Published in final edited form as:

Nature. 1996 November 21; 384(6606): 225. doi:10.1038/384225a0.

An essential bipolar mitotic motor

A. S. Kashina,

Department of Molecular and Cellular Biology, University of California, Davis, California 95616, USA

J. M. Scholey,

Department of Molecular and Cellular Biology, University of California, Davis, California 95616, USA

J. D. Leszyk, and

Worcester Foundation for Biomedical Research, Shrewsbury, Massachusetts 01545, USA

W. M. Saxton

Department of Biology, Indiana University, Bloomington, Indiana 47405, USA

J. M. Scholey: jmscholey@ucdavis.edu

Sir — Separation of the mitotic spindle at the poles during cell division depends on the action of members of the bimC family of proteins. These proteins are part of the kinesin superfamily of ‘motor’ proteins; molecules that bind microtubules and produce mechanical force from chemical energy¹. The mechanism of action of bimC motors is not yet known. We report here that the bimC-related *KLP61F* gene, an essential mitotic gene of the fruitfly *Drosophila melanogaster*, encodes subunits of the bipolar kinesin, KRP₁₃₀, a homotetrameric complex of four motor subunits, each of relative molecular mass 130,000 (M_r 130K), assembled into a bipolar ‘minifilament’². This result suggests that bipolar bimC motors work by crosslinking and sliding apart antiparallel microtubules, causing the separation of duplicated spindle poles and allowing bipolar spindle assembly.

To identify the gene encoding the KRP₁₃₀ subunit, we performed protein microsequencing of purified *Drosophila* embryonic KRP₁₃₀ (refs 2, 3). Proteolysis of KRP₁₃₀ yielded tryptic peptides, which we fractionated, and we selected the seven best-resolved fractions for further analysis. One of these fractions contained a 17-residue peptide, whose sequence we determined. A database search revealed a 100% match of this peptide with a segment of the nonconserved stalk of a previously identified *Drosophila* bimC-related protein, KLP61F (see figure).

Further analysis of the peptide fractions revealed that they contain a total of nine peptides whose masses match exactly those of the predicted tryptic fragments derived from the *Drosophila* KLP61F protein sequence^{4,5}. To confirm that KRP₁₃₀ is identical to KLP61F, we sequenced all nine of these peptides and found that they display 100% identity with the corresponding segments of the deduced KLP61F protein sequence, but differ in many positions from the bimC family consensus sequence (figure). We therefore conclude that a KLP61F polypeptide is identical to a subunit of the bipolar kinesin, KRP₁₃₀.

This discovery contradicts the hypothesis that KLP61F and KRP₁₃₀ differ⁶, and bridges the gap between genetic evidence concerning the biological function of KLP61F (ref. 5), studies of its expression and localization on spindle microtubules^{5,6}, and biochemical and electron-microscope studies of the recombinant polypeptide⁶ and the native bipolar holoenzyme^{2,3}. For example, in *Drosophila* *KLP61F* mutants, the absence of KLP61F function leads to the formation of monopolar spindles with unseparated spindle poles⁵. This observation, together with the results described here, indicates that the essential mitotic *KLP61F* gene might

encode a slow plus-end-directed microtubule motor polypeptide^{3,6} that self-assembles into a bipolar homotetrameric holoenzyme² capable of crosslinking and sliding apart antiparallel microtubules, thereby pushing apart the associated spindle poles during spindle assembly and function.

References

1. Walczak CE, Mitchison TJ. *Cell*. 1996; 85:943–946. [PubMed: 8674121]
2. Kashina AS, et al. *Nature*. 1996; 379:270–272. [PubMed: 8538794]
3. Cole DG, Saxton WM, Sheehan KB, Scholey JM. *J Biol Chem*. 1994; 269:22913–22916. [PubMed: 8083185]
4. Stewart RJ, Pesavento PA, Woerpel DN, Goldstein LSB. *Proc Natl Acad Sci USA*. 1991; 88:8470–8474. [PubMed: 1924306]
5. Heck MS, et al. *J Cell Biol*. 1993; 123:665–679. [PubMed: 8227131]
6. Barton NR, Pereira A, Goldstein LSB. *Mol Biol Cell*. 1995; 6:1563–1574. [PubMed: 8589456]
7. Fernandez J, Andrews L, Mische SM. *Anal Biochem*. 1994; 218:112–117. [PubMed: 8053543]

1.

Map based on the amino-acid sequence of *Drosophila* KLP61F (ref. 5), indicating positions (grey boxes) and amino-acid sequences of KRP₁₃₀-derived peptides (top rows of sequences), corresponding regions of KLP61F (middle rows), and bimC family consensus sequence (bottom rows). Numbers above indicate amino-acid residues bordering the motor (residues 1-354), stalk (354-960) and tail (960-1,066). Single-letter amino-acid code; X indicates unresolved residues. The indicated peptide sequences correspond to residues 123-136, 231-242, 257-270, 339-352, 391-402, 492-516, 542-552, 669-685 and 880-896 for KLP61F. We found 100% identity between KRP₁₃₀ and KLP61F sequences, but little or no homology with the bimC family consensus sequence. Residues highlighted in red are identical in all bimC motors. We immobilized sucrose gradient-purified KRP₁₃₀ on membranes and performed *in situ* proteolysis with trypsin⁷. We analysed selected peptides by matrix-assisted laser desorption-ionization time-of-flight mass analysis, using α -cyano-4-hydroxycinnamic acid as the matrix. The nine unique peptides thus identified matched the predicted masses of KLP61F tryptic fragments (using the MSFIT program at the UCSF mass spectrometry facility to search *Drosophila melanogaster* proteins in the SwissProt.r33 database). These peptides were analysed by automated Edman degradation, yielding reliable sequences (illustrated). bimC consensus sequences in the indicated regions were determined by lineup comparison of *Drosophila* KLP61F, *Xenopus* Eg5, human HsEg5, *Aspergillus* bimC, *Saccharomyces pombe* Cut7 and *S. cerevisiae* CIN8 and KIP1. Full methodological details available on request from J. M. S.