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The Kar3p and Kip2p motors function antagonistically at the spindle poles to influence cytoplasmic microtubule numbers

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SUMMARY

Microtubules provide the substrate for intracellular trafficking by association with molecular motors of the kinesin and dynein superfamilies. Motor proteins are generally thought to function as force generating units for transport of various cargoes along the microtubule polymer. Recent work suggests additional roles for motor proteins in changing the structure of the microtubule network itself. We report here that in the budding yeast *Saccharomyces cerevisiae* microtubule motors have antagonistic effects on microtubule numbers and lengths. As shown previously, loss of the Kar3p motor stimulates cytoplasmic microtubule growth while loss of Kip2p leads to a sharp reduction in cytoplasmic microtubule numbers. Loss of both the Kip2p and Kar3p motors together in the same cell produces an intermediate phenotype, suggesting that these two motors act in opposition to control cytoplasmic microtubule density. A Kip2p-GFP fusion from single gene expression is most concentrated at the spindle poles, as shown previously for an epitope tagged Kar3p-HA, suggesting both of these motors act from the minus ends of the microtubules to influence microtubule numbers.

Key words: Kinesin, Spindle, Microtubule, Polymerization

INTRODUCTION

Microtubules are involved in many types of cellular motility-related events, and the ability of the cell to control the number and lengths of its microtubules is essential to the efficient orchestration of these events. One example of regulatory influence on microtubules is their association with the kinetochore, which is known to stabilize kinetochore microtubule bundles in the spindle (Nicklas, 1988). Another has been observed in neurons, where high molecular weight MAPs bind along the length of the polymer and provide long term stabilization for directional transport to the synaptic complex (Olmstead, 1986).

Recent results suggest a possible additional role for microtubule-based motor proteins in regulating microtubule turnover. Motors are uniquely suited to influence microtubule length. They are mobile and as such are capable of moving to the plus or minus ends of the microtubules, the major sites of polymerization and depolymerization of the polymer. A GST fusion of the *S. cerevisiae* Kar3p motor was shown to move to the minus ends of microtubules and preferentially depolymerize the polymer at the minus ends in vitro (Endow et al., 1994). Deletions of the *KAR3* gene led to a threefold increase in the number of cytoplasmic microtubules observed in arrested or synchronized mitotic cells (Saunders et al., 1997a) and an increase during the blocked meiosis of *kar3* mutants (Bascom-Slack and Dawson, 1997), showing Kar3p function is required to limit cytoplasmic microtubule growth in arrested cells. (In *S. cerevisiae* the nucleus remains intact throughout the cell cycle, and the microtubules of the cytoplasm are functionally related to astral microtubules of vertebrate cells.) Similarly loss of the *S. cerevisiae* dynein motor (Carminati and Stearns, 1997), or of Num1p or Jmn1p, proteins which may function in the dynein pathway (Farkasovsky and Kuntzel, 1995; McMillan and Tatchell, 1994), or the Kip3p kinesin-related motor (Cottingham and Hoyt, 1997; DeZwaan et al., 1997) also produced an increase in cytoplasmic microtubule numbers. When both dynein and Kip3p together are lost in the same cell there is a very dramatic increase in cytoplasmic microtubule lengths (Cottingham and Hoyt, 1997). Currently it is unclear to what extent these proteins directly influence microtubule polymerization and to what extent the phenotype is due to indirect causes such as perturbations of the cell cycle or disruption of spindle structure.

An opposite phenotype is seen with loss of the Kip2p motor (Cottingham and Hoyt, 1997). *kip2* null mutants produced a sharp reduction in the number and length of the cytoplasmic microtubules. *kip2* mutants were also more sensitive to the microtubule-destabilizing drug benomyl, consistent with a microtubule polymerization defect. To further investigate the relationship of motor function and microtubule dynamics a *kar3 kip2* double mutant was constructed. In this strain the microtubules show an intermediate microtubule phenotype from that of the *kar3* or *kip2* mutants alone. These results

suggest that the Kip2p and Kar3p motors function antagonistically in the cell to influence microtubule numbers. An integrated *KIP2-GFP* fusion product was found predominantly at the spindle poles, the site of the previous localization of a Kar3p-HA fusion protein, suggesting both of these motors may function at the minus ends of the microtubules.

MATERIALS AND METHODS

Strains and cell culturing

All strains are derivatives of S288C and are listed in Table 1. *kar3*-Δ *(kar3-102::LEU2*; Meluh and Rose, 1990) and *kip2*-Δ (Roof et al., 1992) mutant alleles have been described previously. The media used were YPD or minimal medium lacking histidine with glucose or galactose added to 2%, or raffinose added to 5% (Sherman et al., 1983). Typically streak cultures on plates were used to inoculate liquid medium. Cultures were grown overnight at 30°C to log phase as determined by visual inspection, but with OD_{600} typically less than 1.0.

α-factor-arrest was achieved by treating cells with pheromone (Bachem California, Torrance, CA) at 6 μg/ml in selective medium at pH 4.0, 30°C, for 3 hours. Typically, more than 80% of the cells arrested without buds, as determined by DIC microscopy. Release from pheromone was achieved by resuspending the centrifuged and washed cells in the same medium without inhibitor. Hydroxyurea arrest was achieved with 0.2 M hydroxyurea in pH 5.8 medium by growing a log phase culture at 30°C for 4 hours. The arrest was confirmed by examining cells fixed in 70% ethanol after staining with the DNA-specific fluorescent dye 4,6-diamidino-2-phenylindole (DAPI; Sigma, St Louis, MO) at 1 μg/ml. Typically more than 70% of the cells accumulate as large budded cells with a single nucleus.

Plasmids

The *KAR3* overexpression plasmid is a derivative of pMR1682 (*URA3-GAL1-KAR3-CEN*; P. Meluh and M. Rose, Princeton) by insertion of the *HIS3* gene into the *URA3* marker of pMR1862. A 1762 bp *Bam*HI fragment of pUC-*HIS3* (a gift of R. Rothstein, Columbia University) containing the *HIS3* gene was filled at the ends with the Klenow enzyme and inserted into pMR1682 cut with *Eco*RV, creating pXZB21.

For the *KIP2-GFP* integration, two plasmids (pJK123 and 124) were constructed. pJK123 is a centromeric *TRP1*-containing vector with a fusion of full-length *KIP2* to *GFP*. The 5′ promoter region (585 bp 5′ of the ATG) and entire open reading frame were amplified using *Bam*HI and *Nhe*I-linked primers. The PCR product was cut with *Bam*HI and *Nhe*I and ligated into a similarly cut pJK52 backbone. pJK52 is a *TRP1 CEN* vector with a fusion of the *Nuf2* gene (flanked by *Bam*HI and *Nhe*I sites) to a cDNA encoding the s65t, v163a mutant of *GFP* (flanked by *Nhe*I and *Hin*DIII sites) followed by the *Nuf2* 3′ UTR sequence (flanked by *Hin*DIII and *Kpn*I sites). pJK124 was constructed as an integrating vector for the expression of *KIP2-GFP*. pJK123 was digested with *Eco*RV and *Kpn*I. The 1976 bp fragment was then ligated into pRS304 (*TRP1* integrating) and cut with *Sma*I and *Kpn*I to create pJK124.

Table 1. Genotypes and plasmids used in this study

Yeast strain	Relevant genotype
WSY32	Wild type
WSY719	*kip2::URA3*
WSY69	*kar3::LEU2*
WSY752	*kar3::LEU2 kip2::URA3*
JKY240	*kip2::KIP2-sGFP:TRP1* (integrated *KIP2-GFP*)
JKY238	*kip2::TRP1*
JKY225	Wild type

Plasmid name	Relevant plasmid loci
pXZB21	*GAL1 KAR3 CEN HIS3*
pJK123	*KIP2-sGFP NUF23′UTR CEN URA3*

Double colon refers to a deletion of the preceding gene with the marker gene that follows, single colon refers to linkage without disruption.

Microscopy

Anti-tubulin immunofluorescence was performed on formaldehyde-fixed cells using monoclonal antibodies YOL 1/34 (Serotec, Oxford, UK) and rhodamine-conjugated secondary antibodies (Jackson Immunoresearch, West Grove, PA) as described (Hoyt et al., 1992; Pringle et al., 1991). Cells were also stained with DAPI to identify the position of the nucleus. The slides were examined with an Olympus B60 epifluorescence microscope using a 100× oil immersion objective, and digital images were captured with a Hammatsu Argus 20 CCD camera and image processor. In order to increase the percentage of cells in the correct focal plane, photo images of all anti-tubulin staining are composites made by combining relevant portions of selected captured images taken from a single sample using cut-and-paste features of the Adobe Photoshop software (Adobe Systems Incorporated, Mountain View, CA). Microtubule arrays were chosen based on clarity and uniform plane of focus throughout the spindle, and care was taken to pick a representative sample. Images were processed within the Photoshop program to make a uniform background. GFP images were taken with Nikon Diaphot 300 microscope with a 100× oil immersion objective.

Microtubule length measurements were made from captured anti-tubulin fluorescent images using the Argus 20 image processor cursor-based measuring capability. Distances were measured from the approximate outside edge of the spindle pole bodies. Calibration of the measurement software program was performed by use of an engraved slide (Olympus Optical Co., Tokyo, JA).

RESULTS

To further investigate the relationship between the roles of the Kip2p and Kar3p kinesin-related motors in the maintenance of the cellular microtubule arrays, deletion mutants of the *KAR3* (*kar3*-Δ; Meluh and Rose, 1990) and *KIP2* (*kip2*-Δ; Roof et al., 1992) genes and the double deletion mutant were examined by anti-tubulin immunofluorescence in synchronized cultures. Cells were arrested at START, near the G_1/S boundary, with the mating pheromone α-factor, and released as a synchronous culture by washing with fresh medium without pheromone. After 30 minutes of release from pheromone, the cells were most likely to be in S phase, but most had not yet assembled spindles (Fig. 1). At this time *kip2* mutants had relatively short microtubules, while in the *kar3* mutant culture the microtubules were longer then in wild-type cells as shown previously (Cottingham and Hoyt, 1997; Saunders et al., 1997a). The *kip2 kar3* double mutants, however, showed an intermediate phenotype between *kar3* and *kip2* single mutants, and the microtubules were generally similar to wild-type cells in number and length (Figs 1, 2). As the synchronized culture continued to progress through the cell cycle, short preanaphase spindles were observed after 60-90 minutes of release. These are visible in Fig. 1 (G_2/M) as short brightly fluorescent bars. At this time the cytoplasmic microtubules (visible as faintly staining lines originating from the edge of the spindle) were observed to be reduced in the *kip2* mutant and increased in the

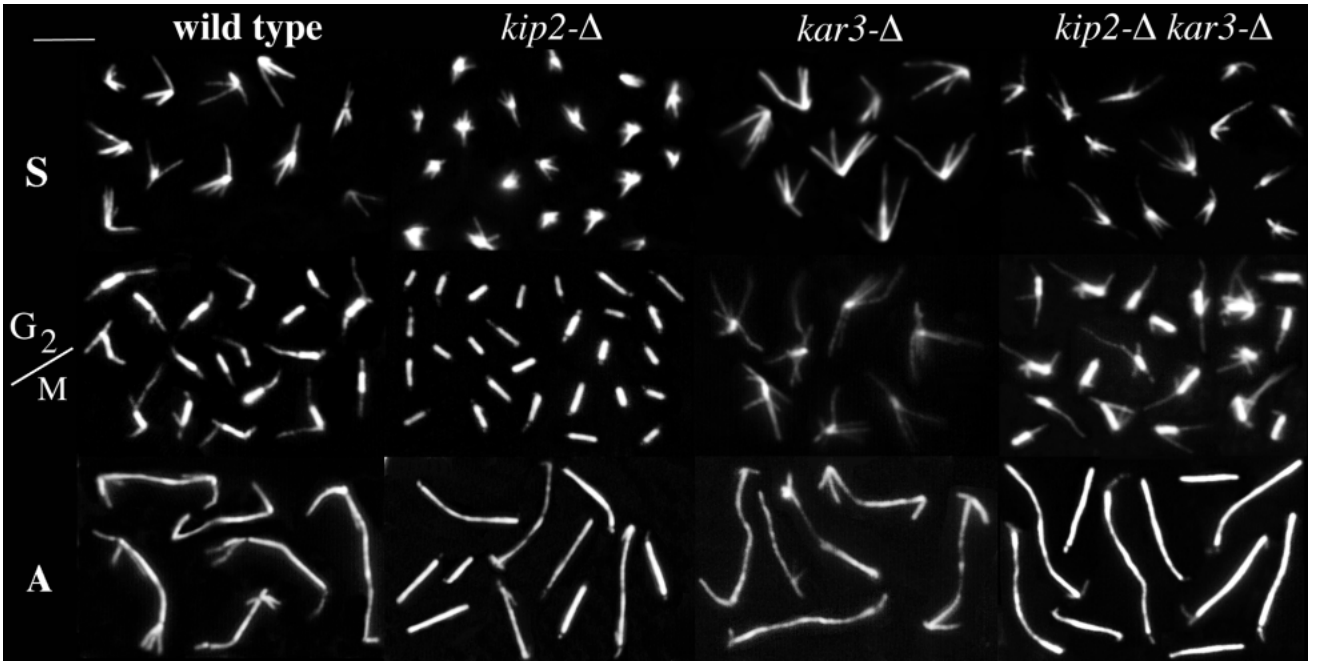
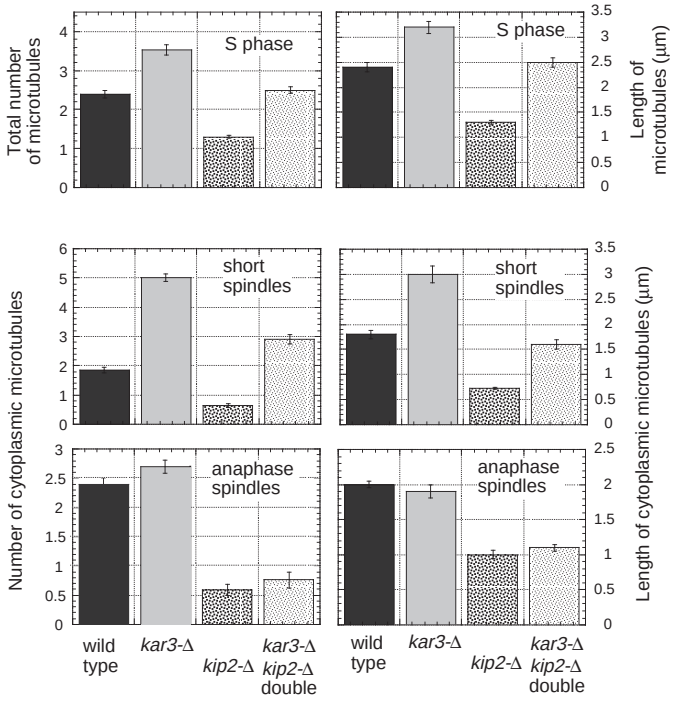


Fig. 1. Change in microtubule densities in *kip2* and *kar3* mutants during the cell cycle. Wild type or single or double *kip2::URA3 kar3::LEU2* disruption mutants were arrested with α-factor at 6 μg/ml in YPD, pH 4.0, for 3 hours at 30°C. Cultures were released in fresh YPD for 30 minutes (S phase), 60 or 90 minutes (G_2/M), or 90 or 120 minutes (A, anaphase) and samples treated for anti-tubulin immunofluorescence as described (Hoyt et al., 1992; Pringle et al., 1991). The assigned phases of the cell cycle are estimates based on the time of release and the appearance of the microtubules. Shown are composite images of representative microtubule arrays from a single sample. At the chosen timepoints more than 90% of the cells contained monastral arrays or more than 60% short or long spindles, as shown. All cultures were arrested and released at the same time. Bar, 5 μm.

kar3 mutant. Cytoplasmic microtubules in the *kar3 kip2* double mutants were intermediate in number and length compared to the single mutants and similar in length to wild-type cells. The intermediate phenotype of the double mutant suggests that these two motors function antagonistically in the cell. After 90-120 minutes of release from alpha factor many of the cells were in anaphase, as determined by the large increase in spindle length (and separation of chromatin, not shown). *kip2* mutants continued to show a reduced cytoplasmic microtubule phenotype, while by late anaphase the spindles of *kar3* mutants and wild-type cells were indistinguishable (Saunders et al., 1997a). Late anaphase spindles in the double mutant were similar in appearance to the *kip2* single mutant. These results indicate that the loss of the Kar3p and Kip2p motors have contrasting effects on the cell but that these effects are mostly eliminated when both motors are lost.



The *kip2 kar3* double mutants were viable, as reported previously (Roof et al., 1992), but in our hands were not wild type for growth. They were temperature-sensitive and had irregular colony size, similar to *kar3*-Δ single mutants (not shown). *kip2 kar3* double mutants also had an increase in mitotically delayed cells with 46% (*n*=400) of the double

Fig. 2. Quantitation of microtubule changes in *kar3* and *kip2* mutants. The average microtubule length and number of cells prepared as in Fig. 1 are shown. At the 30 minute timepoint (S phase), nuclear and cytoplasmic microtubules could not be readily distinguished and all microtubules were included. For G_2/M and anaphase only cytoplasmic microtubules were included. For each sample 100-200 microtubule lengths were measured, or the number of microtubules from 100 spindles or monoastral arrays were counted. Error bars show s.e.m.

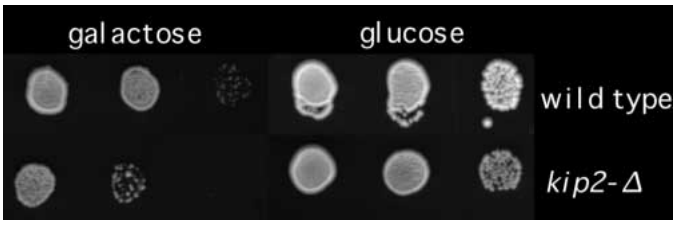


Fig. 3. Increased *KAR3* sensitivity of *kip2* mutant. Wild-type or *kip2*-Δ mutant cells were transformed with a plasmid containing *KAR3* under the control of the *GAL1* promoter (pXZB21). Transformants were selected on synthetic medium lacking histidine and grown overnight on –his medium containing 5% raffinose as the carbon source to derepress the *GAL* promoter. Serial dilutions were plated on glucose and galactose –his plates at 23°C for 8 days.

mutant cells having large buds greater than half the size of the mother and a single nucleus compared to 11% of the wild type (*n*=200). This number is similar to the approx. 40% preanaphase arrest observed in *kar3* single mutants (Meluh and Rose, 1990; Saunders et al., 1997a).

To further test for an antagonistic relationship between these two motors, wild-type cells and *kip2* mutants were transformed with a plasmid expressing the *KAR3* gene under the control of the *GAL1* promoter. Overexpression of *KAR3* slightly inhibits the growth of wild-type cells, as reported previously (Saunders et al., 1997b). *kip2* mutants were more sensitive to *KAR3* overexpression than wild-type cells, consistent with an antagonistic relationship between these two motors (Fig. 3). *kip2* mutants were also slightly more sensitive to the microtubule inhibitor benomyl, as shown previously (Cottingham and Hoyt, 1997), but in our hands this was only seen at concentrations approaching the limit of tolerance for wild-type cells (not shown). This is in contrast to *kar3* mutants, which have previously been shown to grow better when provided with low concentrations of benomyl (Roof et al., 1991; Saunders et al., 1997a). These results further support an antagonistic relationship between these motors.

While *kip2* mutant cultures grew at rates equal to wild-type cells (Roof et al., 1992; our unpublished observations), they did show a novel nuclear migration defect. Chromatin staining with the DNA fluorescent probe DAPI was used to examine formaldehyde-fixed wild-type and *kip2* mutant cells for the timing of nuclear segregation into the bud. In both wild-type cells and the *kip2* mutant the separating chromatin seemed eventually to move completely to the far end of both the mother and bud (not shown). However the early phases of separation differed in the mutant and wild-type cells. In wild type the chromatin usually divided quickly into the bud at early anaphase, as demonstrated previously (Yeh et al., 1995), although apparent exceptions were noted (arrow in Fig. 4). *kip2* mutants frequently exhibited chromatin dividing in the mother cell alone. Initial chromatin movement into the bud in the mutant was typically accompanied by contact of the dividing nucleus with the wall of the mother cell (arrows Fig. 4). 20% of the wild-type cells and 86% of the *kip2* mutants appeared to make contact with the back of the mother cell wall prior to entry of the chromatin into the bud. While the exact sequence of events of division in the *kip2* mutants could not be firmly established in these fixed cells, it appeared that in the absence

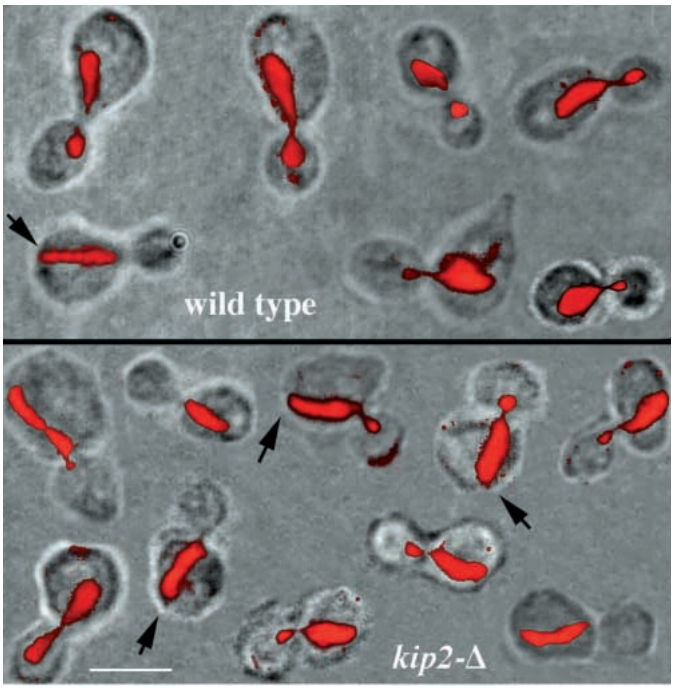


Fig. 4. Delayed entry of chromatin into the bud in *kip2* mutants. Wild-type and *kip2* mutants were grown to log phase, fixed with formaldehyde and stained with DAPI and anti-tubulin antibodies (the latter not shown). Cells were examined for instances of early chromatin division by comparing the DAPI and DIC images. Shown are composites of superimposed DIC and fluorescent DAPI staining of chromatin in red pseudocolor. In wild-type cells chromatin rapidly entered the bud, as determined by the low frequency of division in the mother alone (e.g. A, arrow). *kip2* mutants on the other hand often began dividing in the mother and seemed to show delayed entry into the bud, often following contact between the dividing nucleus and the mother cell wall. Arrows indicate circumstances that are interpreted as contact with the dividing nucleus and the wall of the mother cell prior to or around the time of chromatin entry into the bud.

of long cytoplasmic microtubules, early entry into the bud was delayed, possibly until contact with the cell wall allowed motors pushing from within the spindle to force chromatin through the neck restriction. Binucleates were observed in less than 5% of the *kip2* mutant and wild-type cells at 30°C.

The location of the Kip2p motor in the cell was investigated by fusing *GFP* to the amino terminus of the *KIP2* open reading frame and examining the position of the GFP fluorescence in live cells. To eliminate artifacts from variation in copy number of the tagged gene, the fusion construct was integrated into the genome. Under this condition most of the fluorescence was seen as one or two dots in the cell (Fig. 5). Co-staining with antibodies to a 90 kDa spindle antigen (Rout and Kilmartin, 1990) suggested these dots represent spindle pole bodies, and under these conditions the GFP fluorescence was reduced (not shown). Staining was occasionally observed as well separated dots as shown, suggesting that the Kip2p-GFP remains at the spindle poles during anaphase. To test for Kip2p function from the GFP-tagged fusion protein, cells with the integrated construct were arrested with hydroxyurea, and the numbers of spindle-associated cytoplasmic microtubules compared to wild

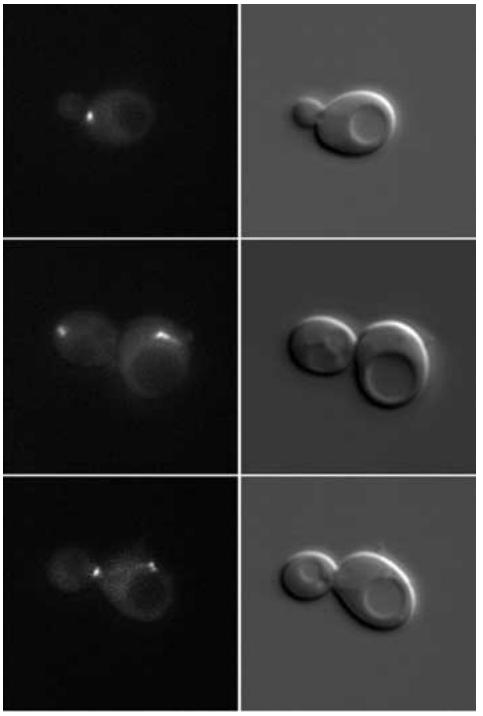


Fig. 5. Kip2p-GFP in single copy is found predominantly at the spindle pole bodies. The *KIP2* gene was fused at the carboxyl terminus to *GFP* as described in Materials and methods. The plasmid was integrated into the genome by linearization and transformation into wild-type cells. Transformants were viewed on a Nikon Diaphot 300 microscope equipped for fluorescence and DIC optics. The left column shows GFP staining, the right the corresponding DIC image.

Table 2. Cytoplasmic microtubule numbers in cells with the *KIP2:GFP* fusion

Genotype (see Table 1)	Plasmid	Average cytoplasmic MT number	Strain
Grown in rich medium:			
kip2-Δ integrated *KIP2:GFP*	None	1.7	JKY240
Wild type	None	1.9	WSY32
Grown in selective medium (–uracil):			
kip2-Δ	Vector (YCp50)	0.23	JKY238
kip2-Δ	*KIP2:GFP*	1.7	JKY238
Wild type	Vector alone	1.7	JKY225
Wild type	*KIP2:GFP*	2.2	JKY225

Cells of the indicated genotype were arrested for 3.5 hours at 30% in 0.2 M hydroxyurea to increase the number of cells with short spindles and treated for immunofluorescence with anti-tubulin antibodies (Materials and methods). The numbers of cytoplasmic microtubules from 100 spindles for each cell type were determined. With rich medium there was no plasmid present. The –uracil medium selects for the indicated plasmid.

type and *kip2* mutants. Cells with the integrated construct had 89% of the cytoplasmic microtubules of wild-type cells (Table 2). When a low-copy centromere-containing (*CEN*) plasmid with the *KIP2-GFP* fusion was introduced into *kip2* mutants, it restored the microtubule numbers to equal those of the wild-type strain with vector alone. These results indicate that the Kip2p-GFP fusion has a similar activity to the wild-type Kip2p protein and that the poleward staining probably reflects the position of the untagged Kip2p in the cell. When the *KIP2-GFP* plasmid was introduced into wild-type cells, additional staining was seen on the cytoplasmic microtubules (not shown), and there was a slight increase in cytoplasmic microtubule numbers to 130% of wild type (Table 2). We interpret these results to mean that *KIP2-GFP* at single copy is found predominately at the spindle poles but can also bind cytoplasmic microtubules, especially when present in higher copy.

CONCLUSIONS

Requirement for Kip2p for cytoplasmic microtubule stability

Loss of *KIP2* caused a reduction in cytoplasmic microtubules while extra copies of *KIP2-GFP* produced a slight increase in cytoplasmic microtubule numbers, suggesting that Kip2p is required to stabilize cytoplasmic microtubules, in agreement with previous observations (Cottingham and Hoyt, 1997). In mitotic metazoan cells a kinetochore association is known to stabilize microtubules from depolymerization. Most of the

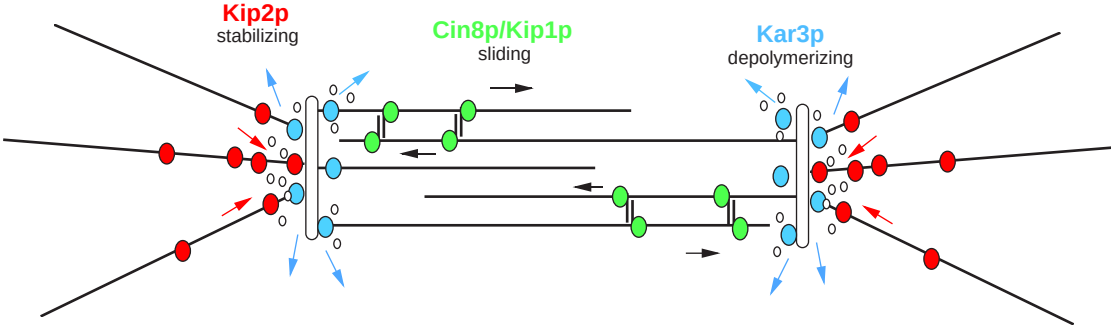


Fig. 6. Model for kinesin motor function in the spindle. Kip2p (shown in red) is proposed to function primarily from the spindle poles to stabilize the cytoplasmic microtubules, as indicated by the red arrows, suggesting incorporation of tubulin dimers (small circles). Kar3p (shown in blue) is believed to also function primarily in the vicinity of the spindle poles but acting to depolymerize both nuclear and cytoplasmic microtubules as depicted by the blue arrows. This represents two types of antagonistic relationships in the spindle, Kar3p and Cin8p and Kip1p (green circles), as discussed previously (Saunders and Hoyt, 1992), and Kar3p and Kip2p, as discussed in the present manuscript.

nuclear microtubules in *S. cerevisiae* are associated with kinetochores (Winey et al., 1995) and therefore may not require Kip2p for stability. In contrast, the ends of cytoplasmic microtubules are not known to be capped and are generally dynamic in yeast (Carminati and Stearns, 1997; Shaw et al., 1997). We believe that Kip2p may function specifically to stabilize cytoplasmic microtubules, similar to the stabilization of nuclear microtubules by association with kinetochores. However, unlike kinetochore association that stabilizes microtubules at the plus ends, Kip2p may function primarily at the spindle poles, the site of the minus ends of the microtubules.

Recent analysis of GFP-based microtubule staining in live cells revealed more cytoplasmic microtubules than typically observed in fixed preparations, about 4-6 per spindle pole (Shaw et al., 1997). Therefore it is possible that the cytoplasmic microtubules are shrinking with fixation and *kip2* mutants are more sensitive and *kar3* mutants perhaps less sensitive than wild-type cells to this fixation artifact. A determination of the exact numbers of microtubules in these mutants may require further examination in live cells.

Kar3p and Kip2p function antagonistically to influence microtubules

Like *kar3* mutants, deletions of *KIP3* or dynein also stimulated increased cytoplasmic microtubule numbers (Cottingham and Hoyt, 1997). When these motor deletion mutations were combined with deletion of *KIP2*, the double mutants showed mostly a *kip2* mutant phenotype. As shown here the *KAR3* gene was different in that loss of Kar3p was completely able to bypass the need for Kip2p for normal cytoplasmic microtubule density. The reduction in the number of cytoplasmic microtubules observed in the preanaphase *kip2* mutants required Kar3p. Conversely most (but not all) of the increase of microtubules observed in the preanaphase *kar3* mutant required the activity of Kip2p. This is strong genetic evidence that these two motors act in a direct antagonistic relationship to influence microtubule stability. This conclusion is supported by the observation that overexpression of *KAR3* inhibits the growth of *kip2* mutants more than the growth of wild type. This antagonistic relationship changes during the cell cycle. Once anaphase is underway Kar3p no longer seems essential to limit the number of cytoplasmic microtubules while Kip2p is still required for cytoplasmic microtubule stability throughout mitosis. Consistent with these observations the staining of Kip2p-GFP appeared to be retained during anaphase while Kar3p-HA staining is lost during spindle elongation (Saunders et al., 1997a).

While the Kip2p and Kar3p motors appear to act antagonistically, the deletion mutants are not strictly complementary. The double mutant still retains the temperature sensitivity and general slow growth characteristics of the *kar3* single mutant. The Kar3p motor is believed to act on both the nuclear and cytoplasmic microtubules (Saunders et al., 1997b), while the Kip2p motor is apparently limited to the cytoplasmic microtubules, based on the Kip2p-GFP localization and the phenotype. Therefore the double mutant probably continues to show the consequence of the loss of Kar3p activity on nuclear microtubules.

An important question is to what extent these phenotypes of increased or decreased microtubules represent a direct consequence of loss of motor activity or are an indirect consequence of another mutational defect. For example, microtubule dynamics change during the cell cycle, and it is known that arrest can stimulate microtubule growth. It is possible that the microtubule irregularities observed with these mutants represent perturbation of the cell cycle, indirectly leading to growth or shrinkage of the microtubules. This is especially relevant for interpretation of the *kar3* microtubule phenotype, which is clearly enhanced by a cell cycle arrest (Saunders et al., 1997a), and since *kar3* mutants have a known cell cycle delay (Meluh et al., 1990).

There are several features of the microtubule phenotypes in these mutants that indicate the phenotype is not due solely to perturbations of cell cycle. (1) If *kar3* or *kip2* mutants are arrested at START or S phase of the cell cycle, they show much more severe phenotypes than wild-type cells arrested under the same conditions (Saunders et al., 1997a; our unpublished observations). (2) In synchronized cycling populations the phenotypes of the *kar3* and *kip2* mutants are observed in nearly all preanaphase cells even though the population as a whole is actively cycling. (3) *kar3 kip2* double mutants show a similar increase in mitotic arrest to the *kar3* single mutants but do not show the accumulation of cytoplasmic microtubules, suggesting the phenotype is not due to the arrest. *kip2* mutants show no obvious cell cycle defect. (4) A Kar3p-GST fusion protein has been reported to preferentially depolymerize microtubules in vitro (Endow et al., 1994). We believe these results indicate that changes in the cell cycle may influence the extent of the phenotype (especially for the *kar3* mutants), but a perturbation of the cell cycle cannot be the sole explanation for the *kip2* or *kar3* microtubule phenotypes.

It is also possible that unidentified cytoskeletal changes, perhaps related to loss of force-generating motor activity, could indirectly produce the change in microtubule structures seen in the *kar3* and *kip2* mutants. For example loss (or an imbalance) of force acting on the microtubules could change the association of the ends of the microtubules with a stabilizing activity of the kinetochores or spindle poles, indirectly leading to the observed increased or decreased growth of microtubules. A further analysis in vitro will be necessary to confirm a direct role of these motors in microtubule polymerization.

Motors acting to influence microtubule lengths

Both Kar3p and Kip2p may function primarily at the spindle poles, based on localization experiments. If the motors function to directly influence polymerization, the preferred model, it is expected that they would act at the ends of the polymer where most or all of the polymerization and depolymerization is occurring (Fig. 6). Therefore we believe it likely that the additional staining of Kar3p on the nuclear microtubules (Page et al., 1993; Saunders et al., 1997a) and Kip2p on the cytoplasmic microtubules represent motor molecules in transit to the ends of the polymer, their main and perhaps exclusive site of action. In this way Kar3p and Kip2p may use their motor domains to position themselves rather than to transport a distinct cargo.

Why would the cell need to regulate cytoplasmic microtubule length? Cytoplasmic microtubules in yeast are thought to play an important role in positioning the nucleus and the spindle at the neck region between the mother and bud and for chromatin separation into the bud, possibly through

association of the cytoplasmic microtubules with the cell cortex (Palmer et al., 1992; Sullivan and Huffaker, 1992; Carminati and Stearns, 1997). While *kip2* mutants showed a severe cytoplasmic microtubule defect, their growth rate was unchanged, therefore we believe it unlikely that long-range association with the distal cell cortex is essential for nuclear migration, spindle positioning, nuclear division or any other essential process, all of which occur in the *kip2* mutants with very reduced or absent cytoplasmic microtubules. However, it is likely that a cortical association of the cytoplasmic microtubules plays at least an auxiliary role in nuclear positioning and chromatin entry into the bud. Kip2p and Kar3p may contribute to the regulation of cytoplasmic microtubule length to facilitate cortical association by making the microtubules dynamic enough to reach the cortex or perhaps make contact at the appropriate sites. One can imagine that cortical association involves a capturing event similar to the chromosome capture during mitosis. One prediction of this model is that the microtubules in the *kip2 kar3* double mutants, while resembling microtubules in wild-type cells in number and length, should be much less dynamic.

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REFERENCES

Bascom-Slack, C. A. and Dawson, D. S. (1997). The Yeast Motor Protein, Kar3p, Is Essential for Meiosis I. *J. Cell Biol.* **139**, 459-67.

Carminati, J. L. and Stearns, T. (1997). Microtubules orient the mitotic spindle in yeast through dynein-dependent interactions with the cell cortex. *J. Cell Biol.* **138**, 629-641.

Cottingham, F. R. and Hoyt, M. A. (1997). Mitotic spindle positioning in *Saccharomyces cerevisiae* is accomplished by antagonistically acting microtubule motor proteins. *J. Cell Biol.* **138**, 1041-1053.

DeZwaan, T. M., Ellingson, E., Pellman, D. and Roof, D. M. (1997). Kinesin-related *KIP3* of *Saccharomyces cerevisiae* is required for a distinct step in nuclear migration. *J. Cell Biol.* **138**, 1023-40.

Endow, S., Kang, S., Satterwhite, L., Rose, M., Skeen, V. and Salmon, E. (1994). Yeast Kar3 is a minus-end microtubule motor protein that destabilizes microtubules preferentially at the minus ends *EMBO J.* **13**, 2708-2713.

Farkasovsky, M. and Kuntzel, H. (1995). Yeast Num1p associates with the mother cell cortex during S/G_2 phase and affects microtubular functions. *J. Cell Biol.* **131**, 1003-1014.

Hoyt, M. A., He, L., Loo, K. K. and Saunders, W. S. (1992). Two *Saccharomyces cerevisiae* kinesin-related gene-products required for mitotic spindle assembly. *J. Cell Biol.* **118**, 109-120.

McMillan, J. N. and Tatchell, K. (1994). The *JNM1* gene in the yeast *Saccharomyces cerevisiae* is required for nuclear migration and spindle orientation during the mitotic cell cycle. *J. Cell Biol.* **125**, 143-158.

Meluh, P. B. and Rose, M. D. (1990). Antibodies to the kinesin motor domain and CENP-E inhibit microtubule depolymerization-dependent motion of chromosomes in vitro. *Cell* **60**, 1029-1041.

Nicklas, R. B. (1998). The forces that move chromosomes in mitosis. *Annu. Rev. Biophys. Chem.* **17**, 431-449.

Olmstead, J. B. (1986). Microtubule-associated proteins. *Ann. Rev. Cell Biol.* **2**, 421-457.

Page, B. D., Satterwhite, L. L., Rose, M. D. and Snyder, M. (1993). Localization of the KAR3 kinesin heavy chain-like protein requires the CIK1 interacting protein. *J. Cell Biol.* **124**, 507-519.

Palmer, R. E., Sullivan, D. S., Huffaker, T. H. and Koshland, D. (1992). Role of astral microtubules in spindle orientation and migration in the budding yeast, *Saccharomyces cerevisiae. J. Cell Biol.* **119**, 583-594.

Pringle, J. R., Adams, A. E. M., Drubin, D. G. and Haarer, B. K. (1991). Immunofluorescence methods for yeast. *Meth. Enzymol.*, 19**4**, 565-602.

Roof, D. M., Meluh, P. B. and Rose, M. D. (1991). Multiple kinesin-related proteins in yeast mitosis. *Cold Spring Harbor Symp. Quant. Biol.* **56**, 693-703.

Roof, D. M., Meluh, P. B. and Rose, M. D. (1992). Kinesin-related proteins required for assembly of the mitotic spindle. *J. Cell Biol.* **118**, 95-108.

Rout M. P. and Kilmartin J. V. (1990). Components of the yeast spindle and spindle pole body. *J. Cell Biol.* **111**, 1913-1927.

Saunders, W., Hornack, D., Lengyel, V. and Deng, C. (1997a). The *Saccharomyces cerevisiae* kinesin-related motor Kar3p acts at preanaphase spindle poles to limit the number and length of cytoplasmic microtubules. *J. Cell Biol.* **137**, 417-431.

Saunders, W., Lengyel, V. and Hoyt, M. A. (1997b). Mitotic spindle function in *Saccharomyces cerevisiae* requires a balance between different types of kinesin-related motors. *Mol. Biol. Cell* **8**, 1025-1033.

Saunders, W. S. and Hoyt, M. A. (1992). Kinesin-related proteins required for structural integrity of the mitotic spindle. *Cell* **70**, 451-458.

Shaw, S. L., Yeh, F., Maddox, P., Salmon, E. D. and Bloom, K. (1998). Astral microtutule dynamics in yeast: A microtubule-based searching mechanism for spindle orientation and nuclear migration into the bud. *J. Cell Biol.* **139**, 985-994.

Sherman, F., Fink, G. R. and Hicks, J. B. (1983). *Methods in Yeast Genetics.* Cold Spring Harbor Press, Cold Spring Harbor, NY.

Winey, M., Mamay, C. L., O'Toole, E. T., Mastronarde, D. N., T. H. Giddings, J., McDonald, K. L. and McIntosh, J. R. (1995). Three-dimensional ultrastructural analysis of the *Saccharomyces cerevisiae* mitotic spindle. *J. Cell Biol.* **129**, 1601-1616.

Yeh, E., Skibbens, R., Cheng, J., Salmon, E. and Bloom, K. (1995). Spindle dynamics and cell cycle regulation of dynein in the budding yeast *Saccharomyces cerevisiae*. *J. Cell Biol.* **130**, 687-700.