Cell division

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In creating the mitotic spindle and the contractile ring, natural selection has engineered fascinating precision machines whose movements depend upon forces generated by ensembles of cytoskeletal proteins. These machines segregate chromosomes and divide the cell with high fidelity. Current research on the mechanisms and regulation of spindle morphogenesis, chromosome motility and cytokinesis emphasizes how ensembles of dynamic cytoskeletal polymers and multiple motors cooperate to generate the forces that guide the cell through mitosis and cytokinesis.

During the nineteenth century, the discovery that cells reproduce themselves by dividing into two illuminated the very origin of cells and became a cornerstone of the cell theory[1,2]. Today, research on cell division flourishes because an improved understanding of its mechanism could lead to improvements in the treatment of diseases such as cancer[3] and because we are fascinated by the cytoskeletal 'nanomachinery' that is responsible for mitosis and cytokinesis[4–14].

The pathways by which the microtubule (MT)-based mitotic spindle and the actin-based contractile ring use cytoskeletal proteins to coordinate mitosis and cytokinesis are well understood[4–14] (Fig. 1). During mitosis, the spindle uses MTs and multiple mitotic motors to distribute identical copies of the replicated genome to the products of each division[2,4–9]. Usually this process begins during prophase (Fig. 1a) with the migration of duplicated centrosomes around the nuclear envelope. The envelope breaks down at the onset of prometaphase, allowing spindle MTs to capture chromosomes and move them to the equator (congression; Fig. 1b), so that by metaphase (Fig. 1c), pairs of sister chromatids lie on the spindle equator facing opposite spindle poles. Upon the onset of anaphase[10], cohesion between sister chromatids is lost, which allows sister chromatids to be moved to opposite spindle poles (anaphase A; Fig. 1d) as the spindle poles themselves move further apart (anaphase B; Fig. 1e). Also during anaphase, the spindle delivers a signal to the cortex (Fig. 1d inset) that defines the position and orientation of the contractile ring, the machine that uses actin and myosin-II to drive cytokinesis (Fig. 1e inset)[11]. The contraction of this ring causes the furrow to ingress as the nuclear envelopes reassemble around sets of decondensing segregated sisters. Finally, the furrow 'seals', completing the separation of the daughter cells (Fig. 1f).

Cells use a significant fraction of their proteins to divide — functional proteomics[12] indicates that *Caenorhabditis elegans* uses 6% of its open reading frames to encode proteins required for cell division and an important subset of these proteins comprise actin filaments, MTs, motor proteins and accessory proteins[13,14]. MTs and actin filaments are linear, polar, multistranded polymers, built from 13 strands of αβ-tubulin heterodimers and 2 strands of G-actin monomers, respectively. These polymers can generate pushing and pulling forces as they grow and shrink by addition and loss of subunits from their ends, and they also serve as tracks for motor proteins that use ATP hydrolysis to generate force and motility[7] (Box 1). At the single-molecule level, cytoskeletal proteins generate piconewton-scale forces and nanometre-scale movements[7,13,14], but during cell division they function as ensembles that are capable of generating forces in the range of nanonewtons and serve to accurately move intracellular components and rearrange areas of the cell surface over distances of tens of microns[7,13–17]. How do these cytoskeletal force generators cooperate to drive the motility events underlying the mechanics and regulation of cell division?

Spindle morphogenesis and elongation

The purpose of mitosis is to segregate sister chromatids by moving them to opposite poles. To this end, spindle MTs become oriented into a bipolar array whose dyad axis divides the structure into two half spindles (Fig. 1c). Within each half-spindle, the MTs lie on trajectories that point their minus-ends towards a focus at the poles, allowing spindle forces to accomplish their goal by translocating chromatids along these trajectories (Fig. 1d). Bipolar spindles can form by two pathways, the centrosome-directed assembly pathway[4], in which MT assembly is nucleated by centrosomes, or the chromosome-directed pathway[5,6], in which chromosomes induce MT assembly (Fig. 2a). The relationship between these pathways is unresolved, as is the question of why some cells (such as *Drosophila* embryos) use the centrosome-directed pathway whereas others (for example, *Drosophila* female oocytes) lack centrosomes and use the alternate chromosome-directed pathway.

Centrosomes consist of a pair of cylindrical centrioles surrounded by pericentriolar material that contains the MT-nucleating γ-tubulin ring complex (γ-TuRC). Electron microscopy suggests that the γ-TuRC acts as a helical template for new MTs which grow by subunit addition at their plus ends[18–21]. Recent work suggests that a MT-associated protein (MAP) called XMAP215 is important for MT nucleation at centrosomes[22]. Perhaps the γ-TuRC and XMAP215 play complementary roles, stabilizing lateral and longitudinal bonds between subunits, respectively[22].

In the chromosomal pathway, the guanine nucleotide-exchange factor of the small GTPase, Ran, generates a spatial gradient of active Ran–GTP around chromosomes[23]. Ran–GTP promotes the release of factors that induce MT assembly from a pool that is sequestered in an inactive form by importin-β, thereby activating spindle assembly around chromosomes[24]. Spindles that lack conventional centrosomes may contain 'pseudo-centrosomes' consisting of various MAPs that are important for spindle pole formation and stability[25]. One of these MAPs is XMAP215, which may be transported to the poles by a minus-end-directed C-terminal kinesin, where it could nucleate MT assembly as in the centrosome-directed pathway[25].

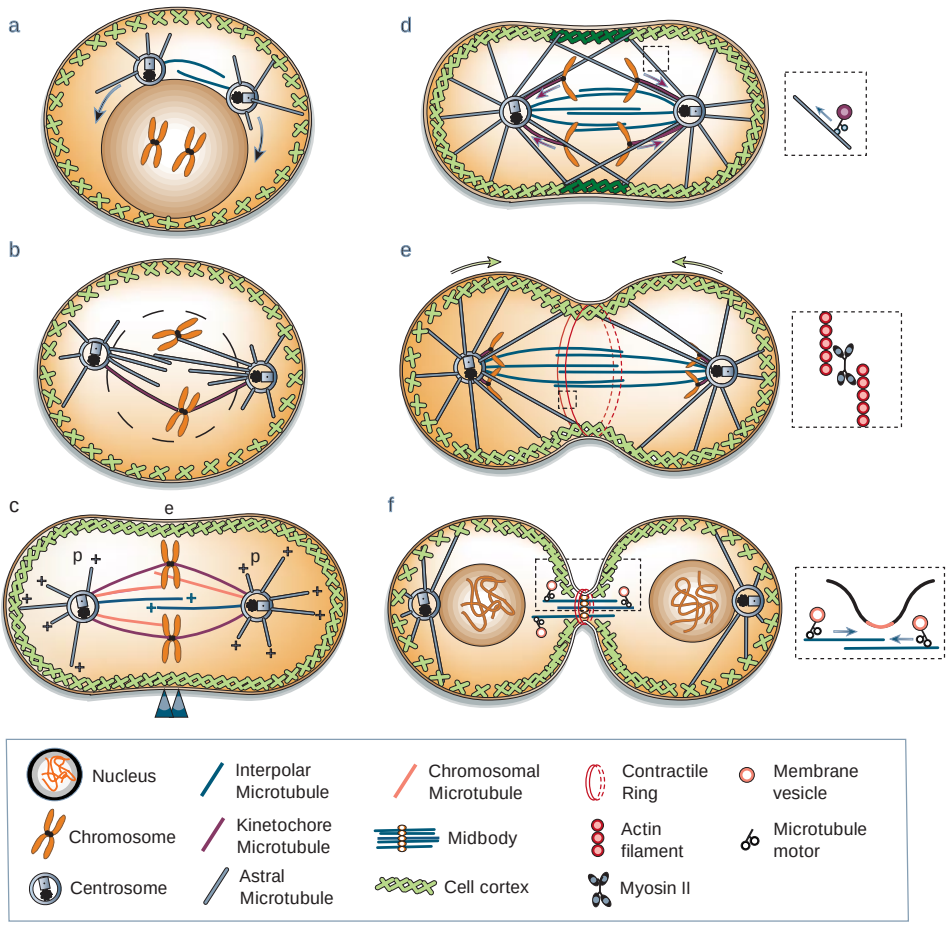


Figure 1 Mitosis and cytokinesis. **a**, Prophase. Duplicated centrosomes migrate around the nucleus. (Centrosomes, consisting of a pair of previously replicated centrioles surrounded by pericentriolar material, nucleate MT assembly and organize spindle poles.) **b**, Prometaphase. The nuclear envelope breaks down allowing MTs to move chromosomes to the equator (e) in a process termed congression. **c**, Metaphase. Sister chromatids (double arrowheads) face opposite poles (p). MTs are oriented with their plus ends distal to the poles, and are organized into four sets, namely: astral MTs, which link spindle poles to the cell cortex; chromosomal MTs, which link chromosome arms to poles; kinetochore MTs (kMTs), which link poles to kinetochores; and interpolar MTs (ipMTs), which link the two poles. **d**, Anaphase A. Chromatids are moved to opposite poles (segregation). **e**, Anaphase B. Pole–pole spacing increases. During late anaphase the division plane is determined by a mechanism involving spindle–cortex interactions and the cleavage furrow containing a contractile ring assembles from actomyosin-II and begins to contract. **f**, Telophase/cell–cell scission. Nuclear envelopes reassemble around decondensing segregated sisters. The contractile ring contracts (furrow ingression) developing a barrier between the daughter cells and constricting the spindle mid-zone (the array of ipMTs lying between separated chromatids) into a structure called the midbody (the remnant of the mid-zone). During abscission, the furrow 'seals' by a mechanism thought to involve vesicle transport/exocytosis, completely separating the daughter cells.

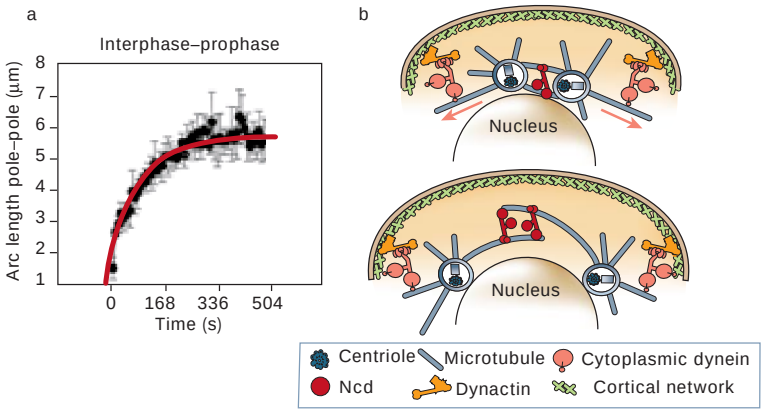
MT motors have subtly different roles in the centrosome- and chromosome-directed assembly pathways[4–6]. In the latter case, MTs randomly organized around chromosomes become crosslinked into antiparallel bundles by bipolar kinesins, then plus-end-directed chromokinesins reorganize these MTs to position their minus ends distal to chromosomes, and finally minus-end-directed motors (dynein or Ncd) crosslink the MT minus ends into focused poles[26]. In the former case, duplicated centrosomes are moved apart by shifts in a balance of outward and inward forces generated by the cooperative action of dynamic MTs, cortical dynein and multiple MT sliding motors localized to interpolar MT (ipMT) bundles[4,27]. Bipolar (plus-end-directed) and C-terminal (minus-end-directed) kinesins acting on ipMTs are candidates for generating some of the antagonistic outward and inward forces that position spindle poles[4]. Recent computer simulations have suggested that bipolar and C-terminal kinesins could generate outward and inward forces on the poles, as expected, but various mixtures of these motors were unable to produce a robust isometric spacing of two spindle poles unless the two kinesins were organized into co-polymers[28].

The formation of such motor co-polymers might be one function of the hypothetical 'spindle matrix' that is proposed to serve as a substrate for the organization and activity of MTs and motors[29,30]. Another possible function of this matrix, if it exists, is to strengthen the spindle machinery, because physical estimates suggest that the large forces developed by spindles (in the nanonewton range)[15] would cause MT buckling unless the MTs are stabilized by a matrix and/or by MT–MT crosslinkers[7]. Definitive evidence for a spindle matrix is lacking, but perhaps matrix proteins are lurking undetected in spindle pole and mitotic MT preparations, which can be characterized by powerful matrix-assisted laser desorption/ionization mass spectroscopy[31,32].

Spindle MTs are highly dynamic (Fig. 2b) and several accessory proteins influence spindle MT dynamics (Fig. 2c). For example, the MAP XMAP215, which promotes MT polymerization, and the kinesin XKCM1, which promotes MT depolymerization, can act together to confer physiological dynamic properties upon purified tubulin[33]. These proteins could regulate the length of spindle MTs, thereby contributing to the control of steady-state mitotic spindle

Box 1
Force-balance model for spindle pole motility

Motility in the spindle depends upon force generation by dynamic MT polymers and kinesin and dynein motors. MTs polymerize or depolymerize to generate pushing or pulling forces on an attached object such as a chromosome or spindle pole. Kinesin and dynein motors can step along the polymer lattice to exert forces on such a load. Simple physical arguments and, in some cases, experimental measurements suggest that the forces generated by MT dynamics and motor protein action are within the range of one to a few tens of piconewtons (refs 7,14). Because the spindle is capable of generating forces in the range of 1,000 pN (ref. 15), the question becomes: how do multiple force-generating elements (tens to thousands) cooperate as ensembles to generate forces and movements characteristic of the spindle?



Insights into this question emerge from a consideration of spindle pole motility during spindle morphogenesis in *Drosophila* embryos; in this system, bursts of spindle pole separation are punctuated by periods of stasis when pole–pole spacing is constant (isometric)[4,27]. For example, plots of spindle pole separation (S) versus time (t) during early (interphase–prophase) mitosis (panel **a** in the figure above) reveal a burst of rapid motility that gradually slows to a stop at a constant (isometric) spacing of 6 μm. This spindle pole motility is proposed to depend upon a balance of opposing forces, as proposed for chromosome motility by Ostergren[36], such that outward forces (F_{out}) drive pole–pole separation, while inward forces (F_{in}) draw the poles together[4,27,37]. In this model, the periods of stasis (for example, the 6-μm isometric state) are 'equilibrium points' when inward and outward forces balance one another.

It is proposed that the outward force during prophase, F_{out}, results from cortical dynein pulling on astral MTs (F_{dyn}) plus polymerizing MTs nucleated from one centrosome exerting pushing forces on the opposite centrosome (F_{pol}). The opposing inward force, F_{in}, is due to the minus-end-directed C-terminal kinesin Ncd acting on interpolar MTs to draw the poles together (F_{ncd})[4,37]. Therefore, the net force driving spindle pole separation is $F(S) = F_{out} - F_{in} = \{F_{dyn}(S) + F_{pol}(S) - F_{ncd}(S)\}$. Intracellular motility events occur under conditions of low Reynolds number[7,13,14,79], and consequently the net force is proportional to the velocity[7,79]. Thus, an equation of motion describing the dynamics of spindle pole motility is: $dS/dt = (1/\mu)\{F_{dyn}(S) + F_{pol}(S) - F_{ncd}(S)\}$, where μ is the effective drag coefficient at the nucleus, and dS/dt is the rate of spindle pole separation[37]. When reasonable parameters and the geometry of the cytoplasm are used, a solution of this equation produces a reasonable fit to the experimental data[37] (in panel **a**; in the figure above, experimental data are shown in black, with the theoretical curve in red).

length[34], and indeed, a balance of activity between XMAP215 and XKCM1 in yeast is required for proper pole–pole separation during anaphase B[35]. Thus, it seems likely that MAPs that control MT polymer dynamics, together with motors that crosslink and slide ipMTs outwards or inwards, could exert forces on spindle poles that control spindle length (Fig. 2c), as pole–pole spacing increases during spindle morphogenesis and elongation, for example[4,27].

The separation of the spindle poles that accompanies the morphogenesis and elongation of the spindle is an example of mitotic motility that reveals some of the basic principles by which cytoskeletal force generators drive the motility events underlying spindle mechanics (Box 1). Ostergren[36] proposed that shifts in a balance of antagonistic forces serve to move and position structures in the spindle, and evidence has accumulated showing that forces generated by growing and shrinking MTs and by antagonistic mitotic motors provide a molecular explanation for such a balance[2,4,6,27,28]. Indeed a quantitative model (Box 1) can explain how a balance of opposing forces generated by ensembles of dynamic MTs and mitotic motors drives spindle pole motility[4,27,37], and similar models are likely to be relevant to other forms of motility (for example, chromosome motility).

Chromosome motility

During mitosis, pairs of sister chromatids associate with the spindle (Fig. 1b), congress to the spindle equator (Fig. 1c), and then segregate to opposite spindle poles (Fig. 1d, e)[8,9].

In the chromosome-directed spindle-assembly pathway, spindle morphogenesis and initial chromosome attachment are coupled, but in the centrosome-directed pathway, centrosome-nucleated MTs display dynamic instability, growing and shrinking in an exploratory fashion to capture chromosomes in a timescale of minutes[38]. Captured chromosomes often attach initially to the wall of spindle MTs by one sister kinetochore and move rapidly polewards at rates of 0.1 μm s^{-1}, possibly using dynein[39], before assuming a bi-oriented end-on configuration.

Once chromosomes have become bi-oriented, they congress to the equator to assume the metaphase configuration (Fig. 1b, c). During congression, bi-oriented chromosomes display 'directional instability', oscillating back-and-forth as episodes of poleward (P) motion and antipoleward (AP) motion at constant velocity are punctuated by rapid reversals, with the frequency of the reversals being biased so as to bring the chromosomes to the equator[40] (Fig. 2d). This bidirectional chromosome motility requires the elongation and shortening of kinetochore MTs (kMTs) and consequently much attention has focused on the important role played by MT dynamics[9]. However, the inhibition of motors such as dynein, chromokinesins and CENP-E can interfere with chromosome alignment, so motors must have some role[41,42] (although the precise role of motors such as dynein in generating P forces remains controversial[27,41,43]).

It is proposed that the bidirectional motility of chromosomes involves the integration of antagonistic P forces and AP forces acting along the pole–pole axis[8,9,44] (Fig. 2e). P forces are likely to depend on the functional coordination of poleward MT flux, the depolymerization of MTs at kinetochores, minus-end-directed kinetochore–dynein motors, and kinetochore motors that couple kinetochore motility to MT dynamics. AP forces depend upon chromokinesins that push chromosome arms towards the spindle equator and generate a force gradient that diminishes with increasing distance from the spindle pole (Fig. 2b, d, e)[8,9,40–42,44–47]. These opposing P and AP forces could produce tension in the kinetochore, the

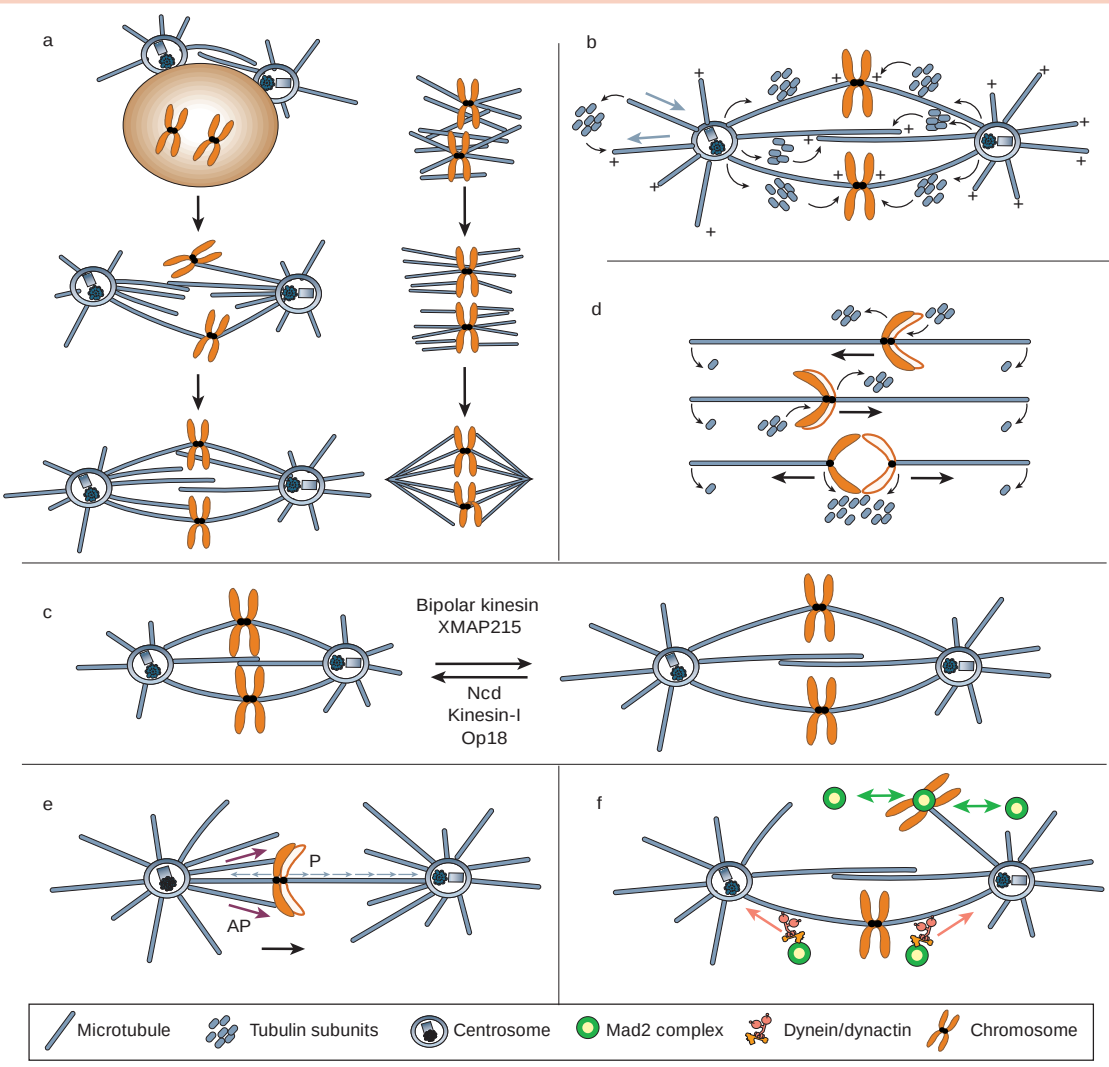


Figure 2 Spindle behaviour. **a**, In some systems, spindles assemble by a centrosome-directed pathway[4] and in others by a chromosome-directed pathway[5,6]. In the first case (left), two duplicated centrosomes nucleate the assembly of MTs, which produce a balance of outward and inward forces that drive pole–pole separation[4,27,37]. In the latter pathway (right), condensed chromosomes direct the assembly of MTs, which are 'sorted' into a bipolar array by MT sliding motors and are crosslinked at their minus ends to form focused poles. **b**, Spindle MT dynamics coupled to GTP hydrolysis. Astral MTs grow and shrink by subunit addition or loss at their plus ends (dynamic instability). Kinetochore MTs (kMTs) and interpolar MTs (ipMTs) display poleward flux owing to subunit addition at their plus ends and loss at their minus ends, coupled with motor-dependent poleward translocation. Flux may exert poleward forces, *F*, that pull sister kinetochores towards opposite poles, where *F* is proportional to distance *S* from a pole[36]. **c**, Control of spindle length by MT-associated proteins (MAPs) and motors. XMAP215 is a MAP that pushes the poles apart by promoting MT polymerization, whereas the kinesin-I motor XKCM1, and the MAP Op18 depolymerize MTs and shorten spindles. Bipolar kinesins crosslink and slide ipMTs outwards, thus lengthening the spindle, whereas Ncd, a C-terminal kinesin motor, slides ipMTs inwards. **d**, Kinetochore motility and MT dynamics. Prior to anaphase, bi-oriented chromosomes display kinetochore directional instability[40] in which kinetochore movement towards its facing pole (thick arrow) is coupled to MT disassembly at the leading kinetochore (upper panel). The kinetochore abruptly reverses its direction of movement, which becomes coupled to MT polymerization at the now trailing kinetochore (centre). During anaphase A, chromatid-to-pole motility is coupled to MT disassembly at the kinetochore and pole (lower panel). **e**, Shifts in a balance of pole-directed (P) and polar ejection (AP) forces control chromosome position. In one model[44] the ejection force within a half spindle forms a gradient that decays with distance from the pole and is opposed by the poleward force acting on the kinetochore. As a kinetochore is pulled polewards, the increasing AP force acts on chromosome arms to increase kinetochore tension, increasing the probability of MT dissociation from the leading kinetochore. Above a maximal tension, the leading kinetochore loses all its kMTs and, as a consequence, there is an abrupt reversal of direction as the sister kinetochore takes the lead. **f**, The spindle-assembly checkpoint. Once chromosomes are held under bipolar tension, dynein turns off the checkpoint by transporting Mad2 from kinetochores to poles.

magnitude of which increases as the chromosome moves polewards until, above a certain maximal tension, the direction of chromosome motion reverses abruptly; modulation of the frequency of reversals would then allow a chromosome to find the equator[8,44].

Anaphase is initiated after the bi-oriented chromosomes have aligned at the metaphase spindle equator, whereupon the links between sister chromatids dissolve and the separated sisters move to opposite poles. Prior to the onset of anaphase, chromosomes are held at the equator by cohesin-mediated sister-chromatid cohesion and by chromokinesin-generated AP forces that push chromosome arms towards the equator[48,49]. The cell-cycle dependent proteolysis of cohesin complexes allows the separation of the sister chromatids, while the degradation of the chromokinesin downregulates the AP forces, allowing the separated chromatids to be transported to

opposite spindle poles at speeds of 0.01–0.1 μm s^{-1} (refs 8,9,40,41,43). A small force of magnitude ~ 1 pN is sufficient to move a chromosome at these speeds[7], although spindles are capable of exerting forces that are a few orders of magnitude greater than this[7,15,50], suggesting that tens to thousands of cytoskeletal force generators must be able to act cooperatively to generate the maximum forces acting during anaphase. Presumably the generation of the required P forces involves the functional coordination of minus-end-directed kinetochore motors, MT depolymerization at kinetochores and poleward MT flux[27,41,43].

Regulation of mitotic progression

Mitotic force generators located at the kinetochores do more than simply move and position chromosomes in the spindle, for they are also components of the spindle-assembly checkpoint, which delays the transition from metaphase to anaphase until all chromosomes are correctly aligned at the metaphase spindle equator (Fig. 1c). To do this, the checkpoint needs to detect a single unattached kinetochore among several properly attached ones.

Sister chromatid separation and exit from mitosis are controlled by the anaphase-promoting complex (APC), a ubiquitin-protein ligase that targets key proteins for proteolysis, for example, the cohesins and chromokinesins, whose destruction facilitates chromatid-to-pole motility. The checkpoint uses a network of proteins to inhibit the activity of the APC until all chromosomes are properly aligned, at which point the checkpoint is silenced allowing the APC to promote anaphase onset. Silencing depends upon the attachment of MTs to kinetochores, the formation of kMTs, and the establishment of bipolar tension at kinetochores[10,51].

A key player is the checkpoint protein Mad2, which is activated as a result of binding transiently to unattached kinetochores[52], and is released as an active inhibitor of APC activity. Unattached kinetochores contain many other checkpoint proteins as well as tension-sensitive phospho-epitopes[53]. Proper bipolar attachment of kinetochores leads to their dephosphorylation, and a dramatic re-localization of Mad2 (ref. 54). Transient kinetochore binding by Mad2 creates high steady-state levels of the protein on unattached kinetochores, but when kinetochores become properly aligned, Mad2 is depleted by translocation along kMTs to the spindle poles by the minus-end-directed dynein–dynactin complex[55] (Fig. 2f). Even a single unattached kinetochore can yield sufficient active Mad2 to inhibit the APC, but once they are all properly attached, Mad2 is sufficiently depleted to silence the checkpoint.

It should be emphasized that the kinetochore is a macromolecular complex containing tens, or possibly hundreds, of polypeptides, several of which may be involved in the spindle-assembly checkpoint. For example, ZW10 and Rod are required for correct targeting of dynein–dynactin to kinetochores and for checkpoint activation[56,57], although their precise role is unclear. In the absence of these proteins, the checkpoint is not activated even though high levels of Mad2 persist on unattached kinetochores, leading to proposals that they may normally serve to release activated Mad2 (ref. 10).

The CENP-E motor is proposed to be a mechanosensor of kinetochore tension, based on the observation that its depletion leads to a failure of checkpoint activation[58]. However, it should be noted that it is difficult to decipher the precise function of CENP-E and other kinetochore motors during anaphase A, because they may participate directly in chromosome motility and also as components of the checkpoint regulatory system[41,42,55,58]. Another fascinating area of uncertainty concerns whether the checkpoint detects MT attachment to kinetochores, tension on the kinetochore, or both. This issue is difficult to resolve experimentally because MT attachment and tension are inter-related — MT attachment leads to tension and tension can lead to more stable MT attachments[59], although only tension can discriminate between monopolar and bipolar attachment[51]. Accordingly, different experiments have led to different conclusions and further work is required[54,59–62].

The spindle-assembly checkpoint acts during metaphase when the spindle is maintained in an isometric state. In *Drosophila* embryonic spindles, isometric steady-state structures are also maintained by a balance of forces acting on astral and ipMTs at prophase, prometaphase and telophase[4]. We speculate that, as in metaphase, these three periods of stasis also allow the spindle to assess its mechanical status and to provide a stable framework to support MT-dependent breakdown of the nuclear envelope[63], chromosome capture and correct nuclear spacing, respectively.

How does the spindle determine the site of cytokinesis?

Cytokinesis, the final stage of division, creates two daughter cells from one parent cell (Fig. 1d–f). The position of the mitotic spindle during anaphase determines the location of the furrow, raising the question, what positions the spindle? Normally the spindle lies at the cell centre with its pole–pole axis parallel to the long axis of the cell, but sometimes the spindle is asymmetrically positioned leading to developmentally important asymmetric divisions. It is plausible that a balance of forces is responsible for cell centring of the spindle and that a shift in this balance leads to asymmetric spindle positioning[64,65].

Spindle MTs determine the position of the cleavage plane midway between the poles (Fig.1d), and early micromanipulation experiments suggested that the astral MTs are responsible for inducing the cleavage furrow[66] with the corresponding signal being proportional to the number of astral MTs reaching the cortex. But other data suggest that ipMTs and kMTs, rather than astral MTs, are responsible for furrow positioning[67]. Some evidence suggests that chromosomes play a role, because some proteins — called chromosome passenger proteins — translocate from chromosomes to spindle MTs, and could therefore control the position of the furrow. Thus, it seems that the whole spindle mediates furrow induction, but there are system-specific differences in the parts of the spindle that are important[68].

The initiation of cytokinesis begins a few minutes after anaphase onset. There is no obvious tight temporal coupling between the completion of mitosis and the beginning of cytokinesis, but there seems to exist a permissive time interval lasting a few minutes after mitotic exit when cytokinesis can occur. In echinoderm eggs, it has been estimated[66] that the signalling event occurs approximately 5 min prior to furrow formation, taking about a minute for the signal to travel from the asters to the furrow, and a further 2.5 min for the furrow to develop. These timescales suggest that motor-mediated transport of signalling molecules is involved. For example, a motor protein moving at ~ 0.1 μm s^{-1} would travel ~ 6 μm over 1 min, which is roughly the thickness of the egg cortex.

It is tempting to speculate that the delivery of this signal as well as other spindle-associated transport events discussed below depends upon a two-step transport system involving kinesin-driven motility along astral MTs followed by myosin-driven motility through cortical actin (Fig. 1d inset). In this way it would be analogous to the pathway of vesicle recruitment for exocytosis during cell membrane resealing and neurotransmission[69]. Elegant experiments have focused on a class of vesicles that seem to be delivered by astral MTs specifically to the contractile ring[70], although this is a late event in cytokinesis. Other factors that may be involved in the spatiotemporal control of furrow initiation include the small GTPase RhoA, and cell cycle-regulated myosin light-chain kinases, which may contribute to the timing of cytokinesis[71]. Functional proteomic approaches to cytokinesis[12,72] may uncover the molecules involved in this and other aspects of cytokinesis.

Once the division plane has been established, the assembly of the actomyosin-based contractile ring is crucial for subsequent ingression (Fig.1e and inset). The accumulation of actin and myosin II in the region of the furrow occurs during late anaphase by an uncertain mechanism. Some pre-existing actin filaments are recruited into the cleavage furrow by directed transport in the plane of the cortex, probably powered by myosin[67], but additional actin

polymerization, as well as recruitment of myosin filaments from the underlying cytoplasm, may occur as well.

Force generation for furrow ingression

The nature of the mechanical process that underlies ingression has been a topic of intense debate, specifically whether relaxation at the poles of the cell or contraction at the equator is responsible. The myosin-dependent equatorial contraction model (Fig.1e) has prevailed[73–75]. Structural studies showed an abundance of organized actomyosin bundles in the contractile ring aligned along the cell equator consistent with a 'purse-string' sliding-filament mechanism. It is estimated that hundreds to thousands of myosin molecules must be localized within this structure where they cooperate to generate the maximal contractile force of 10^3 to 10^5 pN that is proposed to be developed by the contractile ring[16,17,66,75]. However, there are reasons to question the generality of the equatorial contraction model, because highly ordered actomyosin bundles are not a universal feature of the contractile ring, and perhaps contraction could be spread globally throughout the cortex[75], rather than being restricted to the ring.

During ingression, the cell membrane deforms owing to its attachments with an underlying actomyosin network, and consequently ingression is often accompanied by the fusion of membrane vesicles with the ingressing cell membrane behind the leading edge of the furrow[70,76]. This targeted vesicle insertion contributes to narrowing the distance between the tip of the furrow and the spindle mid-zone, in addition to supplying proteins and lipids. It is plausible that a motor transport system may move the vesicles along spindle MTs and cortical actin filaments to the cell surface[69] for fusion with the plasma membrane[70,76].

Completion of cytokinesis

Conventional models of cytokinesis posit that once the furrow is positioned, ingression proceeds independently of the spindle or other MT structures by means of the self-enhancing contractility of the actomyosin network. There is growing evidence, however, of a dynamic interplay between the spindle and the actomyosin cortex: successful completion of cytokinesis requires a host of proteins of uncertain function that localize to the central spindle, including MT-based motors, septins, formin-homology proteins, components of the telophase disc, kinases and GTPases and their regulators[14,71,74]. Among these, a kinesin motor (variously named MKLP1/CHO1/PavKLP/ZEN4) is perhaps best understood as it is crucial in organizing the arrays of antiparallel MTs that form the spindle mid-zone and, as a consequence, it is essential for the completion of cytokinesis[11].

Ingression continues until the contractile ring compresses the central spindle into a compact midbody containing an electron-dense 'matrix' at its midline (Fig. 1f). It is proposed that midbody MTs could then serve as tracks for the motor-mediated transport of Golgi-derived vesicles and signalling molecules to the furrow during cell–cell abscission (Fig. 1f)[70,76]. The reasoning behind this is that, during the process of abscission, the midbody is severed and a secreted membrane barrier partitions the cells to form two daughter cells in a process that is thought to resemble the recruitment of vesicles for Ca^{2+}-regulated exocytosis[69] (Fig. 1f inset).

Future work

The prodigious amount of high quality work, much of which could not be cited, bears witness to our persistent fascination with cell division. For example, we did not mention work on plant cell division, where studies of the phragmoplast have pioneered thinking about cell–cell abscission[77], or work on bacterial cell division, where a tubulin-like protein, FtsZ and an actin-like protein, Par-M seem to drive cytokinesis and plasmid segregation, respectively[78], in an apparent reversal of the eukaryotic paradigm. Despite this effort, much needs to be done to understand mitosis and cytokinesis. The important role that cytoskeletal proteins play as force-generating elements in these processes is clear. The identities of many players are known[4–6,10,13], and there exists a reasonable understanding of the physical mechanisms by which polymer dynamics and motor proteins generate force for motility at the individual level[7,14]. But how these force-generating elements function cooperatively within the ensembles that form the cell division machinery is much less clear[7,37], and improved understanding will require quantitative biophysical and biochemical analysis combined with theoretical modelling in normal and experimentally manipulated dividing cells. These are exciting times for students of mitosis and cell division. □

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1. Harris, H. *The Birth of the Cell* (Yale Univ. Press, New Haven, 1999).
2. Mitchison, T. J. & Salmon, E. D. Mitosis: a history of division. *Nature Cell Biol.* **3,** E17–E22 (2001).
3. Wood, K. W., Cornwell, W. D. & Jackson, J. R. Past and future of the mitotic spindle as an oncology target. *Curr. Opin. Pharmacol.* **1,** 370–377 (2001).
4. Sharp, D. J., Rogers, G. C. & Scholey, J. M. Microtubule motors in mitosis. *Nature* **407,** 41–47 (2000).
5. Karsenti, E. & Vernos, I. The mitotic spindle, a self-made machine. *Science* **294,** 543–547 (2001).
6. Wittman, T., Hyman, A. & Desai, A. The spindle, a dynamic assembly of microtubules and motors. *Nature Cell Biol.* **3,** E28–E34 (2001).
7. Scholey, J. M. & Mogilner, A. in *Molecular Motors* (ed. Schliwa, M.) 327–355 (Wiley-VCH, Weinheim, 2003).
8. Kapoor, T. M. & Compton, D. A. Searching for the middle ground: mechanisms of chromosome alignment during mitosis. *J. Cell Biol.* **157,** 551–556 (2002).
9. McIntosh, J. R., Grishunk, E. L. & West, R. R. Chromosome-microtubule interactions during mitosis. *Annu. Rev. Cell Dev. Biol.* **18,** 193–219 (2002).
10. Shah, J. V. & Cleveland, D. W. Waiting for anaphase: Mad2 and the spindle assembly checkpoint. *Cell* **103,** 997–1000 (2000).
11. Glotzer, M. Animal cell cytokinesis. *Annu. Rev. Cell Dev. Biol.* **17,** 351–386 (2001).
12. Gonczy, P. *et al.* Functional genomic analysis of cell division in *C. elegans* using RNAi of genes on chromosome III. *Nature* **408,** 331–336 (2000).
13. Bray, D. *Cell Movements*(Garland, New York, 2001).
14. Howard, J. *Mechanics of Motor Proteins and the Cytoskeleton* (Sinauer, Sunderland, MA, 2001).
15. Nicklas, R. B. Measurements of the force produced by the mitotic spindle in anaphase. *J. Cell Biol.* **97,** 542–548 (1983).
16. Rappaport, R. Cell division: direct measurement of maximum tension exerted by furrow of echinoderm eggs. *Science* **156,** 1241–1243 (1967).
17. Burton, K. & Taylor, D. L. Traction forces of cytokinesis measured with optically modified elastic substrata. *Nature* **385,** 450–454 (1997).
18. Gunawardane, R. N., Lizarraga, S. B., Wiese, C., Wilde, A. & Zheng, Y. Gamma-tubulin complexes and their role in microtubule nucleation. *Curr. Top. Dev. Biol.* **49,** 55–73 (2000).
19. Moritz, M., Braunfeld, M. B., Guenebaut, V., Heuser, J. & Agard, D. A. Structure of the γ-tubulin ring complex: a template for microtubule nucleation. *Nature Cell Biol.* **2,** 365–370 (2000).
20. Keating, T. J. & Borisy, G. G. Immunostructural evidence for the template mechanism of microtubule nucleation. *Nature Cell Biol.* **2,** 352–357 (2000).
21. Erickson, H. P. γ-tubulin nucleation: template or protofilament? *Nature Cell Biol.* **2,** E93–E96 (2000).
22. Popov, A. V., Severin, F. & Karsenti, E. XMAP215 is required for the microtubule-nucleating activity of centrosomes. *Curr. Biol.* **12,** 1326–1330 (2002).
23. Kalab, P., Weis, K. & Heald, R. Visualization of a Ran-GTP gradient in interphase and mitotic *Xenopus* egg extracts. *Science* **295,** 2452–2456 (2002).
24. Walczak, C. E. Ran hits the ground running. *Nature Cell Biol.* **3,** E1–E3 (2001).
25. Theurkauf, W. E. TACCing down the spindle poles. *Nature Cell Biol.* **3,** E159–E161 (2001).
26. Walczak, C. E., Vernos, I., Mitchison, T. J., Karsenti, E. & Heald, R. Model for the proposed roles of different microtubule based motor proteins in establishing spindle bipolarity. *Curr. Biol.* **8,** 903 (1998).
27. Brust-Mascher, I. & Scholey, J. M. Microtubule flux and sliding in mitotic spindles of early *Drosophila* embryos. *Mol. Biol. Cell* **13,** 3967–3975 (2002).
28. Nedelec, F. Computer simulations reveal motor properties generating stable antiparallel microtubule interactions. *J. Cell Biol.* **158,** 1005–1015 (2002).
29. Kapoor, T. M. & Mitchison, T. J. Eg5 is static in bipolar spindles relative to tubulin: evidence for a static spindle matrix. *J. Cell Biol.* **154,** 1125–1133 (2001).
30. Wells, W. A. Searching for a spindle matrix. *J. Cell Biol.* **154,** 1102–1104 (2001).
31. Wigge, P. A. *et al.* Analysis of the *Saccharomyces* spindle pole by matrix assisted laser desorption/ionization (MALDI) mass spectroscopy. *J. Cell Biol.* **141,** 967–977 (1998).
32. Mack, G. J. & Compton, D. A. Analysis of mitotic microtubule-associated proteins using mass spectrometry identifies astrin, a spindle-associated protein. *Proc. Natl Acad. Sci. USA* **98,** 14434–14439 (2001).
33. Kinoshita, K., Arnal, I., Desai, A., Drechsel, D. N. & Hyman, A. A. Reconstitution of physiological microtubule dynamics using purified components. *Science* **294,** 1340–1343 (2001).
34. Heald, R. A dynamic duo of microtubule regulators. *Nature Cell Biol.* **2,** E11–E12 (1999).
35. Severin, F., Haberman, B., Huffaker, T. & Hyman, A. A. Stu2 promotes mitotic spindle elongation in anaphase. *J. Cell Biol.* **153,** 435–442 (2001).
36. Ostergren, G. The mechanism of co-orientation in bivalents and multivalents. The theory of orientation by pulling. *Hereditas* **37,** 85–156 (1951).
37. Cytrynbaum, E., Scholey, J. M. & Mogilner, A. Force-balance model for early spindle pole separation in *Drosophila* embryonic mitotic spindles. *Biophys. J.* **84,** 757–769 (2003).
38. Holy, T. E. & Leibler, S. Dynamic instability of microtubules as an efficient way to search in space. Proc. Natl Acad. Sci. USA **91,** 5682–5685 (1994).
39. Alexander, S. P. & Rieder, C. L. Chromosome motion during attachment to the vertebrate spindle: initial saltatory-like behavior of chromosomes and quantitative analysis of force production by

nascent kinetochore fibers. *J. Cell Biol.* 113**,** 805-815 (1991).
40. Skibbens, R. V., Skeen, V. P. & Salmon, E. D. Directional instability of kinetochore motility during chromosome congression and segregation in mitotic newt lung cells: a push-pull mechanism. *J. Cell Biol.* **122,** 859–875 (1993).
41. Sharp, D. J., Rogers, G. C. & Scholey, J. M. Cytoplasmic dynein is required for poleward chromosome movement in *Drosophila* embryos. *Nature Cell Biol.* **2,** 922–930 (2000).
42. Yucel, J. K. *et al.* CENP-meta, an essential kinetochore kinesin required for the maintenance of metaphase chromosome alignment in *Drosophila*. *J. Cell Biol.* **150,** 1–12 (2000).
43. Maddox, P., Desai, A., Oegema, K., Mitchison, T. J. & Salmon, E. D. Poleward microtubule flux is a major component of spindle dynamics and anaphase A in mitotic *Drosophila* embryos. *Curr. Biol.* **12,** 1670–1674 (2002).
44. Joglekar, A. P. & Hunt, A. J. A simple, mechanistic model for directional instability during mitotic chromosome movements. *Biophys J.* **83,** 42–58 (2002).
45. Levesque, A. A. & Compton, D. A. The chromokinesin Kid is necessary for chromosome arm orientation and oscillation, but not congression, on mitotic spindles. *J. Cell Biol.* **154,** 1135–46 (2001).
46. Maney, T., Ginkel, L. M., Hunter, A. W. & Wordeman, L. The kinetochore of higher eucaryotes: a molecular view. *Int. Rev. Cytol.* **194,** 67–131 (2000).
47. Desai, A., Verma, S., Mitchison, T. J. & Walczak, C. E. Kin-I kinesins are microtubule-destabilizing enzymes. *Cell* **96,** 69–78 (1999).
48. Nasmyth, K., Peters, J. M. & Uhlmann, F. Splitting the chromosome: cutting the ties that bind sister chromatids. *Science* **288,** 1379–1384 (2000).
49. Funabiki, H. & Murray, A. W. The *Xenopus* chromokinesin, Xkid is essential for metaphase chromosome alignment and must be degraded to allow anaphase chromosome movement. *Cell* **102,** 411–424 (2000).
50. Marshall, W. F., Marko, J. F., Agard, D. A. & Sedat, J. W. Chromosome elasticity and mitotic polar ejection force measured in living *Drosophila* embryos by four-dimensional microscopy-based motion analysis. *Curr. Biol.* **11,** 569–578 (2001).
51. Zhou, J., Yao, J. & Yoshi, H. C. Attachment and tension in the spindle assembly checkpoint. *J. Cell Sci.* **115,** 3547–3555 (2002).
52. Howell, B. J., Hoffman, D. B., Fang, G., Murray, A. W. & Salmon, E. D. Visualization of Mad2 dynamics at kinetochores, along spindle fibers, and at spindle poles in living cells. *J. Cell Biol.* **150,** 1233–1250 (2000).
53. Li, X. & Nicklas, R. B. Tension-sensitive kinetochore phosphorylation and the chromosome distribution checkpoint in praying mantid spermatocytes. *J. Cell Sci.* **110,** 537–545 (1997).
54. Hoffman, D. B., Pearson, C. G., Yen, T. J., Howell, B. J. & Salmon, E. D. Microtubule-dependent changes in assembly of microtubule motor proteins and mitotic spindle checkpoint proteins at PtK1 kinetochores. *Mol. Biol. Cell* **12,** 1995–2009 (2001).
55. Howell, B. J. *et al.* Cytoplasmic dynein/dynactin drives kinetochore protein transport to the spindle poles and has a role in mitotic spindle checkpoint inactivation. *J. Cell Biol.* **155,** 1159–1172 (2001).
56. Chan, G. K. T., Jablonski, S. A., Starr, D. A., Goldberg, M. L. & Yen, T. J. Human Zw10 and ROD are mitotic checkpoint proteins that bind to kinetochores. *Nature Cell Biol.* **2,** 944–947 (2000).
57. Wojcik, E. *et al.* Kinetochore dynein: its dynamics and role in the transport of the Rough deal checkpoint protein. *Nature Cell Biol.* **3,** 1001–1008 (2001).
58. Abrieu, A., Kahana, J. A., Wood, K. W. & Cleveland, D. W. Cenp-E as an essential component of the mitotic checkpoint in vitro. *Cell* **102,** 817–826 (2000).
59. Nicklas, R. B., Waters, J. C., Salmon, E. D. & Ward, S. C. Checkpoint signals in grasshopper meiosis are sensitive to microtubule attachment but tension is still essential. *J. Cell Sci.* **114,** 4173–4183 (2001).
60. Skoufias, D. A., Andreassen, P. R., Lacroix, F. B., Wilson, L. & Margolis, R. L. Mammalian mad2 and bub1/bubR1 recognize distinct spindle-attachment and kinetochore-tension checkpoints. *Proc. Natl Acad. Sci. USA* **98,** 4492–4497 (2001).
61. Shannon, K. B., Canman, J. C. & Salmon, E. D. Mad2 and BubR1 function in a single checkpoint pathway that responds to a loss of tension. *Mol. Biol. Cell* 10.1091/mbc.E02-03-0137 (2002).
62. Zhou, J., Panda, D., Landen, J. W., Wilson, L. & Joshi, H. C. Minor alteration of microtubule dynamics causes loss of tension across kinetochore pairs and activates the spindle checkpoint. *J. Biol. Chem.* **277,** 17200–17208 (2002).
63. Beaudouin, J., Gerlich, D., Daigle, N., Eils, R. & Ellenberg, J. Nuclear envelope breakdown proceeds by microtubule-induced tearing of the lamina. *Cell* **108,** 83–96 (2002).
64. Reinsch, S. & Gonczy, P. Mechanisms of nuclear positioning. *J. Cell Sci.* **111,** 2283–2295 (1998).
65. Grill, S. W., Gonczy, P., Stelzer, E. H. & Hyman, A. Polarity controls forces governing asymmetric spindle positioning in the *C. elegans* embryo. *Nature* **409,** 630–633 (2001).
66. Rappaport, R. *Cytokinesis in Animal Cells* (Cambridge Univ. Press, Cambridge, 1996).
67. Cao, L. G. & Wang, Y. L. Mechanism of the formation of contractile ring in dividing cultured animal cells. I. Recruitment of preexisting actin filaments into the cleavage furrow. *J. Cell Biol.* **110,** 1089–1095 (1990).
68. Wang, Y. L. The mechanism of cytokinesis: reconsideration and reconciliation. *Cell Struct. Funct.* **26,** 633–638 (2001).
69. Bi, G. Q. *et al.* Kinesin- and myosin-driven steps of vesicle recruitment for Ca^{2+} regulated exocytosis. *J. Cell Biol.* **138,** 999–1008 (1997).
70. Shuster, C. B & Burgess, D. R. Targeted new membrane addition in the cleavage furrow is a late, separate event in cytokinesis. *Proc. Natl Acad. Sci. USA* **99,** 3633–3638 (2002).
71. Robinson, D. N. & Spudich, J. A. Towards a molecular understanding of cytokinesis. *Trends Cell Biol.* **10,** 228–237 (2000).
72. Somma, M. P., Fasulo, B., Cenci, G., Cundari, E. & Gatti, M. Molecular dissection of cytokinesis by RNA interference in *Drosophila* cultured cells. *Mol. Biol. Cell* **13,** 2448–2460 (2002).
73. Satterwhite, L. L. & Pollard, T. D. Cytokinesis. *Curr. Opin. Cell Biol.* **4,** 43–52 (1992).
74. Guertin, D. A., Trautmann, S. & McCollum, D. Cytokinesis in eukaryotes. *Microbiol. Mol. Biol. Rev.* **66,** 155–178 (2002).
75. Robinson, D. N., Cavet, G., Warrick, H. M. & Spudich, J. A. Quantitation of the distribution and flux of myosin-II during cytokinesis. *BMC Cell Biol.* **3,** 4–16 (2002).
76. Finger, F. P. & White, J. G. Fusion and fission: membrane trafficking in animal cytokinesis. *Cell* **108,** 727–730 (2002).
77. Liu, B. & Lee, Y.-R. Kinesin-related proteins in plant cytokinesis. *J. Plant Growth Regul.* **20,** 141–150 (2001).
78. Moller-Jensen, J., Jensen, R. B., Lowe, J. & Gerdes, K. Prokaryotic DNA segregation by an actin-like filament. *EMBO J.* **21,** 3119–3127 (2002).
79. Purcell, E. Life at low Reynolds number. *Am. J. Phys.* **45,** 3–11 (1977).

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