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Report

Kif18A Uses a Microtubule Binding Site in the Tail for Plus-End Localization and Spindle Length Regulation

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Summary

The mitotic spindle is a macromolecular structure utilized to properly align and segregate sister chromatids to two daughter cells. During mitosis, the spindle maintains a constant length, even though the spindle microtubules (MTs) are constantly undergoing polymerization and depolymerization [1]. Members of the kinesin-8 family are important for the regulation of spindle length and for chromosome positioning [2–9]. Kinesin-8 proteins are length-specific, plus-end-directed motors that are proposed to be either MT depolymerases [3, 4, 8, 10, 11] or MT capping proteins [12]. How Kif18A uses its destabilization activity to control spindle morphology is not known. We found that Kif18A controls spindle length independently of its role in chromosome positioning. The ability of Kif18A to control spindle length is mediated by an ATP-independent MT binding site at the C-terminal end of the Kif18A tail that has a strong affinity for MTs in vitro and in cells. We used computational modeling to ask how modulating the motility or binding properties of Kif18A would affect its activity. Our modeling predicts that both fast motility and a low off rate from the MT end are important for Kif18A function. In addition, our studies provide new insight into how depolymerizing and capping enzymes can lead to MT destabilization.

Results and Discussion

Kif18A Perturbation Differentially Affects Spindle Length and Chromosome Congression

Spindle morphogenesis is important because defects in spindle organization often perturb the correct timing or the accuracy of chromosome segregation. One critical parameter of spindle morphogenesis is spindle length. For example, in *Xenopus* embryos, spindle lengths correlate with cell size in small cells; however, in larger cells, spindle length reaches an upper limit [13]. There are several models for how the spindle maintains a constant length, including regulation of microtubule (MT) dynamics, a balance of pushing and pulling forces, and a spatial gradient of diffusible morphogens [1].

It was previously shown that proteins that control spindle MT dynamics play a more critical role in spindle length determination than do proteins that mediate MT sliding [2]. Depletion of the human kinesin-8 Kif18A results in an increase in spindle length in addition to its more well-characterized role in mediating chromosome congression [4, 7, 9]. In contrast, depletion of the kinesin-14 HSET results in shorter spindles, which is mediated by HSET crosslinking and sliding of spindle MTs [14]. We therefore asked how Kif18A and HSET coordinately regulate the length of the spindle. Kif18A and HSET were depleted from HeLa cells individually and in combination, and the spindle lengths were measured (Figures 1A–1C). As previously described, depletion of Kif18A resulted in abnormally long bipolar spindles relative to control ($p < 0.001$) [4], whereas depletion of HSET caused shorter spindles ($p < 0.01$) [14]. When HSET was knocked down in combination with Kif18A, the long spindle length phenotype of Kif18A depletion was rescued to levels similar to control ($p = 0.06$). These results support the idea that MT dynamics and MT sliding are both important for spindle length regulation.

Previous studies suggest that the effect of Kif18A on chromosome oscillations is not a result of a change in spindle length but on threshold amounts of the protein [7]. Consistent with this idea, we found that although codepletion of Kif18A and HSET could restore spindle length, the chromosome misalignment phenotype caused by Kif18A knockdown alone could not be mitigated (Figure 1D). One possibility is that although the spindles in the double depletions are near control lengths, the MT organization in these spindles could be highly perturbed and indirectly affect chromosome congression. In addition, Kif18A acts upstream of astrin and Kif2B, which suggests that the effects of Kif18A on chromosome congression may be indirect by regulating astrin localization to kinetochores [15]. These results are consistent with the idea that Kif18A may use different mechanisms to control spindle length and congression at different times during mitosis and highlight the need for a better understanding of the role of Kif18A in spindle length regulation.

Full-Length Kif18A Is Needed for Microtubule Plus-End Targeting and Spindle Length Regulation

To ask how Kif18A controls spindle length, we generated a series of truncated versions of Kif18A to identify the protein domain requirements for proper spindle association. GFP-tagged Kif18A domain deletion constructs were expressed in HeLa cells and then analyzed for their ability to target to spindles and to regulate spindle length and chromosome alignment (Figure 2). Full-length Kif18A (G:Kif18A-FL) localized to the plus ends of MTs (Figure 2A) similar to endogenous Kif18A [4]. Mitotic cells with overexpressed Kif18A-FL had shorter spindles than control cells transfected with GFP-H2B (Figure 2B; $p < 0.05$), but overexpression did not affect the percentage of cells with aligned chromosomes (Figure 2C; $p = 0.48$). G:Kif18A-CD+Neck, which contains only the catalytic domain and the neck, was primarily localized in the cytoplasm of both interphase (data not shown) and mitotic cells with only a faint localization on spindle MTs, suggesting that domains within the stalk and tail are important in mediating proper localization of Kif18A to MT plus ends. Both the length of the spindle ($p = 0.37$) and the percentage of chromosome alignment ($p = 0.53$) in G:Kif18A-CD+Neck cells were similar to control cells.

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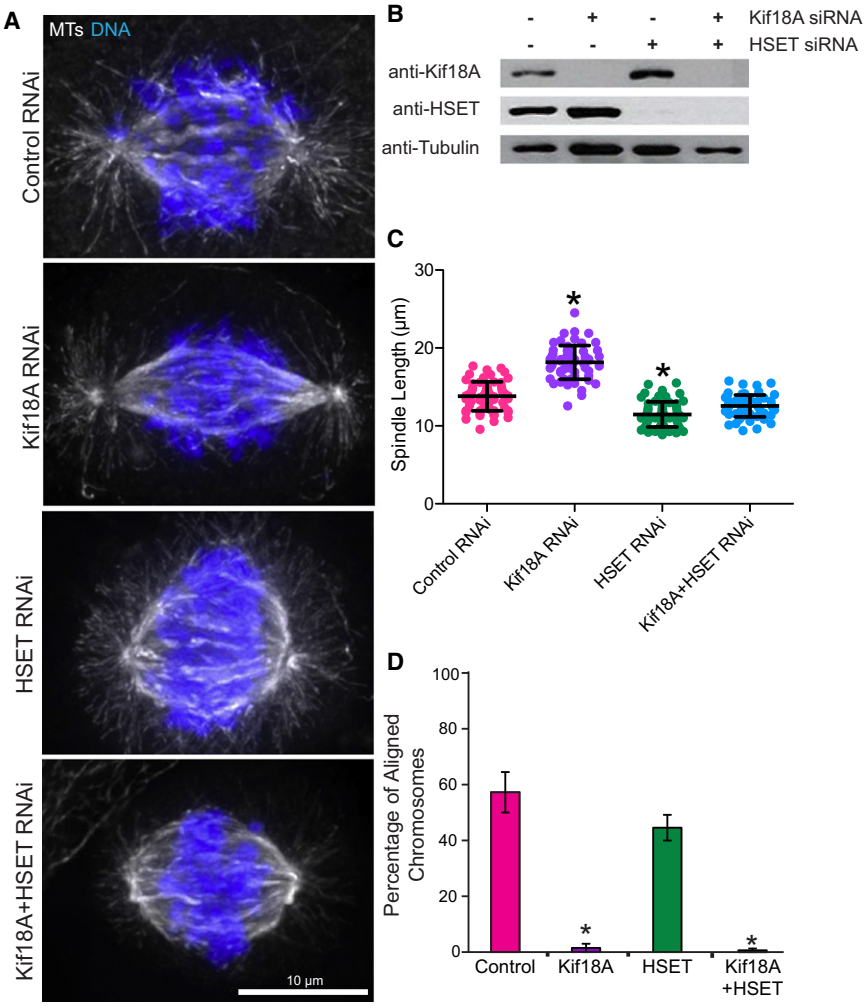

Figure 1. Kif18A and HSET Antagonistically Control Spindle Length

(A) HeLa cells transfected with luciferase (control) or the indicated siRNAs were stained for microtubules (MTs) (white) and DNA (blue). Scale bar represents 10 μm.

(B) Western blot of cells treated with luciferase, Kif18A, HSET, or Kif18A+HSET siRNAs and then probed with anti-Kif18A, anti-HSET, or anti-tubulin antibodies.

(C) Quantification of the spindle lengths from at least three independent experiments. For each knockdown condition, a total of at least 60 cells were scored for spindle length, and dot plots showing the lengths are graphed with the mean ± standard deviation (SD) indicated by the bar and whiskers.

(D) The percentages of cells containing bipolar spindles with aligned chromosomes for each condition were determined from >100 total cells in three independent experiments, and the mean ± standard error of the mean (SEM) is graphed. *p < 0.05 is relative to control.

G:Kif18A-CD, which contains only the catalytic domain, localized to MTs in interphase (data not shown). However, in contrast to G:Kif18A-CD+Neck, G:Kif18A-CD localized robustly along the length of the MTs in mitotic cells with no effect on spindle length ($p = 0.71$) or on chromosome alignment ($p = 0.95$). The construct containing only the stalk and tail domains (G:Kif18A-ST) was nuclear during interphase (data not shown) and surprisingly localized along MTs in mitotic cells. This result indicates the presence of an additional MT binding domain within Kif18A besides the catalytic domain. G:Kif18A-ST did not alter spindle length ($p = 0.26$) or chromosome alignment ($p = 0.11$) relative to control, suggesting that the stalk-tail domain may be necessary but not sufficient to target Kif18A to the plus ends of MTs. Together, these results show that proper targeting of Kif18A to the plus ends of MTs and proper spindle length control require both the catalytic domain and a second MT binding domain within the stalk-tail region. This requirement of proper localization for function was also observed for the *Drosophila* kinesin-8, Klp67A [6]. Although mediated by different mechanisms, proper localization of both Kif18A and Klp67A is required for regulation of spindle length.

The Additional Kif18A Tail MT Binding Domain Is Important for MT Plus-End Targeting and Spindle Length Regulation

The data above suggest that Kif18A contains an additional MT binding domain in its stalk or tail domain. Because other kinesins have second MT binding domains within the tail [16–18], we generated three tail deletion constructs of Kif18A and tested their ability to bind MTs in vitro (Figures 3A and 3B). All three of these proteins exhibited saturable binding to MTs in the absence of nucleotide with apparent K_d values that were indistinguishable (Figures 3C and 3D). These results suggest that amino acids 802–898 are sufficient for MT binding in vitro and constitute an ATP-independent MT binding site.

Based on the observation that G:Kif18A-CD+Neck was unable to localize to the plus ends of MTs, we hypothesized that the additional MT binding site in the tail domain was important for Kif18A localization and function. To test this idea, we compared the cellular localization and spindle lengths of HeLa cells transfected with the minimal MT binding domain alone, G:Kif18A(802–898), to cells transfected with Kif18A without the minimal MT binding domain, G:Kif18A(2–801) (Figures 3E and 3F). Consistent with our in vitro data, G:Kif18A(802–898) bound robustly to spindle MTs and did not alter spindle length relative to control G:H2B transfection ($p = 0.54$). Truncation of the additional MT binding domain of Kif18A in G:Kif18A(2–801) prevented MT localization and did not alter spindle length ($p = 0.17$). These results support the idea that the additional MT binding site in the tail domain is needed for proper localization of Kif18A at MT plus ends and suggest that proper localization is necessary for Kif18A regulation of spindle length.

Mathematical Modeling Predicts that High MT Plus-End Association Is Required for Kif18A Regulation of Spindle Length

It was shown previously that Kif18A, similar to the yeast ortholog Kip3p, pauses at the plus ends of MTs [11, 12], which may be important in the cooperative behavior of kinesin-8 proteins to induce MT destabilization [11]. We postulated that the tail domain of Kif18A would be important in facilitating Kif18A