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Saccharomyces cerevisiae Mer3 Is a DNA Helicase Involved in Meiotic Crossing Over

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Crossing over is regulated to occur at least once per each pair of homologous chromosomes during meiotic prophase to ensure proper segregation of chromosomes at the first meiotic division. In a *mer3* deletion mutant of *Saccharomyces cerevisiae*, crossing over is decreased, and the distribution of the crossovers that occur is random. The predicted Mer3 protein contains seven motifs characteristic of the DExH box type of DNA/RNA helicases. The *mer3G166D* and the *mer3K167A* mutation, amino acid substitutions of conserved residues in a putative nucleotide-binding domain of the helicase motifs caused a defect in the transition of meiosis-specific double-strand breaks to later intermediates, decreased crossing over, and reduced crossover interference. The purified Mer3 protein was found to have DNA helicase activity. This helicase activity was reduced by the *mer3GD* mutation to <1% of the wild-type activity, even though binding of the mutant protein to single- and double-strand DNA was unaffected. The *mer3KA* mutation eliminated the ATPase activity of the wild-type protein. These results demonstrate that Mer3 is a DNA helicase that functions in meiotic crossing over.

During meiosis, two successive rounds of chromosome segregation take place following a single round of DNA replication. At the first meiotic division (meiosis I), pairs of homologous chromosomes (homologs) are synapsed and then segregated to opposite poles. In the prophase of meiosis I, DNA double-strand breaks (DSBs) are formed in a genetically programmed way, and two types of recombination events occur between homologs (3, 31, 45). Crossing over results in reciprocal exchanges of chromosome arms and serves to link the homologs to ensure proper segregation. The other type of recombination, gene conversion, is defined as nonreciprocal exchange of genetic information. To ensure that every pair of homologs sustains at least one crossover, the distribution of crossovers along and among chromosomes is regulated. The regulation of the distribution of crossovers along a chromosome is inferred from the observation of crossover interference, where multiple crossovers are less frequent than expected based on the frequency of single crossovers (38). The distribution of crossovers along and among chromosomes likely represents different manifestations of the same underlying regulation (10, 13, 29, 58). Despite the fact that crossing over is a key process required for faithful segregation of chromosomes during meiosis, the molecular mechanism of the process and its regulation remain elusive.

In *Saccharomyces cerevisiae*, there are a number of genes required for normal frequencies of crossing over that are not required for gene conversion, including *ZIP1*, *ZIP2*, *ZIP3*, *MSH4*, *MSH5*, *MLH1*, *MLH3*, and *MER3* (1, 11, 25, 26, 41, 47, 57, 63). In addition to being required for normal frequencies of crossing over, *ZIP1* and *MER3* appear to be required to regulate the distribution of crossovers (41, 58). Meiosis-specific DSBs appear for a prolonged period of time in the absence of *ZIP1* and *MER3*, suggesting that they have a role in the transition of DSBs to later intermediates (41, 54). Among these genes, the *MER3* gene is of particular interest because the predicted Mer3 amino acid sequence contains the seven motifs characteristic of the DExH box type of DNA/RNA helicases (41). The Mer3 helicase motifs show significant homology to the DNA helicases encoded by yeast *SGS1* and human *BLM* (the gene mutated in Bloom syndrome) (15, 18, 65). Previous studies have shown that such DNA helicases are involved in DNA recombination, replication, and cell cycle control (16, 19, 34, 64). These observations suggest that Mer3 is likely a DNA helicase that functions in meiotic recombination.

Here, we have characterized the phenotype of *mer3* point mutants that have amino acid substitutions in a putative nucleotide-binding domain thought to be required for helicase activity and found that these mutants show a defect in the transition of DSBs to later intermediates, a decreased frequency of crossing over, impaired crossover interference, and a partial reduction in spore viability. These effects are similar to those caused by a *mer3* deletion mutation, which in addition causes severe defects in progression through meiosis and in the production of viable spores. The Mer3 protein was purified and was found to have DNA helicase activity. The mutant Mer3GD protein was found to lack helicase activity, but it retained normal binding activity to single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA). The mutant mer3KA proteins showed no detectable ATPase activity. These results indicate that the Mer3 helicase activity plays a critical role in crossover control during meiosis.

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MATERIALS AND METHODS

Yeast strains, media, and genetic procedures. Yeast strains used are listed in Table 1. General genetic manipulations and preparation of media were performed according to the method of Sherman et al. (52). Transformation with

TABLE 1. Yeast strains used in this study

Strain[a]	Genotype
RKY1293	*MATα ura3-52 trp1 leu2Δ1 his3Δ200 pep4::HIS3 prb1Δ1.6R can1 GAL*
RKY4220	*MAT***a** *leu2::hisG ho::LYS2 lys2 can1 URA3 HOM3 TRP2 MER3* *MATα leu2::hisG ho::LYS2 lys2 CAN1 ura3 hom3-10 trp2 mer3::hisG*
RKY4221	RKY4220, except *mer3::hisG* *mer3::hisG*
RKY4222	RKY4220, except *mer3GD* *mer3::hisG*
RKY4223	RKY4220, except *mer3KA* *mer3::hisG*
RKY4227	RKY4220, except *MER3-FLAG* *mer3::hisG*
TNY821	*MAT***a** *leu2::hisG ho::LYS2 lys2 his4X::LEU2-URA3 arg4-bgl MER3* *MATα leu2::hisG ho::LYS2 lys2 his4B::LEU2 arg4-nsp mer3::hisG*
TNY822	TNY821, except *mer3::hisG* *mer3::hisG*
TNY823	TNY821, except *mer3GD* *mer3::hisG*
TNY824	TNY821, except *mer3KA* *mer3::hisG*

[a] RKY1293 was obtained from the Yeast Genetic Stock Center, Berkeley, Calif., where it is identified as BJ5464. RKY4220, RKY4221, RKY4222, RKY4223, and RKY4227 are congenic with SK1. RKY4221 is identical to TNY368 (41). TNY821, TNY822, TNY823, and TNY824 are derived from SK1.

plasmids and gene disruption and substitution constructs was performed by the lithium acetate procedure (27), and the correct genotype was verified by PCR analysis and DNA sequencing of the substitution site. To introduce *mer3GD-URA3-mer3*, *mer3KA-URA3-mer3*, and *mer3-URA3-mer3-FLAG*, relevant yeast haploid strains were transformed with *Dra*I-digested pRDK4153, *Eco*RI-digested pRDK4154, and *Afl*II-*Sal*I-digested pRDK4155, respectively. To obtain *URA3* popouts, uracil auxotrophs were selected by plating cells on SD plates supplemented with 5-fluoroorotic acid. Sporulation medium was prepared, and synchronous meiotic cultures were obtained as previously described (41), except that to obtain 23°C meiotic cultures, the temperature was shifted from 30°C to 23°C when the cells were transferred from YPA to sporulation medium.

Detection of meiosis-specific DNA DSBs. DNA samples were prepared, and meiosis-specific DSBs in the *his4*::*LEU2* region of chromosome III were detected by Southern blotting as essentially described before (41). DNA was digested with *Pst*I, separated by electrophoresis on a 0.7% agarose gel in TAE buffer (40 mM Tris acetate, 1 mM EDTA), and transferred to Hybond-N^+ nylon membranes (Amersham Pharmacia Biotech). A 1.5-kb *Pst*I-*Eco*RI fragment from pNKY291 (8) labeled with [α-^{32}P]dATP by the random primer method was used as a probe for Southern hybridization. The signals were detected with a Fuji BAS2000 phosphorimager (Fuji) and were quantified with Image Gauge V3.3 (Fuji).

Plasmids. The plasmids containing the *mer3GD-URA3-mer3* (pRDK4153), *mer3KA-URA3-mer3* (pRDK4154), and *mer3-URA3-mer3-FLAG* (pRDK4155) constructs were created by using the pRDK4151 vector. To create pRDK4151, a 2.5-kb *Eco*RI-*Cla*I fragment containing the *MER3* N-terminal region was cloned between the *Eco*RI and *Cla*I sites of pTN149 (41), yielding the *mer3-URA3-mer3*. The *mer3GD* mutation was created by PCR-based mutagenesis (24) with the primers 5′-CCCTCGAGATGAAAACAAAGTTTGATCGCCTCGG, 5′-CCAACCGGATCAGACAAGACTGTATTATTTGAATTGGC, 5′-GCCAATTCAAATAATACAGTCTTGTCTGATCCGGTTGG, and 5′-CCAAGCTTCGGCATGCTTTTCAATTATTTCAATCAATTTGG (the altered nucleotide is underlined) and pRDK4157 template DNA (described below). The resulting 1.1-kb PCR product was digested with *Xho*I and *Hin*dIII and cloned between the *Xho*I and *Hin*dIII sites of pBluescriptII KS^+ (Stratagene) to create pRDK4152. A 1.1-kb *Xho*I-*Sph*I fragment which had the *Xho*I site filled in with Klenow fragment (New England Biolabs) was excised from pRDK4152 and cloned between the *Sna*BI and *Sph*I sites of pRDK4151, yielding pRDK4153. The *mer3KA* mutation was created by site-directed mutagenesis as described by Kunkel (32), with the primer 5′-AAATAATACAGTGGCGGCTGATCCGGTTGG and a pBluescriptII SK^+ (Stratagene)-based plasmid in which a 0.4-kb *Spe*I fragment containing the helicase motif I of *MER3* was cloned at the *Spe*I site of the vector. The 0.4-kb *Spe*I fragment containing the *mer3KA* mutation was excised and substituted for the corresponding region of pRDK4151, to create pRDK4154. A 1.6-kb *Xba*I-*Sal*I fragment containing the Flag sequence at the *MER3* C terminus and *Sal*I site introduced 173 bp downstream from the stop codon was cloned between the *Xba*I and *Sal*I sites of pRDK4151, yielding pRDK4155. The Mer3 protein expression plasmid, pRDK4157, which contains the *GAL10-MER3-FLAG* gene, was created as follows. By using the reverse transcription-PCR (RT-PCR) product of *MER3,* which does not contain the *MER3* intron (41), the *MER3* cDNA was constructed. At the 5′ end of the *MER3* cDNA, an *Xho*I site was introduced by PCR-based mutagenesis (CTCGAG**ATG**; the *Xho*I site and the first codon are underlined and in boldface, respectively). At the 3′ end, *Mlu*I and *Hin*dIII sites were introduced by the same method (ACGCGT**TAA**GCTT; the restriction sites and the stop codon are underlined and in boldface, respectively). The sequence of the stop codon has also been changed from TGA. A DNA fragment constructed by annealing the oligonucleotides 5′-CGCGGGACGTACAAGGACGACGATGACAAGA and 5′-CGCGTCTTGTCATCGTCGTCCTTGTAGTCC was cloned into the *Mlu*I site, and the orientation resulting in the addition of TRDYKDDDDKTR (12 amino acids) containing the Flag epitope sequence at the Mer3 C terminus was confirmed by DNA sequencing. A putative URS1 element, which may repress the transcription in vegetatively growing yeast cells (17), present at position 109 to 117 (AGCCGCCAA) in the *MER3* open reading frame (ORF), was changed by site-directed mutagenesis to AGTCGACAA, which did not change the Mer3 amino acid sequence. The resulting 3.6-kb *Xho*I-*Hin*dIII fragment containing *MER3-FLAG* with the mutated URS1 was cloned between the *Xho*I and *Hin*dIII sites of pRDK249 containing a *GAL10* promoter, a 2 μm origin, and the *URA3* marker (28), yielding pRDK4156. Then a 4.4-kb *Bam*HI-*Hin*dIII fragment containing the *GAL10-MER3-FLAG* region from pRDK4156 was cloned between the *Bam*HI and *Hin*dIII sites of YEplac112 containing a 2 μm origin and *TRP1* (20), yielding pRDK4157. To express the mutant Mer3GD protein, a 1.1-kb *Xho*I-*Sph*I fragment containing the *mer3GD* mutation was excised from pRDK4152 and cloned between the corresponding sites of pRDK4157 to obtain pRDK4158. To express the Mer3KA protein, a 1.0-kb *Sal*I-*Sph*I PCR fragment, which contains the *mer3KA* mutation, amplified from pRDK4154 was inserted between the corresponding sites of pRDK4152 to obtain pTN386, and then a 1.1-kb *Xho*I-*Sph*I fragment from pTN386 was inserted between the corresponding sites of pRDK4157 to create the expression construct pTN483. All modified plasmids were sequenced to confirm that no additional mutations were introduced.

Purification of Mer3 protein. The pRDK4157 plasmid was introduced into the protease-deficient yeast strain RKY1293 by transformation. An initial culture was made by growing cells overnight at 30°C in minimal medium lacking tryptophan and supplemented with 3% (vol/vol) glycerol and 2% (wt/vol) lactic acid. This culture was used to inoculate 1 liter of the same medium, except that lactic acid was present at a concentration of 0.02% (wt/vol) instead of 2% (wt/vol). When the culture reached an optical density at 600 nm of 1 to 1.3, the *GAL10* promoter was induced by the addition of galactose to 2% (wt/vol), and cell growth was continued for 8 h at 30°C. All subsequent operations were performed at 4°C unless otherwise indicated, and the buffers used for all steps up to the elution of the Mer3 protein from the Flag-affinity gel were supplemented with a mixture of protease inhibitors (phenylmethylsulfonyl fluoride and benzamidine at 1 mM each; pepstatin A, aprotinin, and leupeptin at 1 μg/ml each).

Cells from 4 liters of culture were harvested by centrifugation (11,000 × *g* for 5 min), washed with cold water, and suspended in 100 ml of buffer P (50 mM $NaPO_4$ [pH 8.0], 200 mM NaCl, 5 mM EDTA, 1 mM β-mercaptoethanol). After standing on ice for 30 min, the cell suspension was centrifuged as described above and resuspended in 0.2 ml of buffer P per g of cells. The cells were lysed by the addition of an equal volume of acid-washed glass beads (425 to 600 μm in diameter; Sigma) followed by blending in 1.5-ml-tubes for four periods of 2 min each with a Bead-beater (Biospec Products). The cell extract was separated from the glass beads by centrifugation, and the beads were washed with 0.7 ml of buffer P per 1.5-ml tube. The cell extract and washes were combined and centrifuged at 15,000 × *g* for 30 min, and the supernatant was mixed with 1 ml of anti-Flag M2 affinity gel slurry (Sigma) in two 15-ml tubes and incubated for 1.5 h with rocking. The gel was collected by centrifugation (500 × *g*, 3 min) and washed twice with 20 ml of buffer T (20 mM Tris HCl [pH 7.4], 200 mM NaCl, 5 mM EDTA, 10% [vol/vol] glycerol, 1 mM dithiothreitol [DTT]). The gel was then transferred to a 1.5-ml tube and washed with 1 ml of buffer T four times. The bound protein was eluted by incubation of the Flag gel in 1 ml of buffer T containing 0.2 mg of Flag peptide per ml (Sigma) for 1.5 h with rocking. The elution step was repeated twice. The Flag elution fraction was clarified by centrifugation (15,000 × *g* for 10 min) and loaded at 0.5 ml/min onto a MonoQ HR5/5 column (Pharmacia) equilibrated in buffer T. After washing the column with 5 ml of buffer T, the bound protein was eluted with a 10-ml linear gradient from 0.2 to 1 M NaCl in buffer T. The peak of the Mer3 protein, which eluted at ~280 mM NaCl, was directly applied at 1 ml/min onto a 1-ml HiTrap heparin column (Pharmacia) equilibrated with buffer T containing 300 mM NaCl. After washing with 5 ml of buffer T containing 300 mM NaCl, the protein was eluted with a 5-ml linear gradient of 0.3 to 1 M NaCl in buffer T. The Mer3 protein eluted at ~440 mM

was dialyzed for 1 h against 100 volumes of buffer S (20 mM Tris HCl [pH 6.9], 250 mM NaCl, 1 mM EDTA, 10% glycerol, 1 mM DTT) and then loaded at 0.5 ml/min onto a MonoS HR5/5 column (Pharmacia) equilibrated in buffer S. After washing the column with 5 ml of buffer S, the bound protein was eluted with a 5-ml linear gradient from 0.5 to 1 M NaCl in buffer S. The Mer3 protein eluted at ~440 mM NaCl and was dialyzed twice against 100 volumes of storage buffer (10 mM Tris HCl [pH 7.6], 400 mM NaCl, 50% glycerol, 1 mM β-mercaptoethanol) and stored at −80°C. Fractions containing Mer3 protein were identified by SDS-PAGE, and the protein concentration was determined by the Bradford method (7) with the Bio-Rad protein assay. The resulting protein was >95% pure as assessed by SDS-PAGE followed by staining the gels with Coomassie blue. The mutant Mer3GD and Mer3KA proteins were purified exactly as described for the wild-type Mer3 protein.

DNA substrates. Oligonucleotides (high-performance liquid chromatography [HPLC] purified) were purchased from CyberSyn, Inc. For DNA-binding assays, the 50-nucleotide (nt) oligonucleotide T70 (5′-TCGATAGTCTCTAGACAGC ATGTCCTAGCAAGCCAGAATTCGGCAGCGTC) was first 5′ end labeled with [γ-^{32}P]ATP (NEN Life Science Products) by using T4 polynucleotide kinase (New England Biolabs). The labeled oligonucleotide T70 was mixed with a one- to twofold molar of the 50-nt oligonucleotide T74 that is the complementary strand of T70 in annealing buffer (10 mM Tris HCl [pH 7.6], 1 mM EDTA, 0.5 M NaCl), the mixture heated to 94°C for 5 min, and then cooled to 20°C over a 2-h period. The annealed DNAs were separated by PAGE on a 10% polyacrylamide gel run in TBE (90 mM Tris borate [pH 7.6], 2 mM EDTA). The band containing annealed DNAs was excised, and the DNA was eluted by soaking the gel slice in elution buffer (0.5 M NaCl, 0.6 M Na acetate, 1 mM EDTA [pH 8.0]) overnight at 4°C followed by extraction with phenol-chloroform and precipitation with ethanol. The DNA was then dissolved in buffer containing 10 mM Tris HCl (pH 7.6) and 250 mM NaCl. To prepare an ssDNA substrate, the 5′-end-labeled oligonucleotide T70 was purified as described above. For DNA helicase assays, HPLC-purified oligonucleotide M13-50 (5′-CATGCCTGCAGGTCGACTCTA GAGGATCCCCGGGTACCGAGCTCGAATTC; complementary to nt 6230 to 6279 of M13mp18 circular ssDNA) was 5′ end labeled and purified with a QIAquick nucleotide removal kit (Qiagen). The labeled DNA was mixed with an equal molar amount of M13mp18 circular ssDNA in annealing buffer (10 mM Tris HCl [pH 7.6], 1 mM EDTA, 100 mM NaCl), and the mixture was heated to 95°C for 5 min and then incubated at 65°C for 20 min, followed by incubation at 23°C for 20 min. The annealed DNA was purified by gel filtration through a 0.12-cm^2 by 25-cm Bio-Gel A 5m (Bio-Rad) column equilibrated and run in the annealing buffer. The concentration of purified DNAs was determined with a Beckman DU 640B spectrophotometer.

Gel mobility shift assays. Indicated amounts of the Mer3 protein and 1 nM DNA (concentrations are in terms of moles of molecules) were incubated in 20-μl volumes of DNA binding buffer (20 mM Tris HCl [pH 7.6], 50 mM NaCl, 2 mM DTT, 100 μg of bovine serum albumin [BSA] per ml). Prior to assembling the reaction, the ssDNA substrate was incubated at 100°C for 3 min to disrupt secondary structure. After incubation for 30 min on ice, 5 μl of loading buffer A (40 mM Tris HCl [pH 8.0], 25% glycerol, 400 μg of BSA per ml) was added to the binding reaction mixture. The DNA-protein complexes were analyzed by electrophoresis through nondenaturing 5% polyacrylamide gels (19:1) run in low-ionic-strength buffer (6.7 mM Tris HCl [pH 8.0], 3.3 mM Na Acetate, 2 mM EDTA), and the DNA-containing bands were visualized with a phosphorimager (445 SI; Molecular Dynamics).

ATPase assays. Reaction mixtures containing 20 mM Tris HCl (pH 7.6), 50 mM NaCl, 5 mM $MgCl_2$, 2 mM DTT, 100 μg of BSA per ml, 0.02 Ci of [γ-^{32}P]ATP per ml (ICN Biomedical, Inc.), 1.5 μg of poly(dA) (Amersham Pharmacia Biotech) or M13mp18 RFI (Life Technologies Inc.) per ml, and 0.5 mM ATP (Amersham Pharmacia Biotech) were preincubated at 30°C for 5 min, and the reaction was initiated by the addition of 4 nM Mer3 protein. At the indicated times after the addition of Mer3, 5-μl portions of the reaction mixtures were removed and terminated by the addition of 2 μl of 0.2 M EDTA. One microliter of each reaction mixture was spotted onto polyethyleneimine cellulose thin-layer chromatography plates (Merck). The plates were developed in a mixture of 1 M formic acid and 0.5 M LiCl and dried, and the amounts of $^{32}P_i$ and [γ-^{32}P]ATP in the reaction mixture were determined with a phosphorimager (445 SI; Molecular Dynamics).

DNA helicase assays. The indicated amounts of protein and DNA (concentrations are in terms of moles of nucleotides) were incubated in 20-μl volumes containing DNA helicase buffer (20 mM Tris HCl [pH 7.6], 50 mM NaCl, 2 mM DTT, 100 μg of BSA per ml, 2 mM $MgCl_2$, 2 mM ATP). All reaction mixtures were preincubated at 30°C for 5 min, started by the addition of the Mer3 protein, and incubated for the times indicated in the individual experiments. Reactions were stopped by the addition of 5 μl of stop buffer (50 mM Tris HCl [pH 7.6], 50 mM EDTA, 2.5% SDS) and 0.5 μl of 25 mg of proteinase K per ml, followed by incubation at 37°C for 10 min. The DNA products were then analyzed by electrophoresis through nondenaturing 8% polyacrylamide gels run in TBE. The gels were dried, and the radiolabeled DNA was visualized with a phosphorimager. For time course experiments, a single reaction mixture (100 μl) was prepared and incubated, 10-μl aliquots were removed at each time point, and 2.5 μl of stop buffer and 0.25 μl of proteinase K at 25 mg/ml were added to each 10-μl aliquot prior to analysis.

RESULTS

Amino acid substitutions in the nucleotide-binding domain of Mer3 cause defects in meiotic progression, DSB transitions, and viable spore formation. The predicted Mer3 amino acid sequence contains seven motifs characteristic of DNA/RNA helicases (21). Motif I, which is also called a P-loop or Walker motif A, constitutes a nucleotide-binding domain in many proteins containing this motif (22, 62). To determine whether the Mer3 motif I plays an important role in meiosis, conserved amino acids in motif I were altered by site-directed mutagenesis, and the mutations were introduced into the *MER3* genomic locus (Fig. 1A, *mer3G166D* and *mer3K167A*). Such mutations are known to eliminate ATP hydrolysis by the Walker A type of ATPases (23, 55). Interestingly, it has been observed that a *mer3* deletion causes more severe defects in meiotic nuclear divisions and in the transition of DSBs to later intermediates at 30°C than at to 23°C, although a similar reduction of crossovers is observed at both temperatures (41; see below). Isogenic diploid strains containing the mutations were synchronously sporulated at 30 and 23°C, and meiotic nuclear divisions were monitored by DAPI (4′,6′-diamidino-2-phenylindole) staining (Fig. 1B, 30°C; and C, 23°C). As was seen before (41), a deletion of *MER3* resulted in approximately 10% of the wild-type level of the meiotic divisions at 30°C, while at 23°C, the *mer3* deletion delayed the appearance of the meiotic divisions, but eventually reached the wild-type level. In the *mer3GD* and *mer3KA* mutants, ~3- and ~4-h delays in the appearance of meiotic divisions were observed at 30 and 23°C, respectively, although eventually wild-type levels of meiotic divisions occurred, consistent with a similar meiotic progression defect at both temperatures. The delay in the induction of meiotic divisions observed in *mer3GD* and *mer3KA* mutants prompted us to test whether these mutations effect meiosis-specific DSBs. Chromosomal DNA was prepared from synchronously sporulating cultures and DSBs formed in the *his4*::*LEU2* hot spot (Fig. 1D) were detected by Southern blotting (see Materials and Methods). Figure 1E shows the Southern blot demonstrating the formation of DSBs at 30°C. In wild-type cells, DSBs appeared at 5 h after transfer of the cells into sporulation medium and gradually disappeared thereafter. In *mer3GD* and *mer3KA* cells, the DSBs appeared at 5 h, were present through the 9-h time point, and then disappeared. The amount of DSBs formed at site I was measured (Fig. 1F, 30°C; and G, 23°C). These data show that in the *mer3GD* and *mer3KA* mutants, the DSBs were present for a longer period of time and accumulated to higher levels than were seen in the wild-type control, indicating that the transition of DSBs to later intermediates is impaired in the *mer3GD* and the *mer3KA* mutants. However, the defect in the DSB transition in the *mer3GD* and the *mer3KA* mutants was not as severe as that previously seen for the *mer3*Δ mutant (41).

A high incidence of nondisjunction of homologs at meiosis I and a decrease in spore viability have been observed in the *mer3* deletion mutant (41). Tetrads of the *mer3GD* and *mer3KA* mu-

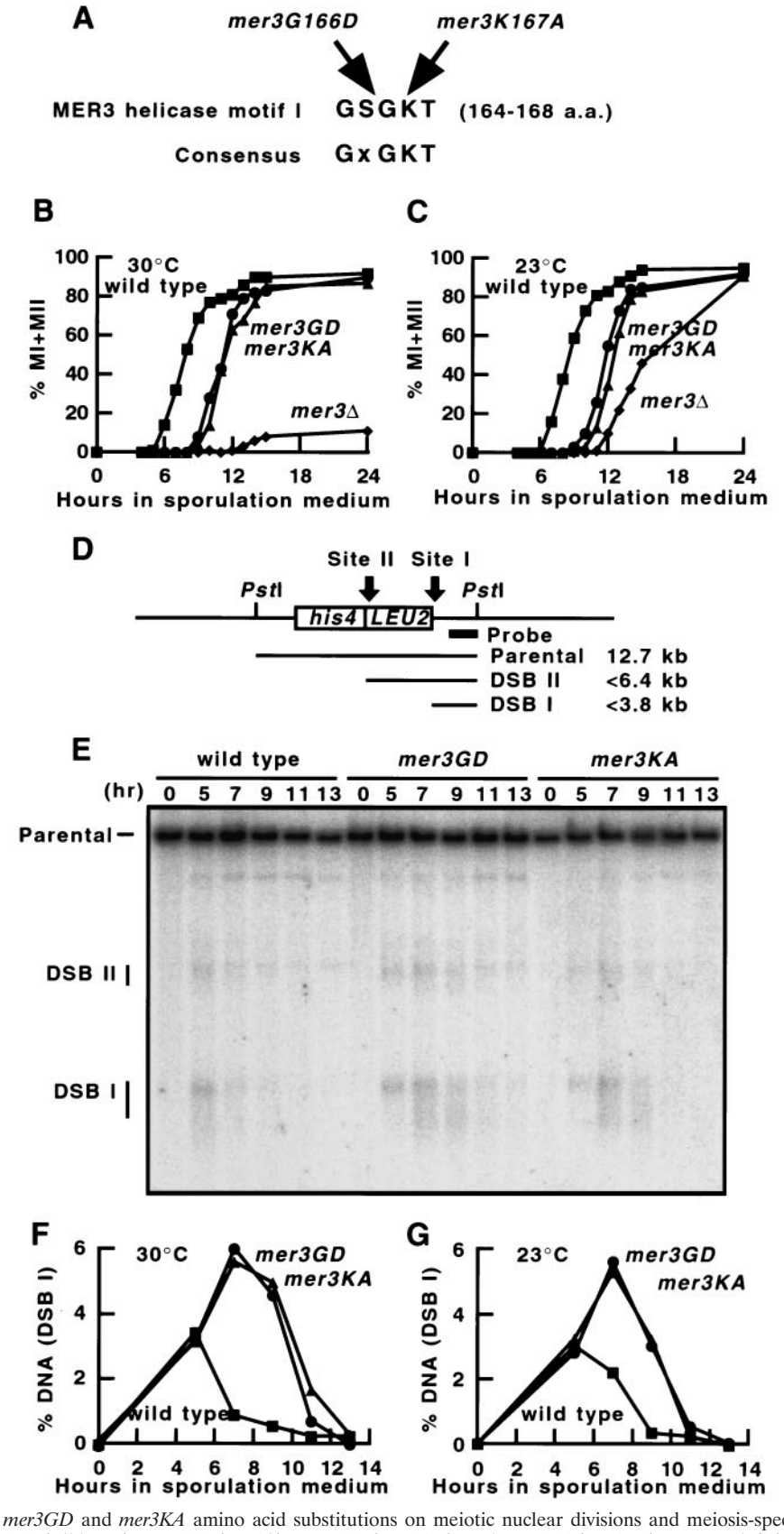

FIG. 1. Effect of the *mer3GD* and *mer3KA* amino acid substitutions on meiotic nuclear divisions and meiosis-specific DSB formation. The isogenic SK1 strains TNY821 (wild type), TNY822 (*mer3*Δ), TNY823 (*mer3GD*), and TNY824 (*mer3KA*) were used. (A) Positions of *mer3GD* and *mer3KA* amino acid substitutions in the Mer3 helicase motif I are indicated. (B and C) The wild-type (squares) and *mer3GD* (circles), *mer3KA*

TABLE 2. Proportion of tetrad types in *mer3* mutant strains

Relevant genotype[a]	Result at:													
	30°C							23°C						
	Tetrad type (%)					% Spore viability	Total no. of tetrads	Tetrad type (%)					% Spore viability	Total no. of tetrads
	4-sv	3-sv	2-sv	1-sv	0-sv			4-sv	3-sv	2-sv	1-sv	0-sv		
MER3	91	7	2	0.2	0.3	97	587	89	9	2	0.2	0.5	96	609
mer3GD	75	7	10	0.5	8	85	730	49	7	19	3	22	65	1,070
mer3KA	70	6	13	0.9	11	81	785	45	8	20	4	24	62	1,170
*mer3*Δ[b]	20	6	30	6	38	41	50	23	5	20	4	48	37	5,952

[a] Isogenic wild-type (RKY4220), *mer3*Δ (RKY4221), *mer3GD* (RKY4222), and *mer3KA* (RKY4223) strains were utilized.
[b] The data for *mer3*Δ at 23°C have been presented before (41).

tants formed at 30 and 23°C were dissected, and the spore viability and the proportion of tetrad types (4-, 3-, 2-, 1-, and 0-sv for four-spore-viable, three-spore-viable, etc.) were determined (Table 2). At 30°C, the *mer3GD* and the *mer3KA* mutant had decreased spore viability compared to the wild-type strain ($P < 10^{-10}$). The proportion of 4-sv tetrads was decreased, and the proportions of 2- and 0-sv tetrads were increased in the *mer3GD* and *mer3KA* mutants compared to the wild-type control. These results are consistent with the idea that nondisjunction of homologs at meiosis I is elevated in the *mer3GD* and *mer3KA* mutants. Essentially similar phenotypes were observed in the *mer3* point mutants at 30 and 23°C, although the spore viability was further decreased at 23°C. In comparison to the *mer3GD* and the *mer3KA* mutants, the *mer3*Δ mutant showed both more pronounced decreases in the proportion of 4-sv tetrads and more pronounced increases in the proportion of 2- and 0-sv tetrads. While the *mer3*Δ mutant has a more severe phenotype, both the *mer3GD* and the *mer3KA* mutations do cause significant defects in progression through meiosis, in the DSB transition, and in the formation of viable spores. A similar level and time course of Mer3 expression were observed in wild-type, *mer3GD*, and *mer3KA* cells during meiosis by Western blotting with anti-Mer3 antibodies (data not shown), indicating that the *mer3GD* and *mer3KA* mutant phenotypes were due to impairment of some enzymatic activity of Mer3 rather than a defect in Mer3 expression or stability.

The *mer3G166D* and *mer3K167A* mutations decrease the frequency of crossing over and impair crossover interference. To determine whether the *mer3GD* or the *mer3KA* mutations effect crossing over, the frequency of reciprocal exchanges in the *CAN1-URA3*, *URA3-HOM3*, and *HOM3-TRP2* intervals on chromosome V (Table 1) were examined by tetrad analysis. At 30°C, the frequency of crossing over in the *CAN1-URA3* and *URA3-HOM3* intervals, but not in the *HOM3-TRP2* interval, was decreased by both the *mer3GD* and *mer3KA* mutations to approximately 60% of the wild-type level ($P < 10^{-10}$) (Table 3). Similar but slightly more severe phenotypes were observed at 23°C. These crossover defects were essentially the same as previously observed for the *mer3*Δ mutant (41). The effect of either of these two point mutations on crossover interference was analyzed in two ways. First, the frequency of nonparental ditype tetrads (NPDs), which is indicative of double-crossovers in an interval, was compared to the frequency expected in the absence of crossover interference, where crossovers would occur randomly (Table 4). At 30°C, in the wild-type strain, the frequency of NPDs observed in the *CAN1-URA3* and *URA3-HOM3* intervals was less than the expected frequency ($P < 10^{-5}$), showing that there was crossover interference. In the case of the *mer3GD* and *mer3KA* mutants, there was almost no difference between NPDs observed and NPDs expected ($0.03 < P < 0.12$), indicating that these two *mer3* mutations impair crossover interference. Second, to confirm that the *mer3KA* and *mer3GD* mutations impair crossover interference, the distribution of zero-, one- and double-crossover events in the *CAN1-URA3* and *URA3-HOM3* intervals was examined (Table 5). In the wild-type strain, the distribution of crossovers observed was different from the distribution predicted in the absence of crossover interference according to a Poisson distribution ($P < 10^{-13}$). In the *mer3GD* and *mer3KA* mutants, the distribution of crossovers observed was close to a Poisson distribution ($10^{-2} > P > 10^{-4}$). In addition, the distribution in these two *mer3* mutants was completely different from the wild-type distribution ($P < 10^{-22}$). Interestingly, in the *HOM3-TRP2* interval, crossover interference was not observed in the wild-type strain, and the *mer3GD* and *mer3KA* mutations did not cause a significant reduction of crossing over in this interval. Similar defects in crossover interference were observed in the *mer3GD* and *mer3KA* mutants at 23°C (Tables 4 and 5). Previous studies with the *mer3*Δ mutant showed a somewhat stronger defect in crossover interference than that observed here with the *mer3GD* or the *mer3KA* mutant (41).

(triangles), and *mer3*Δ (diamonds) strains were sporulated at 30°C (B) and 23°C (C) in liquid medium and analyzed as described in Materials and Methods. Meiotic nuclear divisions were monitored by staining the cells with DAPI and by examining >200 cells at each time point (0, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 24 h) by fluorescence microscopy. Plotted is the percentage of cells that had undergone one or both nuclear divisions (MI ± II) at the indicated times after transfer of the cells to sporulation medium. (D) Positions of major (site I) and minor (site II) DSB sites and *Pst*I restriction sites in the *his4*::*LEU2* region are shown. (E) DNA was prepared from wild-type and *mer3GD* and *mer3KA* cells sporulated at 30°C at the indicated times after transfer of the cells to sporulation medium, digested with *Pst*I, separated by agarose gel electrophoresis, and transferred to a nylon membrane. A probe, the position of which is indicated in panel D, was used to detect fragments of interest by Southern hybridization. (F and G) The steady-state levels of DSBs at site I produced in wild-type (square), *mer3GD* (circle), and *mer3KA* (triangle) cells at 30°C (F) and 23°C (G) were detected by Southern blotting and quantified with a phosphorimager. The percentage of the DSBs in the total DNA at each time point is shown.

TABLE 3. Frequency of crossing over in *mer3* mutant strains

Relevant genotype	Interval	Result at[a]: 30°C No. of events PD	TT	NPD	Map distance (cM)	23°C No. of events PD	TT	NPD	Map distance (cM)
MER3	*CAN1-URA3*	206	312	15	38	204	316	17	39
	URA3-HOM3	217	306	11	35	195	325	18	40
	HOM3-TRP2	438	94	2	10	438	95	1	9.5
mer3GD	*CAN1-URA3*	313	214	9	25 (0.66)	325	192	3	20 (0.51)
	URA3-HOM3	346	191	5	20 (0.57)	359	159	3	17 (0.43)
	HOM3-TRP2	428	102	5	12 (1.2)	433	83	1	8.6 (0.91)
mer3KA	*CAN1-URA3*	336	205	5	22 (0.58)	337	177	10	23 (0.59)
	URA3-HOM3	352	183	5	20 (0.57)	377	146	3	16 (0.40)
	HOM3-TRP2	447	81	3	9.3 (0.93)	436	83	2	9.1 (0.96)

[a] Tetrads obtained in Table 2 were examined. Only 4-sv tetrads that did not show aberrant segregation of the relevant markers were used to calculate map distances. Numbers in parentheses show relative frequencies of crossing over in the *mer3GD* and *mer3KA* mutants compared to those in the corresponding intervals in the wild-type strain. PD, parental ditype; TT, tetratype; NPD, nonparental ditype.

Purification of the Mer3 protein. The genetic analyses described above suggest that the putative nucleotide-binding domain of the Mer3 helicase motifs has an important role in crossing over. To aid in the purification of the Mer3 protein, a Flag epitope was introduced at the C terminus of Mer3 (Fig. 2A). To determine if the *MER3-FLAG* gene encoded a functional protein, the strain RKY4227 was constructed by introducing the *MER3-FLAG* gene at the *MER3* chromosome locus under control of the native promoter. At 30°C, RKY4227 showed a spore viability of 97% (193/200, viable/total spores), similar to that seen in wild-type cells (Table 2), indicating that the tagged Mer3 protein was fully functional. The Mer3 -FLAG protein was then overexpressed from a multicopy plasmid under control of the *GAL10* promoter in vegetatively growing yeast cells by the addition of galactose to the medium. The Mer3-FLAG protein was purified to >95% homogeneity by affinity chromatography on an anti-Flag antibody matrix followed by conventional chromatography through MonoQ, heparin, and MonoS columns (Fig. 2B). The purified protein had a molecular mass of ~140 kDa determined by SDS-PAGE (6.5% polyacrylamide), which is consistent with the predicted molecular weight of 136,574 for the Mer3-FLAG protein. Western blot analysis with anti-Flag monoclonal antibody confirmed that this protein species was the Mer3-FLAG protein (Fig. 2C). The mutant Mer3GD and Mer3KA proteins were also purified by this procedure, resulting in protein preparations of purity similar to that of the wild-type Mer3 protein (data not shown).

DNA-binding properties and ATPase activity of the Mer3, Mer3GD, and Mer3KA proteins. The ability of the Mer3 protein to bind to 50-nt ssDNA and to 50-bp dsDNA with blunt ends was characterized by gel mobility shift assays. The Mer3 protein was able to form stable complexes with both ss- and dsDNA, although there was a slight preference for ssDNA at a high concentration of Mer3 (Fig. 3A and B). Denaturing the dsDNA prior to the addition of the Mer3 protein resulted in increased formation of protein-DNA complexes, confirming the slightly higher preference of Mer3 for ssDNA (Fig. 3E and F). For each substrate, approximately 50% of the DNA was converted to protein-DNA complexes at a ratio of four Mer3 molecules per one DNA molecule. The mutant Mer3GD protein had the same DNA-binding properties as the wild-type Mer3 protein (Fig. 3C and D).

ATPase activity of the Mer3 protein was examined in the

TABLE 4. Analysis of NPD events

Relevant genotype	Interval	Result at[a]: 30°C No. of NPDs Observed	Expected	Total no. of tetrads	Probability	23°C No. of NPDs Observed	Expected	Total no. of tetrads	Probability
MER3	*CAN1-URA3*	15	45	533	7.7×10^{-6}	17	46	537	1.9×10^{-5}
	URA3-HOM3	11	42	534	1.7×10^{-6}	18	51	538	3.8×10^{-6}
mer3GD	*CAN1-URA3*	9	15	536	0.12	3	12	520	9.4×10^{-3}
	URA3-HOM3	5	11	542	0.07	3	8	521	0.08
mer3KA	*CAN1-URA3*	5	13	546	0.03	10	10	524	1.00
	URA3-HOM3	5	10	540	0.11	3	6	526	0.22

[a] Tetrads obtained in Table 2 were examined. The number of NPDs expected was calculated according to the Papazian equation (41a): $NPD = 1/2[1 - TT' - (1 - 1.5TT')^{2/3}]$, where TT′ is the frequency of TT shown in Table 2. Chi-square tests were performed to express the likelihood that the difference between the observed and expected numbers was attributable to chance.

TABLE 5. Distribution of zero-, one-, and double-crossover events in *mer3* mutant strains

Relevant genotype	Interval	Result at[a]: 30°C No. of events Observed 0-CR	30°C Observed 1-CR	30°C Observed 2-CR	30°C Expected 0-CR	30°C Expected 1-CR	30°C Expected 2-CR	30°C Probability	23°C Observed 0-CR	23°C Observed 1-CR	23°C Observed 2-CR	23°C Expected 0-CR	23°C Expected 1-CR	23°C Expected 2-CR	23°C Probability
MER3	*CAN1-URA3*	191	282	60	251	189	71	4.1×10^{-14}	187	282	68	247	192	75	3.7×10^{-13}
	URA3-CAN1	206	284	44	266	185	65	1.7×10^{-16}	177	289	72	241	194	78	1.1×10^{-14}
mer3GD	*CAN1-URA3*	304	196	36	325	163	41	1.2×10^{-2}	322	186	12	347	140	28	2.1×10^{-6}
	URA3-CAN1	341	181	20	361	147	30	2.2×10^{-3}	356	153	12	371	126	21	5.2×10^{-3}
mer3KA	*CAN1-URA3*	331	195	20	355	153	33	1.1×10^{-4}	327	157	40	333	151	34	5.0×10^{-1}
	URA3-CAN1	347	173	20	364	144	28	9.7×10^{-3}	374	140	12	385	120	19	4.9×10^{-2}

[a] Tetrads obtained in Table 2 were examined. The observed number of zero-, one- and double-crossover (0-, 1-, and 2-CR, respectively) events in an interval was derived from the number of PD, TT, and NPD events as 0-CR = PD − NPD, 1-CR = TT − 2NPD, and 2-CR = 4NPD, since only one class gives NPDs among four classes of 2-CR tetrads, assuming no chromatid interference. The expected number of 0-, 1-, and 2-CR events was predicted by a Poisson distribution from the frequency of crossing over observed in Table 2. Chi-square tests were performed to express the likelihood that the difference between the observed and expected patterns was attributable to chance.

presence of poly(dA) or M13mp18 replication form I (M13 RF) and in the absence of DNA by thin-layer chromatography (Fig. 3G). Addition of poly(dA) or M13 RF significantly increased the amount of ATP hydrolyzed by Mer3 in the absence of DNA, indicating that ATPase activity of Mer3 is highly stimulated by either ss- or dsDNA. The Mer3GD protein had almost no significant dsDNA-stimulated ATPase activity and had significantly reduced (threefold reduced versus wild type) ssDNA-stimulated ATPase activity, while the Mer3KA protein had no detectable ATPase activity in the presence or absence of DNA (40) (Fig. 3H and I).

The Mer3 protein has DNA helicase activity. The Mer3 protein was tested for DNA helicase activity by using a DNA substrate containing a ^{32}P-labeled 50-nt fragment annealed to M13mp18 circular ssDNA (Fig. 4A). Displacement of the labeled fragment by the addition of the Mer3 protein was observed as increasing amounts of Mer3 were added to the assay (Fig. 4A, lanes 3 to 5). When Mg^{2+} or ATP was omitted from the reaction, no activity was observed (Fig. 4A, lanes 9 and 10). In studies published elsewhere, we have shown that the Mer3 helicase can displace 100- and 631-nt fragments in a reaction that is almost entirely dependent on the ssDNA binding protein RPA and have shown that Mer3 translocates along ssDNA in the 3′-to-5′ direction (40). The mutant Mer3GD protein displaced only a small amount of the labeled fragment (Fig. 4A, lanes 6 to 8), indicating that the ability to bind to ss- and dsDNA was insufficient for helicase activity. The amounts of the fragment displaced in a similar reaction mixture containing different concentrations of Mer3 were measured (Fig. 4B). At approximately 2 nM Mer3 protein, which corresponds to about seven Mer3 molecules per DNA substrate, strand displacement reached a plateau. Figure 4C shows a time course of the strand displacement reaction. When the Mer3 protein was present, over 50% of the labeled fragments were displaced within 5 min, and the reaction was essentially complete within 10 min. In contrast, the initial velocity of the strand displacement in the presence of the Mer3GD protein was less than 1% of the wild-type level. These results show that the Mer3 protein has DNA helicase activity and that this activity was highly impaired by the *mer3GD* mutation. The Mer3KA protein was not tested for helicase activity, since the Mer3 helicase activity is completely dependent on ATP hydrolysis, and hence the Mer3KA protein would be unlikely to have helicase activity, since it is completely defective for ATPase activity.

DISCUSSION

Meiotic crossing over is regulated to take place at least once between each pair of homologs to ensure proper segregation of

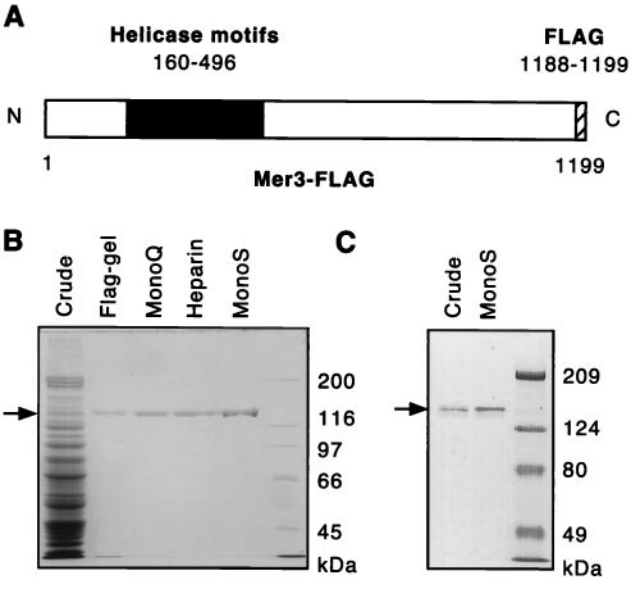

FIG. 2. Purification of the Mer3 protein. (A) Schematic structure of the Mer3 protein. The helicase domain and Flag tag and their respective amino acid coordinates are illustrated as solid and hatched boxes, respectively. (B) SDS-PAGE (6.5% polyacrylamide) analysis of different Mer3 protein fractions generated during the purification of Mer3 protein as described in Materials and Methods. A representative gel stained with Coomassie brilliant blue is shown. The position of the Mer3 protein is indicated by an arrow on the left. Sizes of molecular standards are shown on the right. One microgram of protein was loaded in the MonoS lane. (C) Western blotting analysis of the crude extract (20 μg) and MonoS (40 ng) fractions with anti-Flag M2 antibodies.

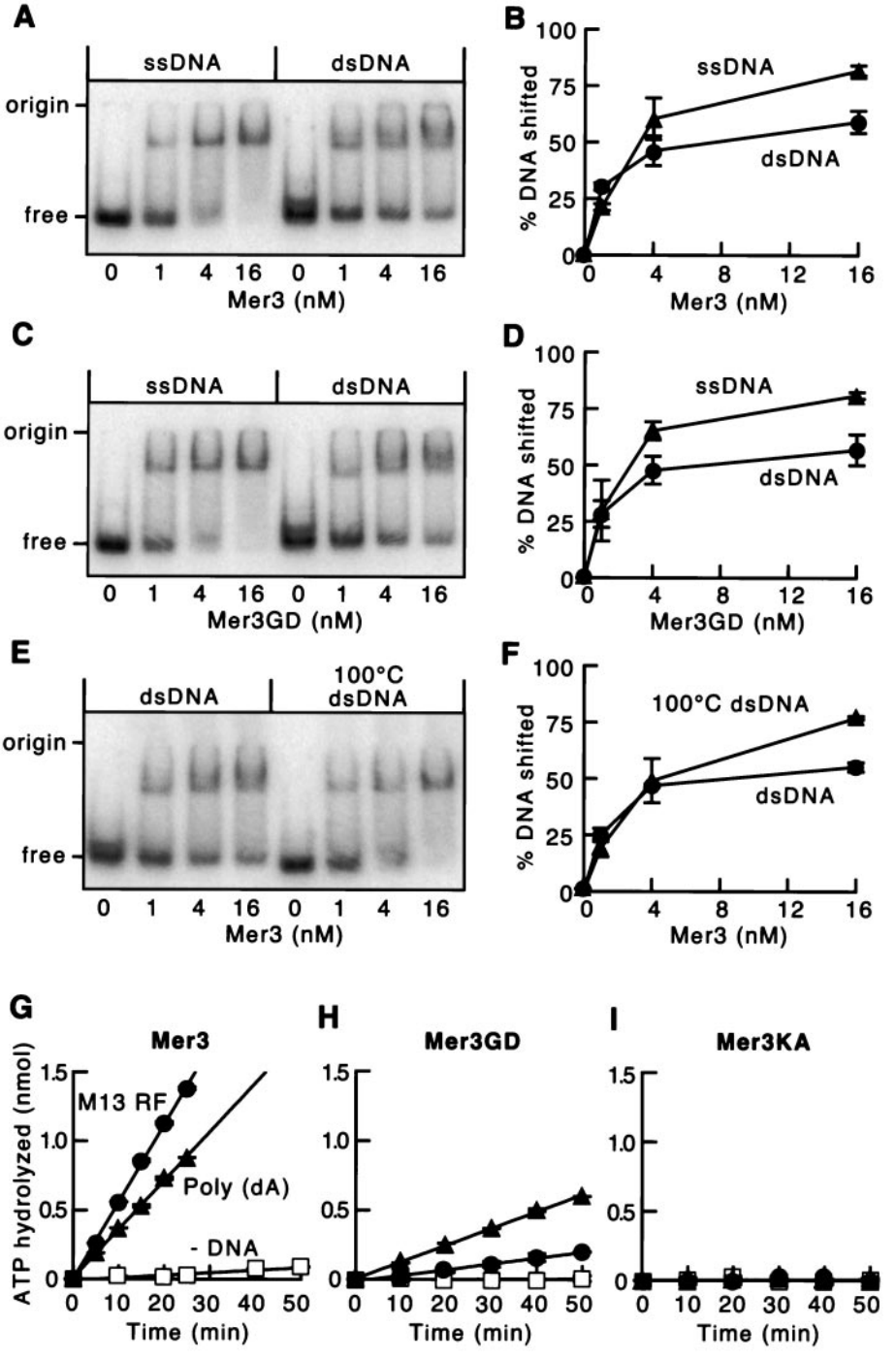

FIG. 3. DNA binding and ATPase activities of the Mer3 protein. Binding of Mer3 protein to oligonucleotide substrates was detected by gel mobility shift assays as described in Materials and Methods. Amounts of the Mer3 or Mer3GD protein are indicated. (A) Binding of the Mer3 protein to 50-nt ssDNA and 50-bp dsDNA. (C) Binding of the Mer3GD protein to ssDNA and dsDNA. (E) Binding of the Mer3 protein to dsDNA, with or without boiling of the DNA for 3 min prior to addition of the protein. The percentage of labeled DNA present in protein-DNA complexes in reactions is plotted in panels B (dsDNA or ssDNA with Mer3), D (dsDNA or ssDNA with Mer3GD), and F (dsDNA or boiled dsDNA with Mer3). The value plotted is the mean of the values obtained in at least two experiments, and the bar shows the standard deviation. (G, H, and I) ATPase activity of Mer3, Mer3GD, and Mer3KA proteins in the absence of additional DNA and in the presence of poly(dA) or M13mp18 replication form I (M13 RF) was examined by thin-layer chromatography as described in Materials and Methods.

chromosomes at meiosis I. Meiotic crossing over is a highly regulated process involving a number of features, including programmed induction of recombination proteins (9, 61) and recombination initiation structures (i.e., DNA DSBs) (56), double Holliday junctions (4, 12, 50), and specific enzyme systems required for crossing over and for prevention of excessive crossovers, called interference (31, 39, 45). Although a number of gene products have been found to be specifically

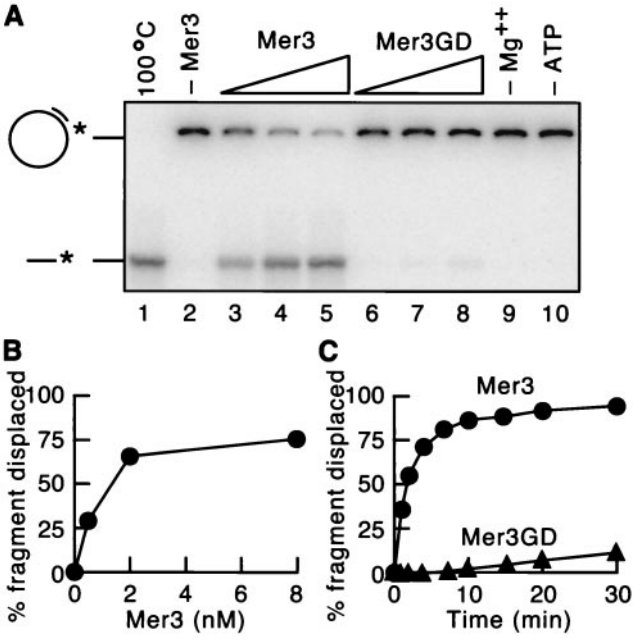

FIG. 4. DNA helicase activity of the Mer3 protein. (A) Displacement of a ^{32}P-labeled 50-nt fragment annealed to M13mp18 circular ssDNA was measured as described in Materials and Methods. Reaction mixtures contained 1 μM (in nucleotides) DNA substrate in helicase buffer and were incubated at 30°C for 30 min after the addition of Mer3. Increasing amounts of Mer3 and Mer3GD protein were present in lanes 3 to 5 (0.5, 2, and 8 nM) and 6 to 8 (0.5, 2, and 8 nM), respectively. In lane 9, 2 mM EDTA was added instead of $MgCl_2$. In lane 10, ATP was omitted from the helicase buffer. The Mer3 protein (8 nM) was added in lanes 9 and 10. (B) The 50-nt fragment displaced in the presence of different concentrations (0, 0.5, 2, and 8 nM) of Mer3 was quantified. Reactions were essentially as described for panel A, except that the reaction mixtures contained 2 μM DNA substrate. (C) Time course of the helicase reaction. Reaction mixtures were essentially as described for panel A, except that they contained 2 μM DNA substrate and 8 nM Mer3 or Mer3GD.

required for crossing over, the exact function of these gene products and the molecular mechanism of crossover control have remained elusive. The *S. cerevisiae* meiosis-specific gene *MER3*, which has been suggested to encode a DNA helicase on the basis of its amino acid sequence, is an important component of the crossover control system (41). A *mer3* deletion mutation decreases sporulation, and among the cells that proceed through meiosis, there are decreased crossing over and an absence of crossover interference, leading to a high incidence of nondisjunction of homologs at meiosis I.

In the present study were constructed two site-directed mutations, *mer3GD* and *mer3KA*, which cause amino acid substitutions of conserved residues in a putative nucleotide-binding domain of the conserved helicase motifs of Mer3. After a delay in induction of meiotic nuclear divisions, the *mer3GD* and *mer3KA* mutant diploid cells sporulated normally, but showed a defect in the DSB transition, nonrandom spore death, decreased frequencies of crossing over, and a nearly randomized distribution of crossovers. The Mer3 protein was purified and shown to bind to both ss- and dsDNA, have DNA-stimulated ATPase activity, and have DNA helicase activity. In studies published elsewhere, we have shown that the Mer3 helicase can displace 100- and 631-nt fragments in a reaction that is almost entirely dependent on the ssDNA binding protein RPA and have shown that Mer3 translocates along ssDNA in the 3′-to-5′ direction (40). The mutant Mer3GD protein was almost entirely defective (<1% wild-type activity) for the DNA helicase activity, but it retained the DNA-binding properties of the wild-type protein. The mutant Mer3KA protein was completely defective for ATPase activity and is unlikely to have helicase activity, since DNA unwinding by Mer3 was dependent on ATP hydrolysis and Mg^{2+}. These genetic and biochemical results indicate that the Mer3 protein functions as a DNA helicase in crossover control and suggest that Mer3 protein may also play a second role that depends on its DNA-binding ability, but not its DNA helicase or ATPase activity.

The data presented here demonstrate that Mer3 is a DNA helicase that can catalyze the displacement of a 50-nt fragment paired with a circular ssDNA; other studies have more extensively characterized the Mer3 helicase and demonstrated it can displace much longer fragments (631 nt) paired with a circular ssDNA, particularly in the presence of RPA (40). Similar strand displacement reactions have been shown to be carried out by comparable amounts of other helicases thought to function in recombination, such as *E. coli* RuvAB and RecQ, yeast Sgs1 and Srs2, and human BLM (5, 30, 35, 46, 59, 60). In *S. cerevisiae*, there are a number of genes encoding predicted DNA helicases that have been shown to be involved in DNA recombination, including *SGS1*, *SRS2*, *RAD54*, *TID1*/*RDH54*, and *MER3* (14, 33, 41, 49, 53, 64, 65). Of these proteins, Sgs1, Srs2, and Mer3 have been shown to be helicases (35; this study), whereas, thus far, Rad54 and Tid1/Rdh54 have only been demonstrated to have an unwinding activity that changes the topology of dsDNA (37, 43, 44). Each of these proteins appears to function in a different type of recombination or at a different step in recombination. Among these proteins, the Mer3 protein is unique in that it is expressed only in meiosis and is required for meiotic progression, DSB transitions, crossing over, and crossover interference. There are several steps during recombination at which a DNA helicase could act, including unwinding of dsDNA during initial strand invasion, strand exchange at early steps of recombination, and branch migration of Holliday junctions later during recombination (42, 48, 51). The observation that *mer3* mutants exhibit similar defects in crossing over, as seen in *msh4* or *msh5* deletion mutants, suggests that Mer3 could function in branch migration and/or resolution of Holliday junctions, as has been suggested for Msh4 and Msh5 (25, 39, 47). In contrast, the observations that a subset of DSBs produced in meiosis appear for a prolonged period of time (*mer3GD*, *mer3KA*, and 23°C *mer3*Δ) or persist (30°C *mer3*Δ) and that there is either a delay (*mer3GD*, *mer3KA*, and 23°C *mer3*Δ) or arrest (30°C *mer3*Δ) of meiotic progression in *mer3* mutants suggest that Mer3 could function at an early step of the recombination pathway that promotes crossing over. We favor the idea that Mer3 functions at an early step in recombination and is required for DSB transitions. Correct transition of DSBs to later intermediates would be required for crossing over and could be a focus for crossover control. This would explain why DSBs accumulate in *mer3* mutants and why there are defects in both crossing over and crossover control. We cannot exclude that Mer3 also acts late in recombination and is required for maturation of a late recombination intermediate that, in the absence of Mer3, is

unstable and dissociates into DSB intermediates. Clearly a more detailed analysis of Mer3 helicase activity, its substrate specificity, and relationships with other recombination proteins will be required to address the question of at which steps of recombination the Mer3 helicase acts.

An interesting feature of the data presented here is the difference in phenotype caused by the *mer3GD* and *mer3KA* mutations compared to the *mer3*Δ mutation. Both the *mer3GD* and *mer3KA* mutations cause a defect in the DSB transition, decrease crossing over, impair crossover interference, and delay progression of meiosis, but they do not reduce the proportion of cells that ultimately complete meiosis. In contrast, the *mer3*Δ mutation confers more severe defects in meiotic progression and in the formation of viable spores (Fig. 1 and Table 2). As described in the section above, a *mer3*Δ mutant shows a temperature-sensitive phenotype. This could be due to the fact that temperature affects the stability of the recombination intermediate (e.g., DNA strand exchange products; DNA-protein complex) or even a higher-order protein complex that can be formed in the absence of Mer3. Nonetheless, the *mer3GD* and *mer3KA* mutations confer recombination defects at both temperatures, suggesting that the helicase and ATPase activities are crucial functions of Mer3. The Mer3GD protein might have a level of residual DNA helicase activity that is sufficient for meiotic progression and for viable spore formation, although this seems unlikely in the case of the Mer3KA protein. Thus, it is possible that the Mer3 protein has another function besides DNA unwinding. Since the Mer3 and Mer3GD proteins have the same ability to bind DNA, this DNA binding activity by itself may be required for meiotic progression. Thus, it is possible that the absence of Mer3 bound to DNA may trigger the meiotic checkpoint that results in meiotic arrest prior to meiosis I (2, 6, 36, 66). Alternatively, it is possible that Mer3 bound to DNA may stabilize recombination intermediates and/or recruit other factors, even in the absence of helicase activity. Similarly, Mer3 protein could stabilize a critical higher-order protein complex, a function that might not require helicase activity. If this is the case, then it is possible that it is the inability to properly assemble such structures or protein complexes or process recombination intermediates formed in this context that triggers meiotic arrest. It is also formally possible that the helicase and ATPase activities are not required for recombination at all and that the *mer3GD* and *mer3KA* mutations cause partial defects in some other function of Mer3, although we consider this unlikely.

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