Microtubules in action

Multiple mechanisms of mitosis?

from Stephen King

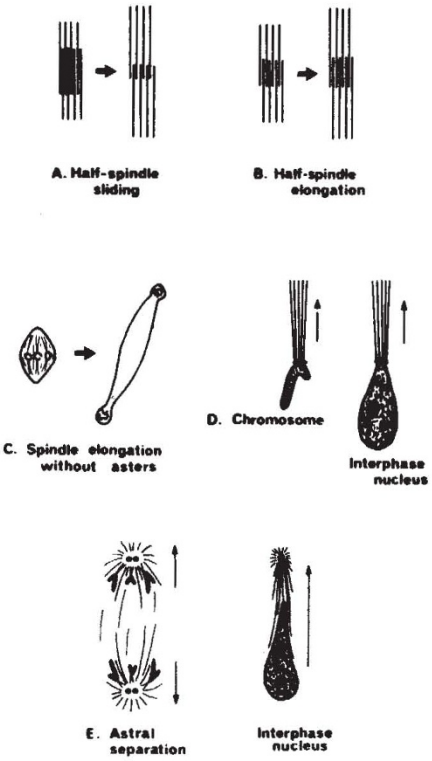
DURING mitosis chromosomes are associated with a framework of microtubules, the varying arrangements of which form a plethora of different spindle designs. It is a matter of some controversy as to whether these differences in spatial organization are reflected in a comparable mechanistic diversity or whether the popular idea of a 'unified mitotic mechanism' (one applicable to all eukaryotic cells) is, in fact, correct. Recently, data have been obtained from several different laboratories which suggest that microtubules of the mitotic appartus may be involved in several distinct motile reactions.

One particularly intriguing result has been obtained from the examination of mitosis in endosperm of the plant *Haemanthus*[1] during treatment with taxol (a drug that stabilizes microtubules and also promotes their assembly[2]). In these experiments, the anaphase movement of chromosomes was desynchronized and for periods of up to 10 min the direction of motion was reversed before polar migration resumed. As shown by immunogold staining, microtubule elongation occurred in the direction of chromosome movement. The data thus suggest that taxol-induced elongation produces a pushing force *in vivo*. However, whether microtubules do, in fact, exert a significant 'push' in the absence of taxol still remains open to question. But bearing in mind this caveat these experiments do provide evidence for chromosome movement as a result of microtubule growth.

The reversible anti-microtubule drug nocadazole has been used to arrest chromosome movement in early anaphase mammalian (PtK_1) cells[3]. Almost complete disassembly of non-kinetochore microtubules (those not directly attached to the chromosomes) results but following release from drug-induced arrest, spindle elongation (anaphase B) and chromosome-to-pole movements (anaphase A) both restart without the reappearance of the disrupted microtubules. This is a highly interesting result as it strongly suggests that anaphase B does not require interzonal microtubules and also that anaphase A motions are not based on an interaction between kinetochore and non-kinetochore microtubules.

Other studies on this cell line have also suggested that interzonal microtubules do not generate the motive force required for anaphase B (ref.4). When microtubule continuity between the two half-spindles was disrupted by pushing the dorsal and ventral membranes of the cell together, the rate of anaphase B essentially doubled. Although reservations have been raised concerning this experiment it is interesting that the results obtained are compatible with those from nocadazole-treated cells.

Both sets of results are apparently at odds with those obtained using permeabilized models of PtK_1 cells[5]. In this system, anaphase B movements continued in conditions similar to those that bring about the sliding disintegration of flagella axonemes, suggesting the presence of a dynein-like Mg^{2+}-ATPase in the spindle[6]. Further evidence for the active involvement of spindle microtubules in anaphase B has been obtained following microbeam experiments on diatoms[7] where interdigitating microtubules slide apart to allow for the observed elongation.



Some of the mechanisms suggested to be involved in anaphase movements. These models are supported by evidence obtained from many different organisms. Thus it appears improbable that a single force-generating mechanism could be responsible for the anaphase separation of chromosomes in all eukaryotes.

In another series of microbeam experiments, however, a laser was used to disrupt the spindle of the fungus *Fusarium solami* and brought about a threefold increase in the velocity of anaphase B (ref.8). This led to the hypothesis that the microtubules function as a governor regulating some unidentified force-generating component (perhaps the astral microtubules). A similar suggestion has also been advanced following an analysis of anaphase B in *Saccharomyces cerevisiae*. Here, the central spindle is composed solely of continuous microtubules and as spindle length increases from 1 to 4 μm the number of microtubules rapidly decreases from 10 to 1. This single microtubule spindle then continues to elongate, reaching a final length of 8 μm (ref.9). Correlation of the decrease in microtubule number with the timing and velocity of anaphase B (ref.10) has shown that at the final transition from two microtubules to one there is an 11-fold increase in the rate of spindle elongation — strong evidence of a passive regulatory role for the continuous microtubules.

Recently it has been hypothesized that in diatoms, chromosome movement is achieved by a motile kinetochore (the chromosome–microtubule attachment site) which utilizes the spindle microtubules as a vectorial framework[11]. Results of experiments using metabolic inhibitors to deplete intracellular ATP levels[12] suggest that it is the establishment of the metaphase configuration of chromosomes that requires ATP. Thus metaphase is proposed to be a 'high-energy' state and anaphase A motions might be achieved by the release of this tension (after chromosome splitting) via the kinetochore. Whether such a hypothesis may be applied to other system is uncertain as diatoms have unusual chromosome and spindle arrangements, but the possibility should not be ignored.

In conclusion, the proposed ubiquity of models based on interactive phenomena is being increasingly challenged. Indeed, the concept of phylogenetic universality[13] has proved to be the most difficult hurdle for any model of mitosis. But it should be remembered that many current hypotheses already invoke two different roles for the spindle microtubules — sliding and assembly/disassembly[5] — and there is no *a priori* reason why other mechanisms using microtubules as a vectorial framework[11,12] or as a regulatory system[8,10] might not also have evolved. Indeed, as more functionally significant information is gathered, the possibility of multiple mechanisms of mitosis becomes increasingly attractive. □

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