Studies on the ejection properties of asters: astral microtubule turnover influences the oscillatory behavior and positioning of mono-oriented chromosomes

JEFFREY G. AULT[1,*], ANTHONY J. DEMARCO[1], EDWARD D. SALMON[2] and CONLY L. RIEDER[1,3]

[1]*Wadsworth Center for Laboratories and Research, Empire State Plaza, P.O. Box 509, Albany, NY 12201-0509, USA*
[2]*Department of Biology, University of North Carolina, Chapel Hill, NC 27599, USA*
[3]*School of Public Health, State University of New York, Albany, NY 12222, USA*

* Author for correspondence

Summary

The position of a mono-oriented chromosome changes as it oscillates to and from the pole to which it is attached. Such oscillatory behavior reveals that the net force on a mono-oriented chromosome is constantly changing. Fluctuations may occur in both the polewardly directed force acting at the kinetochore and the opposing outwardly directed force associated with the aster. We have examined the ejection properties of the aster – as well as the oscillatory behavior and positioning of mono-oriented chromosomes – in relation to astral microtubule turnover. We treated cells containing monopolar spindles with drugs that affect microtubule turnover, either by promoting the depletion of dynamically unstable astral microtubules (nocodazole and colcemid) or by augmenting their numbers and stability (taxol). Both types of drugs stopped the oscillatory behavior of mono-oriented chromosomes within seconds. The final position of the chromosomes depended on how microtubule turnover was affected. In the case of nocodazole and colcemid, non-kinetochore astral microtubules were depleted first and the kinetochore-to-pole distance shortened. In these cells chromosome fragments generated by laser microsurgery were no longer expelled from the center of the aster. By contrast, with taxol the number of non-kinetochore microtubules increased and the astral ejection force became stronger as shown by the finding that the chromosomes moved away from the pole to the periphery of the monaster. Moreover, arms severed from chromosomes at the periphery of the taxol monaster failed to move further away from the aster's center. From these observations we conclude that the oscillatory movements and changing position of a mono-oriented chromosome relative to the pole are mediated by changes in the number of astral microtubules. The dynamic instability of astral microtubules that leads to a rapid turnover may contribute to the astral ejection force by allowing the continual growth of microtubules out from the aster. Growing astral microtubules may exert a pushing force that their rigidity maintains until their depolymerization.

Key words: chromosome oscillations, chromosome positioning, astral ejection force, monopolar spindles, microtubule turnover, laser microsurgery, taxol, nocodazole, *Taricha granulosa*.

Introduction

Mono-oriented chromosomes are frequently positioned many micrometers from the pole to which they are attached. As a rule, these chromosomes exhibit oscillatory behavior, continually moving towards and then away from the pole (Bajer, 1982). These oscillations occur in the absence of antagonistic pulling forces directed at the sister kinetochores towards opposite poles (Rieder *et al.* 1985). As a result, at least two opposing forces are needed for this oscillatory behavior and positioning: a polewardly directed force (i.e. a poleward force) and an outwardly directed force (i.e. an outward force).

Much has been learned in recent years about the force that moves chromosomes towards a pole. The formation of kinetochore fibers, which attach the kinetochore to the spindle pole, is essential for poleward movement (Church and Lin, 1985; Nicklas and Kubai, 1985; Rieder *et al.* 1990). Studies of differentially marked kinetochore fibers (Mitchison *et al.* 1986; Gorbsky *et al.* 1987) and of truncated spindles (Nicklas, 1989) demonstrate that the pole-directed motor is at or near the kinetochore. The initial movement of a prometaphase kinetochore along the surface of a single astral microtubule suggests that the motor resides in the kinetochore corona (Rieder *et al.* 1990). Moreover, high-resolution kinetic analyses reveal that this motion is similar in character to the saltatory motions driven by cytoplasmic dynein (Alexander and Rieder, 1991). Indeed, cytoplasmic dynein has been recently localized at the primary constriction of chromosomes (Pfarr *et al.* 1990; Steuer *et al.* 1990) and, in particular, at the kinetochore corona itself (Wordeman *et al.* 1991).

In contrast to the poleward force, little is known concerning the force that causes chromosomes to move away from a pole. In a bipolar spindle, such movement can

Printed in Great Britain © The Company of Biologists Limited 1991

be at least partially attributed to antagonistic pulling forces directed at opposing sister kinetochores towards opposite poles (Östergren, 1951; Hays *et al.* 1982; Hays and Salmon, 1990). That is, the outward 'pushing' force apparently exhibited by one pole is actually a poleward pulling force towards the opposite pole. Under these conditions the oscillatory behavior of a chromosome may simply be a manifestation of a tug-of-war between two poleward pulling forces, and only poleward forces may be needed to generate oscillations. However, this hypothesis does not explain the oscillatory behavior and positioning of mono-oriented chromosomes on both bipolar and monopolar spindles.

To explain the behavior and positioning of mono-oriented chromosomes, Rieder *et al.* (1986) proposed that movement away from the pole is mediated by the ejection properties of the aster, long known to expel particles and acentric chromosome fragments away from the pole and towards the equatorial plate and spindle periphery (e.g. see Carlson, 1938). This ejection force, coupled with the well-characterized poleward force at the kinetochore, offers an attractive explanation for the oscillatory behavior, and relative position from the pole, of mono-oriented chromosomes.

Although the ejection properties of the aster were first described in 1938 (Carlson, 1938), the mechanism behind this force remains elusive (for review, see Rieder, 1990). Astral microtubules (MTs) appear to be an important component (Rieder *et al.* 1986; Salmon, 1989*a*). Indeed, while it is generally agreed that small membranous particles are translocated within the aster along MTs (Freed and Lebowitz, 1970; Hayden *et al.* 1983), larger particles, inclusions lacking membranes (e.g. nucleoli) and acentric chromosome fragments appear to simply 'float' much more slowly away from the pole (Molè-Bajer *et al.* 1975; Bajer, 1982; Rieder *et al.* 1986). The dynamic instability of astral MTs (Mitchison and Kirschner, 1984*a*,*b*; Hayden *et al.* 1990), which leads to a constant turnover and growth of MT ends away from the center of the aster, may contribute to the astral ejection property (Rieder *et al.* 1986).

To investigate the role of astral MT turnover in the origin of the astral ejection properties, and on the oscillatory behavior and positioning of chromosomes, we treated monopolar spindles with drugs that either depolymerize astral MTs (colcemid and nocodazole) or enhance their polymerization and 'stability' (taxol). During such treatments we examined the effects these drugs had on the oscillatory behavior and positioning of mono-oriented chromosomes, and on the ability of the aster to expel acentric chromosome arms created by laser microsurgery. Monopolar spindles were used in this study to eliminate any possible influences of a second pole on chromosome behavior.

Materials and methods

Newt care and pneumocyte cultures

The behavior of mono-oriented chromosomes on monopolar spindles was studied in cultured lung pneumocytes of the northwestern rough skinned newt, *Taricha granulosa*. The care and feeding of these newts as well as the culturing of their lung cells have been described in detail elsewhere (Rieder and Hard, 1990).

Monolayers of newt lung cells were cultured from explants in Rose Chambers (Rose, 1954) and pneumocytes with optically favorable flat monopolar spindles were initially located using an inverted phase-contrast microscope. Coverslips with the selected pneumocytes were remounted on perfusion chambers (e.g. see McGee-Russell and Allen, 1971) and the chambers were filled with standard newt culture medium consisting of one-half strength L-15 medium (with 10% fetal bovine serum, 100 i.u. ml^{-1} penicillin, 100 $\mu g\,ml^{-1}$ streptomycin and 50 i.u. ml^{-1} mycostatin, pH 7.2–7.4) supplemented with 5–10 mM 4-2-hydroxyethyl-1-piperazine-ethanesulfonic acid (Hepes). The perfusion chambers allowed for the easy administration of drugs and fixatives while continuously observing the cell.

Laser cutting of chromosomes

This portion of the study was done at the National Institutes of Health's Laser Microbeam Program (LAMP) facility located at the University of California at Irvine. The use of the laser microbeam system (Berns *et al.* 1981) for severing newt chromosome arms has been previously described in detail by Rieder *et al.* (1986).

Chromosome arms were severed from the kinetochore region of mono-oriented chromosomes in control cells, and in cells treated with either colcemid (0.15 μM) or taxol (10 μM), using a neodymium–YAG (yttrium–aluminum–garnet) laser. Since the cultures were treated with the drug several hours prior to laser cutting, each mitotic cell contained a spindle characteristic of the treatment. The spindles in cells treated with threshold doses of colcemid (0.15 μM) consisted of 'chromosomal spheres' (see Rieder, 1982) in which all of the chromosomes were mono-oriented and grouped around the single polar area. Taxol-treated cells had multiple asters with various numbers of associated mono-oriented chromosomes (see DeBrabander *et al.* 1986). In these spindles the chromosome arms did not form the characteristic V shape found in controls, but lay at various angles to one another.

Video analysis and immunofluorescence of nocodazole- and taxol-treated cells

The behavior of mono-oriented chromosomes on monopolar spindles was continuously observed and recorded before, during, and after perfusing nocodazole (20 μM in standard newt culture medium) or taxol (10 μM in standard newt culture medium) into the chamber. The light-microscopic system consisted of a Nikon Microphot-FX microscope equipped with differential-interference-contrast optics, a Dage-MTI model 70 Newvicon camera system, a Hamamatsu DVS-3000 image processor, and a Panasonic TQ-2025F optical disc recorder.

The staining procedure for indirect immunofluorescence microscopy was as follows. Control and experimental cells followed *in vivo* were initially fixed for 1–3 min in the perfusion chamber with 1.0% glutaraldehyde in PHEM buffer (Schliwa and van Blerkom, 1981) at pH 7.0. The coverslip cultures containing the cells of interest were then transferred sequentially through: 0.1% glutaraldehyde in PHEM (15 min); 1% Triton X-100 in PHEM (5 min); PHEM (three rinses, 5 min each); $NaBH_4$ (0.5 mg ml^{-1}) in PHEM (two times, 5 min each); $NaBH_4$ (0.5 mg ml^{-1}) in phosphate-buffered saline (PBS) (Weber *et al.* 1978) at pH 7.0 (5 min); PBS (three rinses, 5 min each); 5% fetal bovine serum and 0.1% Tween-20 in PBS (20–30 min at 37°C); a monoclonal antibody (Tu27B) against beta tubulin (a kind gift from Dr L. Bender, University of Alabama at Birmingham) in PBS (overnight at 4°C); PBS (three rinses, 5 min each); a fluorescein-conjugated goat anti-mouse IgG (Sigma Chemical Co., St Louis) in PBS (30 min at 37°C); PBS (five rinses, 5 min each); Hoechst 33342 (0.02 mg ml^{-1}) in PBS (5 s); PBS (three rinses, 5 min). Cultures were mounted on slides in an anti-bleach medium of *p*-phenylenediamine (0.3 mg ml^{-1}) and 50% glycerol in PBS. The cells of interest were subsequently photographed with a Nikon Optiphot microscope, equipped with Epi-fluorescence optics, using Ilford XP1 400 film at an ASA setting of 800.

Results

Effects of nocodazole and taxol on monopolar spindles

Monopolar spindles are formed in newt lung cells when the

two poles fail to separate (Fig. 1A,B) or when they separate too far prior to nuclear envelope breakdown to form a conventional bipolar spindle (Fig. 1C,D) (see Bajer *et al.* 1980; Bajer, 1982; Rieder and Hard, 1990). Under the latter condition two monopolar spindles are formed within the same cell, each of which has a variable number of mono-oriented chromosomes (this is sometimes described as anaphase-like prometaphase, see Bajer, 1982). Chromo-

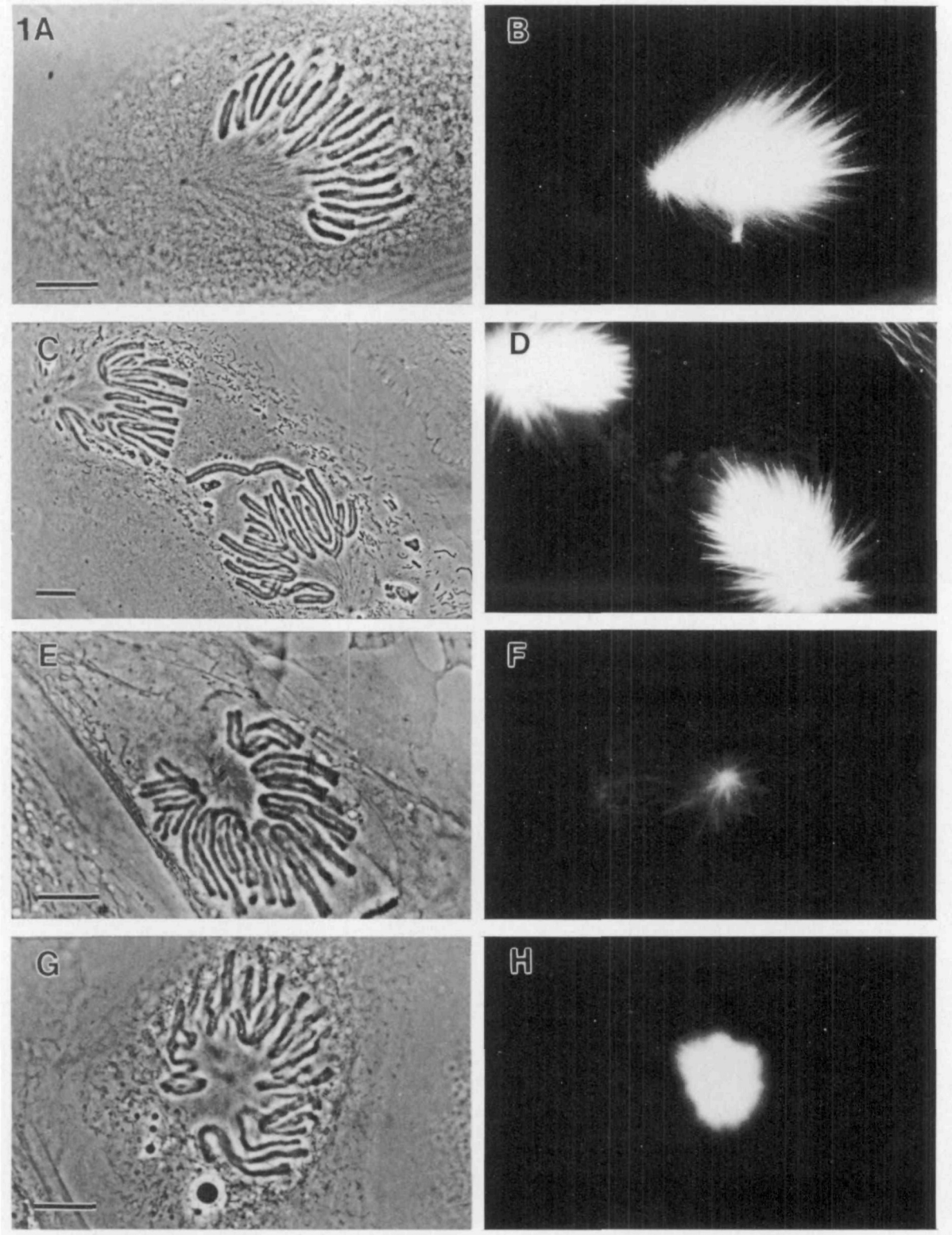

Fig. 1. Phase-contrast (A,C,E,G) and anti-tubulin immunofluorescence (B,D,F,H) photomicrographs of monopolar spindles. (A,B) A monopolar spindle caused by the two poles failing to separate. (C,D) Two monopolar spindles within the same cell caused by the two poles separating too far to form a conventional bipolar spindle. (E,F) A monopolar spindle treated with nocodazole for 4 min prior to fixation. (G,H) A monopolar spindle treated with taxol for 15.5 min prior to fixation. Bars, 10 μm.

somes attached to monopolar spindles are, by definition, always mono-oriented, usually with one attached (active) and one unattached (unactive) kinetochore (reviewed by Rieder, 1990). As a rule the chromosomes on a monopolar spindle are positioned well within the dense array of non-kinetochore astral MTs, i.e. all of the chromosome except the ends is within the aster (cf. Fig. 1A–D).

Two types of drugs were used in this study. Both affect astral MTs, but in different ways. Nocodazole and colcemid bind to the free tubulin dimer at the same site as

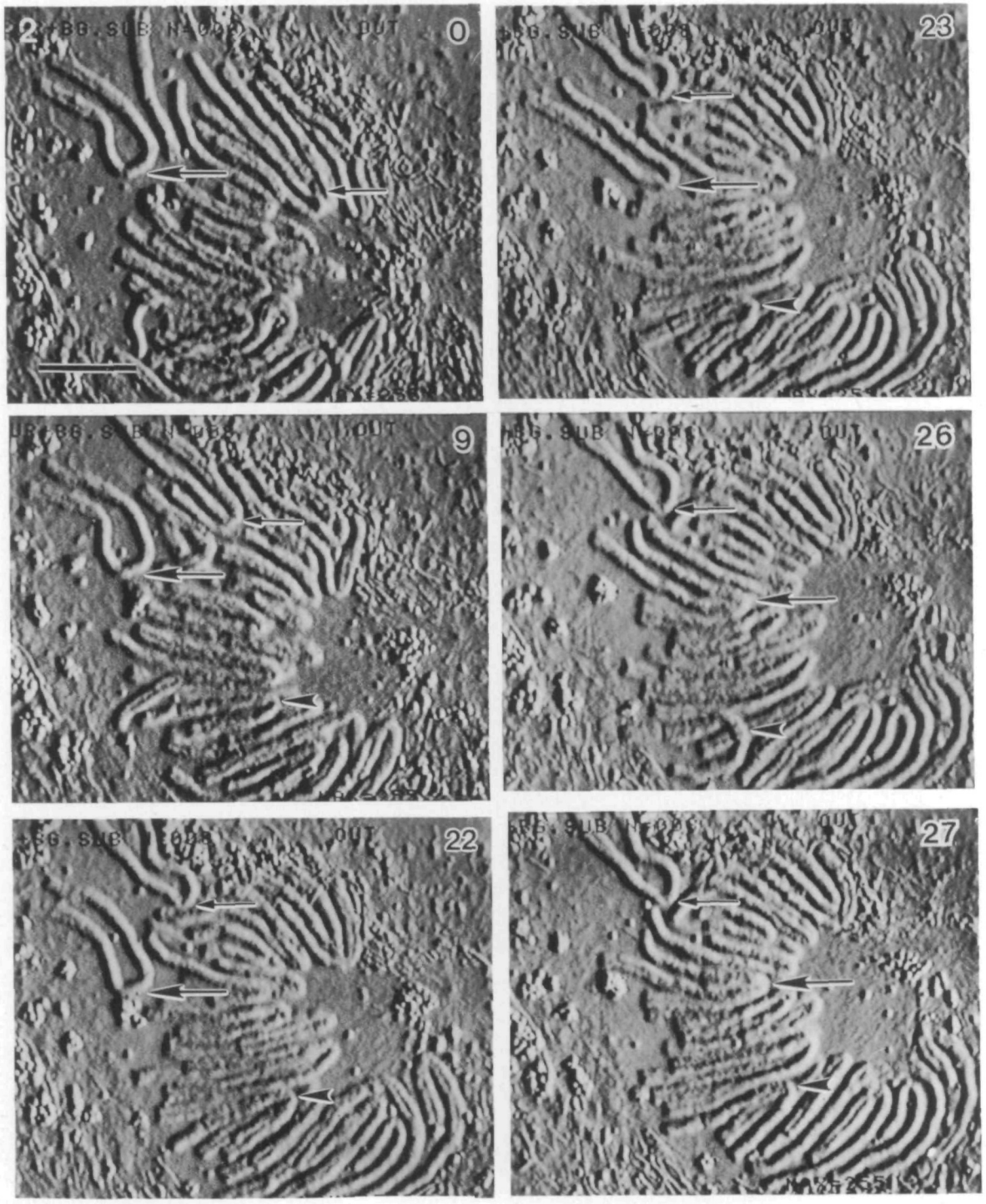

Fig. 2. The ejection properties of the aster are clearly revealed when a mono-oriented chromosome completely loses its attachment to the pole. Here, an oscillating chromosome (small arrow; 0 min) on an untreated monopolar spindle completely loses its attachment to the pole and is ejected to the periphery of the aster (9 min, 22 min). Detachment is evident by the finding that the kinetochore region no longer faces the pole (22–27 min) and that the chromosome is beyond the distal ends of the other chromosomes, which denote the edge of a monopolar spindle (note the unattached chromosome in Fig. 1C,D). Another unattached chromosome (large arrow) is also at the periphery of this monopolar spindle (0–22 min) until it attaches to the pole and moves poleward (23–27 min). A chromosome displaying typical oscillatory behavior is noted by the arrowhead. Time in min is shown at top right corners. Bar, 10 μm.

colchicine (Hoebeke *et al.* 1976; Margolis and Wilson, 1977; Lee *et al.* 1980). This blocks MT assembly, but not disassembly. As a result, owing to the dynamically unstable nature of astral MT growth, the number of MTs associated with the aster rapidly diminishes; the more labile non-kinetochore MTs disappear first, followed by the more stable kinetochore MTs (Salmon *et al.* 1984; Cassimeris *et al.* 1986; Cassimeris *et al.* 1990; this study). A monopolar spindle treated with 20 μM nocodazole for 4 min prior to fixation is shown in Fig. 1E,F. The kinetochore regions of the chromosomes are near the pole and only those MTs within the remnant kinetochore fibers are present.

Taxol has the opposite effect on MTs from nocodazole and colcemid. It promotes MT assembly and the 'stability' of already polymerized MTs (Parness and Horwitz, 1981). Taxol has been shown *in vitro* to lower the critical concentration of tubulin required for MT polymerization and to shorten the lag time for MT assembly (Schiff *et al.* 1979). Monopolar spindles treated with 10 μM taxol for 15.5 min prior to fixation appear to have more, but shorter, MTs than untreated control monopolar spindles (cf. Fig. 1G,H with B,D). The taxol-treated monopolar spindle appears as a dense ball of similar length MTs (Fig. 1H) in which the chromosomes do not penetrate, but are at the edge of the ball (cf. Fig. 1G,H).

Effects of colcemid and taxol on the ejection properties of the aster

The capacity of the aster to eject chromosomes away from its center, in untreated or non-experimentally perturbed monopolar spindles, was clearly revealed in those rare occasions when a mono-oriented chromosome completely lost its attachment to the pole (Fig. 2). Detachment was evident in that the chromosome behaved like an acentric chromosome fragment. The detached chromosome stopped oscillating and moved at 1–2 μm min^{-1} away from the pole to the periphery of the aster. During this movement the kinetochore region trailed the arms, i.e. the V shape of the chromosome was maintained as it moved away from the pole. Once at the astral periphery, the kinetochore region of the detached chromosome no longer faced the pole (attached kinetochores always face the pole to which they are attached when an opposing force – such as that generated by a manipulation needle or by the aster itself – is present). Detached chromosomes remained at the astral periphery until they re-established attachment to the pole. At this time they initiated the rapid pole-directed motion previously described in detail by Alexander and Rieder (1991) for attaching newt pneumocyte chromosomes.

The ejection properties associated with the monaster can be readily demonstrated by cutting off the arms of a mono-oriented chromosome using a neodymium–YAG

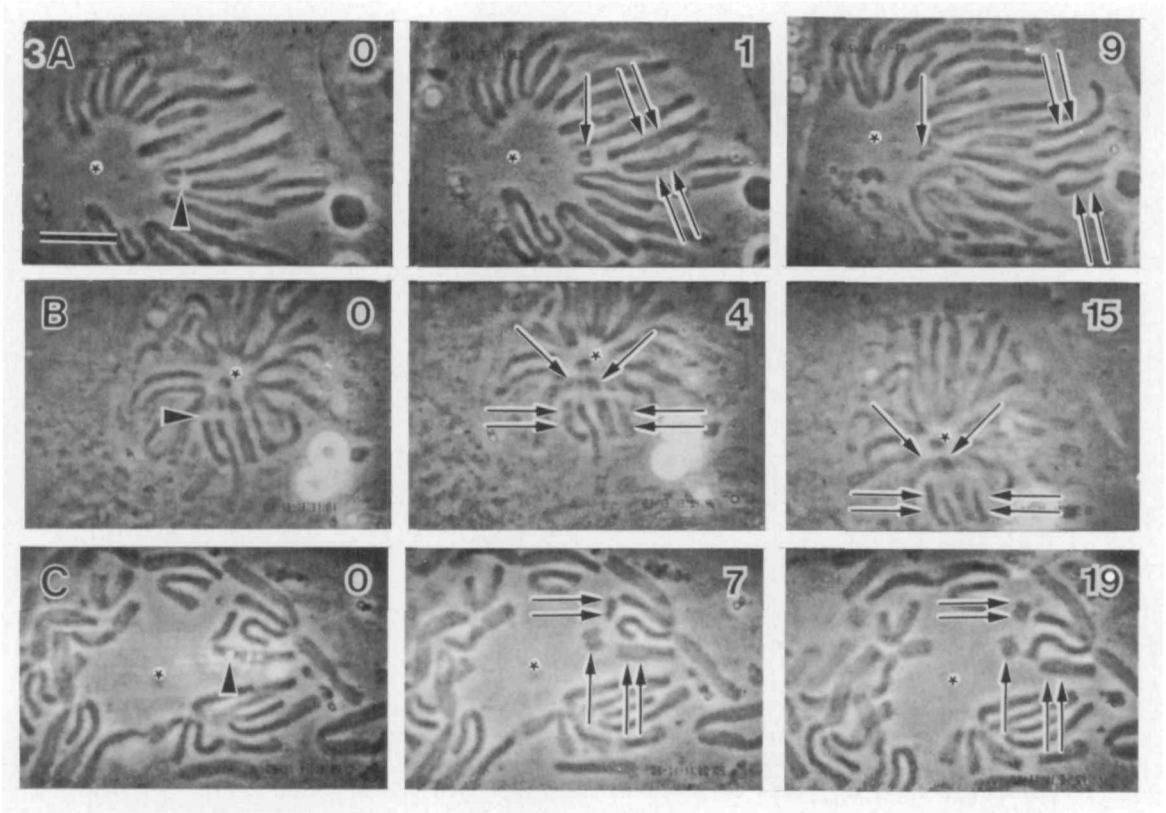

Fig. 3. The astral ejection properties of monopolar spindles. Phase-contrast photomicrographs from video recordings of: (A) an untreated monopolar spindle in which the chromosome arms are cut (arrowhead) from the kinetochore region (0 min) and move away from the pole (1 min) to the periphery of the spindle (9 min). The kinetochore region (single arrow) moves closer to the pole (asterisk) after the arms (double arrows) are removed. The chromosome featured was actually the second one cut in the cell; all four severed arms of both chromosomes can be seen at 9 min (between the pair of double arrows). (B) A monopolar spindle formed in the presence of colcemid. After the chromosome arms are cut off (0 min), they (double arrows) remain close to the kinetochore region (single arrow at 4 min, 15 min). The spindle (pole denoted by asterisk) is not in the same position in each of the three photomicrographs due to movement in the microscope stage during filming. (C) A cell that entered mitosis in the presence of taxol. After the arms of a mono-oriented chromosome are cut (0 min, the laser beam can be seen), they (double arrows) remain close to the kinetochore region (single arrow at 7 min, 19 min; pole denoted by asterisk). Bar, 20 μm.

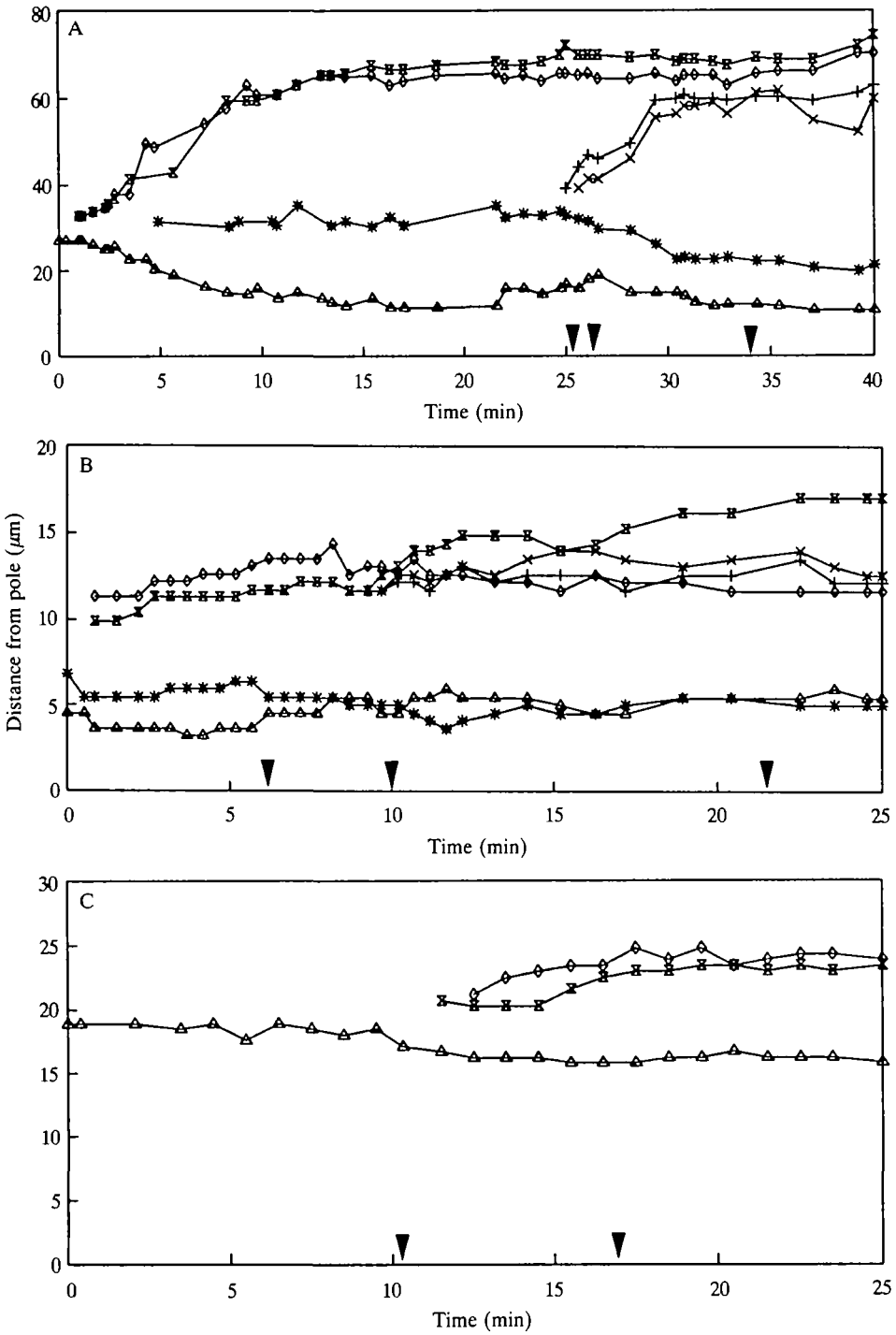

Fig. 4. Graphical representation of the laser-dissected chromosomes shown in Fig. 3. The behavior of kinetochore regions (△——△ and *——*) and their corresponding severed arms (◇——◇, ⧖——⧖ and +——+, ×——×) are illustrated for: (A) the untreated cell in Fig. 3A; (B) the colcemid cell in Fig. 3B; (C) the taxol cell in Fig. 3C. The arrowheads indicate when the photomicrographs in Fig. 3 were taken. Note that the chromosome featured in Fig. 3A is actually the second chromosome dissected in Fig. 4A. Fig. 3C, 19 min is not indicated because it was taken outside the time frame of the graph.

laser. The severed arms always moved slowly ($1–2\,\mu m\,min^{-1}$) to the periphery of the aster where they remained throughout the duration of mitosis (Figs 3A, 4A). The kinetochore region, on the other hand, moved closer to the pole after the arms were cut off (Figs 3A, 4A).

The ability of the aster to eject acentric chromosome fragments was greatly diminished or nonexistent in spindles formed in the presence of colcemid or taxol. In both cases the severed arms of mono-oriented chromosomes remained relatively stationary (Figs 3B,C; 4B,C), and any movement (or turning) could be attributed to Brownian forces. It should be noted, however, that in taxol-treated cells the chromosomes were already positioned at the astral periphery (Fig. 1G,H) where the ejection properties of the aster are not observed, even in untreated cells (see above).

Effects of nocodazole and taxol on the oscillatory behavior and positioning of mono-oriented chromosomes

Before treatment with either nocodazole or taxol, mono-oriented chromosomes on monopolar spindles exhibited oscillatory behavior, moving towards and away from the pole (Figs 5B, 6B) Seconds after the administration of either drug, the oscillations stopped and the position of the chromosomes changed (Figs 5, 6). The kinetochore-to-pole distance rapidly diminished in response to 20 μM nocodazole (Fig. 5). This usually occurred *via* the pole moving

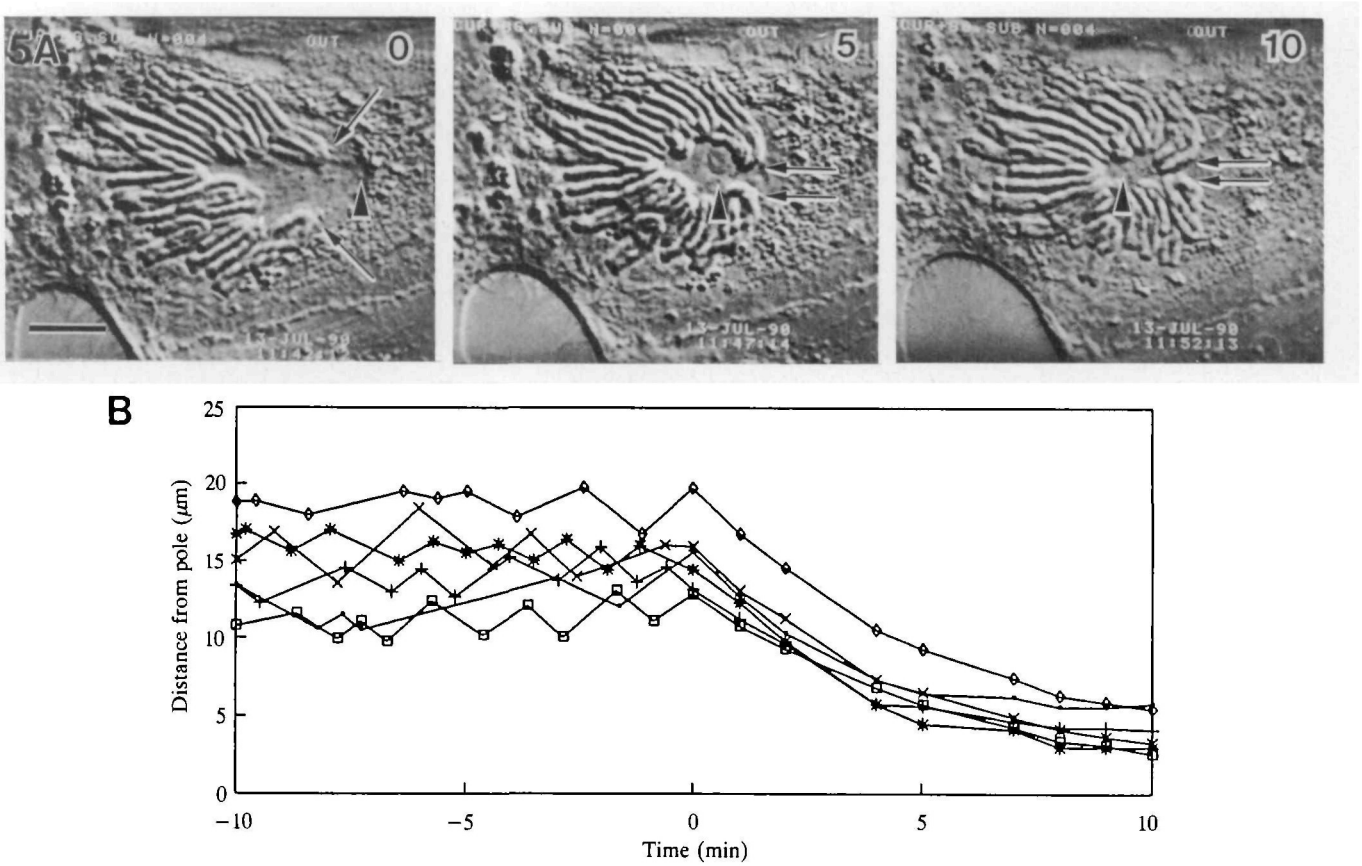

Fig. 5. The effects of nocodazole on chromosome oscillations and positioning. (A) Differential-interference-contrast photomicrographs of a video recording showing the chromosome-to-pole distances shortening after the administration of nocodazole (0 min). The pole (arrowhead) moves into the mass of chromosomes, dragging other chromosomes (single arrows) with it (5 min, 10 min). Bar, 10 μm. (B) Graphical representation of the behavior of six of the chromosomes shown in A. The oscillatory behavior of all six chromosomes stops and they move closer to the pole shortly after the administration of nocodazole (0 min).

closer to the bulk of the chromosomes, while chromosomes at the periphery moved towards the pole (e.g. see Fig. 5A, arrows). When treated with 10 μM taxol the chromosomes on a monopolar spindle moved further away from the pole (Fig. 6). The V shape of the chromosome did not change during the initial stages of this movement, indicating that the force for the movement was not directed only at the kinetochore, but all along the chromosome. Once the chromosome reached the periphery of the aster its arms often became more relaxed and spread out (Fig. 6A; also Figs 1G, 2).

Discussion

Mono-oriented chromosomes in vertebrate epithelial cells exhibit conspicuous and constant oscillatory movements towards and away from the pole to which they are attached. As a result, at any point in time the chromosomes are positioned at variable distances from the pole. Since only one kinetochore of a mono-oriented chromosome is usually active (Rieder *et al.* 1985, 1986; Salmon, 1989*b*; Rieder, 1990), this behavior cannot be explained by models based on a tug-of-war between antagonistic pulling forces directed at sister kinetochores towards opposite poles (reviewed by Salmon, 1989*a,b*). To explain the oscillatory behavior and positioning of mono-oriented chromosomes it has been proposed that the poleward force acting at the only active kinetochore is opposed by an outward force produced by the spindle pole or aster (Rieder *et al.* 1986; Salmon, 1989*a,b*). Under these conditions a chromosome is positioned where the two opposing forces are equal and moves when they are not. Importantly, this model is also consistent with the behavior of bipolarly oriented chromosomes on bipolar spindles. Here, chromosomes are positioned by two opposing poleward forces acting at the sister kinetochores as well as two opposing pushing forces directed away from the two asters that act on the whole chromosome (Rieder *et al.* 1986; Salmon, 1989*a,b*; Rieder, 1991).

We have shown in this study that drugs affecting the stability and numbers of astral MTs affect the ejection properties of the aster, and also the oscillation and positioning of chromosomes, in a predictable manner. When the normal turnover of astral MTs is disrupted, the ejection properties of the aster change, and chromosome oscillations stop. The subsequent behavior and final position of a chromosome depends on whether MT turnover was affected by promoting MT depolymerization or stability. With nocodazole and colcemid, non-kinetochore astral microtubules are depleted first, which eliminates the astral ejection force. As a result the kinetochore-to-pole distance shortens in response to the poleward force generated at the kinetochore, which moves the chromosome poleward in association with the more stable kinetochore MTs (Fig. 5). The pole, which is depleted of most of its microtubules, has less drag than the larger chromosomes and, therefore, is the one usually

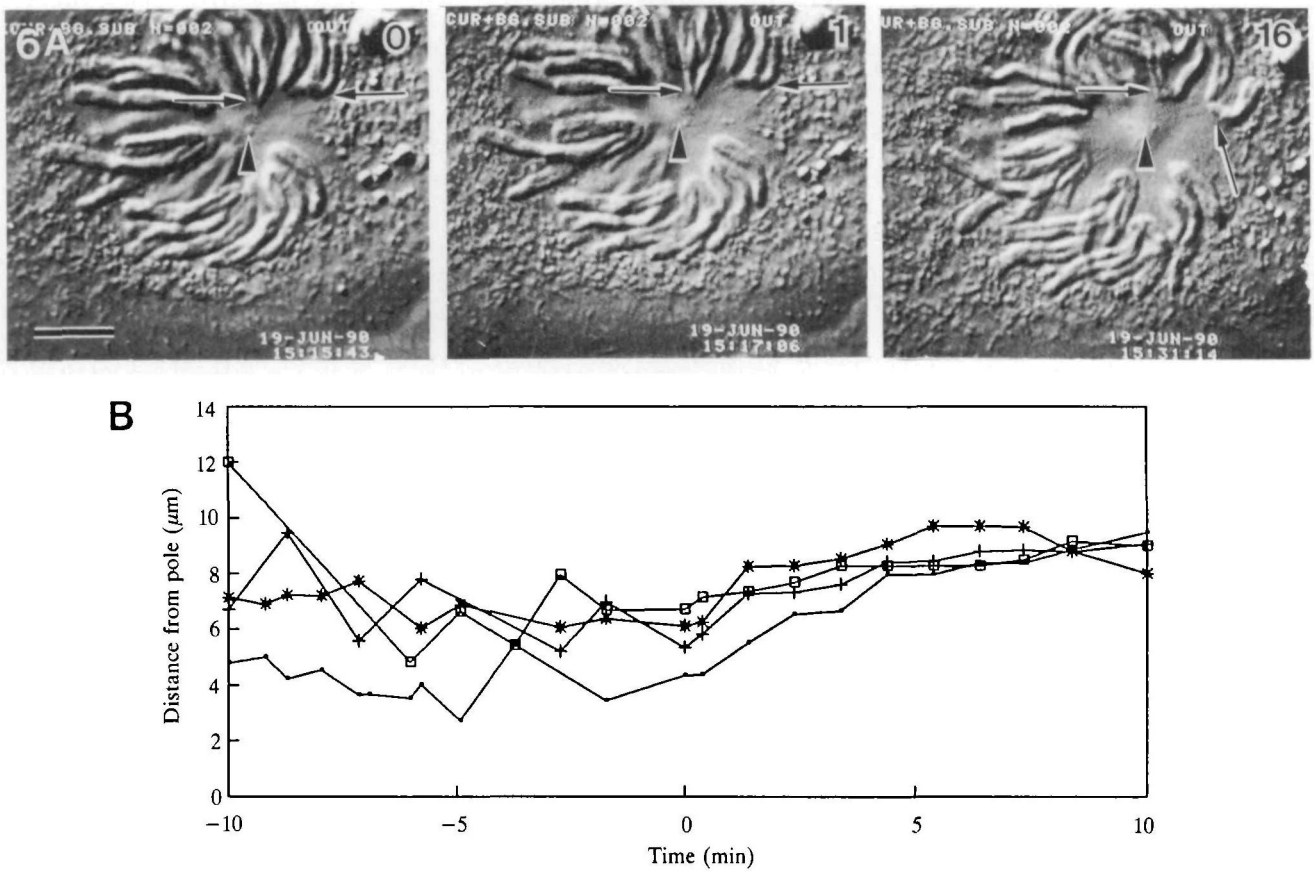

Fig. 6. The effects of taxol on chromosome oscillations and positioning. (A) Differential-interference-contrast photomicrographs of a video recording showing mono-oriented chromosomes backing away from the pole (arrowhead) after the administration of taxol (0 min). The V-shaped configuration of chromosomes (arrows) does not change (1 min) until they reach the periphery of the spindle where the arms may spread out (16 min). Bar, 10 μm. (B) Graphical representation of the behavior of four chromosomes shown in A. The oscillatory behavior of all four chromosomes stops and they move further away from the pole shortly after the administration of taxol (0 min).

displaced. However, if a chromosome was in an area by itself where its drag was against the drag of the other chromosomes then it moved towards the pole and the other chromosomes (Fig. 5A). In contrast, taxol promotes MT polymerization and stability, which leads to an increase of astral MTs. As a result, the ejection force increases and the chromosomes move away from the pole to the periphery of the aster. Clearly, the astral ejection force can be manipulated by changing the number of astral MTs.

There are several conceivable and not mutually exclusive ways by which non-kinetochore astral MTs could produce an outward force on a chromosome. For example, their polymerization may exert a pushing force (Miyamoto and Hotani, 1988). Alternatively, they may penetrate a chromosome and particles translocating along them may impact the chromatin to exert an outward force (here a MT plus-end-directed motor molecule such as kinesin would be required). Finally, the chromosome arms could be studded with MT plus-end-directed motor molecules (e.g. kinesin), which, upon interacting with an astral MT, move the chromosome arm away from the pole (Zhang *et al.* 1990). All of these mechanisms require non-kinetochore MTs to impinge on the chromosome and this has been observed in those organisms in which spindle MTs have been carefully tracked (reviewed by Bajer and Molè-Bajer, 1972; see also Lin *et al.* 1981; Ault, 1986).

In lieu of data implicating MT motor molecules in the astral ejection force (Rieder, 1991), we favor the simpler idea that the constant growth of MTs out from the pole creates a moving steric wall that pushes chromosomes and other large organelles away from the pole. In this context, as theorized by Hill (1981), growing MTs have been shown to exert a force against membranes (Miyamoto and Hotani, 1988) and perhaps kinetochores (Bajer *et al.* 1982). Indeed, growing astral MTs push through the cytoplasm (Cassimeris *et al.* 1988; Hayden *et al.* 1990) and do demonstrate a noticeable force when they push up against and distort the nuclear envelope (Bajer and Molè-Bajer, 1972). Thus, it seems reasonable that growing astral MTs would exert a similar pushing force against chromatin and that, the more MTs impinging on a chromosome, the further the chromosome would be from the pole (if the poleward force remained constant).

A positive correlation between the number of impinging non-kinetochore MTs and the kinetochore-to-pole distance has been reported for the mono-oriented X chromosome univalents in the two grasshopper species, *Melanoplus sanguinipes* and *Melanoplus differentialis*. In *M. sanguinipes*, non-kinetochore astral MTs directly affect chromosome movement by preventing the X chromosome from reorienting and moving towards the opposite pole (Ault, 1986). The X chromosome in this species is unusual in that it forms a stable mono-orientation during meiosis I (Ault, 1984). Orientation stability is attributed to the increased number of impinging non-kinetochore MTs that terminate on or in the chromosome (Ault, 1986). Data from both

species together showed a positive correlation between the number of impinging non-kinetochore MTs from the near pole and the distance the chromosome was from that pole. This correlation is opposite to that predicted with the general MT distribution of a spindle (where the density of MTs is greatest at the pole and less further from it), suggesting that indeed impinging MTs keep chromosomes away from the pole. Interestingly, there was no correlation between the number of kinetochore MTs and the kinetochore-to-pole distance. It is worth noting that the ejection of acentric chromosome fragments by the aster was first described in grasshoppers (Carlson, 1938).

In addition to an outward force directed against the chromosome by impacting growing astral MTs, the chromosome may simultaneously experience additional non-kinetochore-directed forces that contribute to its position. For example, particles translocating along MTs that penetrate through the chromosome (Hayden *et al.* 1990) may exert a force on the chromosome arm upon impact. In the case of minus-end-directed movement, such a force would be manifested as a sudden poleward motion (similar to neocentric behavior). Alternatively, particles moving towards the plus end of a MT that penetrates the chromosome may similarly contribute to the outward ejection force.

The presence of the astral ejection force is temporally correlated with aster size, MT dynamics, and when non-kinetochore MTs impinge on chromosomes. The asters are most robust and their ejection properties appear most active during prometaphase and metaphase (Rieder, 1990). Likewise, astral MT turnover is greater during this time (Gorbsky and Borisy, 1989; Wadsworth *et al.* 1989). Similarly, more non-kinetochore MTs are observed impinging on chromosomes during prometaphase and metaphase than in anaphase (Lin *et al.* 1981; Church and Lin, 1982). Interestingly, if taxol is added during anaphase and spindle MTs are induced to grow, the ejection properties of the aster appear and the arms of the polewardly moving chromosomes are kept away from the pole by impinging non-kinetochore microtubules (Bajer *et al.* 1982; Molè-Bajer and Bajer, 1983). This results in the stretching and breaking of chromosome arms.

We have shown that experimentally manipulating the number of astral MTs affects the astral ejection force, which in turn affects the behavior and positioning of mono-oriented chromosomes. However, in untreated cells fluctuations in the poleward force may actually be the more dominant factor affecting chromosome oscillations and position, particularly if the astral ejection force turns out to be relatively constant. Poleward force may be affected by the elongation of kinetochore MTs at the kinetochore (Bajer, 1982; Mitchison, 1989), by the number and turnover of those kinetochore MTs that support force production (Rieder, 1991), and/or by changes in the functional efficiency of the actual motor itself. It is important to note that since mono-orientations are usually unstable (Dietz, 1958; Nicklas, 1961), such chromosomes may be constantly losing their microtubule attachments to the pole (Ault and Nicklas, 1989). A gradual net loss of force-supporting MTs (see Rieder, 1991) would most likely decrease the poleward force, allowing the astral ejection force to push the chromosome further away from the pole. In newts, it is rare for mono-oriented chromosomes to completely lose their attachment to the pole, but it does happen occasionally (Fig. 2). When this occurs the detached chromosome behaves like an acentric chromosome fragment and is expelled to the periphery of the aster. It remains there until attachment to the pole is re-established, which allows it to move poleward, against the astral ejection force, and resume oscillatory behavior.

We thank Richard Cole for his technical advice throughout the course of this investigation. This work was supported in part by the National Institutes of Health (NIH) through GMS grants R01–40198 (to C.L.R.) and R01–24364 (to E.D.S.), and by Biotechnological Resource grants RR 01219 (to the Wadsworth Center's Biological Microscopy and Image Reconstruction facility) and RR 01192 (to the University of California at Irvine's Laser Microbeam Program).

References

ALEXANDER, S. P. AND RIEDER, C. L. (1991). Chromosome motion during attachment to the vertebrate spindle: initial saltatory-like behavior of chromosomes and a quantitative analysis of force production by nascent kinetochore fibers. *J. Cell Biol.* **113**, 805–815.

AULT, J. G. (1984). Unipolar orientation stability of the sex univalent in the grasshopper *(Melanoplus sanguinipes)*. *Chomosoma* **89**, 201–205.

AULT, J. G. (1986) Stable *versus* unstable orientations of sex chromosomes in two grasshopper species. *Chromosoma.* **93**, 298–304.

AULT, J. G. AND NICKLAS, R. B. (1989) Tension, microtubule rearrangements, and the proper distribution of chromosomes in mitosis *Chromosoma* **98**, 33–39.

BAJER, A. S. (1982). Functional autonomy of monopolar spindle and evidence for oscillatory movement in mitosis. *J. Cell Biol* **93**, 33–48.

BAJER, A. S., CYPHER, C., MOLÈ-BAJER, J. AND HOWARD, H. M. (1982). Taxol-induced anaphase reversal evidence that elongating microtubules can exert a pushing force in living cells. *Proc natn. Acad. Sci. U.S A.* **79**, 6569–6573.

BAJER, A. S., DEBRABANDER, M , MOLÈ-BAJER, J , DEMEY, J. AND PAULAITIS, S. (1980) Mitosis: the mitotic aster, interzone and functional autonomy of monopolar half-spindle. In *Microtubules and Microtubule Inhibitors* (ed. M. DeBrabander and J. DeMey), pp. 399–425 Amsterdam· Elsevier/North Holland.

BAJER, A. S. AND MOLÈ-BAJER, J. (1972). Spindle dynamics and chromosome movement *Int. Rev Cytol* **3** (Suppl.), 1–271.

BERNS, M. W., AIST, J., EDWARDS, J., STRUHS, K., GIRTEN, J., MCNEIL, P., RATTNER, J. B., KITZES, M., HAMMER-WILSON, M., LIAW, L. -H., SIEMENS, A., KOUNCE, M., PETERSON, S., BRENNER, S , BURT, J , WALTER, R., BRYANT, P. J., VANDYK, D., COULOMBE, J , CAHILL, T. AND BERNS, G S (1981) Laser microsurgery in cell and developmental biology. *Science* **213**, 505–513.

CARLSON, J. G. (1938). Mitotic behavior of induced chromosomal fragments lacking spindle attachments in the neuroblasts of the grasshopper. *Proc. natn. Acad. Sci. U.S.A.* **24**, 500–507.

CASSIMERIS, L., PRYER, N. K. AND SALMON, E. D. (1988). Real-time observations of microtubule dynamic instability in living cells *J. Cell Biol.* **107**, 2223–2231.

CASSIMERIS, L., RIEDER, C. L., RUPP, G AND SALMON, E D. (1990). Stability of microtubule attachment to metaphase kinetochores in PtK1 cells. *J. Cell Sci.* **96**, 9–15

CASSIMERIS, L. U., WADSWORTH, P. AND SALMON, E. D. (1986). Dynamics of microtubule depolymerization in monocytes. *J. Cell Biol.* **102**, 2023–2032.

CHURCH, K. AND LIN, H. P. (1982). Meiosis in *Drosophila melanogaster* II. The prometaphase–I kinetochore microtubule bundle and kinetochore orientation in males. *J. Cell Biol.* **93**, 365–373.

CHURCH, K. AND LIN, H. P. (1985). Kinetochore microtubules and chromosome movement during prometaphase in *Drosophila melanogaster* spermatocytes studied in life and with the electron microscope. *Chromosoma* **92**, 273–282.

DEBRABANDER, M , GEUENS, G., NUYDENS, R , WILLEBRORDS, R., AERTS, F., DEMEY, J. AND MCINTOSH, J. R. (1986). Microtubule dynamics during the cell cycle. the effects of taxol and nocodazole on the microtubule system of Pt K2 cells at different stages of the mitotic cycle. *Int. Rev Cytol.* **101**, 215–274

DIETZ, R. (1958). Multiple Geschlechtschromosomen bei den cypriden Ostracoden, ihre Evolution und ihr Teilungsverhalten. *Chromosoma* **9**, 359–440.

FREED, J. J. AND LEBOWITZ, M. M. (1970). The association of a class of saltatory movements with microtubules in cultured cells. *J. Cell Biol.* **45**, 334–354.

GORBSKY, G J AND BORISY, G. G (1989). Microtubules of the kinetochore fiber turn over in metaphase but not in anaphase. *J. Cell Biol.* **109**, 653–662.

GORBSKY, G. J., SAMMAK, P. J. AND BORISY, G. G. (1987) Chromosomes move poleward in anaphase along stationary microtubules that

coordinately disassemble from their kinetochore ends. *J. Cell Biol.* **104**, 9–18.

Hayden, J. H., Allen, R. D. and Goldman, R. D. (1983). Cytoplasmic transport in keratocytes: direct visualization of particle translocation along microtubules. *Cell Motil.* **3**, 1–19

Hayden, J. H., Bowser, S. S. and Rieder, C. L (1990). Kinetochores capture astral microtubules during chromosome attachment to the mitotic spindle: direct visualization in live newt lung cells. *J. Cell Biol.* **111**, 1039–1045.

Hays, T. S. and Salmon, E D. (1990). Poleward force at the kinetochore in metaphase depends on the number of kinetochore microtubules. *J. Cell Biol.* **110**, 391–404.

Hays, T. S., Wise; D and Salmon, E. D. (1982). Traction force on a kinetochore at metaphase acts as a linear function of kinetochore fiber length. *J. Cell Biol.* **93**, 374–382.

Hill, T. L. (1981). Microfilament or microtubule assembly or disassembly against a force *Proc. natn. Acad. Sci. U.S A.* **78**, 5613–5617.

Hoebeke, J., Van Nijen, G. and DeBrabander, M. (1976). Interaction of nocodazole (R17934), a new antitumoral drug, with rat brain tubulin. *Biochem. biophys. Res. Commun* **69**, 319–324.

Lee, C., Field, D. J. and Lee, L. L. Y (1980) Effects of nocodazole on structures of calf brain tubulin. *Biochemistry* **19**, 6209–6215.

Lin, H. P., Ault, J G. and Church, K. (1981). Meiosis in *Drosophila melanogaster.* I. Chromosome identification and kinetochore microtubule numbers during the first and second meiotic divisions in males. *Chromosoma* **83**, 507–521

Margolis, R L. and Wilson, L. (1977). Addition of colchicine–tubulin complex to microtubule ends: the mechanism of substoichiometric colchicine poisoning. *Proc. natn. Acad Sci U S A.* **74**, 3466–3470.

McGee-Russell, S. M. and Allen, R. D. (1971) Reversible stabilization of labile microtubules in the reticulopodial network of *Allogromia. Adv. Cell molec. Biol* **1**, 153–184.

Mitchison, T. J. (1989). Chromosome alignment at mitotic metaphase: balanced forces or smart kinetochores? In *Cell Movement,* vol. 2. *Kinesin, Dynein, and Microtubule Dynamics* (ed. F. D. Warner and J. R. McIntosh), pp. 421–430. New York A. R. Liss.

Mitchison, T. J., Evans, L., Schulze, E. and Kirschner, M. (1986) Sites of microtubule assembly and disassembly in the mitotic spindle. *Cell* **45**, 515–527.

Mitchison, T. J. and Kirschner, M. W. (1984*a*) Microtubule assembly nucleated by isolated centrosomes. *Nature* **312**, 232–237.

Mitchison, T J. and Kirschner, M. W. (1984*b*). Dynamic instability of microtubule growth. *Nature* **312**, 237–242

Miyamoto, H. and Hotani, H. (1988). Polymerization of microtubules in liposomes produces morphological changes of shape. *Proc. Taniguchi Int. Symp* **14**, 220–242.

Molè-Bajer, J. and Bajer, A S (1983). Action of taxol on mitosis· modification of microtubule arrangements and function of the mitotic spindle in *Haemanthus* endosperm. *J. Cell Biol* **96**, 527–540.

Molè-Bajer, J , Bajer, A. S. and Owczarzak, A. (1975). Chromosome movements in prometaphase and aster transport in the newt *Cytobios* **13**, 45–65.

Nicklas, R B (1961). Recurrent pole-to-pole movements of the sex chromosome during prometaphase I in Melanoplus differentialis spermatocytes *Chromosoma* **12**, 97–115.

Nicklas, R. B. (1989). The motor for poleward chromosome movement in anaphase is in or near the kinetochore. *J Cell Biol.* **109**, 2245–2255.

Nicklas, R. B. and Kubai, D. F (1985). Microtubules, chromosome movement, and reorientation after chromosomes are detached from the spindle by micromanipulation. *Chromosoma* **92**, 313–324.

Östergren, G (1951). The mechanism of co-orientation in bivalents and multivalents. The theory of orientation by pulling *Heredutas* **37**, 85–156.

Parness, J and Horwitz, S. B. (1981). Taxol binds to polymerized tubulin *in vitro. J. Cell Biol.* **91**, 479–487.

Pfarr, C. M , Coue, M., Grisson, P. M., Hayes, T. S., Porter, M E and McIntosh, J. R. (1990) Cytoplasmic dynein is localized to kinetochores during mitosis. *Nature* **345**, 263–265.

Rieder, C. L. (1982). The formation, structure, and composition of the mammalian kinetochore and kinetochore fiber. *Int. Rev. Cytol.* **79**, 1–58.

Rieder, C. L (1990) Formation of the astral mitotic spindle· ultrastructural basis for the centrosome-kinetochore interaction. *Electron Microsc. Rev.* **3**, 269–300.

Rieder, C. L. (1991). Mitosis: towards a molecular understanding of chromosome behavior. *Cur. Opinion Cell Biol.* **3**, 59–66.

Rieder, C. L., Alexander, S. P. and Rupp, G. (1990). Kinetochores are transported poleward along a single astral microtubule during chromosome attachment to the spindle in newt lung cells. *J. Cell Biol.* **110**, 81–95.

Rieder, C. L., Davison, E. A., Jensen, L. C. W., Cassimeris, L. and Salmon, E. D. (1986). Oscillatory movements of monooriented chromosomes, and their position relative to the spindle pole, result from the ejection properties of the aster and half-spindle. *J. Cell Biol.* **103**, 581–591.

Rieder, C. L., Davison, E. A., Jensen, L. C. W. and Salmon, E. D. (1985). The distal kinetochore is not responsible for the oscillation of monooriented chromosomes in newt lung cells. In *Microtubules and Microtubule Inhibitors* (ed. M. DeBrabander and J. DeMey), pp. 253–260. Amsterdam: Elsevier Sci. Pub.

Rieder, C. L. and Hard, R. (1990). Newt lung epithelial cells: cultivation, use and advantages for biomedical research. *Int. Rev. Cytol* **122**, 153–220.

Rose, G. G. (1954). Separable and multipurpose tissue culture chamber. *Texas Reps. on Biol. and Med.* **12**, 1074–1083.

Salmon, E. D. (1989*a*). Metaphase chromosome congression and anaphase poleward movement. In *Cell Movement,* vol 2: *Kinesin, Dynein, and Microtubule Dynamics* (ed. F. D. Warner and J. R. McIntosh), pp. 431–440. New York: A. R. Liss.

Salmon, E. D (1989*b*) Microtubule dynamics and chromosome movement. In *Mitosis: Molecules and Mechanisms* (ed. J. Hyams and B R Brinkley), pp. 118–181. New York. Academic Press.

Salmon, E. D , McKeel, M. and Hays, T. (1984) Rapid rate of tubulin dissociation from microtubules in the mitotic spindle *in vivo* measured by blocking polymerization with colchicine. *J. Cell Biol* **99**, 1066–1075.

Schiff, P. B , Fant, J and Horwitz, S B. (1979). Promotion of microtubules assembly *in vitro* by taxol *Nature* **277**, 665–667.

Schliwa, M. and van Blerkom, J (1981). Structural interaction of cytoskeletal components. *J. Cell Biol.* **90**, 222–235.

Steuer, E R , Wordeman, L., Schroer, T. A. and Sheetz, M P (1990). Localization of cytoplasmic dynein to mitotic spindles and kinetochores. *Nature* **345**, 266–268

Wadsworth, P., Shelden, E., Rupp, G. and Rieder, C. L. (1989). Biotin-tubulin incorporates into kinetochore fiber microtubules during early but not late anaphase. *J. Cell Biol.* **109**, 2257–2265.

Weber, K., Rathke, P. C. and Osborn, M. (1978). Cytoplasmic microtubular images in glutaraldehyde-fixed tissue culture cells by electron microscopy and by immunofluorescence microscopy. *Proc. natn. Acad. Sci. U.S.A* **75**, 1820–1824

Wordeman, L., Steuer, E. R., Sheetz, M. P. and Mitchison, T. (1991). Chemical subdomains within the kinetochore domain of isolated CHO mitotic chromosomes. *J. Cell Biol.* (in press)

Zhang, P., Knowles, B A , Goldstein, L. S B and Hawley, R S (1990). A kinesin-like protein required for distributive chromosome segregation in *Drosophila. Cell* **62**, 1053–1062

(Received 12 February 1991 – Accepted 16 April 1991)