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Report

Nonredundant Functions of Kinesin-13s during Meiotic Spindle Assembly

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Summary

Spatiotemporal control of microtubule depolymerization during cell division underlies the construction and dynamics of mitotic and meiotic spindles. Owing to their potent ability to disassemble microtubules, Kinesin-13s constitute an important class of microtubule destabilizing factors. Unfertilized *Xenopus* eggs, similar to other metazoan cells, contain the prototypical Kinesin-13 MCAK as well as a second family member, XKIF2. Here, we compare the roles of MCAK and XKIF2 during spindle assembly in *Xenopus* extracts. We find that although MCAK and XKIF2 have similar localization and biochemical properties, XKIF2 is not required for spindle assembly and, further, cannot substitute for MCAK. Altering dosage of the two kinesins demonstrates that spindle length is exquisitely sensitive to MCAK concentration but not XKIF2 concentration. Finally, we demonstrate that the rate of poleward microtubule flux in *Xenopus*-extract spindles is unaffected by XKIF2 depletion and is only modestly sensitive to reduction of MCAK action. We suggest that, in contrast to models proposed for mammalian somatic cell and embryonic *Drosophila* spindles, Kinesin-13s do not play a central role in poleward flux by depolymerizing minus ends. Rather, MCAK, but not XKIF2, plays a central role in regulating dynamic instability of plus ends and controls spindle length by that mechanism.

Results

To compare the functions of the *Xenopus* microtubule depolymerizing Kinesin-13s MCAK (KIF2C) and XKIF2 (Kif2A), we developed polyclonal XKIF2 antibodies. On immunoblots of *Xenopus* extracts, our antibodies recognize a specific 97 kDa band, whereas our MCAK antibodies [1, 2] recognize a distinct 85 kDa band (Figure 1A). By immunoblotting, we estimate extract concentrations of XKIF2 and MCAK to be 5 nM and 50 nM, respectively.

Immunofluorescence of XKIF2 in *Xenopus*-extract spindles showed discrete chromosome and spindle-pole labeling (Figure 1B). Double labeling of spindles with XKIF2 and CENP-A antibodies [3] revealed that XKIF2 localizes to centromeres (Figure S1 in the Supplemental Data available online), similar to MCAK [2, 4]. We confirmed the centromere localization of XKIF2 by imaging recombinant GFP-XKIF2 in extract spindles (Figure S2 and Movie S1). Notably, comparison of anti-MCAK and anti-XKIF2 immunofluorescence indicates that their centromere and spindle-pole localizations are distinct (Figure 1C). At centromeres in particular, anti-XKIF2 staining is more filamentous than that produced by anti-MCAK.

To compare the activities of MCAK and XKIF2 in vitro, we purified baculovirus-expressed MCAK and XKIF2 (Figure S3) and analyzed the abilities of each kinesin to disassemble a fixed amount of GMPCPP-microtubules (1 μM tubulin) by using a light-scattering assay [5]. Although both motors fully disassembled microtubules in this assay, they catalyzed depolymerization at slightly different rates. A total of 5 nM XKIF2 disassembled 50% of its substrate in 112 s, whereas 5 nM MCAK took 204 s to depolymerize an equivalent amount of polymer (Figure 1D).

Given the observed similarities between XKIF2 and MCAK, we tested whether XKIF2 activity is affected by the Aurora B kinase. In vitro, MCAK activity is inhibited by Aurora B; this control mechanism is thought to promote spindle assembly and chromosome biorientation [1, 6–8]. In in vitro kinase assays, XKIF2 was radiolabeled by Aurora B-INCENP (Figure 1E). In light-scattering microtubule-depolymerization assays, preincubation of 15 nM XKIF2 with Aurora B-INCENP led to 50% microtubule disassembly in 609 s, whereas untreated XKIF2 depolymerized 50% of its substrate within 40 s. We conclude that, similar to MCAK, XKIF2 activity is inhibited by Aurora-B-dependent phosphorylation.

We investigated the function of XKIF2 during spindle assembly by immunodepleting the protein from extracts. Our antibodies remove all detectable amounts of XKIF2 without affecting MCAK protein levels (Figure 2A). In contrast to MCAK depletion, which produces large microtubule arrays through inhibition of plus-end dynamic instability [2, 9], XKIF2 immunodepletion had no effect on spindle morphology or length. In ΔXKIF2 extracts, spindles were 37.7 ± 1.3 μm long (n = 56), similar to those in control extracts (37.6 ± 2.0 μm, n = 61). XKIF2 depletion did cause a mild (~20%) but reproducible defect in metaphase chromosome alignment (Figures 2B and 2C). Similar effects were observed in XKIF2-antibody-blocking experiments (unpublished data).

Despite having similar biochemical properties, our results suggest that MCAK and XKIF2 have distinct roles during spindle assembly. However, it is possible that XKIF2 immunodepletion caused a mild microtubule-depolymerization phenotype simply because it is 10-fold less abundant than MCAK. To test their redundancy during spindle assembly, we immunodepleted MCAK from extracts and replaced it with either MCAK or XKIF2 to a concentration of 50 nM, the physiological concentration of MCAK. XKIF2 protein levels were unaffected in MCAK-depleted extracts (Figure 2D). As

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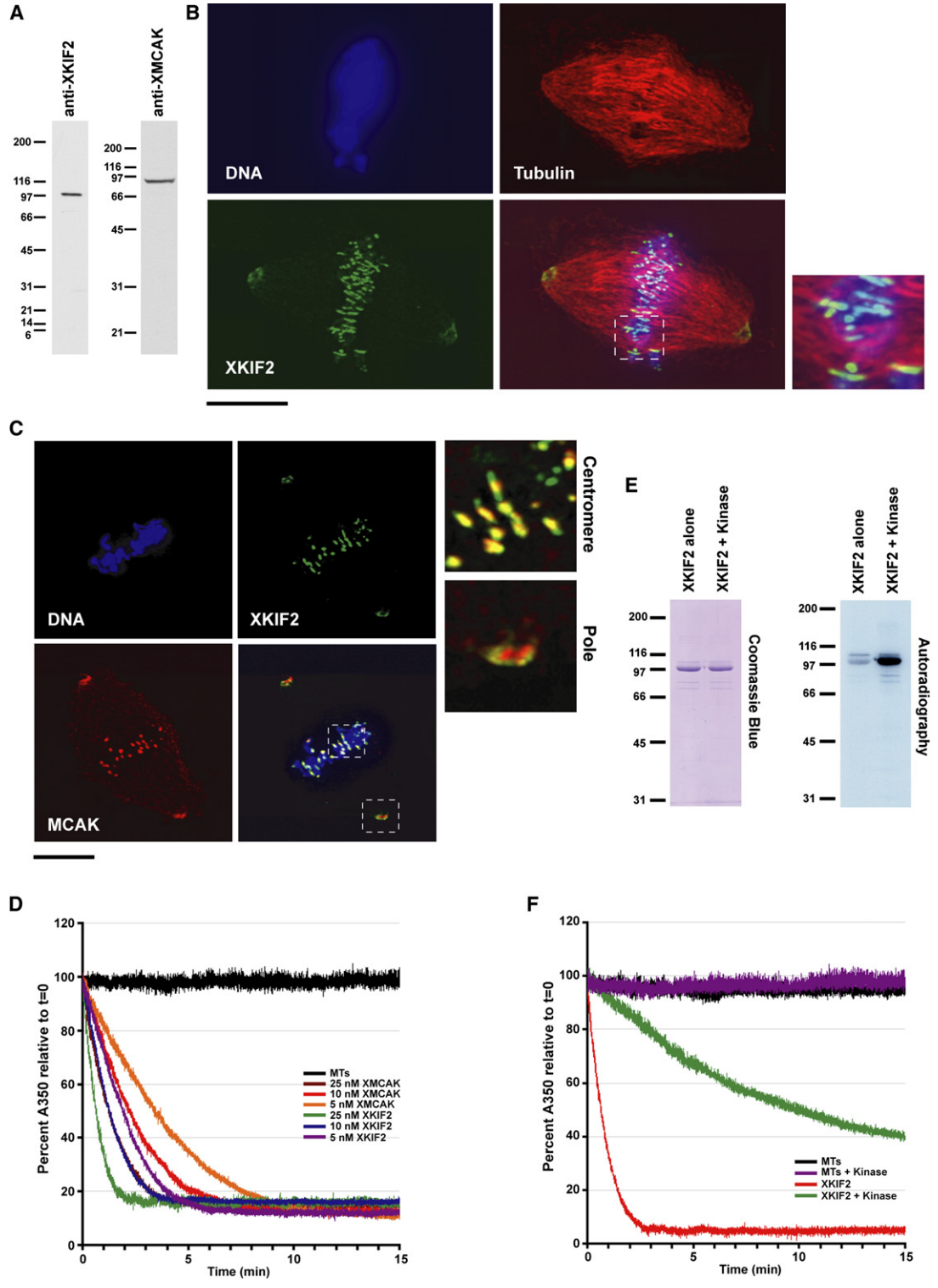

Figure 1. Two *Xenopus* Kinesin-13s Have Similar Cytological and Biochemical Properties

(A) Immunoblot of CSF-egg extract with XKIF2 and XMCAK antibodies.

(B) Localization of XKIF2 in spindles assembled in vitro. Rhodamine-tubulin-labeled metaphase spindles were fixed and stained with XKIF2 antibodies and Hoechst 33342. The scale bar represents 10 μm.

(C) XKIF2 and MCAK can occupy spatially distinct regions at centromeres and spindle poles. Metaphase spindles were fixed and stained with MCAK (red) and XKIF2 (green) antibodies. The scale bar represents 10 μm.

(D) Microtubule depolymerization activities of MCAK and XKIF2 in vitro. A total of 1 μM tubulin assembled into microtubules with GMPCPP was mixed with 5, 10, and 25 nM of either MCAK (orange, red, and dark-red curves, respectively) or XKIF2 (purple, blue, and green curves, respectively) and monitored by light scattering.

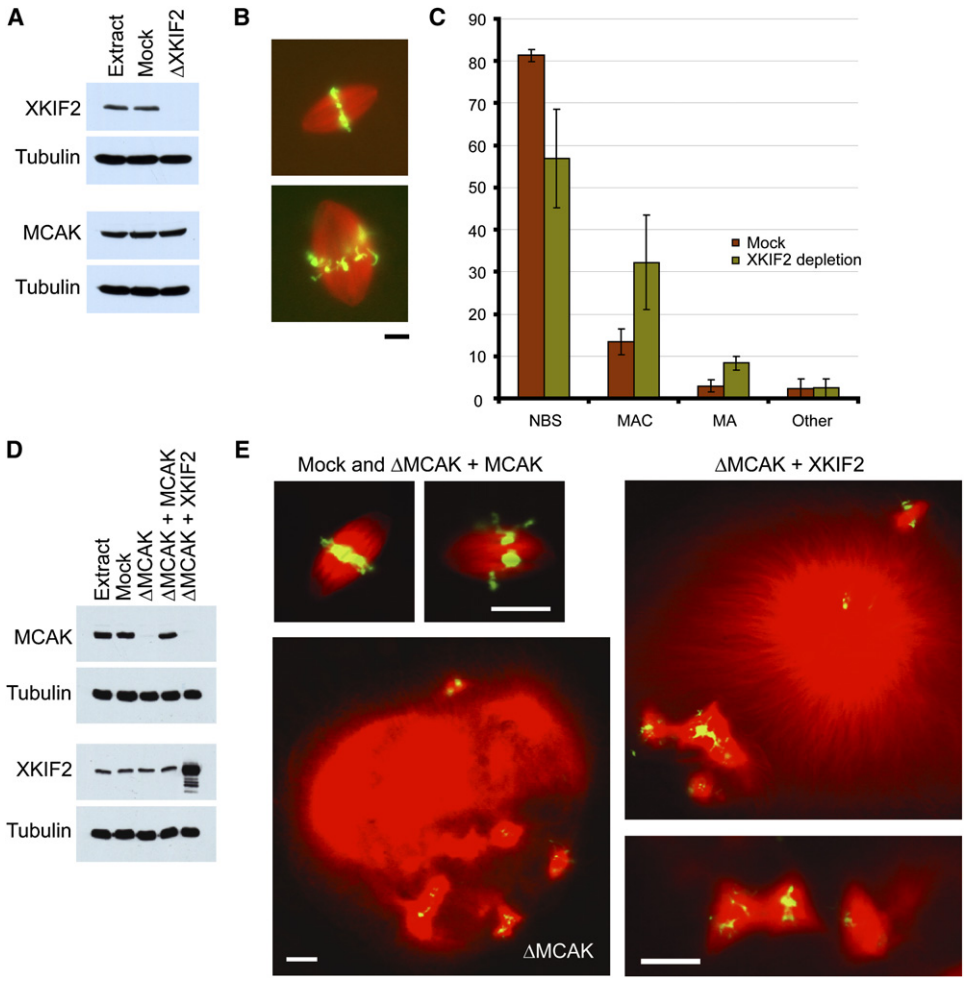

Figure 2. Specific Requirement of MCAK during Spindle Assembly

(A) XKIF2 immunodepletion. Immunoblots of untreated, mock-depleted, and XKIF2-depleted extracts probed with XKIF2, MCAK, and tubulin antibodies are shown.

(B) Predominant phenotypes observed in mock- and XKIF2-depleted extracts. The top panel shows a normal bipolar spindle (NBS); the bottom panel shows a spindle with misaligned chromosomes (MAC). Microtubules (red) were visualized with rhodamine-tubulin, and DNA (green) was visualized with Hoechst 33342. The scale bar represents 10 μm.

(C) Quantitation of structures assembled in XKIF2-depleted extracts. Abbreviations are as described in (B) with the addition of MA, which stands for monoasters. Data are averaged from four independent experiments where error bars represent SD from the mean.

(D) MCAK immunodepletion and add-back of MCAK or XKIF2. Egg extracts were depleted with IgG (mock) or MCAK antibodies and supplemented with recombinant MCAK or XKIF2. Immunoblots of these extracts probed with XKIF2, MCAK, and tubulin antibodies are shown.

(E) XKIF2 fails to substitute for MCAK during spindle assembly. Representative structures formed in spindle-assembly reactions with control, MCAK-depleted, and MCAK-depleted extracts that were rescued with either MCAK or XKIF2 are shown. Scale bars represent 20 μm.

expected, mock-depleted and MCAK-depleted extracts rescued with MCAK formed bipolar spindles, whereas extracts lacking MCAK assembled large microtubule arrays. A total of 50 nM XKIF2 partially rescued the gross microtubule-length defect of MCAK-depleted extracts, but these extracts were incapable of supporting spindle assembly (Figure 2E).

We considered that XKIF2 was incapable of rescuing spindle assembly in MCAK-depleted extracts because this process is sensitive to small changes in the amount of depolymerization activity provided by MCAK. To test this, we reconstituted MCAK-depleted extracts with varied concentrations of MCAK (Figure 3A), and these extracts were used to drive spindle assembly. Although spindle formation in extracts containing 12.5 nM MCAK occurred with reduced efficiency, extracts containing 25–100 nM MCAK readily assembled spindles (Figure 3B). At >100 nM MCAK, spindle assembly failed and the majority of structures were monoasters with short microtubules (data not shown). Thus, spindle assembly tolerates ~2-fold changes in MCAK concentration and makes it unlikely that the failure of XKIF2 to

(E) XKIF2 is a substrate for Aurora B-INCENP in vitro. XKIF2 was incubated with [γ-^{32}P] ATP in the presence or absence of Aurora B-INCENP, and the products of the kinase reactions were monitored by autoradiography and Coomassie blue staining.

(F) XKIF2 is inhibited by Aurora-B-dependent phosphorylation. GMPCPP microtubules (1 μM) were mixed with Aurora B (purple), unmodified XKIF2 (red), or XKIF2 phosphorylated by Aurora B (green) and monitored by light scattering. Black represents microtubules alone.

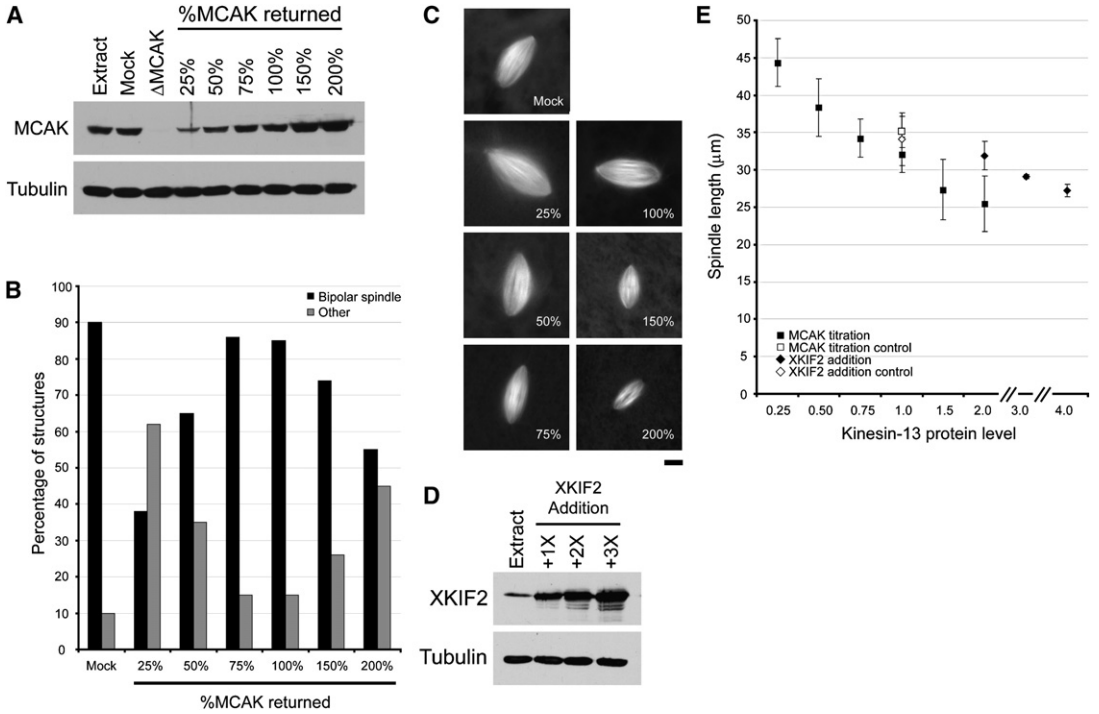

Figure 3. MCAK but Not XKIF2 Influences Spindle Length

(A) Titration of MCAK protein levels. Egg extract was immunodepleted with IgG (mock) or MCAK antibodies and supplemented with various amounts of recombinant MCAK. A total of 100% MCAK is equal to 50 nM. Immunoblots of these extracts probed with MCAK and tubulin antibodies are shown.

(B) Quantitation of structures assembled in extracts from (A).

(C) Representative spindles assembled in the presence of various MCAK concentrations. The scale bar represents 10 μm.

(D) Addition of excess XKIF2 to egg extracts. Recombinant XKIF2 was added to final concentrations of 50 nM (+1× XKIF2), 100 nM (+2× XKIF2), and 150 nM (+3× XKIF2), and these extracts were probed with XKIF2 and tubulin antibodies.

(E) Quantitation of spindle lengths as a function of [MCAK] and [XKIF2]. Average spindle lengths ± SD are shown. Approximately 60 spindles from three independent experiments were analyzed for each MCAK and XKIF2 concentration.

rescue MCAK depletion is simply because of a small variation in the depolymerization activity provided by XKIF2.

We observed that spindle length is more sensitive to the amount of MCAK in extract than XKIF2. Spindles were longer upon MCAK dilution and shorter when excess MCAK was present (Figure 3C). Compared to control spindles (35.1 ± 2.1 μm, n = 60), spindles in 12.5 nM MCAK extracts were elongated by 26% (44.3 ± 3.2 μm, n = 32), whereas those assembled at 100 nM MCAK were shortened by 27% (25.5 ± 3.7 μm, n = 58) (Figure 3E). Spindle shortening occurred with reduced efficiency at high XKIF2 concentrations (Figure 3E), although excess XKIF2 did partially impair spindle assembly similar to high (>100 nM) levels of MCAK (Figure S5). A total of 50 nM excess XKIF2 reduced spindle length by only 6% (control: 34.1 ± 3.5 μm, n = 60; +50 nM XKIF2: 31.9 ± 1.9 μm, n = 65). Even the addition of 150 nM XKIF2 shortened spindles by 20% (27.3 ± 0.8 μm, n = 65); this was an effect observed with 75 nM MCAK (Figure 3E).

Kinesin-13s are posited to play a central role in flux mechanism by depolymerizing microtubules from their minus ends at spindle poles [10, 11]. In *Xenopus*-extract spindles, flux requires Eg5 [12], but the role of depolymerizing factors remains unclear. As such, we examined flux rate in spindles assembled in XKIF2-depleted extracts, in the presence of variable MCAK concentrations or in the presence of MCAK-function-blocking antibodies. Flux rates were determined, principally at the spindle equator, by cross-correlation analysis [12] of spindles labeled with small amounts of fluorescent tubulin [13].

Flux rates in extracts lacking XKIF2 or containing variable concentrations of MCAK were relatively unchanged compared to controls. Similarly, flux rates in anti-MCAK-perturbed spindles were reduced by only ~15% compared to control spindles. Because it was possible that MCAK and XKIF2 act synergistically to generate flux, we treated ΔXKIF2 spindles with MCAK antibodies. Such spindles fluxed at rates identical to those of anti-MCAK-treated spindles (Table 1). These results indicate a lack of synergy between XKIF2 and MCAK in driving flux and suggest that neither depolymerases are central to flux mechanisms in extract spindles.

Discussion

Kinesin-13s depolymerize microtubules [14] to ensure that they only grow to a finite length [15, 16]. Additionally, by acting on plus ends near kinetochores, Kinesin-13s may facilitate poleward chromosome transport [11, 17] and correct attachment errors [6, 7, 18, 19]. Kinesin-13s have also been argued to depolymerize minus ends at

Table 1. Flux in *Xenopus*-Extract Spindles Is Not Greatly Affected by Altering Kinesin-13 Activities

	Flux Rate ± SD (μm/min)	n
MCAK Titration		
Control	2.03 ± 0.46	34
25 nM MCAK	2.11 ± 0.36	24
50 nM MCAK	1.85 ± 0.51	29
100 nM MCAK	1.85 ± 0.49	21
XKIF2 Immunodepletion		
Control	1.71 ± 0.33	25
ΔXKIF2	1.69 ± 0.34	30
α-XMCAK Addition		
Control	2.35 ± 0.26	16
α-XMCAK	1.98 ± 0.42	6
XKIF2 Immunodepletion + α-XMCAK Addition		
Mock depletion	1.72 ± 0.17	6
Mock depletion + α-XMCAK	1.41 ± 0.24	5
ΔXKIF2 + α-XMCAK	1.47 ± 0.28	12

spindle poles, thus promoting flux in kinetochore fibers as well as regulating metaphase spindle length [10, 11, 20–22]. It is unclear whether Kinesin-13s promote all of these activities in all spindle types and also the extent to which Kinesin-13 is specialized for these functions.

In *Xenopus*-extract spindles, MCAK and XKIF2 display similar localizations, suggesting overlapping functions. Consistent with this notion, both motors disassemble GMPCPP microtubules in vitro with quantitatively similar potencies, and both are inhibited by Aurora B. However, MCAK and XKIF2 are not functionally redundant, even when their differing concentrations are considered. MCAK depletion prevents spindle assembly, whereas XKIF2 depletion has no effect on spindle length or morphology. Microtubule growth defects caused by removal of MCAK can be partially suppressed by excess XKIF2, but XKIF2 cannot complement spindle assembly in the absence of MCAK. That XKIF2 is nonessential during spindle assembly contradicts previous work that found that perturbing XKIF2 function with antibodies caused the formation of monopoles [21]. Although the reason for this discrepancy is unclear, the method we used, i.e., immunodepletion, is more direct and thus may provide a more accurate conclusion. We also note that RNAi phenotypes of fly Klp10A and Klp59C are reminiscent of MCAK and XKIF2 immunodepletion phenotypes, respectively [11], consistent with XKIF2 being nonessential for spindle assembly.

Neither MCAK nor XKIF2 appear to be required for flux in *Xenopus* extracts. Although seemingly inconsistent with previous studies in cultured human cells [23] and *Drosophila* embryos [11], it is crucial to recognize that extract spindles are organized on different principles compared to somatic-cell spindles. It is possible that flux is a different process in the two spindle types, accounting for differential requirements in Kinesin-13 activities (Figure 4). Extract spindles are anastral and assemble through Ran-GTP-induced microtubule nucleation around chromosomes, and this is followed by self-organization [24]. Unlike astral spindles, where minus ends are predominantly clustered at the poles, minus ends are scattered throughout the extract spindle [25]. We envision that flux in anastral spindles involves poleward sliding of microtubules, with their minus ends moving but not depolymerizing, and all depolymerization occurring from plus ends via dynamic instability

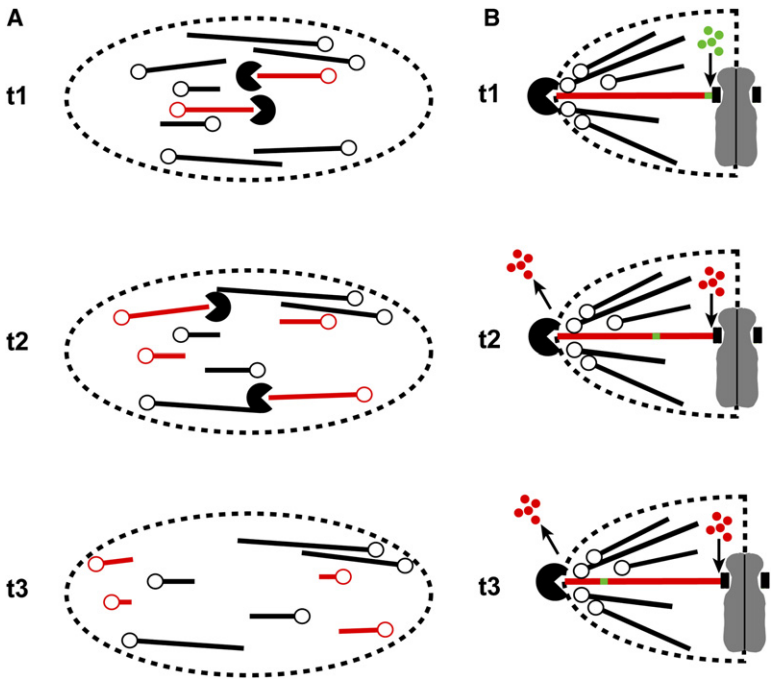

Figure 4. Alternative Models for Kinesin-13 Function in Spindles

Three time points (t1-3) are shown for each model.

(A) Regulation of plus-end dynamic instability. Spindle microtubules are short, their minus ends are capped (open circles), and their plus ends undergo bounded dynamic instability (data not shown). They slide toward the poles under the action of Kinesin-5/Eg5. MCAK (indicated by a Pacman symbol) regulates microtubule length by promoting catastrophes at plus ends (red microtubules). This is the model we currently favor for *Xenopus*-extract spindles and presumably other anastral spindles.

(B) Minus-end depolymerase activity. Only a half spindle is shown. Kinetochore microtubules (red) extend from chromosome to pole, so their flux, indicated by poleward movement of a fiduciary mark (green) on k-fiber microtubules, requires minus-end depolymerization (indicated by red circles) by a Kinesin-13 (indicated by a Pacman symbol). The depolymerase might simply allow metaphase flux, and traction fiber movement in anaphase A, driven by other motors, or might actively promote it by pulling the minus end toward the pole. This model has been proposed for fly, PtK, and *Xenopus*-extract spindles.

(Figure 4A). This model explains why Kinesin-13s are dispensable for microtubule sliding (Table 1). In contrast, kinetochore microtubules in somatic cells run contiguously from kinetochores to poles [26], and thus their minus ends must depolymerize to allow (or promote) flux (Figure 4B).

Whether our conclusion will apply to the less abundant kinetochore microtubules of extract spindles is unclear. Kinetochore microtubules represent a minority (~5%; unpublished data) of microtubules in extract spindles, meaning that cross correlation may have difficulty detecting changes in kinetochores-microtubule flux rates. However, centromere stretch, a read-out of tension at kinetochores [27], is not reduced upon XKIF2 depletion or MCAK dilution nor is it increased when excess MCAK is present (Table S1). Such effects might be expected if Kinesin-13s were critical for flux in kinetochore fibers.

MCAK but not XKIF2 influences metaphase spindle length in our system. Fly Klp10A also regulates spindle length [11, 22], but the Kinesin-13s may act via different mechanisms. Klp10A was proposed to regulate spindle length by actions at minus ends (Figure 4B). Because flux is unaffected by MCAK perturbation, we do not favor a role for MCAK at minus ends in extract spindles. We propose instead that MCAK regulates spindle length by controlling dynamic instability of plus ends (Figure 4A) and thus average microtubule length. Consistently, spindle-tubulin speckles persist longer when MCAK levels are reduced and disappear more rapidly at high MCAK concentrations (D.J. Needleman, A.C. Groen, R.O., L.Mirny, and T.J.M., unpublished data). Furthermore, as observed in cultured cells [28], MCAK decorates microtubule plus ends similar to plus-end-tracking proteins (unpublished data).

Figure 4 highlights current opinions concerning Kinesin-13 functions and organization of spindles more generally. In considering which model is applicable to different spindle types, we point out that, in all flux work to date, minus-end depolymerization rates have been inferred from sliding rates. Reagents are needed to visualize minus ends, thus enabling us to distinguish whether they are moving and capped (Figure 4A) or are instead static and depolymerizing (Figure 4B). Because poleward transport of nonkinetochore microtubules in anastral spindles (Figure 4A) probably differs from dynamics of kinetochore fibers (Figure 4B) in minus-end behavior and in requirement for Kinesin-5/Eg5 activity [12, 20] we propose the name "poleward sliding" for the former, reserving the name "poleward flux" for kinetochore fibers. Elucidating the molecular requirements for, and mechanical consequences of, these different dynamics will help us understand how spindle assembly and function differs between cell types.

Supplemental Data

Experimental Procedures, five figures, and one movie are available online at http://www.current-biology.com/cgi/content/full/17/11/953/DC1/.

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