Differential Regulation of the Kar3p Kinesin-related Protein by Two Associated Proteins, Cik1p and Vik1p

Brendan D. Manning, Jennifer G. Barrett, Julie A. Wallace, Howard Granok, and Michael Snyder

Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut 06520-8103

Abstract. The mechanisms by which kinesin-related proteins interact with other proteins to carry out specific cellular processes is poorly understood. The kinesin-related protein, Kar3p, has been implicated in many microtubule functions in yeast. Some of these functions require interaction with the Cik1 protein (Page, B.D., L.L. Satterwhite, M.D. Rose, and M. Snyder. 1994. J. Cell Biol. 124:507–519). We have identified a Saccharomyces cerevisiae gene, named VIK1, encoding a protein with sequence and structural similarity to Cik1p. The Vik1 protein is detected in vegetatively growing cells but not in mating pheromone-treated cells. Vik1p physically associates with Kar3p in a complex separate from that of the Kar3p-Cik1p complex. Vik1p localizes to the spindle-pole body region in a Kar3p-dependent manner. Reciprocally, concentration of Kar3p at the spindle poles during vegetative growth requires the presence of Vik1p, but not Cik1p. Phenotypic analysis suggests that Cik1p and Vik1p are involved in different Kar3p functions. Disruption of VIK1 causes increased resistance to the microtubule depolymerizing drug benomyl and partially suppresses growth defects of cik1Δ mutants. The vik1Δ and kar3Δ mutations, but not cik1Δ, partially suppresses the temperature-sensitive growth defect of strains lacking the function of two other yeast kinesin-related proteins, Cin8p and Kip1p. Our results indicate that Kar3p forms functionally distinct complexes with Cik1p and Vik1p to participate in different microtubule-mediated events within the same cell.

Key words: kinesin • microtubules • molecular motors • cytoskeleton • Saccharomyces cerevisiae

MICROTUBULES are a component of all eukaryotic cytoskeletons and are essential for many diverse cellular and intracellular movements, such as flagellar motility, organelle positioning, vesicle trafficking, mitotic spindle orientation, and chromosome segregation. Two families of chemomechanical motor proteins, dynein and kinesin-related proteins, are crucial for these microtubule functions (Vale et al., 1985; Schnapp and Reese, 1989; Steuer et al., 1990; reviewed by Vale and Fletterick, 1997; Hirokawa, 1998). These proteins possess highly conserved motor domains responsible for movement along microtubules (Y ang et al., 1990). The nonmotor portion of these proteins links the motor domain to a vast array of cargoes or localizes the motor to various subcellular sites (Y ang et al., 1990; A fishar et al., 1995; Wittmann et al., 1998).

The family of proteins sharing a region of homology with the motor domain of conventional kinesin-heavy chain (KHC)1 has grown enormously in recent years (reviewed by Hirokawa, 1996; M oore and E ndow, 1996). These kinesin-related proteins (KRPs) have been identified in all eukaryotes from yeast to humans. KRPs vary in directionality and the position of their motor domains. Most KRPs, as well as conventional kinesin, have NH2-terminal motor domains and travel toward the dynamic plus ends of microtubules. Others have internally located motor domains and may also have plus-end polarity. A nother subfamily of KRPs have COOH-terminal motors and travel toward the less dynamic minus ends of microtubules, the end generally associated with microtubule-organizing centers. Whereas KRPs share a high degree of sequence similarity within their motor domains, the remainder of their primary sequences are highly divergent. These variable regions are thought to interact with different proteins to mediate the functional specificity of the motor (Page et al., 1994). Recent studies have unveiled many details about the molecular structure and function of kinesin-like motor domains (Hirose et al., 1995, 1996; A rnal et al., 1996; K ull et al., 1996; S ablinski et al., 1996; G ullick et al., 1998), but far

1. Abbreviations used in this paper: HA, hemagglutinin; HAT, HA/ transposon tag; KAP, kinesin-associated protein; KHC, kinesin-heavy chain; KLC, kinesin light chain; KRP, kinesin-related protein; ORF, open reading frame; SPB, spindle-pole bodies.
less is known about how these proteins are regulated and what other components are involved in their function.

A side from the light chains of conventional KHC, few other kinesin-associated proteins (KAPs) have been identified. In many organisms, KHCs associate with various isoforms of kinesin-light chains (KLCs) to form complexes involved in transport of organelle and vesicle populations (Cyr et al., 1991; Beushausen et al., 1993; Gauger and Goldstein, 1993; Wedaman et al., 1993; Kodjakov et al., 1998; Rahman et al., 1998). Additionally, KHC has been shown to tightly associate with both a KLC kinase and a KLC phosphatase that putatively regulate motor function (Mcllvain et al., 1994; Lindesmith et al., 1997). Two KAPs that interact with specific KRCs have also been identified. The KIF3 heterodimeric class of KRCs, also known as kinesin-II, interact with a single KAP to form a heterotrimeric complex (Cole et al., 1993, 1998; Scholey, 1996; Wedaman et al., 1996; Y amazaki et al., 1996). Finally, Saccharomyces cerevisiae KAP, Kar3p, has been shown to associate with the Cik1 protein, which is essential for Kar3p localization and function during mating (Page et al., 1994).

The Kar3 protein is one of six KRCs encoded by the S. cerevisiae genome (Meluh and Rose, 1990). KAR3 encodes a protein containing a kinesin-motor domain at its COOH terminus (Meluh and Rose, 1990). The Kar3p-motor domain possesses minus-end directionality and microtubule-depolymerizing activity in vitro (Endow et al., 1994). In addition to an essential role in nuclear fusion during mating, or karyogamy, Kar3p has been implicated in several microtubule functions during the vegetative cell cycle. These putative functions include spindle assembly, mitotic chromosome segregation, microtubule depolymerization, kinetochore-motor activity, spindle positioning, and as a force opposing the action of other KRCs (Meluh and Rose, 1990; Roof et al., 1992; Hoyt et al., 1993; Saunders and Hoyt, 1992; Saunders et al., 1997a,b; Huyett et al., 1998). This suggests that Kar3p has some KAP-independent functions. Genetic studies support this hypothesis. Kar3p is believed to oppose the force generated by two other S. cerevisiae KAPs, Cin8p and Klp1p, which are involved in spindle pole separation both during spindle assembly and during anaphase B spindle elongation (Hoyt et al., 1992, 1993; Roof et al., 1992; Saunders and Hoyt, 1992; Saunders et al., 1995). Disruption of KAR3 function partially rescues the temperature-sensitive growth defect and spindle collapse phenotype of cin8ts klp1ts mutants (Saunders and Hoyt, 1992; Hoyt et al., 1993). In contrast, disruption of CIK1 does not rescue this mutant (Page et al., 1994; this study). Together, these results suggest that Kar3p may perform some of its vegetative functions alone or in association with a different KAP.

In this study we describe a Cik1p-homologous protein in S. cerevisiae that acts as a second KAP for Kar3p. We demonstrate that this protein, Vik1p (vegetative interaction with Kar3p), is present in vegetatively growing cells but absent from mating-pheromone treated cells. Vik1p forms a complex with Kar3p that is distinct from that between Kar3p and Cik1p. Furthermore, we show that Kar3p and Vik1p are interdependent for their concentration at the poles of the mitotic spindle. Phenotypic and genetic comparisons of cik1ts and vik1ts mutants demonstrate that Cik1p and Vik1p are likely to mediate distinct subsets of Kar3p functions. Our data suggest that Cik1p and Vik1p regulate Kar3p function, at least in part, by targeting the motor to various sites of action within the cell. This is the first example of two distinct associated proteins differentially regulating a single KAP.

Materials and Methods

Strains, Media, and Standard Methods

S. cerevisiae strains used in this study are listed in Table I. Yeast growth media, molecular biological techniques, and genetic manipulations were as described previously (Sambrook et al., 1989; Guthrie and Fink, 1991). Yeast transformation procedures were performed using the lithium acetate method (Ito et al., 1983). Where indicated, rich medium, consisting of yeast extract, peptone, and dextrose, was supplemented with benomyl (DuPont), dissolved in DMSO, to a final concentration of 10, 20, or 30 μg/ml. Sensitivity of wild-type yeast strains to these concentrations of benomyl varied between preparations of benomyl containing agar plates and, hence, growth comparisons were always performed on the same plate. Sensitivity also varies dramatically between growth temperatures (i.e., at 23°C strains are much more benomyl sensitive than at 30°C).

Construction of VIK1::3XHA Strain

A strain containing the VKI1::3XHA allele (Y1744) was constructed by the PCR-epitope tagging method described previously (Schneider et al., 1995). The primers 5'-TATTAACGAGTTTCAAGAAGGTTCAAAACTAAGTTTGGAAGAAAGGCTCACTAGGGATCACAAAAGCTTG-3' and 5'-CTTTATTTTGTCTTATCTCCTATATGATGGTGTCTTTAAAGAAAGCAGAATATGTGACGAGCTTTAAGAAGGGGACTTTG-3' were used in a PCR reaction with pMPY-3XHA as the template. The resulting 1.5-kb PCR product contains the URA3 gene flanked by direct repeats encoding three copies of the hemagglutinin (HA) epitope. They also share genetic interactions with several genes (Manning et al., 1997). Furthermore, Cik1p requires Kar3p for its mitotic spindle localization (Page et al., 1994). The construction of the VIK1::3XHA strain was described previously (Schneider et al., 1995).

The Journal of Cell Biology, Volume 144, 1999 1220
used to transform yeast strain Y 1731, and transformants were selected on synthetic complete medium lacking uracil. Correct integration into the 3′ region of the VIK1 locus was confirmed by PCR analysis with primers to sequences flanking the site of insertion. Transformants with a correct 3XHA-URA3-3XHA α allelic replacement were then incubated on plates containing 5-fluoroorotic acid to select for loss of the URA3 marker by recombination between the repeated 3XHA α regions. The resulting VIK1:3XHA α strain was confirmed by PCR and immunoblot analysis. This strain does not display any vik1Δ phenotypes; growth of VIK1:3XHA α strains in the presence of benomyl is identical to wild-type strains, and the temperature-sensitive growth defect of cik1Δ mutants is the same in a VIK1::HIS3 strain as in 3XHA background.

**Immunoprecipitations and Immunoblot Analysis**

Cells were grown in rich liquid medium to mid-logarithmic phase (OD600 = 0.5–0.8), and a total of 10 OD600 units of cells were collected by centrifugation, washed, and resuspended in 100 μl lysis buffer (1 M NaCl, 10 mM EDTA, 2 mM EGTA, 5% glycerol, 40 mM Tris-HCl, pH 7.5, containing 1 μl yeast protease inhibitor cocktail (Sigma Chemical Co.) and 200 μM PMSF). When indicated, cells were first washed twice with fresh medium, resuspended in medium containing 5 μg/ml α-factor mating pheromone (Sigma Chemical Co.), and incubated for 2 h before harvesting. Cell lysates were prepared in Eppendorf tubes using zirconia/silica beads (Bio-Rad) and incubated for 2 h before harvesting. Cell lysates were brought to a 1-ml vol with the lysis buffer containing detergents (1% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS) without NaCl concentration. The cell lysate was then precelled for 1 h by incubation with 20 μl of a 1:1 slurry of protein A/G-agarose (Pierce) and TBS (150 mM NaCl, 50 mM Tris-HCl, pH 8.0). Immunoprecipitations were performed by incubation of cell lysates with either 2 μl mouse monoclonal anti-HA antibodies (12CA5 from Babco), 10 μl rabbit polyclonal anti-Cik1p antibodies (Page and Snyder, 1992), or 5 μl rabbit polyclonal anti-Kar3p antibodies (gift of L. L. Satterwhite, P. B. Meluh, and M. D. Rose, Princeton University), reconstituted with 1 ml TBS containing detergents and protease inhibitors (1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 μl Sigma yeast protease inhibitor cocktail, 200 mM PMSF) and once with 1 ml TBS plus protease inhibitors. The final pellet was resuspended in 30 μl L-mm l sample buffer (Laemmli, 1970).

Proteins from cell lysates and immunoprecipitations were denatured by incubation at 90°C for 10 min before electrophoretic separation in either 8% or 10% SDS-polyacrylamide gels. For attempts to detect VIK1::3XHA α in mating-pheromone treated cells, a threefold larger volume of cell lysate was loaded than for vegetative cell lysates (data not shown). Proteins were then transferred to Immobilon-P membranes (Millipore) for immunoblot analysis with either mouse monoclonal anti-HA antibodies (12CA5 from Babco), rabbit polyclonal anti-Cik1p antibodies (Page and Snyder, 1992), or a crude IgG fraction of the rabbit polyclonal anti-Kar3p antibodies (M. eluh and R. rose, 1999; Page et al., 1994). Excellent protein bands were then detected with alkaline phosphatase-conjugated secondary antibodies (M. B. mersh) and the CDP Star detection reagent (Boerhringer Mannheim). Overexposure of all blots failed to detect VIK1::3XHA α in cell lysates from mating-pheromone treated cells.

**Fluorescence Microscopy**

Indirect immunofluorescence was performed as described previously (Gehrung and Snyder, 1990; Pringle et al., 1991). Due to sensitivity of the HA-epitope to formaldehyde fixation, mid-logarithmic phase cells were fixed with 3.7% formaldehyde for only 15 min. They were then washed twice with solution A (1.2 M sorbitol, 50 mM potassium phosphate buffer, pH 6.8), and spheroplasts were prepared by incubating cells in solution B containing 5 μg/ml Zymolyase 100T, 0.03% glusulase, and 0.2% 2-mercaptoethanol at 37°C for 15 to 30 min. Spheroplasted cells were then washed and resuspended in solution C and placed onto poly-(L-lysine)-coated slides. VIK1::3XHA α and Kar3p-HA T were detected by incubation overnight at 4°C with preabsorbed mouse monoclonal anti-HA primary
antibodies (16B12 from BABCO) diluted in PBS/BSA. Bound mouse anti-HA antibodies were then detected by incubation for 90 min at room temperature with preabsorbed CV3-conjugated goat anti-mouse secondary antibodies (Jackson Immunoresearch Laboratory, Inc.). Microtubules were detected by incubation with rabbit anti-yeast β-tubulin (Tub2p) primary antibodies (gift of F. Solomon, Massachusetts Institute of Technology; Bond et al., 1986) followed by incubation with FITC-conjugated goat anti-rabbit secondary antibodies. A filter both primary and secondary antibody incubations, slides were washed twice with PBS/BSA and twice with PBS/BSA plus 0.1% NP-40. Finally, slides were mounted in 70% glycerol, 2% n-propyl gallate, and 0.25 μg/ml Hoechst 33258 to preserve the preparation and stain DNA for localization of nuclei.

Photographs of representative cells stained with anti-HA antibodies, anti-Tub2p antibodies, and/or Hoechst 33258 were taken, and composite figures were produced and processed using Adobe Photoshop version 3.0 (A dobe Systems, Inc.). Processing procedures were identical for each photo of a particular staining method within a composite figure.

**Construction of KAR3::HAT Strains**

A strain containing the KAR3::HAT allele was constructed using a transposon insertion technique described previously (Ross-Macdonald et al., 1997). The entire KAR3 coding region was cloned into the vector pH556 and subjected to transposon mutagenesis in Escherichia coli as described (Ross-Macdonald et al., 1997). Plasmid DNA was prepared from selected strains and digested with NotI, producing a fragment containing the mTrn-3XH A/ lacZ2 transposon inserted randomly into the KAR3 gene. These fragments were then used to transform yeast strain Y869, using the URA3 marker encoded by the transposon for selection. Transformants with in-frame transposon insertions into the KAR3 genomic locus were selected by detection of β-galactosidase activity as described (Burns et al., 1994). Cre recombinase-mediated excision, leaving only the 93 amino acid HA transposon tag (HAT) inserted, was induced by growth on galactose (pB227 contains cre under control of a galactose-inducible promoter). Recombinants were selected on 5-fluoroorotic acid. Strains with in-frame HAT insertions were then tested for kar3Δ mutant phenotypes, such as defects in karyogamy and temperature sensitivity. PCR and sequence analysis was performed on DNA from fully complementing strains in order to determine the site of insertion within the KAR3 gene.

Strain Y1870 contains a KAR3 allele with a HAT insertion after the codon for S68, and does not exhibit any kar3Δ phenotypes. Y1870 was then crossed with Y1864 to yield Y1871. Y1871 was then sporulated and a MATα KAR3::HAT cik1Δ::LEU2 segregant (Y1751) was isolated. The VIK1 gene was disrupted with the HIS3 marker in Y1870 yielding Y1752 (see below). Y1752 was then crossed with Y1751 yielding Y1753. Y1753 haploid segregants were used to analyze Kar3p-HAT localization in wild-type, cik1Δ, vik1Δ, and cik1Δ vik1Δ strains.

**Cloning of VIK1 and Disruption of VIK1, CIK1, and KAR3**

Sequence of the VIK1 gene was acquired from the Saccharomyces Genome Database (ORF designation YPL253c; Stanford University). A 2,920-bp region, from the MscI site 9 of the predicted VIK1 translation start site to the Spel site 3 of the VIK1 translation termination site, was PCR amplified from yeast genomic DNA and cloned into pb1uescript SK (Stratagene), replacing its EcoRV-Spel fragment (pSK-VIK1). The Sall-SaclI fragment from this plasmid, containing the VIK1 gene and flanking sequences, was cloned into the Sall-SaclI site of the CEN plasmid pRS316 (Sikorski and Hieter, 1989). This plasmid was linearized by deleting the NruI-AflII fragment containing the entire VIK1 ORF, then gap repaired in a wild-type yeast strain (Guthrie and Fink, 1990). This CEN plasmid encoding wild-type VIK1 was used for subsequent phenotypic analysis. VIK1 disruption constructions were made by replacing the NruI-AflII fragment of pSK-VIK1 with the Smal fragment from either plg A 50 (H153-Km) or plg A 53 (URA3-Km) (EIldeg and D avis, 1988). Sall-Xbal fragments from these plasmids, containing either the H153 or URA3 selectable markers substituted by the S68 and S68 noncoding regions of VIK1, were used to transform yeast strains Y270, Y818, and Y1870 yielding vik1Δ strains Y1733, Y1748, and Y1752, respectively.

CIK1 and KAR3 disruptions were made as previously described (Page and Snyder, 1992; Page et al., 1994). The cik1Δ::LEU2 construct was used to transform strains Y817 and Y1744 yielding cik1Δ strains Y1758 and Y1745, respectively. The kar3Δ::URA3 construct was used to transform strains Y 818 and Y 1744 yielding kar3Δ strains Y 1759 and Y 1750, respectively.

**Results**

**VIK1: A Cik1p Homologue Present in Vegetative Cells but Not in Cells Treated with Mating Pheromone**

A database homology search with the Cik1p amino acid sequence identified an open reading frame (ORF) on chromosome XVI of the S. cerevisiae genome (BLAST search of predicted translation products of S. cerevisiae ORFs; Saccharomyces Genome Database ORF designation Y PL253c) predicted to encode a protein with significant homology to Cik1p. This gene was named VIK1, for vegetative interaction with Kar3p (see below). VIK1 is predicted to encode a protein of 647 amino acids, with an overall sequence identity of 24%, and similarity of 37%, to Cik1p. The VIK1 sequence also shares structural similarity to Cik1p. It contains a predicted α-helical coiled-coil domain (amino acids 80–385) of the same length and location as that found in Cik1p (amino acids 81–388; Page and Snyder, 1992; Fig. 1; predicted with COILS version 2.2; Lupas et al., 1991; Lupas, 1996); both proteins contain a short break of 40 amino acids in their coiled-coil regions. This break and an 80 residue NH2-terminal globular domain are the most divergent regions of the two proteins (18% identity). One notable difference in the amino terminal domain is that VIK1 lacks a recognizable nuclear localization signal found in Cik1p (amino acids 24 to 33; Fig. 1; Barrett, J.G., and M. Snyder, manuscript in preparation). Finally, the COOH-terminal globular domains of the two proteins share two regions of 134 and 43 amino acids in length with 25% identity each. Therefore, VIK1 shares sequence and structural homology to Cik1p which is not confined to their coiled-coil domains.

To characterize the VIK1 protein by immunoblot and immunofluorescence analysis, DNA encoding a triple HA epitope tag was integrated into the COOH-terminal coding region of the VIK1 genomic locus (see Materials and Methods). The resulting VIK1::3XHA fusion allele fully complements VIK1 function (see Materials and Methods). Immunoblot analysis using anti-HA monoclonal antibodies detects a protein of 92-kD in cell lysates and anti-HA immuno precipitations from VIK1::3XHA strains (Fig. 2A). This molecular mass is close to the predicted 76-kD of VIK1p plus the triple HA epitope tag. This fusion protein is not detected in cell lysates or anti-HA immunoprecipitations from VIK1 untagged strains (Fig. 2A). Therefore, the VIK1::3XHA allele is expressed during vegetative growth and produces a functional protein of the expected size.

The VIK1::3XHA protein is not detected in cell lysates or anti-HA immuno precipitations from cultures first exposed to the α-factor mating pheromone (Fig. 2A; see Materials and Methods). In contrast, Cik1p greatly increases in abundance upon exposure to α-factor (Fig. 2B; Page and Snyder, 1992). This consistent with this result, the region upstream of the VIK1 ORF does not contain any predicted pheromone-response elements (Kronstad et al., 1997). However, the pheromone-inducible CIK1 and KAR3 genes each contain multiple pheromone-response
elements in their 5' noncoding regions (Meluh and Rose, 1990; Page and Snyder, 1992). Thus, since Vik1p is not present during mating-pheromone induced differentiation, it is not likely to have a significant role during mating.

**Vik1p Coimmunoprecipitates with Kar3p**

Cik1p physically associates with Kar3p (Page et al., 1994), and Vik1p shares sequence and structural similarity to Cik1p; we therefore tested whether Vik1p could also interact with Kar3p. Cell lysates were prepared from wild-type, kar3Δ, and cik1Δ strains expressing either the Vik1p::3XHA allele or untagged strains (Fig. 3 A). Therefore, Vik1p stability is not affected by the absence of either Kar3p or Cik1p. Anti-HA immunoblots of proteins immunoprecipitated with anti-Kar3p antibodies detects Vik1p-3XHA from a wild-type Vik1p::3XHA strain but not from a kar3Δ Vik1p::3XHA strain or an untagged strain (Fig. 3 B). Therefore, immunoprecipitation data indicate that Kar3p and Vik1p physically interact. Furthermore, the Kar3p-Vik1p complex is quite stable, as cell lysates were prepared in a 1 M NaCl solution (see Materials and Methods).

Vik1p-3XHA is also detected in anti-Kar3p immunoprecipitations from a cik1Δ Vik1p::3XHA strain (Fig. 3 B), indicating that Vik1p is not required for this interaction between Kar3p and Vik1p. Furthermore, an association between Vik1p and Cik1p is not detected by immunoprecipitation experiments. Vik1p-3XHA is not precipitated from Vik1p::3XHA cell lysates by anti-Cik1p polyclonal antibodies (Page and Snyder, 1992; Page et al., 1994), and, reciprocally, Cik1p is not precipitated from these cell lysates with anti-HA antibodies (Fig. 2 B). Protein preparations from vegetatively growing cells or α-fac-
tor-treated cells produce the same results. Thus, Vik1p and Cik1p do not appear to be part of the same complex. Together, these results suggest that Vik1p and Cik1p interact with Kar3p separately, and, therefore, form two different complexes during vegetative growth.

Vik1p Localizes to the Spindle-Pole Bodies

To determine the subcellular localization of Vik1p, asynchronous cultures of VIK1::3XHA and VIK1 untagged strains were fixed briefly with formaldehyde and analyzed by immunofluorescence using anti-HA and anti-tubulin antibodies (see Materials and Methods). VIK1::3XHA cells display anti-HA staining concentrated at the yeast microtubule-organizing centers, or spindle-pole bodies (SPBs), in all stages of the cell cycle (Fig. 4, B and C). The position of the SPBs correspond to the brightest foci of anti-tubulin staining. Anti-HA staining at the SPBs is not detected in VIK1 untagged cells (Fig. 4 A). Moreover, detection of Vik1p-3XHA at the SPBs does not require co-staining with anti-tubulin antibodies; staining of dots at the edges of both preanaphase and anaphase nuclei is still observed in control cells not stained with anti-tubulin antibodies (Fig. 4 C). Vik1p-3XHA staining is brightest in preanaphase cells with short spindles, where localization is clearly concentrated at the spindle poles (pattern observed in 100% of preanaphase spindles; n > 200; Fig. 4 B). Cells in the G1 phase of the cell cycle, scored as unbudded cells with a single SPB, display SPB staining that is more faint and not observed in every cell (staining observed in 60% of G1 cells; n = 100). Finally, cells in early or late anaphase, as scored by elongated spindles and/or elongated nuclei penetrating the bud neck, display faint staining concentrated at the spindle poles (staining of at least one spindle pole is observed in 73% of anaphase cells; n = 100; Fig. 4 C, inset). A nti-HA staining is not detected along the lengths of spindle or cytoplasmic microtubules in VIK1::3XHA cells. Short fixation times due to sensitivity of the HA-epitope to formaldehyde leads to a diminished number of cytoplasmic microtubules. However, in cells that contain cytoplasmic microtubules, only staining at the SPBs is observed; microtubule staining is not evident. These staining patterns are independent of ploidy, and the nature and position of the epitope tag. VIK1::3XHA/VIK1::3XHA homozygous diploids, and cells expressing a VIK1::3Xmyc NH2-terminal fusion allele stained with anti-myc monoclonal antibodies, exhibit identical patterns to those found in VIK1::3XHA haploid cells (data not shown).
The localization of Vik1p at, or near, the SPBs at various levels throughout the vegetative cell cycle is very similar to that reported for fusion proteins of Kar3p with either β-galactosidase or HA (Page et al., 1994; Saunders et al., 1997; see below). However, in pheromone-treated cells, when Kar3p localizes to the SPB and cytoplasmic microtubules (Meluh and Rose, 1990; Page et al., 1994), Vik1p-3XHA staining above background is not detected (data not shown). This is consistent with immunoblot data demonstrating the absence of Vik1p in cell lysates from α-factor–treated cells (Fig. 2A), and further suggests that Vik1p functions in vegetative cells, but not mating cells.

**Spindle-Pole Body Localization of Vik1p is Kar3p Dependent**

Since Kar3p and Vik1p physically associate and have similar localization patterns, we investigated whether the localization of Vik1p to the SPBs was dependent on the presence of Kar3p. Therefore, Vik1p-3XHA localization was analyzed by anti-HA immunofluorescence in kar3Δ cells. Whereas Vik1p-3XHA concentrates at the SPBs in wild-type cells, it does not localize to the SPBs at any stage of the cell cycle in the kar3Δ mutant. Instead, the protein is present in cytoplasmic patches that appear to be excluded from the nucleus (SPB staining was not detected in any cells; n > 300; a representative preanaphase cell is shown in Fig. 5B). This pattern is also observed with antimyc staining in kar3Δ cells containing the VIK1::3Xmyc allele (data not shown). These cytoplasmic patches are not detected in kar3Δ VIK1 untagged strains (Fig. 5A). SPB localization of Vik1p-3XHA can be restored by introducing a CEN plasmid containing KAR3 into the kar3Δ VIK1::3XHA strain (Fig. 5C). This plasmid also restores normal spindle length, since disruption of KAR3 results in accumulation of cells with short spindles compared with wild-type cells (Meluh and Rose, 1990). Therefore, the Kar3p-Vik1p association is required for localization of Vik1p to the SPB.

Cik1p also localizes to the SPB in a Kar3p-dependent manner (Page et al., 1994), and, like kar3Δ mutants, cik1Δ mutants accumulate cells with short mitotic spindles (Page...
Therefore, in order to determine if Cik1p was involved in localizing Vik1p to the SPB or if the short spindle phenotype was responsible for Vik1p mislocalization in kar3Δ mutants, we analyzed Vik1p-3XHA localization in a cik1Δ mutant. In contrast to kar3Δ cells, cik1Δ cells display Vik1p-3XHA staining concentrated at the SPBs during all stages of the cell cycle, as observed in wild-type cells. Furthermore, HA staining is brightest at the poles of the short preanaphase spindles present in cik1Δ cells (a representative cell is shown in Fig. 5 D), indicating that the short spindle phenotype alone is not the cause of mislocalization of Vik1p. Therefore, these immunolocalization data support the immunoprecipitation experiments; Vik1p does not appear to be part of the same complex as Cik1p. Furthermore, Vik1p localization to the SPBs does not require Cik1p function.

Proper Spindle-Pole Body Localization of Kar3p in Vegetative Cells Is Vik1p Dependent

Cik1p is required to localize Kar3p to the SPB and cytoplasmic microtubules during mating but is not required for Kar3p localization to the mitotic spindle poles (Page et al., 1994). This suggests that in vegetative cells, Kar3p can either target to the SPBs by itself or interacts with another protein that is responsible for its concentration at the SPBs. Based on its localization and association with Kar3p, Vik1p could fulfill this function. Therefore, Kar3p was localized in the absence of Vik1p, Cik1p, and both Vik1p and Cik1p.

The subcellular localization pattern of Kar3p has been determined previously using fusion proteins that do not fully complement all of the kar3Δ phenotypes (Meluh and Rose, 1990; Page et al., 1994; Saunders et al., 1997). To create a fully complementing HA epitope-tagged version of Kar3p, we used a transposon insertion technique described previously (Ross-MacDonald et al., 1997; see Materials and Methods). Using this technique we created several strains containing in-frame insertions of the HAT into random regions of the KAR3 genomic locus. We then tested these strains for several kar3Δ phenotypes, such as defects in karyogamy and meiosis, slow growth, temperature-sen-
positive growth, enhanced cytoplasmic microtubules, and the ability to properly localize Vik1p to the SPBs (Mehluh and Rose, 1990; Page et al., 1994; Bascom-Slack and Dawson, 1997; Saunders et al., 1997a). Fully complementing alleles were sequenced and found to lie within the region encoding the NH2-terminal globular domain of Kar3p (data not shown). A strain containing one of these KAR3::HAT alleles (encoding Kar3p with the 93-amino acid HAT insertion at S68) was used for immunofluorescence analysis with anti-HA antibodies.

Localization of Kar3p-HAT was examined in wild-type, cik1Δ, vik1Δ, and cik1Δ vik1Δ strains. A synchronous culture of haploid segregants from a cik1Δ/cik1 Δ vik1 Δ/ vik1 Δ KAR3::HAT/KAR3::HAT diploid strain were fixed and prepared for immunofluorescence with anti-HA and anti-tubulin antibodies (see Materials and Methods). KAR3::HAT wild-type cells, in all stages of the cell cycle, display anti-HA staining concentrated at the SPB region, as well as faint patches confined to the nucleus (as determined by colocalization with anti-tubulin and Hoechst staining, respectively). These fluorescence patterns are not observed in KAR3 untagged strains, in which background staining is restricted to the cytoplasm (Fig. 6A). Consistent with previous Kar3p localization studies (Page et al., 1994; Saunders et al., 1997), Kar3p-HAT staining is brightest at the poles of preanaphase spindles (Fig. 6B; Table II). Most cells in G1 (80%, 100 G1 cells counted) and anaphase (86%, 100 anaphase cells counted) also display Kar3p-HAT localization at the SPBs, but this staining is fainter than that observed in preanaphase cells (data not shown). In contrast to previous studies, Kar3p-HAT localization along spindle microtubules is not detected in any stage of the cell cycle. When cytoplasmic microtubules are observed, in wild-type cells or the mutant cells described be-
Table II. Quantitation of Kar3p Localization in Preanaphase Cells

| Strain   | % Kar3p-HAT localization | N  |
|----------|--------------------------|----|
| WT       | 100                      | 200|
| cik1Δ    | 100                      | 200|
| vik1Δ    | 0                        | 172|
| cik1Δ vik1Δ | 0                     | 174|

Anti-HA staining of asynchronous cells from isogenic Y1753 haploid segregants was used to quantitate Kar3p-HAT localization in preanaphase cells by immunofluorescence. Four different localization patterns were observed:

- **Bright nuclear patches and spindle-microtubule staining.**
- **Bright nuclear patches and faint nuclear patches.**
- **Bright SPB staining with faint nuclear patches.**
- **Bright SPB staining.**

Table II shows the quantitation of Kar3p localization in preanaphase cells.

The effect of disrupting VIK1 and CIK1 function on Kar3p-HAT localization was determined. Since wild-type localization is most obvious in preanaphase cells, we quantified Kar3p-HAT staining patterns in cells from asynchronous cultures at this stage (Table II). Similar to wild-type strains, KAR3::HAT cik1Δ cells display anti-HA staining at the SPBs during all stages of the cell cycle; again, the brightest staining is observed at the poles of the preanaphase spindle (Fig. 6 C; Table II). However, the nuclear patch staining detected in wild-type cells is qualitatively diminished in the cik1Δ mutant. Therefore, as described previously (Page et al., 1994), CIK1 is not required to localize Kar3p to the SPBs during the vegetative cell cycle.

In contrast, Kar3p-HAT localization during vegetative growth is dramatically altered in vik1Δ cells (Fig. 6 D; Table II). Kar3p-HAT is no longer concentrated at the SPBs in these cells; instead, it predominantly displays a bright nuclear patch localization in all vik1Δ cells. Moreover, staining along the lengths of spindle microtubules is detected in many cells (34% of preanaphase cells; Fig. 6 D; Table II). Some vik1Δ cells also display faint staining in the vicinity of the SPBs, but not along spindle microtubules (22% of preanaphase cells; Table II). Therefore, although vik1Δ cells may retain some Kar3p at the SPBs, VIK1 is required to concentrate, or restrict, Kar3p localization to the SPBs.

To determine if the spindle and faint SPB localization of Kar3p detected in vik1Δ cells is due to VIK1 function, we localized Kar3p-HAT in a cik1Δ vik1Δ double mutant. Kar3p-HAT localization is even more aberrant in the double mutant, with the nuclear patch staining distributed more diffusely throughout the nucleus in all cells (representative preanaphase cell shown in Fig. 6 E). Detection of Kar3p-HAT along the spindle or at the SPBs in these cells is greatly reduced compared with the vik1Δ mutant (spindle and faint SPB localization is observed in 7 and 9%, respectively, of cik1Δ vik1Δ preanaphase cells; Table II). Introduction of a CEN plasmid encoding VIK1 into either vik1Δ or cik1Δ vik1Δ mutants completely restores Kar3p-HAT concentration at the SPBs (data not shown). Therefore, during vegetative growth, VIK1 is primarily responsible for SPB targeting, or retention, of Kar3p. Cik1p may mediate Kar3p localization to less concentrated sites within the nucleus, such as the spindle and/or nuclear patches.

**CIK1 and VIK1 Disruptions Result in Distinct Phenotypes**

CIK1 and VIK1 exhibit differences in their expression patterns, and deletion of CIK1 or VIK1 results in unique effects on Kar3p localization. Therefore, the phenotypes resulting from disruption of VIK1 and CIK1 were compared. Suprisingly, vik1Δ mutants grow similar to wild-type strains at all temperatures. Using a sectoring assay described previously (Spencer et al., 1990), we determined that, unlike cik1Δ mutants that have a severe chromosome loss defect (Page and Snyder, 1992), vik1Δ mutants display only a threefold increase in frequency of chromosome loss per cell division relative to wild-type strains (data not shown). Furthermore, consistent with its absence in mating-pheromone treated cells, vik1Δ mutants are not defective in karyogamy, as determined by qualitative mating assays. However, compared with wild-type strains, vik1Δ mutants are resistant to the microtubule-depolymerizing drug benomyl (on plates containing 10, 20, or 30 μg/ml benomyl; 10 μg/ml in Fig. 7 C). The benomyl resistance phenotype always segregates with the vik1Δ mutation (11 tetrads analyzed).

To analyze whether CIK1 and VIK1 are functionally redundant, we tested whether expression of VIK1 from a high copy plasmid could suppress the temperature-sensitive growth defect of a cik1Δ mutant. cik1Δ strains containing either the VIK1 plasmid or vector alone are equally affected by growth at the restrictive temperature (data not shown). A cik1Δ mutant was then mated with a vik1Δ mutant, and the resulting heterozygous diploid strain was sporulated. Haploid segregants from tetratype tetrads were then analyzed (eight tetrads analyzed; results from a representative tetrat are shown in Fig. 7). Wild-type, cik1Δ, vik1Δ, and cik1Δ vik1Δ segregants all display similar growth on rich medium at 37°C (Fig. 7 A). However, cik1Δ mutants are temperature sensitive for growth at 37°C (Page and Snyder, 1992), whereas vik1Δ mutants grow like wild-type strains (Fig. 7 B). Suprisingly, cik1Δ vik1Δ double mutants grow substantially better at 37°C than cik1Δ mutants (Fig. 7 B). This result indicates that disruption of VIK1 partially suppresses the temperature-sensitive growth defect of cik1Δ mutants. The temperature-sensitive growth defect can be restored to a cik1Δ vik1Δ double mutant by introduction of a CEN plasmid encoding VIK1 (data not shown). Deletion of VIK1 also partially suppresses the mitotic delay of cik1Δ mutants, as scored by the percentage of large budded cells with a single preanaphase nucleus in logarithmic phase cultures growing at 30°C (Fig. 7 D). 54% of cells from cik1Δ cultures exhibit the mitotic delay phenotype, whereas only 36% of cells from cik1Δ vik1Δ cultures have this phenotype. Wild-type and vik1Δ cultures each have 22% large budded cells. These strains were also analyzed for growth.
under these conditions, the microtubule-arrested cultures by immunofluorescence with anti-
examined in fixed cells from asynchronous or hydroxy-
cik1p and vik1p are functionally distinct.
their different effects on Kar3p localization, suggest that
sensitive than wild-type strains. Therefore, the phenotypic
wild-type, and have longer, more abundant cytoplasmic microtubules
have very short spindles compared with wild-type strains
have more than 300 total cells were counted for each strain.

---

**Loss of Vik1p Function Suppresses the cin8-3 kip1Δ Mutant**

One vegetative function of Kar3p may be to oppose the action of two other S. cerevisiae KRP s, the redundant Cin8p and Kip1p motors (Hoyt et al., 1992, 1993; Saunders and Hoyt, 1992; Saunders et al., 1997b). This Kar3p function has been proposed primarily due to genetic interactions between mutations in the genes encoding these KRP s. Disruption of Cin8p and Kip1p function, using the conditional cin8-3 kip1Δ mutant, results in a temperature-sensitive growth defect at 35°C that is partially suppressed by disruption of Kar3p function (Saunders and Hoyt, 1992; Hoyt et al., 1993; see below). However, disruption of CIK1, encoding a Kar3p KAP, does not suppress cin8-3 kip1Δ (Fig. 8; Page et al., 1994), suggesting that the Kar3p activity that opposes Cin8p and Kip1p is independent of CIK1.

Since CIK1 was identified as a second KAP for Kar3p during vegetative growth, we tested whether disruption of VIK1 function could suppress the temperature-sensitive growth defect of the cin8-3 kip1Δ mutant. VIK1, CIK1, and KAR3 were deleted individually from the cin8-3 kip1Δ strain, and the resulting mutants were examined for growth defects at 23 and 35°C (Fig. 8). As demonstrated previously, the cin8-3 kip1Δ kar3Δ mutant grows significantly better than the original cin8-3 kip1Δ mutant at the restrictive temperature of 35°C (Saunders and Hoyt, 1992; Hoyt et al., 1993). Likewise, disruption of VIK1 also partially suppresses the cin8-3 kip1Δ temperature-sensitive growth defect, as the cin8-3 kip1Δ vik1Δ mutant grows at 35°C. Introduction of a CEN plasmid encoding VIK1 into the cin8-3 kip1Δ vik1Δ mutant restores temperature sensitivity to levels identical to the cin8-3 kip1Δ mutant (data not shown). In contrast, growth of the cin8-3 kip1Δ ciK1Δ

---

**Figure 8.** The temperature-sensitive growth defect of the cin8-3 kip1Δ mutant is suppressed by disruption of KAR3 or VIK1, but not CIK1. Overnight cultures were grown to stationary phase, diluted down to 5 × 10^6 cells/ml, and 5-μl spots were plated to rich medium then incubated for three days at 23 or 35°C. Pictured are wild-type strain Y1731 and cin8-3 kip1Δ mutant strains Y818, vik1Δ Y1748, ciK1Δ Y1758, and kar3Δ Y1759.
mutant is significantly diminished at all temperatures compared with the cin8-3 kip1Δ mutant, suggesting a possible added defect in the triple mutant. Therefore, like Kar3p, Vik1p functions antagonistically to Cin8p and Kip1p. This result further indicates that Cik1p and Vik1p are involved in distinct Kar3p functions.

**Discussion**

Cik1p is a previously described kinesin-associated protein that interacts with the yeast KRP Kar3p (Page and Snyder, 1992; Page et al., 1994). We have characterized a Cik1p-homologous protein called Vik1p that is present in vegetatively growing cells but, unlike Cik1p, is not detected in mating-pheromone treated cells. Coimmunoprecipitation experiments demonstrate that Vik1p also physically associates with Kar3p and that the Kar3p-Vik1p complex is separate from that of Kar3p and Cik1p. Therefore, Kar3p interacts with two different KAPs to form distinct complexes within the same cell.

**Kar3p and Vik1p Are Interdependent for Proper Localization to the SPBs of Vegetatively Growing Cells**

Vik1p requires Kar3p function for its SPB localization. This suggests that Kar3p-Vik1p complex formation and, presumably, the minus-end directed microtubule-motor activity of Kar3p are required to deliver Vik1p to the SPB. In the absence of Kar3p, Vik1p mislocalizes to cytoplasmic patches and is excluded from the nucleus. In contrast, Cik1p mislocalizes throughout the nucleus in the absence of Kar3p during vegetative growth (Page et al., 1994). Two possible models for Vik1p localization can be invoked. First, Vik1p may require association with Kar3p for its nuclear import. In support of this idea, Vik1p does not have sequences predicting a nuclear localization signal. Moreover, Kar3p can target to the nucleus independent of both Cik1p and Vik1p (Page et al., 1994; this study). This nuclear import of Vik1p by association with Kar3p would be analogous to the nuclear import of the yeast γ-tubulin complex. The import of this complex, and its subsequent binding to the nuclear face of the SPB, requires the nuclear localization signal of one of its components, Spc98p (Pereira et al., 1998). Alternatively, the Kar3p-Vik1p complex may be associated primarily with the cytoplasmic face of the SPB. Therefore, absence of Kar3p would result in release of Vik1p to the cytoplasm specifically, as observed. At this point, we cannot distinguish between these two possibilities, but future studies will address whether the Kar3p-Vik1p complex is on the nuclear, cytoplasmic, or both faces of the SPB.

We demonstrate that, whereas Cik1p is required to localize Kar3p to the SPBs and cytoplasmic microtubules of mating pheromone-treated cells (Page et al., 1994), Vik1p is required for proper concentration of Kar3p at the SPBs of vegetatively growing cells. In the absence of Vik1p, Kar3p mislocalizes to nuclear patches, and can be seen along spindle microtubules in many cells. Therefore, Vik1p is required for proper targeting and/or maintenance of Kar3p at the SPB. Vik1p might mediate interactions between Kar3p and other proteins that tether the complex to the SPB. Alternatively, Vik1p could prevent release of Kar3p from the minus-ends of microtubules, where its motor activity would cause it to accumulate.

In the absence of both Cik1p and Vik1p, Kar3p again mislocalizes but is more diffuse throughout the nucleus. This suggests that Cik1p may be partially redundant with Vik1p for targeting Kar3p to the SPB. However, Cik1p’s primary vegetative function may be to direct localization of Kar3p to other sites within the nucleus, such as nuclear patches and the spindle. This is supported by the observation that the faint nuclear patch staining of Kar3p seen in wild-type cells is diminished in cik1Δ cells. Detection of Kar3p at these sites is greatly enhanced in the absence of its Vik1p-mediated SPB localization.

Finally, since some residual SPB staining can be detected in a small percentage of cik1Δ vik1Δ cells, Kar3p may have an inherent KAP-independent ability to localize to the SPB. This localization is likely to depend on its minus-end directed microtubule motor domain, rather than its non-motor stalk and tail domains. These latter regions of Kar3p have been shown previously to be sufficient for its spindle and SPB localization (Meluh and Rose, 1990; Page et al., 1994). Cik1p and Vik1p presumably target Kar3p to various sites of action through interactions with its nonmotor domain.

**Kar3p Forms Three Functionally Distinct Complexes during the Yeast Life Cycle**

Phenotypic, genetic, and biochemical analysis of Kar3p, by several different groups (Meluh and Rose, 1990; Roff et al., 1991; Saunders and Hoyt, 1992; Hoyt et al., 1993; Endow et al., 1994; Middleton and Carbon, 1994; Cottingham and Hoyt, 1997; DeZwaan et al., 1997; Saunders et al., 1997a; Huyett et al., 1998), has strongly suggested that Kar3p is a multifunctional KRP. An intriguing question in the study of molecular motors is how can one motor protein perform several different functions within a single cell. Our results indicate that Kar3p interacts with two related proteins to form three complexes that are involved in distinct microtubule-mediated cellular processes. We believe that the ability of Kar3p to interact with these associated proteins is crucial to its functional versatility. The exact molecular functions of each of these Kar3p complexes are yet to be defined. However, based on phenotypic and genetic analysis, as well as localization studies, our data reveal some possible general roles for the Kar3p-Cik1p complex during mating and the Kar3p-Cik1p and Kar3p-Vik1p complexes during mitosis (Fig. 9).

The best defined of Kar3p’s functions is its role in the nuclear congression step of karyogamy, during which it associates with Cik1p (Meluh and Rose, 1990; Page et al., 1994). This complex localizes to cytoplasmic microtubules even in the absence of the Kar3p motor domain (Meluh and Rose, 1990; Page and Snyder, 1992; Page et al., 1994), indicating that the nonmotor region of the complex also has microtubule-binding capacity. In the absence of either of these two proteins, microtubules from the SPBs of opposite mating partners fail to interdigitate (Meluh and Rose, 1990; Page et al., 1994). Together, these results suggest a model in which the Kar3p-Cik1p complex acts as a cross-linker between antiparallel microtubules emanating from the SPBs of mating partners. The minus-end directed...
microtubule-motor activity of Kar3p can then create the force that pulls the nuclei together by sliding cross-linked microtubules past one another (Fig. 9A).

Despite many studies on the function of Kar3p during vegetative growth, its role during mitosis remains obscure. Our identification of two Kar3p-interacting proteins with distinct Kar3p-related vegetative phenotypes should help elucidate the exact mitotic functions of this KRP. Disruption of either KAR3 or CIK1 results in similar mitotic phenotypes, including very short spindles indicative of a spindle assembly defect (Meluh and Rose, 1990; Page and Snyder, 1992; Saunders et al., 1997a) and a mitotic delay mediated by the spindle-assembly checkpoint (Roof et al., 1991; M anning, B. D., J. A. Wallace, and M. Snyder, unpublished observation). Unlike during mating, the Kar3p-Cik1p complex is in the nucleus during the mitotic cell cycle, where it associates with the SPBs and, to a lesser extent, spindle microtubules (Page and Snyder, 1992; Page et al., 1994; Saunders et al., 1997a). A nalogous to its role in karyogamy, the complex may act within the spindle to cross-link and slide antiparallel microtubules from opposing SPBs past one another, thereby creating an inward force on the spindle (Fig. 9B). This force may generate a tension important for proper spindle assembly. Alternatively, the Kar3p-Cik1p microtubule cross-linking activity could be crucial to the organization of a bipolar spindle. This spindle assembly defect would account for the chromosome instability phenotype of cik1Δ mutants (Page and Snyder, 1992). A dditionally, it is possible that this complex could play a more direct role in chromosome segregation, perhaps as a kinetochore motor (Hyman et al., 1992; M idleton and Carbon, 1994).

Kar3p is likely to have a separate mitotic function that is mediated by interaction with VIK1 at the SPBs. The benomyl resistance phenotype of vik1Δ mutants, suggests that the Kar3p-Vik1p complex may be involved in microtubule depolymerization (Fig. 9C). The motor domain of Kar3p has been shown to possess minus-end-specific microtubule-depolymerizing activity in vitro (Endow et al., 1994), and has been suggested by phenotypic analysis to depolymerize microtubules in vivo (Saunders et al., 1997). Kar3p complexed with Vik1p, specifically, might posses this activity. A lternatively, Kar3p may require interaction with Vik1p to prevent release from microtubule minus ends where it catalyzes microtubule depolymerization. A t this point, it is unclear whether this complex acts on cytoplasmic microtubules, the spindle, or both. D isruption of VIK1 does not result in any detectable differences in the microtubule structures of fixed cells compared with wild-type strains. I t is possible that accumulation of Kar3p on the spindle, observed in vik1Δ mutants, could stabilize these microtubules and account for the benomyl resistance phenotype.

Based on genetic analysis, Kar3p-Vik1p function is detrimental to mutants lacking the plus-end-directed Cin8p and Kip1p KRP family. T hese proteins are members of the BimC family of KRP s and are believed to act as homotetrameric bipolar motor proteins that generate a SPB separating force during spindle assembly and anaphase B (Hoyt et al., 1992; Saunders et al., 1995; Kas hina et al., 1997; Straight et al., 1998). Disruption of either KAR3 or VIK1 can suppress the temperature-sensitive growth defect of the cin8-3 kip1Δ mutant, suggesting that the Kar3p-Vik1p complex may oppose the function of Cin8p and Kip1p (Fig. 9C; Saunders and Hoyt, 1992; Hoyt et al., 1993). I nterestingly, a TUB2 mutation that stabilizes mi-
mactubules can also suppress this mutant (Saunders et al.,
1997a), suggesting that a defect in the putative microtu-
bule depolymerizing activity of the K ar3p-Vik1p complex
could be sufficient for suppression of cin8-3 kip1Δ.

The functional interactions between the two vegetative
K ar3p complexes may be complicated. For example,
disruption of VIK1 partially suppresses the temperature-sen-
sitive growth defect and mitotic delay of cik1Δ mutants.
One interpretation of this result is that the two K ar3p
complexes partially oppose one another. It is also interest-
ing to note that cik1Δ mutants, in which K ar3p’s SPB lo-
calization is unperturbed, have much stronger phenotypes
(Page and Snyder, 1992) than those of vik1Δ mutants, in
which K ar3p is no longer concentrated at the SPBs. There-
fore, the most critical of K ar3p functions during vegetative
growth may occur at sites other than the spindle poles.
Future studies will address these issues, but it is clear from
our current study that Cik1p and Vik1p are not function-
amally redundant.

**KRP Regulation and Targeting by Kinesin-associated Proteins**

In general, the functional specificity of K RPs is deter-
mined by their nonmotor domains. Some K RPs can target
to their sites of action independent of associated proteins.
For example, the Drosophila Nod protein, involved in chro-
mosome movements during mitosis, contains a DNA bind-
ing motif in its nonmotor domain (Afshar et al., 1995).
However, the targeting of many K RPs will likely be medi-
ated through complex formation with nonmotor subunits
(i.e., K A Ps). The highly divergent nature of the nonmotor
domains of K RPs (reviewed by Goldstein, 1993; Vale and
Fletterick, 1997) suggest that interacting proteins will
also be diverse in sequence. Nevertheless, we expect that
mechanisms of motor targeting by K A Ps will exist that are
universal.

The light chains of conventional kinesin are the most
studied of all K A Ps. Several different K L Cs can exist
within a single cell (Cyr et al., 1991; Beushausen et al.,
1993; Wedaman et al., 1993; Rahman et al., 1998), and
these are thought to control the cargo-binding specificity
of K HC s (K hodjakov et al., 1998; Liao and Gundersen,
1998). Therefore, it is possible that different K L Cs target
kinesin to distinct membranous organelles and vesicles,
and it has been suggested that K L Cs mediate the interac-
tion between kinesin and membranes (Stenoien and Brady,
1997). A covalently, K L Cs might regulate K HC -microtubule
binding and/or motor activity by contacting the motor
domain when kinesin is in a folded confirmation (Hackney
et al., 1992; Verhey et al., 1998).

Little is known about the regulation and targeting of
specific K RPs. The tail domain of the Xenopus mitotic
K R P, X klp2, has recently been shown to require cytoplas-
mic dynein and a microtubule-associated protein, TPX2,
to localize to spindle poles (Wittmann et al., 1998). How-
ever, these proteins are not tightly associated K A Ps. Our
results demonstrate that K ar3p localization is regulated
through its interaction with two different K A Ps, Cik1p
and Vik1p (Page et al., 1994; this study). These K A Ps con-
tral various K ar3p functions, at least in part, by targeting
the motor to discrete sites within the cell. Whether Cik1p
and Vik1p modulate motor activity once K ar3p is at these
sites is not yet known. The study of these proteins should
define regulatory strategies used by other K RPs and help
elucidate general elements underlying the functional di-
versity of K RPs.

We thank C. Horak and J. Vogel for critical comments on
the manuscript. We are grateful to L.L. Satterwhite, P.B. M eluh, and M.D. R ose for pro-
viding the anti-K ar3p antibodies, F. Solomon for the anti-Tub2p antibod-
ies, and M.A. H oyt for the cin8-3 kip1Δ strain.

B.D. M anning and J.G. B arrett were supported by National Institutes of
Health (NIH) training grants and NIH grants GM 3649 and GM 52197,
and this research was funded by NIH grant GM 52197.

Received for publication 24 D ece mber 1998 and in revised form 18 Febru-
ary 1999.

**References**

Afshar, K. J. Scholay, and R.S. H awley. 1995. Identification of the chromo-
some localization domain of the Drosophila Nod kinesin-like protein. J. Cell
Biol. 131:833–843.

Arnal, I., F. M etoz, S. DeB onis, and R. H. W ade. 1996. Three-dimensional
structure of functional motor proteins on microtubules. Curr. Biol. 6:1265–
1270.

Bascom-Slack, C.A., and D.S. D awson. 1997. The yeast motor protein, K ar3p,
is essential for meiosis I. J. Cell Biol. 139:459–467.

Beushausen, S., A. K adakias, and H. J. afe. 1993. Kinesin light chains: identifi-
cation and characterization of a family of proteins from the optic lobe of the
squid Loligo pealei. DNA Cell Biol. 12:901–909.

Bond, J. J. Fridovich-K eil, L. P illus, R. M ulligan, and F. S olomon. 1986. A
chicken-yeast chimeric beta-tubulin protein is incorporated into microtu-
bules in vivo. Cell. 44:461–468.

Burns, N., B. G rimwade, P.B. Ross-M aconald, E.-Y. C hoi, K. F inberg, G. S.
Roeder, and M. S nedy. 1994. Large-scale characterization of gene expres-
sion, protein localization and gene disruption in Saccharomyces cerevisiae.
Genes Dev. 8:1107–1115.

Cole, D.G., S.W. Chinn, K.P. Wedaman, K. H all, T. V snoy, and J. M. S cholay.
1993. Novel heterotrimeric kinesin-related protein purified from sea urchin
eggs. Nature. 366:268–270.

Cole, D.G., D.R. Diener, A.L.H. H imelblau, P.L. B ee ch, J.C. Fuster, and J.L.
R ois enbaum. 1996. Chlamydomonas kinesin-I-like intracellular transport (IFT):
IFT particles contain proteins required for ciliary assembly in Caenorhabditis
elegans sensory neurons. J. Cell Biol. 141:993–1008.

Cotttingham, F.R., and M.A. H oyt. 1997. Mitotic spindle positioning in Saccha-
romyces cerevisiae is accomplished by antagonistically acting microtubule
motor proteins. J. Cell Biol. 138:1041–1053.

Cyr, J.L., K.K. P fister, G.S. B loom, C.A. S laughter, and S.T. B raidy. 1991. Mo-
lecular genetics of kinesin light chains: generation of isoforms by alternative
splicing. Proc. Natl. Acad. Sci. USA. 88:10314–10318.

DeZwaan, T.M., E. E llingson, D. P e lman, and D.M. R oof. 1997. K inesin-
related K I P3 of Saccharomyces cerevisiae is required for a distinct step in
nuclear migration. J. Cell Biol. 138:1023–1040.

Elledge, S.J., and R.W. D amsky. 1998. A family of versatile centromeric vectors
designed for use in the segregating-shuffle mutagenesis assay in Saccharomy-
ces cerevisiae. Gene. 70:303–312.

Endow, S.A., S. K ang, L.L. Satterwhite, M.D. R ose, V.P. S keen, and E.D.
S tenoien. 1994. Yeast K an1p is a minus-end microtubule motor protein that
destabilizes microtubules preferentially at the minus ends. EMBO J. (Eur. M ol.
Biol. Organ.) J. 13:2708–2713.

Gauger, A.K., and L.S.B. G oldstein. 1993. The Drosophila kinesin light chain.
J. Biol. Chem. 268:13657–13666.

Gehring, S., and M. S nedy. 1990. The SPA 2 gene of Saccharomyces cerevisiae
is important for pheromone-induced morphogenesis and efficient mating.
J. Cell Biol. 111:1451–1464.

Goldstein, L.S.B. 1993. With apologies to Scheherazade: tails of 1001 kinesin
motors. Annu. Rev. Genet. 27:319–351.

Guilick, A.M., H. S ong, S.A. E ndow, and J. Rayment. 1998. X-ray crystal struc-
ture of the yeast Kar3 motor domain complexed with Mg.ADP to 2.3 Å res-
olution. Biochemistry. 37:1769–1776.

Guthrie, C., and G.R. F ink. 1991. Guide to yeast genetics and molecular biol-
ogy. Methods Enzymol. 194:1–933.

Hackett, D.D., J.D. L e vitt, and J. S uhan. 1992. Kinesin undergoes a 9 S to 6 S
conformational transition. J. Biol. Chem. 267:8696–8701.

Hirokawa, N. 1996. Organelle transport along microtubules-the role of K I Fs.
Trends Cell Biol. 6:135–141.

Hirokawa, N. 1998. Kinesin and dynein superfamily proteins and the mecha-
nism of organelle transport. Science. 279:519–526.

Hirose, K.A., L. L ockhart, R.A. C ross, and L.A. A mos. 1995. Nucleotide-depen-
dent angular change in kinesin motor domain bound to tubulin. Nature. 376:
277–279.
assembly of kinesin light chain. J. Cell Biol. 110:95–108.

Roof, D. M., P. Meluh, and M. Rose. 1991. Múliple kinesin-related proteins in yeast mitosis. Cold Spring Harbor Symp. Quant. Biol. 61:693–703.

Ross-Macdonald, P., A. Sheahan, G.S. Roeder, and M. Snyder. 1997. A multipurpose transposon system for analyzing protein production, localization, and function in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA. 94:190–195.

Sabin, E. P., F. J. Kull, R. Coker, R.D. Vale, and R.J. Fletterick. 1996. Crystal structure of the motor domain of the kinesin-related motor ncd. Nature. 380:555–559.

Sambrook, J. E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

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

Saunders, W., D. Koshland, D. Sesh, I.R. Gibson, 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., D. Hornack, V. Lengyl, and C. 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., V. Lengyl, and M.A. Hoyt. 1997b. Motor spindle function in Saccharomyces cerevisiae requires regulation of different types of kinesin-related motors. Mol. Biol. Cell. 8:1025–1033.

Schnapp, B.J., and T.S. Reese. 1989. Dynein is the motor for retrograde axonal transport of organelles. Proc. Natl. Acad. Sci. USA. 86:1548–1552.

Schneider, B.L., W. Seufert, B. Steiner, O.H. Yang, and A.B. Futcher. 1995. Use of PCR epitope tagging for protein tagging in Saccharomyces cerevisiae. Yeast. 11:1265–1274.

Schley, J.M. 1996. Kinesin-II, a membrane traffic motor in axons, axonemes, and spindles. J. Cell Biol. 133:1–4.

Sikorski, R., and P. Hieter. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics. 122:19–27.

Spencer, F., S.L. Gerring, C. Connelly, and P. Hieter. 1990. Mitoic chromosome transmission fidelity mutants in Saccharomyces cerevisiae. Genetics. 124:237–249.

Stenoeien, D.L., and S.T. Brady. 1997. Immunochemical analysis of kinesin light chain function. Mol. Biol. Cell. 8:675–689.

Steuer, E.R., L. Wordeman, T.A. Schroer, and M.P. Sheetz. 1990. Localization of cytoskeletal dynein to mitotic spindles and kinetochores. Nature. 345:266–268.

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.

Vale, R.D., and R.J. Fletterick. 1997. The design plan of kinesin motors. Annu. Rev. Cell Dev. Biol. 13:745–777.

Vale, R.D., T.S. Reese, and M.P. Sheetz. 1985. Identification of a novel force generating protein, kinesin, involved in microtubule-based motility. Cell. 42:39–50.

Verhey, K.J., D.L. Lizotte, T. A brazon, L. Barenboim, B.J. Schnapp, and T.A. Rapoport. 1998. Light chain-dependent regulation of kinesin’s interaction with microtubules. J. Cell Biol. 142:1053–1066.

Wademan, K.P., A.E. K night, J. Kendrick-Jones, and J.M. Schley. 1993. Sequences of sea urchin kinesin light chain isoforms. Mol. Biol. Cell. 4:1155–1158.

Wademan, K.P., D.W. Meyer, D.S. Ash, D.G. Cole, and J.M. Schley. 1996. Sequence and submolecular localization of the 115 kD accessory subunit of the heterotrimeric kinesin-II (KRP85/95) complex. J. Cell Biol. 132:371–380.

Wittman, T., H. Boleti, C. Amon, A. Karsenti, and I. Vernos. 1998. Localization and function of cytoplasmic dynein to mitotic spindles and kinetochores. J. Cell Biol. 133:1–4.

Yamazaki, H., T. Nakata, Y. Okada, and N. Hirokawa. 1996. Cloning and characterization of the kinesin-like protein Xklp2 to spindle poles requires a leucine zipper motif. J. Cell Biol. 135:35–44.

Yamazaki, H., T. Nakata, Y. Okada, and N. Hirokawa. 1996. Cloning and characterization of the kinesin-like protein Xklp2 to spindle poles requires a leucine zipper motif. J. Cell Biol. 135:35–44.