# Kinetochores Capture Astral Microtubules during Chromosome Attachment to the Mitotic Spindle: Direct Visualization in Live Newt Lung Cells

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***Abstract.*** When viewed by light microscopy the mitotic spindle in newt pneumocytes assembles in an optically clear area of cytoplasm, virtually devoid of mitochondria and other organelles, which can be much larger than the forming spindle. This unique optical property has allowed us to examine the behavior of individual microtubules, at the periphery of asters in highly flattened living prometaphase cells, by video-enhanced differential interference-contrast light microscopy and digital image processing. As in interphase newt pneumocytes (Cassimeris, L., N. K. Pryer, and E. D. Salmon. 1988. *J. Cell Biol.* 107:2223–2231), centrosomal (i.e., astral) microtubules in prometaphase cells appear to exhibit dynamic instability, elongating at a mean rate of 14.3 ± 5.1 μm/min ($N$ = 19) and shortening at ~16 μm/min. Under favorable conditions the initial interaction between a kinetochore and the forming spindle can be directly observed. During this process the unattached chromosome is repeatedly probed by microtubules projecting from one of the polar regions. When one of these microtubules contacts the primary constriction the chromosome rapidly undergoes poleward translocation. Our observations on living mitotic cells directly demonstrate, for the first time, that chromosome attachment results from an interaction between astral microtubules and the kinetochore.

THE attachment of a chromosome to the nascent mitotic spindle, its poleward orientation, and its subsequent poleward movement correlate with the formation of a birefringent fiber which firmly connects one or both of its kinetochores to the polar regions. Since these kinetochore fibers (K-fibers) produce, transmit, and/or regulate poleward chromosome movement (reviewed in Bajer and Mole-Bajer, 1972; Rieder, 1982; Nicklas, 1988; Salmon, 1989), elucidating their formation, structure, and mechanism of function are of considerable importance to understanding force production within the spindle.

Van Beneden and Neyt are credited (Wilson, 1911, 1925) for first proposing, in the late 1880's, that the fibers which attach chromosomes to the forming spindle are derived from the radial "astral" array of fibers generated by the centrosomes (i.e., spindle poles). Over a half-century later Schrader (1953) argued that "there has never been published a convincing series of stages showing a progressive growth of such fibers from the pole". He then concluded, based on studies of mitosis in *Acroschismus* and *Coccidae*, that the K-fiber "may arise chiefly or entirely through the activity of the kinetochore alone." Subsequent EM studies have shown that microtubules (MTs)[1] are the primary structural constituent of K-fibers (reviewed in Brinkley and Stubblefield, 1970; Rieder, 1982, 1990; McDonald, 1989). As a result, the question of how K-fibers originate has been refined to determining whether those MTs associated with the kinetochore (i.e., K-MTs) are nucleated by the kinetochore, or whether they are generated from the spindle pole(s) and then captured by the kinetochore.

In recent years many investigators have attempted to determine the origin of K-MTs without clearly resolving the question (reviewed in Pickett-Heaps et al., 1982; Rieder, 1982; Nicklas, 1988; McIntosh et al., 1989; McDonald, 1989; Brinkley et al., 1989). It is apparent from these studies that such an analysis is complicated by several factors. First, upon nuclear envelope breakdown (NEB) the chromosomes are exposed to an environment of high MT density generated by the separating spindle poles. Second, it cannot be predicted when a particular chromosome will attach to the spindle, only that it will do so very quickly. Indeed, "all evidence indicates that after the initial stages in the disintegration of the nuclear membrane, the chromosomal fibers appear suddenly within the nuclear area and with their chromosomal connections fully established" (Schrader, 1953). Finally, by convention a spindle MT is considered a K-MT upon terminating in the kinetochore, and it is not possible to determine a priori whether a MT terminating in the kinetochore originated from the kinetochore or the spindle pole.

As a rule, the newt pneumocyte spindle forms in an opti-

1. *Abbreviations used in this paper*: DIC, differential interference-contrast; IMF, immunofluorescence; LM, light microscopic; K-MT, kinetochore microtubule; MT, microtubule; NEB, nuclear envelope breakdown.

cally clear region of cytoplasm, produced by the absence of cytoplasmic organelles within the nucleus at NEB, which is defined during early prometaphase by a barrier of intermediate filaments (Mandeville and Rieder, 1990). These cells remain very flat during mitosis, and chromosomes that are spatially well separated from the spindle poles after NEB are delayed in attaching to the spindle (reviewed in Rieder and Hard, 1990; Rieder, 1990). Recently, Rieder and Alexander (1990) have shown that when such chromosomes finally attach they do so at the periphery of the aster where MT density is extremely low. Using same-cell correlative light microscopic (LM), antitubulin immunofluorescence (IMF), and EM methods, these investigators determined that the attachment and subsequent poleward movement of a chromosome in newt pneumocytes correlated with the association of a single long MT with one of its kinetochores. The fact that this MT traversed the entire distance between the spindle pole and the kinetochore, and often extended well past the kinetochore, indicated that it was of astral origin. These data, when combined with the established polarity of K-MTs (Euteneuer and McIntosh, 1981), strongly support the hypothesis that K-MTs in cells undergoing astral mitosis are derived from the centrosomes.

Although the density of MTs within the newt pneumocyte central spindle is too high to permit the identification of individual MTs with certainty by video-enhanced LM (Cassimeris et al., 1988*a*), within the clear region at the astral periphery MT density is very low and the distal ends of astral MTs are clearly visible by IMF (Rieder and Alexander, 1990). We reasoned that because of the favorable optical conditions, individual astral MTs could be directly examined within the clear region of highly flattened cells by video-enhanced differential interference-contrast (DIC) LM. We report here that individual spindle MTs can indeed be seen in living prometaphase newt pneumocytes using this method. As a result we have been able to examine the behavior of astral MT ends and to directly visualize the initial interaction between astral MTs and the kinetochores of unattached chromosomes.

## Materials and Methods

### Newt Lung Culture

Newt (*Taricha granulosa*) lung primary explants were cultured in Rose chambers as previously described (reviewed in Rieder and Hard, 1990). The cells selected for study were among the largest and flattest cells displaying epithelial characteristics (i.e., pneumocytes). Under our culture conditions these cells were most frequently located near the periphery of the migrating monolayer.

### Light Microscopy and Data Analysis

Mitotically active cultures were screened several times a day using a Nikon Diaphot-TMD inverted phase-contrast microscope. When a prometaphase cell suitable for high-resolution analysis was located, the Rose chamber was quickly dismantled and the culture-containing coverslip was mounted on a microperfusion chamber (McGee-Russell and Allen, 1971).

Perfusion chamber preparations were examined with either a Nikon Microphot-FX or a Zeiss IM 35 microscope, both equipped with DIC optics (objective NA: Nikon = 1.25; Zeiss = 1.4). The illumination, obtained from a 100 W Hg-arc burner, was passed through serial heat, UV-cut, and 546-nm interference filters to minimize specimen irradiation damage. In addition, the Nikon microscope was equipped with a fiber optic pipe to randomize the incident light. Analogue images were acquired with a Dage MTI 70 Newvicon video camera. These images were then digitally processed by background subtraction, averaging eight video frames, and contrast enhancement with a Hamamatsu DVS 3000 image processor. Final images were stored using a Panasonic TQ-2025 optical memory disk recorder operated in time-lapse mode (25–40 frames/min) under the control of custom-designed software. Magnification was calibrated against the 0.62-$\mu$m frustule spacing of the diatom *Pleurasigma angulatum* viewed under the same optical conditions. Still frames were recorded on Kodak Plus-X film either by using a Polaroid freeze-frame video recorder or by directly photographing the monitor.

Frame-by-frame analysis of microtubule elongation and shortening was conducted on optical memory disk images using the Hamamatsu DVS 3000 distance function. In some cases the analysis was facilitated by further contrast enhancement of MT ends.

For correlative DIC and antitubulin IMF, selected cells were fixed during continuous video-DIC observation by perfusion with buffered glutaraldehyde as detailed by Rieder and Alexander (1990). Fixed cultures were then processed for the indirect IMF localization of MTs, as previously described (Rieder and Alexander, 1990), using an mAb against $\beta$-tubulin (TU-27b; kindly provided by Dr. L. Binder, University of Alabama, Birmingham, AL). These cultures were then examined with a Nikon Optiphot LM equipped with a 100× phase-contrast objective (NA = 1.3). Fluorescent and phase-contrast images were photographed on Ilford XP-1 film (ASA 1600), which was developed according to the manufacturer's instructions.

## Results

### Individual Microtubules Projecting from the Polar Region Can Be Visualized in Prometaphase Cells

The mitotic apparatus in highly flattened newt pneumocytes forms in an optically clear region of cytoplasm which is often much larger than the spindle (Fig. 1 *a*; see also Mandeville and Rieder, 1990). In early-to-mid prometaphase cells the distal ends of several longer astral MTs can be seen, after fixation and processing for IMF, to extend past the arms of monooriented chromosomes and to terminate within this optically clear area (Fig. 1 *b*).

When living prometaphase cells were examined by video-enhanced DIC LM, some regions between the distal ends of the chromosome arms and the periphery of the clear area were seen to contain one or more thin linear elements (Figs. 1 *c*, 2, and 3). The contrast of these linear elements varied depending on their orientation relative to the DIC shear plane (compare Figs. 1 *c* and 2 *b*), and the proximal end of each pointed towards the astral center (compare Figs. 1, *a–e*). In some instances several of these thin linear elements could be found within different optical sections of a particular clear region. However, due to their oblique orientation relative to the optical axis, and their random lateral and vertical movements in response to thermal and cytoplasmic forces, it was difficult to maintain focus on the distal ends of these linear elements unless they were positioned near the ventral cell surface. Here the long axis of a linear element was usually perpendicular to the optical axis and its vertical motion appeared restricted, permitting prolonged observation of its distal end.

Same-cell correlative video-DIC and fluorescence LM revealed that each linear element seen within the clear area of a living prometaphase cell correlated, after fixation and antitubulin staining, with a thin fluorescent line extending from the aster (Fig. 1). That each of these thin fluorescent lines at the aster periphery corresponds to an individual MT has been demonstrated previously at the EM level (Rieder and Alexander, 1990). We therefore conclude that the thin linear elements analyzed in this study are individual astral MTs.

When viewed on the monitor in real time, or in time-lapse recordings, many of the MT ends found projecting from near

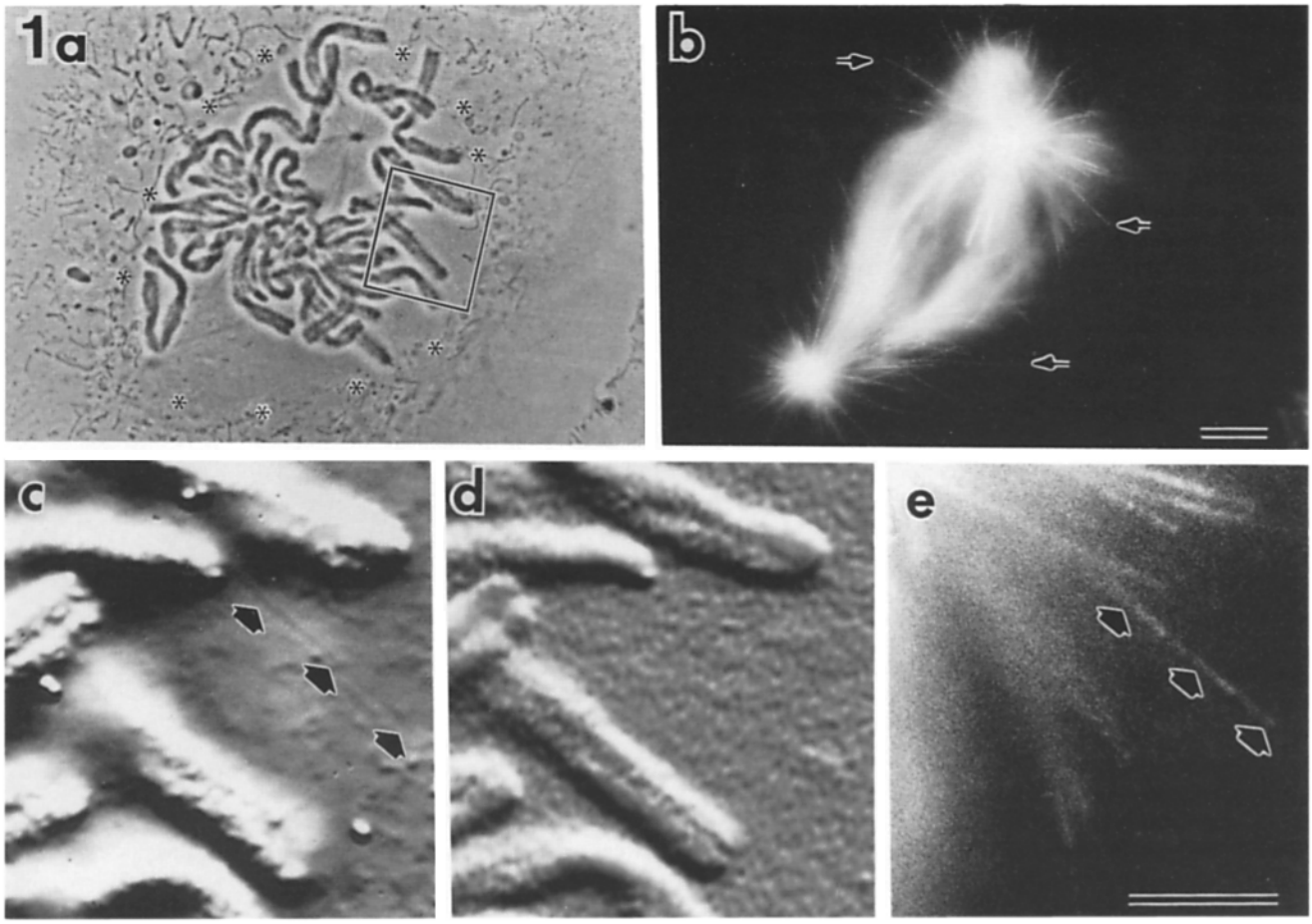



*Figure 1.* (*a–e*). Phase-contrast (*a*) and fluorescence (*b*) micrographs of an early prometaphase newt pneumocyte after antitubulin staining. This cell contains a number of monooriented chromosomes and the spindle is surrounded by a clear area of cytoplasm (*asterisks* in *a*). Note that some of the longer astral MTs (*arrowheads* in *b*) terminate between the distal ends of the monooriented chromosomes and the periphery of the clear area. High magnification video-enhanced DIC (*c*), conventional DIC (*d*), and immunofluorescence (*e*) micrographs, of the region outlined by the box in *a*, just before (*c*) and after (*d* and *e*) fixation and antitubulin staining. Note that the thin linear element seen in the living cell corresponds to a single fluorescent line, i.e., a MT (compare *c* and *d*, *arrows*). Bars: (*a* and *b*) 10 μm; (*c–e*) 5 μm.

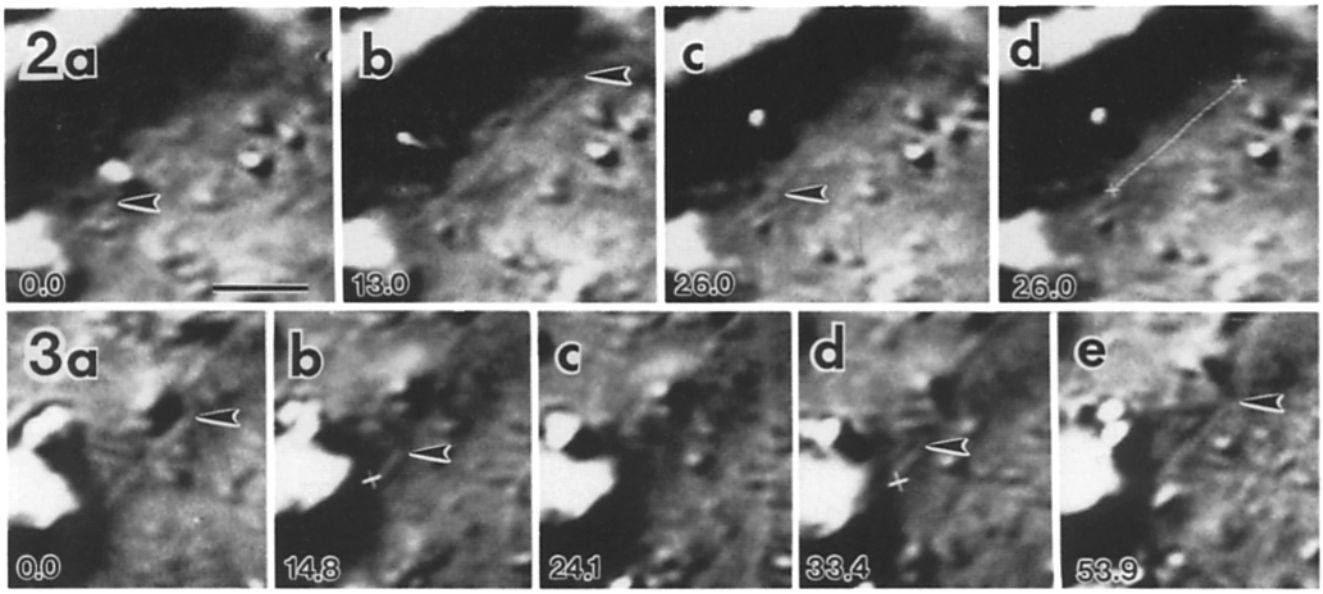
the distal tips of monooriented chromosome arms were subsequently seen to move up to 10 μm into the clear region (Fig. 2). At some point in time thereafter the MT end invariably stopped and began moving back towards the astral center (Figs. 2 and 3). Once the distal end of an MT moved back past a chromosome arm it was lost from view. However, occasionally an MT end reemerged seconds later, from essentially the same location, and reextended into the clear area (e.g., Fig. 3).

Detailed motion analyses were conducted, on time-lapse optical memory disk recordings reprocessed through the Hamamatsu digital processor, only on MT ends imaged without ambiguity. These analyses revealed that the ends of MTs moved away from the astral center at a mean rate of 14.3 μm/min (+/−5.1; $n = 19$). Similar analyses of lamellipodial MTs within interphase pneumocytes revealed a mean "elongation" rate of 7.9 μm/min (+/−2.2; $n = 13$). This rate is similar to the 7.2 μm/min previously reported by Cassimeris et al. (1988*b*) for MT elongation in interphase newt pneumocytes. An analysis of the movement of MT ends towards the astral center proved more difficult since, for unknown reasons, such motion was also usually accompanied by vertical movement of the MT. It was therefore difficult to follow the MT end with certainty and, as a result, we have yet to obtain a statistically significant sample size. However, the rate at which MT ends remaining in focus moved towards the astral center (e.g., Figs. 2 and 3) was ~16 μm/min, a preliminary figure that compares favorably to the 17.3 μm/min shortening rate determined by Cassimeris et al. (1988*b*) for interphase MTs in newt pneumocytes, and the 19.8 μm/min bulk shortening rate reported by Spurck et al. (1990) for newt pneumocyte astral MTs severed by UV irradiation.

### *Kinetochores Capture Microtubules Arising from the Polar Regions*

As previously noted (see Introduction), newt pneumocyte chromosomes spatially well separated from the spindle poles after NEB are delayed in their attachment. Since we could see individual MTs projecting from the polar regions in cells containing such "lost" chromosomes (see above), we reasoned that we might be able to directly observe the initial kinetochore-astral MT interaction that attaches the chromosome to the forming spindle.

It is necessary to briefly outline, at this point, some of the conditions required to optimize the chances of making such




*Figures 2 and 3.* (Fig. 2 *a–d*) Sequential video-enhanced DIC micrographs, taken from a time-lapse series, of an astral microtubule end (*arrows* in *a–c*). The end of this microtubule is distal to the chromosomes but within the clear area of the forming spindle. The microtubule end moved away from the astral center (*a* and *b*) at a rate of 10 μm/min and subsequently moved back (*b–d*) at 16.6 μm/min. The plot generated by the image processor, pictured in *d*, represents the distance by which the microtubule end moved between (*b–d*). Time, in seconds, is at the lower right corner of each micrograph. Bar: (*a*) 2 μm. (Fig. 3 *a–e*). Same optical conditions as in Fig. 2. For the most part, only the distal ends of astral microtubules are found within the clear area surrounding the forming spindle and once these ends move back past the chromosome arms they are no longer visible. In this example an astral microtubule end (*a*) moved back to the chromosome arm at a rate of 15.0 μm/min (*b* and *c*), and 23 s later reextended within a few pixels of its previous location at a rate of 10.6 μm/min (*d* and *e*). The time interval between movement towards and away from the astral center is too short to account for full disassembly of this microtubule and reassembly of a new one. Time, in seconds, is at the lower right corner of each micrograph. Magnification same as in Fig. 2.

observations. After NEB the chromosome must reside 35–50 μm from the closest centrosome to be significantly delayed in attachment. Moreover, it must also be positioned in a well-defined clear area within an extremely flat cell. Next, it must be successfully predicted which kinetochore on the chromosome will attach, and this kinetochore and the closest centrosome must be in approximately the same focal plane (so that MTs arising from the centrosome are oriented perpendicular to the optical axis). In this respect we focused primarily on those kinetochores located closest to the ventral cell surface since previous EM results (Rieder and Alexander, 1990) indicated that these were usually the first to attach. Attachment may be delayed for several hours, during which time focus must be constantly attended to. Finally, even though newt pneumocytes are among the flattest mitotic cells available for such an analysis, they are still several micrometers thick. Even by restricting the focal plane to just above the ventral cell surface, where astral MTs remain visible for extended periods, any movement along the MT (e.g., by cytoplasmic particles or chromosomes) invariably moved it out of focus. As one might expect, these strictures severely limited our number of successful observations.

For this part of the investigation we accumulated high-resolution video records on the attachment of 22 chromosomes. In no case were MTs seen to originate from the kinetochore region. In our best example to date, the arms of an unattached chromosome were probed, over a 90-min period, by several astral MTs (e.g., Fig. 4 *a*). The chromosome was not displaced to any noticeable extent as these MTs impinged on it. Ultimately, the end of one astral MT extended to contact the primary constriction of the unattached chromosome (Fig. 4, *b* and *c*). Upon contacting the primary constriction (Fig. 4 *c*) the chromosome initiated rapid poleward movement (Fig. 4, *d–f*), during which time the MT was no longer clearly visible (presumably because it was deflected from the plane of focus). However, as recently demonstrated by Rieder and Alexander (1990), this MT may have extended well past the moving kinetochore, since a particle initially positioned behind the primary constriction subsequently translocated poleward along the same trajectory taken by the chromosome (Fig. 4, *f–h*). With respect to this event we have clear documentation in three cells of a particle initiating a poleward saltation, along the same trajectory as an attaching chromosome, within seconds after the chromosome begins its poleward transit.

## Discussion

During prometaphase of astral mitosis each centrosome generates a radial array of MTs, i.e., an aster, in which MT density rapidly decreases as a function of distance from the astral center. We demonstrate here that thin linear elements are visible by video-enhanced DIC LM at the aster periphery in living prometaphase newt pneumocytes. We further demonstrate, by correlative LM methods, that each of these linear elements appears as a thin fluorescent line after IMF staining with a tubulin antibody. Since the weakly fluorescent lines observed at the peripheral edges of the aster (e.g., Nicklas et al., 1989) and centrosome (e.g., Osborn et al., 1978; Eckert and Snyder, 1978) after antitubulin IMF are

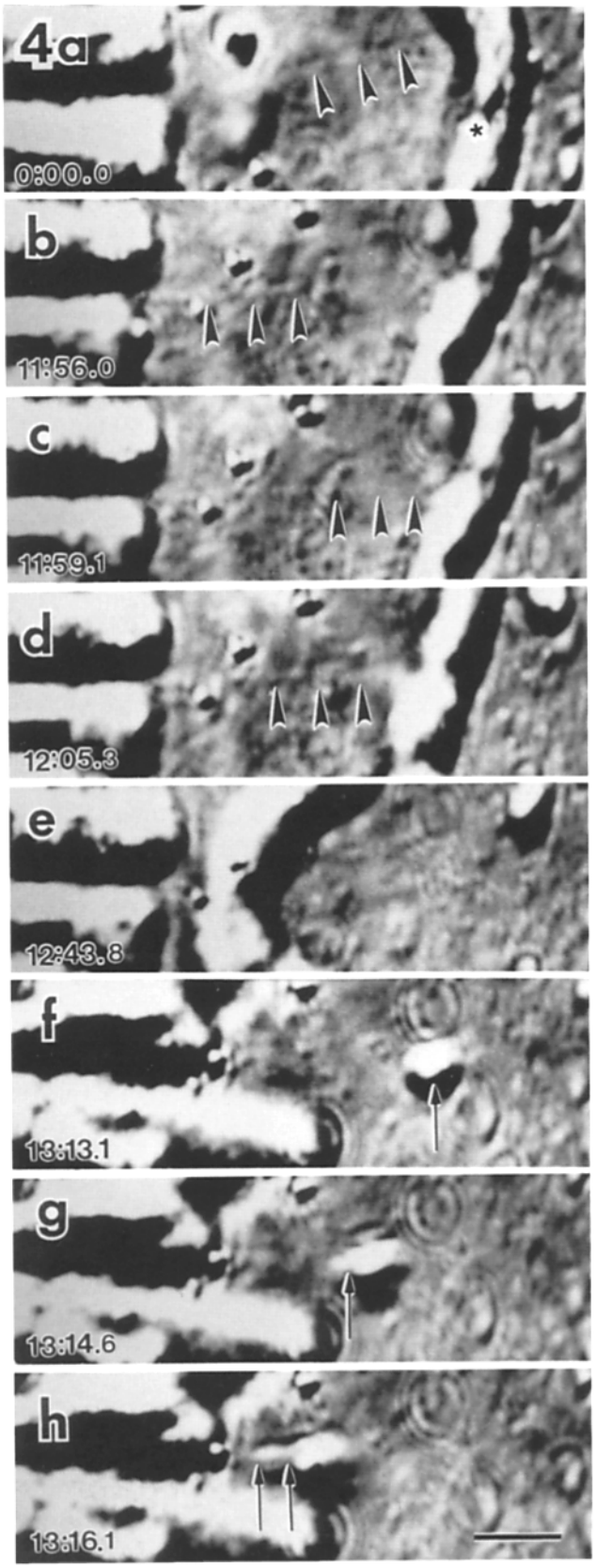



*Figure 4.* Selected video-enhanced DIC micrographs, from a time-lapse recording, of a chromosome attaching to the forming spindle. In this example the chromosome was randomly probed by several microtubules projecting from the aster (e.g., *arrowheads* in *a*). Finally, the end of one astral microtubule (*arrowheads* in *b* and *c*) extended to contact the kinetochore region of the chromosome (*asterisk* in *a*). The chromosome then moved poleward (*d–f*). As the chromosome moved poleward a large particle (*arrows* in *f–h*), initially positioned near the distal kinetochore, underwent rapid poleward transport along the same track followed by the kinetochore. Bar: (*h*) 2.5 μm.

commonly used as a standard for single MTs, and since we have previously shown at the EM level that such lines represent individual MTs in newt pneumocytes (Rieder and Alexander, 1990), we conclude that the thin linear elements we observed at the periphery of the asters by video-enhanced DIC LM are individual astral MTs.

Prometaphase newt lung cells have an appreciable thickness. It can therefore be argued that the moving MT ends we observed represent the movement of MTs in and out of the focal plane. In other words, as a long astral MT moves through the focal plane in a proximal to distal direction, relative to the astral center, it would appear to elongate as the length of the MT progressively comes into focus. However, if this were the case at least some MTs would be expected to move into focus in a distal to proximal direction, yielding images of MT "elongation" towards the aster. This was never seen. More importantly, the end of a linear element seen in live cells by DIC LM always corresponded to the end of a MT when such cells were subsequently stained for the IMF localization of tubulin (see Fig. 1). We are therefore confident that we examined the behavior of MT ends.

The movement of MT ends reported here might be explained by the bidirectional sliding of adjacent astral MTs. Although conceptually possible, such a mechanism requires complex assumptions since half-spindle MTs are of uniform polarity (Euteneuer and McIntosh, 1981). Furthermore, Sammak and Borisy (1988) have recently demonstrated that centrosomal MTs do not slide in interphase fibroblasts. Rather, our data is best explained by the hypothesis that astral MTs are dynamically unstable, i.e., that they coexist in growing and shrinking populations (Mitchison and Kirschner, 1984*a* and *b*). Given this interpretation, our results indicate that astral MTs in prometaphase cells elongate approximately twice as fast as MTs in interphase cells. Since the reported astral MT lifetime in metaphase newt pneumocytes averages 108 s (Wadsworth and Salmon, 1986), and since this represents time spent in both elongation and shortening phases, a growth rate of 14 μm/min yields an average MT length of 14 μm (as calculated according to Cassimeris et al., 1988*b*). It is noteworthy that this calculated average astral MT length is close to the distance that a monooriented chromosome is positioned from the pole in a monopolar spindle (Rieder et al., 1986; Salmon, 1989; Rieder, 1990), suggesting that this distance may be established by the average length of the astral MTs.

Our high-resolution video-enhanced DIC LM observations on living cells directly confirm the century-old hypothesis of van Beneden and Neyt that K-fibers "are simply those astral rays that come into connection with the chromosomes" (Wilson, 1911). This finding is consistent with Rieder and Alexander's (1990) recent conclusion, which was based on less direct IMF and EM evidence obtained from fixed cells followed in vivo, that the MTs comprising the nascent K-fibers

in newt pneumocytes are derived from the spindle poles and not the kinetochore. The formation of K-fibers from astral MTs implies that all of the MTs used to construct the vertebrate mitotic spindle are ultimately derived from the spindle poles (i.e., the asters or centrosomes). Indeed, since two adjacent asters in a mitotic cell do not form a spindle in the absence of chromosomes (e.g., Sluder et al., 1986; Rieder and Hard, 1990), the astral MTs must be organized by the chromosomes (i.e., kinetochores) to form the spindle. Since the proximal ends of these MTs are, for the most part, anchored within the polar MT-organizing center the attachment of a chromosome to the forming spindle and its poleward orientation occurs concomitantly with K-fiber formation.

Regardless of whether individual astral MTs grow and shorten in a dynamically unstable fashion or, less likely, that adjacent astral MTs undergo bidirectional sliding, the net result is that a large volume of cytoplasm surrounding each aster will be continuously probed by MTs (see also Mitchison and Kirschner, 1984*c*; Kirschner and Mitchison, 1986). Consequently, over time, all kinetochores within the "casting" range of the asters will become attached to MTs and transported into the forming spindle. The asters in prometaphase cells therefore function initially to collect chromosomes dispersed during NEB throughout the area previously occupied by the nucleus. In this respect it is significant that the asters in newt pneumocytes (and other vertebrates) are maximally developed at the time of NEB and become progressively diminished as the chromosomes acquire a bipolar orientation and congress to the metaphase plate (e.g., see Fig. 3 in Aubin et al., 1980; Fig. 16 in Rieder and Hard, 1990; McIntosh, 1989). Prometaphase aster size also appears to be correlated with cell size, since the most robust asters are found in oocytes and other large cells (like newt pneumocytes) where chromosomes can become widely dispersed during NEB.

Our direct demonstration that K-MTs are derived from astral MTs explains a number of observations concerning the behavior and structure of attaching chromosomes in vertebrate cells (reviewed in Rieder, 1982, 1990). It also provides a mechanism for how two monopolar spindles can assemble in a common cytoplasm. As previously noted by Rieder and Alexander (1990), astral MTs in prometaphase newt pneumocytes seldom grow longer than 50 $\mu$m. Consequently, if the distance between spindle poles at NEB is >100 $\mu$m no one pair of sister kinetochores can be expected to be probed by MTs growing from both poles, and no chromosome will establish a bipolar attachment. As a result the two asters and their attached monooriented chromosomes migrate within the cell independently of one another, i.e., an anaphase-like prometaphase spindle is formed (e.g., Bajer, 1982). In the vast majority of cells, however, the asters are closer together at NEB, permitting one or more pairs of sister kinetochores to quickly achieve a bipolar attachment. The bipolar attachment of a single chromosome provides an indirect structural connection between the asters, prohibits an anaphase-like prometaphase, and initiates the construction of a bipolar spindle. During this time, monooriented chromosomes undergo congression movements toward the forming metaphase plate, while the interpolar distance progressively decreases from 70 $\mu$m or more to ~45 $\mu$m just before anaphase (Rieder, 1977). An important question is whether these congression movements depend on the acquisition of a MT, extending from the distal pole, by the unattached (i.e., distal) kinetochore of the monooriented chromosome. As noted above, a monooriented chromosome should not be able to acquire an attachment to the distal pole if it is more than 50 $\mu$m from that pole. However, the closer a monooriented chromosome is positioned relative to the distal pole, the more likely it is to encounter MTs arising from that pole. In this context the ejection properties of the aster, which appear to "push" chromosomes away from the pole (Carlson, 1938; Rieder et al., 1986; Salmon, 1989), may significantly facilitate the bipolar attachment of monooriented chromosomes by moving them closer to the distal pole.

Can our conclusion concerning the origin of K-fiber MTs in vertebrate cells be extrapolated to plant cells, or to those spermatocytes (e.g., *Pales*; Dietz, 1964; Steffen et al., 1986) and oocytes (e.g., mouse; Szollosi et al., 1972) that form a bipolar spindle in the absence of clearly-defined spindle poles? We believe so. In plants (reviewed in Bajer and Mole-Bajer, 1972) and oocytes (e.g., Schatten et al., 1985) the prometaphase chromosomes are exposed, upon NEB, to a high density of invading MTs originally associated with the nuclear envelope. In spermatocytes the cytoplasm within the vicinity of the bivalents contains an ample supply of potential K-fiber MTs originally derived from the centrosome (e.g., Janicke and LaFountain, 1984; Church et al., 1986). In each of these cases the polar regions of the fully formed metaphase spindle lack discrete polar organelles and, as a result, appear broad and diffuse. Under these circumstances spindle bipolarity need not necessarily be ascribed to the presence, during prometaphase, of two spindle poles (from which the K-MTs are derived during astral mitosis), but it may partly be the consequence of a parallel aggregation of adjacent K-fibers, constructed from MTs appearing within the vicinity of the chromosomes after NEB. Such an aggregation may be mediated by the lateral interaction of adjacent K-fibers (e.g., see Mole-Bajer, 1969; Cassimeris et al., 1988*a*; Inoué, 1988) and/or by the lateral association of K-fiber polar anchor-points or termini (e.g., Ring et al., 1982). This scheme for bipolar spindle formation in the absence of clearly defined spindle poles may explain why monopolar spindles are never observed in untreated or experimentally manipulated plant cells, whereas multipolar spindles are (e.g., see references in Mole-Bajer, 1969; Schrader, 1953).

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