Localization of the Kar3 Kinesin Heavy Chain-related Protein Requires the Cik1 Interacting Protein

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Abstract. The Kar3 protein (Kar3p), a protein related to kinesin heavy chain, and the Cik1 protein (Cik1p) appear to participate in the same cellular processes in *S. cerevisiae.* Phenotypic analysis of mutants indicates that both *CIK1* and *KAR3* participate in spindle formation and karyogamy. In addition, the expression of both genes is induced by pheromone treatment. In vegetatively growing cells, both Cik1::β-gal and Kar3::β-gal fusions localize to the spindle pole body (SPB), and after pheromone treatment both fusion proteins localize to the spindle pole body and cytoplasmic microtubules. The dependence of Cik1p and Kar3p localization upon one another was investigated by indirect immunofluorescence of fusion proteins in pheromone-treated cells. The Cik1p::β-gal fusion does not localize to the SPB or microtubules in a *kar3*Δ strain, and the Kar3p::β-gal fusion protein does not localize to microtubule-associated structures in a *cik1*Δ strain. Thus, these proteins appear to be interdependent for localization to the SPB and microtubules. Analysis by both the two-hybrid system and coimmunoprecipitation experiments indicates that Cik1p and Kar3p interact, suggesting that they are part of the same protein complex. These data indicate that interaction between a putative kinesin heavy chain-related protein and another protein can determine the localization of motor activity and thereby affect the functional specificity of the motor complex.

MICROTUBULES participate in a wide variety of cellular processes, including mitosis, organelle transport, and cell motility. These processes are mediated by microtubule motors, often kinesin-related proteins (Vale et al., 1985*a*; Zhang et al., 1990; Enos and Morris, 1990; Meluh and Rose, 1990; McDonald et al., 1990; Walker et al., 1990; Roof et al., 1992*a*; Hoyt et al., 1992; Hagan and Yanagida, 1990; Hagan and Yanagida, 1992). Kinesin was originally identified as a tetrameric complex comprised of two heavy chain subunits and two light chains (Vale et al., 1985*a*). The heavy chain contains a motor domain that mediates translocation of the motor along microtubules (Yang et al., 1989); the function of the light chain is unknown.

Recently, a large number of kinesin heavy chain–related molecules have been found and characterized (Vale et al., 1985*a*; Zhang et al., 1990; Enos and Morris, 1990; Meluh and Rose, 1990; McDonald et al., 1990; Walker et al., 1990; Roof et al., 1992*a*; Hoyt et al., 1992; Hagan and Yanagida, 1990; Hagan and Yanagida, 1992). These proteins often contain a central coiled-coil domain flanked by a common motor domain and a nonconserved globular domain. Despite the similarities among the different members of the kinesin family, the various motor proteins participate in many different functions (Vale et al., 1985*b*; McDonald et al., 1990; Zhang et al., 1990; Saxton et al., 1991; Saunders and Hoyt, 1992; O'Connell et al., 1993; Goldstein, 1990). The specificity of function has been speculated to be conferred in part by the nonhomologous region of these proteins (Yang et al., 1989; Meluh and Rose, 1990; Enos and Morris, 1990; Vale and Goldstein, 1990). Through its interaction with light chains or other proteins, this region may determine the localization of the microtubule motor, affect its activity, and/or specify the type of cargo carried (Vale et al., 1985*a*; Cyr et al., 1991; Wedaman et al., 1993). So far no direct evidence supports this hypothesis.

The yeast *Saccharomyces cerevisiae* is an excellent system for the genetic analysis of microtubule-dependent processes such as chromosome segregation during mitosis and nuclear fusion (karyogamy) during conjugation. Molecular characterization of several mutants defective in these processes has uncovered a family of genes encoding kinesin-related proteins. These genes include *KIP1* and *CIN8* which participate in the establishment and maintenance of the mitotic apparatus and *KAR3* which also functions in chromosome segregation and is required for karyogamy (Hoyt et al., 1992; Roof et al., 1992*b*; Meluh and Rose, 1990). Genetic screens that enrich for mutants defective in microtubule processes have identified other genes whose products interact with microtubules but share no or little sequence similarity to previously

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characterized proteins (Berlin et al., 1990; Page and Snyder, 1992). Such genes could encode proteins that function via cooperation with kinesin-like motors. Mutations in such a gene would be expected to result in similar phenotypes as those exhibited in cells mutant for the corresponding kinesin-related gene. Given this assumption, the Cik1 gene product is a candidate for interacting with the Kar3 kinesin-related protein.

Cells deleted for either *CIK1* or *KAR3* exhibit similar phenotypes (Meluh and Rose, 1990; Page and Snyder, 1992). Neither gene is essential for vegetative growth, and mutations in either cause defects in chromosome stability and establishment/maintenance of the mitotic spindle apparatus. *cik1Δ* and *kar3Δ* strains have more prominent microtubule arrays than wild-type cells and both exhibit severe defects in karyogamy. In addition, the expression of both *CIK1* and *KAR3* is induced by treatment with mating pheromone. Kar3p is a kinesin-related protein; its sequence predicts a central coiled-coil domain with a putative microtubule motor domain at the carboxy terminus. Ciklp is not homologous to microtuble motor proteins; however, it does have a putative central coiled-coil domain. Ciklp::β-galactosidase (β-gal)[1] fusion proteins have been localized to the spindle pole body (SPB) and along microtubules in vegetative cells; a Kar3::β-gal fusion lacking the motor domain exhibits a similar localization pattern in pheromone-treated cells. In addition to demonstrating the similar localization patterns of these two proteins, this latter observation indicates that the nonmotor domain of Kar3p directly or indirectly associates with microtubules.

In this article, the phenotypic similarities between *cik1Δ* and *kar3Δ* cells are examined in greater detail, and the results indicate that the corresponding proteins appear to function in the same pathway. Ciklp and Kar3p are shown to interact by the two hybrid system (Fields and Song, 1989) and by co-immunoprecipitation experiments, suggesting that they are part of the same protein complex. Furthermore, Ciklp and Kar3p are interdependent for localization to the SPB and microtubules in pheromone-treated cells. We speculate that Ciklp helps mediate the specialized function of the Kar3p motor complex by interacting with the nonmotor domain and controlling its localization within the cell.

Materials and Methods

Strains, Media, and Microbiological Techniques

Yeast strains are listed in Table I. General genetic manipulations and growth media were as described (Sherman et al., 1986). Yeast transformations were performed by the lithium acetate procedure (Ito et al., 1983). β-gal assays were performed on yeast using a protocol described for *E. coli* (Sambrook et al., 1989). General molecular cloning techniques were as described (Maniatis et al., 1982).

Plasmid Constructs

The *cik1-6::lacZ LEU2 URA3* CEN plasmid (pB20) was described in Page and Snyder (1992). The *kar3::lacZ* fusion was constructed using a *KAR3 URA3 CEN* plasmid. This plasmid was digested at its single MluI site within the *KAR3* coding region, the ends were filled with the large fragment of DNA polymerase and dNTPs. A 3-kb BamHI fragment containing the *lacZ* gene was isolated from the mTn3-*lacZ/LEU2* transposon (Siefert et al., 1986); it ends were filled in and the resulting fragment inserted into the *KAR3* vector that had been treated with calf alkaline phosphatase. The *lacZ* fragment lacks the 5′ sequences necessary for transcription and translation in both *E. coli* and *S. cerevisiae*. In the proper orientation, this construct would result in an in-frame fusion between *KAR3* and *lacZ*. Orientation was determined by restriction mapping and fusion in the proper reading frame was confirmed by β-galactosidase assays in yeast. This construct contains exactly the same amount of *KAR3* upstream of *lacZ* as the *kar3::lacZ* fusion designed by Meluh and Rose (1990) and appears to function identically.

To construct the *lexA::cik1* fusions, the *lexA* vector, pSH2-1 (Fields and Song, 1989; Golemis and Brent, 1992), was digested at EcoRI; this site was filled in and treated with phosphatase. The 1.3-kb XhoI–PstI filled-in *CIK1* fragment or the 0.56-kb XhoI–HpaI filled-in *CIK1* fragment was inserted into the modified *lexA* EcoRI site to generate p46 or p47, respectively. Orientation was determined by restriction mapping, and the proper reading frame was confirmed by DNA sequencing. The construction of the *GAL4::kar3* fusion required an additional step. The 1.5-kb BglII–BglII fragment of *KAR3* was cloned into the BamHI site of the SK$^+$ vector (Stratagene, La Jolla, CA), and a construct in which the *KAR3* 5′ end was proximal to the SmaI site was digested with SmaI and HpaI generating a blunt 1.5-kb fragment. This fragment was inserted into the filled in and phosphatase treated BamHI site of the *GAL4* vector. Orientation and fusion in the proper reading frame was determined by restriction mapping and DNA sequencing. The *lexA::GAL4* fusion plasmid, pl027, was provided by Dr. R. Brent (Brent and Ptashne, 1985).

Disruption of KAR3

The *kar3Δ-4::URA3* deletion was generated through two cloning steps. First, the 3.5-kb *KAR3* PstI–BamHI fragment derived from the pMR794 plasmid (Meluh and Rose, 1990) was inserted into the PstI and BamHI sites of the SK$^+$ vector. This construct (p228) was digested with BglII and the vector DNA was purified from the 1.5-kb BglII fragment of the *KAR3* coding region. The BglII sites of the vector were filled in and treated with phos-

Table I. Strains and Plasmids

Strain	Genotype
Y431	*MATa ura3-52 lys2-801 ade2-101 trp1-901 leu2-98*
Y804	*MATa ura3-52 leu2-3,112 ade2-101*
Y808	*MATa ura3-52 trp1-1 lys2-801 cyh2 kar1-Δ13*
Y815	*MATa ura3-52 leu2-3,112 his3-200 kip1::HIS3*
Y817	*MATa ura3-52 leu2-3,112 ade2-101 his3-Δ200 kip1::HIS3 cin8-3*
Y864	*MATa gal4Δ gal80Δ ura3 his3 leu2 LEXAp-lacZ::URA3**
Y1008	*MATα ura3-52 lys2-801 ade2-101 his3-Δ200 leu2-98 can1-100*
Y1119	*cik1-Δ3::LEU2/CIK1* derivative of Y1131
Y1119-3.7D	*MATa ura3-52 lys2-801 ade2-101 his3-Δ200 cik1-D3::LEU2 can1-100*
Y1119-3.8B	*MATa ura3-52 lys2-801 ade2-101 leu2-Δ98 trp1-901 can1-100 cik1-Δ3::LEU2*
Y1119-3.8C	*MATα ura3-52 lys2-801 ade2-101 leu2-D98 his3-Δ200 cik1-Δ3::LEU2*
Y1131	Y431/Y1008 diploid
Y1137	*kar3-Δ4::URA3/KAR3* derivative of Y1119
Y1137-5C	*MATa ura3-52 lys2-801 ade2-101 leu2-Δ98 kar3-Δ4*
Y1139	*cik1-Δ3::LEU2/CIK1* derivative of the Y814/Y818 diploid
MS10	*MATa ura3-52 leu2-3,112*
MS524	*MATa ura3-52 leu2-3,112 ade2-101 kar3::LEU2*
Plasmid	
pB20	*cik1*$_{(1\text{-}489)}$*-lacZ::LEU2 URA3* (CEN)
pB41	*kar3*$_{(1\text{-}309)}$*-lacZ URA3* (CEN)
pB45	gal4$_{(1\text{-}147)}$-kar3$_{(12\text{-}515)}$ *LEU2* (2 μm)
pB46	lexA$_{(1\text{-}87)}$-cik1$_{(20\text{-}446)}$ *HIS3* (2 μm)
pB47	lexA$_{(1\text{-}87)}$-cik1$_{(20\text{-}207)}$ *HIS3* (2 μm)
pB48	lexA$_{(1\text{-}87)}$-gal4$_{(74\text{-}881)}$ *LEU2* (2 μm)

1. *Abbreviations used in this paper*: β-gal, β-galactose; SPB, spindle pole body.

phatase. A 1.1-kb HindIII fragment containing *URA3* (derived from YEp24) was filled in and inserted into the modified BglII site of the vector, thereby replacing most of the *KAR3* coding sequence. A construct in which *URA3* transcription is oriented opposite to that of *KAR3* was digested with SalI and BamHI and used to replace the *KAR3* allele.

Strain Constructions

MATa kar3Δ and *MATa kar3Δ cik1Δ* strains were constructed by transforming the *kar3-Δ4::URA3* allele into diploid strains Y1131 (a *CIK1/CIK1* strain) and Y1119 (a *cik1-Δ3::LEU2/CIK1* strain). These *kar3Δ/KAR3* diploids were sporulated and subjected to tetrad analysis. For both strains, a typical tetrad generated two large colonies and two smaller colonies. The *URA3* marker always cosegregated with the small colony phenotype, indicating that *kar3Δ* affects cell growth (for each strain, eight tetrads were evaluated).

The *cik1Δ kip1Δ cin8-3* strain was derived through a series of steps. A *kip1Δ/kip1Δ cin8-3/CIN8* diploid was generated by mating Y815 and Y817 (these strains were kindly provided by the A. Hoyt laboratory, Johns Hopkins University, Baltimore, MD) and visually selecting zygotes. Diploids were confirmed as such by verifying that single colony isolates were nonmaters. The diploid strains were transformed with the *cik1-Δ3::LEU2* allele (this deletion allele is described in Page and Snyder, 1992). The transformed strain (Y1139) was sporulated and subjected to tetrad analysis. Eight tetrads were evaluated; within these eight tetrads, six *cik1Δ kip1Δ cin8-3* strains were recovered.

Immunofluorescence Microscopy

Indirect immunofluorescence of yeast cells was performed as described (Adams and Pringle, 1984). Strains with plasmids were grown under selective conditions to 2.5×10^6 cells/ml, pelleted by centrifugation, resuspended in YPD, and grown for 3 h before treating with pheromone or harvesting. For most experiments, samples were stained with rat anti-tubulin monoclonal antibody YOL1/34 (Kilmartin et al., 1982; obtained from Seralab) and rabbit anti-β-galactosidase antibody (Cappel Laboratories, Malvern, PA) that had been preadsorbed with fixed and permeabilized yeast cells before use to remove nonspecific antibodies. For detection of anti-tubulin antibodies and anti-β-gal antibodies, FITC-conjugated goat anti-rat antibodies (Cappel Laboratories) and Texas red-conjugated goat anti-rabbit antibodies (Amersham Corp., Arlington Heights, IL) were used, respectively. For the experiment shown in Fig. 9, a mouse anti-β-gal monoclonal antibody was used (Promega, Madison, WI). Affinity-purified anti-Ciklp antibodies were prepared as described previously (Page and Snyder, 1992). To increase the immunofluorescent signal for the anti-Ciklp antibodies, the biotin-streptavidin amplification system was used (Page and Snyder, 1992). The fluorescent DNA-specific dye, Hoechst 33258, was used to visualize yeast nuclei and mitochrondria.

To ensure that staining of fusion and nonfusion proteins was specific, we performed several types of control experiments which were similar to those described previously (Page and Snyder, 1992). Double immunofluorescence experiments were performed (*a*) on strains without Kar3p::β-gal or Ciklp::β-gal fusions or (*b*) without the β-gal primary antibody. In each case no Texas red staining was evident. Thus, the signals are specific for the fusions and are not due to cross-reactive secondary antibodies.

α-Factor Arrest

MATa cells grown to a mid-log phase in YPD were arrested by adding α-factor (Sigma Chemical Co., St. Louis, MO) to a final concentration of 4 μg/ml. An hour later the same amount of α-factor was added again. After an additional 50 min the culture was examined to evaluate the response. If 80% or more of the cells had formed shmoos, the cells were harvested for immunofluorescence.

Immunoprecipitations

A wild-type strain (MS10) and a *kar3Δ* strain (MS524) were grown to an approximate OD_{600} of 0.4. Cultures were divided and incubated in the presence or absence of 1 μg/ml α-factor (final concentration; Bachem, Torrance, CA) for 2 h at 37°C. Formation of mating projections was verified by light microscopy. Cells in a 10-ml aliquot of each culture were collected by centrifugation, and resuspended in 300 μl ice-cold modified RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% DOC, 0.1% SDS, 50 mM Tris-HCl, pH 8.0, containing 1 mM $Na_4P_2O_7$-$10H_2O$, 2 mM Na β-glycerophosphate, pH 7.0, 0.01 mM Na_3VO_4, 5 mM NaF, 1 mM PMSF, and 10 μg/ml each chymostatin, leupeptin, aprotinin, and pepstatin). Cells were lysed with chilled glass beads (0.75 vol/vol) and the supernatant removed. The beads were washed with 200 ml RIPA buffer, and the wash and lysate combined.

To immunoprecipitate Kar3p, ~400 μl lysate was brought to 1.0 ml with RIPA buffer, 6 μl rabbit polyclonal antiserum to a TrpE fusion of the COOH-terminal domain of Kar3p was added, and the mixture incubated for 90 min. A 1:1 slurry of protein A–Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) and PBS (50 μl) was added and incubated for an additional 45 min. Protein A–Sepharose–antibody complexes were collected at 12,800 g for 1 min, washed twice with 1.0 ml RIPA buffer and once with 1.0 ml 150 mM NaCl, 5 mM EDTA, 50 mM Tris-HCl, pH 7.4, that contained the phosphatase and protease inhibitors in modified RIPA (above). The final pellet was resuspended in 50 μl 4X gel sample buffer, boiled for 4 min, and analyzed on a 10% SDS-polyacrylamide gel. For immunoblotting analysis, proteins were tranferred to ProBlott membrane (Applied Biosystems, Inc., Foster City, CA). Membranes were blocked in buffer A (150 mM NaCl, 100 mM Tris-HCl, pH 7.4) containing 5% dry milk overnight at 25°C.

To detect protein, membranes were incubated with either affinity-purified anti-Ciklp antibodies (Page and Snyder, 1992) or an IgG fraction of polyclonal antiserum to the Kar3 COOH-terminal domain, washed in buffer A, and incubated with a donkey anti-rabbit IgG-HRP conjugate (Amersham Corp.). Blots were developed using the enhanced chemiluminescence detection kit according to manufacturer's instructions (Amersham Corp.).

Results

The Karyogamy Phenotype of cik1Δ Resembles that of kar3Δ

The expression of both *CIK1* and *KAR3* is dramatically induced in response to the mating pheromone α-factor, and *cik1Δ* and *kar3Δ* mutants have several defects in common. Through additional characterization of *cik1* mutants and Ciklp, a number of other CIK1-KAR3 similarities emerged.

cik1Δ and *kar3Δ* mutants both exhibit a severe bilateral mating defect (i.e., very few diploids are formed in matings between two mutant cells) (Meluh and Rose, 1990; Page and Snyder, 1992). During mating of two wild-type cells, the nucleus of each partner migrates proximal to the site of cellular fusion; the two SPBs are oriented toward this fusion site and face one another (Byers and Goetsch, 1975; Meluh and Rose, 1990; Berlin et al., 1990; Cross et al., 1988; Rose, 1991). After cell wall breakdown and cytoplasmic fusion, a microtubule bridge forms between the two SPBs and the nuclei move toward each other and fuse (Fig. 1). Previous studies demonstrated that *cik1Δ* cells failed to fuse their nuclei during conjugation (Page and Snyder, 1992). To examine this nuclear fusion defect more closely, conjugating *cik1Δ* cells were stained with anti-tubulin antibodies. As shown in Fig. 1, the SPBs of the two respective nuclei orient toward each other, yet the bridge of microtubules between the SPBs does not form (Fig. 1). Instead, the cytoplasmic microtubules of each SPB appear distinct and unconnected with those of the other (200 cells examined). This phenotype is similar to that described for *kar3Δ* cells (Meluh and Rose, 1990).

In Pheromone-treated Cells, Ciklp::β-gal Localizes Along Cytoplasmic Microtubules

In yeast cells treated with mating pheromone, a Kar3p::β-gal fusion localizes at the SPB and along microtubules (Meluh and Rose, 1990). We therefore analyzed the localization of a Ciklp::β-gal fusion in cells incubated under similar conditions. In vegetative cells, Ciklp::β-gal fusions are detected at the SPB; weak staining is apparent along nuclear and cytoplasmic microtubules (Page and Snyder, 1992). Previously

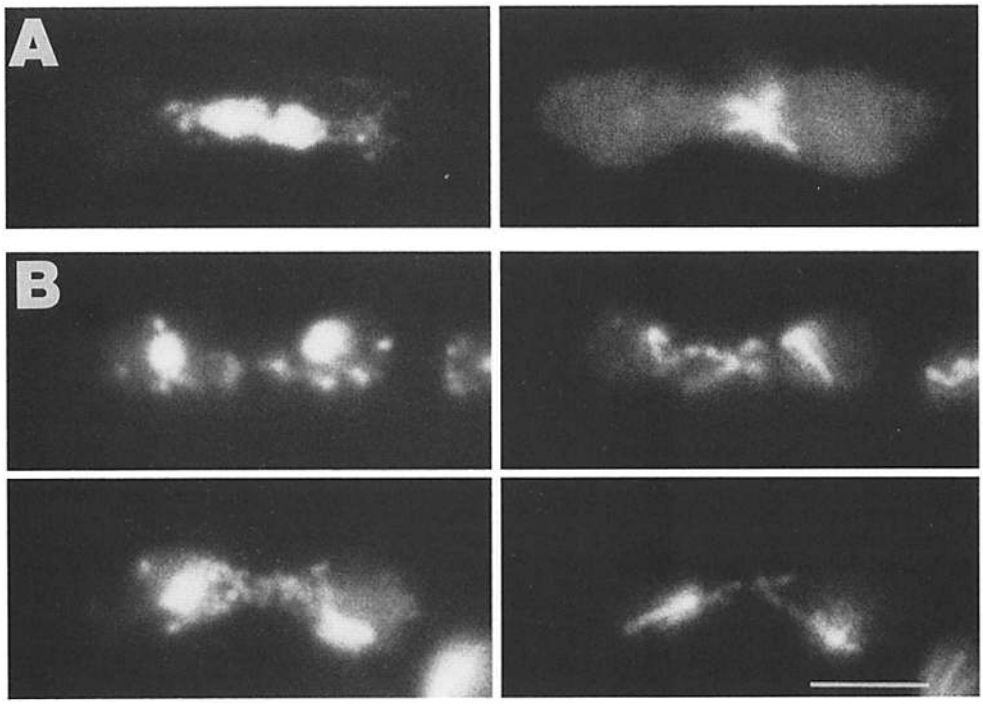

Figure 1. The karyogamy phenotype of wild-type and *cikl*Δ zygotes. Mating mixtures of wild-type strains Y431 and Y1008 (*A*) or *cikl-Δ3* strains Y1119-3.8B and Y1119-3.8C (*B*) were subjected to immunofluorescence analysis. The position of the nuclei was determined by staining with Hoechst 33258 (*left panel*); microtubules were detected with an antibody to yeast β-tubulin (*right panel*). Bar, 4.5 μm.

reported experiments indicated that *CIK1* expression is induced 20-fold in cells treated with mating pheromone (Page and Snyder, 1992). Thus, we expected that the Ciklp::β-gal localization pattern might be more striking in pheromone-treated cells.

MATa cells containing a *cikl::lacZ* fusion were treated with α-factor, which causes cells to arrest in G1 and induces specific conjugation functions (Rose, 1991). The *cikl::lacZ* fusion in this strain encodes the amino terminal 80% of the protein (amino acid residues 1–489) and includes the putative coiled-coil domain. The pheromone-treated cells were then subjected to immunofluorescence with anti–β-gal antibodies. As shown in Fig. 2, SPB/microtubule staining is readily apparent in greater than 90% of the treated cells ($n > 1{,}000$ cells). The Ciklp::β-gal fusion localizes along cytoplasmic microtubules, with more pronounced staining often detected at the SPB and at the end of a microtubule bundle distal to the SPB. This staining pattern is much more intense and slightly different from that of Ciklp::β-gal in vegetative cells (see below, Fig. 7) and from that of the authentic Ciklp localization detected in pheromone-treated cells (see below, Fig. 9 *A*). Nevertheless, the cikl::β-gal localization pattern under pheromone-treatment indicates that Ciklp associates with microtubules (see Discussion). In addition, this Ciklp::β-gal localization pattern is very similar to that reported for a Kar3p::β-gal fusion under identical conditions (Meluh and Rose, 1990).

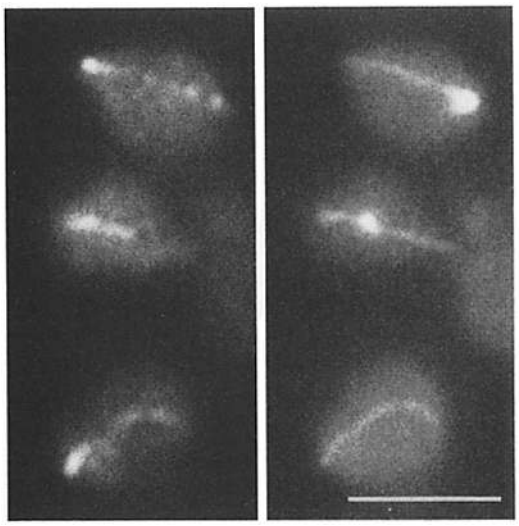

Figure 2. The Ciklp::β-gal fusion localizes to cytoplasmic microtubules in pheromone-treated cells. Strain Y431 containing a *cikl::lacZ* plasmid (pB20) was arrested with α-factor and stained with both a rabbit anti–β-gal antibody (*left panel*) and the rat anti-tubulin antibody YOL1/34 (*right panel*). Bar, 4.5 μm.

After α-factor treatment a Kar3p::β-gal fusion not only localizes to cytoplasmic microtubules, but it also affects their assembly/disassembly dynamics (Meluh and Rose, 1990). In cells expressing a Kar3p::β-gal fusion, microtubules appear to be more stable as determined by their increased resistance to depolymerization by nocodazole. To evaluate whether the Ciklp::β-gal fusion also possesses this microtubule stabilizing phenotype, *MATa* cells containing this fusion were arrested with α-factor and then treated with 15 μg/ml nocodazole (Meluh and Rose, 1990). In control experiments, either cells lacking the Ciklp::β-gal fusion were treated as described above, or cells containing the fusion were subjected to α-factor and nocodazole treatment simultaneously. In Ciklp::β-gal fusion strains that are treated first with pheromone and then nocodazole, a long cytoplasmic microtubule bundle is detected and Ciklp::β-gal is localized along this bundle (Fig. 3). In contrast, in each of the controls, no long microtubules are observed, and the only anti-tubulin staining is detected at the SPB. Thus, the stabilization of microtubules is dependent upon the Ciklp::β-gal fusion, and this fu-

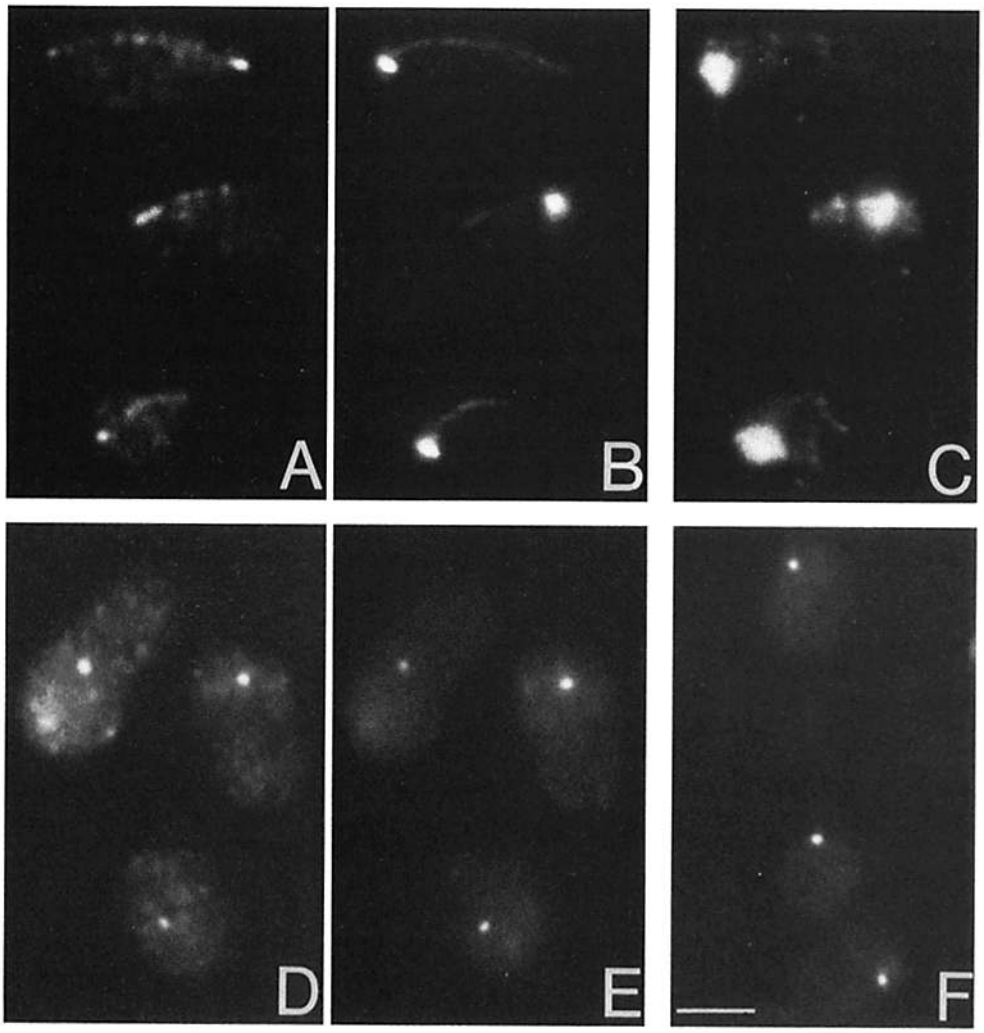

Figure 3. The Ciklp::β-gal fusion protein stabilizes microtubules. Strain Y431 containing a *cikl::lacZ* plasmid (pB20) was arrested with α-factor then treated with 15 μg/ml nocodazole; these cells were stained with anti-β-gal (*A*), anti-tubulin (*B*) and Hoechst 33258 (*C*). As a control, Y431 containing pB20 was treated with α-factor and nocodazole simultaneously. Cells treated in this manner were stained with anti-β-gal (*D*) and anti-tubulin (*E*). (*F*) Y431 was arrested with α-factor then treated with nocodazole and subsequently stained with anti-tubulin. Bar, 3 μm.

sion needs to associate with microtubules before nocodazole treatment to confer stability. These results clearly indicate that, similar to Kar3p::β-gal, the Ciklp::β-gal fusion can stabilize microtubules.

Like Kar3p::β-gal, the Ciklp::β-gal SPB Localization Requires Functional Karlp

In pheromone-treated cells, the SPB localization of Kar3p::β-gal is dependent upon functional Karlp, a protein which is required for nuclear fusion and appears to localize to the SPB (Conde and Fink, 1976; Rose and Fink, 1987; Vallen et al., 1992*a;b*). In wild-type cells arrested with mating-pheromone, Kar3p::β-gal localizes to the SPB and along microtubules; however, in a *karl-Δ13* cell, Kar3p::β-gal localizes along microtubules but not at the SPB.

To determine if the Ciklp::β-gal SPB localization is also dependent upon Karlp, a *MATa karl-Δ13* strain containing *cikl::lacZ* was treated with α-factor and stained with anti-β-gal antibodies. In this strain the microtubules are very long and often appear detached from the SPB. This phenotype is similar to that described for *karl-Δ13* cells containing a Kar3p::β-gal fusion (Vallen et al., 1992*b*). Anti-β-gal staining of *karl-Δ13* cells containing Ciklp::β-gal reveals that the fusion protein accumulates along the cytoplasmic microtubule bundle; no staining is evident at the SPB (Fig. 4). Thus, in pheromone-treated cells, there are a number of similarities between Ciklp::β-gal and Kar3p::β-gal. Both fusions localize to the SPB/microtubules and stabilize microtubules, and SPB localization of both fusion proteins requires Karlp.

In Pheromone-treated Cells, Microtubule Localization of Ciklp::β-gal Is Dependent on Kar3p and Microtubule Localization of Kar3p::β-gal Is Dependent on Ciklp

Since the localization pattern and microtubule stabilizing effect of Ciklp::β-gal and Kar3p::β-gal fusions are identical, the possibility exists that the localization of Ciklp::β-gal might depend on Kar3p and/or Kar3p::β-gal localization might require Ciklp. To test the first of these possibilities, a *MATa kar3Δ* strain containing *cikl::lacZ* was constructed. These cells were arrested with α-factor and subjected to immunofluorescence analysis. In *kar3Δ* cells, Ciklp::β-gal staining is evident throughout the cell and appears to preferentially reside in the cytoplasm (Fig. 5). In contrast, in wild-type and *cikl Δ* cells Ciklp::β-gal staining is primarily along cytoplasmic microtubules (Fig. 2; Page and Snyder, 1992). Therefore, in pheromone-treated cells, the microtubule localization of Ciklp::β-gal requires Kar3p.

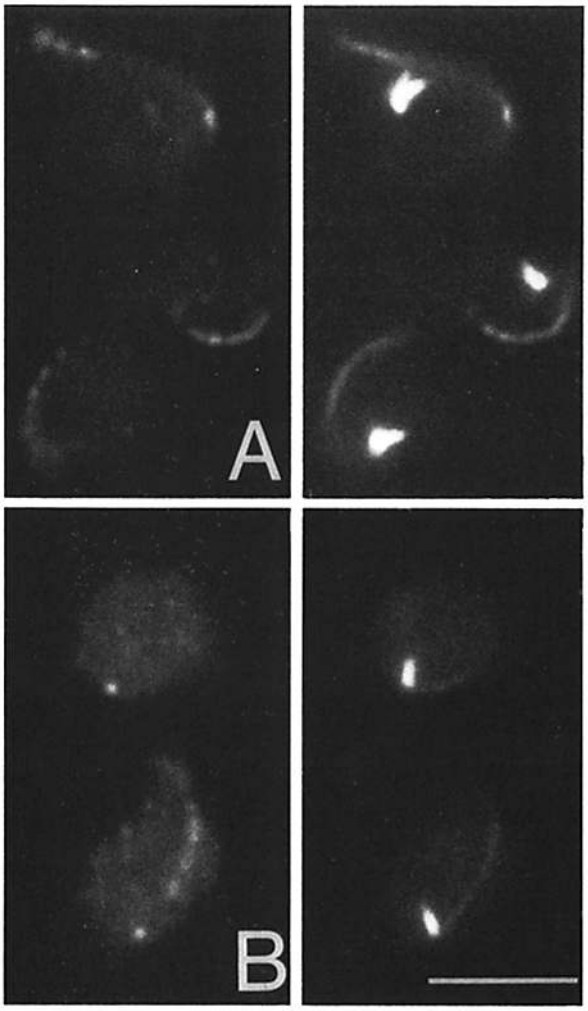

Figure 4. In pheromone-treated cells, the SPB localization of Ciklp::β-gal requires Karlp. Strains Y808, *karl-Δ13*, (*A*) and Y804, *KARI*, (*B*) containing the *cikl::lacZ* plasmid (pB20) were arrested with α-factor and stained with anti-β-gal (*left panel*) and anti-tubulin (*right panel*) antibodies. In these cells the long microtubules are not connected to the SPB (the bright spots in the figure). Bar, 4.5 μm.

To evaluate the dependence of Kar3p::β-gal microtubule localization on Ciklp, immunofluorescence experiments were performed using *kar3::lacZ* constructs (see Materials and Methods). The *kar3::lacZ* fusion contains the *KAR3* upstream regulatory region and the amino terminal half of the coding region; this segment encodes half of the predicted Kar3p coiled-coil domain and lacks the Kar3p putative microtubule motor domain. When wild-type *MATa* cells containing *kar3::lacZ* are treated with α-factor and stained with anti-β-gal antibodies, Kar3p::β-gal is detected along the cytoplasmic microtubules and diffusely in the cytoplasm and nucleus (Fig. 5). This Kar3p::β-gal pattern is identical to that detected in both wild-type and *kar3Δ* cells examined by Meluh and Rose (1990).

The *kar3::lacZ* fusion was transformed into a *MATa cikl Δ* strain, and the resulting strain was arrested with α-factor and subjected to immunofluorescence analysis. Under these conditions, Kar3p::β-gal does not localize to cytoplasmic microtubules (Fig. 5). Instead, the Kar3p::β-gal is primarily dispersed throughout the nucleus and weak cytoplasmic staining is evident. Therefore, Ciklp is required for localization of Kar3::β-gal to microtubules.

In Vegetative Cells, the kar3::β-gal SPB Localization Is Independent of CIK1

Vegetative wild-type cells containing the *kar3::lacZ* fusion were analyzed by immunofluorescence. In ~25% of these cells an anti-β-gal signal is detected at the SPB (Fig. 6 *A*). This SPB staining is not cell cycle-dependent since the signal is detected in both unbudded, small budded and large budded cells. Occasionally weak staining of the spindle microtubules can also be seen (Fig. 6 *B*).

To determine if the Kar3p::β-gal SPB localization is dependent upon Ciklp, the localization pattern of the Kar3p::β-gal fusion in a *cikl Δ* strain was evaluated by staining with anti-β-gal antibodies. Similar to that observed for wild-type cells, Kar3p::β-gal localizes to the SPB in 25% of *cikl Δ* cells, and this localization pattern does not appear to be cell cycle specific (Fig. 6 *B*). Therefore, contrary to the dependence upon Ciklp for the Kar3p::β-gal microtubule localization in pheromone-treated cells, in vegetative cells the Kar3p::β-gal SPB localization appears to be independent of Ciklp. This independence is consistent with the genetic analysis described below.

We also investigated whether the localization of Ciklp::β-gal to the SPB in vegetative cells requires Kar3p. When mitotically growing wild-type cells containing the *cikl::lacZ* fusion are stained with anti-β-gal antibodies, 30% of the cells exhibit a detectable signal in the region of the SPB (Fig. 5; Page and Snyder, 1992). When *kar3Δ* cells with the *cikl::lacZ* fusion are stained with anti-β-gal, ~30% of cells exhibited strong nuclear and weak cytoplasmic staining (>1,000 cells examined; Fig. 7). Cells at different stages of the cell cycle (unbudded, small budded, and large budded cells) exhibit this staining pattern. Because the nuclear signal may obscure detection of staining at the SPB, we cannot conclusively determine whether some Ciklp::β-gal is associated with the SPB.

In Vegetative Cells, cikl Δ and kar3Δ Strains Possess Similar, but not Identical, Phenotypes

Deletion of either *CIK1* or *KAR3* results in several similar vegetative phenotypes which include elevated chromosome loss, temperature sensitivity for growth at 37°C, and defects in spindle establishment and/or maintenance (Page and Snyder, 1992; Meluh and Rose, 1990). Sporulation of a *cikl Δ/CIK1* strain and tetrad analysis after growth at 25°C results in four equally sized colonies, indicating that at this temperature the *cikl Δ* does not have a severe effect upon cell growth (>60 tetrads analyzed). However, when a isogenic *kar3Δ/KAR3* strain is subjected to the same analysis at 25°C, a typical tetrad gives rise to two wild-type colonies and two smaller *kar3Δ* colonies (16 tetrads analyzed). Thus, the *kar3Δ* mutation has a greater effect upon vegetative cell growth than the *cikl Δ* mutation.

Tetrad analysis of a *cikl Δ/CIK1 kar3Δ/KAR3* strain reveals that *cikl Δ kar3Δ* cells are viable, and the colony size of the double mutant is equal to that of a *CIK1 kar3Δ* strain. These results are consistent with Ciklp operating in the same path-

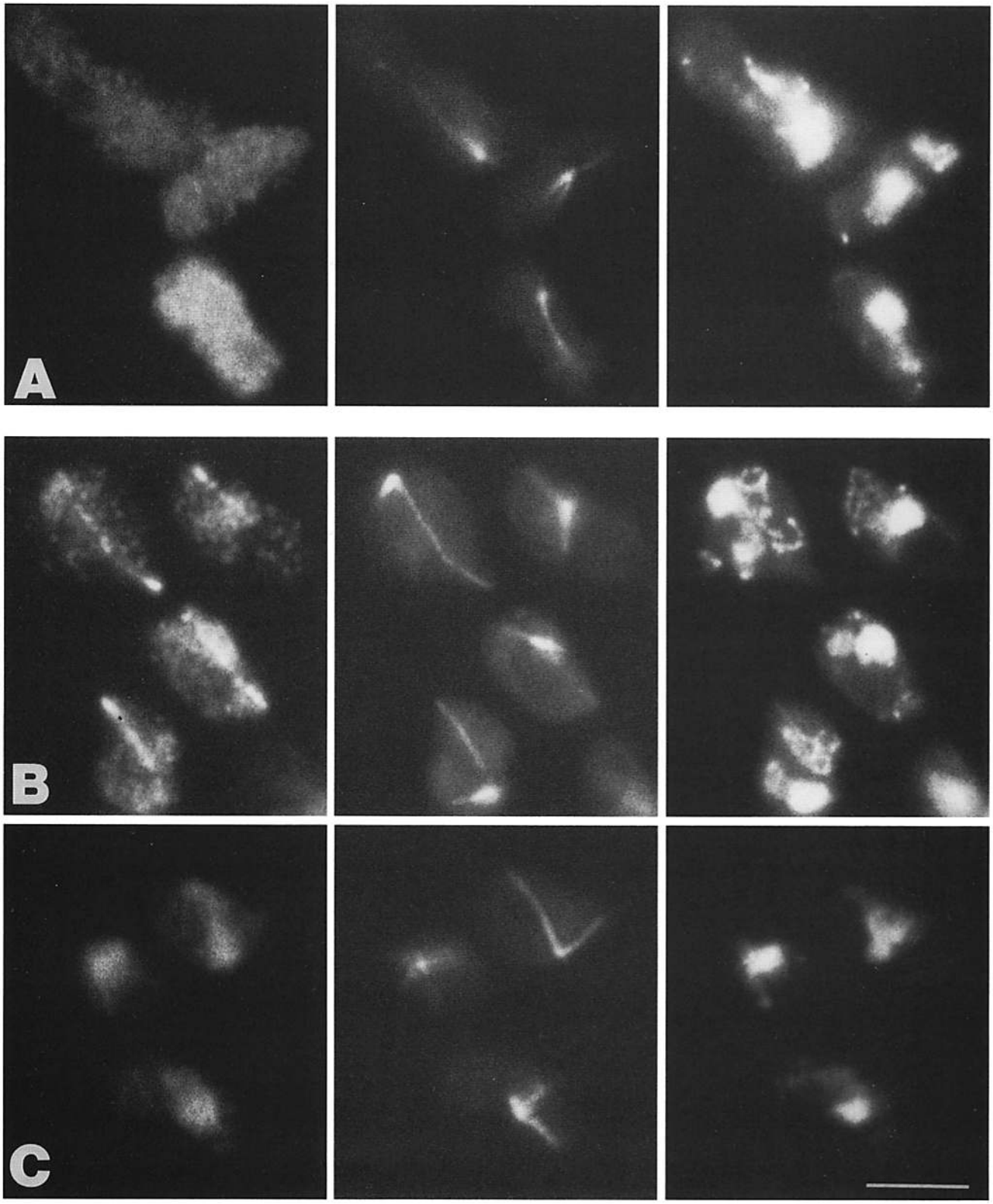

Figure 5. In pheromone-treated cells, the microtubule localization of Cik1p::β-gal and Kar3p::β-gal depend upon *KAR3* and *CIK1*, respectively. A *kar3*Δ strain (Y1137-5C) containing the *cik1::lacZ* plasmid (pB20) (*A*) was treated with α-factor and subjected to immunofluorescence analysis. Wild-type strain Y431 (*B*) and *cik1*Δ strain Y1119-3.7D (*C*), each containing the *kar3::lacZ* plasmid (pB41), were also arrested with α-factor and treated similarly. The left panel shows cells stained with anti-β-gal; the central and right panels exhibit anti-tubulin and Hoechst 33258 staining, respectively. Bar, 4.5 μm.

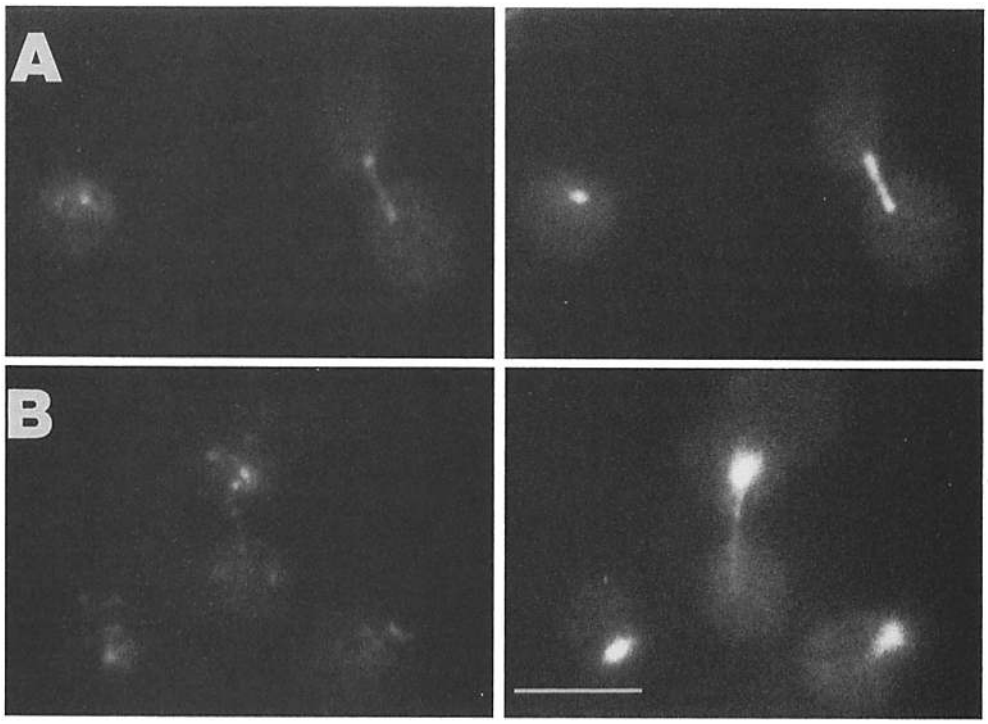

Figure 6. In vegetative cells, the SPB signal of Kar3p::β-gal is independent of *CIK1*. Vegetatively growing wild-type (*A*, Y431) and *cik1*Δ (*B*, Y1119-3.7D) cells containing the *kar3::lacZ* plasmid (pB41) were stained with anti-β-gal antibodies (*left panel*) and anti-tubulin antibodies (*right panel*), respectively. Bar, 4.5 μm.

way with Kar3p. Assuming that Kar3p and Ciklp participate together in some microtubule functions, the greater effect upon cell growth of the *kar3*Δ would suggest that *KAR3* functions in some microtubule processes without *CIK1*. This hypothesis is compatible with the independence of the Kar3p::β-gal SPB localization upon Ciklp in vegetatively growing cells.

To further evaluate the role of Ciklp in Kar3p-dependent processes, a genetic suppression test was used. *CIN8* and *KIP1* are thought to encode functionally redundant kinesin-related proteins (Roof et al., 1992*a*; Hoyt et al., 1992). Deletion of either gene is not lethal; however, a *cin8*Δ *kip1*Δ strain is inviable. A *kip1*Δ *cin8-3* strain is temperature-sensitive for growth at 33°C. Deletion of *KAR3* suppresses this phenotype such that *kar3*Δ *kip1*Δ *cin8-3* cells can form colonies at 33°C (Saunders and Hoyt, 1992). Since Ciklp and Kar3p share several similarities, a *cik1*Δ *kip1*Δ *cin8-3* strain was constructed to determine if deletion of *CIK1* could also suppress the Kip1Δ Cin8⁻ phenotype. Of the six different *cik1*Δ *kip1*Δ *cin8-3* strains examined, all are temperature-sensitive for growth at 33°C. Thus, deletion of *CIK1*, unlike deletion of *KAR3*, does not suppress the Kip1Δ Cin8⁻ phenotype.

CIK1 and KAR3 Interact as Determined by the Two-Hybrid System

Since the Ciklp::β-gal and Kar3p::β-gal localization patterns are similar and localization of each fusion protein is dependent upon the presence of the other wild-type protein, we postulated that Ciklp and Kar3p might interact physically or be part of the same complex. This hypothesis was tested using three different assays: the two-hybrid system, co-immunoprecipitation and localization of authentic Ciklp along Kar3p::β-gal stabilized microtubules.

The two hybrid system utilizes two different fusion plasmids, one encoding the *lexA* DNA binding domain ($lexA_{DB}$) and the other encoding the Gal4 activation domain ($Gal4_{AD}$) (Fields and Song, 1989; Golemis and Brent, 1992). If the $lexA_{DB}$ and the $Gal4_{AD}$ segments are separately fused to two proteins capable of forming a protein–protein complex, the DNA binding and transcriptional activation domain may be brought together and activate transcription of a lexA-responsive *lacZ* reporter construct.

To investigate the potential protein-protein interaction between Ciklp and Kar3p, the appropriate gene fusions were constructed. Two different *CIK1* DNA fragments were fused to sequences encoding the lexA DNA binding domain. The larger fusion contains 71% of the *CIK1* coding region (amino acids 20–446) and includes the entire putative coiled-coil domain. The smaller *lexA::cik1* fusion encodes amino acids 20–207 which includes 75% of the coiled-coil region. The *GAL4::kar3* fusion encodes 70% of the *KAR3* coding sequence from amino acids 12 to 515. This fusion contains the potential coiled-coil domain but lacks the microtubule-motor domain.

The combination of either *lexA::cik1* fusion and the *GAL4* plasmid (without *KAR3* sequences) does not elevate expression of *lacZ* (Table II). This result indicates that the *CIK1* fusions cannot activate transcription or interact with the *GAL4* activation domain. The combination of the *GAL4::kar3* fusion and the *lexA* plasmid is also incapable of increasing *lacZ* transcription above that of the negative control. However, when the reporter strain contains both the *lexA::cik1(20–446)* and *GAL4::kar3* plasmids, the expression of *lacZ*, as determined by β-gal activity, is increased 180-fold (Table II). This activation of transcription indicates that Ciklp and Kar3p interact to form a protein complex. In order to determine the region of Ciklp that interacts with Kar3p, the β-gal activity of a strain containing *GAL4::kar3* and the smaller *lexA::cik1(20–207)* fusion was evaluated. Although

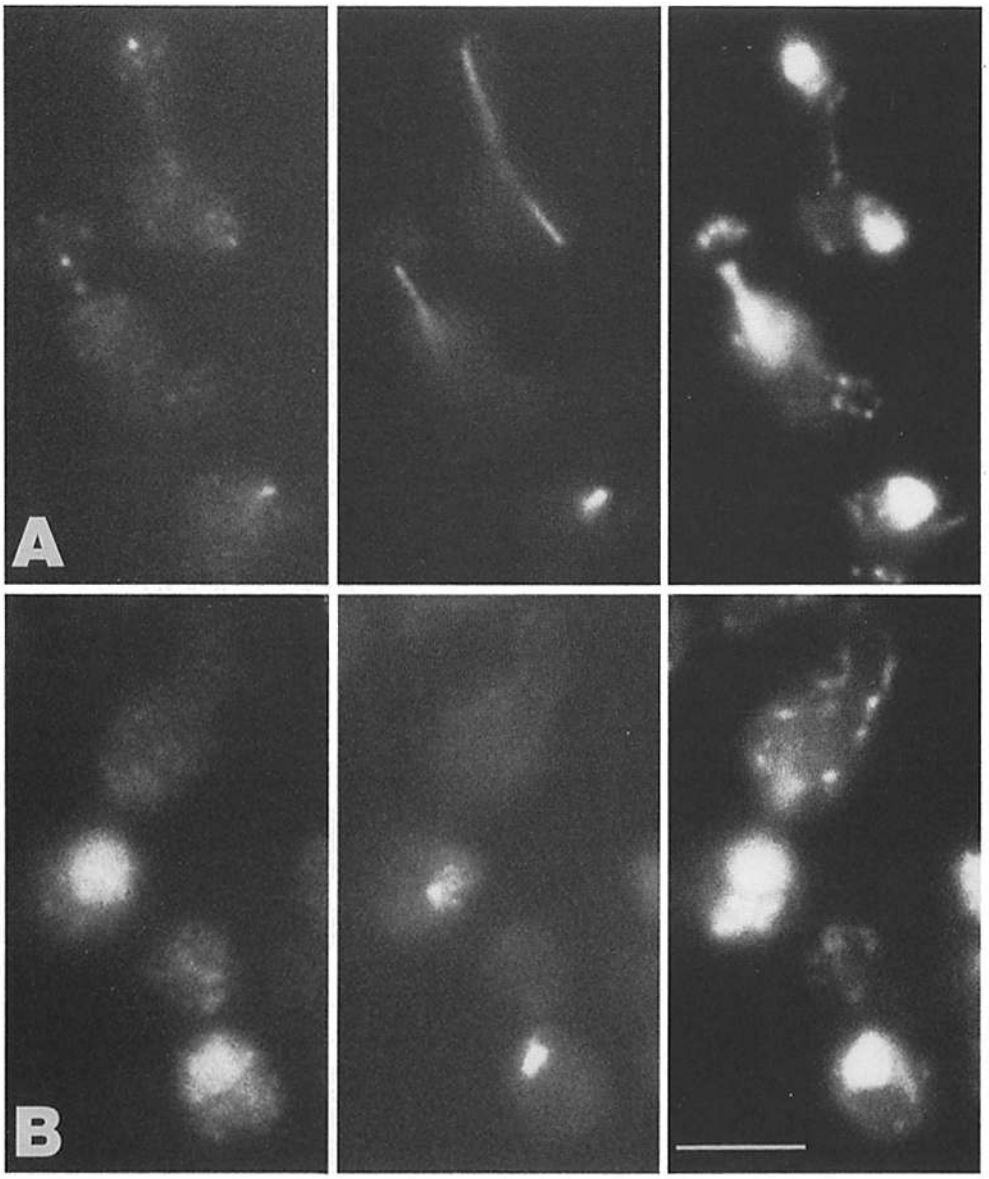

Figure 7. Localization of Ciklp::β-gal in wild-type and *kar3Δ* vegetative cells. Vegetatively growing (*A*) wild-type (Y431) and (*B*) *kar3Δ* (Y1137-5C) cells containing the *cikl::lacZ* plasmid (pB20) were analyzed by indirect immunofluorescence. The left panel contains cells stained with anti-β-gal; the central and right panels correspond to anti-tubulin and Hoechst 33258 staining, respectively. Note that in *B* there are two large budded cells. Bar, 4.5 μm.

the *lexA::cikl(20–207)* fusion includes less than 50% of the *CIK1* present in the larger fusion, this smaller construct in combination with *GAL4::kar3* elevates *lacZ* expression to a comparable level (210-fold). Therefore, as determined by the two hybrid system, Ciklp and Kar3p interact, and this interaction may be facilitated by a region within the amino-terminal half of CIK1.

CIK1 Co-Immunoprecipitates with KAR3

Interaction of Ciklp and Kar3p in vivo was further tested by co-immunoprecipitation experiments. Wild-type and *kar3Δ* strains were incubated in the presence or absence of α-factor and total cellular protein was extracted under nondenaturing conditions. Kar3p complexes were immunoprecipitated from

Table II. CIK1- and KAR3-dependent Transcriptional Activation by the Two Hybrid System

DNA binding domain hybrid	Activation domain hybrid	Colony color	β-gal activity
LEXA(1-87)-CIK1(20-446)	GAL4(768-881)	White	0.1
LEXA(1-87)-CIK1(20-446)	GAL4(768-881)-KAR3(12-515)	Blue	18
LEXA(1-87)-CIK1(20-207)	GAL4(768-881)	White	0.1
LEXA(1-87)-CIK1(20-207)	GAL4(768-881)-KAR3(12-515)	Blue	21
LEXA(1-87)	GAL4(768-881)-KAR3(12-515)	White	0.1
LEXA(1-87)	GAL4(768-881)	White	0.1
LEXA(1-87)-GAL4(87-881)		Blue	188

Numbers adjacent to gene designations refer to codon numbers. β-gal activity is presented in arbitrary units.

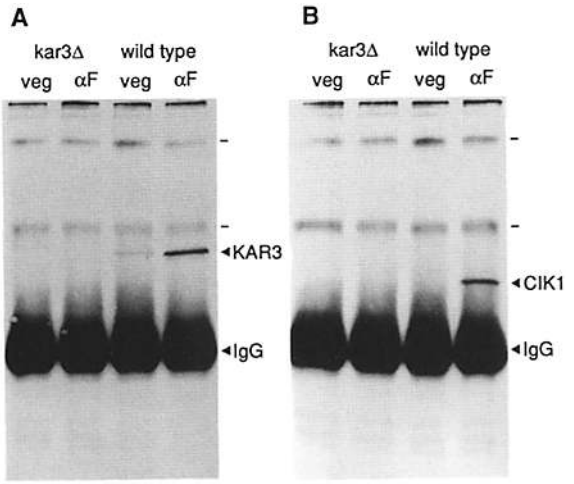

Figure 8. Ciklp co-immunoprecipatates with Kar3p in α-factor–treated cells. Proteins were isolated from a wild-type strain (MS10) or *kar3Δ* strain (MS524) grown vegetatively (*veg*) or treated with α factor (*αF*) and immunoprecipitated with anti-Kar3p antibodies. The immunoprecipitates were analyzed by immunoblot analysis. (*A*) The 88-kD Kar3p (KAR3) was detected by anti-Kar3p antibodies in both vegetative and pheromone-treated wild-type cells and was not evident in *kar3Δ* cells. Immunoglobulin heavy chain (*IgG*) and additional non-specific bands (−) were detected by the secondary antibody in the absence of primary antibody. (*B*) Immunoblots prepared as in (*A*) were probed with anti-Ciklp antibodies. The 77-kD Ciklp (CIK1) co-immunoprecipated from pheromone-treated wild-type cells, and was not detected in immunoprecipitates from vegetative cells or *kar3Δ* cells.

these extracts using anti-Kar3p antibodies, and Kar3p was detected by immunoblot analysis (Fig. 8 *A*). The 88-kD Kar3p is present in vegetative cells, and significantly higher amounts are evident in pheromone treated-cells. The 88-kD protein is not recovered in immunoprecipitates from the *kar3Δ* strain. Additional higher molecular weight bands present on these blots correspond to proteins that are detected by the secondary detection antibodies; they are present when the primary antibody is omitted.

To determine if Ciklp associates with Kar3p, the proteins that co-immunoprecipitate with anti-Kar3p antibodies were probed with affinity-purified anti-Ciklp antibodies. As shown in Fig. 8 *B* the 77-kD Ciklp is readily observed in immunoprecipitated samples from cells treated with α-factor. The protein is not evident in samples from vegetative cells, perhaps because the protein level is below the limit of detection. Ciklp is not detected in immunoprecipitates from the *kar3Δ* strain incubated in the presence or absence of α-factor, indicating that immunoprecipiation of Ciklp requires Kar3p. These results indicate that Kar3p and Ciklp are present in a protein complex in cells arrested with α-factor, consistent with the similar localization of Kar3p and Ciklp to cytoplasmic microtubules in pheromome-treated cells.

The Presence of Kar3p::β-gal Results in Altered Localization of Ciklp

In wild-type *MATa* cells arrested with α-factor, the authentic Ciklp appears associated with the SPB, but is not detected as colocalizing with microtubules (Fig. 9; Page and Snyder,

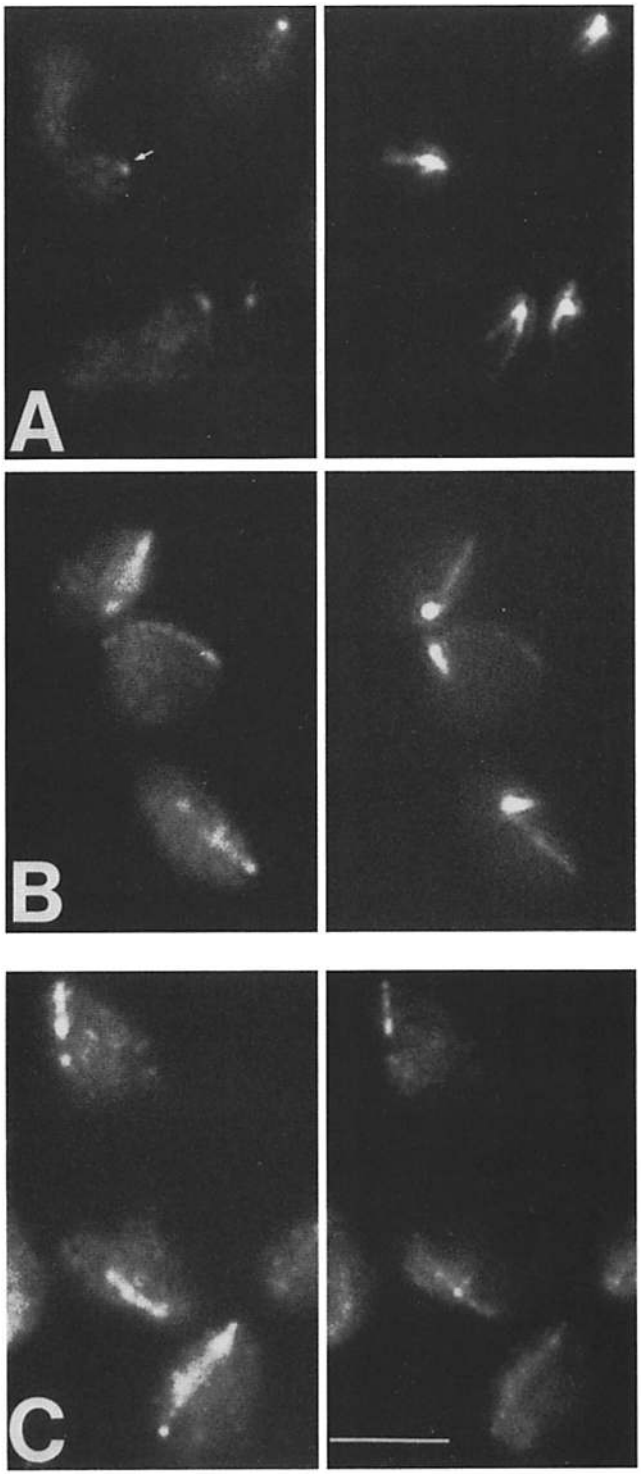

Figure 9. The presence of Kar3p::β-gal alters the localization of authentic Ciklp. Wild-type strain Y431 (*A*) was arrested with α-factor and stained with anti-CIK1 (*left panel*) and anti-tubulin (*right panel*). The strain Y431 containing pB41 (*B* and *C*) was treated identically and stained with anti-CIK1 antibodies (*B* and *C*, *left panel*) and either anti-tubulin (*B*, *right panel*) or anti–β-gal (*C*, *right panel*) antibodies. Bar, 4.5 μm.

1992). This localization differs from that of the anti-β-gal pattern detected in cells containing either *cik1::lacZ* or *kar3::lacZ*. If Ciklp interacts with kar3::β-gal as suggested by several of the above experiments, then one would expect the Ciklp localization pattern to be altered in a strain containing *kar3::lacZ*, such that CIK1 colocalizes with Kar3p::β-gal. To test this possbility, a *MATa* strain containing the *kar3::lacZ* plasmid was treated with α-factor and stained with anti-Ciklp antibodies. Under these conditions, Ciklp not only localizes to the SPB but is also detected along cytoplasmic microtubules (Fig. 9), very similar to the Kar3p::β-gal localization pattern. This result is compatible with an interaction between Ciklp and Kar3p::β-gal.

A possible caveat of this interpretation is that the Kar3p::β-gal fusion causes microtubules to be more stable (Meluh and Rose, 1990). Therefore, the Ciklp microtubule localization pattern could be due to increased microtubule stability instead of an interaction between Ciklp and Kar3p. However, the long microtubules in *kar1-Δ13* strains do not preferentially accumulate the authentic Ciklp (data not shown). If such microtubules are more stable as expected, then microtubule stability does not necessarily result in accumulation of Ciklp along microtubules. In summary, the two-hybrid and coimmunoprecipitation data along with the altered Ciklp localization in Kar3p::β-gal strains indicate that Ciklp interacts with Kar3p.

Discussion

Ciklp and Kar3p are Part of the Same Protein Complex

The results presented above indicate that Ciklp and Kar3p participate in many of the same cellular processes. *cik1* and *kar3* cells exhibit identical karyogamy defects, and the vegetative defects of the two mutants are very similar. Expression of both *CIK1* and *KAR3* is induced by α-factor, and the Ciklp::β-gal and Kar3p::β-gal fusion proteins exhibit similar localization patterns in vegetative and pheromone-treated cells.

Ciklp and Kar3p are likely to interact in vivo as suggested by the two hybrid system, coimmunoprecipitation experiments and the fact that the authentic Ciklp, which is normally detected at the SPB, colocalizes with a Kar3p::β-gal fusion along microtubules in pheromone treated-cells. It is important to emphasize that none of these experiments prove that Ciklp and Kar3p interact directly; it is possible that the interaction is mediated via one or more additional proteins. Nevertheless, the combination of these different results strongly indicate that Ciklp and Kar3p are part of the same multiprotein complex.

Kinesins are comprised of both heavy chains and light chains; by analogy it is possible that kinesin-related motors also contain heavy chains and light chains. Ciklp could be a light chain for Kar3p. Consistent with this hypothesis, Ciklp is predicted to contain a central coiled-coil domain, a feature found in the few kinesin light chains that have been characterized (Cyr et al., 1991; Wedaman et al., 1993; Gauger and Goldstein, 1993). Ciklp and Kar3p might interact via their coiled-coil domains, analogous to heterodimer formation between other coiled-coil proteins (Hatzfeld and Franke, 1985; Coulombe and Fuchs, 1990; Hatzfeld and Weber, 1990; Steinert, 1990; Miller et al., 1993). Alternatively, these two proteins might interact via their amino-terminal domains. Further experiments will be necessary to determine if Ciklp is a light chain and to identify at high resolution the Kar3p-Ciklp interacting sequences.

Localization of Authentic Ciklp vs Ciklp::β-gal Fusion

Authentic Ciklp localizes to the SPB in pheromone-treated cells; whereas, the fusion protein localizes to both the SPB and microtubules. This latter observation indicates that Ciklp associates with microtubules. There are at least two possibilities for where Ciklp might function. Ciklp might interact with microtubules while attached to the SPB. Alternatively, Ciklp, could be part of a minus end motor protein complex, which accumulates at its SPB destination (Page and Snyder, 1993). The motor directionality of Kar3p is not known, but it has been hypothesized to be a minus end motor (Saunders and Hoyt, 1992). The Ciklp::β-gal fusion might be impeded in its transit and thereby allow detection of the protein along microtubules. Regardless of which model is correct, the Ciklp::β-gal fusion indicates an interaction of this protein with microtubules which is not evident by localization of the authentic protein. Thus, the use of fusion proteins, in addition to increasing sensitivity for immunodetection experiments, can help provide information about the function of a particular protein.

β-gal fusions have been extremely useful for determining the localization domain of many proteins (e.g., Silver et al., 1984; Hall et al., 1984; Trueheart et al., 1987). Analysis of Ciklp and Kar3p fusions have determined that the SPB/microtubule localization domain is within the amino terminal half of Ciklp and the amino terminal 42% of Kar3p (Page and Snyder, 1992; Meluh and Rose, 1990). In this latter case, the Kar3p::β-gal fusion is devoid of its putative motor domain, thereby allowing analysis of a separate microtubule interacting domain which requires Ciklp.

Localization of Kar3p::β-gal Requires Ciklp in Pheromone-treated Cells

In wild-type cells treated with pheromone, both Ciklp::β-gal and Kar3p::β-gal fusions localize to cytoplasmic microtubules and the SPB. In contrast, in *kar3Δ* cells treated with pheromone, the Ciklp::β-gal fusion localizes throughout the cell, with a majority of it in the cytoplasm; it does not localize to microtubules. The Kar3p::β-gal fusion does not localize to microtubules or the SPB in pheromone-treated *cik1Δ* cells but instead, appears to be largely present within the nucleus. These results indicate that Ciklp and the nonmotor domain of Kar3p depend upon one another for localization to microtubule-associated structures. Consistent with this hypothesis, loss of Ciklp function yields defects in karyogamy and chromosome segregation that are similar to those of *kar3Δ* defects. Formation of this Ciklp-Kar3p complex and its association with microtubules/SPB are likely to be important for mediating the activities of the putative Kar3p motor. This is the first example of regulated localization of a microtubule motor protein.

The data presented above also suggest that nuclear-cytoplasmic compartmentalization of both Ciklp and Kar3p is regulated by pheromone treatment. In a *kar3Δ* strain, Ciklp::β-gal is detected in the nucleus during vegetative growth, but most of it is evident in the cytoplasm after α-fac-

tor treatment. Furthermore, Ciklp may regulate compartmentalization of Kar3p in pheromone-treated cells. In wild-type cells much of the Kar3p::β-gal protein is present in the cytoplasm; whereas, in a *cik1*Δ strain the fusion protein appears preferentially associated with the nucleus. The nuclear/cytoplasmic compartmentalization of both Ciklp and Kar3p could be regulated by the production of different isoforms of Ciklp in pheromone-treated and vegetative cells. Loss or modification of a Ciklp nuclear localization sequence (NLS) in pheromone treated-cells would retain Ciklp in the cytoplasm. Such a modification could result from production of different peptides or by phosphorylation of the NLS. The Ciklp from pheromone-treated cells migrates slightly faster than that of vegetative cells suggesting that different isoforms of Ciklp do exist under these two growth conditions. Interaction of Ciklp with Kar3p (for example, by binding to the Kar3p NLS) could control the compartmentalization of Kar3p.

In summary, these results indicate that interaction of Ciklp with Kar3p is important for localization of the nonmotor domain of Kar3p to microtubules. The association of an interacting protein (e.g., light chain) with the nonmotor domain of heavy chains may be of general importance for mediating the specialized function of kinesin-related motors.

KAR3 has Functions during Vegetative Growth That Do Not Require CIK1

The phenotypes of *cik1*Δ and *kar3*Δ strains during vegetative growth are very similar, but not identical (see introduction and Results for similarities). *kar3*Δ strains grow slower than *cik1*Δ strains, and *kar3*Δ, but not *cik1*Δ, can partially suppress the Cin8⁻ Kip1Δ phenotype. Furthermore, during vegetative growth the localization of the Kar3p::β-gal fusion does not require Ciklp. Thus, we speculate that another Kar3p interacting protein is present in vegetatively growing cells and that this protein has a function related to Ciklp. The Ciklp redundant function is unlikely to be very important for karyogamy, because *cik1*Δ cells exhibit the same severe defects in bilateral karyogamy assays as *kar3*Δ cells. Redundancy for putative kinesin heavy chains have been previously described for *KIP1* and *CIN8* (Roof et al., 1992*a*; Hoyt et al., 1992); thus, it is possible that kinesin heavy chain interacting proteins (and light chains) are also redundant.

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