# Mitosis: a history of division

focus on
*cell division*

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**Mitosis has been studied since the early 1880s, to the extent that we now have a detailed, but still incomplete, description of spindle dynamics and mechanics, a sense of potential mechanochemical and regulatory mechanisms at a molecular level, and a long list of mitotic proteins. Here we present a personal view of how far we have come, and where we need to go to fully understand the mechanisms involved in mitosis.**

Walther Flemming[1] originally coined the term mitosis in the early 1880s from the Greek word for thread, relfecting the shape of mitotic chromosomes. The basic mechanics of mitosis were described by live imaging from the 1920s to the 1950s and we now have a detailed, although incomplete, overall picture of mitosis. For scholarly reviews, see refs 2–12 and page E27 of this issue.

Chromatin dynamics were well described by early cytologists, partly because chromatin binds to dye molecules so well, hence its name. They described condensation of nuclear material into chromosomes in which the paired sister chromatids are visible (Fig. 1a), and longtitudinal splitting between sister chromatids at anaphase[1,2]. We now understand packaging of DNA into nucleosomes and chromatin fibres fairly well, although how these fibres assemble into mitotic chromosomes remains unknown. The early images revealed, in retrospect, that condensation sorts sister DNA strands into paired chromatids, and that these remain cohered until anaphase. A significant breakthrough in understanding the 'glue' that promotes sister-chromatid cohesion was the identification of the cohesin complex, one subunit of which is proteolyzed to trigger sister-chromatid separation in yeast mitosis and meiosis[13]. However, mysteries remain, particularly in the relationship between condensation and cohesion. During prophase in vertebrates a different complex, condensin, is recruited to chromatin, where it is thought somehow to promote condensation[14] (Fig. 1b). Most of the cohesin is released during condensation, although a small fraction remains between the centromeres, where it presumably promotes cohesion[15] (Fig. 1c). Although condensation and cohesion seem to be mechanistically very different, cohesin and condensin and are thought to share some biochemical function, as both contain heterodimeric pairs of SMC polypeptides[14]. Elucidating this shared function may be one key to understanding chromatin dynamics in mitosis.

The shape of the mitotic spindle, together with evidence for filamentous organization, was described by early cytologists[1,2] (Fig.1),

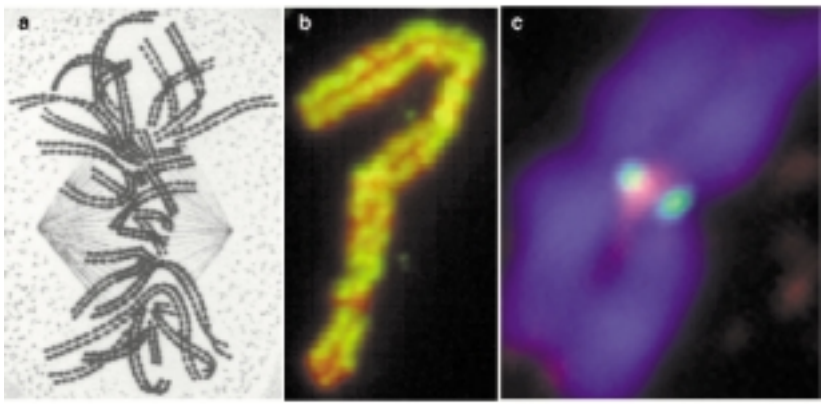


Figure 1 **Old and new views of mitotic chromosomes.** a, **Metaphase salamander cell drawn by Walther Flemming from a stained preparation (adapted from *Views of the Cell*, J. Gall, ASCB, Bethseda, Maryland; 1996). Note the 'thread-like' paired sister chromatids, joined along their lengths, and the spindle fibres.** b, **Replicated mitotic chromosome assembled in *Xenopus* egg extract and stained for DNA (red) and a subunit of the condensin complex (green). Note that condensin, which has a key function in chromatin condensation[14], is targeted to a subset of DNA. (Image courtesy of T. Hirano, Cold Spring Harbor Laboratory, New York.)** c, **Mitotic chromosome from a nocodazole-arrested human cell stained for DNA (blue), kinetochores (green) and a subunit of the cohesin complex (SCC1, red). Note that cohesin is present between the centromeres where sister chromatids are still joined. Cohesin is thought to hold sister chromatids together until anaphase[13,15]. (Image courtesy of S. Hauf and J-M. Peters, IMP, Vienna, Austria.)**

and forces acting parallel to the spindle axis were evident from fixed images and early time-lapse movies of mitosis[2,3,6,16–18] (Fig. 2a). Conclusive evidence that spindles are made of filaments running parallel to the direction of chromosome movement came from polarization microscopy in the early 1950s (ref. 7, Supplementary Information Fig. S1a). The rapid, reversible response of spindle filaments to perturbation led to the proposal that polymerization dynamics might drive chromosome movement[7], a model that is still relevant (Fig. 2b). Introduction of glutaraldehyde fixation subsequently allowed electron-microscopic observation of spindle microtubules and kinetochores in the early 1960s (refs 6, 19). Tubulin was identified in the late 1960s by its prevalence in flagella and ability to bind to radioactive colchicine[5,20,21], and polymerization of brain tubulin *in vitro* was described in 1972 (ref. 22). Tubulin biochemistry gave rise to two models of polymerization dynamics driven by GTP hydrolysis — treadmilling[23] and dynamic instability[24]. Treadmilling was inferred from GTP incorporation at steady state that was, in retrospect, probably due to dynamic instability[25]; treadmilling of pure tubulin has not been directly observed. Photobleaching of fluorescein–tubulin first revealed that spindle microtubules turn over very rapidly at steady state[26,27]. Fast turnover was confirmed by injection of biotin–tubulin[28] and fluorescence photoactivation (Fig. 3c). Dynamic instability of microtubule plus ends is now thought to account for the majority of fast subunit turnover in animal



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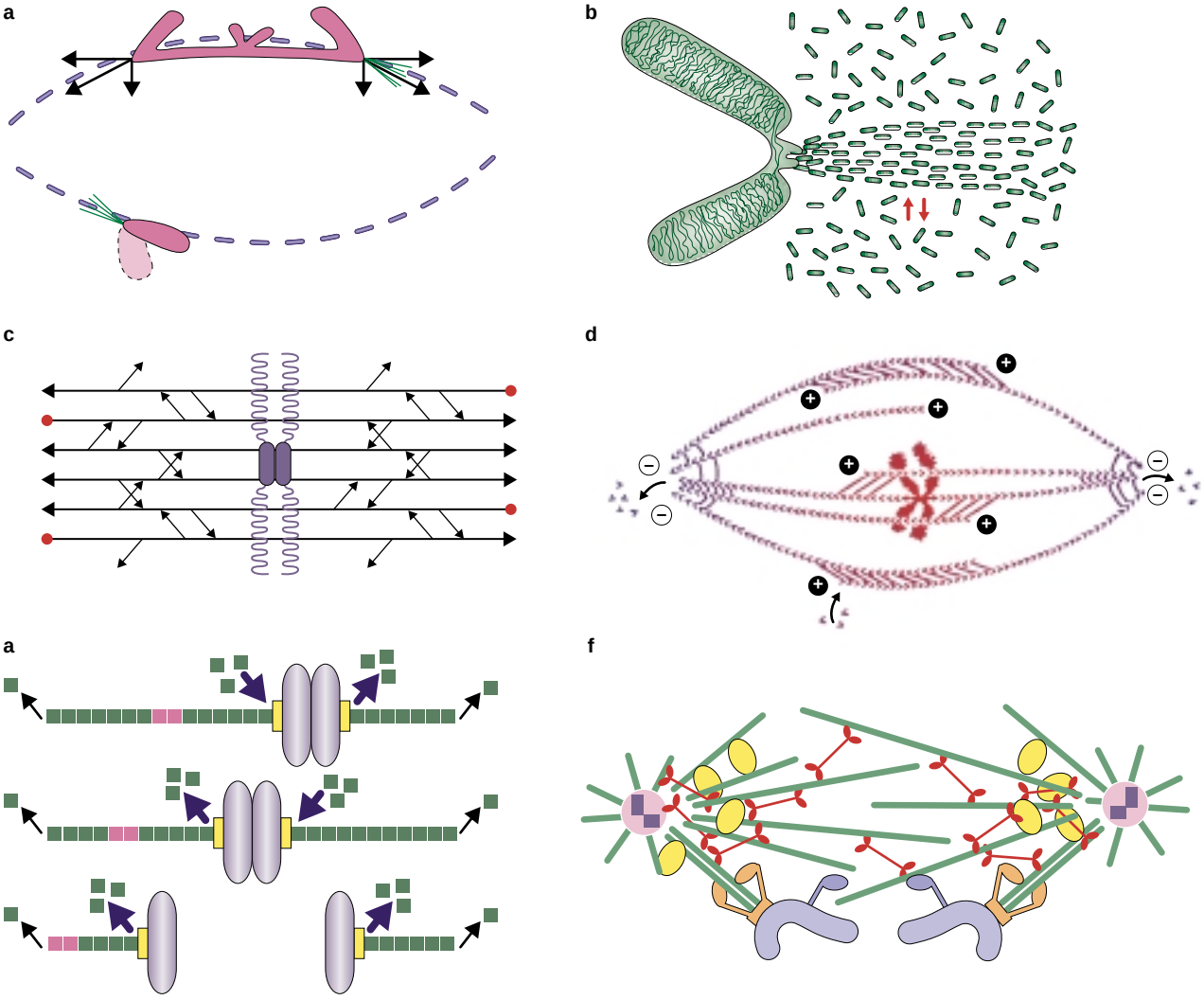


Figure 2 **Evolution of ideas for spindle organization and force generation.** a, **Ostergren's view in the late 1940s, as discussed refs 6, 18. The image represents spindle in an insect spermatocyte. The forces acting on chromosomes (arrows) were inferred from chromsome stretching and time-lapse imaging of movement. Ostergren argued that metaphase alignment was due to equalization of pulling forces when the chromosome was centered on the metaphase plate. (Image adapted from ref. 6.)** b, **Inoue's view in the mid 1960s (ref. 7). Protein-assembly dynamics are central to force production, but the structural details are unclear. Later, Inoue concluded on the basis of experiments (Supplementary Information Fig. S1a) that tubulin must be polymerized at kinetochores during metaphase, and depolymerized near the poles[56]. (Image adapted from ref. 6.)** c, **The model proposed by McIntosh *et al.*[44] in 1969, which explicitly invokes microtubule polarity and motor proteins. Horizontal arrows indicate microtubules and their polarity; short diagonal arrows represent putative mechanochemical enzymes. The predicted microtubule polarity is incorrect, but this model was very important in highlighting the importance of polarity and in stimulating the search for motor proteins involved in mitosis.** d, **Margolis and Wilson's 1981 treadmilling model[59]. Arrows show sites of polymerization at kinetochores and free microtubule plus ends, and depolymerization at poles; they also indicate microtubule polarity. This model combined knowledge of tubulin biochemistry[57] and spindle-microtubule polarity[37] (polarity is indicated with plus and minus signs) and results indicating poleward flux (Supplementary Information Fig. S1a). It predicted poleward flux and postulated functions for dynamics and motors that are still relevant. Whether treadmilling driven by GTP hydrolysis on tubulin has a role in poleward flux remains to be determined.** e, **Current model for polymerization dynamics of kinetochore microtubules, showing the role of dynamics at kinetochore and poles in vertebrate tissue-culture cells. The red mark represents a fiduciary mark such as those shown in Fig. 4c, d. Depolymerization at kinetochores, which was discovered by marking experiments[28,38], now has a key role in chromosome movement. Poleward flux, which has now been directly observed[40,41], is also important.** f, **Current, motor-centric model that emphasizes the role of Eg5 (red) and dynein (yellow) in organizing the spindle[12,47]. Kinetochores (orange) are shown moving polewards through the action of a motor (also orange) that may be dynein or an unknown motor protein[11]. Another motor possibly involved in chromosome movement is attached to chromosome arms (blue). The minus ends of spindle microtubules are shown detached from the centrosomes to allow flux.**



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spindles[9], although the complete life history of spindle microtubules is not known. Photobleaching of tubulin tagged with green fluorescent protein allows turnover to be probed in yeast[29,30] (See Supplementary Information Fig. S1b), where genetics will help to reveal mechanism.

To understand spindle mechanics, it has long been clear that we need to measure the forces acting in the spindle, and also to determine the arrangement and dynamics of the microtubules that are involved in force production. Direct force measurement using microneedles revealed that large forces pull chromosomes towards the poles during anaphase A[31], indicating, alongside other micromanipulation data[32], that the rate of chromosome movement may be limited by a velocity governor, which may be distinct from the force generator(s). A velocity governor in the overlap zone may also limit the rate of spindle expansion (anaphase B) in some systems[33]. The static arrangement of microtubules in animal and yeast spindles is now largely understood as a result of painstaking electron-microscopic analysis[34–36], including the structural polarity of microtubules[37], which is key to modern mechanical models. Our understanding of dynamics in relation to mechanics is still evolving. Progress has been made in part through development of successively improved methods for making fiduciary marks on microtubules (Fig. 3). We now think that in simple spindles, such as those in yeast, microtubules polymerize at kinetochores when chromosomes move away from poles, and depolymerize there when they move polewards[29,30]. In larger, more complex animal spindles, these kinetochore-based mechanisms still operate[28,38], but superimposed on them is a second mechanism for poleward movement in which microtubules flux polewards and depolymerize at poles[39–41]. Prometaphase congression and anaphase B movements have been less studied by microtubule marking. Anaphase B in yeast and diatoms involves antiparallel sliding[29,30,35,36], although how this is powered and regulated is poorly understood. Congression is a particularly interesting problem, as balancing chromosomes at the metaphase plate requires position-dependent forces (Fig. 2a). Two models dominate current discussion — the force per unit length that pulls on kinetochore fibres[18,42], and opposition of kinetochore pulling by pushing on chromosome arms from astral ejection forces[11,43]. Strong arguments exist for both models in different systems, and one model may not apply to all spindles. Overall, we still have a long way to go to understand spindle mechanics, and specific mechanisms may not be suitable for generalization. Progress will require better ways of imaging dynamics, in conjunction with new methods for

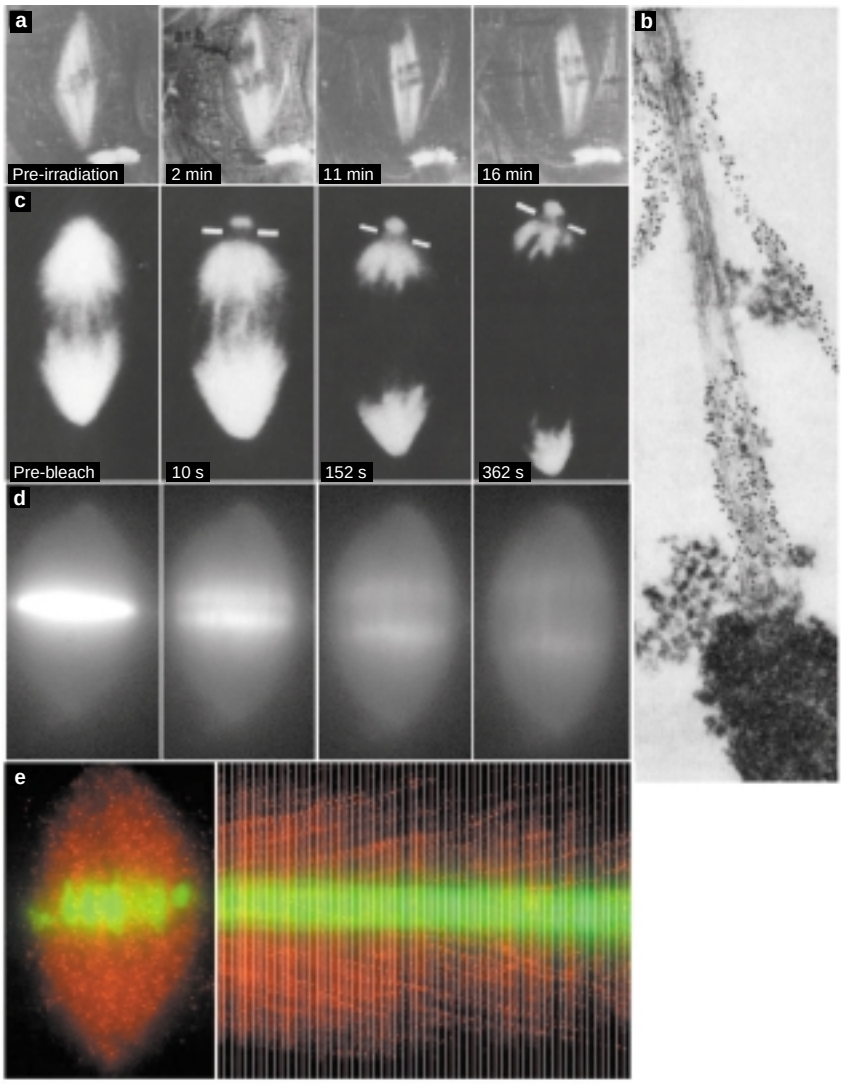


Figure 3 **Evolution of microtubule-marking technology. a, Area of reduced birefringence produced by locally ablating microtubules in an insect spermatocyte with an ultraviolet microbeam[39]. Images were obtained before irradiation and at the indicated times after. The spindle is about 40 mm long. (Adapted from ref. 56.)** b, **Polymerization of biotin-labelled tubulin at a kinetochore in a $PtK_2$ cell (adapted from ref. 28). This cell was fixed 120 s after microinjection. A short segment of biotin-labelled microtubule, visualized with gold particles, has polymerized at the kinetochore; the labelled segments disappeared in anaphase.** c, **Photobleaching of rhodamine–tubulin in an LLC-PK1 cell during anaphase (adapted from ref. 38). As the chromosomes (not shown) move polewards, the bleach mark (bars) remains a constant distance from the pole, indicating that kinetochore microtubules depolymerize primarily at kinetochores. Images were obtained before bleaching and at the indicated times after. The spindle is about 20 mm long.** d, **Photoactivation of caged fluorescein–tubulin incorporated into a *Xenopus* extract spindle. Images were obtained at 60-s intervals, the mark moves at a rate of 2–3 mm $min^{-1}$, and the spindle is about 40 mm long. (Image courtesy of T. Kapoor and the MBL Cell Division Group, Marine Biol. Lab., Woods Hole, Massachusetts).** e, **Fluorescent speckles in a *Xenopus* extract spindle. Imaging of a low density of rhodamine-labelled tubulin (red) generates speckles that can be followed as fiduciary marks[59]. The left panel shows the whole spindle; DNA is shown in green. The right panel shows a series of strips from the centre of this spindle taken at 10-s intervals and displayed side by side (a kymograph). The diagonal streaks represent individual speckles moving polewards at a rate of 2–3 mm $min^{-1}$ as a result of flux. (Image courtesy of A. Desai, P. Maddox and the MBL Cell Division Group.)**



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measuring endogenous forces and applying artificial ones.

How the forces that move chromosomes are generated at a molecular level is one of the oldest questions in mitosis research. Anaphase A and B were shown to be biochemically distinct in their responses to poisons in the 1940s (refs 16, 17). Models from around that time show forces (Fig. 2a), but modern ideas of their molecular basis had to wait for the discovery of the mechanisms of muscle contraction and protein polymerization. Since the 1960s, two lines of thought have predominated — force from polymerization dynamics[7] (Fig. 2b, d, e) and force from motor-protein activity[44] (Fig. 2c, f). We now know that depolymerization can generate forces on chromosomes *in vitro*[9,45,46]. We also know that several different kinesin family members, as well as cytoplasmic dynein, localize to spindles and kinetochores, and have key functions in spindle assembly and perhaps also chromosome movement[11,12,47] (Figs 2c, f and 4). At present, something of a gulf exists between dynamics-centered and motor-centered views of spindle assembly and force generation, although it seems inevitable that the real answer will involve integration of both views. So far neither genetics nor biochemistry has revealed a single key molecular mechanism at the heart of anaphase A or B movement. Imaging the effect on chromosome movement rate of removing candidate motors[12,48,49] is a good start on this problem, but will not be enough. Force-producing systems may be partially redundant, and the likely existence of separate force-generating and velocity-governing systems will complicate interpretation of velocity data. Full understanding of the molecular basis of force generation in mitosis will probably require direct measurement of forces as a function of genetic or biochemical manipulation, as well as biochemical reconstitution.

Colchicine has long been known to arrest cells in mitosis[5] (Fig. 5a), but the idea that spindle damage activates a checkpoint pathway, rather than blocking progression of a series of interdependent cell-cycle events, is a recent one[50]. It took identification of the Mad and Bub proteins, which are required for mitotic arrest but not necessarily for normal mitosis, to make this idea concrete[51,52]. Partly because checkpoint proteins seem to be more conserved between yeast and man than other mitotic components (Fig. 5b), we may understand how the checkpoint works before we understand how chromosomes move[10,11]. The two known motor proteins present at kinetochores, CenpE and dynein, seem to be involved in sensing microtubule attachment[53,54], although they may also function in chromosome movement[11,49,55]. Recruitment of motors to unattached kinetochores may facilitate microtubule capture, and thus repair of spindle defects. Microtubule sensing and force generation at kinetochores must be closely interrelated, and to understand one we may have to understand the other. This is a rich challenge for the future. □

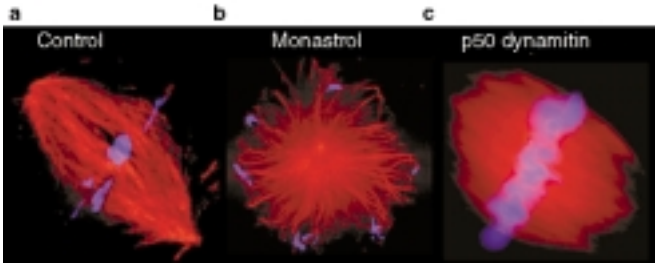


Figure 4 **Motor proteins are important in spindle assembly and function.** a, **Control *Xenopus* extract spindle, showing DNA (blue) and microtubules (red). The spindle is about 40 mm long.** b, **Spindle treated with monastrol, a small molecule that inhibits Eg5 (ref. 61). Eg5 is a mitotic kinesin from the BimC family and is required to make spindles bipolar[12,47].** c, **Spindle treated with p50 dynamitin, a subunit of the dynactin complex, before spindle assembly. This treatment disrupts dynactin and prevents targeting of cytoplasmic dynein to spindle poles. Note the broad, unfocused poles. One function of dynein is in spindle-pole organization[12,47]. (Images courtesy of T. Kapoor and the MBL Cell Division Group.)**

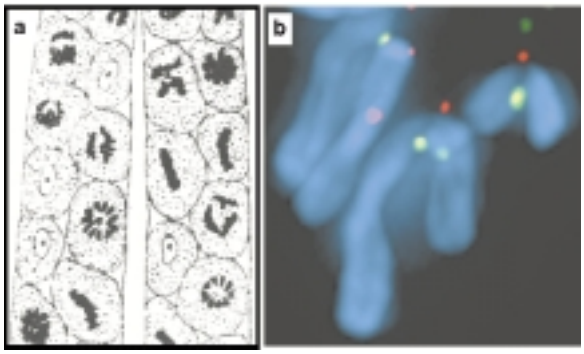


Figure 5 **Mitotic arrest and the spindle-assembly checkpoint.** a, **Image of cells drawn in 1889 by Pernice[60] from a fixed preparation of dog intestine after administration of a lethal dose of colchicine. This was cited by Dustin[5] as the first published observation of mitotic arrest by this tubulin-binding drug. The stain emphasizes chromatin. Note the abundant arrested mitotic cells. (Adapted from ref. 5.)** b, **Recent image of part of a $PtK_2$ cell arrested in mitosis with the Eg5 blocker monastrol[61], stained for DNA (blue), kinetochores (red) and Mad2 (green). The paired kinetochores of three chromosomes can be seen. Each chromosome has one Mad2-negative and one Mad2-positive kinetochore. Recruitment of Mad2 to kinetochores is thought to reflect activation of the spindle-assembly checkpoint that arrests the cell in mitosis[10,11]. (Image courtesy of T. Mayer and T. Kapoor, Dept Cell Biol., Harvard Med. School, Boston, Massachusetts.)**

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