spatial morphogenesis of the mitotic spindle? What are the precise roles of mitotic motors and intracellular transport systems in the spindle? How do force-generating elements contribute to the spindle assembly checkpoint? Is there a spindle matrix? Both experimental and theoretical work is needed to answer these questions and dissect the process of mitosis. Visualizing spindle dynamics, inhibiting motors and imaging the effects on mitotic movements is one of the approaches currently being used, but other approaches will be needed, including better quantization of the mechanical and force-generating properties of spindles before and after genetic and/or biochemical manipulations of the spindle machinery. Additional biochemical approaches, including *in vitro* reconstitution from purified and characterized components will play important roles as well. Finally, as the field is maturing, complementation of these experimental approaches by theoretical modeling is becoming increasingly feasible and important.

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