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REVIEW ARTICLE

Movement of chromosomes with severed kinetochore microtubules

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Abstract Experiments dating from 1966 and thereafter showed that anaphase chromosomes continued to move poleward after their kinetochore microtubules were severed by ultraviolet microbeam irradiation. These observations were initially met with scepticism as they contradicted the prevailing view that kinetochore fibre microtubules pulled chromosomes to the pole. However, recent experiments using visible light laser microbeam irradiations have corroborated these earlier experiments as anaphase chromosomes again were shown to move poleward after their kinetochore microtubules were severed. Thus, multiple independent studies using different techniques have shown that chromosomes can indeed move poleward without direct microtubule connections to the pole, with only a kinetochore 'stub' of microtubules. An issue not yet settled is: what propels the disconnected chromosome? There are two not necessarily mutually exclusive proposals in the literature: (1) chromosome movement is propelled by the kinetochore stub interacting with non-kinetochore microtubules and (2) chromosome movement is propelled by a spindle matrix acting on the stub. In this review, we summarise the data indicating that chromosomes can move with severed kinetochore microtubules and we discuss proposed mechanisms for chromosome movement with severed kinetochore microtubules.

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Chromosomes move poleward after their microtubule connections to the pole are severed by UV microbeam irradiation

Anaphase chromosomes still move poleward after their kinetochore spindle fibres have been severed by ultraviolet light (UV) microbeam irradiation (Forer 1966; Gordon and Inoué 1979; Gordon 1980; Sillers and Forer 1983; Forer and Wilson 1994; Spurck et al. 1997; Forer et al. 2003). Each chromosome moved together with its associated kinetochore 'stub' (the microtubules that remained attached at the kinetochores). In crane-fly spermatocytes, the chromosomes moved with the same speed as before irradiation (Forer 1966; Sillers and Forer 1983; Forer and Wilson 1994; Spurck et al. 1997; Forer et al. 2003), whereas in grasshopper spermatocytes (Gordon and Inoué 1979; Gordon 1980) and newt fibroblasts (Spurck et al. 1997; Pickett-Heaps et al. 1996), the chromosomes moved poleward initially more rapidly than before irradiation and then returned to normal speed. In other experiments, UV microbeam irradiations of kinetochore fibres during anaphase caused chromosomes to stop moving (Bajer and Molè-Bajer 1970; Bajer 1972; Sillers and Forer 1981a, b; Spurck et al. 1990). Whether movements stop or microtubules are severed or both depends on the wavelength of the UV and the total energy delivered to the spindle fibre (Sillers and Forer 1981b, 1983; Hughes et al. 1988).

Results obtained on metaphase spindles were different depending on the cell type. Metaphase chromosomes in crane-fly spermatocytes stayed at the equator after their kinetochore microtubules were severed, while the tips of the kinetochore stubs moved poleward at about the speed of anaphase chromosomes (Forer 1965; Wilson and Forer 1988; Spurck et al.

1997). Metaphase chromosomes in newt fibroblasts (Spurck et al. 1997) and grasshopper spermatocytes (Gordon 1980) on the other hand moved poleward together with their kinetochore stubs after brief movement away from the pole. We still do not know why there is a difference between cell types.

The conclusion that chromosomes move poleward with severed kinetochore microtubules was generally not accepted until just recently. One reason for this scepticism was a concern that the polarised light microscopy used to monitor the effects on kinetochore fibres might not have detected small numbers of remnant microtubules. Thus, when anaphase movements continued after the irradiations, it was suggested that a few microtubules in the irradiated region remained connected with the pole and chromosome movements were due to these few microtubules. Several observations in living cells however indicated that indeed all microtubules were severed by the irradiations. Anaphase and metaphase chromosomes associated with the irradiated stubs in newt fibroblasts (Pickett-Heaps et al. 1996; Spurck et al. 1997) and grasshopper spermatocytes (Gordon and Inoué 1979; Gordon 1980) moved abruptly away from the pole immediately after the irradiation, before moving poleward very shortly thereafter. This indicates that the microtubules were completely severed from the pole. Kinetochore stubs changed angle irregularly (e.g. Spurck et al. 1997), and poleward chromosome movement often occurred when the stub was directed away from the pole (e.g. Figure 11 of Spurck et al. 1997; Figures 2, 4 and 5 of Forer et al. 2003). Both phenomena indicate that the microtubules were completely severed from the pole. Kinetochore stub microtubules splayed out as the stub moved poleward (e.g. Fig. 1; also Spurck et al. 1997), indicating that they were not connected to the pole. Finally, chromosomes would not increase their speed to the pole if there were microtubules remaining in the irradiated area since microtubule depolymerisation—the rate-limiting step for movement (e.g. Nicklas 1975, 1983; Pickett-Heaps et al. 1982)—would govern the speed. Direct observations using electron microscopy and/or immunofluorescence staining for tubulin have confirmed that all microtubules were indeed severed by the irradiation: spindle fibre microtubules did not extend across the irradiated region in cells in which chromosome movement was not monitored (Bajer and Molè-Bajer 1970; Bajer 1972; Wilson and Forer 1988; Snyder et al. 1991) and did not extend across the irradiated region in cells in which anaphase movements continued until fixation several minutes after the irradiations (Forer et al. 2003). Thus, chromosomes can still move poleward after microtubule connections between the kinetochore and pole are completely severed by UV microbeam irradiation.

Chromosomes move poleward after their microtubule connections to the pole are severed by visible light laser microbeam irradiation

Visible light laser microbeam irradiation can sever microtubules in living cells both when the microtubules are fluorescently labelled and when they are not (e.g. Kurachi et al. 1999;

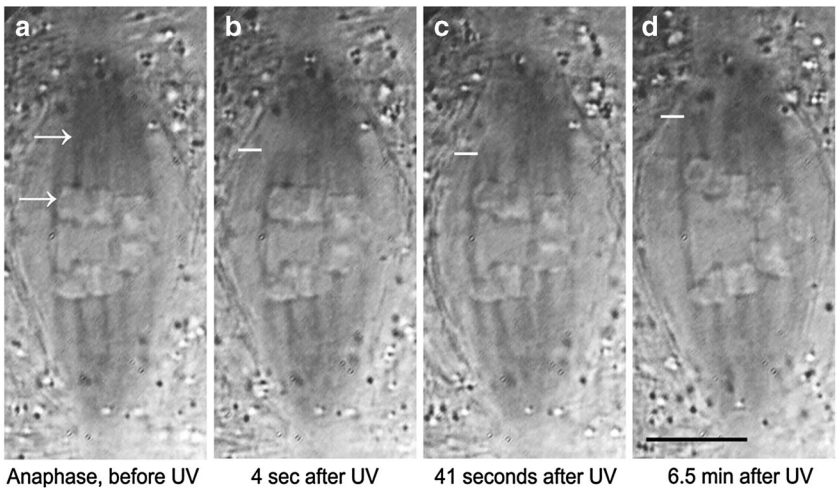

Fig. 1 A series of photographs of a living crane fly taken using a polarising microscope (described in Spurck et al. 1997). It illustrates that kinetochore stubs produced by ultraviolet microbeam irradiation splay out after they are formed and do not point to the pole when the associated chromosome moves poleward. **a** The *top arrow* points to the autosomal spindle fibre that will be irradiated: the fibre looks more or less cylindrical. The *bottom arrow* points to the associated chromosome. **b** Shortly after the ultraviolet microbeam was turned off, the stubs of the autosomal spindle fibre and of the sex chromosome spindle fibre to its left that also was severed were splayed out and were at a changed angle. The *line* indicates the tips of the two kinetochore stubs. **c**, **d** As anaphase proceeded, the kinetochore stub remained splayed out and was not directed to the pole that the associated chromosome moved toward. Granules invaded the spindle in the region in front of the kinetochore stub as the stub moved poleward, as seen in **d** and as clearly seen in the Supplemental video. The *lines* in **c** and **d** point to the tip of the autosomal kinetochore stub. The *bar* in **d** represents 10 μm. This is from the same time-lapse sequence illustrated in Figure 4 of Spurck et al. (1997)

Grill et al. 2001; Botvinick et al. 2004; Chen and Zhang 2004; Khodjakov et al. 2004; Labbé et al. 2004; Heisterkamp et al. 2005; Dick and Gerlich 2013). For fluorescently labelled spindle microtubules, severing was visualised using fluorescence microscopy (Elting et al. 2014) and sometimes confirmed using electron microscopy (e.g. Botvinick et al. 2004; Sikirzhytski et al. 2014). For unlabelled microtubules, severing was sometimes monitored using polarisation microscopy (Chen and Zhang 2004) or otherwise confirmed using immunofluorescence microscopy (Chen and Zhang 2004; Sikirzhytski et al. 2014; Elting et al. 2014; Forer et al. 2013) or inferred from the behaviour of chromosomes after the irradiation (Sikirzhytski et al. 2014; Dick and Gerlich 2013). Several recent articles have studied chromosome movements after their kinetochore microtubules were severed in anaphase and metaphase.

In both anaphase grasshopper spermatocytes and anaphase PtK cells, after laser microbeam irradiation severed kinetochore fibre microtubules, the associated chromosomes abruptly moved backward and shortly thereafter moved poleward faster than prior to the irradiation. They then slowed down to the original velocity (Chen and Zhang 2004; Elting et al. 2014) just as had been observed in UV microbeam experiments. In metaphase PtK cells after laser microbeam irradiation severed kinetochore fibre microtubules, the associated chromosomes abruptly moved a short distance toward the pole associated with the not-irradiated kinetochore fibre, but then quickly reversed direction and moved with the kinetochore stub toward the irradiated pole as the stub changed angle and kinetochore microtubules splayed out (Elting et al. 2014). The same had been observed in UV microbeam experiments on newt fibroblasts. Thus, using visible light laser irradiation instead of UV and studying different cell types, Chen and Zhang (2004) and Elting et al. (2014) confirmed the UV microbeam result that chromosomes move poleward after the microtubules connecting the anaphase chromosomes to the poles are severed.

In other laser microbeam experiments on the same PtK cell line used by Elting et al. (2014), the results obtained were somewhat different in that when the kinetochore fibre microtubules were severed, metaphase chromosomes remained at the equator and anaphase chromosomes 'stopped abruptly' (i.e. without backward movement) (Sikirzhytski et al. 2014)[1]. Sikirzhytski et al. (2014) reported that after stopping for a short time, the anaphase chromosomes moved poleward, first moving at a faster speed than previously and then moving slower, confirming that anaphase chromosomes in PtK cells move poleward after their kinetochore fibre microtubules are severed.

[1] However, in Videos S1 and S4 of Sikirzhytski et al. (2014), illustrating severing of kinetochore microtubules in metaphase and anaphase, the associated chromosomes moved as described by Elting et al. (2014).

Altogether, the visible light laser microbeam experiments agree with the conclusions from the earlier UV microbeam experiments in that anaphase chromosomes can move poleward (at normal speeds or faster) after their kinetochore fibre microtubules are severed. However, there is no agreement on the mechanism by which the chromosomes move poleward with severed kinetochore fibre microtubules.

What propels chromosomes poleward when their connection to the pole is severed?

There are two proposals in the literature to explain how anaphase chromosomes move poleward with only a kinetochore stub: (1) the kinetochore stub is propelled by interacting with non-severed microtubules that extend to the pole (Chen and Zhang 2004; Elting et al. 2014; Sikirzhytski et al. 2014) or (2) the kinetochore stub (and perhaps its chromosome as well) is propelled poleward by spindle matrix component(s) (reviewed in Johansen and Johansen 2007; Johansen et al. 2011).

Kinetochore stubs move because of interactions with non-severed microtubules Fluorescence microscopy observations suggested that there were interactions between kinetochore stubs and other microtubules. Chen and Zhang (2004) stated that the anaphase chromosomes in grasshopper spermatocyte spindles moved poleward only after the stub 'recaptures microtubules from the pole'. Others stated that 'in some cases the initiation of movement coincided with the k-fiber stub end visibly contacting a neighboring microtubule or k-fiber' (Elting et al. 2014) or that movement was 'initiated when the stub appeared to contact neighboring MTs' (Sikirzhytski et al. 2014). In addition to visual observations, both Elting et al. (2014) and Sikirzhytski et al. (2014) presented evidence that kinetochore stubs are propelled poleward by dynein that accumulates at the tips of kinetochore stubs. Dynactin components (p150 glued and Arp1A) accumulated at kinetochore stub tips within seconds after the microtubules were severed (Elting et al. 2014) as did NuMA (Elting et al. 2014, Sikirzhytski et al. 2014), and these proteins moved to the pole with the kinetochore stub. Laser microbeam irradiation of the tip of a moving kinetochore stub caused the new tip to temporarily stop moving, showing that the tip components (that include dynein and NuMA) are required for movement (Elting et al. 2014). After inhibition of dynein activity by overexpression of a dominant-negative p150 fragment, the stub tips did not move poleward although kinetochore stubs behaved as usual in that they changed orientation and the stub microtubules splayed out (Elting et al. 2014). After cells were treated with the dynein inhibitor ciliobrevin at a concentration that did not disrupt spindle bipolarity and was assumed to only partially inhibit dynein, the kinetochore stubs moved poleward

with velocities not significantly different from those in untreated cells. However, the time from creation of the kinetochore stub until it started poleward movement increased from 5 to 19 s (Sikirzhytski et al. 2014). Thus, Elting et al. (2014) and Sikirzhytski et al. (2014) have proposed that chromosomes connected to kinetochore stubs move poleward because the stub tips interact via dynein with other spindle microtubules that presumably connect to the pole and that this interaction propels them to the pole.

Kinetochore stubs move because of interactions with components of a spindle matrix Johansen and Johansen (2007) and Johansen et al. (2011) have summarised the arguments for the concept of a spindle matrix as an elastic structure that embeds the spindle microtubules and may also provide force to move chromosomes. The spindle presumably forms by the interactions of matrix proteins with microtubules and other spindle components including cell cycle regulators (Johansen et al. 2011; Yao et al. 2012). The initial arguments for the existence of a spindle matrix were physiological, based on how chromosomes behaved under various experimental conditions. Later experiments identified specific proteins (e.g. Skeletor, Chromator, EAST and Megator) that embed and are stretched pole to pole by the microtubule spindle (Johansen et al. 2011). Blocking Skeletor via injection of antibodies alters spindle shape and function (Johansen et al. 1996; Walker et al. 2000), while depolymerisation of microtubules by colchicine causes the spindle matrix to contract and coalesce around the chromosomes, suggesting that the microtubules act as struts to stretch out the spindle matrix (Johansen et al. 2011; Yao et al. 2012). Chromator has been directly implicated in spindle function: spindle shape alterations and faulty chromosome movement occur after RNAi depletion of Chromator and after mutation of *Chromator* (Rath et al. 2004; Ding et al. 2009). Since Chromator binds to microtubules, it may serve as the physical bridge between microtubules and the spindle matrix (Yao et al. 2014).

How does the concept of a spindle matrix explain how chromosomes move with only a kinetochore stub? Poleward forces from the spindle matrix propel chromosomes and kinetochore microtubules poleward with the microtubules acting as 'governors': microtubules resist the movement, and the speed of chromosome movement is therefore limited by the rate of depolymerisation of kinetochore microtubules [e.g. Johansen and Johansen (2007); Pickett-Heaps and Forer (2009)]. Chromosomes therefore should increase their speed when the microtubules are severed because the resistance from microtubules is removed[2]. The chromosomes would slow down when the kinetochore stub microtubules encounter other resistance (in the form of other microtubules or the pole). These experimental observations are thus predicted from the spindle matrix model. This interpretation helps to explain other phenomena as well. For example, kinetochore stubs often change orientation after they are formed; chromosomes nonetheless move poleward when their kinetochore stub is not oriented to the pole because the matrix forces continue to act in the poleward direction.

In addition to the family of matrix proteins that includes Skeletor and Chromator, other non-microtubule components in the spindle produce force, including actin, myosin and titin (e.g. as discussed in Johansen et al. 2011; Sheykhani et al. 2013a, Sheykhani et al. 2013b). These could act either as part of the spindle matrix or in conjunction with it (Johansen et al. 2011). Evidence that this is so includes:: actin and myosin inhibitors block tubulin flux in kinetochore microtubules in crane fly spermatocytes (Silverman-Gavrila and Forer 2000) and block normal chromosome movements in crane fly spermatocytes (discussed in Johansen et al. 2011; Sheykhani et al. 2013b). Using a variety of methods, both actin and myosin have also been implicated in spindle function in other cells (e.g. Sandquist et al. 2011; Sheykhani et al. 2013a, Sheykhani et al. 2013b). Perhaps more relevant to the present discussion are the observations that actin and myosin inhibitors block poleward movement of the kinetochore stub in crane fly spermatocytes (Forer et al. 2007) and also block movement of severed microtubules in PtK cells (Sheykhani et al. 2013b). The actin/myosin components of the spindle matrix thus could cause chromosomes to move poleward when kinetochore microtubules are severed.

Can the two models be reconciled?

How are different aspects of the data explained by the two proposals, that the stubs move because they interact (via dynein) with other microtubules or that they are propelled by the spindle matrix? One aspect is the movement of chromosomes with severed kinetochore microtubules. In the spindle matrix hypothesis, the kinetochore microtubules are not motors but governors of movement so poleward movement with severed microtubules is 'built in' to the model. The dynein/microtubule explanation is that the usual PacMan mechanism of anaphase is halted when microtubules are severed (because the kinetochore stub does not shorten during poleward motion), that NuMA and dynein move to the minus ends of the severed microtubules, and that the motor proteins at the microtubule tips slide along intact microtubules that extend to the pole (on the side of the irradiation).

The conclusion that the stub tips contact intact microtubules is not solid. For one, there are so many non-

[2] Crane-fly spermatocyte chromosomes do not change speed when kinetochore microtubules are severed; possible reasons [discussed in Forer et al. (2003)] may relate to the physical connections ('tethers') between separating half-bivalents and to signalling between half-bivalents.

kinetochore microtubules in PtK cells and there is so much lateral and vertical movement of chromosomes and spindle fibres that it is difficult from fluorescence microscopy to determine whether there is direct contact between individual microtubules or bundles. Using quantitative fluorescence measurements, Sikirzhytski et al. (2014) suggest that only a few of the kinetochore stub tip microtubules could contact other microtubules. They expected an increase in fluorescence intensity when there is contact (or close proximity) between two 'point' sources of fluorescence (below the limit of resolution), but because there was no such increase, they suggest that only a few of the stub tip microtubules contact unsevered microtubules. Serial section reconstruction of electron microscope images of severed fibres indicated that the majority of severed ends were free and 'only some resided in the immediate proximity of other spindle MTs' (Sikirzhytski et al. 2014). Those illustrated were usually at an angle to the kinetochore stubs, so it is difficult to see how microtubules at severe angles to each other could slide against each other. There is counter evidence from crane-fly spermatocytes irradiated using an ultraviolet microbeam: there is no indication from fluorescence microscopy or serial section electron microscopy that kinetochore stub tip microtubules interact with any other microtubules that extend to the pole (Forer et al. 2007). Thus, it is not at all clear that the stub tips directly interact with other microtubules.

The dynein model does not explain another aspect of movement with severed microtubules: as the stub moves poleward, intervening microtubules do not remain straight, as they would do if they were producing pulling force. Rather, they become bent as the stub and chromosome move poleward (Pickett-Heaps et al. 1996). As discussed previously (e.g. Pickett-Heaps et al. 1996; Sheykhani et al. 2013b), such bending of microtubules would be expected to occur only if forces propelling kinetochore stubs were external to the microtubules, e.g. arising from a spindle matrix. Similar arguments apply to bending of microtubules as the poles move toward the equator after all intervening microtubules have been severed (Sheykhani et al. 2013b), or after drug treatment (Mitchison et al. 2005), or after release from pressure-induced spindle elongation (Dumont and Mitchison 2009). Bending of spindle microtubules as poles move inward most likely arises from 'some mechanical element inside or outside the spindle' (Dumont and Mitchison 2009) such as a spindle matrix (Johansen et al. 2011).

Nor does the model explain yet another aspect of kinetochore stub movement to the pole, namely that poleward movement of the kinetochore stub is inhibited by inhibitors of actin and myosin (Forer et al. 2007). This would not occur if the poleward movement was due solely to dynein interacting with other microtubules. Another aspect of the data is that poleward movement speeds up when kinetochore microtubules are severed and slows down some time thereafter before the chromosome reaches the pole (e.g. Spurck et al. 1997; Chen and Zhang 2004; Elting et al. 2014). The matrix model predicts both increased speed of the kinetochore stub and its slowing down: movement speeds up when the governor is lost and movement returns to normal speeds when the governors return, for example, when the stub encounters the pole (or other resistance). In the dynein stub tip model, the increased speed could arise by the stubs sliding along other microtubules faster than PacMan, but it is not apparent why chromosome movement speeds would return to normal and there was no discussion of this point at all in Elting et al. (2014) or Sikirzhytski et al. (2014).

The two different conclusions, that the chromosome and kinetochore stub movements are due to dynein interactions with unsevered microtubules or that the movements are due to spindle matrix/actin/myosin interactions, are not mutually exclusive. The matrix explanation fits the data on movement, including possible roles for actin and myosin, but it is silent about dynein involvement or dynein/NuMa accumulating at the tips of the kinetochore stubs. The data showing that these proteins accumulate at stub tips are compelling, although the interpretation that the stub tips slide along other microtubules is not, as discussed above. However, NuMA and dynein might play other roles. If we consider that NuMA (which rapidly accumulates at the tips of kinetochore stubs) is part of the spindle matrix (discussed in Dionne et al. 1999; Johansen et al, 2011) and that the spindle matrix interacts with spindle components to generate elastic force, then perhaps NuMA and dynein act in concert with the spindle matrix to contribute to spindle forces. Or they might provide 'feedback' to spindle matrix/microtubule interactions. The current challenge is to unravel the complex, multi-component contributions to force production in the spindle.

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