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Review

The *bimC* family of kinesins: essential bipolar mitotic motors driving centrosome separation

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Contents

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1. Introduction

Cell division is a complex and important process that involves the formation of a mitotic spindle, a self-organizing bipolar microtubule-based protein machine that uses energy from ATP hydrolysis to ensure the correct segregation of chromosomes into two daughter cells. The spindle consists of a bipolar array of microtubules emanating from two opposite foci called spindle poles or spindle pole bodies,

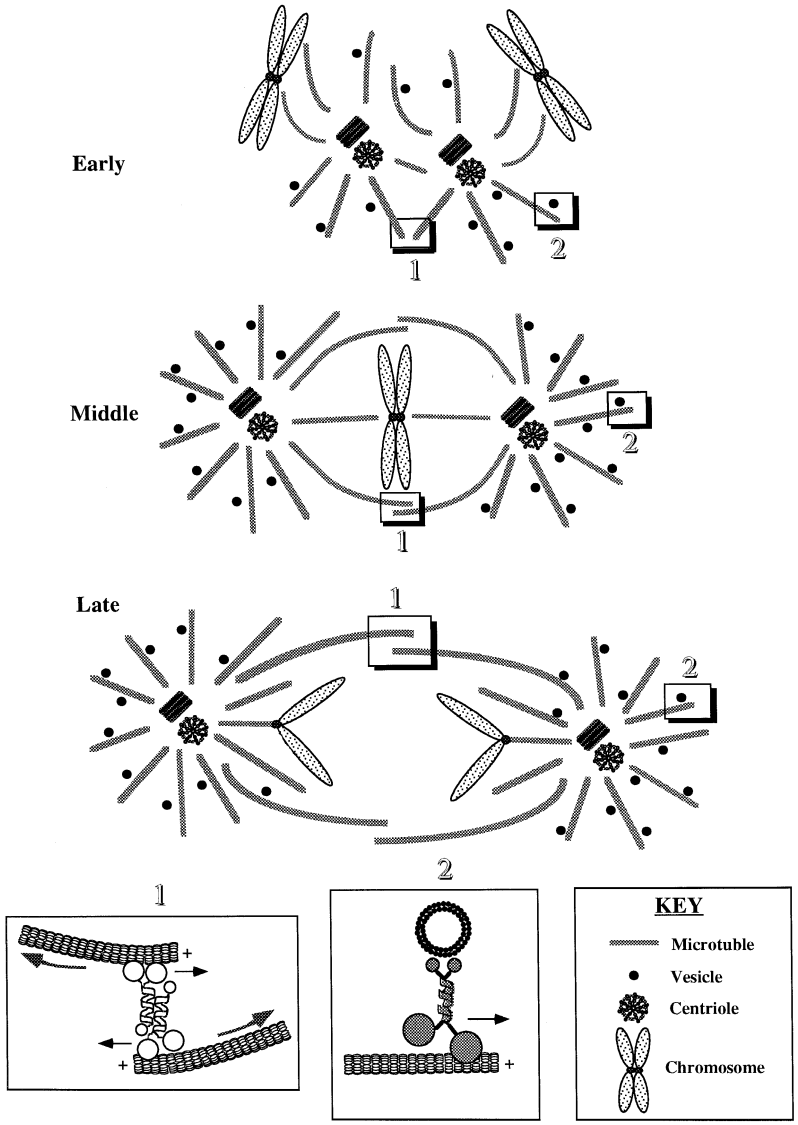

Fig. 1. Simplified diagram showing the assembly and function of a bipolar mitotic spindle. The diagram shows a schematic representation of the motile events occurring during the formation (early), maintenance (middle), and elongation (late) of a normal bipolar mitotic spindle. The different parts of the spindle are identified in the key. During early mitosis the two centrosomes consisting of pairs of centrioles move apart and nucleate arrays of microtubules, some of which capture chromosomes and align them on the spindle equator. By metaphase (middle) the spindle has assumed a bipolar structure with centrosomes at opposite spindle poles and chromosomes aligned at the metaphase plate. During anaphase (late) sister chromatids migrate slowly towards opposite spindle poles (anaphase A) and the poles themselves are pushed apart (anaphase B). Boxes 1 and 2 show the proposed location and mechanism of function of microtubule-microtubule sliding motors (box 1) and vesicle motors (box 2) in the spindle. Microtubule-microtubule sliding motors, including members of the *bimC* family, could exert forces that push apart the spindle poles in the manner indicated (box 1).

together with a number of microtubule-associated proteins that are involved in the attachment of chromosomes and membrane vesicles, and in the positioning and movement of various spindle components (Fig. 1). Critical microtubule-dependent events that occur in the spindle during cell division include the formation, maintenance and elongation of the bipolar spindle, and the segregation of chromosomes into two daughter cells. These events are associated with the movement of different structures, including chromosomes, vesicles, and microtubules along spindle microtubules, and biochemical, genetic and cytological studies suggest that they are mediated by microtubule-based motor proteins [1]. One important series of motor-dependent mitotic events concerns centrosome organization and motility; the concerted action of several families of microtubule motor proteins are currently thought to participate in centrosome movements and spindle pole organization [2,3], and this review focuses on one of these families, namely the *bimC* family of kinesin motor proteins that are thought to participate in the assembly, maintenance and elongation of bipolar mitotic spindles [4]. Recent studies indicate that *bimC* family proteins are bipolar motor proteins that exert their function by crosslinking and sliding apart antiparallel microtubules.

2. Microtubule motor proteins

There are two major groups of microtubule motors identified so far – dyneins and kinesins. Dyneins are known to mediate movement towards the 'minus' ends of the microtubules and there is evidence for the presence of cytoplasmic dynein on spindle fibers and kinetochores [5,6]. While direct evidence that dynein participates in chromosome-to-pole motion is currently lacking, antibody microinjection and immunodepletion experiments suggest a role for dynein in spindle pole organization, spindle pole separation, and bipolar spindle assembly [3,7,8]. Recently, Karsenti et al. (1996) [2] have hypothesized that dynein anchored on the cortex and nuclear envelope could exert pulling forces on centrosomes and, in concert with plus-end-directed microtubule motors, could precisely control the position of centrosomes. The plus-end-directed motor proteins that participate in mitotic movements are likely to be members of the kinesin superfamily, most of which mediate movement towards the 'plus' ends of microtubules (the best-known exceptions being the minus-end-directed carboxy-terminal kinesins).

The classification of motor proteins into the kinesin superfamily is based on sequence homology within a ~350 amino acid region called the 'motor' domain. The structure of conventional kinesin, the founding member of the superfamily, is shown in Fig. 2A [9,10]. Kinesin contains two kinesin heavy chains (KHC), each having an N-terminal globular motor domain whose atomic structure is now known

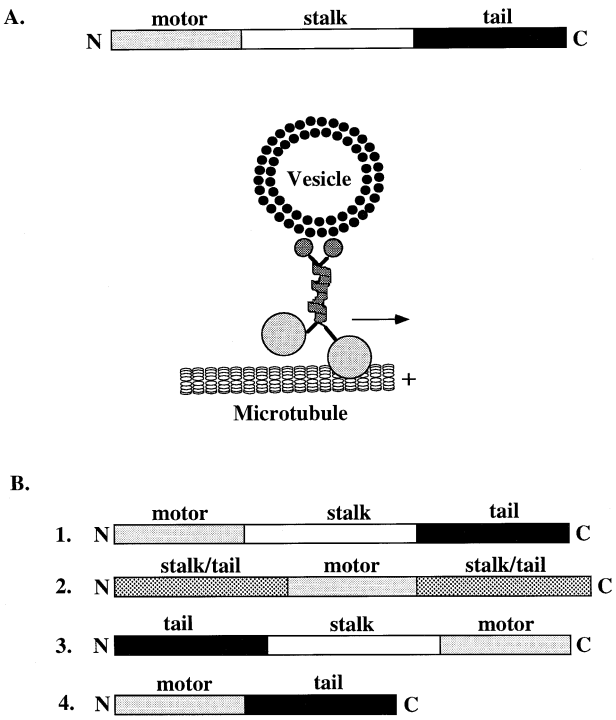

Fig. 2. Structure of conventional kinesin and other members of the kinesin superfamily. (A) Linear map (top) and molecular model (bottom) of kinesin structure. Two motor domains of the kinesin homodimer form globular 'heads' capable of moving along a microtubule (arrow). Two stalk domains dimerize to form a coiled coil rod, followed by a tail domain presumably responsible for cargo (vesicle) attachment that has associated light chains. (B) Maps of domain organization predicted from primary structures of different members of the kinesin superfamily. 1 – motor-stalk-tail structure of conventional kinesin. 2 – stalk/tail-motor-stalk/tail structure of 'central-motor' kinesins. 3 – tail-stalk-motor structure of C-terminal motor kinesins. 4 – motor-tail structure of monomeric kinesins.

[11], followed by an α-helical coiled coil stalk and a globular tail. The two KHCs dimerize in the regions of their coiled coil stalks (below) forming a structure capable of 'walking' along microtubules, with its two globular heads working in a 'hand over hand' fashion. The tail end of the molecule is believed to be responsible for cargo binding [12], and may have light chains associated with it [10]. The role of the light chains is currently unknown.

The motor domains of all kinesins minimally share approximately 35–45% sequence identity with the KHC motor domain and possess conserved ATP-binding consensus sequences. In many cases, the motor domains have been shown to display nucleotide-sensitive binding to microtubules, and they are proposed to couple nucleotide hydrolysis to force generation, thus accounting for the 'motor' functions of kinesins. The kinesin superfamily of motors is divided into 8 families [13] based on amino acid sequence comparison. While the putative motor domains of all members of the kinesin superfamily display at least 35% amino acid sequence identity with the KHC motor domains, the motor domains of members of a particular family typically share a significantly higher level of sequence identity than this, and in some cases, a high level of sequence identity can be found even outside the motor domains as well. The primary structure of members of different families of kinesins often predict differences in the domain organization of the molecules (Fig. 2B), and different kinesin-related polypeptides are believed to self-assemble, with or without accessory subunits, into different oligomeric states ranging from monomer to tetramer [14]. It is hypothesized that these different quaternary structures represent adaptations that allow different kinesin holoenzymes to carry out specific biological functions [14].

It has been shown that many members of the kinesin superfamily are localized to different parts of the mitotic spindle (see [1] and [4] for review). For example, CENP-E is shown to localize to kinetochores and may be involved in chromosome positioning and movement [15], CHO1 localizes to the microtubules in the interzone and is potentially involved in microtubule-microtubule sliding in late stages of mitosis [16], XKLP2 is localized to spindle poles and may participate in centrosome movement [17], and kinesin-II localizes to punctate structures associated with spindle microtubules and may play a role in vesicle transport in the spindle [18]. This review focuses on one family of spindle kinesins, named the *bimC* family after its founding member. Members of the *bimC* family of motors have been shown to localize to spindle microtubules, and it is hypothesized that they, acting in concert with minus-end-directed dyneins and other plus-end-directed kinesins such as CHO1 and XKLP2, serve to control the position of centrosomes and thus play global roles in establishing and maintaining bipolar mitotic spindle structure.

Table 1

Name	Organism	Function	Localization	Motility	Reference
bimC	*Aspergillus nidulans*	Spindle pole separation	–	–	[19]
cut7	*Schizosaccharomyces pombe*	Spindle formation	Spindle microtubules; enriched at spindle poles at early stages of mitosis and in midzone at late stages.	–	[20,40,41]
Eg5	*Xenopus laevis*	Spindle formation	Spindle microtubules	(+)-end directed, 2 μm/min	[23,26,28,38,42–44]
KLP61F/ KRP130	*Drosophila melanogaster*	Spindle pole separation	Spindle microtubules	(+)-end directed, 1 μm/min	[24,25,34,35,45,46]
CIN8 KIP1	*Saccharomyces cerevisiae*	Spindle pole separation and maintenance of the bipolar spindle structure	Spindle microtubules	–	[30,31,47,48]
HsEg5	*Homo sapiens*	Spindle pole separation	Spindle microtubules	–	[22,27,39]

3. The *bimC* family of slow, plus-end directed, spindle-associated kinesins.

The first member of the *bimC* family of kinesins was discovered in a genetic screen for temperature-sensitive lethal mitotic genes in *Aspergillus nidulans* as a mutant that was 'blocked in mitosis' [19]. Temperature-sensitive *bimC* mutants grown at the restrictive temperature failed to separate their duplicated spindle pole bodies during early stages of mitosis, resulting in mitotic defects such as abnormal spindle morphology and failure of nuclear division. The *bimC* gene proved to encode a 132 kDa, 1184 residue polypeptide with an N-terminal putative 'motor' domain sharing 42% sequence identity with the motor domain of the kinesin heavy chain, providing the first direct evidence for the participation of a member of the kinesin superfamily in mitotic spindle function [19].

Mutations in the fission yeast *cut7* gene caused a similar defect to the *Aspergillus bimC* gene, and indeed the cloning and sequencing of the *cut7* gene demonstrated that it also encodes a kinesin-related protein whose N-terminal motor domain shares high sequence identity with the motor domain of the *bimC* protein [20]. The phenotypes of *bimC* and *cut7* mutations suggested a common function, yet, interestingly, the stalk-tail regions of the two proteins (regions of the molecules which are thought to confer functional specificity) showed no significant sequence identity.

Subsequently, another five members of the *bimC* family were identified in different organisms, bringing the total number of *bimC* family members up to seven (Fig. 3 and Table 1), with all seven sharing considerable sequence identity (~50–60%) within their N-terminal motor domains but virtually no similarity within their stalk-tail regions except in a 40 amino acid segment at the very C-terminus, called the '*bimC* box' (see below). (The idea that the silkworm, *Bombyx mori*, contains a *bimC* motor with a C-terminal motor domain requires further work [21]).

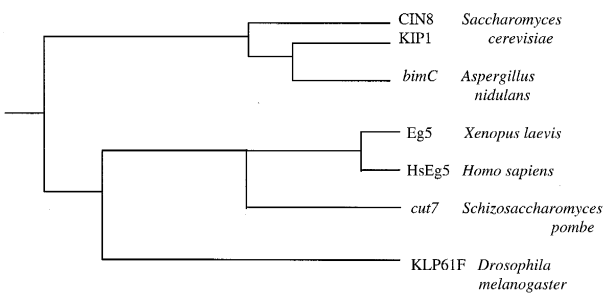

Fig. 3. Phylogenetic tree of the *bimC* family of kinesins. The tree was built based on motor domain sequence alignments as described in ref. [13]. The tree shown is a fragment of a phylogenetic tree of kinesin superfamily shown in ref. [13].

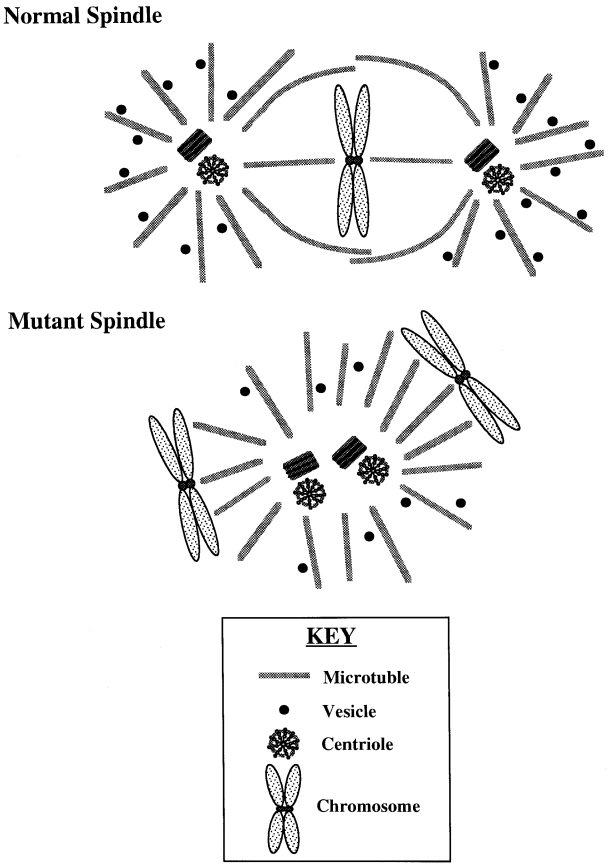

Fig. 4. Simplified diagram showing the effect of mutations in *bimC* family members on the assembly and function of mitotic spindles. The upper panel (normal spindle) shows a normal bipolar mitotic spindle at metaphase, corresponding to the middle stage in figure 1. The lower panel (mutant spindle) shows the abnormal morphology of spindles at a comparable mitotic stage in organisms carrying mutations in *bimC* family motor proteins (see Table 1). While normal cell division involves the formation of a bipolar spindle, *bimC* family mutants are characterized by the formation of monoastral microtubule arrays containing duplicated spindle poles that fail to separate. Different parts of the spindles are identified in the key.

Bona fide members of the *bimC* family of kinesins share several known properties as summarized in

Table 1. In all known cases *bimC* family proteins prove to be localized to spindle microtubules (Table 1), and sometimes an enrichment of the protein at the spindle poles has been noted, but we suspect that at least in some cases this may reflect a higher density of tubulin-associated antigens around the spindle poles. Interestingly, among the anti-*bimC* family member antibodies that stain mitotic spindles are autoantibodies from systemic lupus erythromatosus patients which react with the colied coil rod domain of HsEg5 [22].

Studies of the motor properties of some *bimC* proteins have been performed and in all cases they prove to be slow (+)-end directed microtubule motors, with rates of motility close to the rates of some motile events in the spindle, including centrosome separation during prophase and anaphase [23–26]. *bimC* family members also display unusual microtubule-binding properties; in microtubule cosedimentation assays they bind to microtubules strongly both in the presence of AMPPNP and ATP in low salt buffers, and display the kinesin-like AMPPNP-enhanced, ATP-sensitive microtubule binding only at elevated salt concentrations that are close to physiological [23–25].

Finally, the following types of evidence implicate *bimC* motors in spindle pole separation and in the maintenance of the bipolar spindle structure. Mutations in the *bimC*, *cut7* and *KLP61F* genes appear to block the separation of duplicated centrosomes or spindle pole bodies resulting in the formation of defective 'monoastral' mitotic apparati at early stages of mitosis (Table 1; Fig. 4). Similarly, the microinjection of anti-Eg5 antibodies inhibits centrosome separation leading to the formation of monoastral microtubule arrays with disorganized 'poles' [3,27], and in vitro assays of spindle formation in cell-free mitotic extracts of *Xenopus* oocytes showed that immunodepletion of Eg5 causes defects in spindle formation at early stages of mitosis [3,28].

All of this evidence supports the hypothesis that motors of the *bimC* family participate in the separation of duplicated spindle pole bodies or centrosomes during spindle formation, maintenance, and elongation. Interestingly, genetic studies have shown that in *S. cerevisiae*, the *bimC*-related *cin8* and *kip1* genes redundantly perform this essential mitotic function, despite their lack of sequence conservation outside the motor domains (see [29] for review).

The mitotic function of *bimC* motors is thought to

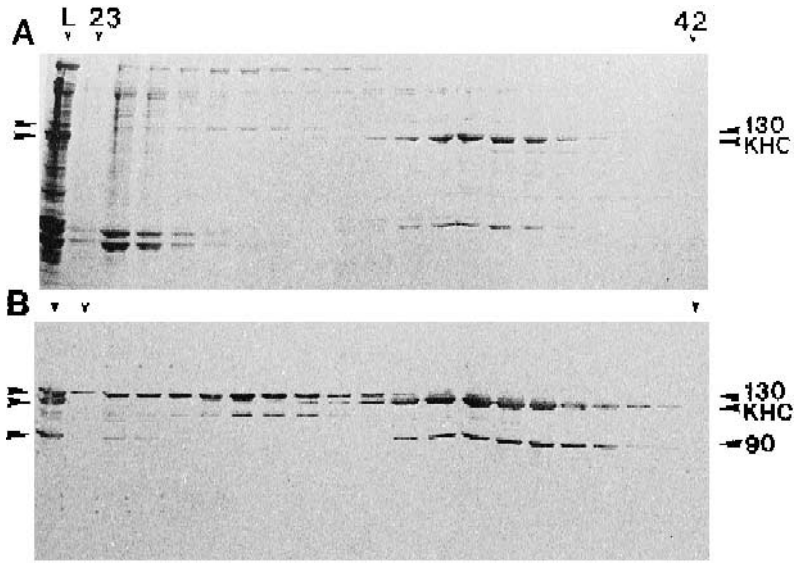

Fig. 5. Pankinesin peptide antibody screen of the *Drosophila* embryonic cytosol. Polyacrylamide gel (A) and corresponding immunoblot with pankinesin peptide antibody (B) of the gel filtration fractions of *Drosophila* MAPs. Pan-kinesin peptide antibody recognizes 3 major bands (indicated on the right as 130, KHC, and 90). Probing with anti-Eg5 and anti-kinesin antibodies showed the 130 kDa band to correspond to an Eg5 homolog (KRP130) and the 120 kDa band (KHC) to correspond to kinesin heavy chain. Lane L shows the ATP-eluted MAPs that were loaded onto the gel-filtration column, numbers on top indicate the beginning and end gel-filtration fractions loaded on the PAGE.

Table 2

	Biochemical properties of KRP130	Biochemical properties of recombinant KLP61F
MT binding	Strong in AMPPNP Strong in ATP-low salt Weak in ATP-high salt	Strong in AMPPNP Strong in ATP-low salt Weak in ATP-high salt
MT-activated ATPase	n/a	0.25 μmol/min/mg K_m (ATP) = 80 μM K_m (MT) = 0.9 μM
MT motility	0.04 μm/s (+)-end directed	0.025 μm/s (+)-end directed
MT crosslinking	a. Bundles MTs in motility assays b. Crossbridges observed in negative staining and rotary shadowing EM	MT bundling not observed in motility assays
Subunit MW	130 000 Da	120 000 Da
R_s	16.2 nm	n/a
S value	7.6 S	n/a
Native MW	490000 Da	n/a
Stoichiometry	4 × 130 000 Da	n/a
Dimensions (in rotary shadowing EM)		n/a
Length:	100 nm	
Heads:	20 × 20 nm	
Stalk:	60 nm	

require the participation of counterbalancing minus-end-directed C-terminal motors of the *kar3* family. For example, it has been shown that a disruption of the *kar3* gene can suppress the phenotype of unseparated spindle poles caused by *CIN8/KIP1* double mutants in *S. cerevisiae* [30,31]. In addition, a mutation of *klpA*, a KAR3 homolog in *A. nidulans*, can suppress a mutant phenotype caused by *bimC* [32] and a disruption of a KAR3 homolog, *pkl1* in *S. pombe*, suppresses mutations of *cut7* [33]. It is therefore believed that the C-terminal minus-end directed kinesins can produce forces that oppose the *bimC*-driven forces in the mitotic spindle, and thus take part in coordination of the complex motile events leading to the bipolar spindle assembly and maintenance.

While genetic and immunological approaches revealed the likely function of the members of the *bimC* family in driving the separation of spindle poles, the molecular mechanism of their action remained unclear. Biochemical studies described below have revealed that at least one member of the *bimC* family, namely the KLP61F polypeptide, self-assem-

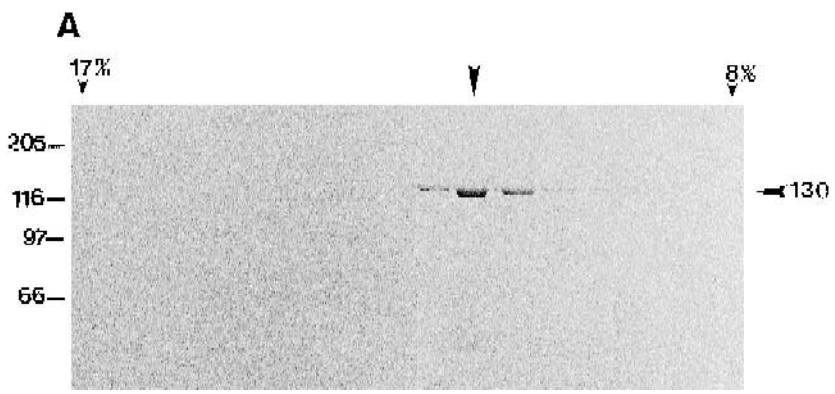

Fig. 6. Polyacrylamide gel of the sucrose gradient purified KRP130. Numbers on left indicate molecular weight standards. Numbers on top show the percentage of sucrose in the upper and lower areas of the gradient. Arrows indicate the peak fraction of KRP130.

bles into a bipolar homotetramer (KRP130) thus providing clues concerning the molecular mechanism by which *bimC* motors can drive centrosome separation.

4. Purification and characterization of KRP130, a bipolar *bimC*-related kinesin from *Drosophila* embryos.

The first purification of a *bimC*-related kinesin-like protein as a native oligomeric holoenzyme was accomplished from *Drosophila* embryonic extracts with the aid of polyclonal antibodies raised against hyperconserved regions within the kinesin motor domain (pankinesin peptide antibodies) ([24] and Fig. 5), and led to the acquisition of information that nicely complements the aforementioned genetic and cytological studies.

In this work, immunoblots of total *Drosophila* microtubule proteins pelleted in the presence of AMPPNP, were probed with a pan-kinesin peptide antibody which revealed several putative kinesin-re-

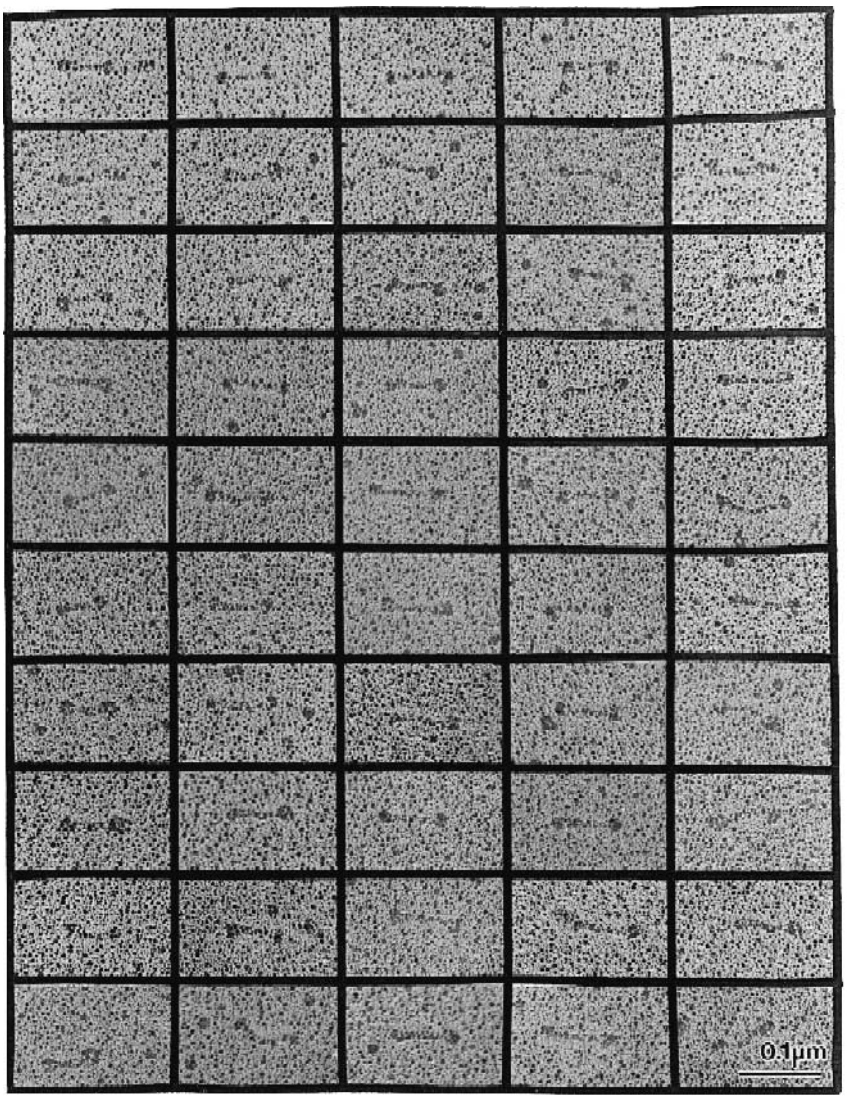

Fig. 7. Electron micrographs of rotary shadowed KRP130 molecules. Shown here is a gallery of images of KRP130 molecules rotary shadowed with platinum.The molecules appear elongated, with enlarged globular ends, generally shaped as dumbells. We hypothesise that the enlarged ends represent two closely juxtaposed motor domains, while the elongated part in between represents two α-helical coiled coils lying adjacent to each other in an antiparallel 4-stranded coiled-coil bundle arrangement (see [34] for details).

lated polypeptides (Fig. 5), one of which, a 130 kDa polypeptide, also showed crossreactivity with polyclonal anti-Eg5 antibody. This protein was purified using a combination of size-fractionation and ATP-sensitive microtubule affinity binding. The new protein did not copurify with any other obvious associated polypeptides through the final sucrose density gradient centrifugation step, leading to the conclusion that it was composed of only one type of polypeptide with a molecular weight of 130 kDa (Fig. 6). It was therefore designated KRP130.

Initial characterization showed that KRP130 shares several distinct biochemical properties with bacterially expressed *Xenopus* Eg5. First, as mentioned above, it was crossreactive with a polyclonal antibody against Eg5. Second, it showed similar motor properties to the bacterially expressed Eg5, turning out to be a slow plus-end directed microtubule motor. Finally, it displayed Eg5-like microtubule binding properties with ATP-sensitivity in high-, but not low-salt buffers [23,24,34]. This evidence led to the proposal that KRP130 was a bona fide *bimC* family member, and therefore data on the properties of the KRP130 holoenzyme should provide insights into the native structure and properties of *bimC* family proteins in general.

The Stokes radius of KRP130, calculated from its behavior on the gel filtration column, was estimated at 16.2 nm, and its sedimentation coefficient, calculated from its position on a sucrose density gradient – 7.6 S. From these numbers it was possible to calculate a native molecular weight of approximately 500 000 Da, and since KRP130 appeared to have no other polypeptides copurifying with it, this estimate of the native molecular weight was most consistent with the notion that four 130 kDa motor subunits self-assemble to form a homotetrameric holoenzyme (Table 2). Since these polypeptides were kinesin-related they were predicted to have a kinesin-like tripartite structure (see Fig. 2A). In addition, it was proposed by various groups that *bimC* family motors could drive spindle pole separation by crosslinking and sliding apart antiparallel microtubules, and therefore it was natural to hypothesize that the four 'motor' polypeptides of KRP130 should assemble in an antiparallel fashion, forming a structure with two motor domains at opposite ends of a bipolar molecule [24].

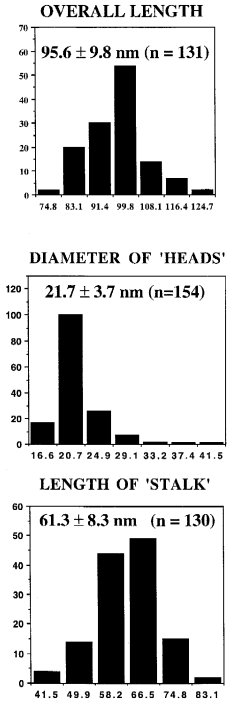

Fig. 8. Statistical analysis of the dimensions of KRP130 molecules. The images of KRP130 molecules as shown in Fig. 7 were measured to determine the overall lengths of the molecules, the length of stalks, and the diameter of globular ends. Each diagram shows number of measurements on *y* axis, and the measurement value on *x* axis. A mean was determined for each parameter based on n individual measurements, and the standard deviation (S.D.) was calculated. The mean, S.D. and number of measurements are presented above each histogram.

To test the hypothesis that KRP130 is a bipolar kinesin, Kashina et al. [34] performed a detailed electron microscopic analysis of purified KRP130 holoenzymes. Rotary shadow electron microscopy (EM) showed KRP130 molecules to be dumbbell-shaped, elongated structures with globular domains on both ends (Fig. 7). Measurements of their size showed an approximate length of 100 nm, consisting of a 60 nm 'stalk' flanked on either end by 20×20 nm 'heads' (Fig. 8). Comparison of these measurements with the measurements of conventional kinesin suggested that KRP130 molecules may be organized as bipolar tetramers, formed by two kinesin-like dimers associated in an antiparallel way with two motor domains exposed on each end of the molecule. To test this hypothesis, rotary shadow EM was per-

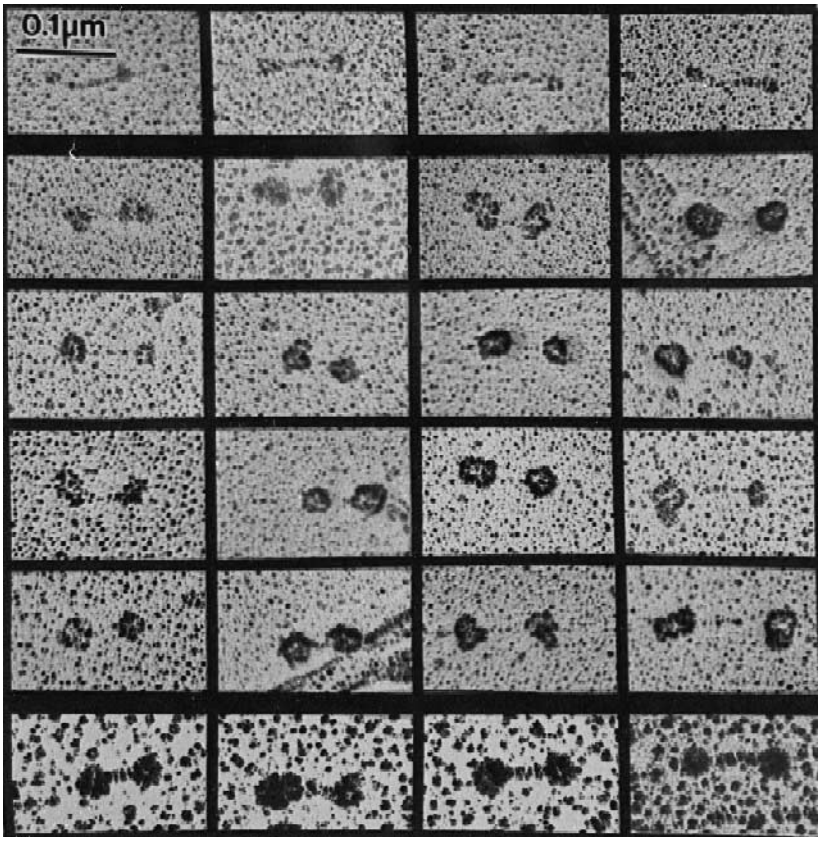

Fig. 9. Antibody against the motor domains decorates the ends of KRP130 molecules. Images of rotary-shadowed, antibody-decorated KRP130 molecules. Top row shows undecorated molecules found on the same grid which appear identical to the images in Fig. 7. Middle rows show molecules decorated with the anti-motor domain antobody. It can be seen that the globular ends of the molecules appear enlarged, while the stalk in between remains unchanged, except for the lighter platinum shadowing due to the shielding of the stalk from the low-angle platinum stream by enlarged heads. Statistical measurements of the enlarged 'heads' indicate two IgG molecules decorating each end of the KRP130 molecules, therefore supporting the hypothesis that two motor domains compose each end of the bipolar kinesin. Bottom row shows heavily shadowed molecules allowing better visualization of the stalk between the decorated 'heads' (see [34] for details).

formed on KRP130 molecules decorated with antibodies reacting with the motor domains of KRP130 (Fig. 9). Under these conditions the globular domains at the ends of the molecules appeared enlarged due to the binding of the motor domain antibodies to the globular 'heads', while the appearance of the central rod remained unchanged, thus supporting the hypothesis that KRP130 has a bipolar homotetrameric structure with two motor domains at each end of the rod. Thus, the structure of KRP130 molecules may be considered as being equivalent to two kinesin-like heavy chain dimers arranged into a bipolar tetramer with two motor domains on each end of the molecule (Fig. 10).

5. Identification of KLP61F as the gene encoding subunits of the bipolar KRP130 holoenzyme

Based on its biochemical and immunological properties, it was proposed that subunits of KRP130 might be encoded by the *Drosophila KLP61F bimC*-related gene [24]. This hypothesis gained strong support from the results of a microsequencing study of

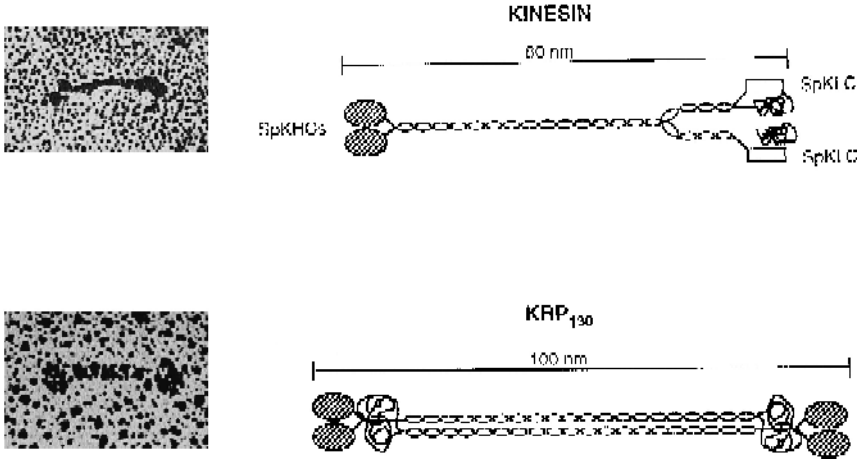

Fig. 10. Structural comparison of conventional kinesin and KRP130 holoenzymes. Left panels show rotary shadow electron micrographs, and right panels show models of the quaternary structures of the heterotetrameric kinesin and homotetrameric KRP130 holoenzymes.

purified KRP130 [35]. Sequence analysis and mass spectroscopic analysis of nine tryptic peptides derived from KRP130 revealed a 100% match with predicted tryptic peptides derived from the deduced sequence of the KLP61F protein, a previously identified *bimC* family member (Fig. 11). The identified peptides were distributed throughout the KLP61F sequence, including the stalk-tail region which is non-conserved among different members of the *bimC* family, indicating beyond reasonable doubt that a subunit of KRP130 and a KLP61F polypeptide are indeed one and the same.

This finding made it possible for the first time to combine biochemical and genetic data on the structure, properties and functions of a *bimC* family kinesin in one system, *Drosophila* (Table 2). It is noteworthy that the properties of recombinant KLP61F protein display striking similarities with the native KRP130 protein. KRP130/KLP61F is a slow, plus-end directed microtubule motor that shows characteristic AMPPNP-enhanced, ATP-sensitive microtubule binding only in physiological salt concentrations. The native holoenzyme is capable of bundling microtubules in in vitro motility assays, and the rate of its movement on microtubules is similar to the rates of mitotic events in the spindle. Immunolocalization studies show that KRP130/KLP61F localizes to all spindle microtubules. Genetic studies reveal that disruption of KLP61F gene function leads to the failure of spindle poles to separate at the beginning of mitosis, which results in the formation of monopolar spindles arrested in metaphase (Fig. 4). Structural studies by electron microscopy and antibody decoration show that KRP130/KLP61F forms a bipolar tetramer with two motor domains exposed on each end of the molecule. These observations are consistent with the hypothesis that KRP130/KLP61F holoenzymes crosslink and slide apart antiparallel microtubules, pushing apart the associated centrosomes and thereby driving centrosome separation as described below (Section 6).

6. Mechanism of bipolar *bimC* kinesin function in centrosome separation during spindle assembly and function

What is the molecular mechanism by which bipolar kinesins exert their function during mitosis? As noted above, different members of the *bimC* family have broadly similar properties, so it is reasonable to hypothesize that all *bimC* motors function by a common mechanism, even though future studies may uncover important differences among them. The mechanism by which the motor domains of *bimC* proteins move along microtubules is not yet known, but the motor properties of four *bimC* protein preparations have been analysed, namely recombinant Eg5, a truncated recombinant Eg5 construct (E437GST), recombinant KLP61F and native KRP130. All of

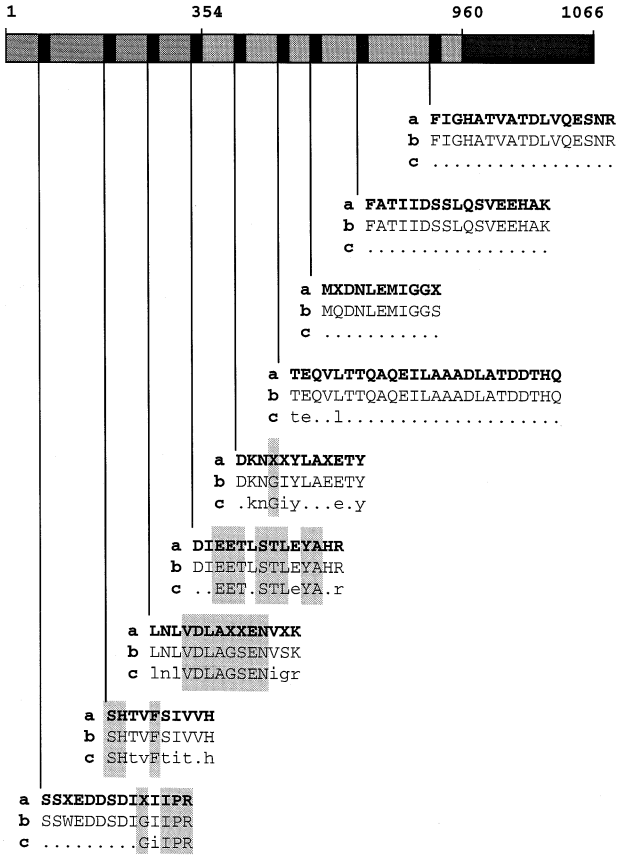

Fig. 11. Subunits of KRP130 are encoded by KLP61F gene. Map based on the amino acid sequence of *Drosophila* KLP61F indicating positions (black boxes) and amino acid sequences (top) of KRP_{130}-derived peptides, (middle) corresponding regions of KLP61F, and (bottom) *bimC* family consensus sequence. Residues identical in KRP130/KLP61F and the consensus sequence are shaded. Numbers above indicate amino acid residues bordering the motor (aa 1–354), stalk (aa 354–960) and tail (aa 960–1066). X indicates unresolved residues. Shaded residues are identical in all *bimC* motors. A 100% match was found between KRP_{130} and KLP61F sequences, whereas little or no homology with the *bimC* family consensus sequence was observed (see [35] for details).

these preparations displayed slow movement towards the plus ends of microtubules at rates of between 0.014 and 0.063 μm/s [24–26,28]. These values are between 10 and 50 times slower than the rates of kinesin-driven motility (0.6 μm/s; [36]). The step size of *bimC* motors has not been determined, but the dimensions of the kinesin and *bimC* motor domains are predicted to be similar, so if we assume a step size of 8 nm for *bimC* motors, similar to that of kinesin, and if we assume that 1 molecule of ATP is hydrolyzed per step, this converts to a Kcat of between 1.7 and 7.9 s^{-1} (which compares to the Kcat of 75 s^{-1} for a kinesin motor moving at 0.6 μm/s along a microtubule taking 8 nm steps).

In two studies, *bimC* motors were shown to display microtubule-activated MgATPase activity [25,26], and as expected from the slow rates of motility, the turnover numbers of the maximally microtubule-activated MgATPase activities were low (Kcat of 0.54 s^{-1} for KLP61F and 1.42 s^{-1} for E437GST). Despite the fact that *bimC* motors function more slowly than kinesin, however, ADP release from the active site is thought to be rate-limiting and accelerated by microtubules in both types of motors (being 10-fold slower for Eg5 than kinesin) and this ADP release step accompanies the transition from a state that is weakly bound to microtubules to a strongly bound state [26]. It is rate limiting ADP release that is thought to limit the rate of physical stepping of the motor domains of *bimC* and kinesin motors along microtubule tracks, accounting for their

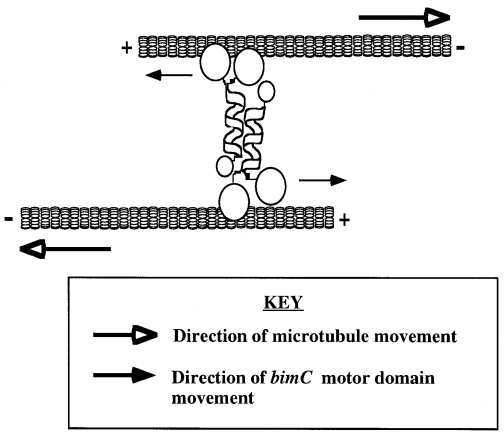

Fig. 12. 'Reverse sarcomere' model of microtubule-microtubule sliding mediated by *bimC* family kinesins. Bipolar homotetrameric *bimC* motors are shown crosslinking antiparallel microtubules (in spindles the microtubules would emanate from duplicated spindle poles, 'minus' ends of microtubules proximal to poles). By walking towards the 'plus' (+) ends of the crosslinked microtubules the *bimC* motors would slide apart the microtubules, 'minus' (−) ends leading (thereby pushing apart the duplicated spindle poles in spindles). The direction of movement of the *bimC* motor domains and the crosslinked microtubules are described by the arrows in the accompanying key.

different rates of microtubule motility [26]. The slow rate of action of the motor domains of *bimC* motors is consistent with rates of spindle pole motility and microtubule-microtubule sliding observed in mitotic spindles, and may allow a high level of precision in the motile events that underlie spindle assembly and chromosome segregation.

It is easy to imagine how the structure of the bipolar KRP130 holoenzyme is adapted to allow four *bimC* motor domains to be arranged so that two motor domains project from opposite ends of the bipolar molecule and interact with antiparallel microtubules emanating from two opposite poles in the mitotic spindle, with their plus ends distal to the poles (Figs. 10 and 12). Thus, two pairs of motor domains on the opposite ends of the motor molecule could crosslink antiparallel spindle microtubules and push them apart by moving towards their 'plus' ends. The simplest model would suggest that *bimC* motors function in centrosome or spindle pole body separation during early stages of mitosis (Fig. 1) – the very event which appears to be disrupted in all identified *bimC* family mutants (Fig. 4). In this case the bipolar *bimC* 'minifilaments' could crosslink microtubules emanating from two unseparated spindle poles, and push them apart by a 'sliding microtubule mechanism', most easily imagined as a geometrical reverse of the sliding filament mechanism of acto-myosin II-driven movements, or a 'reverse sarcomere' (Fig. 12). One could also imagine that such a mechanism operates during spindle maintenance and elongation (Fig. 1). Indeed, recent experiments with CIN8 and KIP1 in yeast suggest that these *bimC* family members can act not only at early stages of mitosis, but at late stages as well, driving spindle elongation in anaphase B [37].

As noted above, however, it is likely that bipolar *bimC* motors do not act in isolation, but rather function in concert with other microtubule motors such as CHO1, XKLP2, the C-terminal kinesins, and cytoplasmic dynein, to provide exquisite control of centrosome positioning that underlies bipolar spindle formation, maintenance and elongation in early, middle, and late stages of mitosis (Fig. 1; ref. [2,3]) The precise nature of the functional interactions of these multiple mitotic motors that operate in spindle assembly and function is a fascinating topic for further research.

7. Regulation of *bimC* subfamily protein function

How is the function of *bimC* motors regulated? Recent studies suggest that phosphorylation of the tail domain of these motors may play an important role. It has been shown that several of the *bimC* family motors contain a conserved 40-amino acid region close to their C-terminus called the *bimC* box (see above), the only known exceptions being the two functionally redundant *bimC* proteins from *S. cerevisiae*, CIN8 and KIP1. The *bimC* box includes a consensus sequence for proline-directed protein kinases, one of which is a mitotic cyclin-dependent kinase, p34cdc2. Two recent studies of *Xenopus* [38] and human [27] Eg5 indicate that phoshorylation of a single threonine within that sequence (T 937 in *Xenopus* Eg5 and T927 in human Eg5) regulates the binding of Eg5 to spindle microtubules. A point mutation of this threonine residue to nonphosphorylatable alanine abolishes the localization of transfected Eg5 to the mitotic spindle. On the other hand, a mutation of threonine to serine, as well as in vitro phosphorylation of Eg5 by p34cdc2, preserves the spindle localization of Eg5.

The mechanisms by which phosphorylation regulates the localization of *bimC* family members to the spindle remain unknown at present but there are several possibilities based on what is known about the regulation of bipolar filamentous myosin II function by light chain phosphorylation [4]. As with myosin II, it is possible that in an unphosphorylated state, a *bimC* polypeptide can fold in such a way that the C-terminal tail becomes juxtaposed to the N-terminal motor head, with the tail inhibiting the self-assembly and motor properties (microtubule binding, ATPase, motility) of the head. This inactive, soluble *bimC* motor would be equivalent to folded 10S myosin II. Phosphorylation of the *bimC* box lying within the tail region of the *bimC* polypeptide may disrupt the association of the tail with the head, allowing the *bimC* rod domain to unfold into a conformation equivalent to 6S myosin II that can self-assemble into bipolar homotetramers whose motor domains are no longer 'turned off' by the tails. These active, bipolar motors could then associate with spindle microtubules, crosslink antiparallel microtubules, and use energy from ATP hydrolysis to move towards the plus ends of the crosslinked micro-

tubules, thereby sliding them apart as shown in Fig. 12.

This model implies considerable conservation in the mechanism of regulation of *bimC* kinesin motors and the class II myosins. At this stage, of course, this model is totally speculative, and it is quite possible that phosphorylation regulates the motor activities and spindle association of *bimC* motors independent of any effect on self-assembly, but it can be tested experimentally. Certainly, the exact role of *bimC* box phosphorylation in regulating the localization and function of *bimC* motors remains an exciting topic for further research.

8. Summary and concluding remarks

The mechanism by which *bimC* motors function in the mitotic spindle is not yet fully understood. We favor the simple model illustrated in Fig. 12, and this view is emphasised throughout the manuscript. In this model, phosphorylated *bimC* polypeptides are assembled into bipolar homotetramers which crosslink antiparallel microtubules emanating from duplicated spindle poles or spindle pole bodies. By walking slowly towards the plus ends of these crosslinked microtubules, *bimC* motors push the microtubules apart, minus ends leading. Consequently, the duplicated poles located at the minus ends of the microtubules are themselves pushed apart.

It is important to note, however, that the current hypothesis for the structure and function of bipolar *bimC* motors needs further detailed testing. For example, the oligomeric state of KRP130 was determined by estimating the native molecular weight from a combination of the Stokes radius (from gel filtration) and the sedimentation coefficient (from sucrose density gradients), but if sufficient pure protein can be obtained, the result should be checked by the more rigorous procedure of analytical ultracentrifugation. While it has been reported that native Eg5 also behaves as a tetramer [2], the truncated recombinant Eg5 protein, E437GST, clearly behaves as a dimer [26]. Moreover, unlike native KRP130, recombinant KLP61F was not observed to bundle microtubules in a motility assay suggesting that, under the conditions used, its motor domains were not organized into bipolar arrays capable of crosslinking microtubules [25]. These results suggest that an important avenue for future research will be to test recombinant KLP61F protein for its ability to assemble into bipolar homotetramers under various conditions, and, if successful, to use protein engineering to determine the sites within the KLP61F polypeptide that are important for bipolar tetramerization.

We also note that some cytological studies indicate that *bimC* motors may function in a more complex manner than suggested by the simple antiparallel microtubule sliding model emphasised here (Fig. 12). In some cells there may be multiple pathways for centrosome movement, involving the association of *bimC* motors with both monastral and antiparallel microtubule arrays leading to proposal that *bimC* motor function does not always require anti-parallel microtubule arrays [39]. In addition, there is evidence that *bimC* motors may somehow organize the minus ends of astral microtubules into highly focused structures, thus contributing to the organization of spindle poles [3,28]. Finally, studies addressing the fascinating problems of *bimC* regulation by mitotic kinases and the nature of the functional interactions that exist between *bimC* motors and other families of microtubule motors are in their infancy [2,3,27,28].

Despite these gaps in our understanding of *bimC* motor function, however, we believe that the simplest interpretation of the results obtained to date, when taken together, is that an important aspect of *bimC* protein function involves the assembly of *bimC* polypeptides into bipolar homotetrameric kinesin molecules that are capable of crosslinking antiparallal spindle microtubules and driving them and their associated spindle poles apart during mitosis. In a broader context, different kinesin holoenzymes consist of kinesin-related polypeptides that share conserved motor domains linked to diverse tail domains, assembled into a variety of quaternary structures ranging from monomers to heterotetramers, and it has been proposed that the quaternary structure of each kinesin motor is adapted to performing a specific biological function [14]. The assembly of four *bimC* polypeptides into a bipolar homotetramer capable of driving centrosome separation by a sliding microtubule mechanism (Fig. 12) represents a striking example of this principle of structural adaptation to biological function among the kinesins.

9. Note added in proof

A member of the *bimC* family of kinesins called TKRP125 has now been characterized in tobacco cells [49].

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