CHAPTER 4

Microtubule Dynamics and Chromosome Movement

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I. Introduction

Traditionally, five stages of mitosis have been defined (Figs. 4.1 and 4.2): prophase, prometaphase, metaphase, anaphase and telophase (Wilson, 1925; Schrader, 1953; Mazia, 1961; Inoué and Sato, 1967; Nicklas, 1971; Bajer and Mole-Bajer, 1971, 1972). There are four phases of chromosome movement: attachment, congression, anaphase A and anaphase B. Chromosomes condense, kinetochores mature, and duplicated spindle poles are usually established during prophase. Prometaphase begins after nuclear envelope breakdown (NEB). At some period after the breakdown of the nuclear envelope, chromosome duplexes usually form a chromosomal fibre between one of their kinetochores and the closest spindle pole; a process termed attachment. These chromosomes are said to be mono-oriented. After attachment, mono-oriented chromosomes move (kinetochore leading) towards the pole. Later, the other kinetochore complex becomes attached to the opposite pole of a bi-polar spindle by a chromosome fibre. These chromosomes are said to be bi-oriented. Bi-oriented chromosomes usually move to a position within the spindle approximately equatorial between the poles, the metaphase plate. The movement of the chromosomes to the metaphase plate is termed congression.

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Figure 4.1  caption overleaf
Bi-oriented chromosomes usually do not sit statically at the spindle equator, but oscillate several micrometres back and forth between the poles. Concurrently, their chromosomal fibres lengthen and shorten. Metaphase is achieved when all chromosomes have bi-oriented and moved close to the spindle equator. Anaphase onset (AO) occurs when chromosome duplexes split into chromatids. Chromosome pairs are separated by two processes in anaphase. Anaphase A is the movement of chromosome pairs to opposite poles as their chromosomal fibres shorten and anaphase B is the elongation of the spindle interpolar length (Inoué and Ritter, 1975). In telophase, the nuclear envelope is re-formed around the chromosomes and cytokinesis occurs.
With respect to the cytoplasmic compartment of the cell, there are two key points of transition in the cell cycle between interphase and mitosis which are not obviously defined by the traditional stages of mitosis. The first occurs at the time of NEB. Before this, the structure and activities of the cytoplasm are typical of interphase. NEB is only one aspect of the global changes that occur in the cell at that time. Extensive rearrangements occur in the organization of microtubules (Fig. 4.2) and other cytoskeletal and cytoplasmic components (Brinkley et al., 1980; Vandre et al., 1984; DeBrabander et al., 1986). Cells generally round-up, pulling away from their neighbours in tissues, and cease much of their synthetic activity. The second major transition occurs at anaphase onset. This is marked by the splitting of paired chromosomes, but, in general, the cell returns to the structural organization and activities typical of interphase cells.

There are three essential structural components required for chromosome segregation: mitotic centres (centrosomes or spindle poles) (Vandre and Borisy, this volume), kinetochores on the chromosomes (Brinkley et al., this volume), and microtubules. The mitotic centres nucleate the growth of spindle microtubules and the microtubules of the chromosome fibres connect the kinetochores on chromosomes to the spindle poles (Inoué, 1981b; Ellis and Begg, 1981; Mazia, 1984, 1987; McIntosh, 1983, 1984, 1985; Nicklas, 1985, 1987a). Thus far, the only known spindle protein essential for chromosome movement is tubulin. A variety of other motility-associated proteins have been found to be associated with the mitotic spindle fibres. These include dynein-like ATPases, kinesin, several other microtubule-associated proteins, calmodulin and actin (Sanger, 1977; Browne et al., 1980; Pratt et al., 1980; Zieve and Solomon, 1982; McIntosh, 1984, 1985; Scholey et al., 1985; Bloom et al., 1986; Leslie et al., 1987; Dinsmore and Sloboda, 1988). Evidence rules out a function for actin–myosin in the generation of mitotic movements (Inoué, 1981b) and there is yet no direct evidence that any of these other spindle-associated proteins actively produce or regulate chromosome movement or spindle assembly. During the past 38 years, various techniques have been developed for the isolation of the mitotic spindle and attached chromosomes free from the cell (Fig. 4.3) (Mazia and Dan, 1952; Kane, 1962; Sakai, 1978; Salmon, 1982; Dinsmore and Sloboda, 1988; Rebhun and Palazzo, 1988). Unfortunately, conditions that permit reactivation of lifelike chromosome movements in isolated spindles have yet to be discovered and the molecular mechanisms that generate the poleward forces for chromosome movement are still uncertain. More progress has been made in partially lysed cell models (Spurck and Pickett-Heaps, 1987) and in the reactivation of interpolar spindle elongation (anaphase B) in isolates (Cande, this volume; Rebhun and Palazzo, 1988).

In the past several years, much has been learned from cellular studies and in
**Figure 4.3.** Mitotic spindle in metaphase isolated from the first-division embryos of the sea urchin *Lytechinus variegatus* photographed using (a) polarization microscopy, (b) phase contrast microscopy, and (c) differential interference microscopy. Bar = 10 \( \mu m \). (From Salmon and Segall, 1980.)

*In vitro* reconstitution experiments about the structural organization and dynamics of microtubule assembly within the spindle and the dynamics of chromosome movement. These results have reoriented the conceptual thinking about the mechanism and regulation of spindle microtubule assembly, the structural basis for the attachment of chromosomes to the spindle fibres, the generation of poleward movements of the chromosomes, the congression of the chromosomes to the spindle equator in metaphase, and the poleward movement of chromosomes in anaphase. It is clear from these studies that the assembly dynamics of microtubules is directly involved with chromosome attachment to the spindle and the production of chromosome movements.

In this chapter, I attempt to integrate recent information on the assembly dynamics of spindle microtubules and the coupling between microtubule dynamics and chromosome movements. I will neglect in-depth discussions of the structure and composition of centrosomes and kinetochores, the mechanisms of interpolar elongation during anaphase, and molecular mechanisms that may regulate changes in microtubule assembly during the cell cycle and mitosis, since they are covered in depth in other chapters in this volume. Mitosis is a complex process and varies significantly between different organisms. The discussion here is primarily focused on the mechanism of

II. The Spindle is Formed From Polarized Arrays of Microtubules

A. Microtubule polarity

Microtubule polarity is important in consideration of the structure of the spindle and the mechanisms of chromosome movement because polarity orients the direction of force production by translocators such as kinesin or dynein (Sale and Satir, 1977; McIntosh, 1984, 1985; Vale et al., 1985b; Mitchison, 1986; Cassimeris et al., 1987a). In addition, the plus and minus ends of microtubules are likely to have different assembly/disassembly kinetics (Leslie and Pickett-Heaps, 1983; Purich and Kristofferson, 1984; Mitchison and Kirschner, 1984c; Horio and Hotani, 1986; Tao et al., 1988; Walker et al., 1988) and binding affinities for other molecular complexes, such as those associated with kinetochores (Huitorel and Kirschner, 1988) or with the centrosome complex.

Microtubules are polarized polymers of tubulin (Mandelkow and Mandelkow, 1985, 1986). Polarity can be identified by growth rate. One end, the plus end, usually grows faster than the minus end at the same tubulin concentration in reconstituted preparations (Bergen and Borisy, 1980; Purich and Kristofferson, 1984; Mitchison and Kirschner, 1984c; Horio and Hotani, 1986; Walker et al., 1988). In addition to growth rate, techniques have been developed to identify microtubule polarity using structural probes (Heidemann and McIntosh, 1980; Telzer and Haimo, 1981; McIntosh and Euteneuer, 1984; McIntosh, 1984, 1985).

B. Orientation of microtubule growth and polarity by MTOCs

Microtubule organization and orientation in both interphase and mitosis (Fig. 4.2) have been shown to be governed by nucleated assembly from microtubule organizing centres (MTOCs) like the centrosome (Borisy and Gould, 1977; Brinkley et al., 1981; Brinkley, 1986; DeBrabander et al., 1980, 1981a, 1986; Evans et al., 1985; McIntosh, 1983; Vandré and Borisy, this volume). The cloud of amorphous material that surrounds the pair of centrioles within the centrosome nucleates the polymerization of microtubules into polar
arrays (McIntosh, 1983; Evans et al., 1985). Polar microtubules have been shown to be oriented with their plus or fast-growing ends distal to the centrosome (Heidemann and McIntosh, 1980; Telzer and Haimo, 1981; McIntosh and Euteneuer, 1984). Immunofluorescence studies have shown that during interphase, most microtubules radiate outward towards the cell surface from a centrosome located near the nucleus (Brinkley et al., 1980; Brinkley, 1986). For example, a mammalian culture cell, such as BSC1, contains several hundred microtubules within the cytoplasmic microtubule complex (CMTC) with mean length near 20 µm (Schulze and Kirschner, 1986, 1987).

Accurate segregation of chromosome duplexes requires a bipolar spindle (Mazia, 1961, 1984, 1987; Mazia et al., 1981; Bajer, 1982; Rieder et al., 1986; Sluder and Rieder, 1985; Sluder et al., 1986). In preparation for mitosis, the centrosome complex is reproduced to establish the bipolarity of the spindle and to generate a bipolar spindle; the centres must also separate (Vandré and Borisy, this volume). This requires some form of microtubule assembly (Brinkley et al., 1967; Brinkley, 1986; Mazia, 1984, 1987). If reproduction or separation fail to occur, then at NEB a monopolar spindle forms (Fig. 4.4).

C. Spindle microtubule organization during mitosis

At NEB, the CMTC disappears and microtubule assembly occurs mainly within the region occupied by the nucleus before NEB (Figs. 4.1 and 4.2). A bipolar spindle is formed from overlapping arrays of microtubules extending...
Figure 4.5. Electron micrographs of adjacent thick sections (A) and (B) showing the cold stable chromosomal fibre microtubules in PtK₁ cells. Cells were cooled to 6°C before fixation and processing for electron microscopy. Bar = 5 μm. Insert is higher magnification view of a kinetochore showing microtubule attachment. Scale = 0.25 μm. (From Rieder, 1981.)
from opposite spindle poles. All polar microtubules are oriented with their plus ends distal from their poles (McIntosh and Euteneuer, 1984; McIntosh, 1984, 1985). In mitosis, the nucleation capacity of the spindle poles is much greater than for the centrosomes during interphase (McIntosh, 1983). There are typically one thousand to several thousand polar microtubules in a metaphase spindle. These are much shorter than the microtubules in the interphase CMTC.

Microtubules attached to kinetochores are termed kinetochore microtubules (McDonald, this volume) (Fig. 4.5). These have been shown to be oriented with their plus ends proximal to the kinetochores (McIntosh, 1984, 1985). The number of kinetochore microtubules at metaphase is typically 15–30 in mammalian cells, but the number can vary considerably between non-homologous chromosomes and cell types (reviewed by Rieder, 1982). Yeast

Figure 4.6. A serial-section reconstruction of the microtubule distribution in a single cold-stable chromosomal fibre from a metaphase Ptk1 cell. K' to K represents the kinetochore region and P' to PCM represents the pericentriolar material. (From Rieder, 1981.)
chromosomes appear to have only one kinetochore microtubule, while large plant chromosomes can have several hundred. In metaphase PtK₁ (Rieder, 1981) and CHO (Witt et al., 1981) cells, ultrastructural analysis (Figs. 4.5 and 4.6) has shown that most kinetochore microtubules extend all the way between the pole and the kinetochore. However in PtK₁, about 25% of the kinetochore microtubules may have a free minus end (see Fig. 4.6). There are only a few microtubules that are attached neither to the kinetochore or the pole.

At AO, the kinetochore microtubules shorten and the mean length of the majority of polar microtubules decreases as the separated chromatids move poleward (Fig. 4.2) (Inoué, 1964; Nicklas, 1975; Salmon and Begg, 1980). Concurrently, the spindle asters begin to elongate out to the cell periphery to re-form the new CMTCs in the daughter cells. It has been shown that the extension of microtubules to the cell surface results in stimulation and orientation of cytokinesis (Rappaport, 1988; Schroeder, 1987; Inoué, 1981b; White and Borisy, 1983), but how this occurs is not well understood. Between the separating chromosomes at the spindle interzone, bundles of microtubules form that are called “stem bodies” (McIntosh et al., 1975; Salmon et al., 1976). These are gathered into a larger bundle, termed the midbody complex, during cytokinesis. Midbody microtubules are oriented with plus ends overlapping in the centre (McIntosh and Euteneuer, 1984). Stem bodies and midbodies may be involved with interpolar elongation (Saxton and McIntosh, 1987).

III. Polar Microtubules Exhibit Rapid Dynamic Instability

A. Spindle dynamic equilibrium assembly

In 1967, Inoué and Sato published their dynamic equilibrium model of spindle fibre assembly and chromosome movement, which was updated to deal more specifically with microtubules by Inoué (Inoué and Ritter, 1975; Inoué, 1976). The dynamic equilibrium model was based upon studies of spindle assembly in living cells, using polarization microscopy techniques to measure the assembly of the spindle fibres. These and subsequent studies showed that the majority of microtubules in the spindle can be rapidly and reversibly depolymerized in the absence of protein synthesis by a variety of physical and chemical agents, including cooling, high hydrostatic pressure, calcium, and tubulin-binding drugs like colchicine and its analogues colcemid and nocodazole (Fig. 4.7) (Inoué, 1952, 1964, 1976, 1981a; Inoué and Sato, 1967; Inoué et al., 1975; Marsland, 1970; Goode, 1973; Salmon, 1975a,b,c; 1976; Salmon et al., 1976, 1984b; Hamaguchi, 1975; Sluder, 1976; Salmon and Segall, 1980; Kiehart, 1981; Izant, 1983; Silver, 1986; Spurck and Pickett-Heaps, 1986a,b). Agents such as D₂O, glycols and DMSO were shown to
promote spindle assembly reversibly at low to moderate concentrations (Inoué and Sato, 1967; Rebhun et al., 1975). Observations like these initially led Inoué (1964) to propose that spindle fibre microtubules were reversibly self-assembled from a cellular pool of tubulin subunits and that microtubule assembly, initiated and organized by the mitotic centres, occurred by entropically driven reactions. These concepts have been largely confirmed by other biochemical and biophysical approaches following the discovery by Weisenberg (1972) of methods for the reversible self-assembly of microtubules in vitro. Inoué suggested that tubulin subunits were able to exchange rapidly at sites within microtubules in order to account for the dynamic behaviour of the spindle fibre assembly. It now appears that microtubule dynamic instability is mainly responsible for the dynamic behaviour of spindle fibres.

B. Microtubule dynamic instability in vitro

In recent years, dynamic instability, as originally conceived by Mitchison and Kirschner (1984a, b, c; Kirschner and Mitchison, 1986a), has been established as the fundamental mechanism of polymerization of microtubules from pure tubulin in vitro (reviewed by Cassimeris et al., 1987a). Real-time observation of the polymerization of individual microtubules demonstrates that dynamic instability involves persistent and distinctly different phases of microtubule elongation and rapid shortening; transitions between these phases are infrequent and abrupt (Horio and Hotani, 1986; Walker et al., 1986, 1988). Microtubules exhibiting dynamic instability never grow to a steady-state length. Instead, they either elongate or rapidly shorten. Following nucleation, a microtubule end usually elongates at constant velocity for a period of time
coextensive with microtubules were seen to elongate at a constant velocity of about 7 μm/min for an average of 71 ± 61 s and rapidly shorten at a constant velocity of about 17 μm/min for an average duration of 22 ± 15 s. At least 70% of the rapid shortening phases were rescued, showing that rescue occurs frequently for microtubules of the CMTC. Direct observations of individual microtubule dynamic instability in interphase mammalian cells has also been obtained from sequential fluorescent images of cells microinjected with fluorescently labelled tubulin (Sammak and Borisy, 1988; Schulze and Kirschner, 1988).

Fluorescent or biotinylated analogues of tubulin have provided substantial information about the pathways of tubulin assembly in living cells. Tubulin, purified from mammalian brain, has been covalently coupled to a fluorophore (DTAF, FITC, and rhodamine) (Keith et al., 1981; Leslie et al., 1984; Wadsworth and Salmon, 1986c; Gorbsky et al., 1987, 1988; Sammak et al., 1987) or biotin (Mitchison et al., 1986; Kristofferson et al., 1986; Schulze and Kirschner, 1986, 1987) in a way that preserves in vitro reassembly capabilities, then microinjected into living cells to serve as a labelled tracer in the cell’s tubulin pool. The distribution of fluorescent tubulin can be recorded in cells using fluorescence microscopy, low-light-level video cameras or CCD detectors coupled to digital image processors (Salmon and Wadsworth, 1986). Higher-resolution light and electron microscopy images have been obtained by lysing and fixing the cells. The hapten-labelled subunits were located within the microtubules using antibodies against the hapten (Solty and Borisy, 1985; Schulze and Kirschner, 1986, 1987; Mitchison and Kirschner, 1986).

Forward incorporation studies have shown that tubulin incorporation occurs at the plus ends of polar microtubules in the CMTC and the mitotic spindle and by nucleated elongation from the centrosome or spindle poles (Solty and Borisy, 1985; Schulze and Kirschner, 1986, 1987; Mitchison and Kirschner, 1986). There is no evidence that tubulin exchanges at sites along the lengths of microtubules in either of these arrays. In addition, about 80% of the microtubules in the CMTC become fully labelled with a half-time of about 5–10 min depending on cell type (Saxton et al., 1984; Schulze and Kirschner, 1986; Sammak et al., 1987). The non-kinetochore microtubules of mammalian spindles are fully labelled within 1 min after labelled tubulin injection (Saxton et al., 1984; Mitchison et al., 1986).

These forward incorporation rates correlate well with the rates of tubulin turnover within microtubules determined by measurement of fluorescence redistribution after photobleaching (FRAP) (Salmon et al., 1984a; Saxton et al., 1984; Salmon and Wadsworth, 1986; McIntosh et al., 1986; Wadsworth and Salmon, 1986a,b). In this approach, cells are microinjected with fluorescently labelled tubulin and allowed to equilibrate so that the fluorescent tubulin becomes uniformly distributed within the cellular tubulin pool and
Figure 4.9. Fluorescence and polarization micrographs of a first-division embryo of the sea urchin *Lytechinus variegatus* demonstrating that fluorescence redistribution after photobleaching (FRAP) is rapid and that photobleaching does not significantly alter the normal amount and distribution of spindle and astral fibre birefringence, the morphological changes in assembly during mitosis, or the timing of mitotic events. The cell was microinjected with DTAF-tubulin about 20 min before mitosis. Frames (a), (c), (e), (f) and (h) are fluorescence micrographs and frames (b), (d) and (g) are polarization micrographs. The contrast seen in the spindle in the polarization micrographs is produced by the birefringence of the parallel array of microtubules. Time in hours: minutes: seconds is given in the lower left-hand corner of each frame. Bleaching for 8 seconds with a 12 μm diameter argon laser microbeam ended at 14:06:03. (From Salmon et al., 1984a.)
incorporated throughout the length of microtubules (Fig. 4.9). A brief (0.1 s) pulse from a focused laser beam is then used to rapidly bleach a fraction (30–50%) of the fluorophores in a local region of the microtubule array. The rate and pattern of FRAP is measured using either photomultiplier spot methods (Fig. 4.10) or by analysis of digitized images (Figs. 4.9 and 4.11). FRAP studies initially showed that fluorescence recovery was rapid, extensive and uniform throughout the bleached region (Salmon et al., 1984a; Saxton et al., 1984). Although photobleaching fluorescently labelled microtubules can cause microtubule fragmentation (Vigers et al., 1988), we found that extensive photobleaching one half of a metaphase spindle in sea-urchin embryos did not alter the birefringence of the spindle fibres (Fig. 4.9) (Salmon et al., 1984a). This result demonstrated that photobleaching destroyed the fluorophores attached to the tubulins within the spindle fibre microtubules in vivo, but did not cut microtubules as occurs with UV irradiation (Forer, 1965, 1966; Wilson and Forer, 1988). Thus, fluorescence recovery was a measure of the steady-state turnover of tubulin within the dynamic spindle fibre microtubules. Fluorescence recovery within a bleached region occurred in three phases, as shown in Fig. 4.10. There is an initial very rapid phase produced by the diffusion of unpolymerized tubulin. The second phase is termed the rapid incorporation phase. Fluorescence recovery during this phase is exponential.

![Figure 4.10](image.png)

Figure 4.10. Computer records of spindle FRAP in a sea-urchin metaphase spindle obtained by photometric techniques. Fluorescence excitation and bleaching were produced by a laser microbeam (488 nm, 4.5 µm diameter) focused in a central half-spindle of first-mitotic Lytechinus variegatus embryos that had been microinjected before mitosis with DTAF–tubulin. Spindle fluorescence was bleached by 50–60% by removing an attenuating filter (5 × 10^-4) from the path of the laser beam. Photon counts were acquired every second using a photomultiplier. The first five fluorescence values were used to normalize the data. A total of 382 s of normalized data were acquired, and data for 0–100, 227–237, and 367–382 seconds are plotted. (From Wadsworth and Salmon, 1986b.)
with a half-time of 16–60 s depending on cell type (Wadsworth and Salmon, 1986b) and represents typically 70–80% of the non-diffusible bleached fluorescence. In the third phase, the remaining 20–30% of the bleached fluorescence recovers more slowly and spindles usually looked fully recovered after about 10 min. These results show that tubulins throughout 70–80% of the microtubules in the spindle—most probably the labile polar, non-kinetochore microtubules—exchange with tubulins in the cellular pool with a half-time of tens of seconds. The slow phase of fluorescence recovery is probably due to the slower turnover of tubulin within the differentially stable kinetochore microtubules, as discussed below.

These results provide strong support for the dynamic instability of polar microtubule assembly. The rapid rate of fluorescence recovery and forward incorporation is several orders of magnitude too fast to be produced by simple equilibrium diffusional exchange of tubulin at microtubule ends (Salmon et al., 1984a).

There is also no pattern to the recovery of fluorescence during the rapid incorporation phase (Salmon et al., 1984a; Saxton et al., 1984; Wadsworth and Salmon, 1986a,b; McIntosh et al., 1986; McIntosh and Vigers, 1987; Cassimeris et al., 1988a). As shown in Fig. 4.11, bar patterns bleached across the middle of a half-spindle in metaphase PtK₁ cells, recover fluorescence without movement of the pattern. This type of experiment showed that plus to minus treadmilling (Margolis et al., 1978; Margolis and Wilson, 1981) did not produce the fluorescence recovery. Since tubulin does not appear to exchange at sites along the lengths of microtubules, whole microtubules must be rapidly
polymerizing and rapidly depolymerizing completely (Fig. 4.12) in order to achieve the rapid rates and uniform pattern observed of fluorescence recovery as illustrated in Figs. 4.9, 4.10 and 4.11.

Another way to look at the dynamics of microtubules is to determine how fast they depolymerize when elongation is abruptly blocked. Colchicine and other drugs, like colcemid and nocodazole, that bind to the colchicine-binding site on tubulin have been used to abruptly block microtubule elongation in cells (Salmon et al., 1984b; DeBrabander et al., 1986). These drugs act by binding to a site on tubulin not exposed when tubulin is assembled into microtubules (Margolis and Wilson, 1977; Margolis et al., 1980; Bergen and Borisy, 1983). It is the tubulin–drug complex which inhibits microtubule
elongation and blocks nucleation. This can be demonstrated by microinjecting tubulin–colchicine complex into cells. The tubulin–colchicine complex is very stable and produces similar rates of spindle microtubule depolymerization to those produced by high concentrations of colchicine or nocodazole (Wadsworth and Salmon, 1986b; McIntosh et al., 1986). In the presence of high concentrations of colchicine, colcemid, or nocodazole, non-kinetochore, polar spindle microtubules depolymerize with a half-time of 6–10 s depending on cell type (Fig. 4.4) (Salmon et al., 1984b). The rates of microtubule depolymerization have been estimated to be of the order of 500–1000 dimers/s (Salmon et al., 1984b) as predicted for rapid shortening during microtubule dynamic instability (Mitchison and Kirschner, 1984b; Horio and Hotani, 1986; Walker et al., 1988).

Although the real-time behaviour of individual microtubules within the spindle has yet to be observed, the above observations leave little doubt that dynamic instability is the dominant mechanism of polar (non-kinetochore) microtubule assembly in the spindle as well as the CMTC. Thus, consideration of mechanisms that may produce or regulate the assembly of the mitotic spindle (Harris, 1978; Vandré et al., 1984) must now be considered in terms of frequencies of nucleation, catastrophe and rescue as well as the rate constants of elongation and rapid shortening (Cassimeris et al., 1987a). This is somewhat complicated. The amount of microtubule polymer can be increased by increasing the frequency of nucleation and/or the mean length of microtubules. Microtubule length can be increased by increasing the rate of elongation or the frequency of rescue and/or by decreasing the frequency of catastrophe or the rate of rapid shortening. Comprehensive information about all these parameters is not yet available. The mean elongation time for a polar spindle microtubule in newt cells is about 100 s based on a measured half-time of FRAP of 87 s (Wadsworth and Salmon, 1986a). The mean length of elongation before catastrophe is likely to be in the range of 10 μm in these spindles, whose interpolar length is 40–50 μm. These values are similar to the mean time of elongation and the mean length of elongation measured for the microtubules in the interphase newt CMTC (Cassimeris et al., 1988b). It now appears that the velocity of elongation, the frequency of catastrophe and probably the rate of rapid shortening are similar during mitosis and interphase in the newt. This suggests that, during mitosis, the short mean length and life-time of spindle microtubules appears to be produced by changes in cell physiology that substantially reduce the frequency of rescue in comparison to the rate occurring during interphase. These arguments (see also Kirschner and Mitchison, 1986a) predict that polar spindle microtubules in general elongate at several micrometres per minute for about a minute before rapidly shortening and disappearing. This cycle would be repeating continuously, but asynronously, among the population of polar microtubules, so that at all times there are growing microtubule plus ends throughout the spindle (Fig. 4.12).
IV. Chromosome Fibre Formation by Microtubule Attachment to Kinetochores

A. Kinetochore capture of microtubules

Initially, it was thought that kinetochore microtubules predominantly formed by nucleated microtubule assembly and that chromosomal fibres resulted from the interdigitation of kinetochore and non-kinetochore microtubules (McIntosh et al., 1969; Nicklas, 1971; McIntosh et al., 1975). In lysed cell preparations, kinetochores as well as centrosomes were shown to initiate the assembly of microtubules (Snyder and McIntosh, 1975; Pepper and Brinkley, 1979; Telzer and Rosenbaum, 1979; Bergen et al., 1980; Telzer et al., 1980; Brinkley et al., 1981; Brinkley, 1986; Mitchison and Kirschner, 1985ab; McIntosh, 1985). However, nucleation of microtubules from kinetochores in vitro required much higher tubulin concentrations than required for centrosomes. Nucleation of microtubules from kinetochores in vivo was also demonstrated immediately following reversal of drug treatments that had induced complete spindle microtubule disassembly and high free tubulin concentrations (Witt et al., 1980; DeBrabander et al., 1981ab,c, 1986). Once the spindle was reassembled and the free tubulin concentration was lowered, it was difficult to tell whether these nucleated microtubules persisted. Nevertheless, the above evidence makes it clear that kinetochores can initiate the growth of microtubules at elevated tubulin concentrations.

However, as initially stressed by Pickett-Heaps et al. (1982) and Rieder (1982), attachment, not nucleation, now appears to be the major mechanism of kinetochore microtubule formation during mitosis (Brinkley et al., this volume). When chromosomes initially form connections to a spindle pole and begin moving towards that pole (orientation), microtubules are seen extending from the kinetochore polewards (Nicklas and Kubai, 1985; Nicklas, 1987a). As seen in Fig. 4.13(a), mono-oriented chromosomes have kinetochore microtubules only at the kinetochore facing the pole. The sister kinetochore faces away from the pole and is devoid of microtubules. This occurs for mono-oriented chromosomes close to or many micrometres away from the pole in either monopolar or bipolar spindles (Rieder et al., 1986). If kinetochore microtubules normally formed by nucleation at kinetochores, microtubules should be evident at the kinetochores on mono-oriented chromosomes facing away from the pole. The concentration of tubulin is not expected to be much different between adjacent sister kinetochores. When a mono-oriented chromosome begins moving toward the opposite pole (when it bi-orients), microtubules are then seen extending towards that pole from the sister kinetochore facing that pole (Fig. 4.13(b)).

There are several other observations consistent with the capture concept (see also Pickett-Heaps et al., 1982; Rieder, 1982). The plus ends of kinetochore
Figure 4.13. Electron micrographs comparing the attachment of microtubules to kinetochores of (A) mono-oriented and (B) bi-oriented newt chromosomes. In (A), the pole is to the lower right. There are no microtubules on the kinetochore facing away from the pole. Bar = 0.25 μm. (Original micrographs provided by Lynne Cassimeris and Conly Rieder.)