Chromosome movement: **Dynein-out at the kinetochore**

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Cell biologists have long speculated that a minus end-directed motor localized at kinetochores contributes to the poleward movement of chromosomes during mitosis. Two recent studies provide direct evidence that cytoplasmic dynein can perform this function.

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During mitosis and meiosis, the movement of chromosomes is linked to the structural and dynamic polarity of spindle microtubules. Paired sister chromatids attach via their kinetochores to the plus ends of microtubules emanating from opposite spindle poles, where the minus ends are focused. Three distinct mechanisms are thought to contribute to kinetochore movements during chromosome alignment in metaphase and segregation to opposite poles in anaphase. First, microtubule plus ends terminating at the kinetochore could shrink and grow without losing their attachment, thereby generating chromosome movement towards and away from the pole, respectively [1]. Second, the disassembly of microtubule minus ends at the spindle poles, called poleward flux, could reel in attached kinetochores [2]. And third, microtubule-based motor proteins localized to the kinetochore could move the chromosome along the surface of spindle microtubules, toward their plus or minus ends. While the functional contribution of a plus end-directed kinetochore motor to chromosome movement has previously been established [3,4], two recent studies [5,6] provide the first direct evidence that the minus end-directed motor cytoplasmic dynein plays a role in driving poleward movement of chromosomes.

Cytoplasmic dynein is no stranger to those familiar with the spindle field. A single isoform of dynein is localized to the cell cortex, at spindle poles and kinetochores, where it is thought to function exclusively in conjunction with dynactin, a multisubunit activating complex [7]. Dynein appears to play key roles at almost every step in the assembly and function of the mitotic spindle. Dynein at the cell cortex is thought to associate with astral microtubules that extend from spindle poles. From this location, dynein could provide an outward force to separate spindle poles during spindle assembly [8] and anaphase B [9]. Dynein can also act to move the spindle within the cell, playing an important role in maneuvering the spindle through the bud neck in the yeast *Saccharomyces cerevisiae* [10], as well as rotating the spindle during oogenesis in the fruitfly *Drosophila* [11] and the early embryonic divisions of the nematode *Caenorhabditis elegans* [12]. In *Xenopus* egg extracts and mammalian tissue culture cells, dynein localizes to the spindle poles, where it focuses the poles of the spindle by cross-linking and sliding microtubule minus ends together [13,14].

In addition to these structural roles, dynein has long been speculated to have a function in chromosome attachment and movement within the spindle. Dynein is the obvious candidate to drive kinetochore-to-pole movement in both prometaphase and anaphase, as the other motors known to localize to the kinetochore, the kinesins MCAK/XKCM1 and CENP-E, do not have minus end-directed activity [4,15,16]. The maximum rate of poleward movement of a mono-oriented chromosome immediately after microtubule attachment is similar to the *in vitro* rate of dynein movement [17]. Additionally, disruption of dynein has been shown to cause defects in anaphase chromosome segregation in *Tetrahymena* [18], and misalignment of chromosomes at the metaphase plate in mammalian tissue culture cells [19].

Direct evidence that dynein is responsible for kinetochore movement *in vivo* has been lacking, however, as dynein disruption affects upstream processes required for spindle assembly, making kinetochore-specific roles difficult to distinguish. Furthermore, research on the kinesin MCAK/XKCM1 in the last several years has led to an alternative model for kinetochore-mediated poleward movement of segregating chromosomes, which does not require a minus end-directed motor [20]. Unlike other motors, MCAK/XKCM1 does not move along microtubules, but instead couples ATP hydrolysis with the depolymerization of microtubule ends [15,16]. CENP-E, on the other hand, has been shown to be important for attachment of kinetochores to the plus ends of shrinking microtubules [21,22]. Thus, through the coordinated activities of these motors, depolymerization of the plus ends of microtubules could propel chromosomes poleward.

Two recent studies [5,6] have demonstrated directly, for the first time, that cytoplasmic dynein at the kinetochore does in fact contribute to chromosome movements in prometaphase and anaphase. The key to the success of both sets of experiments was the ability to examine disruption of dynein at the kinetochore independently of its other functions. Savoian *et al.* [5] specifically interfered with kinetochore-associated dynein during meiosis in *Drosophila* spermatocytes by preventing expression of ZW10 or ROD, proteins responsible for localizing dynein

to kinetochores but not to the cortex or spindle poles [23,24]. They found that the rate of poleward chromosome movement during prometaphase was greatly reduced in these cells, as compared to controls. In addition, the cells exhibited both asynchronous and delayed anaphase initiation, and some chromosome pairs never separated. Savoian *et al.* [5] measured a constant velocity, 2 μm per minute, for chromosome movement throughout anaphase, which was attenuated to 0.5 μm per minute in the dynein-disrupted cells.

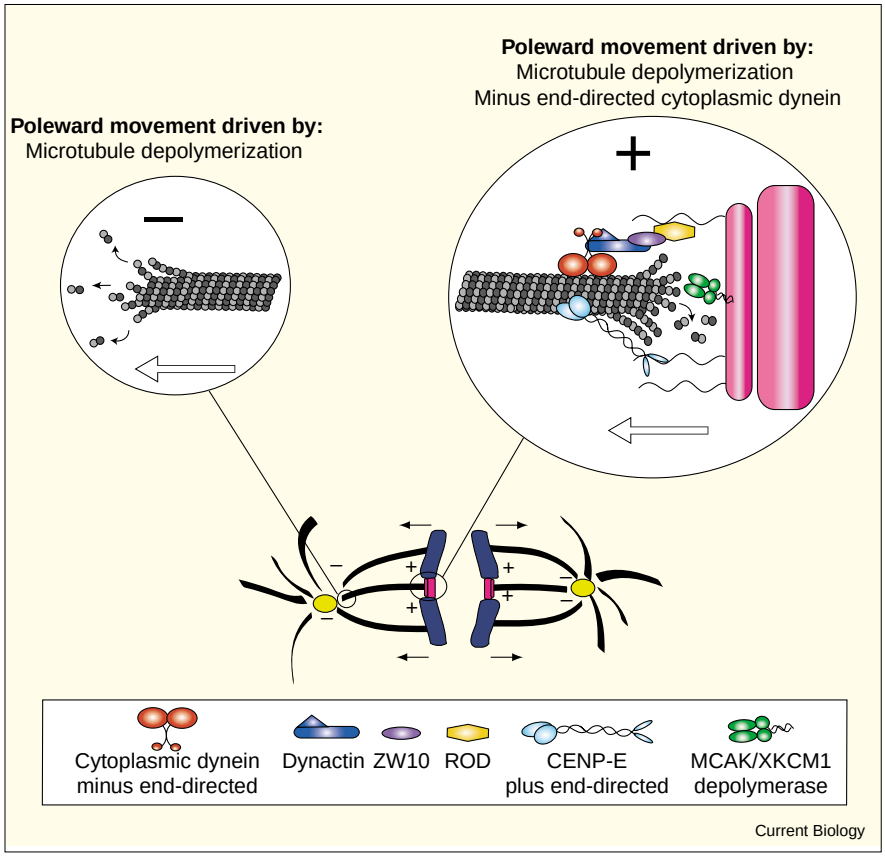
Although these observations provide compelling evidence that dynein has a direct role in poleward chromosome movement, distinct from any structural role, one other explanation has to be considered. If dynein disruption inactivated the mitotic checkpoint, then cells would enter anaphase prematurely, causing both the inactivation of cyclin B/Cdk1 kinase activity and the dramatic changes in cytoskeletal organization that accompany the transition to interphase, potentially affecting chromosome-to-pole motility. Indeed, a checkpoint role for dynein is indicated by precocious sister separation in *ZW10* mutants [23,25]. Furthermore, the amount of dynein at kinetochores decreases at metaphase, similar to the localization pattern of known checkpoint components [17,26,27] and correlating with bipolar attachments that would induce tension [23]. However, the mechanism that regulates dynein association with kinetochores is unclear. A detailed study of grasshopper spermatocytes by King *et al.* [28] demonstrated that, in this system, the loss of dynein at the kinetochore is regulated by microtubule attachment and not tension.

Regardless, to rule out the possibility that dynein inhibition caused premature exit from meiosis, Savoian *et al.* [5] used laser microsurgery to separate the two kinetochore regions on bivalents positioned near the spindle equator during prometaphase, when cyclin B/Cdk1 levels are guaranteed to be high [5]. Chromosomes in control cells moved poleward at near anaphase rates, while separated kinetochores in *ZW10* mutant cells failed to move, or moved at greatly reduced rates. The attenuated rate of poleward chromosome movement caused by dynein disruption thus does not appear to result from a mitotic checkpoint defect.

In a study of *Drosophila* syncytial blastoderm embryos, Sharp *et al.* [6] also measured a reduced rate of poleward movement when they disrupted dynein function by microinjecting either an antibody against dynein heavy chain or the p50 subunit of dynactin, dynamitin. Interestingly, the authors

Figure 1

A model showing poleward movement of a kinetochore in anaphase driven by depolymerization at the plus and minus ends of microtubules, and dynein-dependent minus end-directed kinetochore motility. At the pole, depolymerization of microtubule minus ends (poleward flux) reels in attached chromosomes. At the inner region of the kinetochore [15], MCAK/XKCM1 induces microtubule depolymerization, while CENP-E localized to the outermost region of the kinetochore, the fibrous corona [17], maintains attachment to the plus ends of shrinking microtubules. Cytoplasmic dynein, in association with dynactin, ZW10 and ROD, also localizes to the fibrous corona [17] where its minus end-directed motility drives chromosomes poleward.



saw a gradient of effects that reflected the entire spectrum of dynein function in mitosis. Spindles closest to the point of injection had severe defects including incomplete centrosome separation and prophase arrest, likely due to disruption of dynein function at the cortex [9]. Further from the site of injection, spindles appeared to form normally, but chromosomes did not congress to the metaphase plate, and some spindles initiated anaphase but contained lagging chromosomes. Using real-time imaging of live embryos, Sharp *et al.* [6] found that the rate of anaphase poleward movement of chromosomes was reduced in the embryos that had been injected with p50 or anti-dynein heavy chain antibodies, as compared to controls. In control cells, the measured rate of poleward chromosome movement was biphasic, with an initial slow rate of approximately 2 μm per minute, followed by faster movement of approximately 9 μm per minute. When dynein function was blocked, however, chromosomes maintained the slower speed throughout anaphase. Therefore, taking advantage of the differential sensitivities of mitotic dynein functions to perturbation, the authors could distinguish kinetochore-specific effects on chromosome movement in prometaphase and anaphase.

Together, these studies [5,6] suggest a model for poleward movement of *Drosophila* chromosomes during prometaphase and anaphase involving both microtubule depolymerization and motor function at the kinetochore (Figure 1). Cytoplasmic dynein, tethered to the kinetochore by ZW10 and ROD, contributes to the poleward movement of chromosomes through its minus end-directed motility. At the same time, kinetochores are driven poleward as a result of microtubule depolymerization at plus ends by MCAK/XKCM1 motors while CENP-E serves to maintain the attachment of the kinetochore to the ends of the shrinking microtubules. The movements of the plus end-directed motor CENP-E could directly counteract those of dynein, and may be responsible for maintaining the kinetochore within close proximity to the ends of shrinking microtubules. In addition to motor-driven kinetochore movements, poleward microtubule flux provides a constitutive source of minus end-directed movement for attached chromosomes.

The presence of dynein at the kinetochore in many cell types suggests that its role is conserved. But as illustrated above, different rates of anaphase poleward chromosome movement are observed within different cell types of the same organism, and even within a single cell these movements may be biphasic, suggesting that multiple independent mechanisms are at work. Furthermore, inhibition of dynein function attenuates, but does not altogether block, poleward movement. How and to what extent are these different mechanisms coupled? The continuous end-on attachment indicates that motor function is linked to microtubule polymerization dynamics at the kinetochore, but the relative contribution of these mechanisms could vary. The wild card here is likely to be microtubule depolymerization at the pole, which contributes to varying degrees to anaphase chromosome movement in different systems. For example, the poleward flux of microtubules in *Xenopus* egg extract spindles is thought to be the primary mechanism driving anaphase chromosome movement [2]. In contrast, poleward flux is less rapid in vertebrate somatic cells, where an estimated 75% of the poleward movement is derived from depolymerization at microtubule plus ends [17].

A major obstacle in determining the precise contribution of microtubule depolymerization to chromosome movement in systems such as *Xenopus* and *Drosophila* is the inability to distinguish kinetochore-attached microtubules from other microtubules in the spindle. The use of new high-resolution techniques, such as fluorescent speckle microscopy, may overcome this difficulty, with the added benefit that marked microtubule lattices will allow measurement of microtubule depolymerization at both plus and minus ends. Poleward chromosome movement, like many aspects of spindle function, appears to have redundant mechanisms that are differentially favored in different systems, and may provide a fail-safe system to increase the fidelity of chromosome segregation. The development of more precise molecular tools and *in vitro* reconstitution of kinetochore activities will be instrumental to the further dissection of these mechanisms.

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