Novel Roles for *Saccharomyces cerevisiae* Mitotic Spindle Motors

Frank R. Cottingham, Larisa Gheber, Dana L. Miller, and M. Andrew Hoyt

Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218

Abstract. The single cytoplasmic dynein and five of the six kinesin-related proteins encoded by *Saccharomyces cerevisiae* participate in mitotic spindle function. Some of the motors operate within the nucleus to assemble and elongate the bipolar spindle. Others operate on the cytoplasmic microtubules to effect spindle and nuclear positioning within the cell. This study reveals that kinesin-related Kar3p and Kip3p are unique in that they perform roles both inside and outside the nucleus. Kar3p, like Kip3p, was found to be required for spindle positioning in the absence of dynein. The spindle positioning role of Kar3p is performed in concert with the Cik1p accessory factor, but not the homologous Vik1p. Kar3p and Kip3p were also found to overlap for a function essential for the structural integrity of the bipolar spindle. The cytoplasmic and nuclear roles of both these motors could be partially substituted for by the microtubule-destabilizing agent benomyl, suggesting that these motors perform an essential microtubule-destabilizing function. In addition, we found that yeast cell viability could be supported by as few as two microtubule-based motors: the BimC-type kinesin Cin8p, required for spindle structure, paired with either Kar3p or Kip3p, required for both spindle structure and positioning.

Key words: kinesin • dynein • motor proteins • microtubules • mitotic spindle

MICROTUBULES and their associated motor proteins accomplish many intracellular movements in eukaryotic cells. Two superfamilies of microtubule-based motor proteins have been identified, the kinesins and the dyneins. Members of each superfamily are defined by distinct force-producing domains that are conserved in amino acid sequence. Since all eukaryotic cells express multiple microtubule motors, a major research question concerns the cellular roles performed by each motor.

The budding yeast *Saccharomyces cerevisiae* has been particularly useful for the experimental dissection of microtubule-based motor roles (Hoyt and Geiser, 1996). In addition to the experimental tractability of this organism, it possesses a relatively small and easy to manipulate set of microtubule motor genes. The *S. cerevisiae* genome encodes six kinesin-related gene products (Cin8p, Kip1–3p, Kar3p, and Smy1p) and a single dynein heavy chain (Dyn1p, a member of the cytoplasmic dynein subclass). Although these motor gene products are each distinct in amino acid sequence, none are individually essential for cell viability. All single deletion alleles are viable. This is due to extensive overlaps in functions between these motors. *S. cerevisiae* uses microtubules for only a single process essential for vegetative growth, mitotic spindle function. Previous studies have implicated six of these seven motors in spindle function (Hoyt et al., 1997; Stearns, 1997). The single exception is kinesin-related Smy1p, which is involved in polarized cell growth (Lillie and Brown, 1994, 1998). *smy1* mutants display neither mitotic defects nor genetic interactions with any other microtubule motor gene deletion alleles (Cottingham and Hoyt, 1997; Lillie and Brown, 1998). In contrast, mutations in the remaining six motor genes cause mitotic phenotypes and display extensive genetic interactions. Examples of cooperative (or overlapping functions) and antagonistic interactions have been described and experimentally exploited to reveal essential spindle functions performed by these six motors (for reviews see Hoyt and Geiser, 1996; Stearns, 1997).

The mitotic division of *S. cerevisiae* cells requires microtubule-based activities operating on both sides of the nuclear envelope. The nuclear microtubules assemble into a bipolar spindle structure that separates replicated chromosomes in anaphase, primarily by elongation (anaphase B–type movement). The major and essential role for the cytoplasmic microtubules is positioning the spindle within the cell at the neck between mother and bud. Since this process results in the movement of the entire nuclear envelope and its contents, it is often referred to as nuclear migration. Proper spindle positioning ensures that anaphase spindle elongation segregates one progeny nucleus to the mother cell and the other to the bud.

Two of the *S. cerevisiae* kinesin-related proteins, Cin8p and Kip1p, are members of the BimC sequence subclass. BimC motors are required for bipolar spindle assembly in *S. cerevisiae* and other eukaryotic cell types (Hoyt and Geiser, 1996; Kashina et al., 1998). Cin8p and Kip1p also make a major contribution to anaphase B spindle elongation (Saunders et al., 1995; Straight et al., 1998). These roles are most likely accomplished by the ability of BimC motors to cross-link and slide spindle midzone microtubules within the nucleus. Roles for Kar3p and Kip3p in the bipolar spindle have been implicated, but are not well understood. Kar3p clearly performs numerous but poorly defined roles during mitosis. *kar3Δ* mutant cultures display many aberrant spindles (Meluh and Rose, 1990; Saunders et al., 1997a). Kar3p also antagonizes the pole-separating activity of the BimC motors during the process of spindle assembly (Saunders and Hoyt, 1992; Hoyt et al., 1993; Saunders et al., 1997b). Kar3p accomplishes some of its mitotic functions in association with one of two homologous accessory subunits, Cik1p and Vik1p, which apparently target this motor to distinct cellular roles (Manning et al., 1999; Page et al., 1994). Kip3p has been localized to the nuclear microtubules (as well as cytoplasmic microtubules) and deletion mutants make slightly longer spindles (Cottingham and Hoyt, 1997; DeZwaan et al., 1997; Miller et al., 1998; Straight et al., 1998).

Dynein is an important spindle positioning motor, but is not essential for cell viability (Eshel et al., 1993; Li et al., 1993). Functional overlap for spindle positioning between dynein and Kip3p was described previously (Cottingham and Hoyt, 1997; DeZwaan et al., 1997). Interestingly, both *dyn1 kip3* and *dyn1 kar3* double mutants were severely compromised for growth, and in both cases the growth defect was suppressed by the deletion of *KIP2*, encoding another kinesin-related protein. This suggested that Kar3p may also overlap with dynein and Kip3p for spindle positioning roles. The studies presented here verify this hypothesis. In addition, functional overlap between Kar3p and Kip3p for a nuclear role essential for the structural integrity of bipolar spindles is described. Therefore, Kar3p and Kip3p overlap for essential roles both inside and outside the nucleus. Finally, our studies reveal that *S. cerevisiae* cells can perform mitosis with as many as five of the seven microtubule-based motor genes deleted.

Materials and Methods

Yeast Strains and Media

The *S. cerevisiae* strains used in these experiments are derivatives of S288C and are listed in Table I. The *dyn1::HIS3* (Geiser et al., 1997), *dyn1::URA3* (Eshel et al., 1993), *kar3::LEU2* (Meluh and Rose, 1990), *kip2::URA3* (Roof et al., 1992), *kip3::kan* and *kip3-14* (Cottingham and Hoyt, 1997), *cik1::LEU2* (Page and Snyder, 1992), and *vik1::HIS3* (Manning et al., 1999) alleles were described previously. The unmarked *dyn1-Δ10* allele was created by transforming a *dyn1::URA3* strain with a DNA fragment of *DYN1* that spanned the *URA3* insertion but harbored an internal deletion. Ura⁻ were selected on media containing 5-fluoro-orotic acid (5-FOA)[1] (US Biological). The same strategy was used to create the

---

1. *Abbreviations used in this paper:* DAPI, 4,6-diamidino-2-phenylindole; GFP, green fluorescent protein; SPB, spindle pole body; 5-FOA, 5-fluoro-orotic acid.

unmarked *kip2-Δ10* allele. The *smy1-Δ10* unmarked deletion was created by PCR-targeted gene disruption (Wach et al., 1994). The PCR template DNA was *URA3* flanked by a directly repeated sequence. Following transformation and selection for *smy1::URA3*, we were able to select, using 5-FOA, for segregants that had looped out *URA3* by homologous recombination between the repeated sequence.

Multiple motor gene deletion mutants were constructed by standard genetic techniques. Strains with reduced spindle motor function often displayed evidence of aneuploidy (i.e., non-Mendelian segregation of genetic markers). Therefore, we routinely verified all seven microtubule motor gene alleles by PCR. For *DYN1* and *KIP2*, we were able to use a primer set that flanked the genes and yielded distinct PCR products from both the wild-type and deletion alleles. For the remaining five microtubule motor genes, two PCR reactions were used to test each allelic form. Both reactions used an upstream primer with a sequence present in both wild-type and deletion mutant alleles. This was paired with downstream primers that were specific for either the wild-type allele DNA in one reaction or the deletion DNA in the other.

Rich (yeast extract, peptone, dextrose [YPD]) and minimal (synthetic dextrose [SD]) media were as described (Sherman et al., 1983). Benomyl (DuPont) was added to solid YPD medium from a 10 mg/ml stock in DMSO. Nocadazole (Sigma Chemical Co.) was added to liquid YPD medium from a 3.3 mg/ml stock in DMSO. For G1 synchronization, α-factor (Bachem Bioscience) was added to 4 μg/ml to log-phase cells in liquid YPD, pH 4.0, and incubated at 26°C until >80% of cells were unbudded. For arrest in S phase, hydroxyurea (Sigma Chemical Co.) was added to 0.1 M to log-phase cells in liquid YPD, pH 5.8, and incubated at 26°C until >70% of cells were large-budded.

DNA Manipulations

Temperature-sensitive alleles of *KAR3* were generated by a mutagenic PCR procedure (Staples and Dieckmann, 1993). The entire *KAR3* gene, including 412 bp of upstream sequence and 560 bp of downstream sequence, was amplified under mutagenic PCR conditions. We used *Taq* polymerase according to the supplier's instructions (Stratagene), except that Mn²⁺ and Mg²⁺ were added to final concentrations of 0.25 and 4.5 mM, respectively. The PCR products were concentrated using QIAquick spin columns (Qiagen). pMA1428 was digested with NruI and StuI to create a gapped construct in which all but the first 210 bp of *KAR3* coding sequence was removed. The gapped pMA1428 construct was gel-purified and cotransformed with the concentrated PCR products into strain MAY4619. This strain is unable to survive loss of a plasmid carrying *KAR3* and *URA3*, and is therefore rendered sensitive to 5-FOA. Approximately 6,600 His⁺ transformants were selected at 26°C and then replica plated to 5-FOA–containing media at 26 and 35°C. Six colonies were selected that were viable on 5-FOA at 26 but not 35°C. Plasmids were isolated from the six temperature-sensitive transformants and retransformed into MAY4619. After growth on 5-FOA at 26°C, four of the six retransformed strains displayed reduced growth on YPD at 35°C. These four plasmids were designated pMA1428*kar3-61* through pMA1428*kar3-64*. Of these, pMA1428*kar3-64* caused the greatest reduction of growth for *kar3Δ kip3Δ* strains at 35°C, and was chosen for study in all subsequent experiments. pMA1428*kar3-64* did not alter the temperature resistance of either wild-type or *kip3Δ*, *dyn1Δ*, and *kar3Δ* single mutant strains. Therefore, *kar3-64* is a recessive and nonepistatic mutant.

Microscopy

To stain for DNA, cells were pelleted out of liquid media, resuspended in 70% ethanol, and stored on ice for 30 min. The ethanol-fixed cells were washed once with water and then resuspended in 0.3 μg/ml 4,6-diamidino-2-phenylindole (DAPI) containing 1 mg/ml *p*-phenylenediamine to prevent fading (Sigma Chemical Co.). To stain for chitin-containing bud scars (Pringle, 1991), cells were fixed as above, washed once with water, resuspended in 1 mg/ml calcofluor solution (Sigma Chemical Co.), and stored at room temperature in the dark for 5 min. The cells were washed four times with water and then resuspended in 0.3 μg/ml DAPI plus 1 mg/ml *p*-phenylenediamine before viewing. Mother cell bodies were defined as those which possessed at least two calcofluor-stained chitin rings on their surface. For antitubulin immunofluorescence microscopy, cells were fixed by adding formaldehyde directly to the medium to a final concentration of 3.7%. Microtubules were visualized by a procedure described previously (Pringle et al., 1991) using the rat antitubulin antibody YOL1/34 (Harlan Bioproducts for Science) and rhodamine-conjugated goat anti–rat second-

Table I. Yeast Strains and Plasmids

	Genotype*
Yeast strains	
MAY589	*MAT*a *ade2 his3 leu2 ura3*
MAY591	*MAT*α *lys2 his3 leu2 ura3*
MAY1089	*MAT*a *ade2 lys2 his3 leu2 ura3*
MAY2268	*MAT*α *lys2 his3 leu2 ura3 kar3::LEU2*
MAY3189	*MAT*α *lys2 his3 leu2 ura3 dyn1::URA3*
MAY2269	*MAT*a *ade2 his3 leu2 ura3 kar3::LEU2*
MAY4360	*MAT*a *ade2 his3 leu2 ura3* (pRS313)
MAY4435	*MAT*α *lys2 his3 leu2 ura3 cyh2 dyn1::HIS3*
MAY4518	*MAT*α *lys2 his3 leu2 ura3 kip3::kan*
MAY4560	*MAT*a *ade2 lys2 his3 leu2 ura3 cyh2 kip3::kan kip2::URA3 dyn1::HIS3*
MAY4619	*MAT*a *ade2 lys2 his3 leu2 ura3 kar3::LEU2 kip3::kan* (pMA1223)
MAY4809	*MAT*a *ade2 lys2 his3 leu2 ura3 kar3::LEU2 kip3::kan* (pFC51)
MAY4908	*MAT*a *ade2 lys2 his3 leu2 ura3 kar3::LEU2 kip3::kan* (pFC51*kip3-14*)
MAY4921	*MAT*a *lys2 his3 leu2 ura3 dyn1::HIS3 kip3::kan* (pFC51*kip3-14*)
MAY4924	*MAT*a *lys2 his3 leu2 ura3 dyn1::HIS3 kip3::kan* (pFC51)
MAY5001	*MAT*a *ade2 lys2 his3 leu2 ura3 kar3::LEU2 kip3::kan* (pMA1428)
MAY5022	*MAT*a *ade2 his3 leu2 ura3 kar3::LEU2* (pMA1428)
MAY5218	*MAT*a *ade2 his3 leu2 ura3 kar3::LEU2 dyn1-Δ10* (pMA1428)
MAY5269	*MAT*a *ade2 lys2 his3 leu2 ura3 cyh2 kar3::LEU2 kip3::kan kip2::URA3* (pMA1428)
MAY5360	*MAT*a *ade2 his3 leu2 ura3 cik1::LEU2*
MAY5398	*MAT*a *ade2 his3 leu2 ura3 kar3::LEU2* (pMA1428*kar3-64*)
MAY5399	*MAT*a *ade2 lys2 his3 leu2 ura3 kar3::LEU2 kip3::kan* (pMA1428*kar3-64*)
MAY5400	*MAT*a *ade2 his3 leu2 ura3 kar3::LEU2 dyn1-Δ10* (pMA1428*kar3-64*)
MAY5464	*MAT*a *ade2 his3 leu2 ura3 kar3::LEU2 dyn1-Δ10 kip2::URA3* (pMA1428)
MAY5465	*MAT*a *ade2 his3 leu2 ura3 kar3::LEU2 dyn1-Δ10 kip2::URA3* (pMA1428*kar3-64*)
MAY5522	*MAT*a *ade2 lys2 his3 leu2 ura3 kar3::LEU2 kip3::kan kip2::URA3* (pMA1428*kar3-64*)
MAY5742	*MAT*a *ade2 his3 leu2 ura3 kar3::LEU2* (pRS313)
MAY5774	*MAT*a *ade2 lys2 his3 leu2 ura3 kar3::LEU2 kip3::kan dyn1-Δ10 kip2-Δ10* (pMA1428)
MAY5775	*MAT*a *ade2 lys2 his3 leu2 ura3 kar3::LEU2 kip3::kan dyn1-Δ10 kip2-Δ10* (pMA1428*kar3-64*)
MAY5776	*MAT*a *ade2 his3 leu2 ura3 nuf2::NUF2-sGFP* (pRS313)
MAY5777	*MAT*a *ade2 his3 leu2 ura3 nuf2::NUF2-sGFP kar3::LEU2* (pMA1428*kar3-64*)
MAY5778	*MAT*a *ade2 lys2 his3 leu2 ura3 kar3::LEU2 kip3::kan nuf2::NUF2-sGFP* (pMA1428*kar3-64*)
MAY5779	*MAT*a *ade2 his3 leu2 ura3 kar3::LEU2 dyn1-Δ10 nuf2::NUF2-sGFP* (pMA1428*kar3-64*)
MAY5802	*MAT*a *lys2 his3 leu2 ura3 dyn1::HIS3 cik1::LEU2 kip2::URA3*
MAY5903	*MAT*a *ade2 lys2 his3 leu2 ura3 dyn1-Δ10 kip2-Δ10 smy1-Δ10 kip1::URA3 kip3::kan kar3::LEU2* (pMA1428)
MAY5904	*MAT*a *ade2 lys2 his3 leu2 ura3 dyn1-Δ10 kip2-Δ10 smy1-Δ10 kip1::URA3 kip3::kan kar3::LEU2* (pMA1428*kar3-64*)
MAY5905	*MAT*a *ade2 lys2 his3 leu2 ura3 dyn1-Δ10 kip2-Δ10 smy1-Δ10 kip1::URA3 kip3::kan kar3::LEU2* (pFC82)
MAY5906	*MAT*a *ade2 lys2 his3 leu2 ura3 dyn1-Δ10 kip2-Δ10 smy1-Δ10 kip1::URA3 kip3::kan kar3::LEU2* (pFC83)
MAY5940	*MAT*a *ade2 lys2 his3 leu2 ura3 dyn1-Δ10 kip2-Δ10 smy1-Δ10 kip1::HIS3 kip3::kan cin8::LEU2* (pMA1260)
MAY5941	*MAT*a *ade2 lys2 his3 leu2 ura3 dyn1-Δ10 kip2-Δ10 smy1-Δ10 kip1::HIS3 kip3::kan cin8::LEU2* (pTK199)
MAY6030	*MAT*a *ade2 his3 leu2 ura3 kip3::kan nuf2::NUF2-sGFP* (pFC83)
MAY6332	*MAT*a *ade2 his3 ura3 dyn1::URA3 vik1::HIS3*
MAY6341	*MAT*a *ade2 his3 leu2 ura3 trp1 kip3::kan vik1::HIS3*
MAY6417	*MAT*a *ade2 his3 leu2 ura3 trp1 kip3::kan vik1::HIS3 cik1::LEU2* (pFC48)
Plasmids	
pFC48	*KIP3 URA3 (CEN)*
pFC50	*KIP2 LYS2 (CEN)*
pFC51	*KIP3 LYS2 (CEN)*
pFC51*kip3-14*	*kip3-14 LYS2 (CEN)*
pFC82	*KIP3 HIS3 (CEN)*
pFC83	*kip3-14 HIS3 (CEN)*
pMA1223	*KAR3 URA3 (CEN)*
pMA1260	*CIN8 LYS2 (CEN)*
pMA1428	*KAR3 HIS3 (CEN)*
pMA1428*kar3-64*	*kar3-64 HIS3 (CEN)*
pRS313	*HIS3 (CEN)*
pTK199	*cin8-3 LYS2 (CEN)*

*Alleles common to these yeast strains: *lys2-801 ade2-101 his3-Δ200 leu2-3,112 ura3-52 trp1-Δ1*.

ary antibodies (Jackson ImmunoResearch Laboratories, Inc.). Stained cells were examined with an inverted microscope (Axiovert 135; Carl Zeiss, Inc.) equipped with epifluorescent optics using a 100× objective. Digital images were captured with a cooled, slow-scan CCD camera. In cells expressing the spindle pole body (SPB)-associated fusion protein Nuf2p–green fluorescent protein (GFP) (Kahana et al., 1995), bipolar spindles were quantitated by counting the number of cells with two clearly separated GFP dots.

Electron microscopic examination of thin sections was performed as described by Byers and Goetsch (1991).

Quantitation of Nuclear Mislocalization

To quantitate nuclear mislocalization in cells, two assays were used. In the first, cells were arrested at 26°C with α-factor as described above. Cells were then washed once with water to remove the α-factor and resuspended in YPD media prewarmed to 35°C. The cultures were incubated at 35°C and at regular intervals samples were removed, fixed with ethanol, and stained with DAPI. The percentage of total cells that were large-budded (bud diameter greater than three quarters the diameter of the mother cell) with the nucleus away from the neck was determined for each time point. "Nucleus away from the neck" cells were defined as those in which the closest distance between the nucleus and the neck was greater than one half the diameter of the entire nuclear DNA mass, as judged by eye. In the second assay, cells were arrested at 26°C with hydroxyurea as described above. Next, cultures were shifted to 35°C, and at regular intervals samples were removed, fixed, and either stained with DAPI alone, or processed for both antitubulin immunofluorescence and DAPI staining. The percentage of large-budded cells with the nucleus away from the neck was determined for each time point.

Results

Characterization of a Temperature-Sensitive KAR3 Allele

In a previous study we obtained evidence of overlap for an essential function between Kar3p, Kip3p, and dynein. Although neither *KAR3*, *KIP3*, nor *DYN1* (encoding the dynein heavy chain) are individually essential for cell viability, the double deletion mutant combinations are inviable, or in the case of *dyn1Δ kip3Δ*, grow very slowly (Cottingham and Hoyt, 1997). To investigate the nature of this overlap and the mitotic role of Kar3p, we generated a recessive temperature-sensitive *KAR3* mutant allele (see Materials and Methods). A plasmid carrying this allele, *kar3-64*, was identified in a strain deleted for *KAR3* and *KIP3*. The *kar3Δ kip3Δ* (p*kar3-64*) strain was inviable at 33°C and higher (Fig. 1; compare row A10 to A9, 0
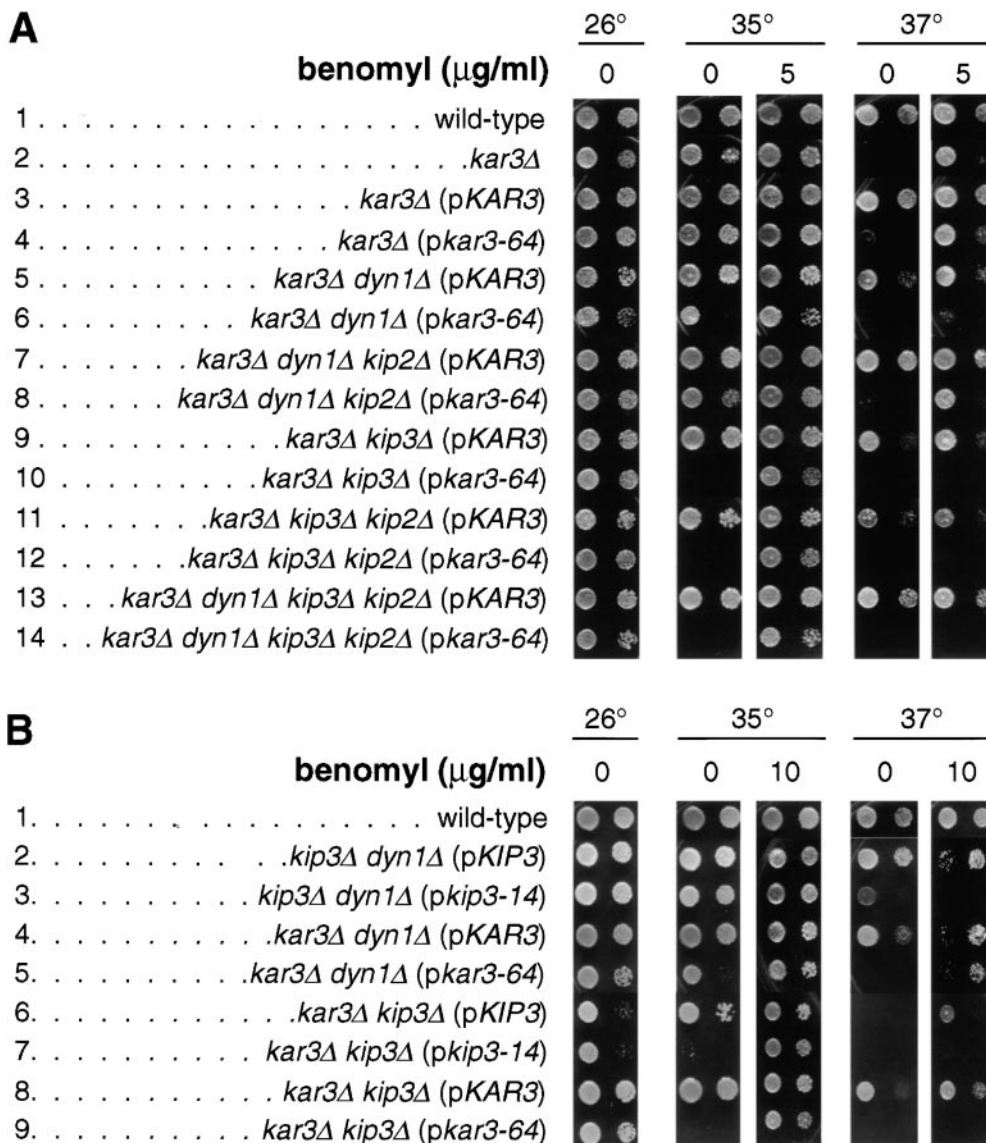
Figure 1. Growth properties of motor mutant strains. Yeast cells of the indicated genotype were spotted onto solid rich media containing the indicated concentration of benomyl, and incubated at the indicated temperature for 3 d. Two spots appear for each sample: the spot to the right is a 40-fold dilution of the sample on the left. Strains used (p indicates a centromere plasmid-carried gene): (A) wild-type (MAY4360), *kar3Δ* (MAY5742), *kar3Δ* (p*KAR3*) (MAY5022), *kar3Δ* (p*kar3-64*) (MAY5398), *kar3Δ dyn1Δ* (p*KAR3*) (MAY5218), *kar3Δ dyn1Δ* (p*kar3-64*) (MAY5400), *kar3Δ dyn1Δ kip2Δ* (p*KAR3*) (MAY5464), *kar3Δ dyn1Δ kip2Δ* (p*kar3-64*) (MAY5465), *kar3Δ kip3Δ* (p*KAR3*) (MAY5001), *kar3Δ kip3Δ* (p*kar3-64*) (MAY5399), *kar3Δ kip3Δ kip2Δ* (p*KAR3*) (MAY5269), *kar3Δ kip3Δ kip2Δ* (p*kar3-64*) (MAY5522), *kar3Δ dyn1Δ kip3Δ kip2Δ* (p*KAR3*) (MAY5774), and *kar3Δ dyn1Δ kip3Δ kip2Δ* (p*kar3-64*) (MAY5775). (B) wild-type (MAY589), *kip3Δ dyn1Δ* (p*KIP3*) (MAY4924), *kip3Δ dyn1Δ* (p*kip3-14*) (MAY4921), *kar3Δ dyn1Δ* (p*KAR3*) (MAY5218), *kar3Δ dyn1Δ* (p*kar3-64*) (MAY5400), *kar3Δ kip3Δ* (p*KIP3*) (MAY4809), *kar3Δ kip3Δ* (p*kip3-14*) (MAY4908), *kar3Δ kip3Δ* (p*KAR3*) (MAY5001), and *kar3Δ kip3Δ* (p*kar3-64*) (MAY5399).

benomyl columns). *kar3Δ dyn1Δ* (p*kar3-64*) cells were also temperature-sensitive for growth, although the effect was not as extreme as for *kip3Δ* (Fig. 1; compare row A6 to A5). p*kar3-64* also failed to complement the slight temperature sensitivity caused by *kar3Δ* (Fig. 1; compare row A4 to A3). In the absence of dynein, kinesin-related Kip2p acts antagonistically to Kar3p and Kip3p. Loss of Kip2p suppresses the growth defects of the *dyn1Δ kip3Δ* and *dyn1Δ kar3Δ* double mutants, but not that of *kip3Δ kar3Δ* (Cottingham and Hoyt, 1997). Accordingly, we found that the *kar3Δ dyn1Δ kip2Δ* (p*kar3-64*) strain showed moderately improved growth at 35°C compared with the *kar3Δ dyn1Δ* (p*kar3-64*) strain (Fig. 1; compare row A8 to A6). However, the *kar3Δ kip3Δ kip2Δ* (p*kar3-64*) strain was just as temperature-sensitive as the corresponding strain possessing the wild-type *KIP2* allele (Fig. 1; compare row A12 to A10).

Log-phase cultures of *kar3Δ kip3Δ* (p*kar3-64*), *kar3Δ dyn1Δ* (p*kar3-64*), and wild-type cells were shifted to 35°C for 2 h and examined by microscopy. The *kar3Δ kip3Δ* (p*kar3-64*) and *kar3Δ dyn1Δ* (p*kar3-64*) showed evidence of reduced proficiency to accomplish mitosis; 57 and 52%,

respectively, of the mutant cells were large-budded and mononucleate, relative to 26% of the wild-type (*n* = 200 cells for each). The *kar3Δ dyn1Δ* (p*kar3-64*) cells often displayed mislocalized nuclei indicative of a spindle positioning defect (see next section).

Cells lacking *KAR3* display a bilateral karyogamy defect; *kar3Δ* cells will form diploids with normal efficiency with *KAR3* cells of the opposite mating type, but not with *kar3Δ* cells (Meluh and Rose, 1990). In addition to its mitotic defects, *kar3-64* caused a temperature-sensitive defect in diploid formation when mated to a *kar3Δ* strain, but not a *KAR3* strain (data not shown).

A Role for Kar3p in Spindle Positioning Revealed in Cells Missing Dynein

In a previous study, the spindle positioning role of Kip3p was clearly revealed in cells missing dynein. Loss of Kip3 function in dynein-deficient cells caused spindle (and nuclear) mispositioning. The lethality of *kar3 dyn1* and *kar3 kip3* cells suggested that Kar3p may also participate in spindle positioning. To investigate this possibility, we as-
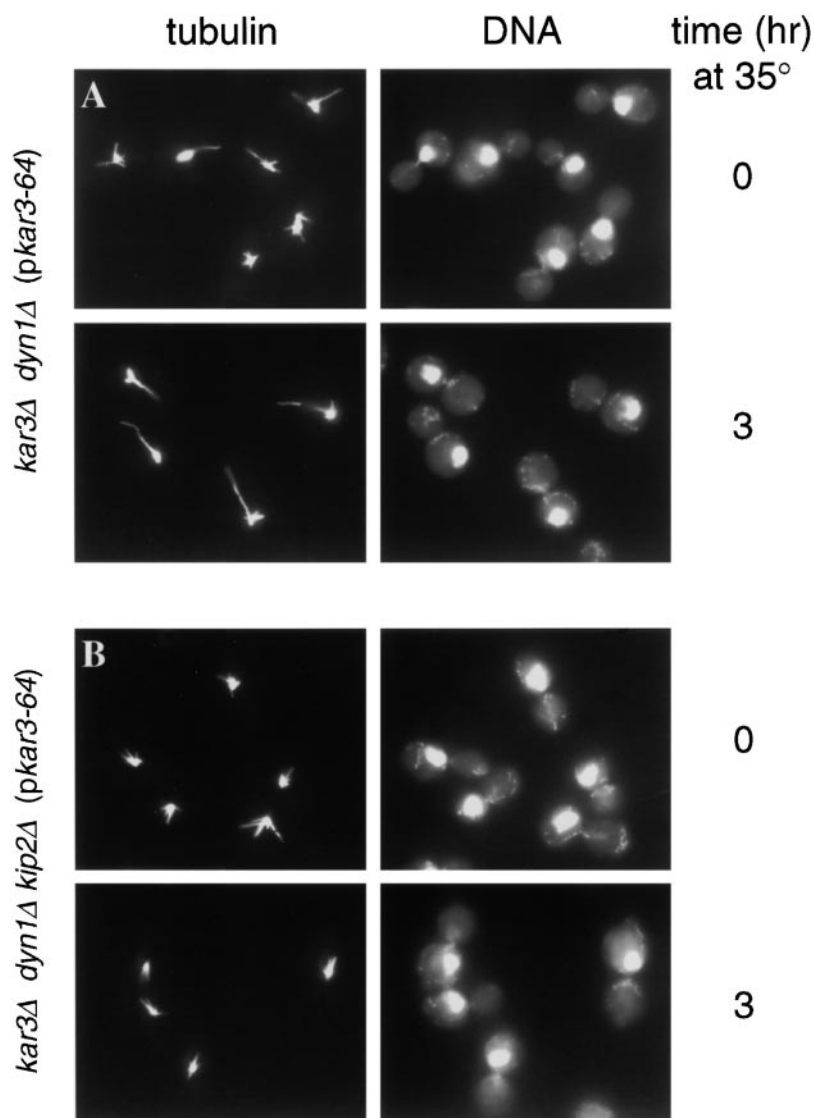

Figure 2. Spindles and nuclei mislocalize in *kar3*-ts *dyn1Δ* cells. The nucleus and spindle were drawn away from the proper position at the mother–bud neck after loss of Kar3p function in the absence of dynein, but not in the absence of dynein and Kip2p. (A) *kar3Δ dyn1Δ* (p*kar3-64*) cells (MAY5400) were arrested with hydroxyurea at 26°C and then shifted to 35°C for 3 h in the continued presence of hydroxyurea. Samples taken before (top row) and after (bottom row) the temperature shift were processed for antitubulin immunofluorescence (left column) and DAPI staining of DNA (right column). (B) *kar3Δ dyn1Δ kip2Δ* (p*kar3-64*) cells (MAY5465) were treated as in A.

sessed the proficiency of *kar3-64* cells at positioning the nucleus at the mother–bud neck (a consequence of proper spindle positioning). Two related assays were used. In the first, cells were arrested in G1 with α-factor at 26°C and released to media at 35°C. The ability of the cells to translocate their nuclei to its proper position at the neck between mother and bud cell bodies was then assessed. In the second, spindle positioning was allowed to first occur at permissive temperature (26°C) in the presence of the DNA synthesis inhibitor hydroxyurea. Yeast cells treated with this inhibitor are still able to accomplish bipolar spindle assembly and spindle positioning (Pringle and Hartwell, 1981). A temperature shift to 35°C was then imposed while the hydroxyurea block was maintained, and the ability of the cells to maintain the nucleus at the neck was assessed. In both assays, the inability to move the spindle or to maintain it at the neck was manifested by the appearance of large-budded cells with the nucleus located away from the neck (Fig. 2) (Cottingham and Hoyt, 1997). Both assays yielded similar results (Fig. 3).

Loss of Kar3p function alone did not cause nuclear mislocalization. In both assays, the *kar3Δ* (p*kar3-64*) strain behaved similar to wild-type (Fig. 3, top panels). This agrees with previous reports, in which the nuclear migration index (the shortest distance between the nucleus and the bud neck divided by the mother cell diameter) for a *kar3Δ* strain was found to be comparable to the nuclear migration index of a wild-type strain (DeZwaan et al.,

1997). Although the *kar3Δ* (p*kar3-64*) strain did not exhibit nuclear mislocalization, this strain did accumulate a large percentage (~50%) of large-budded mononucleate cells at 35°C, as reported previously for strains lacking Kar3p (Meluh and Rose, 1990). In contrast, the elimination of Kar3p function in the absence of dynein resulted in a gradual but significant mislocalization of the nucleus (see *kar3Δ dyn1Δ* [p*kar3-64*] strain in Fig. 2 A and Fig. 3, second row of panels). The extent of this phenotype was always greater than loss of dynein (*kar3Δ dyn1Δ* [p*KAR3*] strain) or Kar3p (*kar3Δ* [p*kar3-64*] strain) alone. As was observed for loss of both Kip3p and dynein activities (Cottingham and Hoyt, 1997), loss of both Kar3p and dynein caused the appearance of long cytoplasmic microtubules that extended into the bud. Bud scar staining with calcofluor was used to identify the mother bodies of cells in which Kar3p and dynein were inactivated (*n* = 200). This analysis revealed that all of the nuclear mislocalization events occurred exclusively in the mother cells, as was previously observed for *kip3 dyn1* cells. Therefore, efficient spindle positioning in the absence of dynein requires Kar3p as well as Kip3p. Furthermore, loss of Kar3p function in dynein-deficient cells that had previously achieved nuclear positioning results in movement of the nucleus away from the neck toward the mother cell cortex. To ensure that the effects of loss of dynein and Kar3p function observed here were not an artifact of the cell cycle block used in the protocols, we also examined temperature-
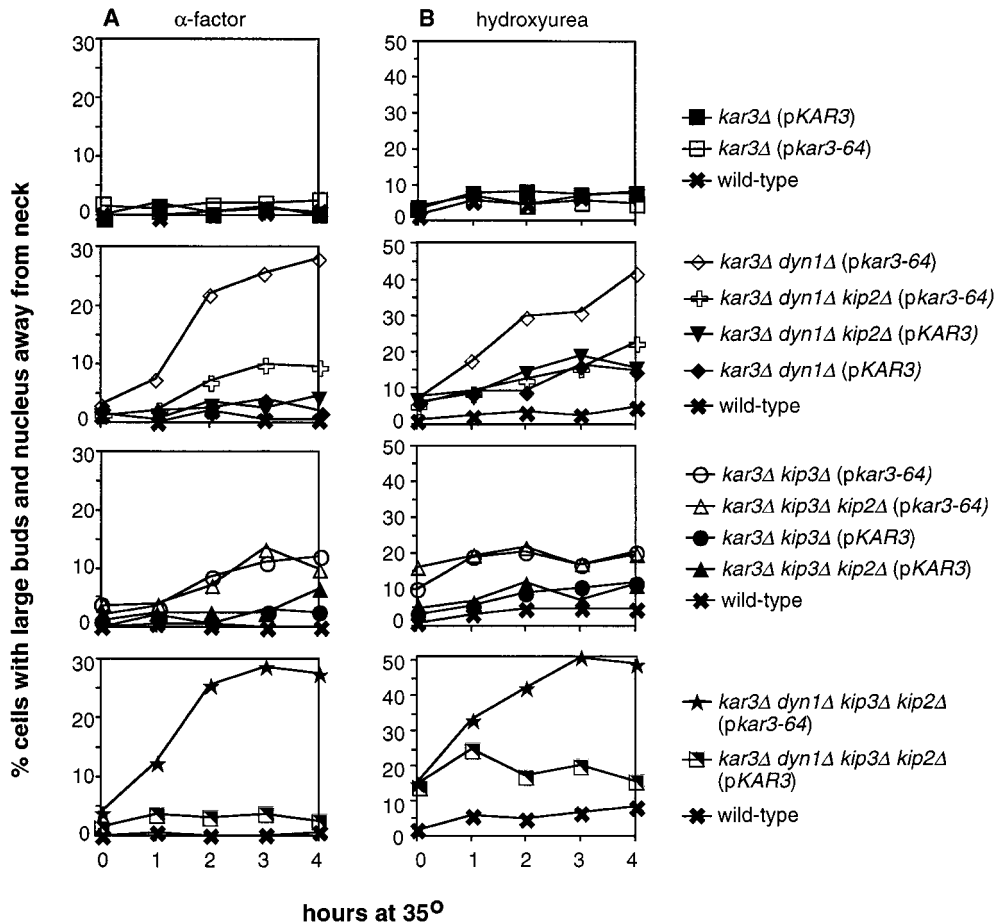
Figure 3. Quantitation of nuclear positioning defects. (A) Nuclear positioning defects following α-factor synchronization at 26°C and release to 35°C. At the time points indicated, samples were removed, stained with DAPI, and the percentage of total cells that were large-budded with the nucleus positioned away from the bud neck was determined (defined as those in which the closest distance between the nucleus and the neck was greater than one half of the diameter of the entire nuclear DNA mass). (B) Nuclear positioning defects after hydroxyurea synchronization at 26°C and a shift to 35°C in the continued presence of hydroxyurea (also see Fig. 2). At the time points indicated, cells were analyzed for nuclear position as in A. Strains used are listed in legend of Fig. 1.

shifted log-phase cultures. After 2 h at 35°C, 28% of large-budded *kar3Δ dyn1Δ* (p*kar3-64*) cells exhibited mislocalized nuclei or two nuclei in one cell body, compared with 6, 8, and 0% of *kar3Δ* (p*kar3-64*), *dyn1Δ*, and wild-type, respectively (*n* = 200 cells for each).

The ability of the deletion of *KIP2* to suppress the growth defect of *kar3 dyn1* cells (see above) was also reflected in its effects on spindle positioning proficiency. In both assays, the *kar3Δ dyn1Δ kip2Δ* (p*kar3-64*) strain displayed a greatly attenuated nuclear positioning defect relative to the *kar3Δ dyn1Δ* (p*kar3-64*) strain (Fig. 2 B and Fig. 3, second row of panels). This indicates that the spindle mislocalization observed in the absence of Kar3p and dynein was caused by the action of Kip2p. *kar3Δ dyn1Δ kip2Δ* (p*kar3-64*) cells were found to have significantly shorter cytoplasmic microtubules compared with the *kar3Δ dyn1Δ* (p*kar3-64*) strain (Fig. 2). This is consistent with the previous observation that loss of Kip2p causes a reduction in cytoplasmic microtubule length (Cottingham and Hoyt, 1997).

The spindle positioning defect caused by the absence of Kar3p and dynein, and its suppression by *kip2Δ*, was very similar to the defect observed previously for cells missing Kip3p and dynein (Cottingham and Hoyt, 1997). Cells missing both Kar3p and Kip3p are inviable, possibly also due to a spindle positioning defect (i.e., eliminating any two of either dynein, Kar3p, or Kip3p would prevent spindle positioning). To test this possibility, we performed the two nuclear position assays on a *kar3Δ kip3Δ* (p*kar3-64*) strain. Both assays demonstrated that loss of Kar3p in the absence of Kip3p caused only relatively mild nuclear mislocalization (Fig. 3, third row of panels). However, this effect was greater than those observed in the absence of either Kar3p or Kip3p alone. Similar findings were obtained in experiments using a temperature-sensitive *KIP3* allele in a *kar3Δ* strain (Cottingham, F.R., and M.A. Hoyt, unpublished observations). The minor nuclear position defect observed in the absence of Kar3p and Kip3p did not appear to be affected by the presence or absence of Kip2p, i.e., the *kar3Δ kip3Δ kip2Δ* (p*kar3-64*) strain behaved similarly to the *kar3Δ kip3Δ* (p*kar3-64*) strain in both nuclear position assays. These findings indicate that Kar3p and Kip3p together make only a minor contribution to spindle positioning when dynein is present. However, when dynein is absent, the contributions of both Kip3p (Cottingham and Hoyt, 1997) and Kar3p (this study) become apparent. The fact that loss of both Kar3p and Kip3p caused only a mild spindle positioning defect, but that lethality was not suppressed by *kip2Δ*, suggests that they overlap for a different essential process. The nature of this process is addressed in the following section.

The findings presented here and elsewhere (Cottingham and Hoyt, 1997) implicate four microtubule-based motors in the process of spindle and nuclear positioning: dynein, Kar3p, Kip3p, and Kip2p. To investigate the consequences of loss of all four of these motors, we performed the two nuclear position assays described above on a strain deleted for *DYN1*, *KAR3*, *KIP3*, and *KIP2* and carrying the *kar3-64* allele on a plasmid (Fig. 3, bottom row of panels). In both assays, the *kar3Δ dyn1Δ kip3Δ kip2Δ* (p*kar3-64*) strain exhibited a nuclear positioning defect at 35°C; it was not able to move the nucleus to the neck efficiently or

maintain it there following hydroxyurea synchronization. The movement of nuclei away from the neck after hydroxyurea treatment was found to occur independent of microtubule function (Cottingham, F.R., and M.A. Hoyt, unpublished observations). Therefore, in this experiment, Kar3p was acting as a solitary and essential spindle positioning motor required to resist a microtubule-independent force that operates on the nucleus. In the last Results section, we demonstrate that either Kar3p or Kip3p alone is sufficient to perform spindle positioning.

Spindle Assembly and Integrity Requires Either Kar3p or Kip3p

As demonstrated above, loss of function of both Kar3p and Kip3p caused only a mild spindle positioning defect. Therefore, the inviability of *kar3 kip3* double mutant cells may be due to a defect in another essential cellular process. In the course of our spindle positioning experiments, microscopy revealed frequent abnormal spindle morphologies in *kar3* cells, as has also been observed in *kar3* cells by others (e.g., Meluh and Rose, 1990; Saunders et al., 1997a). The abnormal spindle phenotype appeared more severe when both Kar3p and Kip3p were absent. To assess bipolar spindle assembly proficiency, cells of various genotypes were synchronized in G1 with α-factor at 26°C and released into media at 35°C. Fig. 4 shows examples of spindles fixed and stained 1 h after release from the α-factor block. Most wild-type cells possessed spindles that were clearly bipolar; a bright bar of spindle microtubules was visible in the nuclei (in some cases the bar was long, indicating anaphase had initiated). Fewer bar structures were found in *kar3Δ* (p*kar3-64*) cells and even fewer in *kar3Δ kip3Δ* (p*kar3-64*) cells. Instead of bars of microtubules, *kar3Δ kip3Δ* (p*kar3-64*) cells often had a bright small mass of nuclear microtubules from which the cytoplasmic microtubules radiated. As described previously for cells deficient for Kar3p and Dyn1p or Kip3p and Dyn1p, the cytoplasmic microtubules of cells missing the functions of both Kar3p and Kip3p grew to much longer lengths than wild-type.

To quantitate the effects of loss of function of Kar3p and Kip3p on bipolar structure, we examined cells whose spindle poles were marked with the GFP. A construct encoding a GFP-tagged SPB protein, Nuf2p, was integrated into our motor mutant strains (Kahana et al., 1995). At various times after release from the α-factor block into 35°C, live cells were observed under the microscope and the percentage of cells with two clearly separated GFP dots was determined (Fig. 5, A and B). Relative to the wild-type, the *kar3Δ kip3Δ* (p*kar3-64*) strain was severely compromised for its ability to generate cells with two distinct GFP dots. The majority of *kar3Δ kip3Δ* (p*kar3-64*) cells were large-budded, with a single GFP dot located at the bud neck (Fig. 5 A, bottom row). Therefore, Kip3p makes an important contribution to spindle assembly that is revealed when Kar3p activity is compromised. However, the activity provided by Kip3p cannot completely compensate for loss of Kar3p, since *kar3-64* and *kar3Δ* (not shown) cells exhibited an intermediate reduction in spindle assembly proficiency, even when Kip3p was present. Dynein, on the other hand, does not appear to overlap
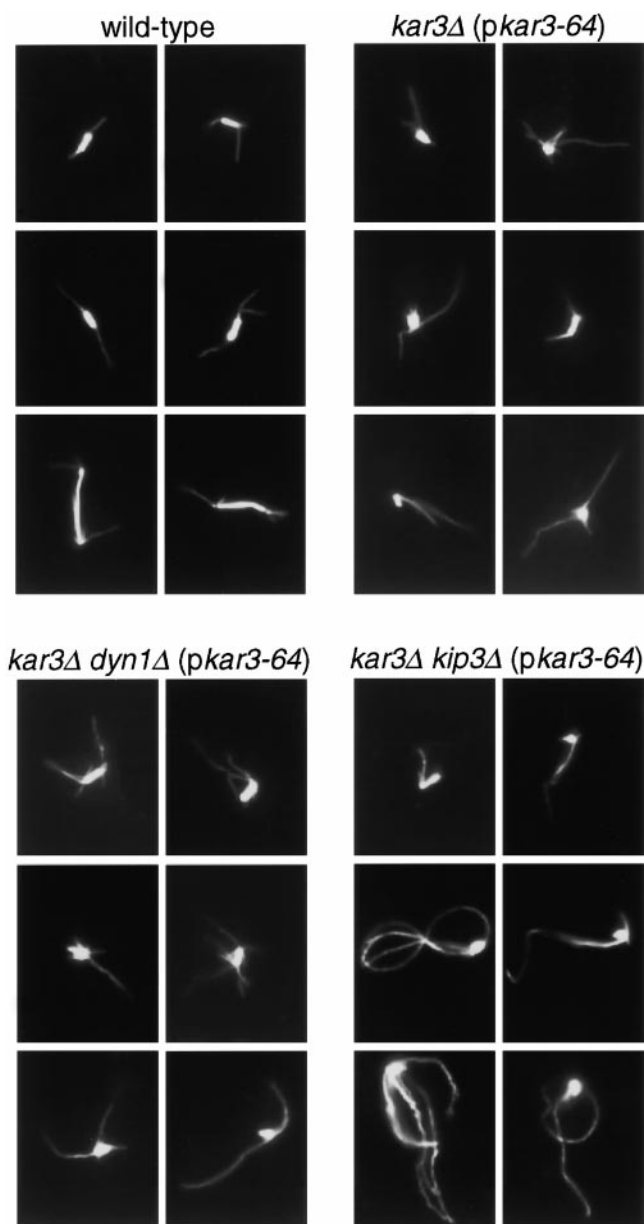
Figure 4. Spindle defects caused by loss of Kar3p function. Cell cultures of the genotypes indicated were synchronized with α-factor at 26°C and then released into medium at 35°C. After 60 min, samples were processed for antitubulin immunofluorescence microscopy. Microtubules of six representative cells are shown for each genotype. Note that the top left example of the *kar3Δ kip3Δ* (p*kar3-64*) strain represents a rare bipolar spindle observed for cells of this genotype. Strains used are listed in legend of Fig. 1.

with Kar3p for establishing spindle structure, since the *kar3Δ dyn1Δ* (p*kar3-64*) strain was no more severely affected than the *kar3Δ* (p*kar3-64*).

To assess the ability of bipolar spindles to maintain their integrity after motor loss, we arrested the mutants with hydroxyurea at 26°C (a condition that allows bipolar spindle assembly) and then shifted to 35°C. Observation of the Nuf2p-GFP dots as a function of time revealed that wild-type and *kip3-14* (a temperature-sensitive allele) cells maintained bipolar spindle structure (Fig. 5 C). In contrast, ~80% of the *kar3Δ* (p*kar3-64*) cells displayed two Nuf2p-GFP dots before the temperature shift, but only ~45% displayed two dots after 3 h at 35°C. The presence or absence of dynein in the *kar3-64* cells made no apparent difference. Spindles also did not lose bipolarity when both Kip3p and dynein were inactivated (Cottingham and Hoyt, 1997). However, the absence of Kip3p significantly enhanced the loss of bipolarity phenotype exhibited by *kar3-64* cells; after 3 h, only ~25% of *kar3Δ kip3Δ* (p*kar3-64*) cells had two clearly separated dots. Therefore, Kar3p and Kip3p overlap for a function required to maintain bipolar spindle integrity.

Thin-section electron microscopy was used to examine the spindle morphology of *kar3Δ kip3Δ* (p*kar3-64*) and wild-type cells treated with hydroxyurea and shifted to 35°C for 3 h (Fig. 6). The wild-type spindles were short and bipolar, with parallel SPBs separated by ~1.5 μm and joined by a bundle of microtubules. No normal-appearing spindles were found in the *kar3Δ kip3Δ* (p*kar3-64*) culture. Of the spindles in which two poles could be visualized in one section, nine had SPBs located adjacent to one another. Six spindles had SPBs that were separated from between 0.2 and 1.2 μm (average = 0.53 ± 0.37 μm, SD), but were clearly defective. The poles of these spindles were not parallel. These findings agree with those from the Nuf2p-GFP analysis. Loss of Kar3p and Kip3p function caused preformed bipolar spindles to break with SPBs frequently moving close together.

Genetic Interactions with Kar3p Accessory Factors

Kar3p forms distinct complexes with two homologous accessory factors, Cik1p and Vik1p (Page et al., 1994; Manning et al., 1999). *cik1* and *vik1* mutants display different phenotypes that each overlap in part with the phenotypes of *kar3* cells. It is likely that these factors target Kar3p to different cellular roles, the precise nature of which have not been determined. We found that, similar to a *kar3Δ* mutant (Cottingham and Hoyt, 1997), *cik1Δ* is lethal in combination with *dyn1Δ*, and this lethality is suppressed by *kip2Δ*. Viable *cik1Δ dyn1Δ* cells could only be created when *KIP2* was deleted. Fig. 7 demonstrates that introduction of *KIP2* by transformation into a *cik1Δ dyn1Δ kip2Δ* triple mutant prevented colony formation, indicating that lethality is due to the activity of *KIP2*. In this case, the similarity in genetic behavior of *cik1Δ* to *kar3Δ* suggests that it is the Cik1p–Kar3p complex that overlaps with dynein for an essential spindle positioning role. However, unlike *kar3Δ*, *cik1Δ* could be combined with *kip3Δ* yielding viable, healthy cells. Therefore, Cik1p–Kar3p complexes do not uniquely perform the function required for spindle integrity that overlaps with Kip3p. This supports the hypothesis that Cik1p is only required for a subset of Kar3p's roles (Manning et al., 1999).

vik1Δ dyn1Δ and *vik1Δ kip3Δ* double mutant cells were also viable and healthy. Therefore, Vik1p–Kar3p complexes do not uniquely overlap for essential functions with either Dyn1p or Kip3p. However, *cik1Δ vik1Δ kip3Δ* triple mutants were inviable. Strain MAY6417 (*cik1Δ vik1 kip3Δ* [p*KIP3 URA3*]) could not survive loss of the *KIP3 URA3*
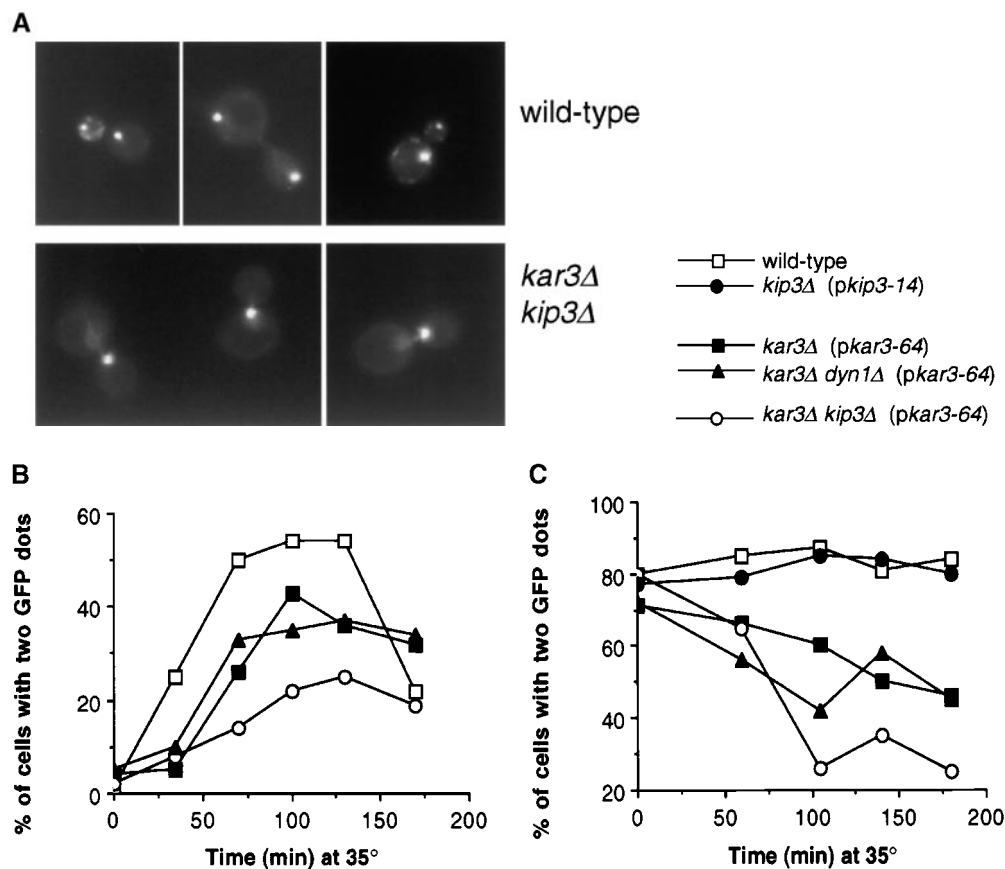
Figure 5. Bipolar spindle assembly and structural integrity requires either Kar3p or Kip3p. (A) Cells of the indicated genotypes also expressed the SPB component Nuf2p tagged with GFP. Cultures of the indicated genotypes were synchronized at 26°C with α-factor and released into media at 35°C. After 60 min, samples of live yeast cells were immediately observed under the microscope. Examples of wild-type (top row) and *kar3Δ kip3Δ* (p*kar3-64*) (bottom row) cells. (B) Quantitation of spindle assembly proficiency. Cells released from α-factor at 35°C, as in A, were observed and the percentage of cells exhibiting two clearly separated GFP dots was determined. The decreases observed in the later time point are due to cells that have entered the next cell cycle. (C) Quantitation of ability to maintain spindle structural integrity. Cells of the indicated genotype (and expressing Nuf2p-GFP) were synchronized at 26°C with hydroxyurea and shifted to 35°C for the indicated times, at which point the percentage of cells exhibiting two clearly separated GFP dots was determined. Strains used: wild-type (MAY5776), *kar3Δ* (p*kar3-64*) (MAY5777), *kar3Δ kip3Δ* (p*kar3-64*) (MAY5778), *kar3Δ dyn1Δ* (p*kar3-64*) (MAY5779), and *kip3Δ* (p*kip3-14*) (MAY6030).

plasmid, as evidenced by its inability to segregate cells capable of growth on 5-FOA. This may indicate that the essential spindle integrity function that overlaps with Kip3p is performed by both Cik1p–Kar3p and Vik1p–Kar3p complexes, and that either alone is sufficient. Alternatively, the lethality of the *cik1Δ vik1Δ kip3Δ* triple mutant may reflect a nonspecific additive effect of combining three deleterious mutations.

Benomyl Can Partially Substitute for the Essential Function Performed by Either Kar3p or Kip3p

Kar3p can act as a microtubule destabilizer in vitro (Endow et al., 1994), and *kar3Δ* vegetative growth defects can be suppressed by the presence of the microtubule-destabilizing reagent benomyl (Roof et al., 1991; Saunders et al., 1997a) (Fig. 1, row A2; 37°C). We found that the temperature sensitivity of *kar3Δ kip3Δ* (p*kar3-64*) cells could be partially suppressed by the presence of 5–10 μg/ml benomyl in the media (Fig. 1, rows A10 and B9). The temperature sensitivity of *kar3Δ kip3Δ* (p*kip3-14*) cells could be suppressed by the same treatment as well (Fig. 1, row B7), similar to the suppression of *kip3-30* (temperature-sensitive) *kar3Δ* reported by others (DeZwaan et al., 1997). This finding suggests that both Kar3p and Kip3p act

to destabilize microtubules, and that this is an important aspect of the essential function for which they overlap (see Discussion). Indeed, all multiple motor mutant genotypes that are dependent upon either *kar3-64* or *kip3-14* for viability were suppressed for temperature-sensitive growth by benomyl (Figs. 1 and 8; also see below).

Characterization of Minimal Microtubule-based Motor Strains

The *S. cerevisiae* genome encodes six kinesin-related motor genes and a single dynein heavy chain. In this and a previous study (Cottingham and Hoyt, 1997) we described two viable triple mutant combinations. Cells deleted for *DYN1* and either *KAR3* or *KIP3* were viable when the antagonistically acting *KIP2* was deleted as well. Since a stripped-down or minimal system can help reveal how a process is accomplished, we attempted to reduce further the number of functional microtubule-based motor genes. In generating strains with reduced spindle motor function, evidence of aneuploidy was sometimes detected (i.e., non-Mendelian segregation of genetic markers). Therefore, we routinely checked all seven motor gene alleles by a PCR assay to verify that the genotypes constructed were correct (see Materials and Methods). We also note that for practi-
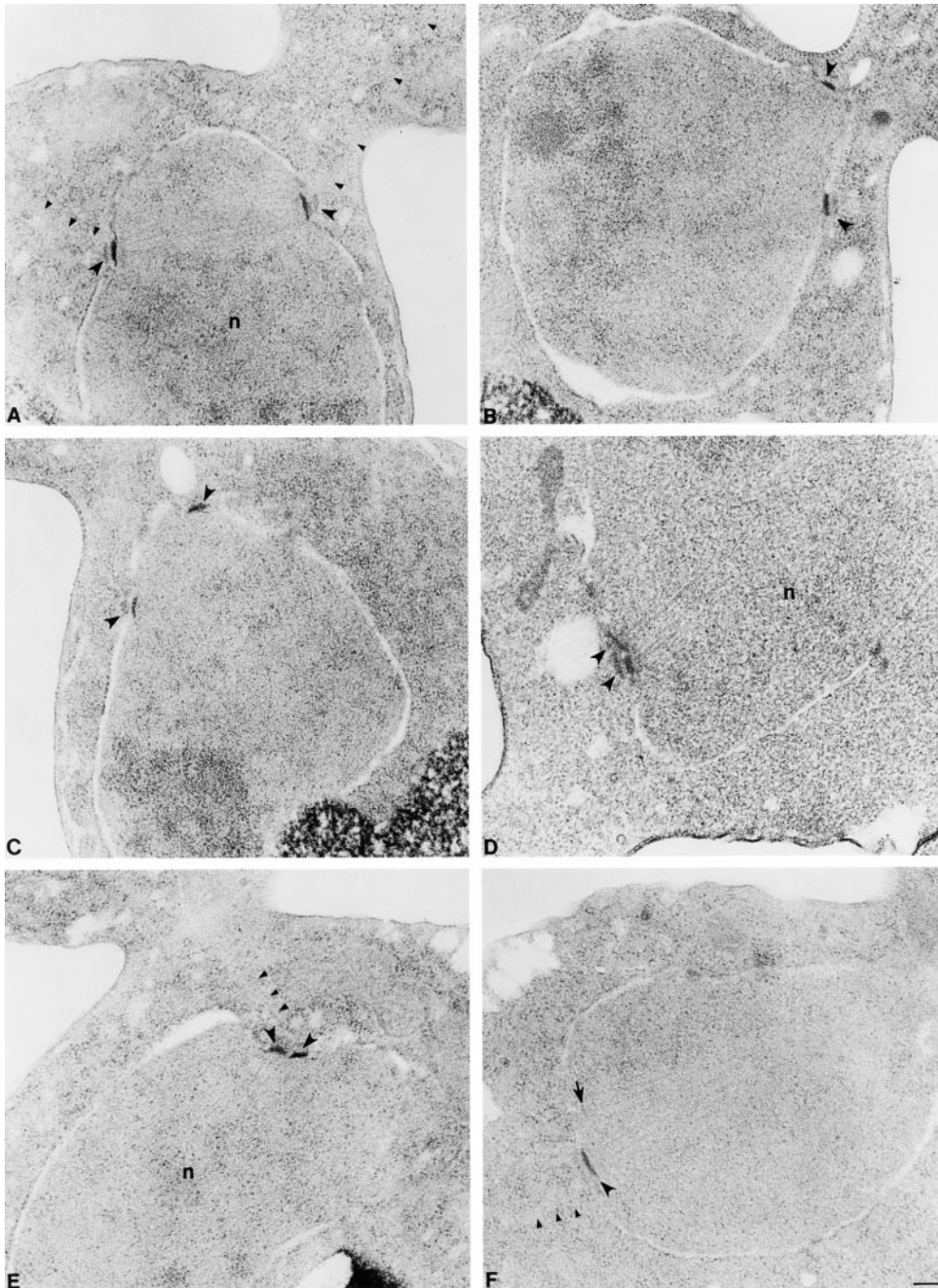
Figure 6. Electron microscopic analysis of *kar3-64 kip3Δ* cells. Wild-type (A) and *kar3Δ kip3Δ* (p*kar3-64*) cells (B–F) were arrested with hydroxyurea at 26°C, shifted to 35°C for 3 h, fixed, and sectioned for electron microscopy. A shows a normal preanaphase bipolar spindle. All the mutant spindles (B–F) appeared defective. In B and C, spindles have lost bipolarity. Although the poles are still slightly separated, they are not parallel. In D, E, and F spindles have collapsed with poles in a side by side orientation. All SPBs are marked with large arrowheads. Arrow in F marks a pole whose edge was just caught in this section. Small arrowheads mark cytoplasmic microtubules. n, nucleus. Bar, 0.2 μm. Strains used are listed in legend of Fig. 1.

cal reasons, the minimal motor strains we created carried one of the active motor genes on a low-copy centromere plasmid. Centromere plasmids are usually present in one to two copies per haploid cell, but can increase slightly if selective pressure is applied (Futcher and Carbon, 1986).

From the two viable triple mutants described above (*dyn1Δ kip3Δ kip2Δ* and *dyn1Δ kar3Δ kip2Δ*) we were able to delete an additional two genes encoding kinesin-related proteins, *KIP1* and *SMY1*. *KIP1* encodes the BimC family motor that is phenotypically less important (the more important BimC motor is encoded by *CIN8*) (Hoyt et al., 1992; Roof et al., 1992). *SMY1* probably has no mitotic spindle role (see Introduction) but was deleted

on account of its sequence relationship to kinesins. The strains created expressed only two of seven microtubule-based motor genes; *CIN8* with either *KAR3* or *KIP3*.
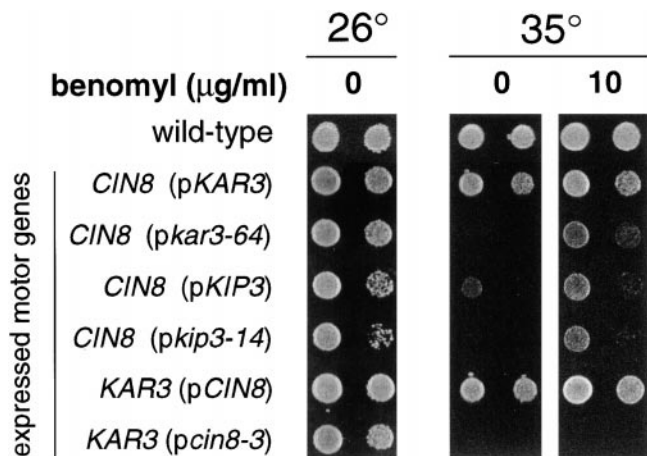
Cells expressing only *CIN8* and *KAR3* were quite healthy relative to wild-type, as judged by doubling time and other cell cycle criteria (Table II). Some difficulty in progression through mitosis was evident from the approximately twofold elevation of large-budded, mononucleate cells in log-phase cultures. Spindle positioning errors were also evident (by the appearance of bi and anucleate cell bodies), but at a level no higher than a *dyn1* single mutant (Cottingham and Hoyt, 1997). Cells expressing only *CIN8* and *KIP3* were considerably less well off. Their doubling
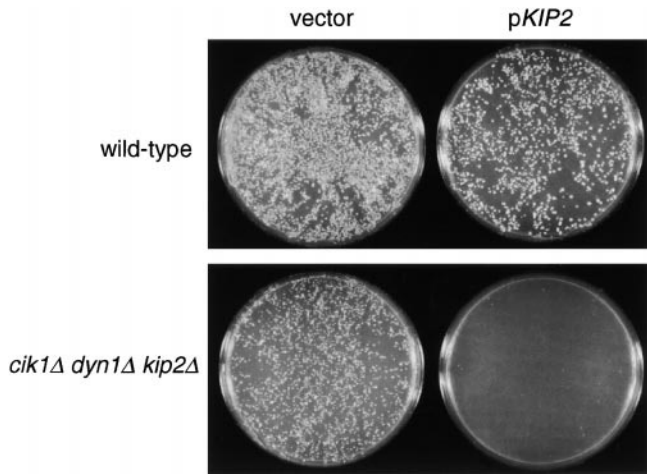
Figure 7. KIP2 is responsible for the growth defect of *cik1Δ dyn1Δ* cells. Cultures of wild-type (MAY591) and *dyn1Δ cik1Δ kip2Δ* (MAY5802) cells were split in two and transformed with either the *KIP2*-containing plasmid pFC50 or an empty *LYS2* vector control (pRS317). Selective media (minus lysine) plates were incubated at 26°C for 3 d.

Table II. Phenotypes of Strains Expressing Only Two Microtubule Motor Genes

Genotype*					
Active genomic motor gene	Motor gene on plasmid	Doubling time‡	Plating efficiency§	% Large-budded, mononucleate cells‖	% Bi or anucleate cells¶
		h	*%*		
wt	–	2.0	100	15	0
CIN8	*KAR3*	2.5	89	37	6
KAR3	*CIN8*	2.2	114	34.5	3.5
CIN8	*KIP3*	3.4	41	32	12

*The wild-type strain (wt) has all normal chromosomal alleles for the seven microtubule-based motor genes. The other strains express only the two motor genes indicated, one from its normal genomic configuration and the second from its normal promoter on a centromere-based (low-copy) plasmid. Strains used are listed in the legend for Fig. 8.

‡Determined by optical density (at 600 nm) for cells grown in rich media at 26°C.

§Colony forming units/cells counted under microscope, then normalized to wt = 100%. Log-phase cultures grown in rich media at 26°C were sampled.

‖Percentage of total cells grown in rich media at 26°C to log-phase that were large-budded (bud diameter greater than or equal to half the mother diameter) and possessed a single DAPI-stained nuclear DNA mass. $n \geq 200$.

¶Percentage of total cells, grown in rich media at 26°C to log-phase, that exhibited two DAPI-stained nuclear DNA masses in one cell body or exhibited no nuclear DNA. $n \geq 200$.

time was significantly increased, and spindle positioning errors were approximately twice that of the *CIN8 KAR3* cells. The plating efficiency of the *CIN8 KIP3* cells was roughly half that of wild-type, indicating many dead cells in the culture. However, this figure is close to that observed for *kar3* single mutants (Meluh and Rose, 1990).

To determine if Cin8p Kar3p or Cin8p Kip3p represent the lowest possible motor complements, we created strains in which one of the two active motor genes was replaced

Figure 8. Benomyl rescues the growth defects of cells expressing only *CIN8* and either *kar3-64* or *kip3-14*. Yeast cells were spotted onto solid rich media, with or without 10 μg/ml benomyl, and incubated at the indicated temperature for 3 d. The genotypes indicate the only two microtubule motor genes expressed in these cells (except for wild-type). Strains used (Note: p indicates a centromere plasmid-carried gene): wild-type (MAY1089), *CIN8* p*KAR3* (MAY5903), *CIN8* p*kar3-64* (MAY5904), *CIN8* p*KIP3* (MAY5905), *CIN8* p*kip3-14* (MAY5906), p*CIN8 KAR3* (MAY5940), and p*cin8-3 KAR3* (MAY5941).

with a temperature-sensitive allele. While cells surviving with only *CIN8 KAR3* or *CIN8 KIP3* were able to grow at 35°C, neither *CIN8 kar3-64*, *CIN8 kip3-14*, nor *cin8-3 KAR3* cells grew at this elevated temperature (Fig. 8). Therefore, under normal growth conditions, Cin8p–Kar3p or Cin8p–Kip3p were the minimal motor sets that supported cell viability (but see below). We examined the effects on spindle integrity and positioning caused by elimination of the function of one of the two active motors in the two-motor strains. Wild-type, *CIN8 kar3-64*, *CIN8 kip3-14*, and *cin8-3 KAR3* cells were arrested with hydroxyurea at 26°C and then shifted to 35°C for 3 h, maintaining the hydroxyurea block. The motor mutant strains exhibited greatly reduced numbers of bipolar spindles as judged by antitubulin immunofluorescence microscopy (78% for wild-type; 17% for *CIN8 kar3-64*; 9% for *CIN8 kip3-14*; and 7% for *cin8-3 KAR3*). This was an expected finding, because a BimC motor (either Cin8p or Kip1p) plus either Kar3p or Kip3p is required for bipolar spindle integrity. However, examination of the nuclear positioning proficiency of these strains did reveal differences. After hydroxyurea synchronization, the shift to 35°C caused nuclei in *CIN8 kar3-64* and *CIN8 kip3-14* cells, but not *cin8-3 KAR3* cells, to mislocalize (Fig. 9). Therefore, it is reasonable to conclude that Kar3p or Kip3p are the only motors performing spindle positioning in their respective two-motor strains, and that Cin8p makes no detectable contribution to this process.

Finally, we found further support for a microtubule-destabilizing role for Kar3p and Kip3p in vivo. Addition of benomyl to the media could suppress the temperature-sensitive growth of cells surviving with only *CIN8 kar3-64* or *CIN8 kip3-14* (Fig. 8). The slow growth of the *CIN8 KIP3* strain was exacerbated at elevated temperature, a phenotype that was also suppressed by benomyl. In contrast, the temperature sensitivity of the *cin8-3 KAR3* strain was not relieved by benomyl. Therefore, although microtubule destabilization may be an important function of
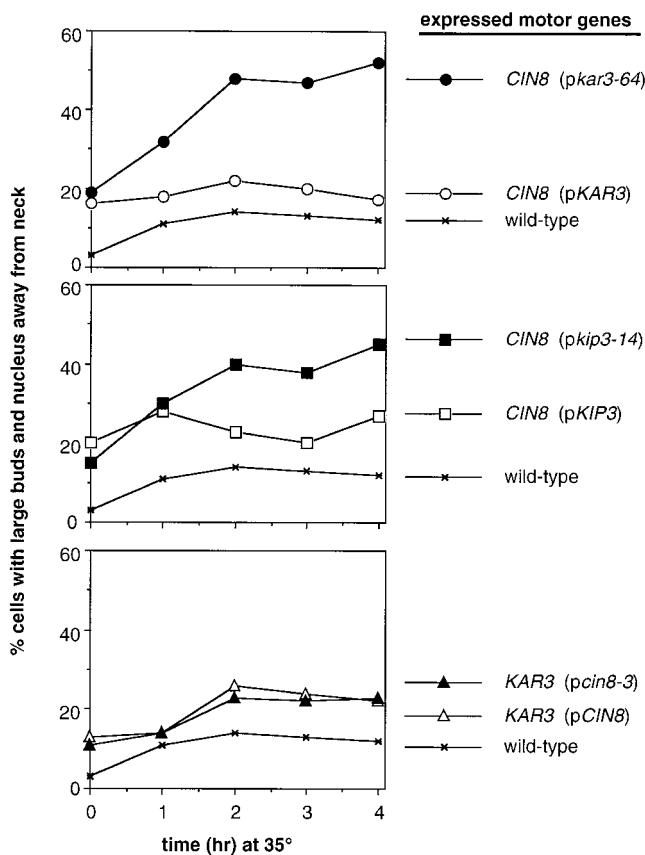
Figure 9. Nuclear positioning proficiency of cells expressing only two microtubule motor genes. Cultures were arrested with hydroxyurea, shifted to 35°C and analyzed as in Fig. 2 and Fig. 3 B. The genotypes indicate the only two microtubule motor genes expressed in these cells (except for wild-type). Strains used are the same as in Fig. 8.

Kar3p and Kip3p, this does not appear to be a role for the BimC motor Cin8p.

Discussion

Kar3p and Kip3p Overlap for Spindle Structural and Positioning Functions

Our findings demonstrate that *S. cerevisiae* Kar3p and Kip3p motors perform overlapping roles contributing to both mitotic spindle positioning and spindle structural integrity. These two activities are primarily accomplished by distinct sets of microtubules, cytoplasmic and nuclear, respectively. Therefore, we conclude that Kar3p and Kip3p act on both sides of the nuclear envelope, a property that may be unique among the seven *S. cerevisiae* microtubule-based motors. We suggest that a major aspect of the nuclear and cytoplasmic functions of both of these motors is to destabilize microtubules.

In the absence of dynein, both Kar3p and Kip3p are required for spindle positioning and vigorous cell growth. Similar to cells in which dynein and Kip3p were eliminated (Cottingham and Hoyt, 1997), we demonstrated here that elimination of dynein and Kar3p caused spindle misposi-

tioning and the appearance of extremely long cytoplasmic microtubules. In both cases, the long microtubule phenotype and the spindle positioning defect were suppressed by the elimination of Kip2p. In cells missing both dynein and Kip2p, either Kar3p or Kip3p would now suffice for viability, probably becoming the sole spindle positioning motor in these cells. We demonstrated that in cells surviving with only Cin8p and Kar3p or Kip3p, all spindle positioning function appeared to be accomplished by Kar3p or Kip3p. It is not clear whether Kar3p and Kip3p normally act in distinct spindle positioning pathways or if they redundantly perform an activity required for the same pathway.

In previous studies, Kar3p was visualized only upon microtubules in the nucleus of mitotic cells (Page et al., 1994; Saunders et al., 1997a). However, our finding that Kar3p participates in spindle positioning indicates that it must be acting upon cytoplasmic microtubules as well. Two other findings indicate that Kar3p can act upon cytoplasmic microtubules: Kar3p influences the dynamics of cytoplasmic microtubules in mitotic cells (this study; Saunders et al., 1997a; Tirnauer et al., 1999), and acts upon cytoplasmic microtubules in karyogamy to draw together the nuclei of mated cells (Meluh and Rose, 1990; Page et al., 1994).

Our studies also demonstrate a role for Kar3p and Kip3p in the nucleus, overlapping for a function essential for bipolar spindle assembly and structural integrity. Previous observations of spindle abnormalities in *kar3Δ* cells suggested a spindle integrity role for Kar3p (Meluh and Rose, 1990; Saunders et al., 1997a). A nuclear role for Kip3p was suggested by its localization to nuclear (as well as cytoplasmic) microtubules (DeZwaan et al., 1997; Miller et al., 1998) and the extra long pre and post-anaphase spindles observed in *kip3Δ* cells (Cottingham and Hoyt, 1997; Straight et al., 1998). We demonstrated here that the structural integrity of the nuclear bipolar spindle requires the actions of at least one of these motors. Since loss of both motors is lethal, but causes only modest effects on spindle positioning, it is likely that spindle integrity is the essential activity for which they overlap. When both motors were inactivated, bipolar spindle assembly and the structural integrity of preformed spindles were compromised. The collapse of preformed spindles was slow, requiring over an hour to reach the maximal effect (Fig. 5 C). Spindle poles moved together, but many poles were still slightly separated. This contrasts with the rapid spindle collapse observed when the two BimC motors, Cin8p and Kip1p, were inactivated; all spindle poles were pulled to an adjacent position after as little as 5 min at the nonpermissive temperature (Saunders and Hoyt, 1992). This indicates that Kar3p and Kip3p probably function differently from the BimC motors in maintaining bipolar spindle structure.

Kar3p forms functionally distinct complexes with two homologous accessory factors, Cik1p and Vik1p (Page and Snyder, 1992; Page et al., 1994; Manning et al., 1999). Cik1p, but not Vik1p, is required for karyogamy, and Kar3p and Cik1p are interdependent for localization to cytoplasmic microtubules in mating cells. We found that, similar to a *kar3Δ* mutant, *cik1Δ* is lethal in combination with *dyn1Δ*, and this lethality is suppressed by *kip2Δ*. This overlap in function between Cik1p and dynein suggests that Kar3p complexed to Cik1p performs the cytoplasmic

spindle positioning function. Our genetic studies indicated that neither Cik1p–Kar3p nor Vik1p–Kar3p complexes uniquely perform the function essential for spindle integrity that overlaps with Kip3p. However, since *cik1Δ vik1Δ kip3Δ* triple mutants were inviable, it remains possible that these two complexes redundantly provide this activity. Our findings lend further support to the hypothesis that Cik1p and Vik1p target the Kar3p motor to distinct cellular functions (Manning et al., 1999).

Spindle Motors Regulate Microtubule Dynamics

The molecular nature of the spindle positioning and structural defects caused by loss of Kar3p and Kip3p is likely to involve a defect in microtubule polymer length regulation. Recent studies have implicated microtubule motors as important regulators of microtubule dynamics. Notably, members of the XKCM1/MCAK family of vertebrate kinesins have been demonstrated to cause microtubule instability by promoting catastrophe events (Desai et al., 1999; McNally, 1999). A microtubule-destabilizing activity for Kar3p has been detected in vitro (Endow et al., 1994). Findings reported here, combined with those of others, clearly demonstrate that Kar3p, Kip3p, and dynein either directly or indirectly act to shorten microtubules in vivo (Roof et al., 1991; Carminati and Stearns, 1997; Cottingham and Hoyt, 1997; DeZwaan et al., 1997; Saunders et al., 1997a; Huyett et al., 1998; Miller et al., 1998; Straight et al., 1998; Tirnauer et al., 1999). These observations can be generally summarized as follows: mutants deficient for any one of these motors display elongated microtubules and/or phenotypes that are suppressible by the antimicrotubule compound benomyl. Double mutants are much more severely affected; they are either inviable or very sick and display extremely long microtubule structures. In the studies reported here, numerous multiple motor mutant genotypes were created that were missing Kar3p or Kip3p and relied upon a temperature-sensitive form of the other for viability. All of these strains were suppressed for temperature-sensitive growth by the addition of the antimicrotubule compound benomyl to the media. Although the mechanism of suppression could be indirect, it is probable that benomyl is substituting for an activity normally accomplished by Kar3p and Kip3p. Depending upon the genotype tested, benomyl suppressed either the lethal spindle structural defect, the lethal spindle positioning defect, or both (i.e., for cells surviving only with *CIN8* and *kar3-64*). Therefore, both the spindle structural and positioning defects caused by *kar3* and *kip3* mutations involve effects on microtubule stability.

It is not clear how increased microtubule stability or length caused the observed *kar3 kip3* mutant spindle structural and positioning defects. The dynamic behavior of the ends of microtubules is probably important for functions such as interactions with kinetochores, antiparallel microtubules, and the cell cortex. Any of these processes may be disrupted by the inability to control the growth of microtubules. A less direct possibility for the *kar3 kip3* spindle structural defect is that the longer or less dynamic spindle microtubules do not form proper interactions with the BimC motors, leading ultimately to spindle collapse. For the spindle positioning defect, it is possible that the ex-

tremely long cytoplasmic microtubules act to prohibit migration of the nucleus up to the cell neck or actually push the nucleus away.

The functional overlap between Kar3p and Kip3p could not have been predicted from the amino acid sequence of these gene products, since each is distinct. Kar3p defines a unique subclass of kinesin-related proteins characterized by their COOH terminus–located motor domain and their minus end–directed force production on microtubules (Meluh and Rose, 1990; Endow et al., 1994; Middleton and Carbon, 1994). The motor domain of Kip3p is located at its NH$_2$ terminus. The directionality of Kip3p has not been determined, but a related motor from *Drosophila* has been found to exhibit plus end–directed activity (Pereira et al., 1997). The directionality of these motors may relate to activities for which they need not necessarily overlap. We predict that the only essential overlap between these motors is their ability to shorten both nuclear and cytoplasmic microtubules.

In contrast to Kar3p, Kip3p, and dynein, the Kip2p motor acts to increase the length of cytoplasmic microtubules. Loss of function causes extremely short cytoplasmic microtubules (Cottingham and Hoyt, 1997; Huyett et al., 1998; Miller et al., 1998), whereas overexpression causes an extra long microtubule phenotype (Huyett et al., 1998; Cottingham, F.R., and M.A. Hoyt, unpublished observations). In addition to shortening the microtubules of *kar3 dyn1* and *kip3 dyn1* cells, *kip2Δ* also suppresses their lethal spindle positioning defect (this study; Cottingham and Hoyt, 1997). The shortened microtubules may be the direct cause of the suppression, allowing spindles now to migrate unimpeded to the neck region. However, the possibility that other activities of Kip2p may antagonize spindle positioning in the absence of dynein cannot be ruled out. In contrast, the elimination of Kip2p did not suppress the lethal spindle structural defect of *kar3 kip3* cells. This is consistent with its localization to cytoplasmic microtubules only (Huyett et al., 1998; Miller et al., 1998), as well as our failure as yet to detect any nuclear role for this motor.

The Minimal Motor Requirement for S. cerevisiae

Cytological descriptions of mitotic spindle function have revealed a complex series of motility events that affect the segregation of replicated chromatids. These events include bipolar spindle assembly, kinetochore capture and congression, and two chromosome-separating movements, anaphase A (movement of kinetochores towards spindle poles) and anaphase B (separation of spindle poles). In considering the minimal microtubule-based motor set required for *S. cerevisiae* viability, it first must be recognized that unlike higher eukaryotic cells, this yeast requires microtubules for only one essential role, mitotic spindle function. Nonetheless, our finding that *S. cerevisiae* can survive with only two microtubule-based motors is unexpected in light of the observed complexities of spindle motile behavior.

We were able to create viable strains expressing only two of seven microtubule-based motors; the BimC-type motor Cin8p combined with either Kar3p or Kip3p. Attempts to reduce motor function further caused expected deleterious phenotypic consequences and revealed that

these represented the minimal sets required for viability. Removing the function of Kar3p (from Cin8p Kar3p-ts cells) or Kip3p (from Cin8p Kip3-ts cells) caused spindles to both collapse and misposition. Removing the function of Cin8p caused spindle collapse, an expected consequence since these cells were missing the other BimC motor, Kip1p (Saunders and Hoyt, 1992). However, elimination of Cin8p function caused no detectable spindle positioning defect. Therefore, it seems likely that in these two-motor strains, all nuclear microtubule motor functions were accomplished by the combination of Cin8p and either Kar3p or Kip3p, and cytoplasmic microtubule motor functions accomplished by either Kar3p or Kip3p.

A few caveats must be considered regarding the conclusion that only two motors are required for viability. First, we cannot exclude the possibility that the observed vigorous growth of the two-motor cells could have been aided by suppressing mutations. The genotypes of created strains were verified with respect to the seven motor loci, so suppressors would necessarily affect genes that do not encode dyneins or kinesins. Second, we must strongly consider a contribution from microtubule-based motility mechanisms that do not include dyneins and kinesins. We are not aware of any force-producing microtubule-based ATPases outside of the dynein and kinesin families. However, an undiscovered class of motors cannot be excluded. All kinesins, dyneins, and myosins (as well as many other proteins) possess a domain with a nucleotide-binding sequence (G-X-X-X-X-G-K-T/S; (Saraste et al., 1990) and a distinct region of coiled α-helical coil sequence. An examination of the *S. cerevisiae* genome reveals gene products of unknown function that contain both of these sequence motifs (i.e., the gene products specified by YLR106C and YPL217C). Another possibility is a motility mechanism that uses a different form of force generation. For example, a protein that is able to couple the dynamic behavior at the ends of microtubules to force production (Koshland et al., 1988; Coue et al., 1991). Such a coupler need not necessarily be a nucleotidease. Therefore, the proper conclusion for our findings is that of the six kinesins plus one dynein encoded in the genome, only two kinesins are required for *S. cerevisiae* viability.

The presence of benomyl in the media partially suppressed the temperature-sensitive growth defects of the Cin8p Kar3p-ts and Cin8p Kip3p ts two-motor strains. However, we have been unable to find a condition allowing vigorous growth in the complete absence of Kar3p and Kip3p (cells surviving on Cin8p alone). Therefore, the temperature-sensitive forms of these motors may retain some activity at the elevated temperature that is required in addition to the microtubule-destabilizing activity of benomyl. Nonetheless, these findings further indicate that microtubule-destabilization is an important activity of Kar3p and Kip3p.

In summary, *S. cerevisiae* cells only require two microtubule-based motors to accomplish mitosis. The BimC motor Cin8p is required to assemble and elongate the bipolar spindle, probably by virtue of its ability to cross-link and slide microtubules. The second motor, either Kar3p or Kip3p, acts to promote microtubule shortening both within the nucleus and in the cytoplasm. Until the possible discovery of other spindle motility-producing mechanisms, we must entertain models in which all essential spindle motile activities can be accomplished by this limited set of motor proteins.

References

Byers, B., and L. Goetsch. 1991. Preparation of yeast cells for thin-section electron microscopy. *Methods Enzymol.* 194:602–608.

Carminati, J.L., and T. Stearns. 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 M.A. Hoyt. 1997. Mitotic spindle positioning in *Saccharomyces cerevisiae* is accomplished by antagonistically-acting microtubule motor proteins. *J. Cell Biol.* 138:1041–1053.

Coue, M., V.A. Lombillo, and J.R. McIntosh. 1991. Microtubule depolymerization promotes particle and chromosome movement in vitro. *J. Cell Biol.* 112:1165–1175.

Desai, A., S. Verma, T.J. Mitchison, and C.E. Walczak. 1999. Kin I kinesins are microtubule-destabilizing enzymes. *Cell.* 96:69–78.

DeZwaan, T.M., E. Ellingson, D. Pellman, and D.M. Roof. 1997. Kinesin-related *KIP3* of *Saccharomyces cerevisiae* is required for a distinct step in nuclear migration. *J. Cell Biol.* 138:1023–1040.

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

Eshel, D., L.A. Urrestarazu, S. Vissers, J.C. Jauniaux, J.C. van Vliet-Reedijk, R.J. Planta, and I.R. Gibbons. 1993. Cytoplasmic dynein is required for normal nuclear segregation in yeast. *Proc. Natl. Acad. Sci. USA.* 90:11172–11176.

Futcher, B., and J. Carbon. 1986. Toxic effects of excess cloned centromeres. *Mol. Cell. Biol.* 6:2213–2222.

Geiser, J.R., E.J. Schott, T.J. Kingsbury, N.B. Cole, L.J. Totis, G. Bhattacharyya, L. He, and M.A. Hoyt. 1997. *S. cerevisiae* genes required in the absence of the *CIN8*-encoded spindle motor act in functionally diverse mitotic pathways. *Mol. Biol. Cell.* 8:1035–1050.

Hoyt, M.A., and J.R. Geiser. 1996. Genetic analysis of the mitotic spindle. *Annu. Rev. Genet.* 30:7–33.

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

Hoyt, M.A., L. He, L. Totis, and W.S. Saunders. 1993. Loss of function of *Saccharomyces cerevisiae* kinesin-related *CIN8* and *KIP1* is suppressed by *KAR3* motor domain mutants. *Genetics.* 135:35–44.

Hoyt, M.A., A.A. Hyman, and M. Bähler. 1997. Motor proteins of the eukaryotic cytoskeleton. *Proc. Natl. Acad. Sci. USA.* 94:12747–12748.

Huyett, A., J. Kahana, P. Silver, X. Zeng, and W.S. Saunders. 1998. The Kar3p and Kip2p motors function antagonistically at the spindle poles to influence cytoplasmic microtubule numbers. *J. Cell Sci.* 111:295–301.

Kahana, J.A., B.J. Schnapp, and P.A. Silver. 1995. Kinetics of spindle pole body separation in budding yeast. *Proc. Natl. Acad. Sci. USA.* 92:9707–9711.

Kashina, A.S., G.C. Rogers, and J.M. Scholey. 1998. The *bimC* family of kinesins: essential bipolar mitotic motors driving centrosome separation. *Biochim. Biophys. Acta.* 1357:257–271.

Koshland, D.E., T.J. Mitchison, and M.W. Kirschner. 1988. Polewards chromosome movement driven by microtubule depolymerization in vitro. *Nature.* 331:499–504.

Li, Y., E. Yeh, T. Hays and K. Bloom. 1993. Disruption of mitotic spindle orientation in a yeast dynein mutant. *Proc. Natl. Acad. Sci. USA.* 90:10096–10100.

Lillie, S.H., and S.S. Brown. 1994. Immunofluorescence localization of the unconventional myosin, Myo2p, and the putative kinesin-related protein, Smy1p, to the same regions of polarized growth in *Saccharomyces cerevisiae*. *J. Cell Biol.* 125:825–842.

Lillie, S.H., and S.S. Brown. 1998. Smy1p, a kinesin-related protein that does not require microtubules. *J. Cell Biol.* 140:873–883.

Manning, B.D., J.G. Barrett, J.A. Wallace, H. Granok, and M. Snyder. 1999. Differential regulation of the Kar3p kinesin-related protein by two associated proteins, Cik1p and Vik1p. *J. Cell Biol.* 144:1219–1233.

McNally, F.J. 1999. Microtubule dynamics: controlling split ends. *Curr. Biol.* 9:R274–R276.

Meluh, P.B., and M.D. Rose. 1990. *KAR3*, a kinesin-related gene required for yeast nuclear fusion. *Cell*. 60:1029–1041.

Middleton, K., and J. Carbon. 1994. *KAR3*-encoded kinesin is a minus-end-directed motor that functions with centromere binding proteins (CBF3) on an in vitro yeast kinetochore. *Proc. Natl. Acad. Sci. USA*. 91:7212–7216.

Miller, R.K., K.K. Heller, L. Frisen, D.L. Wallack, D. Loayza, A.E. Gammie, and M.D. Rose. 1998. The kinesin-related proteins, Kip2p and Kip3p, function differently in nuclear migration in yeast. *Mol. Biol. Cell*. 9:2051–2068.

Page, B.D., and M. Snyder. 1992. CIK1: a developmentally regulated spindle pole body-associated protein important for microtubule functions in *Saccharomyces cerevisiae*. *Genes Dev*. 6:1414–1429.

Page, B.D., L.L. Satterwhite, M.D. Rose, and M. Snyder. 1994. Localization of the Kar3 kinesin heavy chain-related protein requires the Cik1 interacting protein. *J. Cell Biol*. 124:507–519.

Pereira, A.J., B. Dalby, R.J. Stewart, S.J. Doxsey, and L.S.B. Goldstein. 1997. Mitochondrial association of a plus end-directed microtubule motor expressed during mitosis in *Drosophila*. *J. Cell Biol*. 136:1081–1090.

Pringle, J.R. 1991. Staining of bud scars and other cell wall chitin with calcofluor. *Methods Enzymol*. 194:732–735.

Pringle, J.R., and L.H. Hartwell. 1981. The *Saccharomyces cerevisiae* cell cycle. *In* The Molecular Biology of the Yeast *Saccharomyces*: Life Cycle and Inheritance. J.N. Strathern, E.W. Jones, and J.R. Broach, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 97–142.

Pringle, J.R., A.E.M. Adams, D.G. Drubin, and B.K. Haarer. 1991. Immunofluorescence methods for yeast. *Methods Enzymol*. 194:565–602.

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

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

Saraste, M., P.R. Sibbald, and A. Wittinghofer. 1990. The P-loop, a common motif in ATP- and GTP-binding proteins. *Trends Biochem. Sci*. 15:430–434.

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

Saunders, W.S., D. Koshland, D. Eshel, I.R. Gibbons, and M.A. Hoyt. 1995. *Saccharomyces cerevisiae* kinesin- and dynein-related proteins required for anaphase chromosome segregation. *J. Cell Biol*. 128:617–624.

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

Sherman, F., G.R. Fink, and J.B. Hicks. 1983. Methods in Yeast Genetics. Cold Spring Harbor Press, Cold Spring Harbor, NY. 120 pp.

Staples, R.R., and C.L. Dieckmann. 1993. Generation of temperature-sensitive *cbp1* strains of *Saccharomyces cerevisiae* by PCR mutagenesis and in vivo recombination: characteristics of the mutant strains imply that CBP1 is involved in stabilization and processing of cytochrome b pre-mRNA. *Genetics*. 135:981–991.

Stearns, T. 1997. Motoring to the finish: kinesin and dynein work together to orient the mitotic spindle. *J. Cell Biol*. 138:957–960.

Straight, A.F., J.W. Sedat, and A.W. Murray. 1998. Time-lapse microscopy reveals unique roles for kinesins during anaphase in budding yeast. *J. Cell Biol*. 143:687–694.

Tirnauer, J.S., E. O'Toole, L. Berrueta, B.E. Bierer, and D. Pellman. 1999. Yeast Bim1p promotes the G1-specific dynamics of microtubules. *J. Cell Biol*. 145:993–1007.

Wach, A., A. Brachet, R. Pöhlmann, and P. Philippsen. 1994. New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast*. 10:1793–1808.