Kinesin-5 acts as a brake in anaphase spindle elongation

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Bipolar kinesin-5 motors, essential in diverse organisms, can generate positive sliding forces between overlapped interpolar microtubules to push mitotic spindle poles apart. BMK-1, the sole *Caenorhabditis elegans* kinesin-5, is not essential. We have determined, by tracking pole movements in *bmk-1* mutant *C. elegans* embryos, that BMK-1 actually resists pole separation during anaphase. This provides *in vivo* evidence that kinesin-5, when challenged by fast pole separation forces, can serve as a rate-limiting brake for interpolar microtubule sliding.

To organize and then accurately separate duplicated sets of chromosomes, eukaryotic cells construct a spindle. In many cell types, duplication of a centrosome creates two adjacent spindle poles that anchor microtubules by their minus ends to form aster-like radial arrays. Interpolar connections are formed between two asters when their microtubule plus ends associate laterally via cross-linking proteins to overlap in an anti-parallel fashion [1]. A classic model posits that plus-end-directed kinesin-5 type motors, which form bipolar heterotetramers, generate sliding forces between overlapped interpolar microtubules to push spindle poles apart [2,3]. Consistent with this, *in vitro* tests have shown that *Xenopus* kinesin-5 (Eg5) can crosslink antiparallel microtubules and forcefully slide minus-ends away from one another [4]. This agrees with *in vivo* kinesin-5 inhibition, which causes failure of pole separation, convergence of already separated poles, disruption of chromosome segregation and lethality in diverse organisms, from fungi to mammalian cells [5,6].

BMK-1, the sole *C. elegans* kinesin-5, provides a puzzling exception because inhibition does not block mitosis and is not lethal [7]. Such an exception could provide new insights, so we studied BMK-1 distribution and the consequences of disrupting its function on spindle pole behavior in early embryos (see Experimental procedures in Supplemental data published with this article online). BMK-1 distribution in mitosis parallels that of kinesin-5 in other organisms: at the poles and throughout the spindle before anaphase, then most concentrated during anaphase in the 'interzone' between separated chromosomes where interpolar microtubules overlap (Figure 1A,B) [7].

Live imaging of spindles revealed abnormally fast pole separation at the start of anaphase in *bmk-1* deletion mutants (Video S1 in Supplemental data). Anaphase poles in mutants reached half-maximal separation at an average of 34 sec as opposed to 66 sec in wild type (Figure 1C). Although there was a small decrease in GFP-tagged microtubule fluorescence in the anaphase interzones of *bmk-1* mutants (24 ± 10%, n = 10), we observed no disconnection of half-spindles ('pop-apart') like that reported for inhibition of the microtubule depolymerase KLP-7 (MCAK)

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[8]. Similar but less severe phenotypes were seen after depletion of *bmk-1* by RNAi. The implication of the fast initial pole separation in *bmk-1* mutants is that BMK-1 normally resists anaphase pole separation forces generated by other motors.

Outward pulling by astral microtubules in *C. elegans* generates a prominent force in anaphase that can drive a peak pole separation velocity of 0.8–1.5 µm/sec when interzone connections are broken [8]. Kinesin-5 velocity along microtubules is only 0.02–0.10 µm/sec, even when pulled forward toward plus ends with an optical trap [4,9]. Our results suggest that, in the face of fast pole separation forces, BMK-1 regulates the interzone microtubule sliding rate. Although this might be indirect, e.g. via stabilization of interpolar microtubules, it could also reflect a direct effect of BMK-1 as a molecular brake [10], i.e. BMK-1 may govern sliding rates with its slow, but processive, ATP hydrolysis-driven step cycle [9]. Mixed-motor microtubule gliding assays suggest, however, that it also could generate drag via microtubule binding/release kinetics that are independent of ATP hydrolysis [11].

To test regulation of pole separation rate by BMK-1 in a different force-balance environment, we compared pole separation rates in *bmk-1(+)* and *bmk-1* deletion mutant embryos after reducing outward pulling force on spindle poles by inhibition of GPR-1/2 [12,13]. In *bmk-1 (+)* embryos, *gpr-1/2* RNAi dramatically reduced the initial anaphase pole separation rate and the net separation that occurred during anaphase (Table 1). The *bmk-1* mutation suppressed those *gpr-1/2* RNAi phenotypes, allowing a near-normal initial rate and a partial restoration of net separation. This suggests that there is a *gpr-1/2* RNAi-insensitive weak pole separation force that can be unmasked by eliminating the braking activity of BMK-1. It may be generated by a remnant GPR-1/2-dependent mechanism or by a novel GPR-1/2-independent mechanism. In either case, it is evident that pole separation rates are determined by a force-balance relationship [2] and that the *C. elegans* kinesin-5 homolog is a key factor for resisting fast outward pole separation forces.

This role reversal for kinesin-5 in *C. elegans*, from a sliding motor to a sliding brake, supports the view that, despite conservation of basic structural and mechanistic principles in mitosis, the ways in which individual components of the mitotic machinery contribute can be shuffled by evolution. Such divergence may be allowed in mitosis because of the rich "layering" of partially redundant force generation and spindle assembly mechanisms. Where might the selective advantage lie in using BMK-1 as a brake? Perhaps it co-evolved with unusually strong outward astral pulling forces to create a balanced tension that helps ensure the precise control of metaphase-anaphase spindle position that is so critical for determining cell identities during *C. elegans* development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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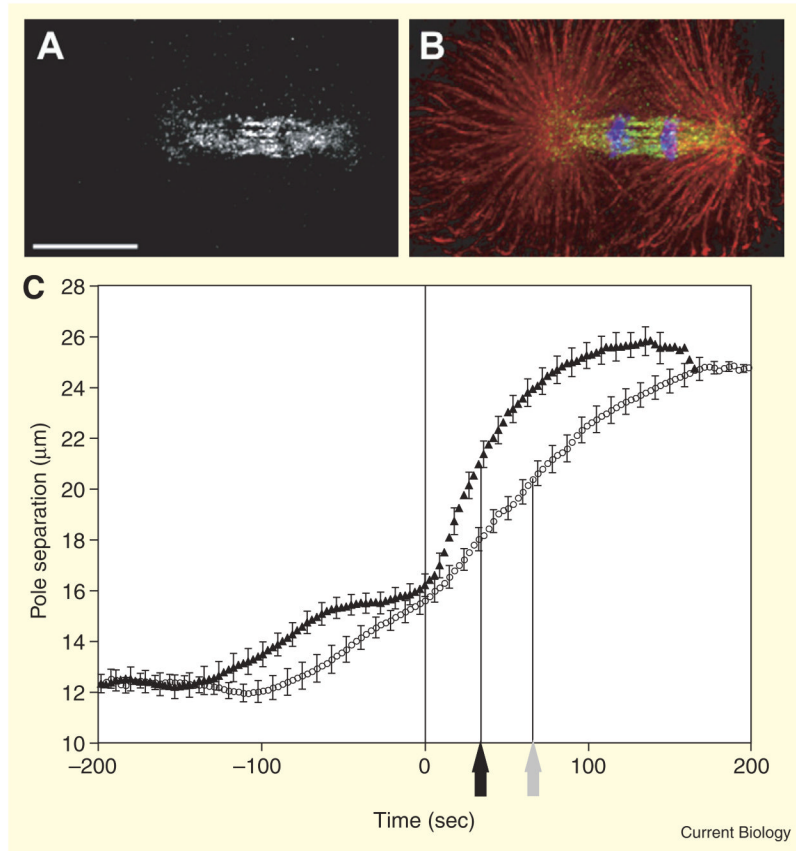
Figure 1.
Distribution of BMK-1 and its influence on spindle pole separation. (A) Anti-BMK-1 immunostaining of a 1-cell anaphase embryo (bar = 10 µm). (B) The same embryo with anti-BMK-1 in green, anti-α-tubulin in red, and DAPI staining of chromosomes in blue. (C) The distance between the centers of mitotic spindle poles as a function of time in living wild-type (circles) and *bmk-1(ok391)* mutant (triangles) embryos. Each data point represents the average pole-to-pole distance at a given time point before or after the last frame before start of anaphase chromosome separation (n = 20 embryos). Error bars indicate 95% confidence intervals (1.96 × σ/√n). Black and grey arrows indicate half-maximal anaphase pole separation for *bmk-1* mutants and wild type, respectively.

Table 1

Effects of *gpr-1/2* RNAi on spindle pole separation in *bmk-1(+)* and *bmk-1* deletion mutant embryos.

Genotype and RNAi treatment	[1]Initial pole separation rate (µm/s) 0-18s	Net increase in separation during anaphase (µm)	Maximum pole separation distance (µm)	n
bmk-1(+)	0.107 ± 0.008	8.33 ± 0.29	24.72 ± 0.21	10
bmk-1(ok391)	0.162 ± 0.012	8.98 ± 0.27	25.08 ± 0.41	10
bmk-1(+) & gpr-1/2(RNAi)	0.049 ± 0.007	4.46 ± 0.23	17.26 ± 0.29	9
bmk-1(ok391) & gpr-1/2(RNAi)	0.102 ± 0.010	7.00 ± 0.52	19.60 ± 0.61	8

[1]All values represent mean ± SEM determined from pole-pole distance measurements starting in the last frame before detectable anaphase chromosome separation.