Kinesin-related Proteins Required for Assembly of the Mitotic Spindle

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Abstract. We identified two new *Saccharomyces cerevisiae* kinesin-related genes, *KIP1* and *KIP2*, using polymerase chain reaction primers corresponding to highly conserved regions of the kinesin motor domain. Both KIP proteins are expressed in vivo, but deletion mutations conferred no phenotype. Moreover, *kip1 kip2* double mutants and a triple mutant with kinesin-related *kar3* had no synthetic phenotype. Using a genetic screen for mutations that make *KIP1* essential, we identified another gene, *KSL2*, which proved to be another kinesin-related gene, *CIN8*. *KIP1* and *CIN8* are functionally redundant: double mutants arrested in mitosis whereas the single mutants did not. The microtubule organizing centers of arrested cells were duplicated but unseparated, indicating that *KIP1* or *CIN8* is required for mitotic spindle assembly. Consistent with this role, KIP1 protein was found to colocalize with the mitotic spindle.

THE accurate segregation of chromosomes during mitosis is accomplished by a combination of multiple microtubule-based movements (for review see McIntosh and Pfarr, 1991; Sawin and Scholey, 1991). These include migration of the microtubule organizing centers to form a bipolar spindle, chromosome congression to the metaphase plate, chromosome movement along the pole-to-kinetochore microtubules (anaphase A) and spindle elongation due to sliding of the pole-to-pole microtubules (anaphase B).

Although a number of mechanisms have been proposed for the generation of forces that induce movement, it is likely that at least some of the movements are mediated by mechanochemical "motor proteins." One prototype motor protein, kinesin, was identified in squid axons as a protein capable of directing movement of vesicles and organelles toward the plus ends of microtubules in an ATP-dependent fashion (Vale et al., 1985). More recently a superfamily of kinesin-related genes has emerged (for review see Goldstein, 1991; Rose, 1991) whose members each encode a region of high sequence similarity to a 450-residue amino-terminal domain of *Drosophila* kinesin heavy chain (khc).[1] We use the term kinesin-related to denote genes showing sequence similarity in the motor domain, suggesting a common ancestry. This domain is sufficient to confer microtubule-based movement in vitro (Yang et al., 1990). These kinesin-related genes have been identified in several organisms either by mutations that affect chromosome and nuclear movements in mitosis and meiosis, or by DNA sequence homology to khc. Remarkably, in each case the sequence similarly does not extend beyond the "motor domain"; all the presently known genes encode "tail" sequences that are unrelated to khc and to other members of the kinesin superfamily. Moreover, the motor domain has been found at either the NH_2 or COOH terminus. In addition to kinesin, motility has been demonstrated for *Drosophila ncd* (McDonald et al., 1990; Walker et al., 1990). Although it is expected that most of the genes encode motor proteins, it is possible that some of the proteins have nonmotile functions (Roof et al., 1992).

Members of the kinesin superfamily participate in a wide variety of microtubule-mediated processes. For example, the KAR3 protein of *Saccharomyces cerevisiae* functions in at least two distinct movements (Meluh and Rose, 1990). KAR3 is required for nuclear fusion, where it probably serves to move the two nuclei of a zygote together via the cytoplasmic microtubules. *kar3* mutants also accumulate inviable cells that are blocked in mitosis. Their morphology suggests that KAR3 participates in spindle elongation. Mutations in kinesin-related genes *bimC* of *Aspergillus nidulans* (Enos and Morris, 1990) and *cut7* of *Schizosaccharomyces pombe* (Hagan and Yanagida, 1990) prevent the separation of the spindle pole bodies necessary to form a bipolar mitotic spindle. Mutations in the *Drosophila* genes *ncd* and *nod* principally affect meiotic chromosome transmission, but *ncd* may also play a role in mitosis (Davis, 1969; Carpenter, 1973; Sequeira et al., 1989; Zhang and Hawley, 1990). *Drosophila khc* mutations affect neuromuscular functions (Saxton et al., 1991), as does the *Caenorhabditis elegans unc-104* mutation (Hall and Hedgecock, 1991). These are only a subset of the kinesin-related genes, since in *Drosophila* the existence of 11 kinesin-related genes has been verified (Steward et al., 1991), but as many as 35 may exist (Endow and Hatsumi, 1991).

Since several different motors and a variety of movements occur within a single cell, some degree of spatial and temporal specificity of each motor exist. Specificity determinants have been postulated to lie in the tail sequences

1. *Abbreviations used in this paper*: 5FOA, 5-fluoroorotic acid; khc, kinesin heavy chain; PCR, polymerase chain reaction.

which are unique to each protein, but sequences in the motor domain could confer specificity as well. However, the multiplicity of kinesin-related proteins within a single organism raises the possibility of functional overlap wherein several different motor proteins power a single movement. For example, KAR3 cannot be the sole force-generating protein that participates in mitotic spindle elongation, because *kar3* null mutants are viable although slow growing (Meluh and Rose, 1990). Presumably other force-generating proteins also serve to power this movement. Such functional redundancy might explain the absence of kinesin-related genes among the collection of cell division cycle (CDC) genes in yeast. It is not yet known to what extent individual motors are restricted to a single movement, and to what extent several different motors can overlap in function.

We report here the existence of multiple kinesin-related proteins in *S. cerevisiae*. *KIP1* and *KIP2* were identified by their homology with khc and *KAR3*. Using a genetic screen for mutations that make *KIP1* essential for viability, we identified another gene, *KSL2*. *KSL2* was found to be allelic with *CIN8*, another kinesin-related gene (Hoyt et al., 1992). *KIP1* and *CIN8* exhibit functional redundancy in spite of the dissimilarity of their tail sequences. We have used conditional double mutants to show that these proteins mediate migration of the duplicated spindle pole bodies at the onset of mitosis. Immunofluorescent localization of KIP1 protein shows that it is present on early mitotic spindles.

Materials and Methods

Strains and Microbial Techniques

The yeast strains used are listed in Table II. Media and genetic techniques were as described in Rose et al. (1990). Yeast transformations were by the lithium acetate method of Ito et al. (1983), with 50 μg of sheared denatured salmon sperm DNA as carrier. Plasmids were recovered from yeast for transformation into *Escherichia coli* by the method of Hoffman and Winston (1987).

Polymerase Chain Reaction

Polymerase chain reaction amplification of kinesin-related genes was done using degenerate primers that correspond to conserved regions of *KAR3*, *bimC*, and khc. Primer 1 encodes the peptide IFAYGQT (5′ gggaattc-AT(ACT)TT(CT)GC(ACGT)TA(CT)GG(ACGT)CA(AG)AC3′) and is 384-fold degenerate. Primer 2 encodes the peptide LVDLAGSE (5′ gggaattc-(CT)T(ACGT)GT(ACGT)GA(CT)(CT)T(ACGT)GC(ACGT)GG(ACGT)(AT)(GC)(ACGT)GA3′) and is 131,000-fold degenerate. Primer 3 is the antisense strand corresponding to the peptide LVDLAGSE (5′gggatcc(CT)TC-(ACGT)(CG)(AT)(ACGT)CC(ACGT)GC(ACGT)A(GA)(GA)TC(ACGT)-AC(ACGT)A3′) and is 131,000-fold degenerate. Primer 4 is the antisense strand corresponding to the peptide HIP(Y/F)R(D/E/N)SK (5′ggggatccTT-(ACGT)(CG)(AT)(ACGT)T(TC)(ACGT)C (GT)(AG)(AT)A(ACGT)GG-(AGT)AT(AG)TG 3′) and is 131,000-fold degenerate. Two or more residues in parentheses indicates each was included at that site, and lower case nucleotides indicate restriction sites (EcoRI or BamHI) included to allow directional cloning. Approximately 0.5 μg of yeast genomic DNA (prepared as in Hoffman and Winston, 1987) was amplified with native Taq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT) as recommended by the manufacturer. The initial five cycles were performed at 95°C for 1 min, 37°C for 2 min, 72°C for 1 min, and the subsequent 25 cycles were performed at 95°C for 1 min, 42°C for 2 min, and 72°C for 1 min.

To clone the amplified DNA, the fragments were resolved on 4% NuSieve (FMC Corp., Rockland, ME) agarose gels, excised, and electroeluted. The DNA was digested with EcoRI and BamHI, cloned into pBluescript KS(+) (Stratagene, La Jolla, CA) and sequenced.

Isolation of the KIP1 and KIP2 Genes

A YCp50 plasmid-based yeast genomic library was screened by colony hybridization (Davis et al., 1980) using gel purified cloned polymerase chain reaction (PCR) amplified DNA fragments as probes. Candidate plasmids were analyzed by restriction digest and Southern blotting to identify plasmids with the hybridizing sequences near the middle of the yeast DNA insert. pMR1690, pMR1691, and pMR1692 carry *KIP1*, and pMR1695 and pMR1697 carry *KIP2*.

Plasmid Constructions and DNA Sequencing

For sequencing *KIP1*, the 5,734-bp BglII fragment from pMR1692 was cloned in both orientations into pKS(+) to make pMR1722 and pMR1723. As this fragment was found not to include the *KIP1* NH_2 terminus, a partially overlapping 1,708-bp EcoRI–PstI fragment from pMR1690 was cloned into pKS(+) and pKS(−) to make pMR1703 and pMR1702. For sequencing *KIP2*, the 3,819-bp EcoRI–BglII fragment from pMR1697 was cloned into pKS(+) and pKS(−) to make pMR1705 and pMR1706. To sequence beyond the BglII site which is located 20 bp from the COOH terminus of *KIP2*, the 671-bp PstI–EcoRI fragment from pMR1695 was cloned into pKS(+) to make pMR2362. Nested deletions of the above plasmids were made using exonuclease ExoIII and nuclease S1 (Hoheisel and Pohl, 1986), and single-stranded DNA was sequenced using Sequenase (United States Biochemical, Cleveland, OH). Both strands of the *KIP* coding regions were sequenced.

KIP1 was subcloned into the yeast shuttle vectors constructed by Sikorski and Hieter (1989). The 5,093-bp NheI fragment from the *KIP1* plasmid pMR1691 was cloned into the SpeI site of pRS316 (a *CEN* plasmid marked with *URA3*), pRS315 (*CEN LEU2*) and pRS306 (YIp *URA3*) to make pMR1895, pMR1893, and pMR1891, respectively.

For insertional mutagenesis and immunological detection of *KIP1*, DNA fragments which encode three copies of the hemagglutinin epitope were introduced into four restriction sites of pMR1893, to create in frame insertions. The *kip1-5*::HA insertion was made at *KIP1* amino acid residue 211 using a BglII restriction site, the *kip1-6*::HA insertion was made at residue 754 using MluI, the *KIP1-4*::HA insertion was made at residue 54 using AflIII and the *KIP1-7*::HA was made at residue 1,022 using AccI. Since the AflIII and AccI sites were not unique, pMR1893 DNA was partially digested with the restriction enzyme and singly cut plasmid DNA was purified by agarose gel electrophoresis. The linear DNA fragments were treated with large fragment of DNA polymerase I and 4 dNTPs to generate blunt ends. The DNA fragments encoding the epitope were isolated from plasmid GTEPI, obtained from B. Futcher (Cold Spring Harbor Laboratory, NY). This plasmid contains a NotI fragment that encodes three copies of the sequence YPYDVPDYA, in pBluescript II SK(−). The 143-bp insert DNA for the *KIP1-4*::HA and *kip1-5*::HA alleles was made by digesting GTEPI with BstXI, removing the 3′ extension with T4 DNA polymerase and digestion with SmaI. The 182-bp insert DNA for the *kip1-6*::HA allele was made by digesting GTEPI with SacI, removing the 3′ extension with T4 DNA polymerase, digestion with ClaI, and treatment with DNA polymerase I large fragment to generate the second blunt end. The 151-bp insert DNA for the *KIP1-7*::HA allele was made by digesting GTEPI with SacI, removing the 3′ extension with T4 DNA polymerase and digestion with SmaI. The insert fragments were purified by agarose gel electrophoresis and ligated with the appropriate linearized *KIP1* plasmid DNA. Plasmids containing insertions in the desired restriction sites and in the correct orientation were identified by restriction digestion and gel electrophoresis of plasmid DNA isolated from *E. coli* transformants.

DNA and predicted protein sequence analysis was performed using the Sequence Analysis Software Package by Genetics Computer, Inc.

Construction of kip1 and kip2 Deletion Strains

The null allele *kip1Δ1::HIS3* is an internal deletion of *KIP1* corresponding to amino acids 106 to 1,046, replaced by *HIS3*. pMR1892 was digested with BclI to remove three BclI fragments internal to *KIP1*, and a fragment containing *HIS3* was inserted to create pMR1921. The null allele *kip2Δ2::TRP1* is an internal deletion of *KIP2* corresponding to amino acids 94 to 667, replaced by *TRP1*. pMR1775 was partially digested with XhoI, the fragment that resulted from cutting only at XhoI sites within *KIP2* was gel purified, and a fragment containing *TRP1* was inserted to create pMR1791. The *kip2Δ1::URA3* allele in pMR1790 was made using a fragment encoding *URA3* instead of the *TRP1* fragment. The resulting *kip1* deletion strains are MS2333 and MS2334, and the *kip2* deletion strains are MS2309 and MS2354.

The *kip* deletion mutations were inserted into the wild-type genomic *KIP* genes using the one-step gene replacement technique of Rothstein (1983). pMR1921 was digested with EcoRI plus XhoI, and pMR1790 and pMR1791 were digested with XhoI plus SpeI before transformation. The structure of each deletion mutation was confirmed using Southern blots of genomic yeast DNA prepared from the deletion mutants.

Mapping of KIP1 and KIP2

KIP1 was assigned to chromosome II by hybridization of a *KIP1* probe to electrophoretically separated yeast chromosomes. The *KIP1* gene was localized to a position near *ilsl* on the left arm of chromosome II by hybridization of the *KIP1* probe to dot blots of mapped yeast DNA fragments kindly provided by L. Riles and M. Olsen (Washington University School of Medicine, St. Louis, MO). Linkage to *ilsl* was confirmed using meiotic crosses. The *kip1::URA3* strain MS2305 was crossed to the *ilsl* strain L785 and sporulated. 42 tetrads were parental ditype and one was tetratype, indicating that *KIP1* is 1 cM from *ilsl* (calculated using the formula of Perkins, 1949).

KIP2 was assigned to the left arm of chromosome XVI by hybridization of a *KIP2* probe to electrophoretically separated yeast chromosomes and chromosome fragments. Sequence of the *KIP2* region revealed that the likely *KIP2* initiation codon is located 326 bp downstream of the termination codon of the *PEP4* gene (see Fig. 2).

Immunological Techniques

Total yeast protein for Western blots was extracted from exponentially growing cells by the method of Ohashi et al. (1982), resolved by SDS-PAGE and electrophoretically transferred to nitrocellulose membrane. The membrane was incubated with mAb 12CA5 ascites fluid, which recognizes an epitope (termed HA) from the influenza hemagglutinin protein (Wilson et al., 1984). Alternatively, a polyclonal anti-kinesin peptide antibody was used (Sawin et al., 1992). After incubation with secondary antibody conjugated to HRP (Amersham Corp., Arlington Heights, IL), protein was detected using the ECL detection kit (Amersham Corp.).

Immunolocalization was performed by the methods of Adams et al. (1984) and Kilmartin and Adams (1984) as modified in Rose and Fink (1987). For detection of HA epitope-tagged proteins with the 12CA5 antibody, cells were fixed with formaldehyde for 30 min at 23°C. Tubulin staining was done with rabbit anti-tubulin antibody RAP1/24, a gift of F. Solomon (Massachusetts Institute of Technology, Cambridge, MA). Goat anti-rabbit antibody conjugated to fluorescein isothiocyanate, or conjugated to rhodamine, and goat anti-mouse antibody conjugated to fluorescein isothiocyanate were obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). DNA was stained using the fluorescent dye 4,6-diamidino-2-phenylindole (DAPI) (Boehringer Mannheim Biochemicals).

Electron Microscopy

Cells were pregrown at 23°C and shifted to 37°C for 3 h in YM1 medium (Hartwell, 1967), then fixed and embedded for sectioning by the method of Byers and Goetsch (1991). Cells with a large bud and at least one spindle pole body were identified, and adjacent sections were scored for the presence or absence of a second spindle pole body.

Chemical Mutagenesis

Strains MS2335 and MS2336 were mutagenized for the synthetic lethal mutant screen with ethyl methanesulfonate as described in Rose et al. (1990), plated for single colonies on YPD medium, and replica printed to 5-fluoroorotic acid (5FOA) (Boeke et al., 1987) medium (all at 30°C) to identify 5FOA sensitive derivatives. Temperature sensitive *kipl* mutations were identified using the plasmid shuffle technique (Boeke et al., 1987). For this purpose, plasmid DNA was mutagenized with hydroxylamine as described in Rose and Fink (1987) and used to directly transform yeast. Putative *kipl* (ts) plasmids were recovered from yeast by transformation into *E. coli*, and the BamHI XbaI fragment containing *kipl* was cloned into the *URA3*-marked YIp vector pRS406 (Sikorski and Hieter, 1989). The resulting plasmids were linearized with BglII or MluI and used to replace the chromosomal copy of *KIP1* with the *kipl*(ts) allele by plasmid integration and excision (Scherer and Davis, 1979).

Results

Identification of the Kinesin-related Genes KIP1 and KIP2

Members of the kinesin superfamily possess several regions of sequence conservation within their putative motor regions. To identify new kinesin-related genes, we amplified yeast genomic DNA by PCR using degenerate oligonucleotide primers corresponding to three regions highly conserved between *KAR3*, *khc*, and *bimC*. One of the primer sites includes part of an ATP binding/hydrolysis consensus sequence (Walker et al., 1982), while the other two primer sites are in regions implicated in microtubule binding (Yang et al., 1989). The primer sites flank additional conserved regions, whose presence in an amplified DNA fragment signified identification of a kinesin-related gene.

Two genes with homology to the motor domain of kinesin were identified by cloning and sequencing the DNA fragments generated by PCR amplification. We have designated the genes *KIP1* and *KIP2*, for kinesin-related protein. To obtain the complete genes, the cloned amplified DNA fragments were used as hybridization probes to screen a yeast genomic DNA library. DNA fragments carrying *KIP1* or *KIP2* were identified by hybridization, subcloned, and their DNA sequences were determined. The *KIP1* open reading frame would encode a peptide of 1,111 amino acids (Fig. 1), while the *KIP2* open reading frame would encode a peptide of 706 amino acids (Fig. 2). The chromosomal location of *KIP1*, as determined by hybridization of a *KIP1* probe to a mapped yeast DNA library and by meiotic crosses, is on the left arm of chromosome II near *ILS1*. *KIP2* was located by DNA sequencing of a known adjacent gene and resides on the left arm of chromosome XVI next to *PEP4* (see Materials and Methods). Neither gene has been previously described.

The NH_2-terminal region of the predicted KIP1 protein and a central region of the predicted KIP2 protein show extensive sequence similarity to the force generating domain of khc (Fig. 3 *A*). The *KIP1* and *Drosophila melanogaster* khc sequences show 42% amino acid identity over the central 335 residues of this domain (defined in Rose, 1991), and the *KIP2* sequence shows 38% identity. The sequence conservation includes the consensus sequence GX_4GKT proposed to contribute to ATP binding and hydrolysis (Walker et al., 1982), as well as other sequences present in the domain of kinesin thought to be required for microtubule binding (Yang et al., 1989). *KIP1* shows particularly high sequence similarity with the putative motor domains of *bimC*, *cut7*, and *Xenopus Eg5* (Le Guellec et al., 1991) (65, 54, and 52% identity, respectively, over 335 amino acids), suggesting that these proteins comprise a subgroup of highly conserved kinesin-related genes.

In contrast to the motor domain, the COOH-terminal 690 residues of *KIP1* show little or no sequence similarity with the nonmotor regions of any of the kinesin-related proteins, nor has substantial sequence similarity been found with sequences in the current GenBank database using the TFASTA search program. Like many members of the kinesin superfamily, the nonmotor region of *KIP1* encodes several regions containing heptad repeats of hydrophobic and charged amino acids (Fig. 3 *B*). Such regions are associated with the forma-

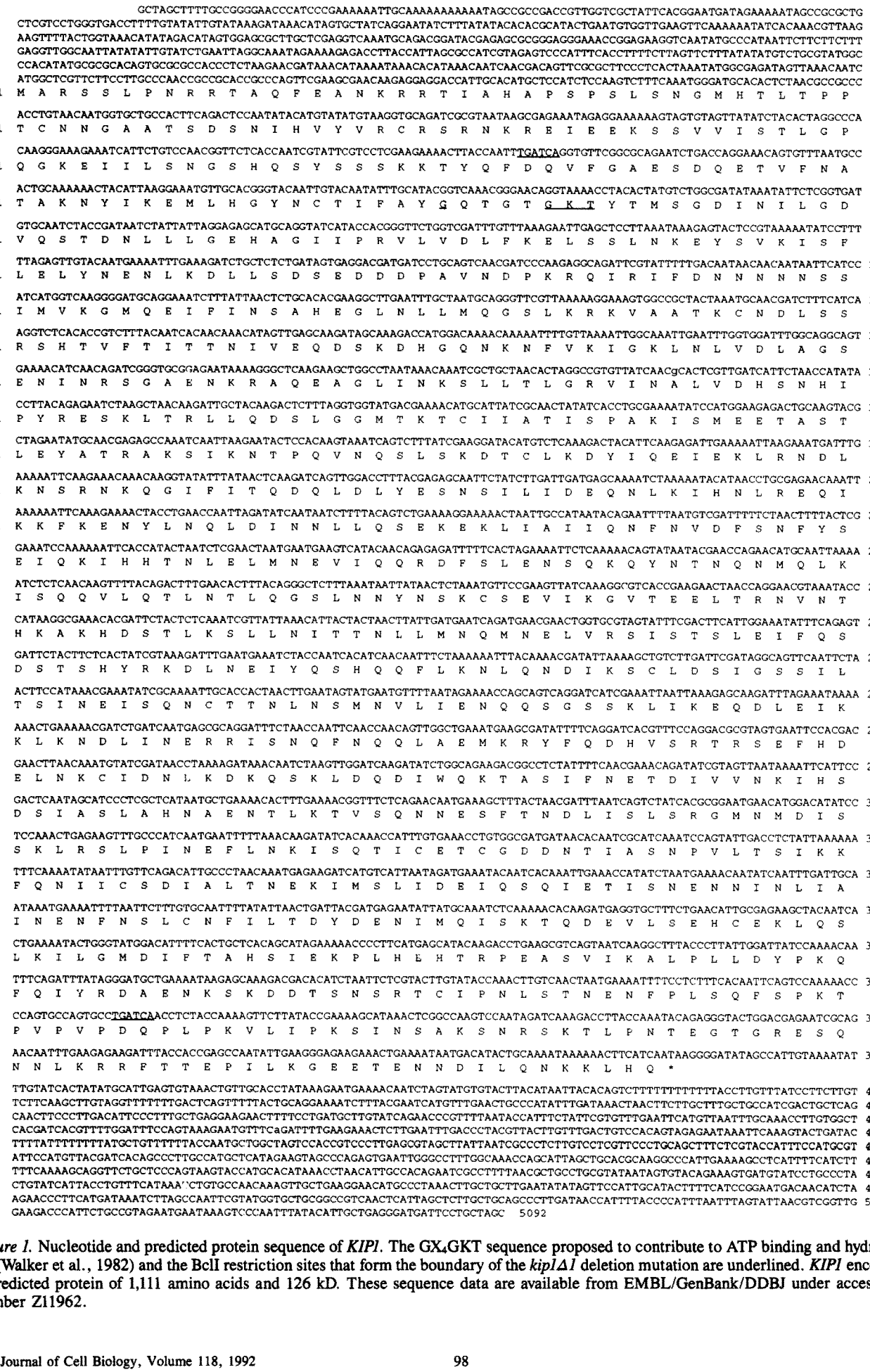


Figure 1. Nucleotide and predicted protein sequence of *KIP1*. The GX_4GKT sequence proposed to contribute to ATP binding and hydrolysis (Walker et al., 1982) and the BclI restriction sites that form the boundary of the *kip1Δ1* deletion mutation are underlined. *KIP1* encodes a predicted protein of 1,111 amino acids and 126 kD. These sequence data are available from EMBL/GenBank/DDBJ under accession number Z11962.

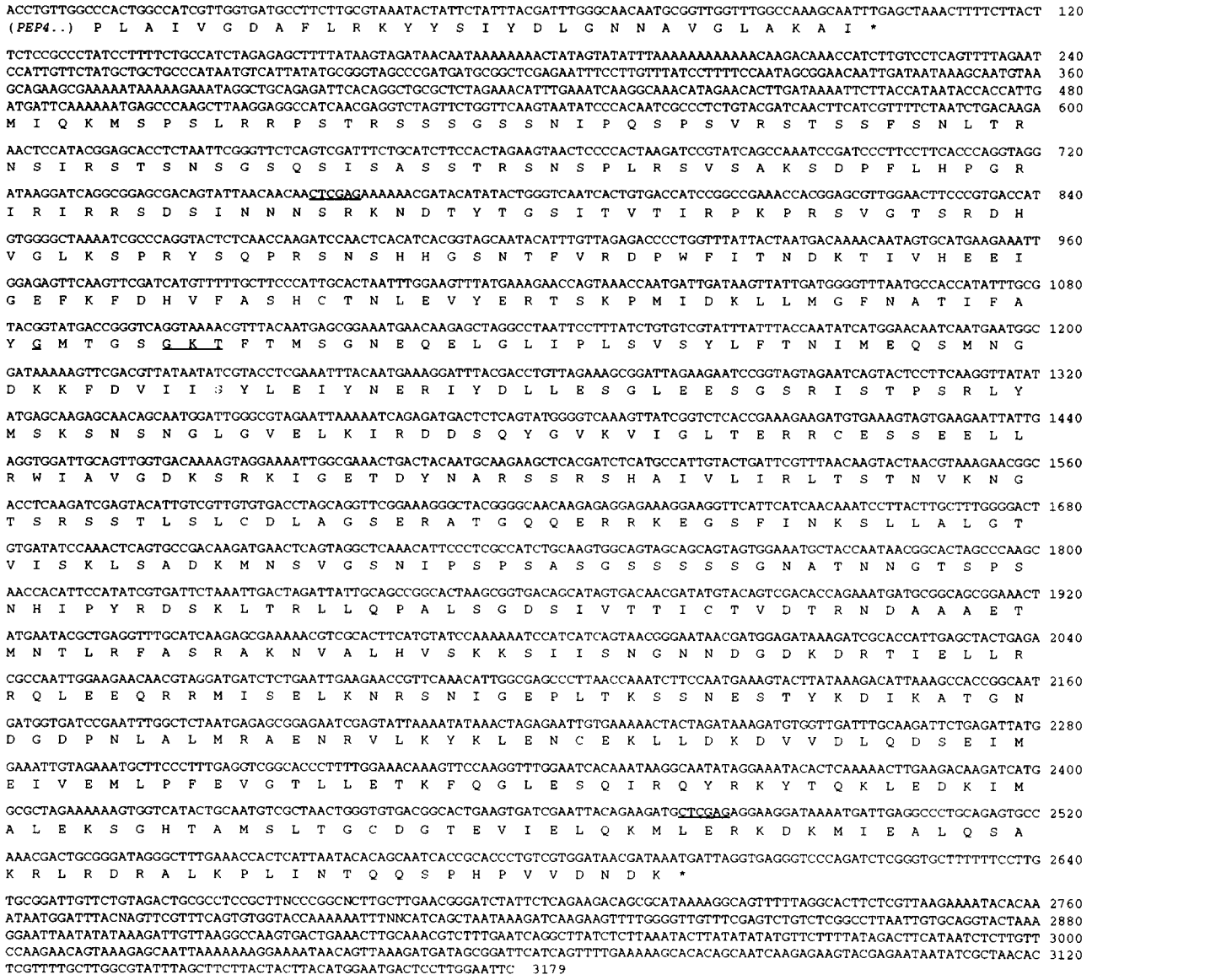


Figure 2. Nucleotide and predicted protein sequence of *KIP2*. The GX_4GKT sequence proposed to contribute to ATP binding and hydrolysis (Walker et al., 1982) and the XhoI restriction sites that form the boundary of the *kip2Δ* deletion mutations are underlined. *KIP2* encodes a predicted protein of 706 amino acids and 78 kD. The COOH-terminal predicted protein sequence of *PEP4* is shown. These sequence data are available from EMBL/GenBank/DDBJ under accession number Z11963.

tion of an alpha helical-coiled coil and suggest that KIP1 protein is dimeric.

Unlike the other superfamily members in which the motor domain is at either the NH_2 or COOH terminus, the *KIP2* kinesin-related domain is almost centrally located. A 90 amino acid NH_2-terminal extension precedes the motor domain and is serine-rich (33%). It shows no substantial homology to other proteins in the current GenBank database. The 137 amino acid *KIP2* COOH-terminal extension is predicted to be alpha helical and includes three 30–amino acid regions of heptad repeats consistent with the formation of an alpha helical-coiled coil (Fig. 3 *B*). Thus, KIP2 protein might also be dimeric.

kip1 and kip2 Deletion Mutants Are Viable

To determine the functions of *KIP1* and *KIP2* we constructed deletion mutations and examined their effects after replacement of the wild-type chromosomal allele. The *kip1* deletion mutation removes residues 106 through 1,046 and the *kip2* deletion mutation removes residues 94 throughout 667 (Figs. 1 and 2). The resultant *kip1* and *kip2* null mutants were then examined for defects in the three known microtubule-dependent functions of yeast: mitosis, meiosis, and karyogamy.

KIP1 and *KIP2* individually play no obvious role in mitotic growth, meiosis, or mating. The deletion mutations of both genes could be recovered in haploid as well as diploid yeast and mutants showed wild-type growth rates on rich medium at 16, 23, 30, and 37°C. No pronounced effect on sensitivity or resistance to the antimicrotubule drug benomyl was detected at 30°C. Furthermore, both *kip1* and *kip2* mutants were unimpaired for karyogamy and meiosis. Crosses of *kip1* X *kip1* and *kip2* X *kip2* generated diploids at the wild-type frequency when measured using a qualitative limited mating assay (Conde and Fink, 1976). Sporulation of the resulting

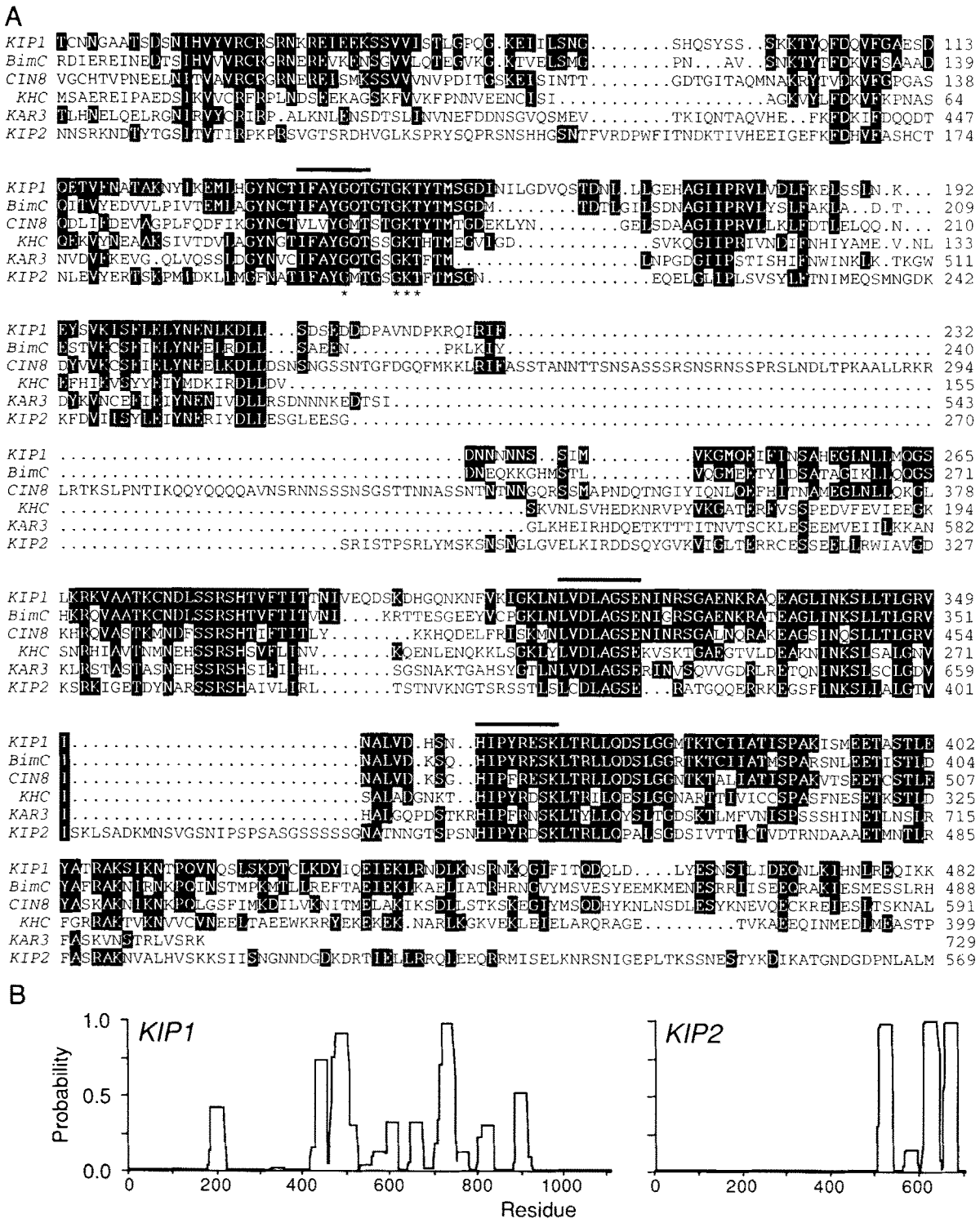


Figure 3. Sequence comparison of motor domains and probabilities of coiled-coil formation by KIP1 and KIP2. (*A*) Sequence comparison of the motor domains of *S. cerevisiae KIP1*, *KIP2*, *KAR3*, and *CIN8*, *A. nidulans bimC* and *D. melanogaster khc.* Amino acids identical to *KIP1* are indicated as white characters on black, and gaps introduced to facilitate alignment are indicated with periods. The sites of PCR primers used to identify *KIP1* and *KIP2* are marked with heavy lines (—), and the GX_4GKT sequence for ATP binding is marked with asterisks (*). The spacing of the conserved regions in the predicted *KIP2* sequence differs from other kinesin family members due to an insertion of 28 amino acids at residue 402, and *CIN8* differs due to an 84-amino acid insertion at residue 253. The sequences were aligned using the Pileup computer program (Genetics Computer, Inc.). The *bimC* sequence (amino acids 69–488) is from Enos and Morris (1990), *CIN8* (63–591) is from Hoyt et al. (1992), khc (1–399) is from Yang et al. (1989), and *KAR3* (375–729) is from Meluh and Rose (1990). (*B*) The probability that each residue of KIP1 and KIP2 is part of a coiled-coil structure was calculated using the algorithm of Lupas et al. (1991), using a window size of 28 amino acids.

homozygous null diploid strains yielded tetrads with >90% spore viability.

The lack of an obvious phenotype conferred by the *kipl* and *kip2* mutations was surprising because both *KIP1* and *KIP2* are expressed in wild-type strains during vegetative growth (data shown below). One possible explanation is that these kinesin-related proteins overlap in their essential function, so that a mutation in a single gene has little effect. Functional redundancy was tested with the *KIP1*, *KIP2*, and *KAR3* genes by constructing double and triple mutant strains. All of the double and triple mutants were viable. The *kipl kip2* double mutant showed a wild-type growth rate, while all of the mutants containing the *kar3* deletion mutation grew at a reduced rate, due to a defect in mitosis as described previously (Meluh and Rose, 1990). The *kipl* and *kip2* mutations did not exacerbate the growth defect of *kar3* strains. Several possibilities could explain the lack of an observable phenotype. One is that *KIP1*, *KIP2*, and *KAR3* are not the sole members of a functionally redundant group. Alternatively the group may not be required for an essential process.

Like the single mutants, the *kipl kip2* double mutants mated and formed diploids at wild-type frequencies when crossed either with wild-type or mutant strains. The homozygous diploids could be sporulated to yield tetrads with >90% spore viability. Thus *KIP1* and *KIP2* are not conjointly required for either karyogamy or meiosis. The various *kar3* mutants could not be tested because *KAR3* is essential for both nuclear fusion and meiosis.

ksl Mutants Make KIP1 Essential for Growth

As suggested above, the lack of a phenotype conferred by the *kipl* and *kip2* deletion mutations can be explained by proposing the existence of yet other force generating protein(s) that can substitute or compensate for the loss of *KIP1* or *KIP2*. In that case, loss of either gene individually might cause no gross defect, but simultaneous loss of both genes would be lethal (synthetic lethality). We therefore designed a genetic screen to identify mutants in which *KIP1* is essential for mitotic growth. This genetic screen could potentially identify the genes for force generating proteins which might not have been detected using our PCR primers, as well as other genes required for the activity of an additional motor protein.

The synthetic lethal mutant screen is based upon an assay in which the requirement for a given gene is assessed by determining whether a mutant strain is able to segregate a plasmid bearing that gene as its sole copy (Kranz and Holm, 1990; Bender and Pringle, 1991). Specifically, the parent strains (MS2335 and MS2336) were deleted for the chromosomal *KIP1* gene and were also *ura3*$^-$, but carried a functional *KIP1* gene on a centromere-based plasmid marked with *URA3* (pMR1895). The parent strains frequently segregate the plasmid during vegetative growth and consequently become *ura3*$^-$. Ura3$^-$ but not Ura3$^+$ strains can grow on medium containing 5FOA (Boeke et al., 1987). Colonies of the parental strains grown on rich medium contain many Ura$^-$ segregants; upon replica plating to 5FOA these clones appear to be drug resistant owing to the large number of Ura$^-$, 5FOA resistant cells. In contrast, putative synthetic lethal mutants cannot remain viable after plasmid segregation; these appear as 5FOA sensitive colonies owing to lack of viable Ura$^-$ cells.

Screening of 22,000 mutagenized cells identified 18 independent 5FOA^S mutants. These were potential *ksl* mutants (*kip* synthetic lethal). All of the mutants were found to be recessive for the *Ksl* phenotype. The mutations fell into four complementation groups; *kslI* had 14 alleles, *ksl2* had two alleles, and two mutations were unique.

To determine whether the 5FOA^S mutants now required a functional *KIP1* gene, or were 5FOA^S for reasons unrelated to *KIP1* function, we performed a secondary screen. Potential *ksl* mutants were transformed with a second *KIP1* plasmid which carried a *LEU2* selectable marker instead of *URA3* (pMR1893). In mutants which require *KIP1* for viability, the newly introduced *KIP1* gene should complement the *ksl*$^-$ *kipl*$^-$ defect, rendering the original *URA3*-marked *KIP1* plasmid dispensable. This would consequently permit accumulation of *ura3*$^-$ cells and the strains would again appear to be 5FOA^R. Representatives of each *ksl* complementation group were tested, and it was found that only the two *ksl2* alleles fulfilled the criteria of becoming 5FOA^R. The remaining mutants remained 5FOA^S and were not studied further.

Meiotic crosses were used to demonstrate the synthetic lethality between *kipl* and *ksl2* independent of the plasmid segregation test. The *kipl*Δ*1 ksl2* mutant MS2839 was crossed to the *kipl*Δ*1* strain MS2336 and sporulated. Since a *KIP1*$^+$ plasmid was present, tetrads with three and four viable spores could be recovered because the plasmid *KIP1* gene complemented the defect of *kipl*Δ*1 ksl2*$^-$ spores. In the four spore tetrads, two spores were 5FOA^S and two were 5FOA^R, indicating that the Ksl$^-$ phenotype is caused by a mutation at a single locus. The *KIP1* plasmid in the above heterozygous diploid strain could be segregated, confirming that the *ksl2* mutation is recessive. When the diploid strains without the *KIP1*$^+$ plasmid were sporulated, two spores in each tetrad were viable and two were inviable, indicating that in the absence of *KIP1* function, the *ksl2* mutation is lethal. Thus mutants singly defective in *KIP1* or *KSL2* are viable, but the double mutant is inviable.

KSL2 and Kinesin-related CIN8 Are the Same Gene

During the course of this work, the *S. cerevisiae CIN8* gene was cloned and identified as another kinesin-related gene (Hoyt et al., 1992). Certain mutations in the *CIN8* gene result in an increased rate of chromosome loss and temperature sensitive growth. *KIP1* and *CIN8* show particularly high sequence identity within their putative motor domains (Fig. 3 *A*, 56% identity), but show no substantial similarity in their COOH-terminal regions. Like *KIP1*, *CIN8* is a member of the *bimC/cut7/Eg5* subgroup, although *KIP1* is more closely related to *bimC* and *cut7* than it is to *CIN8*.

Functional redundancy between *kipl* and *cin8* was tested by crossing *kipl*Δ1::*HIS3* strain MS2333 to *cin8*Δ::*LEU2* strain MAY2058 and examining the meiotic products. The spores inferred to carry both the *kipl* and *cin8* mutations were inviable, indicating synthetic lethality. When the *KIP1 URA3* plasmid pMR1895 was present, the double mutants were viable but 5FOA^S, confirming that *KIP1* is essential in a *cin8* background.

Complementation and allelism tests were used to determine whether the *ksl2* and *cin8* mutations define the same gene. As described above, *ksl2 kipl* and *cin8 kipl* double mutants are viable when a *KIP1 URA3* plasmid is present; these stains appear as 5FOA^S colonies due to the lethality of plasmid loss. For complementation testing, these double mutants

Table I. Cell Type Distribution of Single and Double Mutants

Strain	Relevant genotype	Time at 37°C	Cell morphology (%)*					
			No bud	Small bud	Medium bud	Large bud, 2 nuclei, elongated spindle	Large bud, 1 nucleus, short spindle	Anucleate
		h						
MS10	WT	0	41	24	19	16	0	0
MS10	WT	3	46	19	20	13	1	0
MS10	WT	5	44	23	19	15	0	0
MS2333	*kip1Δ1*	0	45	27	13	15	0	0
MS2333	*kip1Δ1*	3	44	28	16	11	0	0
MS2333	*kip1Δ1*	5	49	26	14	10	0	1
MS2909	*cin8-101*	0	67	14	7	7	1	0
MS2909	*cin8-101*	3	17	14	8	9	50	3
MS2909	*cin8-101*	5	25	20	9	5	36	6
MS2911	*kip1*(ts) *cin8-101*	0	48	20	15	17	1	0
MS2911	*kip1*(ts) *cin8-101*	3	10	7	2	0	75	7
MS2911	*kip1*(ts) *cin8-101*	5	18	4	1	0	45	33

* Bud size was scored by Nomarski microscopy, nuclei were visualized by staining DNA with DAPI, and microtubule organization was examined by indirect immunofluorescent staining of tubulin. At least 150 cells were scored for each time point.

were used to make diploid strains. The *cin8Δ kip1Δ1*/p*KIP1 URA3* strain MY2875 was crossed to the *ksl2-1 kip1Δ1*/p*KIP1 URA3* and *kisl2-2 kip1Δ1*/p*KIP1 URA3* strains MS2839 and MS2868. The diploids were 5FOA^S, indicating that the plasmid-based *KIP1*$^+$ gene remained essential because *ksl2* and *cin8* did not complement. Sporulation of these diploids yielded no spores that could become 5FOA^R, indicating that the *ksl2* and *cin8* mutations are tightly linked. We have redesignated *ksl2-1* to be *cin8-101* and *ksl2-2* to be *cin8-102*.

Conditional kip1 cin8 Double Mutants Arrest before Spindle Pole Body Migration

The synthetic lethality between the *kip1Δ1* and the *cin8*$^-$ mutations indicates that either *KIP1* or *CIN8* is sufficient to perform a function essential for viability. To determine the nature of the essential function, we generated temperature-sensitive alleles of *KIP1* in the *cin8-101* background, and examined the phenotype of the double mutant at the nonpermissive temperature.

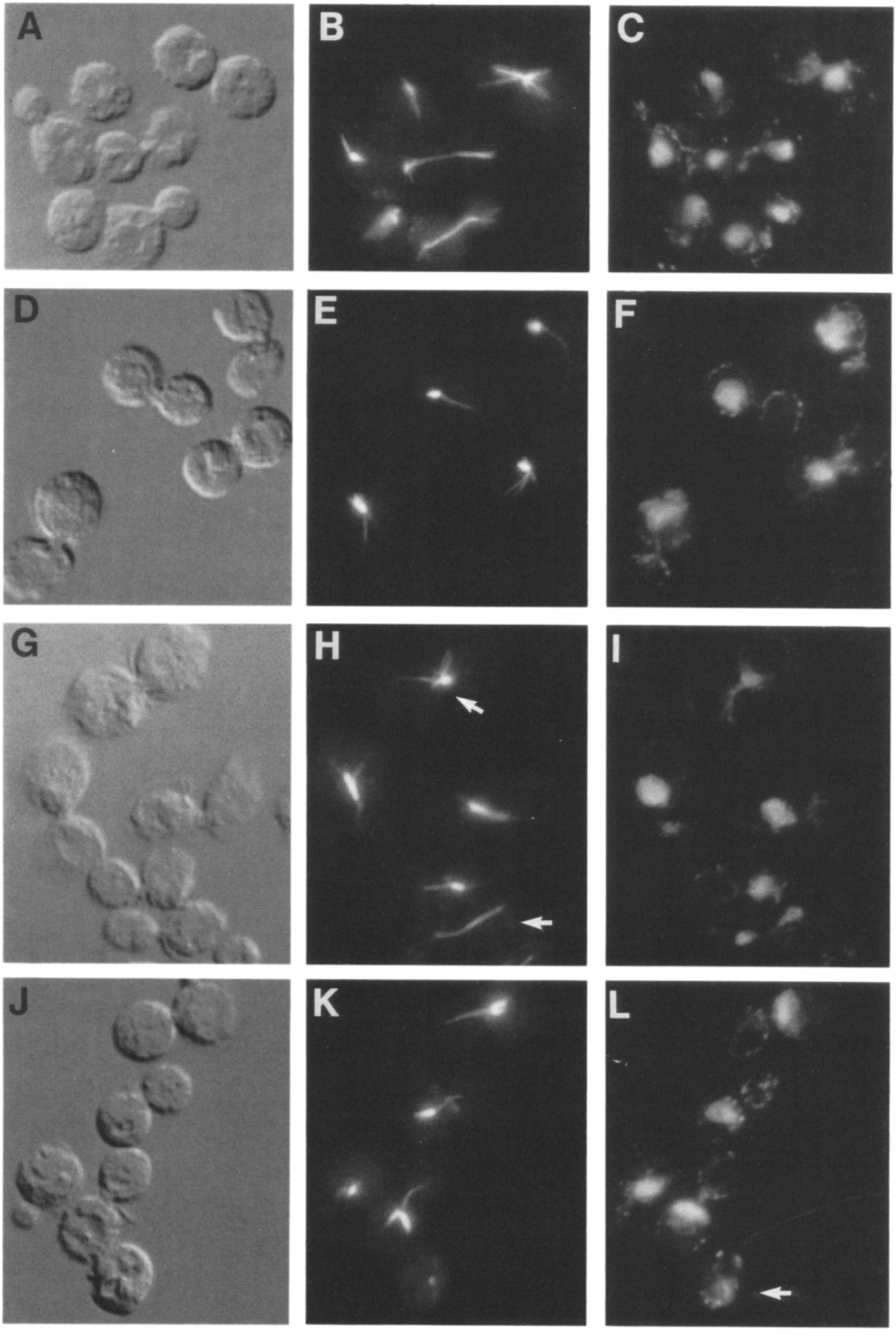
To isolate *kip1* temperature-sensitive alleles, we mutagenized the wild-type *KIP1* gene on plasmid pMR1893, and used the "plasmid shuffle" procedure (Boeke et al., 1987) to identify plasmids that were conditional for their ability to complement the *kip1Δ1 cin8-101* defect of strain MS2879. Three temperature-sensitive alleles, *kip1-101*(ts), *kip1-102*(ts), and *kip1-103*(ts) were isolated. The *kip1*(ts) genes from these plasmids were recloned in an integrating vector and used to replace the wild-type chromosomal *KIP1* gene of several yeast strains by plasmid integration and excision. The temperature-sensitive phenotype could be recovered after plasmid excision in the *cin8-101* point mutant background (MS2909) as well as in a *cin8* deletion background (MAY2059).

The gene replacement procedure allowed dominance or recessivity of the *kip1*(ts) alleles to be easily determined because integration of the *kip1*(ts) plasmids created a *kip1*(ts)/*KIP1*$^+$ merodiploid. The three merodiploids were temperature resistant in both the *cin8-101* and *cin8* deletion backgrounds, indicating that the *kip1*(ts) alleles are recessive.

The *kip1-101*(ts) *cin8-101* double mutant cells were examined by light microscopy after incubation at 37°C to determine whether the mutant arrests at a specific stage of the cell cycle. The *kip1-101*(ts) *cin8-101* strain was pregrown at 23°C, shifted to 37°C for 3–5 h, then fixed and stained. A nearly uniform arrest morphology was observed after incubation at 37°C for 3 h. About 75% of the cells in the arrested culture had a single large bud (Table I). Staining of DNA with the fluorescent dye DAPI showed that the large budded cells contained only a single nucleus often located in or near the bud neck (Fig. 4). In comparison, the nucleus in wild-type large budded cells had already divided and segregated into the mother and daughter cells (data not shown).

Microtubule organization was examined using indirect immunofluorescent staining of tubulin. The microtubules of the arrested cells appeared to emanate from a single pole and did

Figure 4. Uniform arrest morphology of *kip1* (ts) *cin8-101* double mutant. Cells were examined by phase contrast (*A, D, G* and *J*), anti-tubulin staining (*B, E, H,* and *K*), and DAPI staining to localize nuclei (*C, F, I,* and *L*). (*A–C*) *cin8-101* single mutant cells (MS2909) incubated at 37°C for 3 h. Large-budded cells with long anti-tubulin staining bars and two nuclei are present. An abnormal large-budded cell with a short anti-tubulin staining bar and a single nucleus is also present. (*D–F*) *kip1* (ts) *cin8-101* double mutant cells (MS2883) arrested at 37°C for 3 h. The large-budded cells have short anti-tubulin staining bars and a single nucleus. (*G–I*) *cin8-101* single mutant cells incubated at 37°C for 5 h. Note the large-budded cell with a short anti-tubulin staining bar and a single nucleus (*upper arrow*), and a large-budded cell with a long anti-tubulin staining bar and two nuclei (*lower arrow*). (*J–L*) *kip1* (ts) *cin8-101* double mutant arrested at 37°C for 5 h. The large-budded cells have short anti-tubulin staining bars and a single nuclei. An anucleate cell is also present (*arrow*).


A
B
C
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not span the width of the nucleus. The vertex of the microtubules at the edge of the nucleus was usually located near the bud neck, and appeared brighter and thicker than the microtubules characteristic of unbudded wild-type cells (Fig. 4). In some cells, two distinct bundles of microtubules converged at the pole, forming a "V" structure. This phenotype is suggestive of a block after spindle pole body duplication but before the formation of the bipolar mitotic spindle.

In addition to the large-budded cell type, the arrested culture contained a significant number of abnormal unbudded anucleate cells. The fraction of anucleate cells increased from 7 to 33% when incubation at 37°C was prolonged to 5 h, suggesting that the anucleate cells were derived from the large budded cells when cytokinesis continued in the absence of nuclear division. This phenotype is similar to that seen with the *cut* mutants of *S. pombe*, including mutants defective in a kinesin-related gene, *cut7*, which is required for bipolar spindle formation (Hagan and Yanagida, 1990).

We also examined the effect of a single *kipl*Δ*1* or *cin8-10l* mutation on the distribution of cell types. The *kipl*Δ*1* strain cell type distribution was similar to wild type at 23 and 37°C (Table I), suggesting that under these growth conditions *CIN8* can completely compensate for loss of *KIP1*. In contrast, the *cin8-10l* single mutant showed an abnormal cell type distribution at 37°C. After 3 h at 37°C, about 50% of the cells had a large bud and a single nucleus, and 3% of the cells were anucleate (Table I). Many of the cells with a large bud and a single nucleus contained microtubules that appeared to emanate from a single pole with a morphology similar to that of the arrested *kipl*(ts) *cin8-10l* double mutant. However, the *cin8-10l* single mutant culture also contained cells with more mature spindles than those seen in the *kipl*(ts) *cin8-10l* double mutant. The microtubules of a few of the large-budded cells with a single nucleus appeared to emanate from two poles, forming a short bright-staining bar, indicating that a bipolar spindle had formed. The *cin8-10l* culture also contained 5–9% cells with a large bud and two separate nuclei (Table I). In these cells the microtubules spanned the distance between the nuclei, a characteristic of normally fully elongated mitotic spindles. The fraction of *cin8-10l* cells in the 37°C culture with elongated spindles was similar to that seen for the wild-type strain, consistent with the wild-type growth rate exhibited by the *cin8-10l* mutant at 37°C. The presence of cells with elongated spindles suggests that the *cin8-10l* mutation causes a pause at the onset of mitosis, or that some cells bypass the abnormal state. However, the large number of abnormal cells in the *cin8-10l* culture indicates that *KIP1* does not duplicate all *CIN8* functions.

Since the *kipl*(ts) *cin8-10l* double mutants appeared to be defective in converting the short monopolar spindle into a bipolar spindle, we wanted to determine whether the *KIP1/CIN8* function is required before or after spindle pole body duplication. Spindle pole bodies were examined by EM of arrested double mutant cells. The diploid double mutant MS2923 was pregrown at 23°C, shifted to 37°C for 3 h, and the cells were prepared for EM. Several serial sections were examined for 11 large-budded cells with at least one spindle pole body. A second spindle pole body was observed in 8 cells (Fig. 5). The two spindle pole bodies were usually located immediately adjacent to each other. These results demonstrated that the spindle pole bodies could be duplicated, but that spindle pole body migration failed to occur, resulting in a monopolar spindle as the terminal structure of the double mutant at the restrictive temperature.

Both the NH_2-terminal and COOH-terminal Domains Are Required for KIP1 Activity

Since *KIP1* and *CIN8* exhibit functional redundancy in spite of the dissimilarity of their tail sequences, we investigated whether mutations in the *KIP1* tail affected *KIP1* function. The effect of mutations in the *KIP1* NH_2-terminal and COOH-terminal domains was tested by introducing in frame insertions of DNA fragments at four sites (Fig. 6 *A*) and testing the ability of the altered *KIP1* genes to complement the *kipl*Δ*1 cin8-10l* growth defect. To allow immunological identification of the proteins, we inserted DNA fragments encoding three repeats of the hemagglutinin peptide recognized by the mAb 12CA5 (Wilson et al., 1984). The insertions introduced 49–61 codons into *KIP1*, carried on plasmid pMR1893. Complementation was tested using the plasmid shuffle technique after transformation of strain MS2879. The COOH-terminal *KIP1-7*::HA insertion allele complemented the *kipl*Δ*1 cin8-10l* defect at 23, 30 and 37°C, while the NH_2-terminal *KIP1-4*::HA allele complemented only at 23 and 30°C. In contrast, *KIP1* genes bearing insertions in the motor region (*kipl-5*::HA) and near the middle of the COOH-terminal domain (*kipl-6*::HA) failed to complement at any temperature. Similar amounts of full-length KIP1 protein were detected for the functional *KIP1-4*::HA allele and the noncomplementing *kipl-5*::HA and *kipl-6*::HA alleles by Western blotting of protein from cells grown at 23°C, sug-

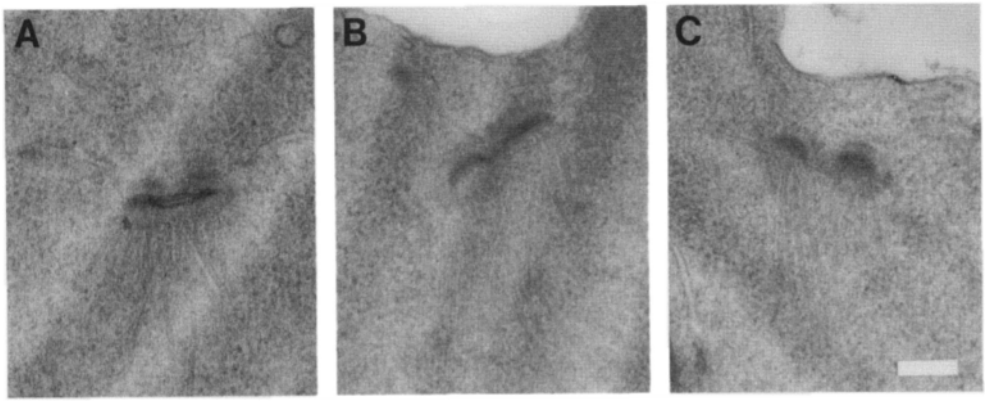

Figure 5. Spindle pole body duplication in *kipl* (ts) *cin8-10l* arrested cells. Electron micrographs (*A–C*) each show a section of different MS2923 diploid cells arrested at 37°C for 3 h. Two spindle pole bodies are visible in each cell. Bar, 200 nm.

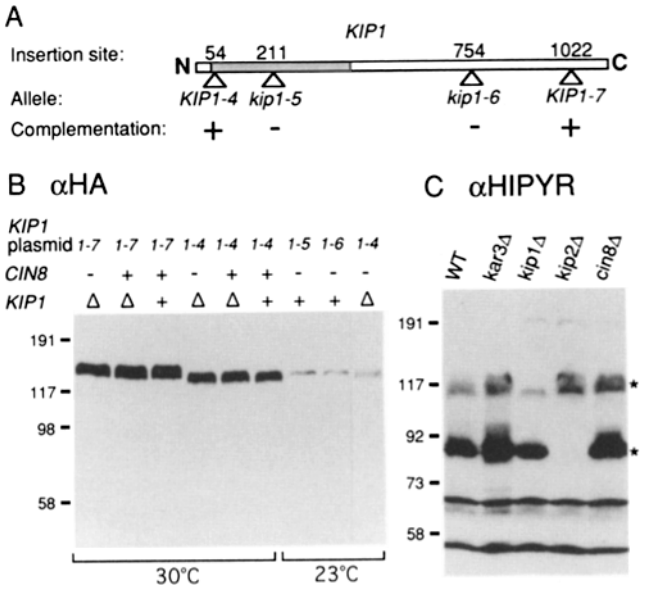

Figure 6. Expression of KIP1 and KIP2 protein. (*A*) The four sites used to insert the hemagglutinin epitope. The motor region of KIP1 is the shaded portion of the bar. The number of the KIP1 amino acid before each insertion is shown above the bar, and the corresponding allele number of each insertion is listed below the bar. The ability of each allele to complement the *kip1Δ1 cin8-10l* defect of strain MS2879 at 30°C is indicated. (*B*) Western blot of epitope tagged KIP1 protein from *CIN8*$^+$ and *cin8-10l* strains. The HA epitope-tagged *KIP1* genes were present on a single copy plasmid, indicated by allele number. (Δ) The chromosomal *kip1* deletion; (−) *cin8-10l* point mutation; (+) and wild-type genes. Identical amounts of KIP1 protein was detected in wild-type, *kip1Δ1*, and *kip1Δ1 cin8-10l* strains, using the *KIP1-7*::HA epitope insertion (first three lanes) and using the *KIP1-4*::HA epitope insertion (4th to 6th lanes). The amount of *KIP1-4*::HA detected from cells grown at 30°C (fifth lane) was greater than the amount detected in cells grown at 23°C (ninth lane), indicating KIP1 levels are temperature-dependent. Both the functional *KIP1-4*::HA epitope insertion strain (ninth lane) and the nonfunctional *kip1-5*::HA and *kip1-6*::HA epitope insertion strains (seventh and eighth lanes) had similar levels of detected KIP1 protein at 23°C. The strains used (from left to right) were MS2936, MS2935, MS2934, MS2930, MS2929, MS2928, MS2924, MS2927, and MS2930. (*C*) Detection of proteins containing a conserved motor domain sequence. Protein was detected using anti-HIPYRESKLT antibody (Sawin et al., 1992) by Western blotting after gel electrophoresis of total yeast protein. The protein inferred to be KIP1 (because it is absent in the *kip1Δ1* strain) is marked with the upper asterisk and the protein inferred to be KIP2 (because it is absent in the *kip2Δ* strain) is marked with the lower asterisk. The strains used were MS10, MS524, MS2333, MS2309, and MAY2059, all grown at 23°C.

gesting the *kip1-5*::HA and *kip1-6*::HA proteins are defective in function rather than expression (Fig. 6 *B*).

KIP1 Expression Is Identical in CIN8$^+$ *and cin8*$^-$ *Strains*

To test whether *KIP1* is induced in response to the defect caused by the *cin8-10l* mutation, we used the hemagglutinin epitope tagged *KIP1* genes described above to measure the level of KIP1 protein in wild-type, *kip1Δ1*, and *kip1Δ1 cin8-10l* strains. The *KIP1* protein level was determined by Western blotting after gel electrophoresis of total protein from yeast harboring the tagged plasmids (Fig. 6 *B*). All of the epitope-tagged *KIP1* plasmid strains contained a single reacting protein species. The *KIP1-4*::HA fusion protein had was ~134 kD, which is consistent with the predicted fusion protein molecular mass of 131 kD. The level of *KIP1* expression was identical in each genetic background (Fig. 6 *B*), indicating that *KIP1* is not induced in response to a defect caused by the *cin8-10l* mutation.

We used an antibody raised against the conserved motor region peptide HIPYRESKLT to visualize potential kinesin-related proteins (Sawin et al., 1992). At least six proteins were detected in a wild-type strain by Western blotting (Fig. 6 *C*). To investigate whether the detected proteins correlate to known genes, total protein was prepared from yeast stains deleted for single kinesin-related genes. A protein of ~121 kD was present in all strains except the *kip1* deletion strain, indicating that this protein is the likely product of *KIP1*. As was the case with epitope-tagged KIP1, the level of KIP1 protein detected with the HIPYR antibody was similar in *CIN8*$^+$ and *cin8* deletion strains, confirming that *KIP1* expression is unaltered by a *cin8* defect.

KIP2 protein was also detected using the HIPYRESKLT antibody. An 83-kD protein was present in all strains except the *kip2* deletion strain, indicating that *KIP2* is expressed during normal growth. No proteins could be positively assigned to the *KAR3* and *CIN8* genes, as all proteins detected in the wild-type strain were also present in the *kar3* and *cin8* deletion strains. The HIPYRESKLT antibody may not recognize these proteins since they are not conserved at the site used to raise the antibody. KAR3 differs in two residues (HIPFRNSKLT) and CIN8 differs in one residue (HIPFRESKLT). The remaining four proteins were ~54, 66, 112, and 190 kD. All four were detected in *kip1*, *kip2*, *kar3*, and *cin8* deletion strains, suggesting that additional *S. cerevisiae* kinesin-related proteins exist.

KIP1 Localizes to Mitotic Spindles

The cellular location of KIP1 was examined using indirect immunofluorescent staining of yeast strains carrying the *KIP1-4*::HA or the *KIP1-7*::HA epitope-tagged gene. The epitope-tagged gene was carried on a single copy *CEN* plasmid and was expressed from its own promoter. In these experiments, the epitope tagged gene was the sole source of KIP1 in both a *CIN8*$^+$ and a *cin8-10l* background. Medium and large-budded cells frequently showed staining of short bars, which coincided with anti-tubulin staining of short mitotic spindles (Fig. 7). In addition, KIP1 staining in unbudded and small-budded cells was often detected as a dot located at, or near the spindle pole body. Overall, ~25% of the cells showed staining using either the *KIP1-4*::HA or the *KIP1-7*::HA epitope-tagged genes. The observed variation in staining intensity may reflect the potential low abundance of KIP1 protein and the sensitivity of the HA epitope to fixation. The same localization pattern was observed in the *CIN8*$^+$ and *cin8-10l* genetic backgrounds. These data suggest that KIP1 is a normal participant in spindle pole body migration, and not simply an auxiliary motor induced or relocalized in response to a *cin8*$^-$ defect.

Discussion

Our results demonstrate the existence of multiple members

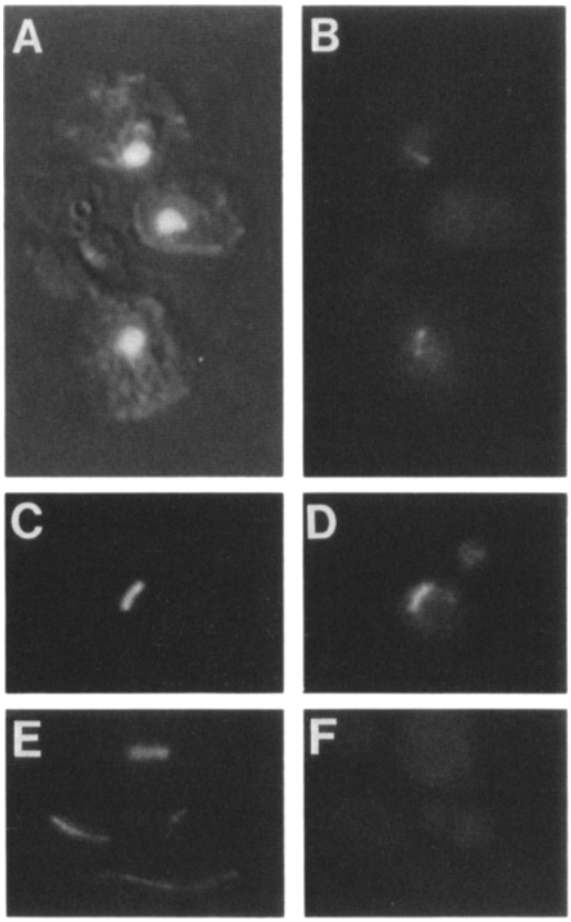

Figure 7. Immunofluorescent localization of epitope-tagged KIP1 protein. (*A*) DAPI staining of DNA to localize nuclei. (*B*) The antibody specific to epitope-tagged KIP1 stains a short bar within the nuclei of budded cells. The cells in *A* and *B* were not stained with the anti-tubulin antibody. (*C* and *D*) Colocalization of KIP1 and tubulin. (*C*) Anti-tubulin staining and (*D*) staining of the same structure with the 12CA5 antibody specific to epitope-tagged KIP1. The *KIP1-4*::HA epitope insertion strain MS2929 was grown at 23°C. Control strain MS2933, which does not carry an epitope-tagged *KIP1* gene, was grown at 23°C and cells were stained as in (*C* and *D*) with anti-tubulin, 12CA5 and both secondary antibodies. The rhodamine fluorescence derived from anti-tubulin staining (*E*) is not detected in the fluorescein isothiocyanate channel (*F*), nor is a signal generated in *F* by cross-reaction of the goat anti-mouse fluorescein isothiocyanate-conjugated secondary antibody with the rabbit anti-tubulin primary antibody.

of the kinesin superfamily in yeast. Two of the kinesin-related genes, *KIP1* and *CIN8*, were shown by mutant analysis to perform an essential role in mitosis. The essential role was evident because mutants defective in both *KIP1* and *CIN8* are inviable. However, mutants singly defective in *KIP1* or *CIN8* are viable, indicating that either wild-type gene can provide the essential function missing in the double mutant. Therefore, *KIP1* and *CIN8* are functionally redundant. Functional redundancy between *KIP1* and *CIN8* is supported by the finding that one or two extra copies of the *KIP1* gene on a plasmid can suppress the temperature sensitivity caused by certain *cin8* point mutations (Hoyt et al., 1992).

The essential function of *KIP1* and *CIN8* was examined using a double mutant containing a *kip1* temperature sensitive allele and a *cin8* point mutation allele. The double mutant showed a nearly uniform cell division cycle arrest morphology at the nonpermissive temperature. Light microscopy showed that the arrested cells have a single large bud, a single nucleus and a short monopolar spindle. EM revealed that most large-budded cells have duplicated spindle pole bodies. The arrest morphology suggests that the KIP1 and CIN8 proteins mediate spindle pole body separation and migration to form a bipolar spindle. Consistent with this role, immunolocalization of KIP1 protein showed that it is a component of short mitotic spindles. The mitotic spindle localization of KIP1 is similar to the mitotic spindle localization of *S. pombe* cut7 protein reported recently (Hagan and Yanagida, 1992), although we rarely detected KIP1 protein on elongated spindles.

Particularly high sequence similarity within the putative motor domains defined a subgroup of kinesin-related proteins. Whereas most kinesin related proteins contain between 30 and 45% identity within the motor region, the motor regions of *KIP1* and *A. nidulans bimC* showed 65% identity, *KIP1* and *S. pombe cut7* showed 54% identity, and *KIP1* and *Xenopus Eg5* showed 52% identity. *CIN8* is also a member of the subgroup; it shows 56% identity with *KIP1*. Presumably, the high sequence similarity within the *bimC/cut7* subgroup reflects a common cellular function for these proteins. Consistent with this suggestion, the *bimC* and *cut7* gene products appear to play similar roles early in mitosis, as mutations in either gene can block separation of the newly duplicated spindle pole bodies (Enos and Morris, 1990; Hagan and Yanagida, 1990).

Redundant Kinesin-related Proteins

Several possible mechanisms could explain how either one of two different motors is sufficient to perform an essential role. One possibility is that only one of the two motors is normally used. A defect in the primary motor might lead to induction or relocalization of a second motor so that it could perform as a substitute. The second "auxiliary" motor could be induced by a mitotic checkpoint arrest mechanism that monitors the progress of mitotic spindle formation similar to that previously described (Hoyt et al., 1991; Li and Murray, 1991). A second possible explanation is that both motors normally act in tandem to power a single movement, but either motor acting alone is sufficient. Finally, it is possible that successful completion of one movement can compensate for the loss of a second movement. For example, incomplete spindle elongation (anaphase B) might be not be lethal when anaphase A proceeds normally, but would be lethal when anaphase A is also defective. The combinatorial use of mitotic motors is probably necessary to achieve high fidelity chromosome transmission.

Contrary to the inducible auxiliary motor hypothesis, *KIP1* is expressed at the same level and shows apparently identical localization in both *cin8*$^-$ and *CIN8*$^+$ strains. The converse of the induction hypothesis is that *CIN8* would be induced in response to a *KIP1* defect. However, *cin8*$^-$ single mutants have an altered cell type distribution at 37°C, with many cells that are arrested or delayed at an early stage of mitosis. These results suggest that *CIN8* is expressed and plays an important role in *KIP1*$^+$ strains. Therefore, *CIN8* does not serve solely as an auxiliary motor that is induced in response to a *kip1* defect. We expect that insights into the

Table II. S. cerevisiae Strains Used

Strain	Genotype*
MS10	a *ade2-101*
MS524	a *ade2-101 kar3-101::LEU2*
MS2305	α *trp1Δ1 kip1-2::URA3*
MS2309	a *ade2-101 his3Δ200 kip2Δ1::URA3*
MS2333	a *ade2-101 his3Δ200 kip1Δ1::HIS3*
MS2334	α *trp1Δ1 his3Δ200 kip1Δ1::HIS3*
MS2335	a *ade2-101 his3Δ200 kip1Δ1::HIS3* pMR1895 (*CEN URA3 KIP1*)
MS2336	α *trp1Δ1 his3Δ200 kip1Δ1::HIS3* pMR1895 (*CEN URA3 KIP1*)
MS2354	α *trp1Δ1 kip2Δ2::TRP1*
MS2839	a *ade2-101 his3Δ200 kip1Δ1::HIS3 cin8-101* pMR1895 (*CEN URA3 KIP1*)
MS2868	a *ade2-101 his3Δ200 kip1Δ1::HIS3 cin8-102* pMR1895 (*CEN URA3 KIP1*)
MS2879	a *trp1Δ1 his3Δ200 kip1Δ1::HIS3 cin8-101* pMR1895 (*CEN URA3 KIP1*)
MS2883	a *trp1Δ1 his3Δ200 kip1Δ1::HIS3 cin8-101* pMR2371 (*CEN LEU2 kip1-101*(ts))
MS2909	α *trp1Δ1 his3Δ200 cin8-101*
MS2911	α *trp1Δ1 his3Δ200 kip1-101*(ts) *cin8-101*
MS2923	a/α *his3Δ200/his3Δ200 +/trp1Δ1 kip1Δ1::HIS3/kip1-101* (ts) *cin8-101/cin8-101*
MS2924	a *trp1Δ1 his3Δ200 kip1Δ1::HIS3 cin8-101* pMR1895 (*CEN URA3 KIP1*) pMR2365 (*CEN LEU2 kip1-5*::HA)
MS2927	a *trp1Δ1 his3Δ200 kip1Δ1::HIS3 cin8-101* pMR1895 (*CEN URA3 KIP1*) pMR2368 (*CEN LEU2 kip1-6*::HA)
MS2928	a *ade2-101* pMR2369 (*CEN LEU2 KIP1-4*::HA)
MS2929	a *ade2-101 his3Δ200 kip1Δ1::HIS3* pMR2369 (*CEN LEU2 KIP1-4*::HA)
MS2930	a *trp1Δ1 his3Δ200 kip1Δ1::HIS3 cin8-101* pMR2369 (*CEN LEU2 KIP1-4*::HA)
MS2933	a *trp1Δ1 his3Δ200 kip1Δ1::HIS3 cin8-101* pMR1893 (*CEN LEU2 KIP1*)
MS2934	a *ade2-101* pMR2370 (*CEN LEU2 KIP1-7*::HA)
MS2935	a *ade2-101 his3Δ200 kip1Δ1::HIS3* pMR2370 (*CEN LEU2 KIP1-7*::HA)
MS2936	a *trp1Δ1 his3Δ200 kip1Δ1::HIS3 cin8-101* pMR2370 (*CEN LEU2 KIP1-7*::HA)
MY2875‡	α *his3Δ200 lys2-101 kip1Δ1::HIS3 cin8Δ::LEU2* pMR1895 (*CEN URA3 KIP1*)
MAY2058	α *his3Δ200 lys2-101 cin8Δ*::LEU2
MAY2059	a *his3Δ200 ade2-101 cin8Δ*::LEU2
L785	a *ils1-1 ura3-52 ade*

* All strains are derivatives of strain S288C, and all contain the *ura3-52* and *leu2-3,112* mutations. Strain MS10 is from the lab collection. MS524 is from Meluh and Rose (1990), MAY2058 and MAY2059 were obtained from M. A. Hoyt (Johns Hopkins University, Baltimore, MD), and L785 was obtained from G. Fink (Whitehead Institute, Cambridge, MA). All other strains were constructed for this study. Yeast genes carried on plasmids are indicated in parentheses after the plasmid name. *KIP1*::HA designates an in frame insertion that encodes three copies of the HA epitope.
‡ Derived from MAY2058.

mechanism of redundancy will be gained by high resolution localization studies of KIP1 and CIN8. Of course either KIP1 or CIN8 could be an auxiliary motor that is regulated at the level of activation rather than expression or localization.

An important issue is what makes *CIN8* and *KIP1* partially interchangeable. Presumably there is a feature common to both KIP1 and CIN8 that allows this pair of proteins to be interchanged. Since the kinesin-related proteins all possess similar motor domain sequences, the unique tail sequences are a likely site to define specificity of function. Indeed, we showed that the *KIP1* COOH-terminal tail domain is required for *KIP1* activity. In this view, the tail sequences of *KIP1* and *CIN8* would share a common feature that allows functional substitution. However, we find little or no sequence similarity between the COOH-terminal tail domains of *KIP1* and *CIN8*. Alternatively, functional specificity could be defined by the motor regions. In this regard, it will be of interest to determine whether the other kinesin-related proteins that show particularly high sequence conservation in the motor domains with KIP1 and CIN8 are capable of complementing the *kip1 cin8* double mutant defect.

The mitotic defect of mutants defective in *CIN8* alone indicates that the KIP1 and CIN8 proteins are not completely interchangeable. No defect was detected in the *kip1* single mutant, indicating that CIN8 can completely substitute for KIP1. However, both KIP1 and CIN8 could perform additional roles which are not redundant with each other, but that do overlap with other force generating proteins. If such additional force generating proteins exist, the genes could potentially be identified using the *ksl* mutant screen. Since the *ksl* mutant screen for synthetic lethal mutants with *KIP1* has yielded only two *cin8* alleles, the screen is not exhausted and could potentially identify other force generating proteins that overlap with a different *KIP1* function.

Functional overlap among force-generating proteins may be a general phenomenon. The multiple movements of mitosis may be powered by force generating proteins that have various degrees of functional overlap with each other. A second *S. cerevisiae* kinesin-related protein that is likely to have partial functional overlap with other force-generating proteins of mitosis is KAR3. The morphology of *kar3* mutants indicates that this protein participates in anaphase spindle elongation (Meluh and Rose, 1990). The viability of *kar3* null mutants suggests a second protein can partially fulfill the mitotic function of *KAR3*. A third potential example of motor redundancy is *KIP2*. The *KIP2* protein is expressed, but no defect in *kip2* null mutants could be detected, raising the possibility that another force-generating protein overlaps *KIP2* function. Recently, synthetic lethal mutants for *KIP2* have been isolated (D. Roof, D. Loayza, and M. Rose, unpublished results). In addition to genetic arguments for the existence of additional force-generating proteins, the spectrum

of yeast proteins recognized by the anti-kinesin peptide antibody suggests that at least four additional kinesin-related proteins exist in *S. cerevisiae.*

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