Saccharomyces cerevisiae MPH1 Gene, Required for Homologous Recombination-mediated Mutation Avoidance, Encodes a 3’ to 5’ DNA Helicase*

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The MPH1 (mutator pheno-type 1) gene of Saccharomyces cerevisiae was identified on the basis of elevated spontaneous mutation rates of haploid cells deleted for this gene. Further studies showed that MPH1 functions to channel DNA lesions into an error-free DNA repair pathway. The Mph1 protein contains the seven conserved motifs of the superfamily 2 (SF2) family of nucleic acid unwinding enzymes. Genetic analyses have found epistasis of the mph1 deletion with mutations in the RAD52 gene group that mediates homologous recombination and DNA repair by homologous recombination. To begin dissecting the biochemical functions of the MPH1-encoded product, we have expressed it in yeast cells and purified it to near homogeneity. We show that Mph1 has a robust ATPase function that requires single-stranded DNA for activation. Consistent with its homology to members of the SF2 helicase family, we find a DNA helicase activity in Mph1. We present data to demonstrate that the Mph1 DNA helicase activity is fueled by ATP hydrolysis and has a 3’ to 5’ polarity with respect to the DNA strand on which this protein translocates. The DNA helicase activity of Mph1 is enhanced by the heterotrimERIC single-stranded DNA binding protein replication protein A. These results, thus, establish Mph1 as an ATP-dependent DNA helicase, and the availability of purified Mph1 should facilitate efforts at deciphering the role of this protein in homologous recombination and mutation avoidance.

DNA helicases are involved in essentially all the metabolic pathways that entail melting of the DNA double helix, such as DNA replication, homologous recombination (HR), and DNA repair reactions (1, 2). These enzymes invariably possess the ability to hydrolyze a nucleoside triphosphate (most often ATP) when DNA (in general ssDNA) is present, and they are capable of utilizing the free energy derived from the nucleotide hydrolytic reaction to translocate on the DNA. Upon encountering a duplex region, the base pairing in the DNA is disrupted to result in the separation of DNA strands. Certain DNA helicase enzymes, e.g. those that belong to the RecQ helicase family, are specific for DNA structures that arise during homologous recombination and when replication forks stall (1, 2). Interestingly, the translocase activity of some helicases enables them to dislodge proteins from their path of movement, and this property of the helicase enzymes in question is believed to be germane for their biological roles (3–6). A number of DNA helicases, including the yeast Srs2 and Sgs1 proteins and the human BLM and WRN proteins, play roles in modulating homologous recombination efficiency and outcome, preventing replication fork demise, and the general maintenance of the genome (1, 2, 7). In humans, inactivation of the BLM and WRN helicases can lead to Bloom syndrome and Werner syndrome, which are marked by cancer predisposition.

These findings aptly underscore the importance for delineating the functions of DNA helicases in homologous recombination and related processes.

The Saccharomyces cerevisiae MPH1-encoded product, a nuclear protein that possesses sequence homology to the superfamily 2 class of helicase enzymes, has been implicated in an HR-dependent pathway of mutation avoidance (8). A deletion mutant of the MPH1 gene was first identified to be a spontaneous mutator (8). In mph1Δ cells, forward mutations at the CAN1 locus and the reversion of trp1-289 harboring an amber mutation are both enhanced. However, frameshift mutations, as gauged by analyzing the mutation spectrum at the URA3 locus and measuring the reversion of hom3-10, are not elevated (9). Additional analyses involving a rev3Δ mutation, which inactivates the error prone DNA polymerase ζ, revealed that the genetic mutations that accumulate in mph1Δ cells arise from the error-prone bypass of spontaneous DNA lesions by this polymerase. Cells lacking Mph1 are also hypersensitive to the genotoxic agents ethyl methanesulfonate, methyl methanesulfonate, and 4-nitroquinoline 1-oxide and slightly so to camptothecin (9). The DNA damage sensitivity and mutator phenotype of the mph1Δ mutant are epistatic to mutations in genes that belong to the RAD52 epistasis group required for HR, the homology-directed repair of damaged DNA, and the restart of stalled DNA replication forks. Even though the mph1Δ mutation does not impair spontaneous mitotic heteroallelic recombination and in fact elevates spontaneous allelic recombination frequency in a sgs1Δ background (10), unequal sister chroma-
tid recombination events are compromised.2 Taken together, the available genetic data are consistent with the premise that Mph1 protein functions to channel DNA lesions or DNA intermediates that are associated with damaged replication forks into HR or that it is involved in the resolution of such DNA intermediates by HR.

As part of our overall effort at delineating the mechanism of HR pathways, we would like to define the biochemical properties of the Mph1 protein and its interactions with proteins of the RAD52 epistasis group. Toward this goal, we have overexpressed Mph1 in yeast, devised a procedure for its purification to near homogeneity, and carried out initial biochemical characterization of the purified protein to show that it has ssDNA-dependent ATPase and DNA helicase activities. Candidate orthologues of Mph1 are present in other eukaryotes including humans. The availability of purified Mph1 and the demonstration of its catalytic activities should facilitate future efforts aimed at deciphering the role of this factor and its orthologues in homologous recombination and mutation avoidance.

**EXPERIMENTAL PROCEDURES**

**Expression and Purification of Mph1 Protein**—The MPH1 gene, containing an epitope of the Sendai virus L-protein (DGSGLDIEPDSS (11)) and also a His6 affinity tag, was cloned as an SmaI/EcoRI fragment into the EciI3811/EcoRI sites of pYES2 (Invitrogen) containing the GAL1 promoter to yield pYES2-Mph1 (2α, URA3, GAL1-MPH1). This Mph1 expression plasmid was introduced into the Ecl136II/EcoRI sites of pYES2 (Invitrogen) containing the GAL1-MPH1 and URA3, HIS3, PRB1, and H9004). Overnight cultures grown at 30 °C in medium containing galactose and incubated at 30 °C for 18 h to induce complete synthetic media lacking uracil were diluted 8-fold into fresh medium containing galactose and incubated at 30 °C for 18 h to induce the expression of Mph1. Cells were harvested by centrifugation and stored at −80 °C. All the subsequent steps were conducted at 4 °C to prevent loss of activity. 200 μg of yeast paste was thawed in 350 ml of cell breakage buffer (50 μM Tris-HCl, pH 7.5, 150 mM KCl, 10% sucrose, 2 mM MgCl2, 100 mM NaCl) followed by slow cooling to room temperature. Hybridized DNA substrates were separated from un-annealed oligonucleotides in 12% non-denaturing polyacrylamide gels run in TAE buffer (40 μM Tris acetate, pH 7.4, 0.5 mM EDTA) and were recovered from the gel by electroelution in dialysis tubing in TAE buffer.

DNAs and DNA Helicase Substrates—The δX174 replicative form I DNA was purchased from Invitrogen, and the viral (+) strand was from New England Biolabs. Linearized replicative form I DNA was prepared by digestion with ApaI. The oligonucleotides used in this study are summarized in Table I (Invitrogen). Selected oligonucleotides were 5′ end-labeled with [γ-32P]ATP using T4 polynucleotide kinase. The following substrates were constructed from the oligonucleotides; (i) radioactively labeled H1 was hybridized to unlabeled H2 to obtain a substrate with a 40-base pair duplex region and 40-nucleotide 3′ ssDNA overhang, (ii) radioactively labeled H1 was hybridized to unlabeled H4 to obtain a partial duplex with a 40-base pair duplex region and a 40-nucleotide 5′ ssDNA overhang, and (iii) radioactively labeled A2 was hybridized to unlabeled A1 to yield a partial duplex with a 100-base pair duplex region and a 50-nucleotide 3′ ssDNA overhang. The DNA hybridization reactions were conducted by heating equimolar amounts of the indicated oligonucleotide pairs at 95 °C for 10 min in buffer H (50 mM Tris-HCl, pH 7.5, 10 mM MgCl2, 100 mM NaCl) followed by slow cooling to room temperature. Hybridized DNA substrates were separated from un-annealed oligonucleotides in 12% non-denaturing polyacrylamide gels run in TAE buffer.

**ATPase Assay**—Unless stated otherwise, Mph1 (35 nm) was mixed with the DNA cofactor (25 μM nucleotides or base pairs) in 10 μl of buffer A (30 mM Tris-HCl, pH 7.5, 2.5 mM MgCl2, 1.5 μM [γ-32P]ATP, 1 mM dithiothreitol, and 100 μM bovine serum albumin) containing the indicated amounts of KCl. Unless stated otherwise, the reaction mixtures were incubated at 30 °C, and at the indicated times a 2-μl aliquot was removed and mixed with an equal volume of 500 μM EDTA to halt the hydrolysis reaction. The released radiolabeled phosphate was separated from the unhydrolyzed radiolabeled ATP by thin layer chromatography in polyethyleneimine cellulose sheets (13), and the level of these radiolabeled species was determined by phosphorimaging analysis of the chromatography sheets in a Personal Molecular Imager FX (Bio-Rad).

**DNA Helicase Assay**—Mph1 was incubated at 30 °C with the helicase substrates (300 nm nucleotides) in 10 μl of buffer D (30 mM Tris-HCl, pH 7.6, 2.5 mM MgCl2, 2 mM ATP, 50 mM KCl, 1 mM dithiothreitol, and 100 μM bovine serum albumin) and was passed through a 30-ml column of Q-Sepharose (Amersham Biosciences) and was concentrated to 80 μl of buffer K containing 150 mM KCl and 20 mM imidazole. Mph1 was eluted with K buffer containing 200 mM imidazole. The protein-containing fractions were pooled and fractionated on a 0.5-ml Mono S column (Amersham Biosciences) using a 0.5-ml gradient of 150–650 mM KCl in K buffer. Mph1 eluted from the Mono S column at ~350 mM KCl and was concentrated to 80 μl in a Centricon-30 (Millipore). The purified protein was stored in small aliquots at −80 °C. To establish the identity of the purified protein, it was subjected to MALDI-TOF analysis in the W. M. Keck Biomedical Spectrometry Laboratory at the University of Virginia Health System. This analysis confirmed that the purified protein was Mph1.

**DNA Helicase Assay**—The heterotrimetric ssDNA binding factor RPA was purified to near homogeneity from Escherichia coli cells tailored to co-express the three subunits as described (12).

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2 K. Anke Schürer and W. Kramer, unpublished data.
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FIG. 1. Overexpression and purification of Mph1 protein. A, extracts from S. cerevisiae cells harboring plasmid pYES2-Mph1 that overexpresses Mph1 (lane 2) or the empty pYES2 expression vector (lane 1) grown in galactose-containing medium were subject to immunoblot analysis with anti-histidine antibodies. The amount of protein loaded in both the lanes was 6 μg. B, schematic representation of the chromatographic procedure devised for the purification of Mph1. C, purified Mph1 (1 μg) was analyzed by SDS-PAGE and Coomassie Blue staining. Ni-NTA, nickel nitrilotriacetic acid.

RESULTS

Mph1 Overexpression and Purification—For protein purification we attached a carboxyl-terminal His6 epitope to the MPH1-coding sequence and then placed the tagged gene under the galactose inducible GAL1 promoter in the vector pYES. The resulting plasmid, pYES-Mph1 (2μ, URA3, GAL1-MPH1), and the empty vector pYES were introduced into the protease-deficient yeast strain BJ5464. Plasmid-bearing strains were grown in the presence of galactose to induce the GAL1 promoter. When analyzed by immunoblotting with anti-histidine antibodies, lysates prepared from cells harboring pYES-Mph1 showed an immunoreactive band of 130 kDa that was absent in the control cell lysates (Fig. 1A). The observed size of the tagged Mph1 protein in the immunoblot analysis is in exact agreement with the predicted value of 130 kDa. A chromatographic procedure that entails fractionation of extracts on Q-Sepharose, SP-Sepharose, nickel nitrilotriacetic acid-agarose, Source Q, and Mono S was devised (Fig. 1B) to purify the tagged Mph1 protein to near homogeneity (Fig. 1C). Results from a MALDI-TOF analysis of the purified protein helped verify that it was tagged Mph1. The overall yield of Mph1 was 300 μg from 200 g of yeast cell paste. Three separate His6-tagged Mph1 preparations gave very similar results in all of the biochemical assays described below.

Mph1 Has ssDNA-dependent ATPase Activity—The presence of Walker nucleotide binding motifs that could potentially endow Mph1 with the ability to bind and hydrolyze ATP prompted us to examine the purified Mph1 protein for ATPase activity. The ATPase assays were carried out in the absence of DNA or in the presence of the dX174 replicative form I DNA (RF I), or poly(dT) were incubated for the indicated times. B, fractions from the last step of Mph1 purification in Mono S were analyzed by SDS-PAGE and Coomassie Blue staining (panel I) and examined for ATPase activity with the dX174 viral (+) strand as co-factor (panel II). In A and B, the averaged values from at least three independent experiments were plotted.

µg/ml bovine serum albumin). RPA (500 nM) was added to some of the reactions. Aliquots (5 μl) of the reactions were drawn at the indicated times and treated with SDS (0.2% final) and protease K (0.5 mg/ml) at 30 °C for 1 min. The released 32P-labeled DNA fragment was separated from the substrate in 12% nondenaturing polyacrylamide gels in TAE buffer at 23 °C. The gels were dried onto Whatman DE81 paper (Whatman International Limited) and analyzed by phosphorimaging.

The presence of Walker nucleotide binding motifs that could potentially endow Mph1 with the ability to bind and hydrolyze ATP prompted us to examine the purified Mph1 protein for ATPase activity. The ATPase assays were carried out in the absence of DNA or in the presence of the dX174 replicative form I DNA, linear duplex, or viral (+) strand DNA. As shown in Fig. 2A, whereas little or no ATP hydrolysis occurred in the absence of DNA and with the replicative form I DNA or linear duplex, robust ATPase activity was seen with the viral (+) strand DNA. These results indicate that Mph1 has an ATP hydrolytic function that is dependent on ssDNA for activation. Even though oligonucleotides can also serve as a co-factor for ATP hydrolysis by Mph1 (see “Discussion”), poly(dT) was ineffective in this regard. Whether the absence of ATP hydrolysis with poly(dT) is due to a lack of binding of this polynucleotide by Mph1 or to another reason remains to be determined. When measured at 30 °C, pH 7.5, 15 mM KCl, 2.5 mM Mg2+, and with the dX174 viral (+) strand as the DNA cofactor, the kcat of ATP hydrolysis was calculated to be ~1500 min−1. The ATPase activity observed is intrinsic to Mph1 because (i) our Mph1 preparations were highly purified (Fig. 1C) and (ii) this activity co-eluted with the Mph1 protein during the last step of protein purification in Mono S (Fig. 2B).

Next, we examined how the Mph1 ATPase activity is influenced by various biochemical parameters. As shown in Fig. 3A, there was a slight increase in the ATP hydrolysis rate by elevating the incubation temperature from 23 °C. The ATPase activity of Mph1 is somewhat salt-sensitive, such that ~30% inhibition of the activity occurred when the KCl concentration
was increased from 15 to 200 mM (Fig. 3B). The optimal pH for ATP hydrolysis resides within the range of 6.6–8.4 (Fig. 3C).

In terms of metal requirement, maximal activity was obtained with Mg²⁺, whereas Co²⁺ and Ca²⁺ are much less effective in this regard, and no activity was observed with Mn²⁺ or Zn²⁺ (Fig. 3D). The optimal concentration of Mg²⁺ was 2.5 mM.

Minimal DNA Size Needed for the Activation of ATPase Activity—We investigated the minimal length of ssDNA required for activation of the Mph1 ATPase activity. For this purpose we compared oligonucleotides ranging from 5 to 90 nucleotides in length for the ability to support ATP hydrolysis by Mph1. Although lengthening the oligonucleotide to 30 residues resulted in only a slight elevation of ATP hydrolysis, a disproportionately large increase in ATPase activity was seen when the DNA length reached 40 residues (Fig. 4B). However, further increasing the DNA length from 40 to 60 or 90 nucleotides had only a minor stimulatory effect on ATP hydrolysis. The 60-mer substrate is as effective as X viral (5.4 kilobases in length) as a cofactor of ATP hydrolysis.

**Fig. 3.** Biochemical parameters that influence the efficiency of ATP hydrolysis. Mph1 ATPase activity was examined as a function of the reaction temperature (A), KCl concentration (B), pH (C), and divalent metal co-factor (D). The øX174 viral (+) strand was used as the DNA co-factor throughout. The experiments in A, B, and D were carried out in reaction buffer of pH 7.5. MgCl₂ (2.5 mM) was included as the metal cofactor in A, B, and C, and 2.5 mM chloride salt of the indicated divalent metal ions was used in D. The buffers used in C were based on 30 mM Bis-Tris/HCl (pH 6.0–7.0) or Tris/HCl (pH 7.3–8.6). In A–D, the averaged values from at least three independent experiments were plotted.

**Fig. 4.** Minimal length of ssDNA required for the activation of ATPase activity. A, ATPase reactions with 5, 8, 12, 16-mer oligonucleotides and øX174 viral (+) strand were carried out. In B, the DNA species tested in the ATPase assay were 20, 30, 40, 60, 90-mer oligonucleotides and øX174 viral (+) strand. In A and B, the averaged values from at least three independent experiments were plotted.

**Fig. 5.** Mph1 has a 3’ to 5’ DNA helicase activity. A, Mph1 (4 nM) was incubated for the indicated times with substrates that contained a 40-bp duplex region and a 40-nucleotide 3’ or 5’ overhang. HD, heat-denatured substrate; NP, substrate incubated in buffer without Mph1. The asterisk denotes the ³²P label. B, the data from A are plotted. C, the data from another experiment in which Mph1 (2, 4, and 6 nM) was incubated for the indicated times with the substrate that contained a 3’ overhang. All the experiments were repeated at least twice with similar results.

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Mph1 Possesses DNA Helicase Activity—Because Mph1 is related in sequence to the members of the superfamily 2 helicase family, we wished to test for helicase activity in the purified Mph1 protein. For this, we prepared 32P-labeled substrates related in sequence to the members of the superfamily 2 helicase family, we wished to test for helicase activity in the purified Mph1 protein. For this, we prepared 32P-labeled substrates that contained a 40-bp duplex region and a 40-nucleotide 3′ overhang was examined with Mph1 (4 nM) in the presence of ATP or with ATP, ADP, AMP-PCP (PCP), AMP-PNP (PNNP), or ATPγS as indicated. The incubation time was 20 min. The asterisk denotes the 32P label. HD, heat-denatured substrate; NP, substrate incubated in buffer without Mph1. B, ATP hydrolysis by Mph1 was examined with 0.1 mM of [γ-32P]ATP and with or without 0.5 or 2 mM unlabeled ATP, ADP, AMP-PCP, AMP-PNP, or ATPγS, as indicated. C, the helicase substrate (see A) was incubated for 10 min with Mph1 (4 nM) in the ATP-containing buffer with 2.5 mM Mg2+, Mn2+, Ca2+, Cd2+, or Zn2+, as indicated. The symbols are as described in A. All the experiments were repeated at least twice with similar results.

DNA Unwinding Requires ATP Hydrolysis—We wished to establish that the DNA unwinding function of Mph1 is fueled by ATP hydrolysis. As shown in Fig. 6A, no unwinding of the 3′ substrate was seen when ATP was omitted or substituted with ADP or the non-hydrolyzable (or slowly hydrolyzable) ATP analogues AMP-PNP, AMP-PCP, and ATPγS. The lack of DNA unwinding in the presence of these ATP analogues could have been due to an inability of Mph1 to recognize these nucleotides. To help eliminate this caveat, we examined the ability of the three analogues to inhibit the ATPase activity of Mph1. In these assays, the [γ-32P]ATP concentration was at 0.1 mM, whereas the concentration of the three analogues was at either 0.5 or 2 mM. The results, as presented in Fig. 6B, showed that the amount of 32P-labeled ATP hydrolyzed by Mph1 was reduced by all these nucleotides, with the most pronounced inhibition seen with ATPγS. For instance, at 0.5 and 2 mM of ATPγS, the hydrolysis of ATP was reduced by 65 and 80%, respectively. These results indicate that Mph1 binds ATPγS well and the other two analogues moderately well, and they further verify that DNA unwinding by Mph1 requires hydrolysis of the bound nucleotide. The results in Fig. 6C reveal that DNA unwinding by Mph1, like ATP hydrolysis, requires Mg2+, with Ca2+, Zn2+, Co2+, and Mn2+ being ineffective in this regard.

Effect of RPA on Mph1-mediated DNA Unwinding—The helicase substrate used above contains a 40-bp duplex region. We asked whether a substrate that harbors a longer duplex region is also unwound by Mph1. For this purpose we made a 32P-labeled partial duplex that contains a 100-bp duplex region and a 50-nucleotide 3′ ssDNA overhang without (A) or with (B) RPA. The asterisk denotes the 32P label. HD, heat-denatured substrate. C, the data from A and B are plotted for the 5-min time point. Both the experiments were repeated twice with similar results.

FIG. 7. Stimulatory effect of RPA on the DNA unwinding activity of Mph1. The indicated concentrations of Mph1 were incubated with a substrate that contained a 100-bp duplex region and a 50-nucleotide 3′ ssDNA overhang without (A) or with (B) RPA. The asterisk denotes the 32P label. HD, heat-denatured substrate. C, the data from A and B are plotted for the 5-min time point. Both the experiments were repeated twice with similar results.

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ing Mph1 helicase, we added the heterotrimeric ssDNA binding factor RPA to the reaction. Importantly, the presence of RPA significantly enhanced the unwinding ability of Mph1, such that ~25% of the substrate had been dissociated by 50 nM Mph1 after 5 min of incubation (Fig. 7, B and C).

To determine whether Mph1 is capable of unwinding an even longer duplex region, we prepared another helicase substrate consisting of a 32P-labeled 500-base DNA fragment hybridized to the dX174 viral (+) strand (14). The substrate was incubated with increasing amounts of Mph1 with or without a quantity of RPA that would be sufficient to sequester the free ssDNA present in the substrate and generated as a result of DNA strand separation. However, over a wide range of Mph1 concentrations and under a variety of reaction conditions tried, little or no dissociation of this long helicase substrate occurred (data not shown).

**DISCUSSION**

**Mph1 Protein Is a 3’ to 5’ DNA Helicase**—In this study we have overexpressed the MPH1-encoded product as a His6-tagged protein in yeast and have devised a simple method for its purification to near homogeneity. We established the identity of the Mph1 protein by immunoblot analysis with anti-histidine antibodies and mass spectrometry. Because the Mph1 protein exhibits homology to helicase enzymes of the superfamily 2 (9), we examined our purified Mph1 preparations for the ability to hydrolyze ATP and unwind DNA. The results showed a robust ssDNA-dependent ATPase activity in Mph1 and also an ability of Mph1 to unwind partially duplex DNA substrates. These enzymatic activities co-eluted with the Mph1 protein during the last step of purification in Mono S, thus helping establish that the activities are intrinsic to Mph1. The maximal activation of the ATP hydrolytic function requires a relatively long stretch of ssDNA such that a length increase from 30 to 40 nucleotides resulted in greater than a 3-fold elevation of ATP hydrolysis. Whether this reflects a large ssDNA binding site size for Mph1 or another reason remains to be determined. The polarity of DNA unwinding is 3’ to 5’ with respect to the strand on which Mph1 translocates, and as the unwinding reaction is coupled to the hydrolysis of ATP. Although Mph1 is perfectly capable of dissociating a 40-bp duplex region without the aid of another protein factor, significant unwinding of a 100-bp duplex is contingent upon the addition of the heterotrimeric ssDNA binding factor RPA. The role of RPA in the Mph1-mediated DNA unwinding is likely to prevent DNA re-annealing by sequestering single strands of DNA produced as a result of strand separation by Mph1. However, Mph1, even with RPA present, cannot completely unwind a 500-bp duplex region.

**Role of the Mph1 Helicase in Homologous Recombination and Mutation Avoidance**—The mph1Δ mutant shows a spontaneous mutator phenotype and also sensitivity to a variety of DNA damaging agents, and both of these phenotypic manifestations are epistatic to mutations in the RAD51, RAD52, and RAD55 genes (10), members of the RAD52 epistasis group required for chromosome repair and maintenance via HR. The mph1Δ mutant shows hyperrecombination between heteroalleles in the sgs1Δ background (10) but appears to be impaired in sister chromatid-based HR-mediated rescue of damaged replication forks caused by the treatment of cells with the UV mimetic 4-nitroquinoline 1-oxide and the topoisomerase I inhibitor camptothecin.9 It seems possible that Mph1 protein functions to stabilize or process the DNA intermediates that are produced during HR-mediated replication fork rescue and restart. Alternatively or in addition, Mph1 could be involved in the DNA synthesis step of HR-mediated replication fork rescue, and in this regard, the apparently modest processivity of the Mph1 helicase activity would be sufficient for the relatively short new DNA segment that needs to be synthesized.

Like Mph1, several other eukaryotic DNA helicases, including the Srs2 and Sgs1 helicases in *S. cerevisiae* and the BLM and WRN helicases in humans, have been found to exert an important influence on the efficiency of HR reactions and/or the outcome of these reactions. In *S. cerevisiae*, mutational inactivation of the SRS2 gene, which encodes a member of the SF1 helicase family, results in a spontaneous hyperrecombination phenotype and other phenotypic changes because of the deregulation of HR, thus indicative of an anti-recombinase function in the Srs2 protein (5, 6, 15–17). Analysis of the functional interactions between the Rad51 recombinase and purified Srs2 has revealed a robust ability in this helicase protein to disrupt the Rad51-ssDNA nucleoprotein filament, a critical catalytic intermediate in HR reactions (18), thereby providing a biochemical explanation for its anti-recombinase activity (5, 6). Because the mph1Δ mutation elevates allelic recombination in the sgs1Δ background, it remains possible that Mph1 protein also acts in a manner similar to Srs2 in destabilizing nucleoprotein filaments of Rad51 and ssDNA. In addition to its anti-recombinase function, Srs2 also positively influences the repair of double-strand breaks by the synthesis-dependent single-strand annealing sub-pathway of HR. Whether or not Mph1 also affects the efficiency of double-strand break repair via synthesis-dependent single-strand annealing will be a subject of our future investigations. In genetic and biochemical studies, the Sgs1 helicase and human BLM helicases appear to work in conjunction with topoisomerase III to suppress the formation of crossover recombinants by resolving the Holliday intermediate in a non-crossover fashion (19, 20), and the WRN helicase appears to be important for processing recombination intermediates possibly including the Holliday structure (21). It will be of interest to determine whether or not Mph1 interacts with topoisomerase III and resolves specific DNA structures that arise during HR reactions as well.

The development of an expression system and purification protocol for Mph1 as well as the characterization of the ATPase and helicase activities of Mph1 should provide valuable material and basal information for defining its biochemical properties and interactions with other protein factors that function in the formation and resolution of HR intermediates. Because an apparent orthologue of MPH1 exists in humans, our continuing studies with *S. cerevisiae* Mph1 should complement efforts on the human counterpart.

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