The Kar3-Interacting Protein Cik1p Plays a Critical Role in Passage Through Meiosis I in *Saccharomyces cerevisiae*

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ABSTRACT

Meiosis I in *Saccharomyces cerevisiae* is dependent upon the motor protein Kar3. Absence of Kar3p in meiosis results in an arrest in prophase I. Cik1p and Vik1p are kinesin-associated proteins known to modulate the function of Kar3p in the microtubule-dependent processes of karyogamy and mitosis. Experiments were performed to determine whether Cik1p and Vik1p are also important for the function of Kar3p during meiosis. The meiotic phenotypes of a cik1 mutant were found to be similar to those of kar3 mutants. Cells without Cik1p exhibit a meiotic defect in homologous recombination and synaptonemal complex formation. Most cik1 mutant cells, like kar3 mutants, arrest in meiotic prophase; however, in cik1 mutants this arrest is less severe. These data are consistent with the model that Cik1p is necessary for some, but not all, of the roles of Kar3p in meiosis I. vik1 mutants sporulate at wild-type levels, but have reduced spore viability. This loss in viability is partially attributable to vegetative chromosome loss in vik1 diploids. Cellular localization experiments reveal that Kar3p, Cik1p, and Vik1p are present throughout meiosis and are consistent with Cik1p and Vik1p having different meiotic roles.

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MEIOSIS, mitosis, and karyogamy (nuclear fusion) depend upon microtubule function. In the budding yeast *Saccharomyces cerevisiae*, one kinesin-like motor protein, Kar3p, has been reported to function in all of these processes. Kar3p is essential for karyogamy (Meluh and Rose 1990). In newly formed zygotes, Kar3p is thought to function by cross-bridging microtubules between fusing nuclei, drawing them together by minus-end-directed forces (Meluh and Rose 1990). Kar3p is important but not essential for mitosis. Vegetative kar3 mutant cells display a number of phenotypes, including slow growth, which is the consequence of ~40% of cells experiencing cell cycle arrest, temperature sensitivity, short bipolar spindles, and longer, more numerous cytoplasmic microtubules (Meluh and Rose 1990; Roof et al. 1991; Saunders et al. 1997b). The temperature sensitivity of vegetatively growing kar3 mutants is thought to be attributable to the exaggerated cytoplasmic microtubules, as the temperature sensitivity is suppressible by the addition of microtubule-stabilizing drugs, such as benomyl, or by mutations in certain genes required for microtubule assembly and stability, such as *BIM1* (Tirnauer et al. 1999) and *TUB3* (Saunders et al. 1997b). These observations suggest that an important mitotic role of Kar3p is the mediation of microtubule depolymerization (Saunders et al. 1997b), an activity that has been demonstrated for the protein *in vitro* (Endow et al. 1994). In mitosis, Kar3p is thought to oppose the forces of two other kinesin-like motor proteins, Kip1p and Cin8p (Saunders et al. 1997b). Certain mutations in *kar3* can partially suppress the inviable phenotype of *kip1, cin8-3* double mutants at restrictive conditions (Saunders et al. 1997b). *kar3* mutants are dependent on several of the mitotic spindle checkpoint components for viability (Roof et al. 1991; Hardwick et al. 1999). Finally, Kar3p has been shown to have a role in mitotic spindle positioning (Cottingham et al. 1999).

Kar3p is essential for meiosis (Bascom-Slack and Dawson 1997). *kar3* cells arrest reversibly in prophase of meiosis I, with bushy prophase spindles, incomplete synaptonemal complex (SC) formation, modest levels of double-strand breaks, and highly reduced levels of heteroallelic recombination (Bascom-Slack and Dawson 1997).

In mating and vegetative cells it has been demonstrated that the activity of Kar3p is influenced by interactions with other kinesin-associated proteins (KAPs) that specify its function (Manning et al. 1999). Kar3p has two known KAPs, Cik1p and Vik1p, which have been implicated in directing Kar3p function in different microtubule-dependent events in mitotic cells (Page and Snyder 1992; Page et al. 1994). For example, the inviability of *cin8-3, kip1* double mutants is suppressible by a third mutation in either *VIK1* or *KAR3* mutants, but not in *CIK1* (Manning et al. 1999). Conversely, cik1 and kar3 mutants are temperature sensitive, whereas vik1 mutants are not (Manning et al. 1999). While Vik1p has no known role in karyogamy (Manning et al. 1999), Cik1p has been shown to be responsible for the cyto-
plasmid localization of Kar3p during this process (Page et al. 1994). Cik1p and Vik1p also appear to affect the distribution of Kar3p along the mitotic spindle (Manning et al. 1999). Another study showed that the Kar3p-Cik1p complex, but not the Kar3p-Vik1p complex, is involved in spindle positioning; however, both complexes are involved in spindle integrity (Cottingham et al. 1999).

The specific functions of Kar3p in meiosis are unclear. One approach to addressing this issue is to determine the meiotic roles of proteins known to interact with Kar3p in other cell cycle stages. Here we characterize the mitotic phenotypes of cik1 and vik1 mutants. The meiotic phenotypes of cik1 mutants are consistent with the model that Cik1p is critical for the function of Kar3p in prophase of meiosis I. vik1 mutants have a spore viability defect that is at least partially attributable to a mitotic chromosome maintenance defect, suggesting that its role in meiosis is either not essential or can be provided by other proteins. We localized Kar3p, Cik1p, and Vik1p in meiotic cells and observed differential staining between Cik1p and Vik1p, further suggesting that these proteins have different meiotic roles.

MATERIALS AND METHODS

Yeast strains, culture techniques, and cytology: Genotypes of strains used in this study are listed in Table 1. All of these strains are congenic derivatives of S288C (Nicolas et al. 1989) except where noted in text. Strain 2003 was a gift from Dan Burke.

Yeast cell culture and cytological techniques, including immunofluorescence of fixed and spread cells, were as described previously (Bascom- Slack and Dawson 1997; Kamieniecki et al. 2000). The sporulation regimen in this report was performed as described previously (Bascom-Slack and Dawson 1997) with the exception that we added adenine to the sporulation medium to a final concentration of 10 µg/ml. Weakly staining cytoplasmic foci were observed in experiments using anti-Myc primary antibody. These signals were also seen in control experiments with a strain carrying no Myc-epitope-tagged genes. Therefore, weak cytoplasmic staining was ignored in our experimental strains. Images were collected using an Olympus BX60 microscope with a 1.4 NA objective and a Hamamatsu (Bridgewater, NJ) model C4742-95 cooled CCD camera. Images were collected and manipulated using Openlab 2.2 software.

Plasmid construction, gene disruptions, and gene tagging: cik1 was cloned by complementing the cik1 temperature-sensitive phenotype as described by others (Page and Snyder 1992). The complementing plasmid, dubbed pRS1, contains a 9606-bp fragment of chromosome XII/1DNA (Saccharomyces Genome Database, chromosome XII coordinates 657470–667076). This fragment contains the entire open reading frame of CIK1. We have since subcloned a Sac-KpnI fragment containing only the CIK1 open reading frame and surrounding untranslated sequences into pRS415 (Sikorski and Heitter 1989) to create pRS12, which complements the sporulation defect of cik1 mutants as well as pRS1.

pRS4 was made by inserting a Kar3p-containing fragment (Eag-SalI; see below) into pCY204, which is a YCP50 vector containing HO under the control of its own promoter (Russell et al. 1986). The Kar3p-containing insert consists of 2416 bp of Kar3p DNA (205 bp upstream to 2211 bp downstream of the start codon), flanked by EagI and SalI restriction enzyme sites introduced by PCR.

pRS40 and pRS41 were made by ligating a VIK1-containing Ngod-MvKpnI fragment of pRS16-VIK1, a gift from Mike Snyder, into the integrating plasmids pRS404 and pRS406 (Sikorski and Heitter 1989), respectively. These plasmids were digested with Nrdl to direct their integration into the chromosome at the VIK1 locus.

pD168 is composed of a pUN105 vector (Elledge and Davis 1988) that carries a KAR3-GFP fusion gene under the control of the KAR3 promoter. The green fluorescent protein (GFP) sequence (a gift from Aaron Straight) has the S65T, V163A, and S175G mutations that result in a thermoresistant GFP (Siemering et al. 1996).

pRM2 was constructed by ligating a Hpal fragment containing the AR4G open reading frame and promoter into the Hpal site of ADE1 on pXW125 (a gift from Jim Haber).

cik1 deletions were constructed by transforming a haploid strain with a PCR-generated KanMX cassette (Guldener et al. 1996) flanked by 40 bp of CIK1 sequence corresponding to the beginning and end of the CIK1 open reading frame. G418 [200 mg/liter in YPD, geneticin from GIBCO BRL (Gaithersburg, MD), product no. 11811] resistant colonies were selected and tested for temperature sensitivity. Temperature-sensitive candidates were identified, and the correct insertion was confirmed by Southern blot analysis (Southern 1975; data not shown). With regard to temperature sensitivity, inability to mate, and microtubule morphology, strains bearing the cik1::KanMX allele behave phenotypically as reported previously for cik1 null mutants (Page and Snyder 1992).

vik1 deletions were constructed by transforming a haploid strain with a PCR-generated KanMX cassette with 45 bp of VIK1-flanking homology (Guldener et al. 1996). vik1 mutants were confirmed by PCR.

ADE1 was disrupted by AR4G by transforming yeast with pRM2 that had been digested with XhoI. Arg+ prototrophs were screened for red colonies to confirm the ADE1 disruption.

The carboxy termini of Kar3p, Cik1p, and Vik1p were tagged with 13 Myc, using the PCR-based gene modification system of Longtine et al. (1998). The KANMX cassette (from pFA6a-13Myc-KanMX) was used for tagging KAR3, while the HIS3MX cassette (from pFA6a-13Myc-His3MX6) was used for tagging CIK1 and VIK1.

Genetic techniques: Commitment to heteroallelic interhomolog recombination, return to vegetative growth, and the quantitative viable haploid formation analyses were as described previously (Bascom-Slack and Dawson 1997).

Chromosome I nondisjunction frequencies: Chromosome I nondisjunction frequencies were determined in DRS144 and DRS146. In these strains, one copy of chromosome 1 had been modified such that ADE1 was replaced by AR4G. Both diploids are also homozygous for the CAN1 gene, which confers sensitivity to canavanine. Cultures were sporulated in liquid medium for 48–72 hr and then were treated with zymolyase and Triton X-100 to kill nonspores (Rine and Rine 1997). Spores were then plated onto complete medium lacking adenine and arginine. Among cells able to grow on this medium are haploids disomic for chromosome I and unsporulated diploids that survived the zymolyase/detergent treatment. [Spores were also plated onto rich medium to determine the frequency of colony-forming units (CFU) in the detergent-treated cells.] To distinguish between these categories, colonies were tested to determine the ploidy of chromosome V using a previously described method (Zeng and Saunders 2000). Fifty patches of Ade+ Arg+ colonies on a YPD plate were replica plated to medium containing canavanine and...
were then subjected to 0 or 5000 μJ of UV irradiation from a Stratagene (La Jolla, CA) Stratolinker model 2400 and allowed to grow for 2 days at 30°C. Cells are much more likely than haploids to survive on canavanine-containing medium after mild mutagenesis. Candidate Arg⁺ Ade⁺ patches with high rates of papillation on canavanine medium were considered haploid. All colonies classified as haploid on the basis of the chromosome V analysis behaved as maters in mating-type tests. The frequency of chromosome I disomy in haploid spore products of DRS144 and DRS146 was determined as follows: frequency of haploid chromosome I disomy = Ade⁺ Arg⁺ disomes (CFU/ml of Ade⁺ Arg⁺ prototrophs × frequency of chromosome V monosomes)/total haploids (CFU/ml). These experiments were done in parallel strains and the results were averaged.

Diploid vegetative chromosome I loss frequency: The rate of vegetative chromosome I loss in diploid cells was determined in the above-mentioned strains, DRS144 and DRS146. Triplicate cultures of both DRS144 and DRS146 were grown in liquid medium lacking both adenine and arginine to maintain both copies of chromosome I. When these cultures reached 1–2 × 10⁷ cells/ml they were plated onto YPD and allowed to grow for 5 days at 30°C. The frequency of red-sectored colonies was determined (DRS144 had 0 red-sectored colonies/16,963 total colonies, while DRS146 had 38 red-sectored colonies/11,829 total colonies).

Haploid vegetative chromosome I loss frequency: The rate of vegetative chromosome I loss in haploids was determined in haploid strains disomic for chromosome I. A number of steps were performed to construct the strains used in this experiment. First, DRS144 was sporulated and the cultures were plated onto canavanine test (see above) to determine the rate of vegetative chromosome I loss in haploids. Next, the frequency of red-sectored colonies was determined (DRS144 had 0 red-sectored colonies/11,829 total colonies, while DRS146 had 38 red-sectored colonies/11,829 total colonies).

<table>
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<tr>
<th>Strain</th>
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<td>Dd513.3A</td>
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<tr>
<td>DRS49.6B</td>
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<tr>
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<tr>
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<tr>
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<td>DRS142</td>
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<td>2003 × TRK80</td>
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<td>DRSK28.2A × DRSK28.2B</td>
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from diplods. Chromosome XVI ploidy could also be deduced because the two VIK1 loci were differentially marked, one by TRP1 and the other by URA3 (see Table 1). Colonies that were disomic for chromosome I, monosomic for chromosome V, exhibited a or α mating behavior, and carried the URA3 (but not the TRP1) copy of chromosome XVI were used to generate vik1::KANMX and VIK1 isogenic strains carrying the chromosome I disome. These cells were treated with 5-fluorouracil (5-FOA) to identify cells that had “looped out” the URA3 gene integrated at the VIK1 locus. Because the URA3 gene was flanked on one side by the wild-type VIK1 and on the other side by a vik1::KANMX allele, the FOA derivatives could be either Vik1− or Vik1+. The VIK1 genotype of the FOA derivatives was determined, and an isogenic VIK1 and vik1 pair was used to determine the loss frequency of chromosome I. The assay was the same as in the chromosome loss assay used for diplods discussed above (vik1Δ had 38 red-sector CFU/9667 total CFU, while VIK1 had 2 red-sector CFU/8145 total CFU).

RESULTS

cik1 mutants are defective in spore formation: To determine the sporulation phenotype conferred by a cik1 null mutation, a diploid cik1 strain (DRS52) was induced to undergo meiosis. The same strain bearing a CIK1 centromeric plasmid was evaluated in parallel as an isogenic control. Cells that had been incubated in sporulation medium for 96 hr were adjusted to 106 cells/ml and observed with bright-field microscopy. While CIK1 cells exhibited 25.4% ascus formation, cik1 strains formed no ascii at all (n > 210). The complete absence of sporulation observed here contrasts with a previous report that cik1α cells, also in an S288C-derived strain background, exhibited a considerably reduced sporulation efficiency and the formation of dyads rather than tetrads for those cells that did form ascii (Kurihara et al. 1996). A more sensitive genetic experiment was performed to test the severity of the meiotic defect in cik1 mutants by directly selecting for viable haploid meiotic products formed by a diploid cik1 strain (DRS52). DRS52 is heterozygous for alleles that confer resistance to the drugs canavanine and cyclohexamide (can1/can1, CYH2/CYH2). For both of these genes, the allele conferring sensitivity to the drug is dominant, such that the diploid (DRS52) will die on medium containing either of these drugs. If DRS52 were to sporulate, 25% of its spores would inherit both the can1 and cyh2 alleles, allowing them to live in a medium containing both drugs. Additionally, DRS52 is heterozygous at the ADE2 locus (ADE2/ade2) so that it produces white colonies, while one-half of its haploid products would inherit the ade2 allele and would produce red colonies. To determine the frequency of haploid production, we scored production of Can−, Cyh−, red colonies in aliquots of cells harvested from synchronous meiotic cultures of cik1 (DRS52) and CIK1 (DRS52 + pRS1) strains (Figure 1). The cik1 strain produced Ade−, Can−, and Cyh− CFUs at 0.2% the efficiency of the isogenic strain harboring a CIK1 plasmid. To test further whether the rare Ade−, Can−, and Cyh− colonies produced by the cik1 strains were indeed haploids, we tested the mating types of five isolates. All five that were tested mated with either a MATα or a MATa strain, suggesting that they contained a single copy of chromosome III and supporting the conclusion that cik1 mutants are unable to efficiently form viable haploid products. Consistent with an earlier report (Bascom-Slack and Dawson 1997), the kar3 strain yielded no haploid colonies among >106 cells tested.

cik1 mutants arrest in prophase I of meiosis: To determine the meiotic stage at which cik1 mutants are blocked, we monitored the fraction of cells that would progress beyond meiotic prophase I. Cells categorized as beyond prophase I included un budded cells containing two or more 4,6-diamidino-2-phenylindole (DAPI) dihydrochloride (a DNA staining dye)-stained chromatin masses and/or bipolar spindles. In CIK1 cells, 34.5% of cells were postprophase at 46 hr. By 46 hr only 2.5% of cik1 mutants had proceeded beyond prophase I (Figure 2A). Of the cells that had gone beyond prophase I, DAPI staining revealed equal numbers of binucleate and tetranucleate cells, although no spores were ob-
served. The majority of cik1 cells arrested at prophase I with a single chromatin mass and monopolar tubulin arrays (see Figure 2, B and C).

The cik1 meiotic arrest is reversible: To test whether the cik1 meiotic arrest is the consequence of irreversible damage we determined whether cik1 cells that were arrested in meiosis could return to mitotic growth when plated on rich medium. The number of CFUs was determined by gathering cells throughout meiosis and plating dilutions on rich medium (YPD). The viability of cik1 strains remains high for the first 24 hr (by which time the cells have reached their prophase arrest), after which there is a gradual drop in viability while an isogenic CIK1 strain retains high levels of viability for at least 72 hr (Figure 3A). Ability of the cik1 mutants to resume growth if returned to rich medium indicates that meiotic arrest is not attributable to irreversible cellular damage. Instead this result suggests the block in meiotic progress is a regulatory arrest rather than a lethal event. Consistent with this notion, rare cik1 cells are observed to proceed beyond prophase I and form bi- or tetranucleate cells with normal-looking spindles and DAPI masses (Figure 2).

Heteroallelic interhomolog recombination is impaired in cik1 mutants: A landmark of prophase of meiosis I is the commitment to meiotic levels of interhomolog recombination (Sherman and Roman 1963; Esposito and Esposito 1974). To assay for this landmark, isogenic cik1 (DRS65) and CIK1 (DRS65 + pRS1) strains were constructed that contained arg4 heteroallelic mutations (Bascom- Slack and Dawson 1998). These strains allowed us to assay the frequency of recombination between the two mutations by selecting for Arg+ prototrophs. The frequency of prototroph formation was measured by returning aliquots of the meiotic culture to vegetative growth on medium with and without arginine. The cik1 strain exhibited a strong reduction in prototroph formation, although the defect was not absolute (Figure 3B). By 72 hr after meiotic induction, recombinants were detectable at \( \sim 1\% \) of the level exhibited by the CIK1 control. This result is similar to that seen in kar3 mutants (Bascom- Slack and Dawson 1997).

Strains deficient for Cik1p form an incomplete synaptonemal complex: SC, a tripartite proteinaceous matrix that forms between synapsed homologs, is a defining landmark of meiotic prophase. To determine if cik1 mutants form SC we used indirect immunofluorescence to observe Zip1p, a component of the central element of the SC (Sym et al. 1993), in cik1 and CIK1 cells. Aliquots of synchronous meiotic cultures were harvested at 12.5, 13.5, and 14.5 hr postmeiotic induction. Spread nuclei (\( n > 489 \) per strain) from these cultures reveal that, while approximately equal fractions of nuclei from wild-type and mutant strains had Zip1p staining (10% for cik1, 12% for CIK1), the cik1 strain was severely defective in assembling the Zip1p into the continuous worm-like deposits that are characteristic of mature SC. Among the Zip1p-staining CIK1 cells, 49% showed multiple worm-like structures suggestive of complete SC formation. In contrast, in cik1 cells evidence of complete synopsis of all of the chromosome pairs was never seen. Of the Zip1p-staining cik1 cells, 17% exhibited some worm-like staining (as in Figure 4B), but we never observed more than approximately eight (Figure 4B) continuous complexes per spread. The majority of cik1 strains had a discontinuous, punctate Zip1p-staining pattern (Figure 4C). Many of the Zip1p-staining cik1 cells (22%; 14/64) displayed a bright staining focus that is likely a polycomplex (Figure 4, B and C, arrowheads); this was rare (4%; 2/51) in the CIK1 spreads that had Zip1p staining. Polycomplexes are often seen in meiotic mutants that arrest in prophase of meiosis I without the synopsis of homologous chromosomes (Klapholz et al. 1985; Sym and Roeder 1995). Similarly, kar3 mutants fail to produce mature SC and experience polycom-
**Figure 3.**—*cik1* mutants arrest reversibly in meiosis and are defective in the process of interhomolog recombination. (A) *cik1* mutants arrest reversibly in meiosis. The total number of colony-forming units was determined by inducing isogenic wild-type (DRS65 + pRS1) and *cik1* (DRS65) strains to undergo meiosis, taking aliquots, plating dilutions on YPD, and counting the number of colonies after 4 days of growth at 30°. (B) *cik1* mutants are defective in interhomolog recombination. Isogenic cultures of *CIK1* (DRS65 + pRS1) and *cik1* (DRS65) strains, heterozygous for a pair of *arg4* heteroalleles (*arg4R*V and *arg4*/*H9004*42), were induced to undergo meiosis. Aliquots were removed over the course of 3 days; these were plated onto complete medium without arginine to determine the number of *Arg+* recombinants. Aliquots were also plated onto nonselective medium to determine the total number of colony-forming units. The *cik1* mutants exhibited 1.2% of the wild-type levels of recombination at 72 hr. The same cultures were used for both assays (A and B). These were single experiments that were representative of reproducible phenotypes.

**Figure 4.**—Synaptonemal complex formation in *cik1* mutants. Cultures of a wild-type strain (A, DRS51 + pRS1) and a *cik1* strain (B and C, DRS51) were harvested 12.5–14.5 hr postmeiotic induction. Their nuclei were spread and stained with the DNA-specific dye DAPI (column 1) and anti-Zip1p antibodies (column 2). Wild-type cultures often displayed full SC formation (A). *cik1* mutant cells rarely show partial SC formation (B) and often display the more punctate pattern of Zip1p staining shown in C. Polyc complexes (arrowheads) were observed in 3.9% of wild-type and 21.9% of *cik1* mutant nuclei.

spores disomic for the nondisjoined chromosome and two inviable spores bereft of the chromosome or by loss of a chromosome in either vegetative growth or meiosis prior to anaphase I. The resulting 2n − 1 cell undergoing meiosis would yield two spores with, and two spores without, a copy of the lost chromosome yielding 2:2 tetrads.

**vik1** mutants do not experience elevated levels of meiosis I nondisjunction: Consistent with the hypothesis that Vik1p has a role in meiosis I, its transcription is induced in meiosis (Chu et al. 1998), and the protein is evident in prophase I cells (see below and Figure 8). To determine whether the increased level of two-spore viable tetrads in *vik1* mutants was from a defect in chromosome disjunction at the meiosis I division, we designed a strain for which spore products resulting from meiosis I nondisjunction events could be selected. The *vik1* mutant and control strains were modified so that their two copies of chromosome I were differentially marked with prototrophic genes such that spores diso-
mic for chromosome I could be isolated (see Materials and Methods for details). We found that the rate of chromosome I disomy among vik1 spores was indistinguishable from that observed in wild-type strains (Table 3). Similar results were found for chromosomes III and V (data not shown). We conclude that the reduced spore viability of vik1 mutants is not because of a meiosis I nondisjunction defect.

Chromosome I is lost at high frequency during vegetative growth in vik1 mutant diploids: To determine if lowered spore viability in vik1 mutants stems mainly from a mitotic rather than a meiotic problem, we determined the rates of chromosome I loss in vik1 mutants in the aforementioned diploid strains (DRS144 and DRS146) and in haploid strains disomic for chromosome I (DRS144.1 and DRS144.2; see Table 1). Cells were grown in medium that selected for both copies of chromosome I. These were then plated on rich medium and the loss of one copy of chromosome I was assayed by the generation of red sectors (see Materials and Methods). vik1 mutant haploids had a 16-fold increase in the loss of chromosome I (Table 3). This is slightly higher than the 3-fold increase in chromosome loss monitored using a reporter chromosome III fragment (Manning et al. 1999). The frequency of chromosome I loss in the vik1 diploid strain was at least 50-fold higher than in the isogenic wild type (Table 3). If the loss frequency we measured for chromosome I in diploids is similar to that of the other chromosomes, then the overall decrease in spore viability due to mitotic chromosome loss would be 1.5% (frequency of chromosome I loss × 16 chromosomes × two inviable spores per tetrad), a value that would contribute to the loss of spore viability in vik1 strains.

To determine the temporal and subcellular localization patterns of Kar3p, Cik1p, and Vik1p, Myc-epitope-tagged versions of these genes were constructed (see Materials and Methods). The Myc-tags are C-terminal and the native promoters of the genes are retained. The tagged genes were introduced through multiple backcrosses into the SK-1 background (Kane and Roth 1974) to ensure synchronous and efficient sporulation (Figures 5F, 6E, and 8E). Diploid cells, homozygous for the tagged genes, efficiently formed tetrads with high viability (≥92% viability for Cik1p-Myc and Vik1p-Myc and 86.5% viability for Kar3p-Myc; n ≥ 22 tetrads). These strains, as well as a congenic strain with no Myc-tag, were sporulated, and samples were harvested at timed intervals for microscopic analysis. Cells were fixed and treated with either DAPI and antiu-

### Table 2

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Spore viability (%)</th>
<th>Tetradi viability classes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vik1Δ</td>
<td>78.1</td>
<td>4:0 3:1 2:2 1:3 0:4</td>
</tr>
<tr>
<td>VIKI</td>
<td>90.8</td>
<td>72.5 26.6 0.9 2.8 0</td>
</tr>
</tbody>
</table>

Spores of isogenic strains (DRS142 vik1Δ and DRS143 VIKI) were dissected on rich media (YPD). After 3 days of growth (30°C) viability was assayed. N = 159 for DRS142 and N = 109 for DRS143. Sporulation frequencies were 18.6% for DRS142 and 17.4% for DRS143 when assayed by light microscopy (n > 500).

### Table 3

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Ploidy</th>
<th>Frequency of disomic spore formation*</th>
<th>Frequency of chromosome I loss*</th>
</tr>
</thead>
<tbody>
<tr>
<td>vik1Δ</td>
<td>Diploid</td>
<td>$2.3 \times 10^{-3} \pm 1.7 \times 10^{-3}$</td>
<td>$4.6 \times 10^{-4} \pm 1.1 \times 10^{-4}$</td>
</tr>
<tr>
<td>VIKI</td>
<td>Diploid</td>
<td>$2.1 \times 10^{-3} \pm 1.5 \times 10^{-3}$</td>
<td>$&lt; 8.4 \times 10^{-6}$</td>
</tr>
<tr>
<td>vik1Δ</td>
<td>In + 1</td>
<td>ND</td>
<td>$5.6 \times 10^{-5} \pm 6.7 \times 10^{-5}$</td>
</tr>
<tr>
<td>VIKI</td>
<td>In + 1</td>
<td>ND</td>
<td>$3.4 \times 10^{-5} \pm 5.1 \times 10^{-5}$</td>
</tr>
</tbody>
</table>

ND, not determined.

* Diploids heterozygous for centromeric markers on chromosome I (ADE1/ade1::ARG4) were sporulated (DRS144 VIKI; DRS146 vik1Δ). The frequency of spore products disomic for chromosome I was determined (see Materials and Methods) in duplicate experiments. One standard deviation of error is shown with the frequency of disomy.

The frequency of mitotic chromosome I loss was determined in either diploids (DRS144 and DRS146) or haploids disomic for chromosome I (DRS144.1 and DRS144.2) by assaying for red-sectored colony formation on YPD. We estimate from the observed relative frequency of one-half vs. smaller sectors (observed ratio 7:38; expected ratio 7:42) that chromosome loss in the first three cell generations of colony formation gave visible sectors. Therefore, the loss frequency was determined to be the number of sectored colonies/total colonies × 7 (the number of mitotic divisions in the first three generations of colony formation). Experiments were done in triplicate (diploids) or duplicate (haploids); the average with one standard deviation of error is shown. n > 11,000 total colonies for diploids and n > 8000 colonies for In + 1 haploids.
bulin antibodies to monitor progress of the cultures through meiosis or with DAPI and anti-Myc antibodies to evaluate localization of Kar3p, Cik1p, or Vik1p in meiosis relative to the nucleus.

Kar3p: Kar3p-Myc staining was observed throughout meiosis I and meiosis II until after the breakdown of the spindles (Figure 5). In this experiment and in those with Cik1p and Vik1p, cells that had progressed beyond anaphase II exhibited a natural fluorescence that obscured the fluorescent signal used to detect Myc-staining, making it difficult to draw conclusions about the presence of Kar3p, Cik1p, or Vik1p in the late stages of sporulation. Epitope-tagged versions of Kar3p have been shown previously to exhibit localization to the spindle pole bodies (SPBs) and to spindles of mitotic cells and, to a lesser extent, a pattern of general nuclear staining or “nuclear patches” (Meluh and Rose 1990; Manning et al. 1999). All three types of localization were also seen in meiotic cells (Figure 5). In early meiotic cells that are in prophase (t = 2 and 4 hr), Kar3p typically localized to a single nuclear focus and bright patches distributed throughout the nucleus (Figure 5A). As cells progressed into anaphase I, Kar3p localized in patches throughout the nucleus and to two foci (see Figure 5B; t = 4 hr and beyond). These foci presumably correspond to the spindle poles, and in separate experiments (in S288c-background cells) we found that these foci colocalize with Tub4-GFP, a spindle pole body component (Marschall et al. 1996; data not shown). The localization of Kar3p to both the poles and nuclear patches continues through anaphase I and II (Figure 5, C–E). Throughout meiosis, localization of Kar3p to the region of the spindle between the poles was limited, observed in only 28% of Myc-staining nuclei (see the top spindle in Figure 5D for an example).

Cik1p: Cik1p and tagged versions of Cik1p have been shown to localize to the nucleus, spindle, and SPB of vegetatively growing cells (Page et al. 1994; Manning et al. 1999). In mitotic and meiotic cells we observed the bright nuclear patch staining pattern of Cik1p-Myc staining similar to that of Kar3p-Myc (Figure 6, A–D). We also noted a SPB-like staining pattern, although this was rare (at t = 6 hr, 6% of mononucleate cells with Myc staining exhibited a single focus while 8% had two foci, compared to 54 and 10% for Kar3p-Myc at the same time point n = 50). Throughout meiosis the SPB-like staining could be detected, although it was infrequent and usually faint (indicated by arrowheads in Figure 6, A and B). We were able to confirm that these Cik1p-Myc foci are at SPBs by demonstrating colocalization with Tub4p, a component of the SPB (Figure 7, D–F). The two notable differences between Cik1p-Myc and Kar3p-Myc staining occurred in the frequency and intensity of SPB-like staining (see above) throughout meiosis and in binucleate cells that had just experienced anaphase I, judging by the stretched, bilobed shape of their nuclei. In randomly chosen binucleate cells, 61%
Meiosis in \( cik1 \) Mutants

Figure 7.—Kar3p localization in prophase nuclear spreads and colocalization of Cik1p and the SPB component, Tub4p, in whole meiotic cells. In A, B, and C, meiotic nuclei were spread and prepared for fluorescent microscopy. (A and B) Nuclei (DJ1 + pD168) were stained with antisera for Zip1p (an SC component, shown in red) and Kar3p-GFP (shown in green). Prophase I cells representative of two classes of Kar3p staining patterns are shown (A, a single focus 96%; B, multiple foci 4%; \( n = 50 \)). (C) Nuclei (DRS151) were stained with DAPI (chromatin stain, blue) and antisera against Ndc10p-GFP (a kinetochore protein, green) and Kar3p-Myc (red). D–F are a montage of whole meiotic cells from the same field. Meiotic cells (DRS106) were prepared for immunofluorescent microscopy. (D) Cik1p-Myc staining (green); (E) Tub4p-GFP foci (red); and (F) overlay showing the colocalization of Cik1p and Tub4p in nuclei with bipolar spindles. Bar, 5 \( \mu m \).

The shared pattern of Kar3p and Cik1p staining prior to anaphase, followed by the detection of Cik1p but not Kar3p at the midzone, is consistent with a dissociation of some or all of the Kar3p-Cik1p complexes as cells enter anaphase I. Alternatively, it may be that Kar3p-Myc, but not Cik1p-Myc, is difficult to detect at the midzone for any number of technical reasons.

The nuclear patching phenotype exhibited by Kar3p and Cik1p is consistent with the association of these proteins with chromosomes. To test this hypothesis we looked for association of Kar3p with isolated chromosomes. In spread prophase I nuclei with Kar3p-GFP staining displayed a single focus and several (3–20) smaller foci (Figure 2B). Similar staining patterns were seen for Cik1p-Myc in spread prophase I cells (data not shown). The fact that Kar3p and Cik1p nuclear patches could be observed...
in the majority of whole prophase cells, while they were rarely observed associated with spread chromosomes, suggests that either the nuclear patches are not associated with chromatin in prophase I of meiosis or that the methods used for chromosome spreading disrupt the association of Kar3p and Cik1p with chromatin but not with the SPBs. To determine if the rare Kar3p (or Cik1p) foci that were found associated with spread chromosomes were localized to the kinetochores, we compared the localization of Kar3p with Ndc10p, a known kinetochore protein (Goh and Kilmartin 1993; Jiang et al. 1993) in nuclear spreads (DRS151). We found that neither Kar3p-Myc nor Cik1p-Myc (not shown) colocalized with Ndc10p-GFP during prophase I (Figure 7C; n > 70).

Vik1p: Vik1p-Myc had a staining pattern that was distinct from that of Kar3p and Cik1p in that the nuclear patching phenotype was rarely seen (at t = 6 hr, 6% of cells had nuclear patches of Vik1p, compared to 36 and 85% for Kar3p and Cik1p, respectively; n ≥ 50); the nuclear patching phenotype was not observed in a no-epitope control strain. Instead, depending on the stage of meiotic progression, one to four foci were seen in meiotic cells (Figure 8, A–D). Throughout meiosis, Vik1p appears to be primarily associated with the SPBs, as has been shown in vegetative cells (Manning et al. 1999).

cik1, vik1, and kar3 mutants differ in the length of their meiotic monopolar microtubule arrays: Cik1p has been reported to affect the length and number of microtubules in mitotic cells (Page and Snyder 1992). cik1 mutants display pronounced nuclear microtubule arrays in vegetative and α-factor arrested cells, but shorter bipolar spindles (Page and Snyder 1992; Manning et al. 1999). Similar phenotypes have been reported for kar3 mutants (Meluh and Rose 1990; Saunders et al. 1997a); however, vik1 mutants were reported to have microtubule arrays and bipolar spindles indistinguishable from those of wild-type strains (Manning et al. 1999). In meiosis, kar3 mutants have been shown to arrest with prominent nuclear microtubule arrays (Bascomb-Slack and Dawson 1997). To see if the meiotic phenotypes of cik1 and vik1 were similar to the reported mitotic phenotypes, we assayed the length of nuclear monopolar tubulin arrays in meiotic time courses. To assay the differences among strains, we measured the length of the longest nuclear microtubule bundle in monopolar tubulin arrays of randomly chosen wild-type, cik1, vik1, and kar3 meiotic diploids.

The monopolar arrays in wild-type cells showed a distribution of lengths at t = 0 (when cells were transferred into sporulation medium) with an average length of 1.21 μm. As meiosis progressed, the average length of the arrays increased (Figure 9). Note that most wild-type cells that will complete meiosis have formed bipolar spindles before the 26-hr time point. Thus, the cells that remain with monopolar arrays at 26 hr do not represent cells undergoing normal passage through meiosis. vik1 mutants have vegetative and meiotic monopolar arrays with lengths that are indistinguishable from wild type (Figure 9). cik1 mutant microtubule arrays are similar in length to wild-type arrays in vegetative cells grown in YPAcetate (t = 0); however, in early (t = 6 hr) and middle (t = 12 hr) meiosis cik1 mutants have
mutated for these genes arrest in prophase I with similar more highly elongated nuclear microtubule arrays in *cik1* meiosis I. This conclusion is based upon several meiotic the model that in meiosis, as in mitosis, Kar3p performs

mutants. Over time, the length of the microtubule bun-

bars showing 1 SD reflect this distribution. (n = 25 arrays for T = 0; n = 30 for meiotic samples.)

arrays that are 20% longer than wild type and this difference increases to 40% at t = 26 hr (Figure 9). *kar3* mutants show highly variable and elongated prophase arrays. Vegetative *kar3* mutant nuclear microtubule arrays are, on average, twice as long as those of wild-type cells and the length of their long microtubule arrays is maintained throughout meiosis (Figure 9). The exaggerated length of *kar3* mutant arrays is not an artifact of prolonged arrest in prophase I, because wild-type cells at 26 hr with prophase I microtubule arrays have presumably arrested in meiosis I as well and do not exhibit the greatly elongated microtubule phenotype of *kar3* mutants. Over time, the length of the *cik1* monopolar arrays approaches that of the *kar3* mutants, but even at 26 hr the *kar3* arrays are longer. Unlike the other strains, *kar3* mutants frequently exhibited unusual spindle morphologies, as has been reported previously (Basc-om-Slack and Dawson 1997). These include bushy nuclear monopolar arrays, tandem (side-by-side) nuclear monopolar arrays, cytoplasmic microtubules that span the diameter of the cell, and cytoplasmic accumulations of microtubules into bright-staining foci adjacent to the SPB.

**DISCUSSION**

We have characterized the meiotic phenotypes of *cik1* mutants and found them consistent with the model that Cik1p is critical for the function of Kar3p in prophase of meiosis I. This conclusion is based upon several meiotic phenotypes shared by both *cik1* and *kar3* mutants. Cells mutated for these genes arrest in prophase I with similar defects in the levels of meiotic recombination and the formation of mature synaptonemal complexes. We also show that Cik1p and Kar3p have similar but distinct localization patterns in meiotic cells.

We found that, although Vik1p is present in meiosis, *vik1* mutants have no dramatic meiotic defects. We demonstrated that *vik1* mutants exhibit reduced spore viability, which is due to neither meiosis I nor meiosis II nondisjunction (data not shown), but is caused, at least in part, by the loss of chromosomes during vegetative cell divisions. It is also possible that the elevated levels of two-spore viable tetrads that we observed in the *vik1* mutants are due to loss of chromosomes in meiosis prior to anaphase I, and our assays would not detect this type of error. The loss of chromosomes could occur by a simple failure of chromosomes to attach to the spindle. The Ncd motor protein of Drosophila is homologous to Kar3p and is required for organizing meiotic spindles in Drosophila oocytes. In *ncd* mutants chromosomes can be lost because of their failure to be captured by the disorganized microtubules (Hatsumi and Endow 1992; Endow and Komma 1996). Although the assembly of meiotic spindles in yeast and Drosophila oocytes is drastically different, the Vik1p-Kar3p complex may have an organizational function that parallels that of Ncd, and *vik1* defects may result in mild spindle disorganization and meiotic chromosome loss that yields elevated levels of two-spore viable tetrads.

What are Cik1p and Kar3p doing in meiosis I? The major events of prophase I include formation of double-strand breaks, interhomolog recombination, synopsis of homologous chromosomes, and the establishment of a meiosis I spindle. Cik1p and Kar3p may be directly or indirectly necessary for these events. Many organisms, including *S. cerevisiae*, reorganize the positions of the chromosomes within the cell during prophase I (Dernberg et al. 1995; Zickler and Kleckner 1998). In this process, telomeres become clustered with the chromosome arms radiating outward in an organization referred to as the bouquet. Bouquet formation may facilitate homologous recombination, as agents that disrupt bouquet formation have been demonstrated to disrupt chiasma formation (Loidl 1990). In budding yeast, both genetic and cell biological methods have been used to demonstrate clustering of telomeres in prophase I (Goldman and Lichtten 1996; Hayashi et al. 1998; Trellles-Sticken et al. 1999). Failure in this prealignment is one way to explain how defects in a motor protein might result in a defect in interhomolog recombination. Alternatively, the recombination defects of these mutants might be an indirect consequence of an arrest triggered by defects in spindle assembly or function.

The experiments presented here are consistent with the model that in meiosis, as in mitosis, Kar3p performs some roles independently of Cik1p. *kar3* mutants have more highly elongated nuclear microtubule arrays in
meiosis than do cik1 mutants. Cik1p is deposited at the spindle midzone at anaphase I while this seems not to be true for the majority of Kar3p. kar3 mutants have a more severe prophase I arrest than do cik1 mutants. cik1 mutants produce rare viable haploids and display postprophase spindle morphologies at detectable levels, while kar3 mutants were observed to do neither. In another study (KURIHARA et al. 1996), kar3 mutants produced no spores, while cik1 mutants produced low levels of ascii that contained two spores. We attribute the different cik1 sporulation phenotypes described between these two studies to either the well-documented differences in meiotic mutant phenotypes in different strains or to differences in sporulation regimens. In both strains the kar3 phenotype is more severe than the cik1 phenotype.

In summary, Vik1p appears to play a minor or redundant role in meiosis. The loss in spore viability of vik1 mutants can partially be attributed to chromosome loss in mitotic cell divisions that precede meiosis; meiotic chromosome loss may also contribute to this phenotype. The severe defects in recombination and synapsis exhibited by both cik1 and kar3 mutants are consistent with the hypothesis that Cik1p acts as a KAP to Kar3p in its essential role in prophase I of meiosis. The overall slightly milder meiotic phenotypes of cik1 mutants suggest that in meiosis, as in mitosis, Kar3p can perform some functions independently of Cik1p.

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