

Kinesin-Related Proteins at Mitotic Spindle Poles: Function and Regulation

Minireview

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The mitotic spindle, which consists of a dynamic array of microtubules and associated proteins, is responsible for segregation of chromosomes during mitosis. At least seven different families of kinesin-related proteins (KRPs) have been localized to the mitotic spindle (Figure 1). Many KRPs have been proposed to function in spindle assembly, to drive chromosome movement, to maintain a force that holds the spindle together, to drive microtubule flux, and to control microtubule dynamics within a spindle (reviewed by Sawin and Endow, 1993; Bloom and Endow, 1995; Vernos and Karsenti, 1996). Despite the large number of these microtubule motor proteins within a spindle, there has been no clear demonstration of the function of any of them, although hypotheses have proliferated.

One approach to understanding KRP function in spindle assembly is to look for conservation of function between the simplest and more complex mitotic systems. Only four mitotic KRPs have been identified in *Saccharomyces cerevisiae*, compared with as many as eight in other organisms. The yeast proteins must then represent the minimal subset of KRPs required to build a mitotic spindle. Two of the yeast KRPs, *Cin8* and *Kip1*, are part of a large superfamily of KRPs, the *bimC* family, that have been implicated in spindle assembly and function in many divergent organisms (Hoyt et al., 1992; Roof et al., 1992). Recent papers on homologs of *Cin8* and *Kip1* in higher eukaryotes may reveal the function and regulation of these proteins in building the mitotic spindle;

they form the basis of this review (Blangy et al., 1995; Sawin and Mitchison, 1995; Kashina et al., 1996). The other highly conserved mitotic motor family is the minus-end-directed *Kar3* family, which appears to play an antagonistic role to the *bimC* family of KRPs (reviewed by Sawin and Endow, 1993). Owing to space limitations, we will not discuss this family of KRPs or any of the other motor proteins families diagrammed in Figure 1 (see Sawin and Endow, 1993; Bloom and Endow, 1995; Vernos and Karsenti, 1996, for recent reviews).

The *BimC* Family of KRPs

bimC mutants in *Aspergillus nidulans* have a defect in spindle pole body separation and mitotic spindle formation. The *bimC* gene product was cloned and found to encode a protein with homology to vesicle kinesin (Enos and Morris, 1990). This was the first identification of a KRP involved in mitosis and the beginning of a new era in mitosis research. *BimC* family members are conserved throughout evolution, since homologs have been isolated from widely divergent organisms including yeast, *Xenopus*, *Drosophila*, and humans (Table 1). Presumably, all eukaryotes use a member of the *bimC* family during mitosis. These homologs share approximately 50%–60% identity within the motor domain and little or no homology outside the motor domain, with the exception of a short, interesting stretch in the tail (see below).

In addition to conserved sequence, it is likely that *bimC* homologs share similar biochemical properties. *Xenopus* Eg5, *Drosophila* KRP₁₃₀, and *Drosophila* KLP61F are slow (1–2 μm/min), plus-end-directed motors *in vitro* (Sawin et al., 1992; Cole et al., 1994; Barton et al., 1995). All three motors also show unusually tight binding to microtubules. KRP₁₃₀ was analyzed biochemically and predicted to be a homotetramer, based on its size on both gel filtration chromatography and on sucrose gradients (Cole et al., 1994). A recent electron microscopic study of KRP₁₃₀ has demonstrated that this protein exists as a bipolar homotetramer with pairs of motor domains at opposite ends of the molecule (Kashina et al., 1996). It will be interesting to analyze biochemically other *bimC* homologs to see if this bipolar homotetrameric structure is conserved. We think that it will be, and this opinion is reflected in our models.

The most extensive genetic analysis of *bimC* homologs has been in *S. cerevisiae* on *Cin8* and *Kip1* (Hoyt et al., 1992; Roof et al., 1992). *Cin8* and *Kip1* appear to play a redundant function in spindle assembly. Loss of *Cin8* alone causes a block in mitotic progression, which can be overcome by overexpression of *Kip1*. Loss of *Kip1* alone has no phenotype, although the double mutant is lethal. The *Cin8/Kip1* proteins appear to play a role in spindle pole separation and are required for maintenance of the bipolar spindle structure. A further analysis of *Cin8/Kip1* proteins has shown that their activity is also required later in mitosis for anaphase chromosome segregation (Saunders et al., 1995). *S. pombe cut7* mutants and *D. melanogaster KLP61F* mutants also have defects in spindle pole body separation and form defective mitotic spindles (Hagan and Yanagida, 1990; Heck et al., 1993).

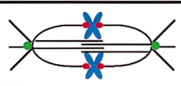
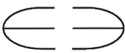
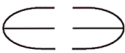





Motor	Role	
BimC Family	Spindle Pole Separation	
Kar3 Family	Spindle/Pole Structure	
Xklp2	Spindle Pole Separation	
MKLP1 CHO1	Antiparallel Microtubule Sliding	
Chromokinesins Nod*	Chromosome Positioning	
Cenp-E	Chromosome Movement	
MCAK/XKCM1	Microtubule Dynamics	

Figure 1. Localization and Putative Function of KRPs Involved in Mitosis

The left column denotes the protein family. The center column denotes the putative function, and the right column diagrams the localization pattern of the protein. Black, microtubules; green, centrosomes; blue, chromosomes; red, kinetochores.

*Nod localization has only been demonstrated in meiotic spindles.

Table 1. Properties of BimC Family Members

Motor	Organism	Motility	Structure	Localization	Phosphorylation
bimC	<i>A. nidulans</i>	ND	ND	ND	Role Unknown
Cin8	<i>S. cerevisiae</i>	ND	ND	Spindle, ↑ Pole	No Site
Kip1	<i>S. cerevisiae</i>	ND	ND	Spindle	No Site
Cut7	<i>S. pombe</i>	ND	ND	Spindle, ↑ Pole	Role Unknown
KLP61F	<i>D. melanogaster</i>	1 μm/min, +end	ND	Spindle	Role Unknown
KRP130	<i>D. melanogaster</i>	2.4 μm/min, +end	Bipolar Tetramer	ND	Not Sequenced
Eg5	<i>X. laevis</i>	2.1 μm/min, +end	ND	Spindle, ↑ Pole	Localization
HsEg5	<i>H. sapiens</i>	ND	ND	Spindle	Localization

ND, Not Determined.

BimC homolog function has also been studied in biochemically tractable systems. *Xenopus laevis* Eg5 function has been analyzed using an in vitro spindle assembly reaction in *Xenopus* egg extracts (Sawin et al., 1992). Either immunodepletion of Eg5 or antibody addition to extracts causes a defect in both half-spindle and bipolar spindle formation. Initially, only half-spindles with defective poles form. This suggests a role for Eg5 in assembly of the spindle pole itself. Eventually, aberrant structures form that look like rosettes of microtubules with DNA at the periphery. These rosettes also form if antibodies are added after spindle formation. This phenotype is consistent with a role for Eg5 in spindle pole separation and for bipolar spindle maintenance. A more recent study has looked at a human bimC homolog called HsEg5 (Blangy et al., 1995). Microinjection of antibodies to HsEg5 causes a striking phenotype: about 80% of the injected cells arrest in mitosis with a prometaphase-like chromosome alignment and no spindle pole separation. Consistent with other bimC homologs, both Eg5 and HsEg5 appear to play similar roles in spindle pole formation and/or separation.

Mechanism of BimC Family Function

The simplest model for bimC homolog function is that the protein cross-links microtubules and walks towards the plus end, thus pushing the two spindle poles apart (Figure 2a). This model is consistent with much of the

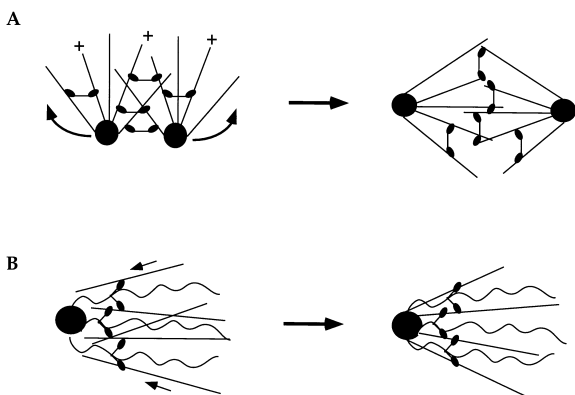


Figure 2. Models for BimC Homolog Function

(A) Plus-end-directed motor cross-links microtubules extending from adjacent poles and pushes poles apart. Arrows indicate the movement of the spindle poles.

(B) Plus-end motor anchored by tail to spindle matrix organizes microtubules at spindle pole. Arrows indicate the direction of microtubule movement.

functional data on bimC homologs; however, it is not totally consistent with the localization data. In cases in which bimC homolog localization has been determined, the protein is found on spindle microtubules, sometimes with an enrichment near spindle poles (Hagan and Yanagida, 1992; Hoyt et al., 1992; Roof et al., 1992; Sawin et al., 1992; Barton et al., 1995; Sawin and Mitchison, 1995; Table 1). One might expect to find a protein important in cross-linking of microtubules to be enriched in the central spindle, where the density of microtubule overlap is the highest. Indeed, *S. pombe* cut7 has been found in central spindles during anaphase, but it is enriched at the spindle pole during the rest of mitosis. It is possible that the spindle pole enrichment is due to an equilibrium distribution in which the protein accumulates at the pole over time because it is carried there by microtubule flux. Another idea is that a bimC homolog is involved in spindle pole organization (Figure 2b). By binding through the tail domain to a nonmicrotubule spindle matrix, a plus-end-directed motor could reel in microtubules to the pole. In this model, failure in pole separation could be a secondary defect due to an improperly formed spindle pole. Alternatively, spindle pole organization and spindle pole separation might be independent processes.

Regulation of Localization by Phosphorylation

Conversion of the interphase microtubule array to the mitotic spindle is a dramatic example of cytoplasmic reorganization that is triggered by activation of cdc2 kinase activity. It seems likely that some, if not all, mitotic motors will be substrates of this kinase or downstream kinases. Several members of the bimC family (bimC, Eg5, KLP61F, cut7, and HsEg5) contain a short 40 amino acid stretch in the tail domain called the bimC box. Within this 40 amino acid sequence is a conserved sequence TGXTPXK/RR, which is a consensus sequence for proline-directed kinases such as cdc2. By transient transfection, it has been shown that mutation of the phosphorylatable threonine to alanine or to aspartic acid abolishes recruitment of the protein to the mitotic spindle; however, mutation of the threonine to serine preserves spindle localization, suggesting that phosphorylation is necessary (Blangy et al., 1995; Sawin and Mitchison, 1995). A more detailed analysis of HsEg5 phosphorylation demonstrated that HsEg5 was phosphorylated in vivo at T-927 during mitosis and that the same site could be phosphorylated in vitro by cdc2 kinase (Blangy et al., 1995). Taken together, these results strongly suggest that phosphorylation of HsEg5/Eg5 by cdc2 kinase at this site is required for recruitment of the protein to the mitotic spindle.

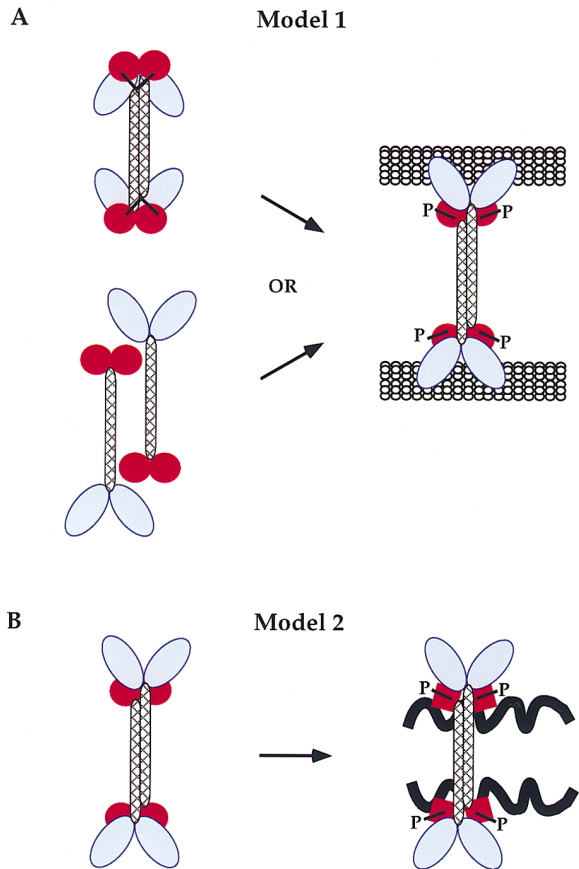


Figure 3. Models for Regulation of BimC Homolog Activity by Phosphorylation

(A) Phosphorylation of the tail (red) regulates the structure of the motor (blue).

(B) Phosphorylation of the tail causes a conformational change of the tail that allows it to bind to the spindle matrix (thick squiggly line).

A further analysis of the requirements for Eg5 localization revealed that the full-length Eg5 sequence was required for proper spindle localization (Sawin and Mitchison, 1995). Expression of either an N-terminal fragment or a C-terminal fragment is not sufficient for localization. This suggests that unlike kinesin or some other KRPs, the tail domain of Eg5 is not sufficient for tethering the protein to microtubules or to other spindle components. Thus, it is possible that the bipolar structure is important for localization.

The potential roles for phosphorylation of bimC homologs can be divided into two classes. In model 1, phosphorylation regulates the structure and/or activity of the motor itself or its interaction with microtubules (Figure 3a). Phosphorylation could control the tetramerization of a bimC homolog, and only the tetramer can bind to microtubules and carry out its function, analogous to the regulation of myosin filament assembly by phosphorylation (Egelhoff et al., 1993). Alternatively, phosphorylation may regulate the interaction of the motor domain of a bimC homolog with microtubules. For example, phosphorylation of the tail domain could cause a conformational change that allowed the motor domain to bind to microtubules. In support of model 1, the Eg5

C-terminus alone does not localize to the mitotic spindle even with an intact phosphorylation site, suggesting that phosphorylation does not generate a simple binding site for another spindle component. The fact that no spindle enrichment is seen in phosphorylation site mutants suggests that the motor heads are inactive in microtubule binding, again consistent with model 1. In model 2, phosphorylation regulates the interaction of the motor with some other unidentified spindle component (Figure 3b). In support of model 2, the enrichment of bimC homologs near mitotic poles, which is not the localization predicted from microtubule interactions alone, suggests that binding to some other spindle component may be regulated. The observation that Eg5 depletion affects the structure of half-spindles, where overlapping antiparallel microtubules are not present, is also consistent with model 2, in that the motor may bridge microtubules and some other spindle component.

The above models require that phosphorylation of bimC homologs is required for mitotic spindle localization, but the *S. cerevisiae* proteins Cin8 and Kip1 do not contain the conserved tail sequence that is shared by other bimC homologs. Given that the timing of spindle assembly and mitosis during the *S. cerevisiae* cell cycle is dramatically different from that in other eukaryotes, it is not surprising that *S. cerevisiae* uses a different mechanism for Cin8/Kip1 localization (reviewed by Page and Snyder, 1993). It will be interesting to examine the requirements for Cin8/Kip1 protein localization during *S. cerevisiae* spindle assembly.

Summary and Future Directions

Clearly, bimC homologs are essential for mitotic spindle assembly. All members that were analyzed functionally appear to play some role in spindle pole formation or separation. Whether all the bimC motors play the same role or whether the protein sequence is conserved and the precise function has diverged slightly to accommodate differences in mitosis between organisms is not known. In the future, it will be important to pursue the exact mechanism of spindle pole function of each family member. Resolution of the role of phosphorylation is likely to tell us a great deal about the function of the bimC family motors. It should be relatively straightforward to determine whether phosphorylation of the tail directly affects motor structure or function, for example by regulating tetramer formation or ATPase activity. Formation of higher order oligomers by polymerizing tetramers is another interesting possibility but one for which there is no evidence at the present time. Determining whether phosphorylation regulates binding to some other spindle component may be more difficult; the interaction need not be strong, since the motor domain itself might tend to concentrate the protein in the spindle. If bimC homologs do indeed interact with another, presumably conserved, spindle component, its identification would then become an important priority for students of spindle structure and function. Analysis of the function of bimC homolog phosphorylation will also set the stage for studying regulation of other mitotic kinesins that are likely to be equally interesting.

Selected Reading

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