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The MER3 DNA Helicase Catalyzes the Unwinding of Holliday Junctions*

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The MER3 protein of *Saccharomyces cerevisiae* is required for crossover in meiosis and has been suggested to act at the initiation of homologous pairing and the resolution of Holliday junctions. The purified MER3 protein is a DNA helicase that translocates along single-stranded DNA in the 3′ to 5′ direction displacing annealed DNA fragments. Here, MER3 was found to be able to unwind various double-stranded DNA (dsDNA) substrates, including a 30-bp dsDNA with a 20-nucleotide 3′-overhang, a 30-bp dsDNA with a 20-nucleotide 5′-overhang, a 50-bp dsDNA with blunt ends, and a Holliday junction with 25-bp arms, each of which had a blunt end. Efficient unwinding of the 3′-overhang substrate appeared to initiate by the binding of MER3 to the 3′ single-stranded tail in a reaction that required six or more unpaired bases. Unwinding of the blunt end and 5′-overhang substrates appeared to initiate at the blunt ends of these substrates. Unwinding of the Holliday junction was more efficient than the unwinding of the blunt and 5′-overhang substrates and was influenced by Mg^{2+} concentrations that cause changes in the structure of the junction. Possible roles for Holliday junction unwinding in meiotic crossover are discussed.

In most organisms at least one crossover between every pair of homologous chromosomes is required for faithful segregation at the first meiotic nuclear division (meiosis I; for reviews, see Refs. 1–3). A number of *Saccharomyces cerevisiae* genes have been shown to be required for normal levels of meiotic crossing over, but not for gene conversion; these include *MSH4*, *MSH5*, *MLH1*, *MLH3*, *ZIP1*, *ZIP2*, *ZIP3*, *MER3*, and *EXO1* (4–11) of which *MLH1*, *MLH3*, and *EXO1* also function in DNA mismatch repair in mitotic cells (12–14). Mutations in any of these nine genes result in a 50% or greater reduction in crossing over and consequently in increased levels of homolog non-disjunction and spore death. Mutations in *ZIP1*, *ZIP2*, *ZIP3*, and *MER3* also cause a defect in meiotic progression and result in cell cycle arrest prior to meiosis I (4–6, 9). In addition, in cases where it has been examined (*i.e. ZIP1*, *MSH4*, and *MER3*), mutations in these genes also cause defects in crossover interference and hence defects in the regulation of the distribution of crossovers along a chromosome (9, 15, 16). Consistent with these results, mutations in the mouse *MSH4*, *MSH5*, *MLH1*, and *EXO1* genes have been shown to cause defects in chromosome pairing and in cell cycle progression in meiosis (17–21).[1] Overall, these results have been taken to suggest that the proteins encoded by *MSH4*, *MSH5*, *MLH1*, *MLH3*, *ZIP1*, *ZIP2*, *ZIP3*, *MER3,* and *EXO1* function in a crossover-specific meiotic recombination pathway. Exactly how these proteins function to promote crossing over is unclear, although two types of mechanisms have been suggested: in one case the proteins act early in recombination to produce the intermediates that potentially result in crossovers, and in the other case they act to modulate the resolution of Holliday junction recombination intermediates to yield crossovers (22–25).

The MER3 protein is specifically expressed during meiosis where it is required for crossing over (9). On the basis of sequence homology with proteins known to be DNA helicases, MER3 was predicted to be a DNA helicase. The purified MER3 protein was found to bind both ssDNA[2] and dsDNA and have potent ATPase activity that was stimulated either by ssDNA or dsDNA (26, 27). The MER3 protein had DNA helicase activity and unwound 50-, 100-, and 631-nt fragments annealed to M13mp18 single-stranded circular DNA in a reaction that required ATP and Mg^{2+}. Unwinding of the 100- and 631-nt fragments required high amounts of MER3, although relatively low amounts of MER3 could unwind the 100- and 631-nt fragments in the presence of ssDNA-binding proteins, either *S. cerevisiae* RPA (28) or *Escherichia coli* SSB (29). The polarity of the MER3 helicase was determined to be 3′ to 5′ relative to the single-stranded regions of the helicase substrates. On the basis of these properties, it was suggested that MER3 could function in recombination by promoting the initial unwinding of recombination substrates, by driving strand exchange promoted by RAD51 (30) and DMC1 (31, 32), or possibly by catalyzing branch migration of Holliday junctions. In the present study we have investigated the interaction between MER3 and defined DNA substrates including a Holliday junction with 25-bp arms with blunt ends, a 50-bp dsDNA with blunt ends, a 30-bp dsDNA with a 20-nt 5′-overhang, and a 30-bp dsDNA with a 20-nt 3′-overhang. MER3 was found to unwind these substrates with a substrate preference of 3′-overhang > Holliday junction > blunt end > 5′-overhang. Possible roles for such unwinding reactions in recombination are discussed.

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[1] K. Wei, A. Clark, M. F. Kane, D. Mazur, E. Wong, T. Parris, N. Kolas, R. Russell, T. Kunkel, R. D. Kolodner, P. Cohen, and W. Edelmann, manuscript in preparation.

[2] The abbreviations used are: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; nt, nucleotide; HJ^{MIS}, Holliday junction derivative with mispaired bases; ATPγS, adenosine 5′-*O*-(thiotriphosphate); RPA, replication protein A; SSB, single-strand DNA binding protein.

TABLE I
Oligonucleotides for the construction of DNA substrates

T70	(50nt) 5′-TCGATAGTCTCTAGACAGCATGTCCTAGCAAGCCAGAATTCGGCAGCGTC-3′
T71	(50nt) 5′-GACGCTGCCGAATTCTGGCTTGCTAGGACATCTTTGCCCACGTTGACCCA-3′
T72	(50nt) 5′-AACGTCATAGACGATTACATTGCTAGGACATGCTGTCTAGAGACTATCGA-3′
T73	(50nt) 5′-TGGGTCAACGTGGGCAAAGATGTCCTAGCAATGTAATCGTCTATGACGTT-3′
T74	(50nt) 5′-GACGCTGCCGAATTCTGGCTTGCTAGGACATGCTGTCTAGAGACTATCGA-3′
T75	(30nt) 5′-GACGCTGCCGAATTCTGGCTTGCTAGGACA-3′
T76	(30nt) 5′-TGCTAGGACATGCTGTCTAGAGACTATCGA-3′
T88	(50nt) 5′-GACGCTGCCGAATTCTGGCTTGCTCTCTGCACTTTGCCCACGTTGACCCA-3′
T89	(50nt) 5′-AACGTCATAGACGATTACATACGTCTGACATGCTGTCTAGAGACTATCGA-3′
T90	(50nt) 5′-TGGGTCAACGTGGGCAAAGTGCAGCTACGTATGTAATCGTCTATGACGTT-3′
BOT	(30nt) 5′-GCATAGGACATGCTGTCTAGAGACTATTCG-3′
TOP	(30nt) 5′-CGAATAGTCTCTAGACAGCATGTCCTATGC-3′
TOP2A	(32nt) 5′-CGAATAGTCTCTAGACAGCATGTCCTATGCAA-3′
TOP3A	(33nt) 5′-CGAATAGTCTCTAGACAGCATGTCCTATGCAAA-3′
TOP4A	(34nt) 5′-CGAATAGTCTCTAGACAGCATGTCCTATGCAAAA-3′
TOP6A	(36nt) 5′-CGAATAGTCTCTAGACAGCATGTCCTATGCAAAAAA-3′
TOP8A	(38nt) 5′-CGAATAGTCTCTAGACAGCATGTCCTATGCAAAAAAAA-3′
TOP12A	(42nt) 5′-CGAATAGTCTCTAGACAGCATGTCCTATGCAAAAAAAAAAAA-3′
TOP20A	(50nt) 5′-CGAATAGTCTCTAGACAGCATGTCCTATGCAAAAAAAAAAAAAAAAAAAA-3′

MATERIALS AND METHODS

Preparation of MER3 Protein—The MER3 protein tagged with a FLAG epitope at the C terminus that was previously shown to complement a *mer3* deletion mutant (27) was overexpressed in yeast cells and purified by binding to FLAG affinity gel (Sigma) followed by sequential chromatography on Mono Q HR5/5, HiTrap heparin, and Mono S HR5/5 (Amersham Biosciences) columns, as previously described (26, 27). The final protein preparations were greater than 98% pure MER3.

DNA Substrates—The sequences of the oligonucleotides used are shown in Table I. To prepare the Holliday junction blunt, 3′-, and 5′-overhang substrates (Fig. 1), and the Holliday junction derivative containing one mispaired base in each arm at the junction (Fig. 5*C*, HJ^{MIS}), oligonucleotide T70 was 5′-end-labeled with [γ-^{32}P]ATP (PerkinElmer Life Sciences) and T4 polynucleotide kinase (New England Biolabs). The labeled DNA was mixed with 2× concentrations of other oligonucleotides (T71, T72, and T73 for the Holliday junction substrate; T74 for the blunt substrate; T76 for the 3′-overhang substrate; T75 for the 5′-overhang substrate; T88, T89, and T90 for the HJ^{MIS} substrate) in annealing buffer (10 mM Tris-HCl (pH 7.6), 1 mM EDTA, 0.5 M NaCl), heated to 94 °C for 5 min, then cooled down to 20 °C over a 2-h period. The annealed DNAs were separated by 10% polyacrylamide gel electrophoresis (PAGE) (60:1 acrylamide/bisacrylamide) in TBE (90 mM Tris borate, 2 mM EDTA). The DNA was recovered by soaking the excised band in elution buffer (0.5 M NaCl, 0.6 M sodium acetate, 1 mM EDTA) overnight at 4 °C followed by extraction with phenol/chloroform and precipitation with ethanol. The recovered DNA pellet was dissolved in suspension buffer (10 mM Tris-HCl (pH 7.6), 250 mM NaCl), and the concentration of the purified DNA was determined using a DNA DipStick™ (Invitrogen). The DNA substrates containing different lengths of a poly(dA) 3′-tail (Fig. 4, *B* and *C*) were prepared as described above, except that oligonucleotide BOT was 5′-end-labeled and annealed to TOP, TOP2, TOP3, TOP4, TOP6, TOP8, TOP12, or TOP20.

Gel Mobility Shift Assays—Indicated amounts of the MER3 protein and 0.8 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 dithiothreitol, 100 μg/ml bovine serum albumin). After incubation for 30 min on ice, 5 μl of loading buffer A (40 mM Tris-HCl (pH 8.0), 25% glycerol, 400 μg/ml bovine serum albumin) was added to the binding reaction. The DNA protein complexes were analyzed by electrophoresis through non-denaturing 5% polyacrylamide gels (19:1) run in low ionic strength buffer (6.7 mM Tris-HCl (pH 8.0), 3.3 mM sodium acetate, 2 mM EDTA), and the ^{32}P-labeled DNA-containing bands were visualized with a PhosphorImager (445 SI, Molecular Dynamics).

DNA Helicase Assays—The indicated amount of protein and 1 nM DNA substrate (concentration is in moles of molecules) were incubated in 20-μl volumes containing DNA helicase buffer (20 mM Tris-HCl (pH 7.6), 10 mM NaCl, 2 mM dithiothreitol, 100 μg/ml bovine serum albumin, 1.5 mM $MgCl_2$, 2 mM ATP). The NaCl and $MgCl_2$ concentrations and both the divalent cation and nucleotide type were varied in individual experiments as indicated. All reactions were preincubated at 30 °C for 5 min, started by the addition of MER3 protein, and then incubation was continued for 30 min. 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/ml proteinase K, followed by incubation at 37 °C for 10 min. For time courses, 110-μl reactions were performed, and aliquots of 5 μl were withdrawn at the indicated time points and mixed with 2.5 μl of stop buffer and 0.25 μl of 25 mg/ml proteinase K. The DNA products were analyzed by electrophoresis through non-denaturing polyacrylamide gels run in Tris borate/EDTA (TBE). The gels were dried, and the radiolabeled DNA was visualized using a phosphorimager.

RESULTS

The ability of MER3 to bind to different DNA substrates was analyzed using gel mobility shift assays. The DNA substrates analyzed were a model Holliday junction with 25-bp arms, each of which had a blunt end, a 50-bp dsDNA with blunt ends, a 30-bp dsDNA with a 20-nt 3′-ssDNA overhang, and a 30-bp dsDNA with a 20-nt 5′-ssDNA overhang (Fig. 1, *A–D*). Incubating each of these DNA substrates with increasing amounts of MER3 resulted in the production of specific, more slowly migrating protein-DNA complexes (Fig. 1, *E–H*). We do not know if the multiple, slow-migrating DNA species formed represent the binding of multiple MER3 molecules or different DNA conformations. However, since the relative proportion of the slowly migrating species increases as the protein concentration increases, where there are excess amounts of MER3 over DNA substrates (Fig. 1, *E–H*, *lanes 3* and *4*), it seems possible that these forms require binding of multiple MER3 molecules. The relative affinity of MER3 for each of the DNA substrates appeared to be similar.

To determine if MER3 could unwind the Holliday junction substrate, MER3 DNA helicase assays were performed with either the Holliday junction substrate (Fig. 1*A*) or the dsDNA substrate with a 3′-overhang (Fig. 1*C*). Reactions were performed with different divalent cations and different nucleotide cofactors (Fig. 2). MER3 converted the Holliday junction to a mixture of partially unwound Y-junctions and completely unwound ssDNA and converted the dsDNA with a 3′-overhang to ssDNA. These unwinding reactions required a divalent cation, of which $MgCl_2$ and $MnCl_2$ were equally effective at the concentration tested. A small amount of unwound product was observed in the presence of $CaCl_2$, and no unwound product was observed in the presence of $ZnCl_2$ at the concentration tested. ATP and dATP were equally effective at supporting the unwinding reaction, whereas ATPγS and ADP did not support unwinding at the concentration tested. No difference in the requirement for divalent cations and nucleotides was observed for MER3-dependent unwinding of the Holliday junction and the 3′-overhang substrates under the reaction conditions used. These results clearly indicate that MER3 can unwind a Holli-

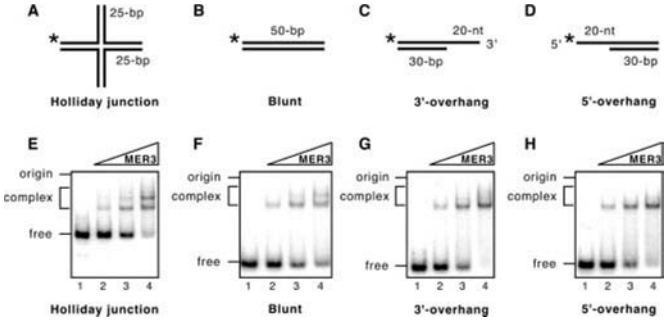

FIG. 1. **Binding of MER3 to model DNA substrates.** Illustrated are the DNA substrates used in this study including the Holliday junction substrate (*A*), the blunt end substrate (*B*), the 3′-overhang substrate (*C*), and the 5′-overhang substrate (*D*). Sequences of oligonucleotides used to prepare the DNA substrates are shown in Table I (see also "Materials and Methods"). The lengths of single- and double-stranded regions are indicated. Positions of ^{32}P-labeled 5′-ends of oligonucleotides are indicated as an asterisk. The Holliday junction substrate has a 12-bp core homology region that allows branch migration of the junction; this substrate is the same as the previously described J12 junction (55), except that an extra base was added to the oligonucleotides, as appropriate, to eliminate the one base 5′-overhang that was originally present at the end of the arms. *E–H*, the interaction between the DNA substrates and increasing amounts of MER3 (0, 0.5, 2, and 8 nM) were examined under conditions described under "Materials and Methods."

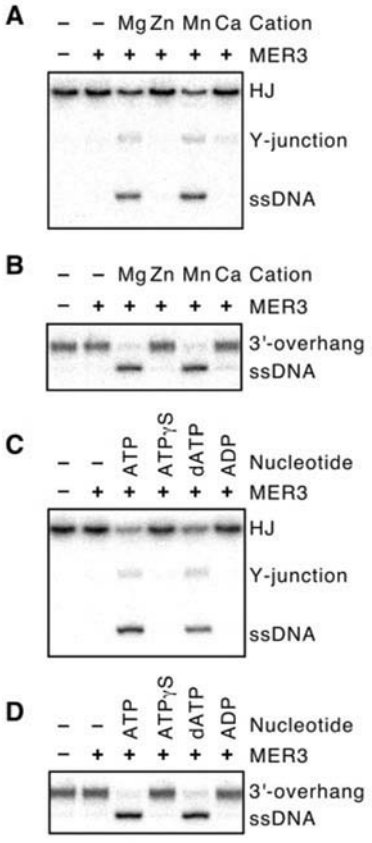

FIG. 2. **Unwinding of the Holliday junction and the 3′-overhang substrates in the presence of different cations and nucleotides.** *A* and *B,* MER3 DNA helicase reactions were carried out in the presence of 0.5 mM $MgCl_2$, $ZnCl_2$, $MnCl_2$, or $CaCl_2$ as indicated. Reaction mixtures contain 1 nM (expressed as moles of molecules) DNA substrates, 2 mM ATP, and 0.05 mM EDTA. After preincubation at 30 °C for 5 min, reactions (20 μl each) were initiated by the addition of 1.6 nM MER3 and further incubated for 30 min. The reaction was terminated by the addition of 5 μl of stop buffer (see "Materials and Methods") and 0.5 μl of 25 mg/ml proteinase K, followed by incubation at 37 °C for 10 min. DNA products were separated by electrophoresis through 10% non-denaturing polyacrylamide gels run in 1× Tris borate/EDTA (TBE) buffer, and the P^{32}-labeled fragments were detected using a phosphorimager. The Holliday junction (*A*) or 3′-overhang (*B*) were used as DNA substrates in helicase reactions. *C* and *D*, MER3 DNA helicase reactions were carried out in the presence of 2 mM ATP, ATPγS, dATP, or ADP as indicated. Reactions are essentially the same as described above, except that all reactions contained 0.5 mM $MgCl_2$. The Holliday junction (*C*) or 3′-overhang (*D*) were used as DNA substrates in helicase reactions. *HJ*, the Holliday junction substrate.

day junction in addition to unwinding a dsDNA with a 3′-overhang.

To better characterize the MER3 DNA helicase activity, the ability of MER3 to unwind the Holliday junction, blunt end dsDNA and 3′- and 5′-overhang substrates (Fig. 1, *A–D*) was studied as a function of MER3 concentration (Fig. 3, *A* and *B*) and incubation time (Fig. 3, *C* and *D*). All four substrates were unwound by MER3, and all of the unwinding reactions showed a relatively similar time course and protein concentration dependence. Under the reaction conditions used (Fig. 3), multiple DNA substrates were unwound per MER3 monomer; note that the active species of MER3 is thought to be an oligomeric form, although the exact species is not known because MER3 appears to exist as a heterogeneous mixture of different oligomers (26, 27). Based on the results, MER3 appeared to have a substrate preference of 3′-overhang > Holliday junction > blunt > 5′-overhang. The same preference was observed in the reactions containing a higher concentration of MER3 (2.5 nM) that were incubated for a longer period of time (60 min) where 100% unwinding of the Holliday junction was observed; under conditions where the Holliday junction was 100% unwound, the extent of unwinding of the blunt-end substrate observed never exceeded 50%, a finding similar to the results shown in Fig. 3 (data not shown). The observation that the 3′-overhang substrate is the preferred substrate is consistent with previously published results showing that the MER3 helicase acts by binding to ssDNA and translocating along it in the 3′ to 5′ direction displacing hybridized ssDNA fragments (26). Given this polarity and the fact that apparently MER3 can initiate unwinding from a blunt end, it is not surprising that the 5′-overhang substrate was the least preferred substrate; compared with the other substrates where each end could serve as an entry site, the 5′-overhang substrate likely has only one end that can serve as an entry site since binding to the 5′-overhang would result in unproductive translocation off of the substrate.

The observation that MER3 could initiate unwinding from a blunt end was possibly unexpected given previous results that MER3 binds ssDNA and translocates along it. The mechanism of unwinding from blunt ends was investigated further by examining the effect of NaCl on the unwinding of each of the four substrates (Fig. 4*A*). Unwinding of the 3′-overhang substrate was unaffected by the addition of 150 mM NaCl. In contrast, unwinding of all of the other substrates was significantly inhibited by the addition of 150 mM NaCl. Since 150 mM NaCl does not inherently inhibit MER3 helicase activity, one explanation for this effect is that unwinding from blunt ends requires transient denaturation (*i.e.* breathing) of the ends, and the inhibitory effect of 150 mM NaCl is caused by stabilization of the blunt ends. Because breathing would not be expected to produce large regions of ssDNA, the size of the 3′ single-stranded overhang required for efficient unwinding of the 3′-overhang substrate was studied in the presence of 150 mM NaCl (Fig. 4, *B* and *C*). Substrates containing 2, 3, or 4 dA residues of 3′-overhang were all unwound with the same efficiency as a blunt-ended substrate, whereas substrates containing 6, 8, 12, and 20 dA residues of 3′-overhang were all unwound with significantly increased, but identical efficiency. This indicates that MER3 requires a short (not more than six nucleotides) single-stranded region to serve as an entry site to initiate unwinding. Such a small entry site size is consistent

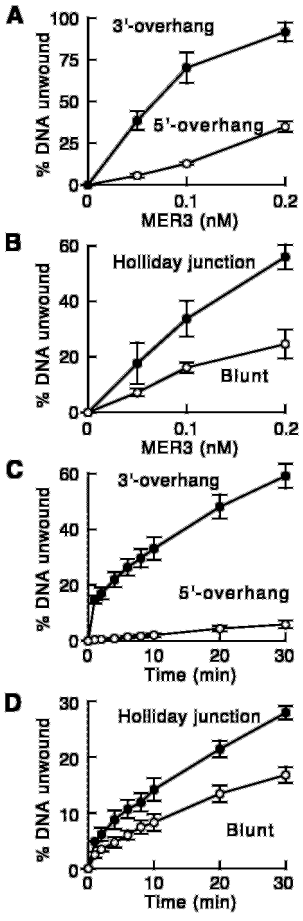

FIG. 3. **Unwinding of model DNA substrates by MER3 helicase.** *A* and *B*, unwinding of the four DNA substrates shown in Fig. 1 was measured at different concentrations of MER3 (0, 0.05, 0.1, and 0.2 nM) in 30-min DNA helicase assays. *C* and *D*, unwinding of the DNA substrates was measured at different incubation times (0, 1, 2, 4, 6, 8, 10, 20, and 30 min) after the addition of 0.2 nM MER3. All DNA substrates were present at a concentration of 1 nM (expressed as moles of molecules). The percentage of DNA unwound using the 3′- and 5′-overhang substrates is shown in *A* and *C*, and that for the Holliday junction and blunt substrates is presented in *B* and *D*. In the case of the Holliday junction, DNA unwound contains Y-junction and ssDNA. The value is the mean of three sets of experiments, and the *error bar* shows the S.D.

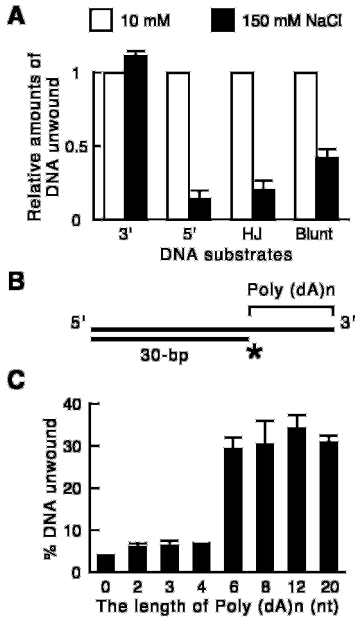

FIG. 4. **The effect of NaCl concentration and the length of 3′ single-stranded overhang on DNA unwinding by MER3 helicase.** *A*, the amount of DNA unwound in the presence of 150 mM NaCl compared with that in the presence of 10 mM NaCl. Reactions contained 1 nM DNA substrates (expressed as moles of molecules) and 0.4 nM MER3. In the case of the Holliday junction, DNA unwound contains Y-junction and ssDNA. The value is the mean of two sets of experiments, and the *error bar* shows the standard deviation. *B*, illustrated is the DNA substrate containing a 30-bp double-stranded region and poly(dA) 3′ single-stranded overhang. The position of the ^{32}P-labeled 5′-end of oligonucleotide BOT is indicated as an asterisk. *C*, the percentage of DNA unwound was measured using the DNA substrate containing various lengths of 3′ single-stranded overhang. Reactions contain 1 nM DNA substrates (expressed as moles of molecules), 150 mM NaCl, and 0.4 nM MER3. The value is the mean of three sets of experiments, and the *error bar* shows the S.D.

with the observation that MER3 can unwind blunt-ended substrates more efficiently at low, but not high, NaCl concentrations. However, it should be noted that high NaCl concentrations also affect the configuration of model Holliday junctions (33–36) raising the possibility that the affect of NaCl on unwinding of the Holliday junction could reflect effects on both the ends of the substrate and the junction itself (see "Discussion").

MER3 unwound the Holliday junction substrate 2–3× more efficiently than the dsDNA with blunt ends (Fig. 3, *B* and *D*). However, this is an underestimate of the relative efficiency of unwinding if unwinding can only initiate from the ends, because unwinding the dsDNA with blunt ends only requires initiating unwinding from one end, whereas fully unwinding a Holliday junction likely requires initiating unwinding from as many as three ends. This analysis suggests that a Holliday junction could be unwound by a different mechanism than a dsDNA with blunt ends. To investigate the mechanism of unwinding further, a titration of $MgCl_2$ concentration *versus* unwinding was performed (Fig. 5, *A* and *B*). Unwinding of both the 3′-overhang and blunt substrates was essentially maximal at 0.5 mM $MgCl_2$, and $MgCl_2$ concentrations up to 5 mM resulted in the same level of unwinding. Unwinding of the 5′-overhang substrate increased in $MgCl_2$ concentrations of up to 2 mM, above which activity would plateau. Unwinding of the Holliday junction showed a completely different dependence on $MgCl_2$ concentration. Unwinding steadily increased until maximal activity occurred at 1.5 mM $MgCl_2$; this level of unwinding was 2–3× higher than observed for the blunt dsDNA substrate at the same $MgCl_2$ concentration. Above 1.5 mM $MgCl_2$, the extent of unwinding decreased until a plateau was reached at $MgCl_2$ concentrations of 3 mM and above; in the plateau region, the efficiency of unwinding the Holliday junction was the same as with the blunt-end substrate. Because the $MgCl_2$-dependent MER3 activity peak observed with the Holliday junction substrate does not correspond to an effect on MER3 helicase activity, it seems likely that the $MgCl_2$ effect reflects an effect of $MgCl_2$ concentration on the conformation of the Holliday junction substrate. Indeed, it is well known that $MgCl_2$ and other salts have significant effects on the conformation of the types of Holiday junctions used here as substrates (33, 34). To further analyze such junction effects, a Holliday junction was constructed by modifying the core sequence of oligonucleotides T88, T89, and T90 so that the junction could not branch migrate and contained one mismatched base pair (C:T) in each arm at the junction. A titration of $MgCl_2$ concentration *versus* unwinding was then performed using the modified Holliday junction substrate (HJ^{MIS}) (Fig. 5, *C* and *D*). Unwinding of the HJ^{MIS} substrate did not show the $MgCl_2$ optimum of the fully paired Holliday junction but, rather, the $MgCl_2$ titration was essentially the same as seen with the 3′-overhang and blunt-end substrates.

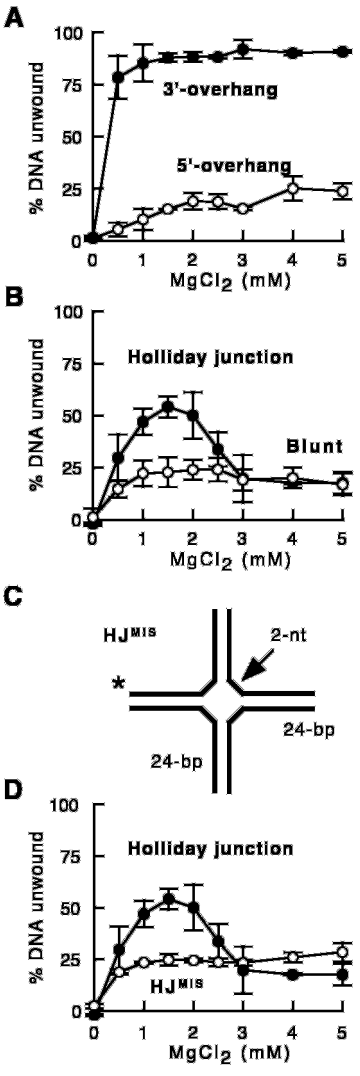

FIG. 5. **The effect of $MgCl_2$ concentration on MER3 helicase activity.** The effects of $MgCl_2$ concentration on MER3 helicase activity was determined using the DNA substrates illustrated in Fig. 1 present at a concentration of 1 nM (expressed as moles of molecules). The percentage of DNA unwound by MER3 (0.2 nM) at different concentrations of $MgCl_2$ (0, 0.5, 1, 1.5, 2, 2.5, 3, 4, and 5 mM) using the 3′- and 5′-overhang substrates (*A*), and the Holliday junction and the blunt substrates (*B*) is presented. *C*, the DNA substrate containing one C:T mispair in each arm at the base of the Holliday junction is illustrated (HJ^{MIS}). The position of the ^{32}P-labeled 5′-end of oligonucleotide T70 is indicated as an asterisk. *D*, the percentage of DNA unwound in the MER3 helicase assay using the HJ^{MIS} substrate shown in (*C*). In the case of the Holliday junction, DNA unwound contains Y-junction and ssDNA. The value is the mean of three sets of experiments, and the *error bar* shows the S.D.

DISCUSSION

In the present study, we have extensively characterized the ability of the MER3 DNA helicase to unwind model DNA substrates constructed with oligonucleotides. The MER3 protein was able to unwind double-stranded DNA substrates, including a 30-bp dsDNA, with a 20-nt 3′-overhang, a 30-bp dsDNA with a 20-nt 5′-overhang, a 50-bp dsDNA with blunt ends, and a Holliday junction with 25-bp arms, each of which had a blunt end. The 30-bp dsDNA with a 20-nt 3′-overhang was unwound with the greatest efficiency consistent with previous results showing that the MER3 helicase acts by translocating along single-stranded DNA in the 3′ to 5′ direction (26). MER3 also appeared to be able to initiate unwinding from blunt ends at reduced efficiency, possibly by binding to transient single-stranded regions produced at the ends by breathing. Consistent with this, only a short 6-nt or longer 3′ single-stranded overhang was required for maximal unwinding of 3′-overhang substrates. The 5′-overhang substrate was also a substrate for MER3, although it was the least efficient substrate. This unwinding was likely due to initiation on the blunt end, because initiation on the 5′-overhang would result in non-productive translocation off of the substrate. MER3 could unwind Holliday junction substrates with a greater efficiency than the 50-bp blunt-end dsDNA substrate. Given that it should require three separate initiation events to unwind a Holliday junction from the ends compared with one initiation event for the blunt end dsDNA substrate, the Holliday junctions were clearly preferred substrates for MER3 compared with the blunt-end dsDNA substrate. The observation that MER3 can unwind Holliday junctions places MER3 in a class of DNA helicases that function in recombination and repair including *E. coli* RuvAB (37, 38) and RecG (39, 40), bacteriophage T4 gene 41 protein in the presence of gene 59 protein (41), *S. cerevisiae* SGS1 (42) and mammalian BLM (43), and WRN (44, 45).

Holliday junctions, like the ones studied here, undergo conformational changes that are modulated by cations like Mg^{2+} (33–36). In the absence of Mg^{2+}, these junctions exist as planar-X structures. As the Mg^{2+} concentration is increased to 80 μM, these structures undergo a change to a stacked-X configuration (34), which is the configuration recognized by Holliday junction resolution enzymes. As the Mg^{2+} concentration is increased, the stability of the stacked-X configuration increases progressively because the Mg^{2+} ions counterbalance the negative charges of the DNA backbone allowing the DNA arms to stack more tightly on each other. Ions such as Na^+ induce the same changes, albeit at a higher concentration. When mispaired bases are introduced at the base of the junction, significantly higher Mg^{2+} concentrations are required to induce the planar- to stacked-X conformational change (46). The titration of $MgCl_2$ concentration *versus* MER3 catalyzed unwinding of the fully base paired Holliday junction showed an unusual optimum at 1.5 mM $MgCl_2$. This does not correspond to an effect of Mg^{2+} on MER3 helicase activity using DNA substrates other than a Holliday junction and is thus likely to represent an effect of Mg^{2+} on Holliday junction structure. Because of the presence of ATP in the DNA helicase reactions, it is difficult to know the free Mg^{2+} concentration in the Mg^{2+} titrations performed here. However, given the ATP concentration of 2 mM, the amount of free Mg^{2+} present at the 1.5 mM Mg^{2+} activity peak is likely to be quite low and in the range where the planar- to stacked-X conformational change occurs. Likewise, the inhibition of unwinding seen at higher Mg^{2+} concentrations likely represents stabilization of the stacked-X structure that occurs at high Mg^{2+} concentrations. The observation that introducing mispaired bases in each arm at the junction significantly alters the Mg^{2+} *versus* activity titration supports this interpretation. The modified Holliday junction would be predicted to require higher Mg^{2+} concentrations for induction of both types of structural changes. These results suggest that unwinding of a Holliday junction by MER3 is highly sensitive to the conformation at the junction. A somewhat similar inhibitory effect by an excess Mg^{2+} was also observed with the bacterial RecQ and RecG helicases (47, 48). Furthermore, combined with the observation that MER3 unwound the Holliday junction substrates more efficiently than the blunt-end dsDNA substrate, the unusual effect of Mg^{2+} concentration on Holliday junction unwinding raises the possibility that MER3 might initiate unwinding of a Holliday junction from the junction rather than from the ends. Alternatively, unwinding could initiate at the ends, and at 1.5 mM $MgCl_2$ the Holliday junction could assume

a conformation that is much more easily unwound than the linear dsDNA substrates.

Previous studies have suggested that MER3 could act during at least two stages during meiotic recombination. It could either act in the initiation of strand invasion or in extending the length of early heteroduplex recombination intermediates and thus promote the formation of a recombination intermediate required for crossing over, or it could function to promote the resolution of Holliday junctions to yield crossovers (26). The results presented here provide more detail about how MER3 could act during recombination. The observation that MER3 can unwind dsDNA from blunt ends suggests that MER3 could be involved in unwinding the ends of meiotic double-strand breaks (DSBs) prior to their resection. However, this is inconsistent with the observation that MER3 is not required for resection of meiotic DSBs and suggests that MER3 acts later in recombination such as in promoting strand exchange after the DSBs are resected. The observation that MER3 can unwind Holliday junctions supports the idea that MER3 could act later in recombination by branch migrating Holliday junctions to extend the regions of heteroduplex DNA formed and thus stabilize early recombination intermediates. In this regard, initiation of branch migration by binding to the Holliday junction or by binding to DNA at a distant site followed by translocation to the Holliday junction would both result in similar branch migration. In addition, the ability to unwind Holliday junctions could also reflect a role of MER3 in the resolution of such intermediates. Studies of the interaction between MER3 and the homologous pairing proteins RAD51 (30) and DMC1 (31, 32) and other proteins like the MSH4-MSH5 complex (49), the MLH1-MLH3 and MLH1-PMS1 complexes (50–53), and MUS81-MMS4 DNA endonuclease (54), which may function in either Holliday junction formation or resolution, should shed further light on the function of MER3.

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REFERENCES

1. Kleckner, N. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 8167–8174
2. Paques, F., and Haber, J. E. (1999) *Microbiol Mol. Biol. Rev.* **63,** 349–404
3. Roeder, G. S. (1997) *Genes Dev.* **11,** 2600–2621
4. Agarwal, S., and Roeder, G. S. (2000) *Cell* **102,** 245–255
5. Chua, P. R., and Roeder, G. S. (1998) *Cell* **93,** 349–359
6. Sym, M., Engebrecht, J., and Roeder, G. S. (1993) *Cell* **72,** 365–378
7. Hollingsworth, N. M., Ponte, L., and Halsey, C. (1995) *Genes Dev.* **9,** 1728–1739
8. Ross-Macdonald, P., and Roeder, G. S. (1994) *Cell* **79,** 1069–1080
9. Nakagawa, T., and Ogawa, H. (1999) *EMBO J.* **18,** 5714–5723
10. Kirkpatrick, D. T., Ferguson, J. R., Petes, T. D., and Symington, L. S. (2000) *Genetics* **156,** 1549–1557
11. Tsubouchi, H., and Ogawa, H. (2000) *Mol. Biol. Cell* **11,** 2221–2233
12. Prolla, T. A., Christie, D. M., and Liskay, R. M. (1994) *Mol. Cell. Biol.* **14,** 407–415
13. Flores-Rozas, H., and Kolodner, R. D. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 12404–12409
14. Szankasi, P., and Smith, G. R. (1995) *Science* **267,** 1166–1169
15. Sym, M., and Roeder, G. S. (1994) *Cell* **79,** 283–292
16. Novak, J. E., Ross-Macdonald, P. B., and Roeder, G. S. (2001) *Genetics* **158,** 1013–1025
17. Kneitz, B., Cohen, P. E., Avdievich, E., Zhu, L., Kane, M. F., Hou, H., Jr., Kolodner, R. D., Kucherlapati, R., Pollard, J. W., and Edelmann, W. (2000) *Genes Dev.* **14,** 1085–1097
18. Edelmann, W., Cohen, P. E., Kneitz, B., Winand, N., Lia, M., Heyer, J., Kolodner, R., Pollard, J. W., and Kucherlapati, R. (1999) *Nat. Genet.* **21,** 123–127
19. de Vries, S. S., Baart, E. B., Dekker, M., Siezen, A., de Rooij, D. G., de Boer, P., and te Riele, H. (1999) *Genes Dev.* **13,** 523–531
20. Baker, S. M., Plug, A. W., Prolla, T. A., Bronner, C. E., Harris, A. C., Yao, X., Christie, D. M., Monell, C., Arnheim, N., Bradley, A., Ashley, T., and Liskay, R. M. (1996) *Nat. Genet.* **13,** 336–342
21. Edelmann, W., Cohen, P. E., Kane, M., Lau, K., Morrow, B., Bennett, S., Umar, A., Kunkel, T., Cattoretti, G., Chaganti, R., Pollard, J. W., Kolodner, R. D., and Kucherlapati, R. (1996) *Cell* **85,** 1125–1134
22. Storlazzi, A., Xu, L., Schwacha, A., and Kleckner, N. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 9043–9048
23. Nakagawa, T., Datta, A., and Kolodner, R. D. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 14186–14188
24. Hunter, N., and Kleckner, N. (2001) *Cell* **106,** 59–70
25. Allers, T., and Lichten, M. (2001) *Cell* **106,** 47–57
26. Nakagawa, T., Flores-Rozas, H., and Kolodner, R. D. (2001) *J. Biol. Chem.* **276,** 31487–31493
27. Nakagawa, T., and Kolodner, R. D. (2002) *Mol. Cell. Biol.* **22,** 3281–3291
28. Alani, E., Thresher, R., Griffith, J. D., and Kolodner, R. D. (1992) *J. Mol. Biol.* **227,** 54–71
29. McEntee, K., Weinstock, G. M., and Lehman, I. R. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 857–861
30. Sung, P. (1994) *Science* **265,** 1241–1243
31. Li, Z., Golub, E. I., Gupta, R., and Radding, C. M. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 11221–11226
32. Hong, E. L., Shinohara, A., and Bishop, D. K. (2001) *J. Biol. Chem.* **276,** 41906–41912
33. Murchie, A. I., Clegg, R. M., von Kitzing, E., Duckett, D. R., Diekmann, S., and Lilley, D. M. (1989) *Nature* **341,** 763–766
34. Duckett, D. R., Murchie, A. I., and Lilley, D. M. (1990) *EMBO J.* **9,** 583–590
35. Duckett, D. R., Murchie, A. I., Diekmann, S., von Kitzing, E., Kemper, B., and Lilley, D. M. (1988) *Cell* **55,** 79–89
36. Clegg, R. M., Murchie, A. I., Zechel, A., Carlberg, C., Diekmann, S., and Lilley, D. M. (1992) *Biochemistry* **31,** 4846–4856
37. Iwasaki, H., Takahagi, M., Nakata, A., and Shinagawa, H. (1992) *Genes Dev.* **6,** 2214–2220
38. Tsaneva, I. R., Muller, B., and West, S. C. (1992) *Cell* **69,** 1171–1180
39. Lloyd, R. G., and Sharples, G. J. (1993) *Nucleic Acids Res.* **21,** 1719–1725
40. Lloyd, R. G., and Sharples, G. J. (1993) *EMBO J.* **12,** 17–22
41. Jones, C. E., Mueser, T. C., and Nossal, N. G. (2000) *J. Biol. Chem.* **275,** 27145–27154
42. Lu, J., Mullen, J. R., Brill, S. J., Kleff, S., Romeo, A. M., and Sternglanz, R. (1996) *Nature* **383,** 678–679
43. Karow, J. K., Chakraverty, R. K., and Hickson, I. D. (1997) *J. Biol. Chem.* **272,** 30611–30614
44. Gray, M. D., Shen, J. C., Kamath-Loeb, A. S., Blank, A., Sopher, B. L., Martin, G. M., Oshima, J., and Loeb, L. A. (1997) *Nat. Genet.* **17,** 100–103
45. Suzuki, N., Shimamoto, A., Imamura, O., Kuromitsu, J., Kitao, S., Goto, M., and Furuichi, Y. (1997) *Nucleic Acids Res.* **25,** 2973–2978
46. Duckett, D. R., and Lilley, D. M. (1991) *J. Mol. Biol.* **221,** 147–161
47. Harmon, F. G., and Kowalczykowski, S. C. (2001) *J. Biol. Chem.* **276,** 232–243
48. Whitby, M. C., and Lloyd, R. G. (1998) *J. Biol. Chem.* **273,** 19729–19739
49. Pochart, P., Woltering, D., and Hollingsworth, N. M. (1997) *J. Biol. Chem.* **272,** 30345–30349
50. Hunter, N., and Borts, R. H. (1997) *Genes Dev.* **11,** 1573–1582
51. Wang, T. F., Kleckner, N., and Hunter, N. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 13914–13919
52. Mechanic, L. E., Frankel, B. A., and Matson, S. W. (2000) *J. Biol. Chem.* **275,** 38337–38346
53. Argueso, J. L., Smith, D., Yi, J., Waase, M., Sarin, S., and Alani, E. (2002) *Genetics* **160,** 909–921
54. Kaliraman, V., Mullen, J. R., Fricke, W. M., Bastin-Shanower, S. A., and Brill, S. J. (2001) *Genes Dev.* **15,** 2730–2740
55. Connolly, B., Parsons, C. A., Benson, F. E., Dunderdale, H. J., Sharples, G. J., Lloyd, R. G., and West, S. C. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 6063–6067