Activation of mitotic kinesin by microtubule bundling

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Kinesin-5 family members cross-link and slide parallel microtubules of opposite polarity, an activity that is essential for the formation of a bipolar spindle during mitosis. In this issue, Kapitein et al. (Kapitein, L.C., B.H. Kwok, J.S. Weinger, C.F. Schmidt, T.M. Kapoor, and E.J.G. Peterman. 2008. *J. Cell Biol.* 182:421–428) demonstrate that microtubule cross-linking triggers the conversion of kinesin-5 motility from a diffusive mode to a directional mode, initiating antiparallel microtubule sliding.

During mitosis, microtubules rearrange and form the mitotic spindle, a highly organized structure necessary for the separation of replicated chromosomes. Spindle assembly is a result of the coordinated actions of two types of microtubule-based motor proteins, the kinesins and dyneins. To understand the underlying mechanism of spindle formation, it is important not only to characterize the motile activities of each motor but also to elucidate how individual motors are regulated. In this issue, Kapitein et al. (see p. 421 of this issue) demonstrate that at physiological ionic strength, the mitotic motor kinesin-5 diffuses along microtubules in an ATP-independent manner. When kinesin-5 cross-links two microtubules, it moves processively along the microtubules and drives antiparallel microtubule sliding (Fig. 1 A). This is an elegant way of turning on and off this type of motor as it functions in vivo to slide two opposing microtubules during bipolar spindle formation.

Kinesin comprises 14 subfamilies. The proteins of each subfamily are composed of a conserved motor domain (or "head"), which binds to microtubules and hydrolyzes ATP, and subfamily-specific stalk and tail domains. Several kinesin subfamilies are involved in intracellular transport, whereas the others work together in spindle assembly. The mechanisms of motility have been extensively studied in different kinesin families, but it is still unclear how motility is regulated in vivo (Fig. 1 B). In solution, the kinesin-1 motor head contains tightly bound ADP, but binding of the head to microtubules promotes exchange of ADP for ATP, enabling kinesin-1 to initiate processive run. Recent studies showed that in the full-length kinesin-1, the release of ADP is suppressed by a direct interaction of the tail domain with the motor head (Cai et al., 2007; Dietrich et al., 2008). Cargo binding to the tail releases the head so that kinesin-1 can move processively along the microtubule to transport cargo. Kinesin-3 (or Unc104/KIF1A) primarily exists as a monomer and diffuses along the microtubule, but, at high motor concentrations, the protein dimerizes and begins to move processively (Tomishige et al., 2002). Local clustering of PIP_2 on the vesicle membrane also triggers dimerization of kinesin-3 to activate vesicle transport (Klopfenstein et al., 2002). Kinesin-13 (or MCAK/KIF2) diffuses along a microtubule until it eventually reaches the end, at which point it processively depolymerizes the microtubule into tubulin subunits (Helenius et al., 2006). Collectively, the mechanisms regulating kinesin movement appear to be diverse and specific to each subfamily. Among the subfamilies, however, there appear to be two common modes of motion: a nonfunctional, diffusive mode and a functional, processive mode (efficiently converting ATP hydrolysis to mechanical work).

Kinesin-5 (also known as Eg5/BimC) is a plus end–directed microtubule-based motor protein that is essential for bipolar spindle formation during the early stages of mitosis and is involved in the growth of axons in developing neurons (Myers and Baas, 2007). Kinesin-5 exists as a homotetramer; two kinesin-5 dimers bind in an antiparallel manner to form a dumbbell-shaped tetramer (Kashina et al., 1996). Kinesin-5 cross-links microtubules of opposite polarity and slides them apart (Kapitein et al., 2005). Dimeric kinesin-5 demonstrates processive motility, but its processivity is relatively low (it detaches after approximately eight steps on average; Valentine et al., 2006), and it often displays one-dimensional diffusion along microtubules (Kwok et al., 2006). A kinetic study of dimers revealed a unique property: upon microtubule binding, one of the kinesin-5 heads releases ADP, but the release of ADP from the second head is slow, even in the presence of ATP, suggesting that kinesin-5 before the processive run is in a state in which ATP binding to the nucleotide-free head is suppressed (Krzysiak et al., 2008). The processive motility mechanism of kinesin-5 appears to be similar to that of kinesin-1; however, it remains unknown how the activity of kinesin-5 is regulated. Specifically, what are the triggers for its activation?

Kapitein et al. (2008) first showed that low ionic strength is one trigger for activation. Using single-molecule fluorescence motility assays, they demonstrate that tetrameric *Xenopus laevis* Eg5 move unidirectionally along microtubules under low ionic strength.

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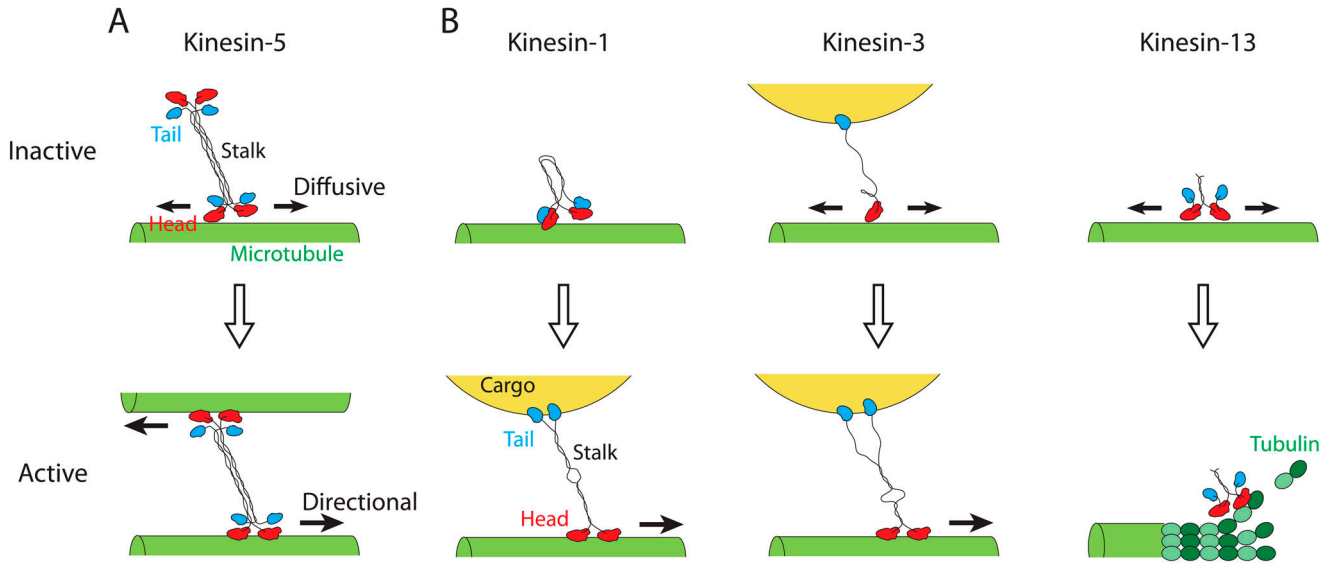

Figure 1. **Regulation mechanisms of the processive activities of kinesin subfamily motor proteins.** (A) Kinesin-5 on a microtubule switches its motional mode from diffusive to directional upon binding to the second microtubule. (B) Activation mechanisms for the other kinesin subfamilies (see text for detail).

At higher ionic strength, Eg5 predominantly displays unbiased diffusional motion along microtubules. Quantitative analysis using mean square displacement shows that the motile activity of tetrameric Eg5 consists of a combination of both diffusive and directional modes. The ratio of the motility modes depends on ionic strength; under higher ionic conditions, Eg5 displays simple Brownian diffusion with an increased diffusional coefficient compared with that at lower ionic strength. The diffusion rates are similar to those in the presence of ADP, suggesting that the diffusive mode is independent of ATP hydrolysis. It is not clear how Eg5 diffuses along microtubules, but using the analogy with other kinesin motors (Okada and Hirokawa, 2000), it might involve weak electrostatic interactions between the negatively charged C terminus of tubulin and positively charged residues on Eg5 (possibly in the head or tail domain because dimeric Eg5 did not display persistent diffusion).

Furthermore, Kapitein et al. (2008) show that microtubule bundling is also a trigger for kinesin-5 activation. They first note that Eg5 displays directional movement along axonemes, which are bundles of microtubules arranged in a regular 9 + 2 pattern, even under high ionic strengths. Then, they use a microtubule–microtubule sliding assay that has previously been used to demonstrate the ability of Eg5 to cross-link and slide two fluorescently labeled microtubules (Kapitein et al., 2005). In this case, however, they simultaneously observe the movements of fluorescent microtubules and of GFP-tagged Eg5 molecules on the microtubules to directly test whether microtubule cross-linking alters the motional modes of individual Eg5 molecules. They found that Eg5 occasionally shows directional movement within the region where two microtubules overlap and slide, whereas outside the overlap zone, Eg5 predominantly displays diffusive motion. These results provide direct evidence that Eg5 switches from diffusive to directional mode upon microtubule cross-linking.

These new results by Kapitein et al. (2008) reveal that the motile activity of kinesin-5 changes between diffusive and directional modes and that the balance between these two modes depends on both ionic strength and microtubule cross-linking (Fig. 1 A). This latter mechanism presents a fascinating possibility for the regulatory mechanism of kinesin-5 in vivo. Kinesin-5 may recognize and accumulate at the region where two oppositely oriented microtubules overlap, and only after cross-linking these microtubules does kinesin-5 initiate antiparallel microtubule sliding by using ATP hydrolysis energy. These experiments also propose important questions for the future: how is kinesin-5 activated though microtubule cross-linking or decreased ionic strength, and what is the structural difference between kinesin-5 in the two motional modes? It is puzzling how the information of microtubule binding in one end of the tetramer is transmitted to the opposite end. A unique conformation of kinesin-5's neck linker observed in the crystal structure may be involved in sensing the force applied toward perpendicular to the microtubule long axis (Turner et al., 2001). Moreover, the regulatory mechanisms of the other mitotic kinesin (i.e., kinesin-14 or ncd) that antagonizes kinesin-5 and chromosomal kinesins (Tao et al., 2006; Kim et al., 2008) also need to be elucidated, and this information would be assembled to describe how these motors coordinate for proper bipolar spindle formation using computer simulations (Nédélec, 2002) and to test using reconstituted or in vivo analysis.

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