The Yeast Motor Protein, Kar3p, Is Essential for Meiosis I

Carol A. Bascom-Slack, and Dean S. Dawson

Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts 02111

Abstract. The recognition and alignment of homologous chromosomes early in meiosis is essential for their subsequent segregation at anaphase I; however, the mechanism by which this occurs is unknown. We demonstrate here that, in the absence of the molecular motor, Kar3p, meiotic cells are blocked with prophase monopolar microtubule arrays and incomplete synaptonemal complex (SC) formation. *kar3* mutants exhibit very low levels of heteroallelic recombination. *kar3* mutants do produce double-strand breaks that act as initiation sites for meiotic recombination in yeast, but at levels severalfold reduced from wild-type. These data are consistent with a meiotic role for Kar3p in the events that culminate in synapsis of homologues.

M EIOSIS is a dynamic process in which the behavior of the chromosomes and spindle is coordinated to allow the generation of haploid gametes from diploid precursor cells. This is especially evident in the dramatic movements of the chromosomes and spindle in anaphase I, when homologous chromosomes are drawn apart. Before this, during prophase, the chromosomes align and recombine with their homologous partners while an organized array of microtubules develops. The relationship of microtubule and chromosome activity in prophase is less clear; however, there is evidence to suggest that these are not independent processes. For example, in many organisms it has been shown that chemical agents or environmental conditions that disrupt meiotic spindle development have profound effects on chiasma formation or chromosome synapsis (for review see Loidl, 1990). The interdependence of microtubule function and chromosome behavior in early meiosis is also seen in certain oocytes in which the highly condensed meiotic chromatin serves as a foundation around which the microtubules are organized (Karsenti et al., 1984; Sawada and Schatten, 1988; Theurkauf and Hawley, 1992; Heald et al., 1996; for review see Rieder et al., 1993; McKim and Hawley, 1995).

Much of early meiosis (prophase) is dedicated to the establishment of a tight association among homologous chromosomes (for review see Bascom-Slack et al., 1997). In yeast, the initial steps of this process are thought to involve pairing interactions between homologous DNA sequences, eventually leading to homologous recombination (Kleckner and Weiner, 1993). The alignment process culminates in synapsis, the tight association of homologues along the proteinaceous structure known as the synaptonemal complex (SC).[1] As these events occur, the early stages of spindle formation are in progress (Moens and Rapport 1971; Byers, 1981; Padmore et al., 1991). The spindle pole bodies (SPBs) duplicate during premeiotic DNA replication and an increasingly dense nuclear microtubule array emanates from the adjacent duplicated SPBs as prophase progresses (Moens and Rapport, 1971). An early landmark in meiotic prophase is the initiation of meiotic recombination by the generation of double-strand breaks (DSBs) in the DNA (Sun et al., 1989; Padmore et al., 1991). Recombination among homologues is important for chromosome alignment, as mutants that fail to initiate recombination do not achieve full SC formation (Engebrecht and Roeder, 1989; Giroux et al., 1989; Hollingsworth and Byers, 1989; Alani et al., 1990; Rockmill and Roeder, 1990; Roeder, 1990; Loidl et al., 1994). After SC assembly, and subsequent disassembly, the SPBs migrate apart to form a short bipolar spindle that rapidly lengthens at anaphase I, moving the homologues away from each other (Moens and Rapport, 1971; Byers, 1981; Padmore et al., 1991). In meiosis II the chromosomes undergo an equational division in which the sister chromatids separate away from one another.

Motor proteins have been implicated as playing critical roles in spindle assembly and function, and in chromosome segregation (for review see McIntosh and Pfarr, 1991; Sawin and Endow, 1993; Hoyt, 1994; Walczak and Mitchison, 1996). Whereas many of these studies have focused on mitotic functions, the analogous meiotic functions undoubtedly

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1. *Abbreviations used in this paper*: CFU, colony forming unit; CM, complete medium; DAPI, 4′, 6-diamidino-2-phenylindole dihydrochloride; DSB, double-strand break; SC, synaptonemal complex; SPB, spindle pole body; YPD, yeast extract, peptone and dextrose medium.

require motor proteins as well. Additionally, the spindle and chromosome behaviors that are unique to meiosis might require meiosis-specific motor protein activities. Among motor proteins with known meiosis-specific roles are two kinesin-like proteins (klps) from Drosophila (Endow et al., 1990; McDonald and Goldstein, 1990; Zhang et al., 1990). The nod protein has been proposed to push nonexchange chromosomes toward the center of the spindle prior to anaphase I (Afshar et al., 1995). The ncd protein has a very different function. In Drosophila oocytes, the microtubules normally assemble around a condensed meiotic chromosome mass and then become organized into a bipolar spindle. ncd mutant oocytes form wide or multipolar spindles in meiosis I (Kimble and Church, 1983; Hatsumi and Endow, 1992*b*). This observation, combined with the fact that the ncd protein bundles microtubules in vitro (McDonald et al., 1990), has led to the hypothesis that, in vivo, the ncd protein works to organize microtubule ends into spindle poles in meiosis I (Hatsumi and Endow, 1992*a*).

The yeast motor protein Kar3p shows structural and functional similarities to ncd protein. Unlike most klps, both Kar3p and ncd protein contain their conserved motor domains at the carboxy terminus and their nonconserved regions at the amino terminus. In addition, both proteins have been shown to move toward the minus ends of microtubules in vitro (McDonald et al., 1990; Walker et al., 1990; Endow et al., 1994). *KAR3* was originally isolated in a screen for mutants defective for the fusion of gametic nuclei (karyogamy) in newly formed zygotes (Polaina and Conde, 1982). During karyogamy, Kar3p, in association with the protein Cik1, is found in the cytoplasm where it localizes to microtubules and SPBs (Meluh and Rose, 1990; Page et al., 1994). Kar3p may facilitate migration of the zygotic nuclei toward one another by cross-linking interdigitated cytoplasmic microtubules that radiate from SPBs within the two nuclei, and moving toward the minus end (the end at the SPB) of one microtubule while maintaining a hold on the other (Meluh and Rose, 1990). Additionally, Kar3p has the ability to depolymerize microtubules in vitro and may act at the SPB to shorten microtubules connecting the two nuclei (Endow et al., 1994). A characterization of spindle behavior in *kar3* cells in mitosis suggests that depolymerization may be an important in vivo function as well (Saunders et al., 1997*a*).

KAR3 is important, but not essential, for mitosis. Mitotic cultures of *kar3* mutants produce inviable large-budded cells containing short mitotic spindles (Meluh and Rose, 1990). Based on these phenotypes, it was proposed that *KAR3* is involved in spindle development. This model is supported by the demonstration that certain *kar3* alleles can suppress *cin8-3 kip1Δ* double mutants that have defects in mitotic spindle development (Saunders and Hoyt, 1992; Hoyt et al., 1993). A *kar3Δ* mutation in combination with a spindle checkpoint mutation (*mad2*, Li and Murray, 1991; *bub*, Hoyt et al., 1991) results in lethality, suggesting that in *kar3* mutants, checkpoint genes must be used to delay entry into anaphase while a functional mitotic spindle is developed (Roof et al., 1992).

Here we demonstrate that *KAR3* is essential for passage through meiosis I. *kar3* mutants exhibit a novel meiotic phenotype that suggests Kar3p participates in meiotic events that culminate in chromosome synapsis.

Materials and Methods

Genetic Procedures

Synthetic complete medium (CM) and rich yeast extract, peptone and dextrose medium (YPD) for growth of yeast strains have been described (Sherman et al., 1986). CM lacking one or more nutrient(s) is denoted by indicating the nutrient missing (e.g., CM-Arg is CM without arginine). Medium used to select for Can^r Cyh^r spores was CM-Arg-supplemented with cyclohexamide (10 µg/ml) and canavanine (60 µg/ml). For synchronous liquid sporulation, cells were grown at 30°C in presporulation medium (2% potassium acetate, 2% peptone, 1% yeast extract) to a concentration of ~5 × 10^7 cells/ml, washed in water, and incubated at the same density in 1% potassium acetate at 30°C with vigorous shaking (200 rpm). Presporulation medium and 1% potassium acetate were supplemented with nutrients for which the strain was deficient at one-fourth and one-half the concentrations recommended for CM, respectively.

Plasmids and Strain Constructions

Genotypes of all strains are as indicated in Table I. All strains used are S288C derivatives (Nicolas et al., 1989). MR820 (a gift from M. Rose, Princeton University, Princeton, NJ) contains a 3,461-bp PstI-BamHI *KAR3* fragment in a YCp50 vector. To construct pD154.12, the 3,692-bp NarI-BamHI fragment from MR820 was inserted between the NarI and BamHI sites of the *LEU2*, and *CEN3* vector, pUN105. pD153.9 was constructed by modifying MR820. A 1,528-bp BglII fragment from MR820 corresponding to the coding sequence for *KAR3* (amino acids 12–571) was replaced by a BamHI fragment that carries the *HIS3* gene.

To construct DJ1 + MR820, **a** and α haploids containing MR820 were transformed with the *Ssp*I-*Eco*RV *kar3Δ::HIS3* fragment from pD153.9. His$^+$ transformants were subjected to Southern blot analysis to confirm that the chromosomal *KAR3* locus had been replaced by the *kar3Δ::HIS3* fragment. These two haploid strains were mated to produce DJ1 + MR820. DJ1 was created by growing DJ1 + MR820 nonselectively at 25°C to allow isolation of derivatives that had lost MR820. DJ1 + pD154.12 was created by transforming DJ1 + MR820 with pD154.12 and then growing nonselectively to allow isolation of derivatives that had lost MR820. Dd519 + MR820 and Dd519 were constructed similarly to DJ1 + MR820 and DJ1. DC99-1, DC99-1C, DC152, and DC152B were constructed by standard genetic crosses.

Cytology

Cells were induced to undergo synchronous sporulation in liquid medium as described in *Genetic Procedures*. At desired times after transfer to 1% KoAc, 7–10 ml of culture were removed and processed for immunofluorescence or EM. Preparation of DJ1 and DJ1 + pD154.12 cells for tubulin immunofluorescence was as described (Pringle et al., 1991). Monoclonal rat anti–tubulin antibody (Accurate Chemical and Scientific Corp., Westbury, NY) was used at a dilution of 1:200. Anti–rat FITC-conjugated secondary antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) was used at a dilution of 1:200. Mounting medium containing 25 µg/ml of 4′, 6-diamidino-2-phenylindole dihydrochloride (DAPI) was added and slides were viewed by fluorescence microscopy on a microscope using a 100× plan fluor lens (Axioscop; Carl Zeiss, Inc., Thornwood, NY).

Preparation of Dd519 and Dd519 + MR820 cultures for chromosome spreads was as previously described (Dresser and Giroux, 1988). The spreading procedure used was that described by (Loidl et al., 1991), except the slides were coated with poly-L-lysine before the addition of cells. Anti-Zip1 immunostaining was performed as in Sym et al. (1993), except that Zip1p antibody was used at a dilution of 1:100 in PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na$_2$HPO$_4$, 1.4 mM KH$_2$PO$_4$, 2% BSA).

For visualization of SCs by EM, 7–10 ml of cells were removed 14–16 h after cells were induced to sporulate, and were prepared for EM as in Loidl et al. (1991).

Commitment to Heteroallelic Interhomologue Recombination

Interhomologue recombination was measured by methods previously described (Sherman and Roman, 1963; Esposito and Esposito, 1974). Isogenic *KAR3* (Dd519 + MR820) and *kar3Δ* (Dd519) strains heteroallelic for mutations at *arg4* were induced to undergo synchronous sporulation. Aliquots were removed at timed intervals following meiotic induction and

Table I. Strains and Plasmids Used in this Study

Strain	Genotype	
DJ1	*MATα leu2::LYS2 PGK::TRP1 trp1-289 CAN1 ura3-52 cyh2 lys2ΔB his3Δ1 ADE2 kar3Δ::HIS3*	
	MATa leu2-3,112 PGK trp1-289 can1 ura3-52 CYH2 LYS2 his3Δ1 ade2 kar3Δ::HIS3	
Dd519	*MATα leu2 PGK::TRP1 trp1-289 CAN1 ura3-52 cyh2 arg4-Δ57,RV his3Δ1 kar3Δ::HIS3*	
	MATa LEU2 PGK trp1-289 CAN1 ura3-52 CYH2 arg4-Δ42 his3Δ1 kar3Δ::HIS3	
TC12	DJ1 carrying pUN105	
DC99-1	*MATa leu2 PGK::TRP1 trp1-289 ura3-52 arg4-ΔHpa rad50SK181::URA3 his3Δ1 ADE2 kar3Δ::HIS3*	[+]pD154.12
	MATα leu2-3,112 PGK trp1-289 ura3-52 arg4-ΔHpa rad50SK181::URA3 HIS3 ade2 kar3Δ::HIS3	
DC99-1C	*MATa leu2 PGK::TRP1 trp1-289 ura3-52 arg4-ΔHpa rad50SK181::URA3 his3Δ1 ADE2 kar3Δ::HIS3*	
	MATα leu2-3,112 PGK trp1-289 ura3-52 arg4-ΔHpa rad50SK181::URA3 HIS3 ade2 kar3Δ::HIS3	

plated onto YPD plates to measure the total cell viability and onto CM-Arg plates to measure the number of Arg[+] recombinants. The frequency of interhomologue recombination was calculated by dividing the total number of Arg[+] colony forming units (CFUs) by the total number of viable cells.

Analysis of Double-Strand Breaks

For experiments using the *rad50S* strains DC99-1 and DC99-1C, cells were sporulated and, at each time point, 25 ml were removed, mixed with 25 ml of 95% ethanol and 1.25 ml of 0.5 M EDTA, and stored at −20°C overnight. Chromosomal DNA was extracted as described (Rocco et al., 1992). Approximately 5 μg of each sample were digested with BglII, subjected to electrophoresis, and then transferred to a nylon membrane (Boehringer Mannheim Corp., Indianapolis, IN) for Southern analysis (Southern, 1975). The *ARG4* probe used was a 3.3-kb PstI fragment from pNPS558 (gift from N. Schultes and J. Szostak, Massachusetts General Hospital, Boston, MA). The *THR4* probe used was a 4.9-kb PstI fragment from pMJ338 (gift from M. Lichten, National Institutes of Health, Bethesda, MD). Probe fragments were radioactively labeled using the Stratagene Prime-It system (La Jolla, CA).

DNA fragments were quantified using a Molecular Dynamics Phosphor Imager (Sunnyvale, CA). The DSB frequency was estimated by dividing the amount of radioactivity in the major DSB fragment by the total amount of radioactivity in that lane. The rates of DSB formation were calculated by determining the slope of the curves after normalizing for the maximal frequency of DSBs.

The approaches described above were also used to examine DSBs in the *RAD50* strains Dd519 and Dd519 + MR820 immediately after transfer to sporulation medium and at intervals after transfer to sporulation medium.

Results

Spindle Morphology of kar3 Mutants

It has been reported that *kar3* null mutants fail to produce spores when assayed using bright field microscopy (Hoyt et al., 1993; Kurihara et al., 1996). To determine the stage of the meiotic block, homozygous *kar3* mutant cells were analyzed by observing the spindle morphology and DNA staining patterns throughout meiosis (Fig. 1). At 5 h after the induction of meiosis, wild-type cells exhibit tubulin arrays typical of yeast prophase spindles (microtubules emanating from duplicated, unseparated SPBs). At the same
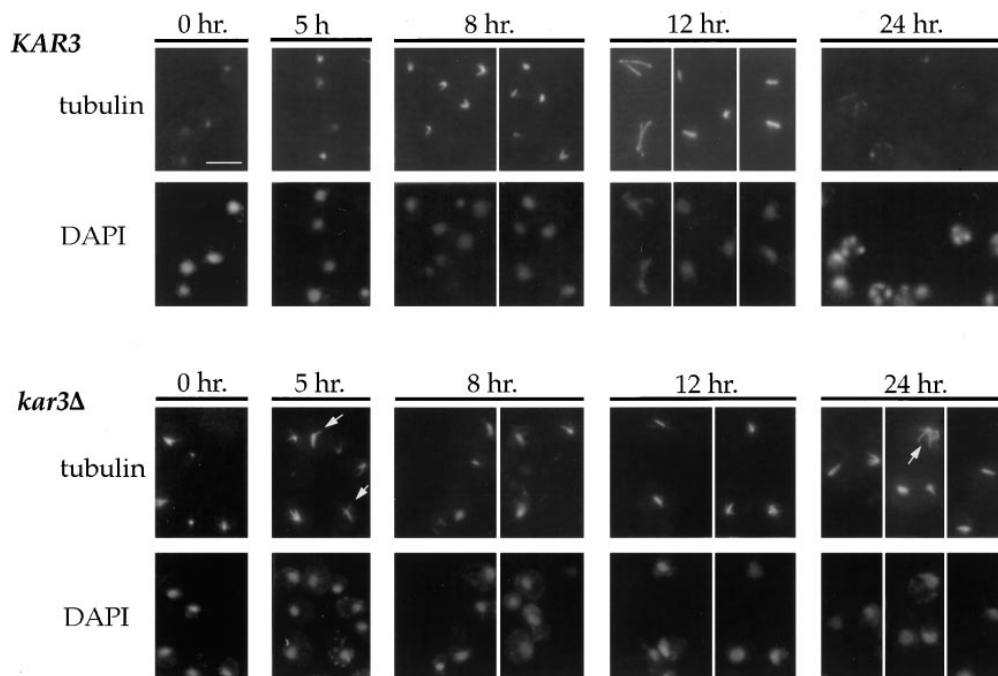

Figure 1. Analysis of tubulin and DNA staining pattern of *KAR3* and *kar3* cells throughout meiosis. Aliquots of isogenic wild-type (DJ1 + pD154.12) and *kar3* (DJ1) cells were fixed at the indicated times after transfer to sporulation medium and stained with antitubulin antibodies and the DNA-specific dye DAPI. Antitubulin and DAPI images of representative cells are shown for both wild-type (*top*) and *kar3* (*bottom*) time courses. Arrows in the 5-h *kar3* samples indicate pronounced cytoplasmic projections. The arrow in the 24-h *kar3* panel identifies a highly abnormal spindle. Small numbers of unusual spindles were observed in all time points for the *kar3* culture. Bar, 10 μm.

time, most mutant cells possess similar tubulin arrays that appear slightly longer than those in wild-type cells. Additionally, the mutants often have elongated cytoplasmic microtubules that are not seen in wild-type cells (Fig. 1, *5 h*), indicating that the *kar3* mutation has an effect early in prophase. By 12 h most wild-type cells have spindles characteristic of metaphase I or later stages of meiosis, and at 24 h the wild-type cells have nearly all formed tetrads. In contrast, *kar3* mutants continue to exhibit bush-like tubulin staining. At late time points (24 h and beyond, not shown), monopolar arrays with numerous and/or very long projections become more common (an example is shown in the 24-h time point, Fig. 1).

Staining of DNA with DAPI was also used to monitor progress through meiosis. Binucleate and tetranucleate cells were extremely rare in the mutant at late time points, indicating that these cells do not progress beyond anaphase I. To quantitatively demonstrate the lack of postprophase spindles in the mutant, we counted the number of recognizable postprophase cells compared to the total number of cells for both the wild type and mutant throughout meiosis. Only cells that exhibited unquestionable postprophase morphologies were classified as postprophase. Generally, anaphase spindles, some metaphase spindles, and binucleate and tetranucleate nuclei were easily distinguished. For wild-type cells, the fraction classified as postprophase was an underestimate as not all cells stained adequately for classification, and for others, critical features were not in the plane of focus in the photographs used for the quantification. The results shown in Fig. 2 indicate that by 12 h there is a significant difference in the number of postprophase cells for the mutant versus the wild type, and at 24 h no postprophase cells are observed in the *kar3* mutants whereas 21.5% of the wild-type cells show unquestionable progression beyond prophase.

kar3 Null Mutants Fail to Produce Haploid Meiotic Products

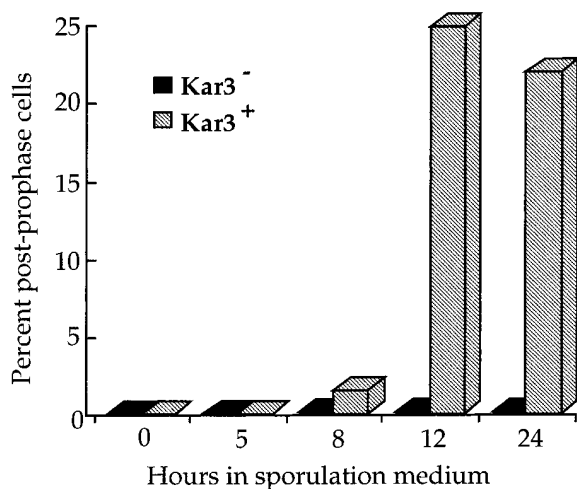To quantitatively measure whether the meiotic block in *kar3*

Figure 2. kar3 mutant cells are blocked before metaphase I. The frequency of cells exhibiting unquestionable postprophase I morphology was determined for both the wild-type and *kar3* cells. $n \geq 28$ for each time point.

mutants was absolute, we designed a strain that would allow us to measure the efficiency with which a *kar3* mutant produces viable haploid meiotic products compared to a *KAR3* strain. Briefly, a homozygous *kar3Δ::HIS3* diploid strain (TC12) was heterozygously marked for both canavanine and cyclohexamide resistances (*CAN1/can1*, *CYH2/cyh2*). In both cases, the sensitive allele is dominant; therefore, with the exception of rare double recombinants, these diploids are unable to grow on medium containing canavanine and cyclohexamide. One quarter of the spores produced by such a diploid will inherit both the *can1* and *cyh2* genes and will grow in the presence of both compounds. In addition, the diploid strain is heterozygous for the *ADE2* gene (*ADE2/ade2*) and produces white colonies, but haploid cells that have inherited only the recessive *ade2* allele will produce red colonies. The haploidization frequency of this *kar3* diploid was measured by inducing a culture to undergo synchronous meiosis and plating cells at timed intervals to measure the ratio of Ade⁻, Cyhʳ, Canʳ cells to the total number of viable cells (Fig. 3). An isogenic strain carrying a single copy number plasmid with the wild-type *KAR3* gene (DJ1 + pD154.12) was used as the wild-type control. The wild-type culture began to produce Ade⁻, Canʳ, Cyhʳ cells at 8 h, and by 48 h 9.18% of the cells had this phenotype (100% sporulation would be expected to yield ~12.5% Canʳ, Cyhʳ, Ade⁻ cells). No Ade⁻, Canʳ, Cyhʳ colonies were ever detected for the mutant even after 3 d in sporulation medium (Fig. 3), indicating that the *kar3* defect causes a robust block of the meiotic process.

kar3 Mutants Arrest Reversibly in Meiosis

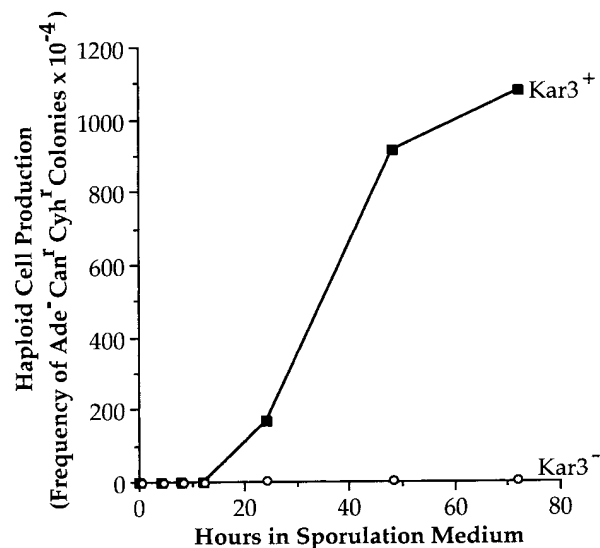Whereas *kar3* mutants appear to arrest during meiosis,

Figure 3. kar3 mutants fail to produce haploid meiotic products. Isogenic *kar3* (TC12) and *KAR3* (DJ1 + pD154.12) cultures heterozygous for *ade2*, *cyh2*, and *can1* were pregrown and induced to undergo meiosis as described in Materials and Methods. At the times indicated, aliquots were removed and plated onto YPD and CM-Arg + cyclohexamide + canavanine. The parameter plotted is the fraction of Ade⁻, Canʳ, Cyhʳ CFUs at each time point relative to the total CFUs on YPD plates. For the *kar3* mutants, at least 13,000 cells were plated at each time point. Ade⁻, Cyhʳ, Canʳ colonies were never observed for the *kar3* mutants.

they remain viable in sporulation medium. The total number of viable cells in the above experiments was determined by plating on rich nonselective medium that supports vegetative growth at each time point. Viability of the *kar3* mutants remains high for the first 8 h of meiosis and then gradually decreases. In a series of experiments, ≥50% of *kar3* cells remain viable between 24–48 h of meiosis (data not shown). This demonstrates that although the *kar3* cells are blocked in meiosis so that they cannot produce viable spores, the meiotic arrest is overcome when the cells return to mitotic growth.

Commitment to Heteroallelic Interhomologue Recombination in kar3 Mutants

Commitment of cells to meiotic levels of interhomologue recombination is a landmark event of meiosis that occurs in prophase I. To assay for interhomologue recombination, the frequency of prototroph formation at heteroallelic loci is measured at timed intervals in meiosis by returning meiotically-induced cells to vegetative growth (Sherman and Roman, 1963; Esposito and Esposito, 1974). Using a strain containing a pair of closely linked *arg4* heteroallelic mutations, we were able to quantify the frequency of interchromosomal recombination events by selecting for Arg⁺ cells. The *kar3* version of this strain was unable to commit to wild-type levels of heteroallelic recombination after 27 h, by which time the *KAR3* control strain had reached maximal levels of recombination (Fig. 4). The mutant exhibited a gradual accumulation of Arg⁺ cells. By 27 h, the *kar3* mutants showed ~5% of wild-type levels of recombinants.

Double-Strand Break Formation in kar3 Mutants

The reduced commitment to recombination could be an indication of a failure to either initiate or resolve recombination events. DSBs in the DNA are the initiating events in meiotic recombination and can be monitored using Southern analysis to detect the appearance of diagnostic restriction fragments. The ability of a *kar3* mutant to form DSBs was measured by monitoring the accumulation of DSB products throughout meiosis in the region upstream of the *ARG4* gene which contains hot spots for meiotic recombination (Sun et al., 1989). Identification of break products is facilitated by the presence of the *rad50S* mutation which allows DSBs to form but prevents their processing, resulting in the accumulation of what would otherwise be a transient product (Alani et al., 1990). The interhomologue recombinants measured in the commitment to recombination assay (above) are thought to arise from events initiated at DSBs. Restriction fragments diagnostic of the DSB products were apparent in both a *KAR3* wild-type and *kar3* mutant strain (Fig. 5 *A*). In the mutant, the breaks accu-
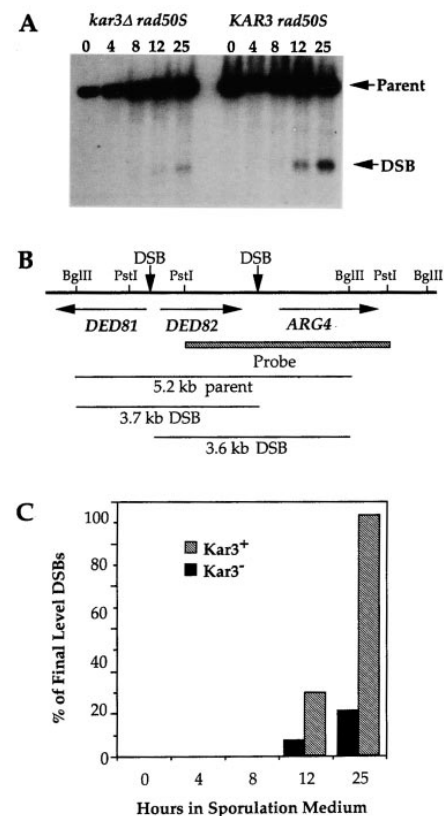
Figure 5. *kar3* cells are capable of initiating DSBs. Isogenic *KAR3* (DC99-1) or *kar3* (DC99-1C) cultures, homozygous for the *rad50S-K181* allele were pregrown and induced to undergo synchronous meiosis. Aliquots were removed at the number of hours indicated after transfer to sporulation medium and DNA was extracted. The DNA was digested with *Bgl*II, subjected to electrophoresis on a 0.7% agarose gel, and analyzed by Southern blot hybridization using as a probe the *ARG4* sequences indicated in (*B*). (*A*) The predominant DSB and parental fragments are labeled with arrows. (*C*) Quantification of the DSB products. The fraction of radioactivity in each lane that was localized to the DSB products was quantified. The percent of DSB product in each lane was normalized to the final level of DSB product produced in the *KAR3* strain. In the *kar3* mutants, the DSBs accumulate to 18.6% of levels detected in the isogenic *KAR3* strain.
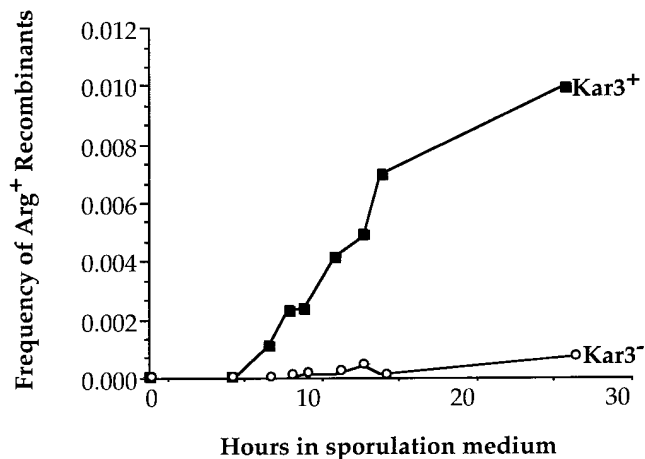
Figure 4. *kar3* cells undergo interhomologue recombination at severely reduced levels. Isogenic cultures of *KAR3* (Dd519 + MR820) and *kar3* (Dd519) strains, heterozygous for a pair of *arg4* heteroalleles (*arg4RV* and *arg4Δ42*), were induced to undergo synchronous meiosis as described in Materials and Methods. Aliquots were removed at the indicated times and plated onto CM-Arg to determine the number of Arg⁺ recombinants. Aliquots were also plated onto YPD or CM to determine total CFUs. Plotted is the ratio of Arg⁺ CFUs to total CFUs. There was little loss of viability throughout the course of this experiment. The *kar3* cells showed ~5% of wild-type levels of recombination at 27 h.

mulated to 18.6% of wild-type levels (Fig. 5 *C*). The appearance of DSBs in the *kar3* mutant demonstrates that, although this strain is unable to commit to wild-type levels of meiotic recombination, it is capable of initiating substantial levels of DSBs in a *rad50S* background. To verify that the differences in DSB accumulation are not unique to the *ARG4* locus, we quantified DSBs upstream of the *ARE1* gene on chromosome *III*, another hot spot for recombination (Goldway et al., 1993; Baudat and Nicolas, 1997). In a *kar3* mutant, breaks accumulate to 33% of the level detected in an isogenic *KAR3* strain (data not shown). For both the *ARG4* and *ARE1* loci, DSBs accumulate to a lesser amount in a *kar3* mutant than in an isogenic *KAR3* strain in the time span over which the breaks were assayed.

Certain mutants (e.g., *rad50S* and *dmc1*) able to form, but not properly process, DSBs accumulate broken DNA strands and exhibit a prophase arrest characterized by reduced levels of commitment to heteroallelic recombination (Alani et al., 1990; Bishop et al., 1992), phenotypes similar to those of *kar3* mutants. To determine whether a *kar3* mutation alone causes accumulation of DSBs, we tested for the appearance of DSBs in a strain that was wild-type at the *RAD50* locus. Low levels of restriction fragments diagnostic of DSB formation appeared transiently in the *kar3* mutants, concomitant with their appearance in the wild-type control (not shown). The transient nature of the diagnostic restriction fragments suggests the DSBs can be repaired or processed to some other intermediate in the *kar3* mutants.

kar3 Mutants Fail to Form Mature SC

Many mutations that prevent interhomologue recombination also prevent complete SC formation. To test the ability of *kar3* mutants to form SC, we immunostained spread meiotic chromosomes harvested 14 h after induction of meiosis with antibody to Zip1, a component of the SC (Sym et al., 1993). Approximately equal fractions of mutant and wild-type nuclei showed Zip1 staining, indicating that the *kar3* mutation does not block progression to this stage. In wild-type samples, most Zip1-stained nuclei (79%, $n = 160$) exhibited worm-like structures indicative of full-length SC. In many of these nuclei ~16 synapsed pairs were clearly evident (Fig. 6 *a*). Mutant nuclei exhibited a range of Zip1 staining patterns, though none showed characteristic full-length SC staining. 46% ($n = 129$) of the *kar3* Zip1-stained nuclei showed numerous dots or elongated signals indicative of partial or discontinuous SC formation (Fig. 6, *b* and *c*; the remaining 56% showed largely homogeneous nuclear staining). ~25% of the *kar3* cells (32 of 129 spreads) with Zip1 staining contained a single brightly stained structure (Fig. 6, *b* and *c*), which probably indicates the presence of a polycomplex. These structures were seen in <3% of the wild-type spreads (4 of 160) at the same time point. Polycomplexes are common in pachytene nuclei of mutants unable to assemble SC (Alani et al., 1990; Bishop et al., 1992; Loidl et al., 1994), or can result from overexpression of Zip1p (Sym and Roeder, 1995). The SC defect of the *kar3* mutants does not improve over time. At a later time point, when >50% of the *KAR3* cells have produced tetrads, the DNA in the *kar3* mutants has
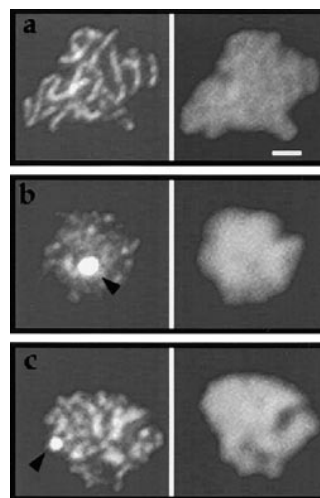

Figure 6. Zip1 staining pattern of *kar3* cells. Aliquots of isogenic *KAR3* (Dd519 + MR820) and *kar3* (Dd519) meiotic cultures were harvested at pachytene, 14 h after induction of sporulation. Nuclei were spread and stained with anti-Zip1 and the DNA-specific dye, DAPI. (*a*) The left panel shows the Zip1 staining pattern of a typical wild-type pachytene nucleus; the corresponding staining with DAPI is shown on the right. (*b* and *c*) The left-hand panels are Zip1-stained nuclei representative of those with dots or elongated signals. The corresponding DAPI images are shown on the right. Brightly stained foci indicative of polycomplexes were observed in 25% of the *kar3* cells, and 2.5% of the wild-type cells (*arrows*). Bar, 1 μm.

decondensed and the Zip1 staining is barely visible (data not shown).

Consistent with the Zip1 staining, observation of silver-stained pachytene spreads with the electron microscope demonstrated a variety of SC phenotypes for the *kar3* mutants, ranging from completely unstructured to nearly wild-type levels of SC formation (Fig. 7). Maximal SC structures in the wild-type and in the *kar3* mutants appear simultaneously. Whether the SC present in the mutants represents small regions of synapsis among homologues or regions of illegitimate synapsis between nonhomologous chromosomes cannot be determined from this experiment. The electron microscopic observation of the mutants also revealed densely-stained bodies in many cells; these may represent polycomplexes. In many of the mutant and wild-type samples, duplicated but unseparated SPBs have been preserved and are visible in the mutant nuclei (Fig. 7, *E* and *F*). The timing of the appearance and subsequent disappearance of SC-related structures is similar in the wild-type and in the *kar3* mutants, yet the mutants fail to assemble metaphase spindles. This indicates that the mutants can proceed through, but cannot exit from, prophase.

Discussion

Kar3p is a versatile protein. It acts in the cytoplasm to mediate nuclear fusion when zygotes are formed (Meluh and Rose, 1990; Page et al., 1994), and in mitosis it localizes to the nucleus where it likely plays an important role in spindle development (Meluh and Rose, 1990; Roof et al., 1992; Page et al., 1994; Saunders et al., 1997*a,b*). Here we demonstrate that *KAR3* is required for progression through meiosis I. Our data are consistent with a model in which Kar3p function is a prerequisite for homologous chromosome synapsis.

The difference between the mitotic and meiotic phenotypes of *kar3* mutants raises the question of whether Kar3p performs the same role in these two types of chromosome segregation. In meiosis, *kar3* cells arrest before the devel-
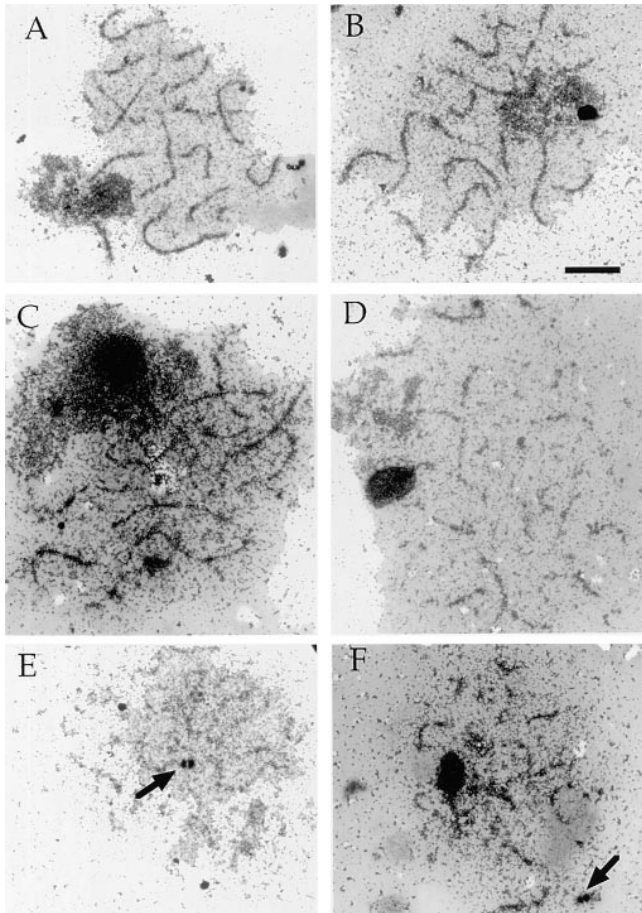
Figure 7. Electron micrographs of pachytene nuclei. Isogenic *KAR3* (Dd519 + MR820) and *kar3* (Dd519) nuclei were harvested at pachytene and spread as indicated in Materials and Methods. (*A* and *B*) SCs from wild-type nuclei. (*C–F*) Examples of representative SC morphologies detected in *kar3* cells. Some cells exhibit a large degree of synapsis (*C*), yet others show little to no SC (*E*). Duplicated, but unseparated SPBs from *kar3* cells are apparent in *E* and *F* (*arrows*). Bar, 1 μm.

opment of bipolar metaphase spindles, whereas the arrest exhibited by a fraction of cells in vegetative *kar3* cultures features metaphase spindles (Meluh and Rose, 1990; Roof et al., 1992). In our strain background, pedigree analysis reveals that about one in three mitotic cell divisions produces a daughter that arrests, usually as a large-budded cell (Shanks, R., and D. Dawson, unpublished observations). If the *kar3* mitotic and meiotic arrests are the same, then one would expect arrested meiotic cells to be inviable when returned to vegetative growth conditions. However, *kar3* mutant cells arrested in meiosis can resume mitotic growth with nearly 100% efficiency. The differences between the mitotic and meiotic phenotypes of *kar3* mutants could be attributable to *KAR3* performing different functions in the two processes or, alternatively, to differences in the way the two processes are affected by a common defect. Regardless, it is clear from our results that *KAR3* affects functions that are unique to meiosis and cause a prophase I arrest.

Though *KAR3* encodes a motor protein, the *kar3* meiotic arrest is most reminiscent of those exhibited by certain

recombination mutants. *kar3* mutants, like meiotic recombination mutants that are able to form but not properly process DSBs, show low commitment to heteroallelic recombination, failure to assemble a complete SC, and failure to complete the first meiotic division. Unlike these mutants, however, *kar3* mutants do not cause DSBs to accumulate. In addition, spindle-associated defects in chromosome metabolism mutants such as *rad50S* and *dmc1* have been reported to occur later than the aberrant tubulin arrays in *kar3* mutants (Alani et al., 1990; Bishop et al., 1992).

DSBs are formed in *kar3* mutants, but the level of DSBs was reduced from that of wild type. The reduction in DSB formation in *kar3* strains seems likely to be an indirect result of the *kar3* defect. One explanation stems from recent demonstrations that DSB levels are affected by homologous pairing interactions. Haploid chromosomes and loci without homologous pairing partners show reduced levels of DSB formation (de Massy et al., 1994; Xu et al., 1995; Rocco and Nicolas, 1996). For the two loci we have observed, our DSB results are consistent with the model that *kar3* mutants are defective for meiotic homologous chromosome interactions that lead to full levels of DSB formation. The reduced frequency of DSBs alone does not explain the reduced level of heteroallelic recombination observed in the *kar3* mutant (19% of wild-type DSB formation at *ARG4*, but 5% of wild-type heteroallelic recombination). Many explanations might account for this. First, this result would be obtained if higher levels of DSBs occur in a *rad50S* background (in which breaks were quantified) than in a wild-type background (in which commitment assays were performed). Second, the recombination machinery might be altered in *kar3* mutants, such that heteroallelic recombinations are less likely to yield an Arg$^+$ product. Finally, the disparity may reflect an inability of DSBs to be converted into interhomologue events in the *kar3* background. This might be expected in a mutant defective in establishing homologous pairing interactions. In the absence of a homologous partner, breaks might be repaired from sister chromatid. Such repair events would go undetected in the commitment assay.

In addition to the recombination defects, *kar3* mutants show a defect in SC formation as assayed by EM and immunofluorescence using antibodies against Zip1, a component of the SC. This indicates that *KAR3* is required for events preceding chromosome synapsis. A relationship between recombination and SC formation has been well established in *Saccharomyces cerevisiae.* SC formation is likely dependent on recombination; mutants unable to initiate interhomologue recombination are also defective in SC formation (for review see Bascom-Slack et al., 1997). The meiotic phenotype of *kar3* mutants is like that exhibited by a number of recombination mutants in that SC formation is incomplete. More SC is formed in *kar3* mutants than in mutants that abolish recombination, such as *rad50* and *spo11* (Giroux et al., 1989; Alani et al., 1990). It has been proposed that synapsis is dependent on the initiation and maturation of recombination among homologous chromosomes (Roeder, 1990; Kleckner and Weiner, 1993). The amount of SC in a given *kar3* nucleus might, therefore, be indicative of the number of successful interhomologue recombination events in that nucleus.

What is the basis of the terminal meiotic phenotype in *kar3* mutants? In mitotic cells, Kar3p has been implicated in spindle development; spindle checkpoint genes are essential for viability of *kar3* mutants. Is the spindle checkpoint mechanism responsible for the arrest? The *kar3* mutants exhibit clear microtubule abnormalities, though it is possible this is partially due to, rather than a cause of, the arrest. Some pachytene-arrested recombination mutants develop bush-like microtubule arrays (Alani et al., 1990); *kar3* mutants may share this tendency of arrested meiotic cells to accumulate aberrant microtubule arrays. The meiotic roles of the known mitotic spindle checkpoint genes have yet to be demonstrated (Hoyt et al., 1991; Li and Murray, 1991). If the mitotic checkpoint genes are responsible for the *kar3* meiotic arrest, then the arrest point is different from their mitotic arrest point; *kar3* mutants are blocked in prophase whereas mitotic spindle checkpoint blocks cell cycle progress at a later stage (onset of anaphase) (Hoyt et al., 1991; Li and Murray, 1991).

The recombination defects of the *kar3* mutants suggest that the terminal phenotype might also be due to a recombination checkpoint mechanism. The meiotic prophase arrest of *dmc1* mutants, which shows several parallels to the *kar3* arrest, has been shown to be mediated by the *RAD17*, *RAD24*, and *MEC1* genes that monitor DNA integrity in mitosis (Lydall et al., 1996).

The demonstration that a microtubule-dependent motor is required to achieve normal meiotic recombination establishes the importance of microtubules for meiotic events that occur well before a bipolar spindle is assembled. Studies in a variety of organisms have provided cytological evidence that chromosome positioning early in meiosis might be important for achieving subsequent synapsis (for review see Loidl, 1990). The bouquet structure, in which the ends of the chromosomes are clustered along a small region of the nuclear envelope, is generally observed during meiotic prophase before full-length SC formation in a variety of organisms (for review see Dernburg et al., 1995). These data have led to the notion that the bouquet arrangement facilitates chromosome pairing by bringing the ends of chromosomes into close proximity. In *Schizosaccharomyces pombe* where meiosis immediately follows karyogamy (zygotic meiosis), the telomeres lead dramatic chromosome movements throughout karyogamy and early meiosis. These movements appear to result in a clustering of the telomeres near the SPBs. In *S. cerevisiae*, homologous chromosomes are roughly aligned in mitosis, lose this organization in premeiotic S phase, and become realigned in meiotic prophase. It is tempting to speculate that a protein required for both karyogamy and meiosis in *S. cerevisiae* may play a role in spindle and chromosome movements analogous to those observed during karyogamy and early meiosis in *S. pombe.*

Though no specific nuclear organization of meiotic chromosomes has been observed in *S. cerevisiae*, EM and fluorescence in situ hybridization (FISH) experiments have indicated that at some meiotic stages, nonhomologous telomeres are in closer proximity than nonhomologous interstitial regions (Dresser and Giroux, 1988; Weiner and Kleckner, 1994). Similarly, genetic experiments that tested the frequency with which pairs of homologous sequences recombined, when placed in assorted locations on homologous and heterologous pairs of chromosomes, provided evidence for chromosomal alignment before recombination (Goldman and Lichten, 1996). The low level of inter-homologue recombination measured in the *kar3* strains is similar to that observed between homologous sequences placed on heterologous chromosomes–in both cases nonalignment of the chromosomes can be used to explain the observed low recombination frequency.

We propose that Kar3p acts in meiotic prophase to mediate homologous chromosome interactions either indirectly, by promoting early microtubule organization, or directly, by positioning chromosomes in a microtubule-dependent manner in the early prophase nucleus. The favorable arrangement of chromosomes in the prophase nucleus might be critical for two key meiotic events. First, the alignment of homologous chromosomes might place homologous sequences in the same subregions, thereby reducing the effective volume that must be searched to achieve the homologous DNA–DNA contacts that lead to meiotic recombination. Second, rough alignment of homologues might be a precondition for efficient synapsis. The alignment of homologues would minimize the movement required for synapsis and would reduce the chance that heterologous chromosomes would be entangled between synapsed pairs.

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