INSTRUCTOR'S GUIDE:

"The Dynamics and Mechanics of Mitosis"

---

This Instructor's Guide provides some basic background information and a few references related to the video sequences. It also covers the two Appendices. The first, "Mitosis in Diatoms", shows how these exceptional microscopic objects have contributed to our understanding of mitosis. The second, "Experiments on Mitotic Cells", illustrates a tiny subset of experimental approaches, some of which show how challenging it can be to interpret and reconcile different experimental results. The video is organised into consecutively numbered Chapters.

---

The mitotic spindle is an extraordinary structure which achieves a very important role in the cell with almost faultless accuracy. If it did not do so, eucaryotic life would be impossible. The literature on mitosis, although vast, still does not adequately explain the cellular mechanisms that organise and separate chromosomes. Schrader summarised progress up to 1944 thus: "Since about 1870, there has been a succession of periods in which triumph seemed to stand on the threshold as, first, observers of the living cell, then students of the morphology of the fixed cell, and lastly the physiologists marshalled the evidence furnished by their different attacks ....each of these periods had a corresponding aftermath of disillusion, always accompanied by a new appreciation of the difficulties of the problem". His comments remain valid today.

Chap 1. "Introduction: A Very Brief History of Mitosis"

In 1858, Virchow enunciated the famous principle: "Omnis cellula e cellula" (All cells come from pre-existing cells). Wilson (1925) states that ".....this terse phrase embodies one of the most important generalizations of modern science"; and later: "....cells have no other mode of origin than by the division of pre-existing cells. Upon cell-division, therefore, depends not alone heredity but the very continuity of life." However, it is not clear why continuity of a cell's life requires cell division. Mazia's (1961) approach to this enigma is to stress what happens when cells stop division: they inevitably die.

Flemming in 1879 first appreciated the significance of chromosomes and he coined the term "mitosis" in 1882. After Mendel's laws were rediscovered around 1900 by Correns, DeVries and Tschermak, Sutton and De Vries in 1903 demonstrated the correlation between the behaviour of chromosomes and transmission of genetic characters. Confusingly, chromosomes disappear after mitosis, inexplicable behaviour in entities supposedly continuous from generation to generation. Rabl and Boveri proposed correctly that chromosomes dispersing after mitosis do not lose their identity, and showed
that upon reforming for the next division, their morphology reflected their disposition from the preceding mitosis.

Analysis of spindle structure was initiated by Van Beneden, Boveri and others in the 1880's; they correctly represented the spindle as two half spindles, and distinguished between chromosomal and continuous (pole-to-pole) fibres. Chromosome movement in living cells was becoming more frequently observed by the 1930's, but generally neglected in historical reviews (e.g. Wilson, 1925) were the superb observations of Lauterborn (1896) on mitosis in living diatoms (Appendix 1).

The advent of electron microscopy in the late 1950's led to controversy over the existence of spindle fibres which illustrated how dependent scientific observations can be on preparative methods. With good reason, osmium tetroxide was regarded as the best fixative available at that time. Then the introduction of glutaraldehyde (Sabatini et al., 1963) showed that osmium fixation destroys most microtubules (MTs). Inoue and Bajer's work on living cells was seminal in showing the reality of spindle fibres.

2: Mitosis and Cytokinesis in Animal (Vertebrate) Cells

Mitosis is a separate process from cytokinesis and requires entirely different cytoskeletal systems. In most familiar cells, cytokinesis occurs during telophase but in many cells, mitosis can continue without cytokinesis, creating multinucleate cells (Chap. 7). Mitosis is divided into five stages: Prophase; Prometaphase; Metaphase; Anaphase and Telophase. A distinctive "Preprophase" stage is recognised in higher plants (Chap. 5). Each stage steadily flows into the next. Only two transitions between stages are sharply defined; these are:

i) the beginning of prometaphase, when chromosome activity suddenly commences as spindle fibres penetrate amongst them; and

ii) the beginning of anaphase, when the paired chromatids comprising the chromosomes, synchronously split and separate polewards.

Animal cells have a centrosome near the nucleus from which cytoplasmic MTs radiate and which contains two centrioles. During S-phase of the cell cycle, two new centrioles appear adjacent to the older ones. These pairs, one new and one older centriole, separate to establish the spindle poles at prophase. Many other cells (e.g., protists, fungi) have similar centrosomes at the spindle poles, but devoid of centrioles. Higher plant cells do not have centrioles and centrosomes (Chap. 5). These observations negate any role of centrioles in directly generating the MTs of the spindle.

Chap. 3. Spindle Fibres: Origin and Organization.

During prophase and prometaphase, centrosomes initiate a phase of MT assembly and elongation, generating increasingly conspicuous asters. The polar MTs display enhanced dynamic instability (Mitchison & Kirschner, 1984; Sammak & Borisy, 1988), shortening and elongating continuously. The MTs are
initially all equivalent but some become overlapped with similar MTs from the other pole, becoming stabilised and creating continuous fibres running between the poles. During prometaphase, breakdown of the nuclear envelope allows other polar MTs to penetrate among the chromosomes. Kinetochores immediately respond (Chap. 4) by attaching to them, forming bundles of MTs called kinetochore fibres. The remaining polar MTs continue to define the aster at each pole. The distinction between these sets of MTs is particularly clear in diatoms (Appendix 1).

As increasing numbers of chromosomes attach to both asters, the previously elongating spindle now shortens in response to the increasing compression on it generated by collective pulling forces acting on paired kinetochores. By true metaphase, the kinetochores are lined up across the middle of the spindle, holding this "metaphase plate" equilibrium because of the balanced forces on the kinetochores. Chromosomes are not quiescent during metaphase (a popular misconception derived from static figures and diagrams), often oscillating gently. Metaphase is prolonged to allow tardy chromosomes time to achieve bipolar attachment.

During anaphase, simultaneous splitting of chromosomes allows single chromatids to move to opposite poles, usually led by kinetochores whose fibres are shortening. This chromosome-to-pole movement is called anaphase A and its mechanism(s) is controversial. The many models proposed over many years are either invalid or limited in their ability to account for chromosome movement. The currently popular paradigm is the "PAC-MAN" model, in which disassembly of kinetochore MTs at the kinetochore is coupled with generation of polewards force (Inoue & Salmon, 1995; Rieder & Salmon, 1994). It, too, has unresolved problems (Pickett-Heaps & Forer, 2001). During anaphase A, the spindle elongates as the poles move further apart. This movement, anaphase B, can be generated by several different mechanisms: elongation of the half spindle MTs (which occurs while adjacent kinetochore MTs are shortening: Tippit et al. 1980b); sliding of the half spindles apart, as in diatoms (Appendix 1); and pulling activity of polar MTs radiating into the cell cortex (Aist & Berns, 1981).

The spindle displays a mechanism for blocking entry into anaphase until all chromosomes are correctly attached (Rieder et al., 1995), although this is not infallible - witness the abnormal anaphase shown in Chap. 4. When the drug cytochalasin blocks correct kinetochore attachment (Appendix 2: Chap. 16), cells cannot enter anaphase A, although anaphase B will eventually occur. Carefully timed treatment can cause anaphase chromosomes to lose connection to spindle fibres, whereupon anaphase immediately stops with the cell apparently able to sense incorrect attachment.

During telophase, many cells undergo cytokinesis by cleavage as a contractile furrow pinches the cell in two. The continuous fibres remaining between the separated chromatids are compressed into a dense, narrow midbody composed of two sets of stable, overlapped MTs. In higher plant cells,
these remaining MTs are incorporated into the phragmoplast (Chap. 5).

During mitosis, sets of MTs appear and disappear, elongate and shorten, in a regulated fashion. How control is exercised over their dynamics is poorly understood. Current opinion suggests that: i) MTs grow and shorten predominantly at their "+" end, distal to the pole; ii) the cell undergoes global changes that stimulate MT assembly during prophase and late telophase, and disassembly during anaphase; iii) when MTs from one pole interact laterally with those from the other pole forming continuous fibres, their "+" end is stabilised; and iv) when MTs are captured by kinetochores, their "+" end is stabilised; thereafter their assembly/disassembly is controlled by the kinetochore.

Chap. 4. Interaction of Kinetochores and Spindle Fibres. Because MTs are inserted into and attached to kinetochores, in the 1970's biochemical and cytological investigations concentrated on their presumed role in nucleating these MTs. This model was misguided: the role of kinetochores in capturing and sliding along polar MTs was first obvious in diatoms (Appendix 1: Chap. 10) and confirmed in animal cells (Rieder & Alexander, 1990). Chromosomes behave individually during prometaphase. Many attach to the nearest pole and then oscillate markedly, an activity that ensures that the second kinetochore in each pair can encounter and then attach to MTs from the opposite pole. Individual chromosomes seem to use tension to tell whether they are correctly attached to both poles (Nicklas & Koch, 1969). This sensing is critically important since it provides the mechanism for ensuring correct bipolar attachment and thus, correct mitosis.

Chap. 5. Mitosis and Cytokinesis in Higher Plant Cells.
Strasburger first used stamen hairs of *Tradescantia* to follow plant cell division and they still are especially useful experimental material, recommended for classroom use. Simple dissection of young flower buds will release the hairs, consisting of a row of single cells with many dividing. Older cells display classic cytoplasmic streaming.

Plant tissues are comprised of highly ordered patterns of cells, often differentiated in linear rows (e.g., in a root tip). The cell wall does not permit cells to move during tissue differentiation. Consequently, these patterns of cells have to be created in the growing regions (meristems) from sequences of cell divisions in which the plane of division is tightly controlled. In three important respects, mitosis and cytokinesis are different in higher plant and animal cells.

i) Higher plant cells display the "Preprophase Band of Microtubules" (Pickett-Heaps and Northcote, 1966). With rare exceptions (e.g., endosperm, pollen cells; Gunning et al. 1978, 1982), prior to mitosis, the MTs dispersed along the wall of interphase cells gather into a tight "Preprophase Band" (PPB) whose location precisely predicts where the cell-plate will join the pre-existing walls, and thus, the plane of impending division. The PPB is transitory and its MTs become incorporated into the forming spindle.

ii) The spindle is "Acentric" ("Anastral"). The spindle of higher plant
cells is devoid of centrosomes and centrioles, displaying broad poles and unfocussed fibres. These spindles can become centric at one stage in the life cycle of primitive land plants which form flagellated sperm for sexual reproduction. The centrioles required for flagella formation arise de novo during the divisions that generate spermatogeninous tissues. Then, once formed, the centrioles are located at the poles of spindle which are now transformed into astral, centric spindles. There is no reason to suppose that these centric and acentric spindles are functionally different.

iii) Strasburger in 1875 described cytokinesis accomplished within the "Phragmoplast" by a new cross-wall called the "Cell-plate". The cell-plate arises as tiny droplets on interzonal spindle fibres migrate centrally to create an uneven diaphragm which rapidly consolidates. The cytoplasm is complex and contains much membrane (endoplasmic reticulum) Golgi-derived vesicles and other ill-defined components (e.g., Gunning, 1982; Samuels et al., 1995). Higher plant cells do not (with few exceptions) cleave like animal cells. (For a comprehensive review of plant cytokinesis, see Gunning, 1982). The phragmoplast normally arises from pole-to-pole fibres (MTs) remaining between daughter nuclei during telophase. Considerable proliferation of these fibres accompanies the lateral growth of the phragmoplast at the margin of the cell-plate. Bajer and Mole-Bajer have exhaustively documented phragmoplast activity (Gunning, 1982). The behaviour of the phragmoplast suggests that its outgrowing edge is seeking the correct site for cross-wall attachment as it nears the parental wall (Gunning 1982) - perhaps responding to cues associated with the PPB. In certain tissues (e.g. multinucleated endosperm), phragmoplasts can form between non-daughter as well as daughter nuclei, and so generation of the phragmoplast is not dependant upon the presence of the interzonal spindle.

Overall, there are several morphological and behavioural differences between animal (Chaps. 4, 5) and higher plant spindles. Chromosomes in plant spindles achieve metaphase rapidly and they do not display much oscillating behaviour. Polar transport (see Appendix 2: Chap. 18) in the half spindles generates a metaphase plate in which chromosome arms are pushed polewards along spindle fibres during metaphase, and some of these arms move ahead of the kinetochores during anaphase. In animal cells, chromosome arms are pushed laterally away from the metaphase plate and chromosome arms trail behind the kinetochores. These differences are discussed further in Appendix 2.

Chap 6. "Closed" Spindles. Many protistal and fungal cells have closed spindles in which the nuclear envelope remains intact. The origin of intranuclear spindle MTs is not always obvious. In some cases, MTs clearly enter through small holes ("fenestrae") in the nuclear envelope. In others, however, no such holes are present but nevertheless, MTs soon become evident near the polar regions.

Experiments on artificially created binucleate cells show that when two open spindles form near each other, they usually fuse into one unit. Thus, closed spindles prevent them from fusing in multinucleate cells. Since mitosis
can occur within the confines of the nuclear envelope, the spindle can be a self-contained system that does not require any anchoring of asters in the cytoplasm (although this often occurs in many cells and tissues). In some multinucleate fungi and algae, nuclei circulate freely around the cytoplasm while undergoing mitosis.

Chap. 7. Mitosis in Multinucleate Cells. Mitosis in multinucleate cells is usually highly synchronous. The example shown, Hydrodictyon, was well known to early microscopists for the way anaphase is initiated as a wave passing through the whole cell.

Appendix 1:

Chap. 8. Mitosis In Diatoms

Diatoms are exceptional organisms for observing mitosis and cellular morphogenesis. Lauterborn's (1896) classic account is accurate down to the smallest details, including the depiction of golgi bodies and MTs (for a partial translation of the original German, see Pickett-Heaps et al., 1984). Lauterborn's work was largely ignored by early microscopists; for example, E.B. Wilson's classic "The Cell in Development and Inheritance" mentions his work only in a couple of footnotes. Recent observations on diatom spindles and their interpretations have also provoked some scepticism. However, many observations on diatom spindles (e.g., the initially controversial observations that kinetochore capture polar MTs) have been confirmed using conventional spindles. The unique cytological value of diatom spindles derives from several characteristics: i) some species are very large and mitosis is rapid, enabling mitotic activity to be seen in living cells with great clarity; ii) they create a conspicuous "central spindle" to which chromosomes attach; thus, unlike conventional spindles, it is conceptually easy to see what structure chromosomes attach to and move over during mitosis; and iii) their spindles are uniquely well organised, allowing their structure to be analysed and correlated with their behaviour. In contrast, the fibrous spindle of conventional cells is far more difficult to interpret structurally.

Cell division in diatoms has been extensively reviewed in Pickett-Heaps (1991a).

Chap. 9. Structure and Function of The Central Spindle. That the central spindle consists of two half spindles has been unambiguously established from 3-D reconstructions derived from serial sections examined by electron microscopy (Tippit et al., 1978; McDonald et al., 1977). This structure is also true of more conventional spindles; for example, the continuous fibres that run from pole to pole each consist of an overlapping set of MTs, sometimes only one from each pole (Tippit et al. 1980b, 1984). Similar analysis confirms that the two half spindles separate by sliding apart, as indicated by in vivo behaviour.
When half spindles are severed from their poles by a UV-microbeam (Chap. 19; Leslie & Pickett-Heaps, 1983; Stonington et al., 1990), the remnant overlap still elongates at anaphase; thus, the sliding force is generated in the overlap. The isolated central spindle will also elongate in the presence of ATP \textit{in vitro} (Cande & McDonald, 1985) and elongation in living cells is stopped by metabolic inhibitors that deplete cytoplasmic ATP levels. MT packing in the overlap is highly ordered with each MT having as closest neighbours 4 MTs from the other pole. This packing maximises the lateral interaction of each MT with MTs of opposite polarity from the other pole (Tippit et al., 1975). It must reflect the manner that MTs of opposite polarity (i.e., from opposite poles) preferentially interact during spindle assembly early in prophase (Chap. 11) and/or the way they slide apart. Analysis of conventional spindles reveals a similar preferred lateral spacing between MTs from opposite poles in continuous fibres (Tippit et al., 1983).

Serial section analysis suggests that in conventional spindles, elongation during anaphase B can be accomplished by sliding plus elongation of the half-spindles (Tippit et al., 1980b, 1984; Ding et al., 1993). Whether sliding occurs universally during anaphase B is uncertain. The way diatom half-spindles disassemble after anaphase B suggests:

\textit{first}, that the ends of MTs are stabilised while overlapped, and disassembly can commence once the half spindles separate. In contrast, the overlapped, interdigitated MTs (remnant continuous fibres) of vertebrate spindles do not slide and separate completely, and instead are transformed into the stable mid-body which persists long after cleavage is over (Byers & Abramson, 1968). Smaller conventional spindles are different again: as the spindle elongates by sliding, the ends of shorter MTs become free and these MTs disassemble, while longer MTs slide and elongate. Thus, the population of spindle MTs changes from numerous overlapped MTs at metaphase to far fewer, greatly elongated overlapped MTs by telophase (Tippit et al., 1980b, 1984; Ding et al., 1993).

and \textit{second}, that unidirectional disassembly (Soranno & Pickett-Heaps, 1982) of half spindles is related to the uniform molecular polarity of their constituent MTs. This directionality is an intrinsic property of the MTs, and is not just due to the other polar end of the half spindles remaining "capped"; if a slot is cut in the half spindle using a UV-microbeam, disassembly occurs at the newly created "+" ends, while the equivalent "-" ends are stable (Leslie & Pickett-Heaps, 1984).

\textbf{Chap. 10. Attachment of Kinetochores to Polar Microtubules.} As soon the central spindle enters the nucleus during prometaphase, chromosomes exhibit rapid and irregular poleward irregular oscillations along invisible tracks corresponding to polar MTs (Tippit et al., 1980a). Each chromosome rapidly achieves bipolar attachment when the second of each pair of kinetochores encounters MTs from the opposite pole; the latter then stretches to that pole, and the pair of chromatids become quiescent, stretched across the central spindle. This behaviour - so clear in diatoms - appears similar to what is seen in animal spindles (Chap. 4), but with at least one structural difference.
In conventional spindles, lateral interaction of kinetochores with polar MTs is soon followed by these MTs terminating in the kinetochore. However, in the diatom spindle, few or none do so (Tippit et al., 1980a). Why this is so is not clear but this discrepancy is significant because some models of chromosome movement (e.g. the "Pac-Man" model) require MTs to terminate in the kinetochore; such models cannot explain diatom mitosis.

Furthermore, electron microscopy reveals a diffuse, unidentified component permeating the MTs of the central spindle, between the poles and tips of stretched chromatids (i.e. the kinetochores); this amorphous structure has been called the "collar" (Tippit and Pickett-Heaps, 1977; Pickett-Heaps et al., 1978). Since the stretched chromosomes appear to spring elastically to the pole at anaphase, this material could be an elastic component in the traction apparatus that moves chromosomes. Our supposition that conventional spindles might contain such a component, is controversial and not widely shared.

Chap. 11. Origin and Positioning of the Spindle in Pennate Diatoms; the Microtubule Center

As Lauterborn discovered, the central spindle arises before prophase from a "spindle precursor" that appears close to, but separate from the cell's interphase Microtubule Center (MC: equivalent to the centrosome). The spindle precursor consists of two plates which clearly act as nucleating centres for MTs. Thus the origin of spindle MTs and assembly into their overall arrangement is particularly clear in diatoms.

In diatoms, the nucleus moves to a specific site on the side wall prior to mitosis (Round et al., 1990). This preprophase migration of the MC, spindle precursor and nucleus is generated by the MT cytoskeleton focussed upon the MC, and is particularly striking in vacuolated cells like Surirella (Lauterborn, 1896; Pickett-Heaps et al., 1984: next chapter) and Cymatopleura (Pickett-Heaps, 1991b). Immediately this premitotic movement is completed, the MT cytoskeleton responsible breaks down as its MTs are mobilised into the rapidly growing spindle. The MC also quickly disappears at this stage.

After cleavage, the plates organising the poles of the spindle disperse while a new MC arises nearby in each daughter cell. These MCs set up a new interphase MT cytoskeleton which soon exerts a profound effect on post-mitotic morphogenesis. In Surirella, it is involved in moving nuclei back to the center of the cell, and then later in complex folding and stretching of the chloroplast into its interphase morphology. In many diatoms, the MC/MT complex is also involved in morphogenesis of the complex silica cell wall (reviewed in Pickett-Heaps et al. 1990).

Chap. 12. Positioning of the Spindle in Centric Diatoms

Centric diatoms display similar precise morphogenetic movements. The MT cytoskeleton is manifested in the cytoplasmic strands traversing the vacuole of larger species. Premitotic movement is analogous to that in pennate diatoms, even down to the "searching" movements preceding prophase. Once the nucleus is correctly positioned, breakdown of the MT cytoskeleton is revealed
by the sudden disorganization of the radially oriented chloroplasts and cytoplasmic strands. During cleavage, re-establishment of the MT cytoskeleton coincides with its interaction with the ingrowing cleavage furrow, drawing the nucleus back to the center of the newly forming silica wall.

Chap. 13. Cleavage in Diatoms

Because diatoms are encased in a rigid wall, cleaving cells can be turned over, offering a unique face view of the cleavage furrow.

====================================
Appendix 2:

Chap. 14. Experiments on Mitotic Cells

This introductory chapter shows the type of equipment needed for experiments recording the response of living cells to exposure to drugs and other reagents.

Chap. 15. Effects of Anti-Microtubule Drugs on the Spindle. Drugs such as colchicine have long been known to block mitosis by disrupting the spindle (Inoue, 1952). Observations on living animal cells show that during spindle breakdown, the poles are rapidly drawn in to the chromosomes. Several interpretations of this movement are possible. Promoting MT disassembly might stimulate a traction mechanism (PAC-MAN) that functions using MT disassembly for force production. Alternatively, the poles and kinetochores could be connected by some other force-generating, possibly elastic component whose energy is released by the disassembly of compression-resistant MTs holding them apart.

The effects of anti-MT agents on mitotic diatoms are revealing because the central spindle is very stable and remains unaffected, presumably because the sensitive "+" ends are embedded in the overlap (cf. Chap. 9). However, polar MTs, including those that run past the kinetochore attachments, are rapidly disassembled. As a result, the central spindle becomes skewed while stretched chromosomes irregularly disengage from the poles under tension. Most release from one pole and then spring to the other where they remain attached. Thus, each pole collects a random collection of doubled chromosomes. If similarly treated cells are examined by electron microscopy, one kinetochore of these polar chromosomes remains attached to the collar material condensed at the pole after release from tension (Pickett-Heaps & Spurck, 1982). Thus, we again conclude that the collar represents an important component that attaches kinetochores to the pole.

Chap. 16. Effects of Anti-Actin Drugs on Mitosis and Meiosis. While anti-actin agents such as the cytochalasins are well known to disrupt cleavage, it is widely accepted (see any cell biology text book) that actin plays no significant role in mitosis. However, actin has been found at the kinetochore of various cells (refs. in Sampson et al., 1996). In the green alga *Oedogonium*, the kinetochores stain with the specific actin-binding agent phalloidin (Sampson & Pickett-Heaps, 2001). Furthermore, cytochalasin D (CD) at high concentrations rapidly and invariably blocks mitosis in *Oedogonium*. Spindle fibres are still present and the chromosomes actively move about, but appear unable to attach to them; the cell remains in a suspended quasi-prometaphase for several hours before undergoing a very slow, highly abnormal anaphase B. Reversal of this block is rapid, even after some hours in the drug (Sampson et al., 1996). If chromosomes drop off an anaphase spindle, anaphase immediately stops, as if
the cell senses that chromosome attachment is incorrect.

Equivalent experiments on meiotic spindles of crane fly spermatocytes give less clear-cut results but almost always abnormalities become evident (Forer & Pickett-Heaps, 1998). Anaphase is often slowed down reversibly, dramatically speeding up when CD is washed out. CD-treated spermatocytes frequently proceed through meiosis when either or both autosomes and sex chromosomes are incorrectly attached or unattached, a situation very rare in untreated cells.

**Chap. 17. Osmotic Shock.** When mitotic PtK cells are treated with hyperosmotic sucrose (0.4-0.5M), chromosomes become almost invisible while the spindle fibres condense into thick bundles of packed MTs (Snyder et al., 1984). Within minutes, the metaphase spindle elongates up to 40-50% as kinetochore fibres appear to release from kinetochores (Mullins et al., 1985). Under the TEM, kinetochores change their morphology, becoming associated with amorphous material (Pover et al., 1985). Upon washing out the sucrose, the spindle immediately recovers and the spindle shortens. Mitosis then proceeds normally. Anaphase spindles react similarly but do not show spindle elongation.

These experiments confirm the impression that the metaphase spindle is under compression, presumably generated as chromosomes attach to each pole and collectively exert tension across the spindle. The stored energy is apparently released during osmotic shock when kinetochore fibres detach from kinetochores. By anaphase, splitting of chromatids and their separation relieves this compression and the spindle does not elongate significantly on treatment with sucrose.

**Chap. 18. Transport Properties of Spindle**

A confusing number of transport systems are detectable in the living spindle. Most conspicuous is a rapid, irregular bidirectional movement of organelles like mitochondria along spindle fibres. This "saltatory" movement (Rebhun, 1967) is a common feature of most MTs arrays in interphase cells too, and may have little relevance to chromosome movement although perhaps prometaphase oscillations are generated by related mechanism(s).

**18.1 Polar Ejection Forces.** The spindle becomes cleared of large organelles by the activity of spindle fibres throughout mitosis, mostly by particles being transported polewards (next para.). However, in vertebrate spindles, larger particles and test objects such as inert fragments, cut off chromosomes using a laser microbeam, are eliminated away from the pole by "polar ejection forces" (Rieder & Salmon, 1994). The forces are attributed to the constantly growing and shortening polar MTs impinging upon the chromosomes and pushing them away from the pole (Rieder & Salmon, 1994). During prometaphase, chromosome oscillations appear to result from the polewards (sliding) movement of kinetochores irregularly counteracted by these polar forces.
18.2 Polar Transport. "Polar transport" is the slow steady transport of small/very small particles along spindle fibres to the poles throughout mitosis (Ostegren et al., 1960; Bajer et al., 1987). It is dependent upon MTs (e.g., the video sequence of Closterium). The rate of polar transport and anaphase A are equivalent and some have suggested that it is involved in generating polewards chromosome movement (e.g., Ostergren et al., 1960).

In the first experiment illustrated in the video, a laser microbeam is used to inactivate one kinetochore on two metaphase chromosomes in Haemanthus endosperm cells. This treatment causes each to move polewards under the influence of the remaining intact kinetochore (Khodjakov et al., 1997), before initiating prometaphase-like oscillations. This experiment supports the idea that during the metaphase equilibrium, chromosomes remain central due to balanced forces pulling them to both poles.

When the laser is directed so as to cut segments off chromosomes, these fragments are also moved polewards during metaphase, now by non-kinetochore-mediated, polar transport (Rieder & Salmon, 1994; LaFountain et al., 2001). But in Haemanthus spindles, a dramatic change takes place after the cell enters anaphase: the fragments stop their polewards movement and instead now move toward the interzone and forming phragmoplast (Khodjakov et al., 1996). Finally, during telophase, spindle transport reverses once again with a mass movement of material, including fragments of chromosomes, away from the phragmoplast, toward the daughter nuclei, as is seen in normal cells (Chap. 5).

This latter behaviour is different to that seen in similar experiments on animal spindles where polar transport of larger objects appears to be overridden by polar ejection forces (Chap. 18.1). Khodjakov et al. (1996) conclude that plant and animal cells are different mechanistically as well as phenomenologically. However, the differences might arise from: i) astral v. anastral spindle organization with the density of focussed polar MTs concentrating polar ejection forces in a manner not possible in anastral spindles; and ii) plant cells undergoing a burst of MT assembly initiating phragmoplast formation and simulating polar ejection forces.

Chap. 19. Microsurgery with a UV-microbeam. MTs are particularly susceptible to irradiation with UV light between 250-290 nM and so a UV-microbeam irradiation can create lesions ("Areas of Reduced Birefringence" or ARBs; Forer, 1965) devoid of MTs within spindle fibres. If ARBs are created across one half spindle in animal cells, the edge of the ARB closest to the kinetochore is relatively stable, leaving a kinetochore fibre "stub" still attached to the kinetochore. At the other edge of the lesion, fibres undergo rapid disassembly polewards (as in the diatom half spindle; Chap. 9). Recovery commences in a few minutes by the pole on the irradiated side of the spindle moving inwards, closing the ARB and creating a shorter half-spindle (Spurck et al., 1990). Remarkably, the other half-spindle then shortens an equivalent amount and the shortened spindle undergoes normal anaphase later.

This response is different to that generated by laser inactivation of the kinetochore (Chap. 18.2); attachment to the pole has apparently not been cut
on the irradiated side by the UV-microbeam since chromosomes remain essentially stationary; some may actually move toward the ARB. This result suggests to us (Spurck et al., 1997) that some other component besides MTs continues to connect kinetochores to the pole. The movement of the pole inwards is reminiscent of the effect of anti-MT agents applied to the spindle (Chap. 15). How is this movement generated? Perhaps MTs growing across the ARB draw the pole inwards, creating a traction fibre connecting pole and kinetochores.

A series of irradiations show how subtle the mechanics of mitotic movement are. When a UV irradiation cuts a few individual kinetochore fibres within one half spindle, the pole usually moves inwards immediately and the unirradiated neighbouring fibres bow slightly in response. Apparently, cutting a few fibres increases compression across the spindle - not the result expected if kinetochores are pulling toward the pole via their MTs. If anaphase fibres are cut, those chromatids adjacent to the ARB continue polewards movement, sometimes even accelerating. Finally, if by chance a remnant fibre is left along the edge of the ARB, this remnant will crumple and break. Collectively, these experiments suggest that there might be an additional component between the pole and kinetochore, separate from MTs and not necessarily cut by the microbeam. In this model, spindle MTs are seen as withstanding compression forces, not generating them.

Similar experiments on spermatocytes raise similar issues (Spurcke et al., 1987). The polewards edge of the ARB rapidly disassembles polewards, but the pole never moves inwards to fill the ARB as it does in newt spindles; consequently, we can follow the longer-term behaviour of the kinetochore fibre stub. It often moves around, indicating that the fibre is truly severed and in metaphase cells, slowly grows to establish a normal fibre. When an anaphase kinetochore fibre is severed, the chromatid(s) keep moving polewards even while the kinetochore stub is concurrently elongating polewards. The latter result is impossible to explain on the basis that disassembly of the kinetochore fibre at the kinetochore is generating polewards motion (PAC-MAN model: Spurck et al., 1997; Pickett-Heaps & Forer, 2001). Perhaps MT assembly in the stub is stimulated by the tension pulling the kinetochore polewards, whereas normally, this fibre would be under compression, stimulating its disassembly.

Conclusion. I hope these experiments and notes provoke viewers to explore the rich, stimulating and deeply puzzling literature on mitosis. The movements of chromosomes are subtle and complex, yet most models attempting to explain them are narrow and simplistic. Multiple mechanisms must be at work in the spindle; not only are these integrated to achieve chromosome motion, but different organisms use these mechanisms to different extents (Pickett-Heaps & Bajer, 1978). Numerous models have been proposed that utilise MT-motors such as dynein (e.g. Sharp et al., 2000) to drive chromosome movement but recent analysis of spindle motility proteins has raised unexpected problems. For example, a search of the genome of Arabidopsis shows that it and presumably other higher plants are devoid of cytoplasmic dyneins and dynactin (Lawrence et al., 2001). Perhaps this absence can be correlated with most higher plants
having lost the capability to form flagellated cells, which in turn might relate to their creation of acentric spindles v. the centric spindles of animal cells. Lower land plants which form flagellated sperm cells, show both types of spindle (centric spindles being a feature of their spermatogenous cells). Apparently, when the genes for flagellar function are activated, their products concurrently change spindle morphology - but surely not its basic mode of function. Thus, an absence of dynein from angiosperm spindles presents problems for some currently popular models of spindle function. In a similar vein, Matsuura et al.: (2002) find that mutants of Chlamydomonas deficient in kinesin II cannot swim, but still undergo normal mitosis. We now have many candidates which might be involved in generating spindle motility, but very little idea of where they are situated and even whether they are needed for mitosis at all.
REFERENCES CITED


Lawrence, C.J., Morris, N.R., Meagher, R.B. & Dawe, R.K. (2001). Dyneins have run their course in


