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 the spindle pole bodies necessary to form a bipolar mitotic spindle. Mutations in the kinesin-related genes, 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.

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

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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 kch 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 kch. Primer 1 encodes the peptide IFAYGQ7 (5'gggattc-AT(ACCTT)TTCGCACGT/GACGT/CAGTAC/A/G'AC3') and is 384-fold degenerate. Primer 2 encodes the peptide LVDLASGEE (5'gggattc-(CT)TGCACGT/GACGT/CAGTAC/A/G'AC3') and is 130,000-fold degenerate. Primer 3 is the antisense strand corresponding to the peptide LVDLASGEE (5'gggattc-(CT)TGCACGT/GACGT/CAGTAC/A/G'AC3') and is 384-fold degenerate. Primer 4 is the antisense strand corresponding to the peptide H15P/F1RD/ER/N3SK (5'gggattc-(CT)TGCACGT/GACGT/CAGTAC/A/G'AC3') and is 130,000-fold degenerate. Primer 5 is the antisense strand corresponding to amino acids 693 to 1,046, replaced by putative Rho1 domain. Primer 6 is the antisense strand corresponding to amino acids 1,047 to 1,992, replaced by the TRP1 fragment.

#### Construction of KIP1 and KIP2 Deletion Strains

The null allele kip1Δ::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 kip2Δ::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 resulting from cutting only at XhoI sites within KIP2 was gel purified, and a fragment containing TRP1 was inserted to create pMR1791. The kip2Δ::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 ild 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 ild was confirmed using meiotic crosses. The kip1::URA3 strain MS3205 was crossed to the ild1 strain IL78S and sporulated. 42 tetrads were parental ditype and one was tetratype, indicating that KIP1 is 1 cM from ild (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. Restriction mapping 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 RAPI/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 YMI 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 MS8353 and MS8336 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-fluorocotic acid (5FOA) (Boeke et al., 1987) medium (all at 30°C) to identify 5FOA sensitive derivatives. Temperature sensitive kip1 mutations were identified using the plasmid shuffle technique (Boeke et al., 1987). For this purpose, plasmid DNA was mutagenized with hydroxyamine as described in Rose and Fink (1987) and used to directy transform yeast. Putative kip1 (ts) plasmids were recovered from yeast by transformation into E. coli, and the BamHI XbaI fragment containing kip1 was cloned into the URA3-marked Ypl 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 kip1(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 domains. 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/hydrolisis 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 NH2-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 GXGKT 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 *KiplI*. The GXGKT sequence proposed to contribute to ATP binding and hydrolysis (Walker et al., 1992) and the BglII restriction sites that form the boundary of the *kipI* deletion mutation are underlined. The predicted protein of 1,111 amino acids and 126 kD. The sequence data are available from EMBL/GenBank/DDBJ under accession number Z11962.
Figure 2. Nucleotide and predicted protein sequence of KIP2. The GXGKT 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/DBJ under accession number Z11963.
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 \textit{S. cerevisiae} KIP1, KIP2, KAR3, and \textit{CIN8}, \textit{A. nidulans} \textit{bimC} and \textit{D. melanogaster} \textit{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 (D), and the \textit{GX4GKT} sequence for ATP binding is marked with asterisks (*). The spacing of the conserved regions in the predicted KIP2 sequence differs from other \textit{ldnesin} family members due to an insertion of 28 amino acids at residue 402, and \textit{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 \textit{bimC} sequence (amino acids 69-488) is from Enos and Morris (1990), \textit{CIN8} (63-591) is from Hoyt et al. (1992), \textit{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.
homzygous 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 homzygous 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-. Ura+ 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 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; ksl1 had 14 alleles, ksl2 had two alleles, and two mutations were unique.

To determine whether the 5FOA mutants now required a functional KIP1 gene, or were 5FOA 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- kip- 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. Representatives of each ksl complementation group were tested, and it was found that only the two ksl2 alleles fulfilled the criteria of becoming 5FOA. The remaining mutants remained 5FOA 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 kiplΔ ksl2 mutant MS2839 was crossed to the kiplΔ 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 kiplΔ ksl2- spores. In the four spore tetrads, two spores were 5FOA and two were 5FOA, 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 kiplΔ::HIS3 strain MS2333 to cinΔ::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, 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 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 | No bud | Small bud | Medium bud | Large bud, 2 nuclei, elongated spindle | Large bud, 1 nucleus, short spindle | Anucleate |
|--------|-------------------|---------------|--------|-----------|------------|--------------------------------------|------------------------------------|-----------|
| MS10   | WT                | 3              | 46     | 19        | 16         | 0                                    | 1                                  | 0         |
| MS10   | WT                | 5              | 44     | 23        | 19         | 15                                  | 0                                  | 0         |
| MS2333 | kipIΔ1            | 0              | 45     | 27        | 13         | 15                                  | 0                                  | 0         |
| MS2333 | kipIΔ1            | 3              | 44     | 28        | 16         | 11                                  | 0                                  | 0         |
| MS2333 | kipIΔ1            | 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 | kipI(ts) cin8-101 | 0              | 48     | 20        | 15         | 17                                  | 1                                  | 0         |
| MS2911 | kipI(ts) cin8-101 | 3              | 10     | 7         | 2          | 0                                    | 75                                 | 7         |
| MS2911 | kipI(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 kipIΔ1/pKIP1 URA3 strain MY2875 was crossed to the ksl2-1 kipIΔ1/pKIP1 URA3 and ksl2-2 kipIΔ1/pKIP1 URA3 strains MS2839 and MS2868. The diploids were 5FOAΔ, indicating that the plasmid-based KIP1Δ1 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 ksl2-1 and cin8 mutations are tightly linked. We have redesignated ksl2-1 to be cin8-101 and ksl2-2 to be cin8-102.

Conditional kipI cin8 Mutants Arrest before Spindle Pole Body Migration

The synthetic lethality between the kipIΔ1 and the cin8Δ 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 kipI 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 kipIΔ1 cin8-101 defect of strain MS2879. Three temperature-sensitive alleles, kipI-101(ts), kipI-102(ts), and kipI-103(ts) were isolated. The kipI(ts) genes from these plasmids were recloned in an integrating vector and used to replace the wild-type chromosomai 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 kipI(ts) alleles to be easily determined because integration of the kipI(ts) plasmids created a kipI(ts)/KIP1Δ1 merodiploid. The three merodiploids were temperature resistant in both the cin8-101 and cin8 deletion backgrounds, indicating that the kipI(ts) alleles are recessive.

The kipI-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 kipI-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 kipI(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) kipI(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) kipI(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 nucleus. An anucleate cell is also present (arrow).
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 kiplΔ1 or cin8-101 mutation on the distribution of cell types. The kiplΔ1 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 cin8-101 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) cin8-101 double mutant. However, the cin8-101 single mutant culture also contained cells with more mature spindles than those seen in the kipl(ts) cin8-101 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 cin8-101 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 cin8-101 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 cin8-101 mutant at 37°C. The presence of cells with elongated spindles suggests that the cin8-101 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 cin8-101 culture indicates that KIP1 does not duplicate all CIN8 functions.

Since the kipl(ts) cin8-101 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 NH2-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 NH2-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 kiplΔ1 cin8-101 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 kiplΔ1 cin8-101 defect at 23, 30 and 37°C, while the NH2-terminal KIP1-4::HA allele complemented only at 23 and 30°C. In contrast, KIP1 genes bearing insertions in the motor region (kip1-5::HA) and near the middle of the COOH-terminal domain (kip1-6::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 kip1-5::HA and kip1-6::HA alleles by Western blotting of protein from cells grown at 23°C, sug-
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 kipl-Δ cin8-10I defect is indicated. (B) Western blot of epitope tagged KIP1 protein from Cin8+ and cin8-10I strains. The HA epitope-tagged KIP1 genes were present on a single copy plasmid, indicated by allele number. (A) The chromosomal kipl deletion; (-) cin8-10I point mutation; (+) and wild-type genes. Identical amounts of KIP1 protein were detected in wild-type, KIP1-4:HA and cin8-10I strains. The amount of KIP1-4:HA detected from cells grown at 30°C (fifth lane) is 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 kipl-5::HA and kipl-6::HA epitope insertion strains (seventh and eighth lanes) had similar levels of KIP1 protein at 23°C. The strains used 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-HIPYRESKL antibody (Sawin et al., 1992) by Western blotting after gel electrophoresis of total yeast protein. The protein inferred to be KIP1 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. All strains grown at 23°C.

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 kipl-Δ cin8-10I defect is indicated. (B) Western blot of epitope tagged KIP1 protein from Cin8+ and cin8-10I strains. The HA epitope-tagged KIP1 genes were present on a single copy plasmid, indicated by allele number. (A) The chromosomal kipl deletion; (-) cin8-10I point mutation; (+) and wild-type genes. Identical amounts of KIP1 protein were detected in wild-type, KIP1-4: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 kipl-5::HA and kipl-6::HA epitope insertion strains (seventh and eighth lanes) had similar levels of detected KIP1 protein at 23°C. The strains used 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-HIPYRESKL 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 kipl-Δ 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. All strains grown at 23°C.

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KIPI Expression Is Identical in CIN8+ and cin8- Strains

To test whether KIP1 is induced in response to the defect caused by the cin8-10I mutation, we used the hemagglutinin epitope tagged KIP1 genes described above to measure the level of KIP1 protein in wild-type, kipl-Δ1, and kipl-Δ1 cin8-10I 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 a Mr of 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 cin8-10I mutation.

We used an antibody raised against the conserved motor region peptide HIPYRESKL 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 strains deleted for single kinesin-related genes. A protein of ~121 kD was present in all strains except the kipl 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 HIPYRESKL 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 HIPYRESKL 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 (HIPFRENSKLT). The remaining four proteins were ~54, 66, 112, and 190 kD. All four were detected in kipl, kip2, kar3, and cin8 deletion strains, suggesting that additional Schizosaccharomyces 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 cin8-10I 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 cin8-10I 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
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 \( kipl \) temperature sensitiv-
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 find little or no sequence similarity between the COOH-terminal tail domains of KIP1 and CIN8.

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

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**Table II. S. cerevisiae Strains Used**

| Strain | Genotype* |
|--------|-----------|
| MS10   | a ade2-101 |
| MS524  | a ade2-101 kar3-101::LEU2 |
| MS2305 | α trpl Δ1 kip1-2::URA3 |
| MS2309 | a ade2-101 hisΔ200 kipΔ1::URA3 |
| MS2333 | a ade2-101 hisΔ200 kipΔ1::HIS3 |
| MS2334 | α trplΔ1 hisΔ200 kipΔ1::HIS3 |
| MS2335 | a ade2-101 hisΔ200 kipΔ1::HIS3 pMR1895 (CEN URA3 KIP1) |
| MS2336 | α trplΔ1 hisΔ200 kipΔ1::HIS3 pMR1895 (CEN URA3 KIP1) |
| MS2354 | α trplΔ1 hisΔ200 kipΔ2::TRP1 |
| MS2839 | a ade2-101 hisΔ200 kipΔ1::HIS3 cin8-101 pMR1895 (CEN URA3 KIP1) |
| MS2868 | a ade2-101 hisΔ200 kipΔ1::HIS3 cin8-102 pMR1895 (CEN URA3 KIP1) |
| MS2879 | a trplΔ1 hisΔ200 kipΔ1::HIS3 cin8-101 pMR1895 (CEN URA3 KIP1) |
| MS2883 | a trplΔ1 hisΔ200 kipΔ1::HIS3 cin8-101 pMR2371 (CEN LEU2 kip1-101(ts)) |
| MS2909 | a trplΔ1 hisΔ200 cin8-101 |
| MS2911 | a trplΔ1 hisΔ200 kip1-101(ts) cin8-101 |
| MS2923 | a hisΔ200/hisΔ200 + trplΔ1 kip1Δ1::HIS3 kip1-101 (ts) cin8-101/cin8-101 |
| MS2924 | a trplΔ1 hisΔ200 kipΔ1::HIS3 cin8-101 pMR1895 (CEN URA3 KIP1) pMR2365 (CEN LEU2 kip1-5::HA) |
| MS2927 | a trplΔ1 hisΔ200 kipΔ1::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 hisΔ200 kipΔ1::HIS3 pMR2369 (CEN LEU2 KIP1-4::HA) |
| MS2930 | a trplΔ1 hisΔ200 kipΔ1::HIS3 cin8-101 pMR2369 (CEN LEU2 KIP1-4::HA) |
| MS2933 | a trplΔ1 hisΔ200 kipΔ1::HIS3 cin8-101 pMR1893 (CEN LEU2 KIP1) |
| MS2934 | a ade2-101 pMR2370 (CEN LEU2 KIP1-7::HA) |
| MS2935 | a ade2-101 hisΔ200 kipΔ1::HIS3 pMR2370 (CEN LEU2 KIP1-7::HA) |
| MS2936 | a trplΔ1 hisΔ200 kipΔ1::HIS3 cin8-101 pMR2370 (CEN LEU2 KIP1-7::HA) |
| MS2945 | α hisΔ200 lys2-101 kipΔ1::HIS3 cinΔΔ::LEU2 pMR1895 (CEN URA3 KIP1) |
| MS2956 | α hisΔ200 lys2-101 cinΔΔ::LEU2 |
| L785  | a hisΔ200 ade2-101 cinΔΔ::LEU2 |

* 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 strain name. KIP1::HA designates an in-frame insertion that encodes three copies of the HA epitope.

† Derived from MAY2058.
of yeasts 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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Conde, I., and G. R. Fink. 1976. A mutant of *Saccharomyces cerevisiae* defective for nuclear fusion. *Proc. Natl. Acad. Sci. USA*. 73:3651-3655.

Davis, D. G. 1969. Chromosome behavior under the influence of claret-nondisjunctional in *Drosophila melanogaster*. Genetics. 61:577-594.

Davis, R. W., D. Botstein, and J. R. Roth. 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 251 pp.

Endow, S. A., and M. Hasegawa. 1991. A multimeric kinesin gene family in *Drosophila*. *Proc. Natl. Acad. Sci. USA*. 88:4442-447.

Enos, A. P., and N. R. Morris. 1990. Mutation of a gene that encodes a kinesin-like protein blocks nuclear division in *A. nidulans*. *Cell*. 60:1019-1027.

Goldstein, L. S. B. 1991. The kinesin superfamily: tails of functional redundancy. *Trends Cell Biol*. 1:93-98.

Hagan, I., and M. Yanagida. 1990. Novel potential mitotic motor protein encoded by the fission yeast cutP gene. *Nature (Lond.)*. 347:563-566.

Hagles, L. H. 1967. Mitochondria in temperature-sensitive mutants of yeast. *J. Bacteriol.* 93:1662-1670.

Hoffman, C. S., and F. Winston. 1985. A ten minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *E. coli*. *Gene (Amst).* 57:267-272.

Hoehfeld, J., and F. M. Pohl. 1986. Simplified preparation of unidirectional deletion clones. *Nucl. Acids Res.* 14:3605.

Hoyt, M. A., L. Totis, and B. T. Robers. 1991. *Saccharomyces cerevisiae* genes required for cell cycle arrest in response to loss of mitotic spindle function. *Cell*. 66:507-517.

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

Ito, H., Y. Fukuda, K. Murata, and A. Kimura. 1983. Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* 153:163-168.

Kilmartin, J. V., and A. E. M. Adams. 1984. Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces*. *J. Cell Biol*. 98:922-933.

Kranz, J. E., and C. Holm. 1990. Cloning by function: an alternative approach for identifying yeast homologs of genes from other organisms. *Proc. Natl. Acad. Sci. USA*. 87:6629-6633.

Le Guével, R., J. Paris, A. Couturier, C. Roghi, and M. Philippe. 1991. Cloning by differential screening of a *Xenopus* cDNA that encodes a kinesin-related protein. *Mol. Cell. Biol*. 11:3395-3398.

Li, R., and A. W. Murray. 1991. Feedback control of mitosis in budding yeast. *Cell*. 66:519-531.

Lupas, A., M. Van Dyke, and J. Stock. 1991. Predicting coiled coils from protein sequences. *Science (Wash. DC).* 252:1162-1164.

McDonald, H. B., R. J. Stewart, L. S. B. Goldstein. 1990. The kinesin-like nod protein of Drosophila is a minus end-directed microtubule motor. *Cell*. 63:1159-1165.

McIntosh, J. R., and C. M. Pfarr. 1991. Mitotic motors. *J. Cell Biol*. 115:577-585.

Meluh, P. B., and M. D. Rose. 1990. *Kar3*, a kinesin-related gene required for yeast nuclear fusion. *Cell*. 60:1029-1041.

Ohashi, A., J. Gibson, I. Gregor, and G. Schatz. 1982. Import of proteins into mitochondria. *J. Biol. Chem*. 257:13042-13047.

Perrins, D. D. 1949. Biochemical mutants of the smut fungus *Ustilago maydis*. *Genetics*. 34:607-629.

Rofd, D. M., P. B. Meluh, and M. D. Rose. 1992. Multiple kinesin-related proteins in yeast mitosis. *Cold Spring Harbor Symp. Quant. Biol*. 61:693-703.

Rose, M. D. 1991. Kinesin-related genes. In *Guidebook to Cytoskeletal Proteins*. R. Vale and T. Kreis, editors. (Sambrook and Tooze Scientific Publishers.) In press.

Rose, M. D., and G. R. Fink. 1987. *Kar1*, a gene required for function of both intrasaccul and extranuclear microtubules in yeast. *Cell*. 48:1047-1060.

Rose, M. D., F. Winston, and P. Hieter. 1990. Methods in Yeast Genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 198 pp.

Rothstein, R. J. 1983. One-step gene disruption in yeast. Methods Enzymol. 101:202-211.

Sawin, K. E., and J. M. Scheyte. 1991. Motor proteins in cell division. *Trends Cell Biol*. 1:122-129.

Sawin, K. E., T. J. Mitchison, and L. G. Wordeman. 1992. Evidence for kinesin-related proteins in the mitotic apparatus using peptide antibodies. *J. Cell Sci*. 101:303-313.

Saxton, W. M., J. Hicks, L. S. B. Goldstein, and E. C. Raff. 1991. Kinesin heavy chain is essential for viability and neuromuscular functions in *Drosophila*, but mutants show no defects in mitosis. *Cell*. 64:1093-1102.

Scherer, S., and R. W. Davis. 1979. Replacement of chromosome segments with altered DNA sequences constructed in vitro. *Proc. Natl. Acad. Sci. USA*. 76:4951-4955.

Sequeira, W., C. R. Nelson, and P. Sautner. 1989. Genetic analysis of the claret locus of Drosophila melanogaster. *Genetics*. 123:511-524.

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

Stewart, R. J., P. A. Pesavento, D. N. Woerpel, and L. S. B. Goldstein. 1991. Identification and partial characterization of six members of the kinesin superfamily in *Drosophila*. *Proc. Natl. Acad. Sci. USA*. 88:8470-8474.

Vale, R. D., T. S. Reece, and M. P. Sheetz. 1985. Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell*. 42:59-68.

Walker, R. E., J. Saraste, M. J. Runswick, and N. J. Gay. 1982. Distantly related sequences in the α- and β-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO J.* 1:945-951.

Walker, R. A., E. D. Salmon, and S. A. Endow. 1990. The Drosophila claret segregation protein is a minus-end directed motor molecule. *Nature (Lond).* 347:780-782.

Wilson, I. A., H. L. Niman, R. A. Houghten, A. R. Cherington, M. L. Connelly, and A. L. Lerner. 1984. The structure of an antigenic determinant in a protein. *Cell*. 37:767-778.

Yang, J. T., R. A. Laymon, and L. S. B. Goldstein. 1989. A three-domain structure of the kinesin heavy chain revealed by DNA sequence and microtubule binding analyses. *Cell*. 56:879-889.

Yang, J. T., W. M. Saxton, R. J. Stewart, E. C. Raff, and L. S. B. Goldstein. 1990. Evidence that the head of kinesin is sufficient for force generation and motility in vitro. *Science (Wash. DC).* 249:42-47.