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Mycobacterium tuberculosis UvrD1 and UvrA Proteins Suppress DNA Strand Exchange Promoted by Cognate and Noncognate RecA Proteins†

Pawan Singh,[‡,⊥] K. Neelakanteshwar Patil,[‡,⊥] Jasbeer Singh Khanduja,[‡,⊥] P. Sanjay Kumar,[‡] Alan Williams,[∥] Franca Rossi,[§] Menico Rizzi,[§] Elaine O. Davis,[∥] and K. Muniyappa*[,‡]

[‡]*Department of Biochemistry, Indian Institute of Science, Bangalore 560012, India,* [§]*Department of Chemical, Food, Pharmaceutical and Pharmacological Sciences (DiSCAFF), University of Piemonte Orientale "A. Avogadro", Novara, Italy, and* [∥]*Division of Mycobacterial Research, MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.* [⊥]*These authors contributed equally to this work*

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ABSTRACT: DNA helicases are present in all kingdoms of life and play crucial roles in processes of DNA metabolism such as replication, repair, recombination, and transcription. To date, however, the role of DNA helicases during homologous recombination in mycobacteria remains unknown. In this study, we show that *Mycobacterium tuberculosis* UvrD1 more efficiently inhibited the strand exchange promoted by its cognate RecA, compared to noncognate *Mycobacterium smegmatis* or *Escherichia coli* RecA proteins. The *M. tuberculosis* UvrD1^{Q276R} mutant lacking the helicase and ATPase activities was able to block strand exchange promoted by mycobacterial RecA proteins but not of *E. coli* RecA. We observed that *M. tuberculosis* UvrA by itself has no discernible effect on strand exchange promoted by *E. coli* RecA but impedes the reaction catalyzed by the mycobacterial RecA proteins. Our data also show that *M. tuberculosis* UvrA and UvrD1 can act together to inhibit strand exchange promoted by mycobacterial RecA proteins. Taken together, these findings raise the possibility that UvrD1 and UvrA might act together *in vivo* to counter the deleterious effects of RecA nucleoprotein filaments and/or facilitate the dissolution of recombination intermediates. Finally, we provide direct experimental evidence for a physical interaction between *M. tuberculosis* UvrD1 and RecA on one hand and RecA and UvrA on the other hand. These observations are consistent with a molecular mechanism, whereby *M. tuberculosis* UvrA and UvrD1, acting together, block DNA strand exchange promoted by cognate and noncognate RecA proteins.

The process of homologous recombination (HR)[1] plays a fundamental role in the genetic diversification of bacterial genomes (*1*−*4*). However, genome rearrangements due to genetic recombination, deletions, and duplications are believed to be the major source of instability of bacterial genomes (ref *5* and references cited therein). To circumvent this problem, eubacteria have evolved unique mechanisms that control the fidelity of genetic recombination, including mismatch repair system (MutSLH), nucleotide excision repair proteins (UvrABCD), recombinational repair proteins (RecFOR), and antirecombinases (RecX, PsiB, and DinI) (refs *2* and (*4*−*6*) and references cited therein). Bioinformatic analyses of completely sequenced mycobacterial genomes including *Mycobacterium tuberculosis* (*7*), *Mycobacterium leprae* (*8*), *Mycobacterium bovis* (*9*), *Mycobacterium avium*, *Mycobacterium paratuberculosis*, and *Mycobacterium smegmatis* [The Institute for Genome Research (http://www.tigr.org)] have disclosed the presence of genes encoding enzymes/proteins that participate in many of the DNA repair/recombination pathways, consistent with the notion that the basic strategy used to repair DNA lesions or HR in the genome is conserved. In *Escherichia coli*, the components of the DNA repair pathways have been isolated and characterized in a series of genetic and biochemical studies and include the mutator proteins (MutL, MutS, MutH, and UvrD/MutU), several exonucleases, including ExoI, ExoVII, RecJ, and ExoX, DNA polymerase III, SSB, DNA ligase, and the Dam methylase (*10*−*12*). Many of these proteins act in concert to correct base pair mismatches and small insertions/deletions. In *E. coli*, UvrD has been shown to act in concert with UvrABC in nucleotide excision repair and with MutHLS in mismatch repair (*13*−*15*). *In vivo*, *uvrD* suppresses HR since *uvrD*-null mutants display increased frequency of HR (*16*−*20*), and an allele of *uvrD*, *uvrD252*, fully abolishes HR in a *recBC sbcBC* background (*21*). Consistent with these studies, UvrD promotes dissolution of the recombination intermediates and abets replication fork reversal by clearing RecA from DNA at forks (*22*−*25*). The action of UvrD during recombination and replication seems to be conserved in other

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*Corresponding author. Tel: (91-80) 2293 2235/2360 0278. Fax: (91-80) 2360 0814/0683. E-mail: kmbc@biochem.iisc.ernet.in.

[1]Abbreviations: ATPγS, adenosine 5′-*O*-(thiotriphosphate); bp, base pair; dsDNA, double-stranded DNA; DTT, dithiothreitol; EcRecA, *Escherichia coli* RecA; EDTA, ethylenediaminetetraacetic acid; HR, homologous recombination; IPTG, isopropyl 1-thio-β-D-galactopyranoside; MMR, mismatch repair; MsRecA, *Mycobacterium smegmatis* RecA; MtRecA, *Mycobacterium tuberculosis* RecA; MtUvrA, *M. tuberculosis* UvrA; MtUvrD1, *M. tuberculosis* UvrD1; NER, nucleotide excision repair; NHEJ, nonhomologous end-joining; ODN, oligonucleotide; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; ssDNA, single-stranded DNA.

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species (*26*). For example, inactivation of *SRS2*, the yeast *uvrD* homologue, increases the frequency of HR and causes cell lethality in the presence of chemicals that perturb replication or in combination with mutations that affect replication (*27*, *28*).

The evolutionary success of *M. tuberculosis* as a pathogen largely relies on its ability to persist in host tissues. To this end, various pathways of DNA repair appear to be important for *M. tuberculosis* pathogenesis and persistence in the host (*29*−*32*). For example, inactivation of *uvrD1* has been shown to attenuate *M. tuberculosis* in a mouse model of tuberculosis infection (*33*). Consistent with this, other studies have shown that expression of *uvr* genes is increased in *M. tuberculosis* growing in human macrophages, implicating the importance of the *uvr* system for its survival in hostile environments (*34*). Additionally, *uvr* genes are involved in the response to H_2O_2 damage (*35*) and are upregulated upon exposure to UV irradiation (*30*). However, our understanding of the roles of various DNA repair pathways in *mycobacteria* is still poor, compared with that of other bacteria, and has advanced more slowly in *M. tuberculosis* because of the technical difficulties in working with this slow-growing pathogen. Consequently, only a few of *mycobacterial* DNA repair genes and/or their enzymatic activities have been characterized, both *in vivo* and *in vitro*.

M. tuberculosis is striking because it lacks the MutHLS genes associated with the MMR repair pathway as well as some of the genes of the HR pathway, including *recJ, sbcBCD*, and *recET*, which are common to *E. coli* and other eubacteria (*36*, *37*). Notably, *M. tuberculosis uvrA* and *uvrD1* appear to be essential for DNA repair and are upregulated by stress and during its growth in human macrophages (*34*, *38*). Previous studies have focused on the biochemical characterization of *M. tuberculosis* UvrD helicases (*39*−*41*). Although the kinetic and mechanistic aspects of HR in *M. tuberculosis* have been studied in some detail (*42*−*47*), an understanding of how the protein effectors regulate HR remains a challenging experimental problem. To this end, recent studies in *M. smegmatis* suggest that *uvrD1* and *uvrB* play an important role in maintaining the fidelity of recombination (*48*). We asked, what role would *M. tuberculosis* Uvr proteins, in particular UvrD1 and UvrA, play in HR promoted by its cognate RecA? Would these helicases regulate strand exchange in a species-specific manner? Our studies show that MtUvrD1 exerts a strong inhibitory effect on DNA strand exchange promoted by its cognate RecA, compared to non-cognate RecA proteins. Furthermore, MtUvrA by itself has no discernible effect on strand exchange promoted by EcRecA; however, it inhibited strand exchange promoted by *M. tuberculosis* and *M. smegmatis* RecA proteins. MtUvrA and MtUvrD1 function together to inhibit DNA strand exchange promoted by *M. tuberculosis* RecA. Taken together, these observations suggest a molecular mechanism of suppression of DNA strand exchange promoted by cognate and noncognate RecA proteins by the combined action of MtUvrA and MtUvrD1 proteins.

EXPERIMENTAL PROCEDURES

Biochemicals, Enzymes, and DNA. All of the fine chemicals were of molecular biology grade, purchased either from GE Biosciences or from Sigma. T4 polynucleotide kinase was purchased from New England Biolabs. Radiolabeled ATP was purchased from BRIT, Mumbai. ATPγS was purchased from Merck whereas dATP was procured from Amersham Biosciences. Fast-performance liquid chromatography columns for protein purification and protein A−Sepharose CL-4B were purchased from GE Biosciences. *E. coli* RecA (*44*), *M. tuberculosis* RecA (*44*), *M. smegmatis* RecA (*45*), and MtUvrD1 (*39*) were purified as described. The concentration of each protein was determined by the dye-binding method (*49*). All of the protein preparations were free of any exo- or endonuclease contamination.

Oligonucleotides were synthesized by Sigma-Genosys, Singapore, and their sequences are as follows: ODN1, 5′-GATCTGTACGGCTGGACAGTGTTGTGAGTGAGTTGAAGATGGGAGGTAGTGTGCTAGGTGGCTTAGGAGAGAGTCGTTAGTGT-3′; ODN2, 5′-ACACTAACGACTCTCTCCTAAGCCACCTAGCACACTACCTCCCATCTTCAACTCACTCACAACACTGTCCAGCCGTACAGATC-3′; ODN3, 5′-GACGTGGGCAAAGGTTCGTCAATGGACTGACAGCTGCATGG-3′; ODN4, 5′-CGAACCTTTGCCCACGTC-3′. The ODN1 and ODN3 were labeled at the 5′ end by using [γ-^{32}P]ATP and T4 polynucleotide kinase. Radiolabeled duplex DNA (83 bp) was prepared by annealing labeled ODN1 with an equimolar ratio of unlabeled ODN2 (*50*) at 95 °C for 5 min followed by gradual cooling to 24 °C, whereas 3′-overhang substrate was prepared by annealing ODN3 with ODN4. The annealed mixture was electrophoresed on a 10% polyacrylamide gel in 44.5 mM Tris−borate buffer (pH 8.3) containing 1 mM EDTA at 150 V for 7 h. The bands corresponding to the annealed substrates were excised from the gel and eluted into TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA). The concentration of the labeled duplex substrate was expressed in moles of nucleotide residues per liter.

Expression and Purification of the M. tuberculosis UvrD1^{Q276R} Mutant. A mutation causing a single amino acid substitution in the *M. tuberculosis* UvrD1 ATPase/helicase (Q276) motif was introduced using the QuikChange site-directed mutagenesis kit (Stratagene) according to the manufacturer's instructions. The expression plasmid pET28b-*uvrD1* (*39*) encoding the wild-type *UvrD1*served as the template, and complementary oligonucleotides except for the mismatch were used for mutagenesis. The rationale underlying the Q276R change was based on studies with *Bacillus stearothermophilus* PcrA (*51*). Briefly, the Q276R mutation was generated using the primers 5′-CGTCGGGGATGCCGATCGGTCGATCTATGCGTTCC-3′ (forward) and 5′-GGAACGCATAGATCGACCGATCGGCATCCCCGACG-3′ (reverse), where the underlined sequences correspond to the changed amino acid codons. The UvrD1 mutant construct was sequenced after mutagenesis to ensure that it contained only the desired mutation.

The *M. tuberculosis* UvrD1^{Q276R} mutant protein was expressed in *E. coli* BL21(DE3) Star cells harboring the expression plasmid. The mutant protein was purified using the same protocol that was used for the wild-type UvrD1 (*39*). The progress of purification was monitored by SDS−PAGE (Supporting Information Figure 1C). The gels were stained with Coomassie brilliant blue R-250. The purified enzyme fractions were combined, dialyzed against a buffer containing 50 mM Tris-HCl buffer, pH 8.5, 0.4 M NaCl, 25% glycerol, and 5 mM β-mercaptoethanol, and then stored in a −80 °C freezer. The concentration of mutant protein was determined by the dye-binding method (*49*). The protein preparation was free of any exonuclease or endonuclease contamination.

Expression and Purification of the M. tuberculosis UvrA Protein. Bioinformatic analysis of the *M. tuberculosis* H37Rv genome revealed the presence of the *uvrA* gene (Rv1638),

which encodes MtUvrA protein consisting of 972 amino acid residues with a molecular mass of 106 kDa. *M. tuberculosis uvrA* was cloned and overexpressed in *E. coli* Rosetta2 (DE3) pLysS strain harboring the plasmid p*Mt*His*uvrA*. The bacteria were grown in LB broth, supplemented with 100 μg/mL ampicillin and 34 μg/mL chloramphenicol, at 37 °C to A_{600nm} = 0.6. Subsequently, the culture was incubated for 20 min at 24 °C, and MtUvrA expression was initiated by the addition of IPTG to a final concentration of 0.5 mM, followed by another 12 h of incubation at 18 °C. Cells were collected by centrifugation, washed in STE buffer (10 mM Tris-HCl, pH 8, 100 mM NaCl, and 1 mM EDTA), and resuspended in the TNG buffer (50 mM Tris-HCl, pH 8, 300 mM NaCl, and 10% glycerol). Cells were lysed by sonication and lysate was treated with DNase I (20 μg/mL) at 37°C for 30 min. The cell debris was removed by centrifugation in a Beckman Ti-45 rotor at 30000 rpm for 1 h at 4°C. Imidazole was added to the supernatant to a final concentration of 40 mM and was applied onto a Ni-NTA agarose column preequilibrated with buffer A containing 40 mM imidazole. The column was washed with 5 bed volumes of buffer A supplemented with 120 mM imidazole. The column was washed with buffer B [50 mM Tris-HCl (pH 8)], containing 1 M NaCl, until the wash buffer was devoid of A_{260nm} absorbing material. The column was reequilibrated with 5 bed volumes of buffer A. Bound proteins were eluted with 3 bed volumes of buffer A containing 600 mM imidazole. Peak fractions, free of endogenously bound DNA, were pooled and 10-fold diluted with buffer B. The protein pool was loaded onto a heparin−agarose column preequilibrated with buffer B containing 100 mM NaCl. The column was washed with 10 bed volumes of buffer B (50 mM Tris-HCl, pH 8, containing 250 mM NaCl). Bound proteins were eluted with 3 bed volumes of buffer B containing 650 mM NaCl. The peak fractions were pooled and dialyzed against storage buffer (50 mM Tris-HCl, pH 8, 300 mM NaCl, 5% glycerol, and 1 mM DTT). MtUvrA protein was purified to homogeneity, as assessed by SDS−PAGE (Supporting Information Figure 1D). The purified protein, which was free of exo- or endonucleases, was stored at −80 °C.

Three-Strand Exchange Assay. The assay was performed as described (*44*, *45*). Briefly, the reaction mixture (10 μL) containing 20 mM Tris-HCl (pH 7.5 for EcRecA and MtRecA; pH 7 for MsRecA), 3 mM dATP or 0.1 mM ATPγS, 8 mM $MgCl_2$, 5 μM ssDNA (ODN1), and 2.5 μM RecA (from the organism indicated) was incubated in the absence or presence of an ATP regeneration system (5 mM phosphocreatine and 10 units/mL creatine phosphokinase) at 37 °C for 5 min and then with MtUvrD1 or MtUvrA, or MtUvrD1 plus MtUvrA, for 5 min at 37 °C. The strand transfer reaction was initiated by the addition of 1 μM ^{32}P-labeled duplex DNA, and incubation was continued for an additional 10 min. Reaction was stopped by the addition of 2.5 μL of 5× stop buffer (5% SDS and 100 mM EDTA) followed by the addition of 1.4 μL of 10× electrophoresis dye [50% glycerol, 0.42% (w/v) bromophenol blue, and 0.42% (w/v) xylene cyanol]. Samples were loaded onto 10% polyacrylamide gel and electrophoresed in 44.5 mM Tris−borate buffer (pH 8.3) containing 1 mM EDTA at 150 V for 7 h. The ^{32}P-labeled DNA substrate and product were visualized by a Fuji FLA-9000 phosphorimager. The bands were quantified in a UVI-Tech gel documentation station using UVI-BandMap software version 97.04 and plotted using Graphpad Prism (version 5.0).

Immunoprecipitation and Immunoblotting. Polyclonal antibodies against EcRecA and MtUvrD1 were raised in rabbits. Anti-EcRecA antibodies cross-react with MtRecA and MsRecA proteins (*47*). Ten micrograms of purified MtUvrD1 (nucleic acid free) was incubated with 10 μg of purified RecA proteins (nucleic acid free) from the specified source in 200 μL of buffer containing 20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, and 1 mM ATP at 37 °C for 30 min. Samples were then incubated at 4 °C for 4 h to stabilize protein complexes, followed with anti-EcRecA or anti-MtUvrD1 antibodies at 4 °C for 8 h with gentle stirring. After the addition of 40 μL of protein A−Sepharose CL-4B (50% slurry), the samples were further incubated for 4 h at 4 °C with gentle stirring. Bead-bound proteins were collected by centrifugation and washed six times, each time with 1 mL of cold TNA buffer (25 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 1 mM ATP) containing 0.1% Triton X-100. Proteins were separated on SDS−PAGE and subsequently transferred to a nitrocellulose membrane for subsequent incubation with the specified antibodies. Control reactions where purified proteins alone were incubated with protein A−Sepharose CL-4B beads or purified proteins incubated with nonspecific antibody and protein A−Sepharose CL-4B beads were processed in a similar fashion. The blots were incubated with the appropriate primary and secondary antibodies and visualized by enhanced chemiluminescence followed by autoradiography.

DNA Unwinding Assay. Unwinding assays were performed as described (*39*). Briefly, reactions (10 μL) were performed in an assay buffer containing 1 nM 5′-^{32}P-labeled 3′-overhang unwinding substrate, 20 mM Tris-HCl (pH 7.5), 1 mM DTT, 5 mM $MgCl_2$, 50 mM NaCl, and increasing concentrations of wild-type MtUvrD1 or MtUvrD1^{Q276R}, respectively. Samples were preincubated at 37 °C for 5 min, and unwinding reactions were then initiated by the addition of 2 mM ATP or 0.1 mM ATPγS. Reactions were further incubated at 37 °C for 30 min and stopped by the addition of 1% SDS, 25 mM EDTA, and 10 nM of trap DNA (cold ODN3). The samples were electrophoresed on 8% native PAGE in 0.5× TBE (45 mM Tris−borate buffer, pH 8.3, containing 1 mM EDTA) buffer at 120 V for 4 h. Gel was dried, and the bands were visualized by a Fuji FLA-9000 phosphorimager.

DNA-Dependent ATPase Aassay. The standard ATPase assay was performed in a 20 μL reaction mixture containing 20 mM Tris-HCl (pH 7.5), 1 mM DTT, 5 mM $MgCl_2$, 50 mM NaCl,10 μM M13 circular single-stranded DNA, and increasing concentrations of wild-type MtUvrD1 or MtUvrD1^{Q276R}, respectively. Samples were preincubated at 37 °C for 5 min. The reaction was initiated by addition of 2 mM α[^{32}P]ATP, incubated for 30 min at 37 °C and stopped by the addition of 25 mM EDTA. Two microliter aliquots were then transferred onto polyethylenimine cellulose F sheets (Merck), which were then developed with a solution containing 0.5 M LiCl and 1 M formic acid. The TLC sheet was air-dried, and the bands were visualized by a Fuji FLA-9000 phosphorimager.

Bioinformatics. *M. tuberculosis* and *M. smegmatis* UvrD1 and UvrA sequences identified as homologues to *E. coli* UvrD and *E. coli* UvrA, respectively, were retrieved from the TIGR Comprehensive Microbial Resource (CMR) database site (http://cmr.jcvi.org/tigr-scripts/CMR/CmrHomePage.cgi). The pairwise sequence alignments were done with the needle program using matrix EBLOSUM 62.

RESULTS

Expression and Purification of RecA, MtUvrA, and MtUvrD1 Proteins. *M. tuberculosis* RecA (MtRecA), *M. smegmatis* RecA (MsRecA), and *E. coli* RecA (EcRecA) proteins were

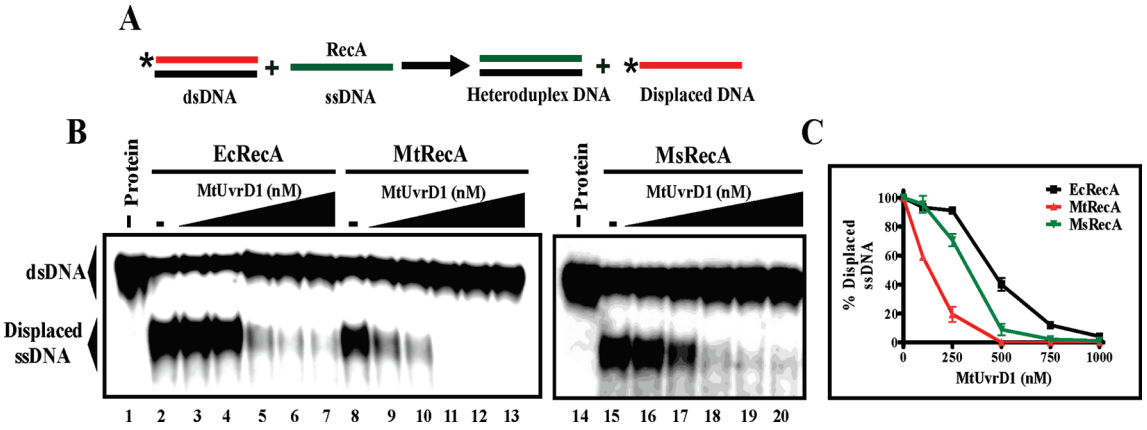

FIGURE 1: Effect of increasing concentrations of MtUvrD1 on strand exchange promoted by cognate and noncognate RecA proteins in the presence of dATP. (A) Schematic depicting the experimental design. (B) Strand exchange reactions were performed with 5 μM ssDNA and 1 μM ^{32}P-labeled linear double-stranded DNA containing EcRecA, MtRecA, or MsRecA in the absence (lanes 2, 8, and 15) or in the presence of 100, 250, 500, 750, and 1000 nM MtUvrD1 (lanes 3−7, 9−13, and 16−20), respectively, as described under the Experimental Procedures. Lanes 1 and 14 show control reactions performed in the absence of the indicated RecA. The positions of double-stranded DNA and displaced ssDNA are indicated on the left-hand side. (C) Graphical representation of the extent of inhibition of strand exchange as a function of increasing concentrations of MtUvrD1. The percent activity (shown as % displaced ssDNA) in the absence of MtUvrD1 has been normalized to 100% reaction. The data points represent the mean ± SE of three independent experiments.

overproduced in *E. coli* and purified to homogeneity as described under Experimental Procedures (Supporting Information Figure 1A). The wild-type and the variant forms of *M. tuberculosis* UvrD1 were overproduced in *E. coli* and purified as described (*39*). Proteins were purified to homogeneity as judged by SDS−PAGE and staining with Coomassie blue R-250 (Supporting Information Figure 1B,C).The *M. tuberculosis* UvrA protein was expressed in the *E. coli* Rosetta2 (DE3) pLysS strain and purified to homogeneity, as judged by SDS−PAGE and staining with Coomassie blue R-250 (Supporting Information Figure 1D). To test whether the purification conditions that we have used produced functionally active proteins, these were tested in standard biochemical assays: RecA proteins were assayed for their ability to promote strand exchange and hydrolysis of ATP to ADP and P_i. Likewise, the MtUvrA protein was tested for its ability to bind a variety of DNA substrates and catalyze ATP hydrolysis (data not shown). We examined DNA-unwinding activity of MtUvrD1^{WT} and MtUvrD1^{Q276R} proteins with the DNA helicase substrate (18 bp duplex bearing 23-nucleotide single-stranded 3′-termini) and DNA-dependent ATPase activity using M13 circular single-stranded DNA as described (*39*). These assays revealed that MtUvrD1^{WT} exhibited the DNA unwinding and ATPase activities in a concentration-dependent manner. Whereas MtUvrD1^{WT} was able to unwind DNA in the presence of either ATP (Supporting Information Figure 2A) or ATPγS (Supporting Information Figure 2C), the MtUvrD1^{Q276R} variant was unable to do so under similar conditions (Supporting Information Figure 2B). Furthermore, quantification of the unwinding activity exhibited by the MtUvrD1^{WT} suggests that UvrD1 is a weak helicase requiring a 500-fold molar excess of enzyme (Supporting Information Figure 2D). The weak helicase activity of MtUvrD1^{WT} may be due to the fact that the assays were performed in the absence of its cognate NHEJ protein Ku (*40*). In addition, the MtUvrD1^{Q276R} mutation, which lies within motif III, was devoid of ATPase activity (Supporting Information Figure 3). However, the DNA-binding activity of the MtUvrD1^{Q276R} mutant to the DNA helicase substrate was unaffected (data not shown). Like the MtUvrD1^{Q276}R variant, mutation (Gln275) in motif III of M. smegmatis UvrD1 also abolished the ATPase and unwinding activities but not its DNA-binding activity (*52*). These results suggest that the purified proteins are active in both DNA-binding and enzymatic assays.

M. tuberculosis UvrD1 Inhibits RecA-Mediated Strand Exchange in Vitro. In *E. coli*, genetic studies have suggested that *uvrD* suppresses HR (*17*−*23*). To understand the mechanistic basis, it seemed important to test whether DNA strand exchange, the central step in HR, is affected by UvrD helicase. To test this hypothesis more directly, we examined the effect of *M. tuberculosis* UvrD1 on strand exchange promoted by its cognate MtRecA. The most studied type of reaction promoted by RecA is the nonreciprocal strand exchange between linear double-stranded and single-stranded DNA (*2*−*4*). We have exploited this system to test the effects of MtUvrD1 and MtUvrA proteins on RecA-catalyzed strand exchange using synthetic substrates. The schematic in Figure 1A provides details about strand labeling and the progress of the reaction. A number of published studies have used a similar length of DNA substrates to understand the mechanistic aspects of strand exchange promoted by EcRecA and its homologues (ref *53* and references cited therein). In this assay, complete strand exchange would result in the displacement of the ^{32}P-labeled noncomplementary strand, which migrates faster in the gel, and the formation of unlabeled heteroduplex DNA. The substrate and product of this reaction were monitored by native PAGE, and the extent of strand exchange was determined by comparing the band intensities of the two radioactive species: displaced ^{32}P-labeled ssDNA and ^{32}P-labeled linear duplex DNA. Reaction mixtures contained 5 μM ssDNA and 2.5 μM indicated RecA protein: these conditions ensured that there was sufficient ssDNA to bind all of the RecA protein present. Strand exchange was initiated by the addition of increasing amounts of MtUvrD1 helicase and a fixed amount of ^{32}P-labeled 83-bp DNA at the same time. After 10 min incubation, samples were deproteinized and processed as described under Experimental Procedures.

First, we tested the effect of MtUvrD1 on the strand exchange activity promoted by its cognate and noncognate RecA proteins. As shown in Figure 1B, we observed a decrease in the extent of strand exchange promoted by MtRecA in the concentration range of 100−250 nM, and complete inhibition occurred at

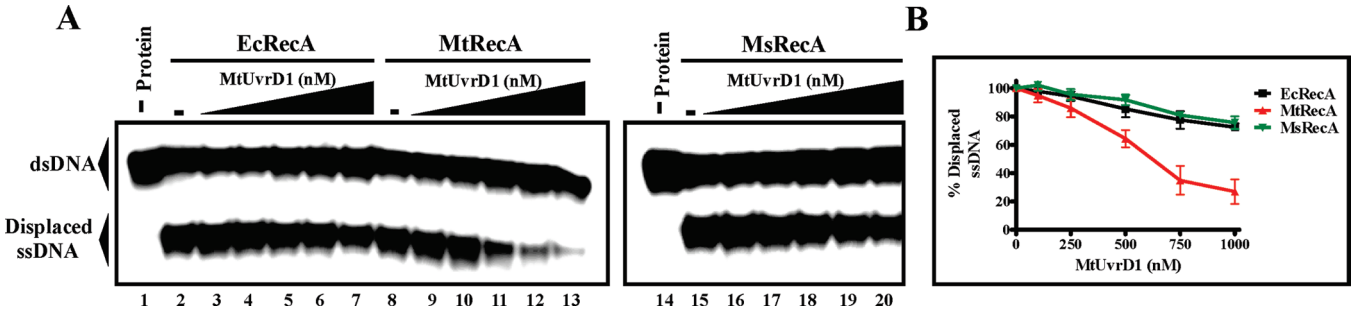

FIGURE 2: Effect of increasing concentrations of MtUvrD1 on strand exchange promoted by RecA proteins in the presence of ATPγS. Assay was performed as described in the legend of Figure 1, except ATPγS replaced dATP. (A) Strand exchange reactions were performed with EcRecA, MtRecA, or MsRecA in the absence (lanes 2, 8, and 15) or presence of 100, 250, 500, 750, and 1000 nM MtUvrD1 (lanes 3−7, 9−13, and 16−20), respectively. Lanes 1 and 14 show control reactions performed in the absence of the indicated RecA. The positions of double-stranded DNA and displaced ssDNA are indicated on the left-hand side. (B) Graphical representation of the extent of inhibition of strand exchange as a function of increasing concentrations of MtUvrD1. The percent activity (shown as % displaced ssDNA) in the absence of MtUvrD1 has been normalized to 100%. The data points represent the mean ± SE of three independent experiments.

500 nM. To assess the generality of the UvrD1 effect, we compared the strand exchange activity of MsRecA and EcRecA under similar conditions. The strand exchange activity of each of these proteins was not significantly affected by MtUvrD1 at concentrations up to 250 nM. However, complete inhibition of DNA strand exchange promoted by noncognate RecA proteins required much higher concentrations of MtUvrD1. Quantification of the concentration-dependent effect suggests that the reaction promoted by MtRecA was relatively more sensitive to inhibition by MtUvrD1, compared to MsRecA or EcRecA (Figure 1C). The different levels of inhibitory effect could be explained by the weak antagonistic interaction between noncognate RecA proteins and MtUvrD1 or due to their inherent ability to resist MtUvrD1 action. This effect was not due to dATP depletion because the reactions were performed in the presence of the ATP regeneration system. A similar pattern of inhibition was found when MtUvrD1 was preincubated with RecA nucleoprotein filaments for 5 min prior to the addition of duplex DNA (data not shown).

One of the biochemical mechanisms proposed for the antirecombinase activity of *E. coli* UvrD involves its ability to disassemble the RecA nucleoprotein filament (*22*, *23*). A number of studies have shown that ATPγS halts dissociation of RecA and stabilizes the RecA nucleoprotein filaments, and it also supports very efficient strand exchange between completely homologous double- and single-stranded oligonucleotide substrates (*2*−*4*). To gain further insights, we asked whether the mechanism of inhibition of strand exchange promoted by MtUvrD1 proceeds in a manner similar to that of *E. coli* UvrD (*22*, *23*). To this end, assays were performed as described above except that ATPγS replaced dATP. The reaction was initiated by the addition of increasing amounts of MtUvrD1 and a fixed amount of ^{32}P-labeled 83-bp DNA. A more complex picture emerged when ATPγS was utilized in the strand exchange assays (Figure 2). The extent of strand exchange promoted by MtRecA progressively decreased with increasing concentrations of MtUvrD1. At 500 nM MtUvrD1, strand exchange promoted by MtRecA was inhibited to an extent of ~50% (Figure 2B); however, this amount is >2.5 times higher than that required to cause 50% inhibition for reactions performed in the presence of dATP (compare Figure 1C with Figure 2B). In contrast to the results obtained with MtRecA, the efficiency of strand exchange promoted by either EcRecA or MsRecA was not significantly affected with a wide range of MtUvrD1 concentrations. These strikingly unexpected results are consistent with the notion that the RecA nucleoprotein filaments differ in their inherent ability to resist MtUvrD1 action. Alternatively, the differences may be attributable to the heterologous nature of the proteins. However, the latter seems unlikely since MtUvrD1 was able to inhibit strand exchange promoted by both EcRecA and MsRecA in the presence of dATP (Figure 1).

The M. tuberculosis UvrD1^{Q276R} Mutant Inhibits DNA Strand Exchange. In this study, complete strand exchange promoted by RecA protein culminates in the displacement of the ^{32}P-labeled single-stranded DNA, which is essentially similar to that of the reaction promoted by UvrD helicase. To assess whether the duplex DNA unwinding or ATPase activities of MtUvrD1, or both, are essential for inhibition of strand exchange, the MtUvrD1^{Q276R} variant was generated. We note that this variant was found to be inactive in ATPase and DNA helicase activity (see Supporting Information Figure 2B and Supporting Information Figure 3). We performed strand exchange assays using increasing concentrations of the *M. tuberculosis* UvrD1^{Q276R} variant under conditions as described above. Figure 3A shows representative strand exchange reactions promoted by MtRecA, EcRecA, or MsRecA in the presence of increasing concentrations of the *M. tuberculosis* UvrD1^{Q276R} mutant. *E. coli* and *M. smegmatis* RecA proteins were included to allow comparison with MtRecA. The extent of inhibition of MtRecA promoted strand exchange by MtUvrD1^{Q276R} increased in a concentration-dependent manner (Figure 3A), but the efficiency was not comparable to that of the MtUvrD1^{WT} (see Figure 1). Quantification revealed that 500 nM MtUvrD1^{Q276R} mutant was able to inhibit ~70% of strand exchange promoted by MtRecA. Similarly, the MtUvrD1^{Q276R} mutant enzyme inhibited MsRecA promoted strand exchange, albeit at slightly higher concentrations. However, MtUvrD1^{Q276R} failed to inhibit EcRecA promoted strand exchange even at concentrations up to1 μM. Thus, these results suggest that although the DNA unwinding and ATPase activities of MtUvrD1^{WT} are not absolutely essential for suppression of RecA promoted strand exchange, they might play a facilitatory role by increasing the inhibition efficiency.

How similar is *M. tuberculosis* UvrD1 to its homologues in *E. coli* and *M. smegmatis*? To address this issue, UvrD1 sequences were aligned across their entire lengths using ClustalW2. The pairwise comparison revealed that *M. tuberculosis* UvrD1 shares 81% overall amino acid identity (and 88% similarity) with its homologue from *M. smegmatis* and 38% overall amino acid

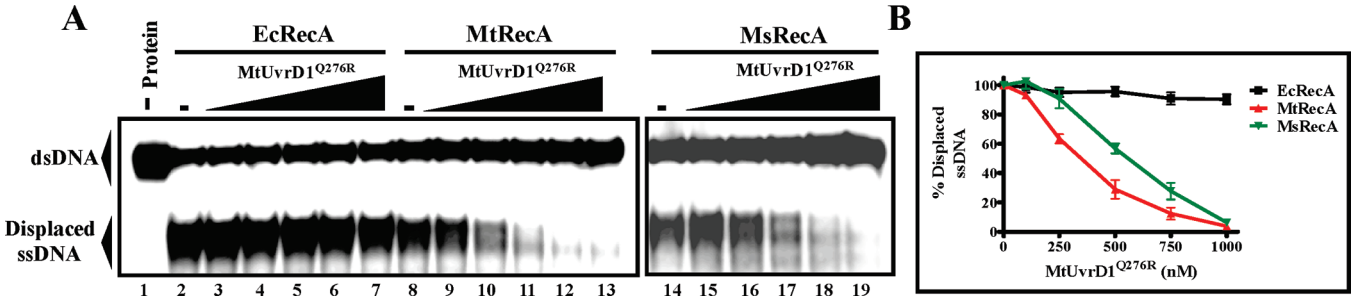

FIGURE 3: Effect of increasing concentrations of MtUvrD1^{Q276R} on strand exchange promoted by cognate and noncognate RecA proteins in the presence of dATP. Assay was performed as described in the legend of Figure 1, except MtUvrD1^{Q276R} was used instead of wild-type MtUvrD1. (A) Strand exchange reactions were performed with 5 μM ssDNA and 1 μM ^{32}P-labeled linear double-stranded DNA containing EcRecA, MtRecA, or MsRecA in the absence (lanes 2, 8, and 14) or in the presence of 100, 250, 500, 750, and 1000 nM MtUvrD1^{Q276R} (lanes 3−7, 9−13, and 15−19), respectively. Lane 1 shows the control reaction performed in the absence of RecA. The positions of double-stranded DNA and displaced ssDNA are indicated on the left-hand side. (B) Graphical representation of the extent of inhibition of strand exchange as a function of increasing concentrations of MtUvrD1^{Q276R}. The percent activity (shown as % displaced ssDNA) in the absence of MtUvrD1^{Q276R} has been normalized to 100% reaction. The data points represent the mean ± SE of three independent experiments.

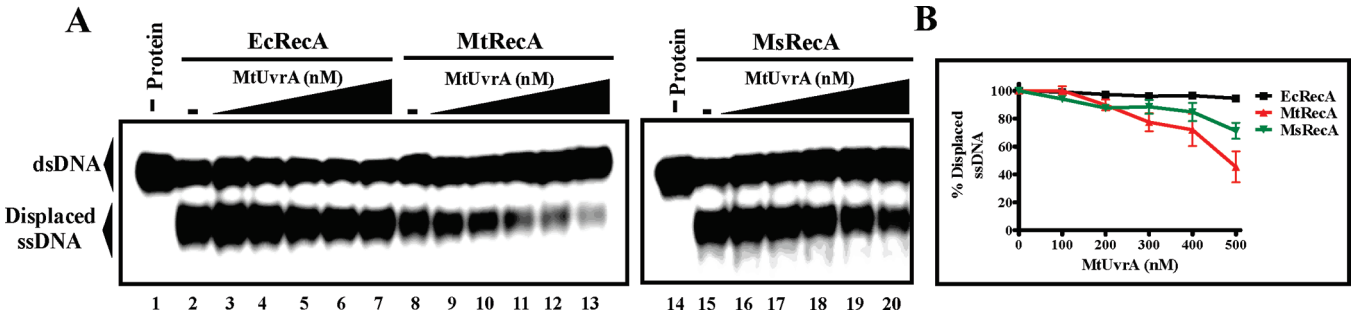

FIGURE 4: Effect of increasing concentrations of MtUvrA on strand exchange promoted by RecA proteins in the presence of dATP. Assay was performed as described in the legend of Figure 1, except MtUvrA replaced MtUvrD1. (A) Strand exchange reactions were performed with EcRecA, MtRecA, or MsRecA in the absence (lanes 2, 8, and 15) or presence of 100, 200, 300, 400, and 500 nM MtUvrA (lanes 3−7, 9−13, and 16−20), respectively. Lanes 1 and 14 show control reactions performed in the absence of the indicated RecA. The positions of double-stranded DNA and displaced ssDNA are indicated on the left-hand side. (B) Graphical representation of the extent of inhibition of strand exchange as a function of increasing concentrations of MtUvrA. The percent activity (shown as % displaced ssDNA) in the absence of MtUvrA has been normalized to 100%. The data points represent the mean ± SE of three independent experiments.

identity (and 53% similarity) with *E. coli* UvrD, respectively (Supporting Information Table 1). In addition, all three sequences possess the conserved helicase domains, with >80% overall amino acid similarity at the conserved motifs, suggesting probable conservation of function. However, *M. tuberculosis* UvrD1 is more closely related to the *M. smegmatis* homologue than to its *E. coli* counterpart. Although the overall sequence identity between the MtUvrD1 and its *E. coli* homologue is low, it is substantially higher than what is often considered a cutoff for predicting functional similarity (20%).

Synergistic Effect of MtUvrA and MtUvrD1 on RecA-Promoted Strand Exchange. Genetic studies have shown 25−50% phage recombination to be u*vrABC*-dependent (*54*). Similar studies have demonstrated that *uvrA* and *uvrB* suppress illegitimate recombination (*55*). Furthermore, illegitimate recombination was synergistically enhanced by the *uvrA uvrB* and *recQ uvrA* double mutations (*55*). A recent study has shown that UvrA modulates the UvrD helicase activity but has no effect on Rep helicase, a UvrD homologue (*56*). However, the mechanistic basis underlying the suppression of HR by *uvrA* and *uvrD* is unknown. To study the relationship between UvrA and UvrD, we first examined the effect of MtUvrA on strand exchange under the conditions described above. The results in Figure 4A indicate that the strand exchange activity of EcRecA was unaffected by MtUvrA even at concentrations up to 500 nM (lanes 3−7). In comparison, the strand exchange activity of MsRecA was slightly affected by MtUvrA at the highest concentration tested (Figure 4, lanes 16−20). By contrast, addition of increasing concentrations of MtUvrA resulted in a consistent and progressive increase in the extent of inhibition of strand exchange catalyzed by MtRecA (Figure 4, lanes 9−13). These observations suggest that physical interaction between UvrA with its cognate RecA may be responsible for the inhibition of strand exchange caused by UvrA.

Next we examined the possible synergistic effect of MtUvrA and MtUvrD1 on strand exchange promoted by all three RecA proteins. We surmised that their physical and/or functional interaction may contribute to the regulation of HR *in vivo*, particularly given the fact that UvrA and UvrD proteins are known to function in the same pathway. At the concentrations of MtUvrA used in this assay, it had no significant inhibitory effect on strand exchange promoted by all three RecA proteins (Figure 5, panels A, C, E; lanes 3−7, Figure 5, panels B, D, F). Strikingly, in the presence of 100 nM MtUvrD1, addition of increasing concentrations of MtUvrA resulted in a proportional decrease in the extent of strand exchange promoted by MtRecA (Figure 5C, lanes 9−13), and the reaction was inhibited to >80% at 200 nM (Figure5D). In contrast, the same concentrations of MtUvrA and MtUvrD1 failed to modulate strand exchange promoted by EcRecA (Figure 5A,B). In the case of MsRecA, however, we observed inhibition at much higher MtUvrA concentrations (Figure 5E,F).

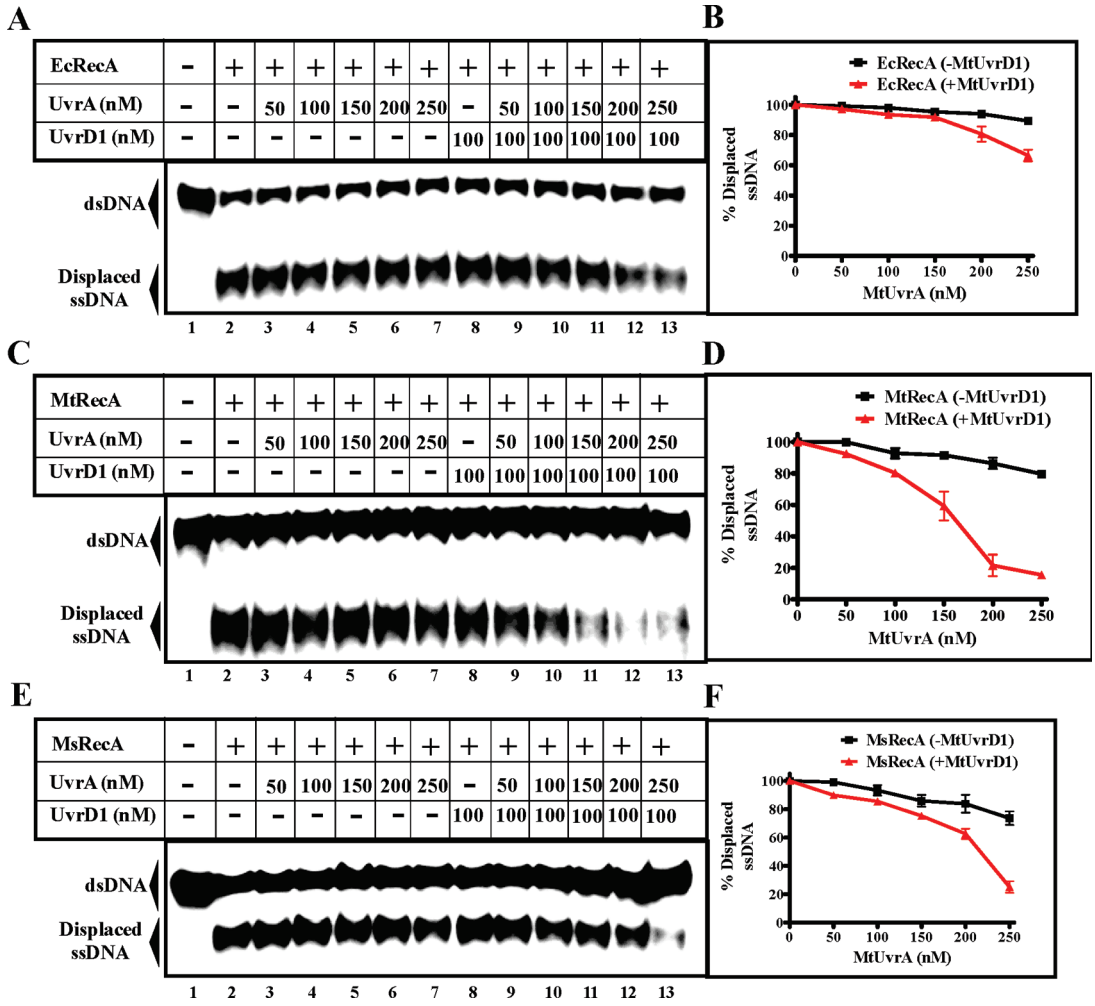

FIGURE 5: MtUvrA augments the inhibition of RecA protein promoted strand exchange by MtUvrD1. Assay was performed as described in the legend of Figure 1. Strand exchange reactions performed with EcRecA (panel A), MtRecA (panel C), and MsRecA (panel E) in the absence (lanes 2−7) or in the presence (lanes 8−13) of UvrD1 (100 nM) and in the presence of 50, 100, 150, 200, and 250 nM MtUvrA (lanes 3−7 and 9−13), respectively. Lane 1 depicts the reaction performed in the absence of proteins, and lane 2 corresponds to a control reaction performed in the absence of MtUvrA and MtUvrD1. The positions of double-stranded DNA and displaced ssDNA are indicated on the left-hand side. Panels B, D, and F show the graphical representation of the extent of inhibition of strand exchange promoted by EcRecA, MtRecA, and MsRecA as a function of increasing concentrations of MtUvrA in the presence of a fixed amount of MtUvrD1. The percent activity (shown as % displaced ssDNA) in the absence of MtUvrD1 or MtUvrA has been normalized to 100%. The data points represent the mean ± SE of three independent experiments.

Given the wide variability in the degree of inhibition exerted by MtUvrA on strand exchange of all three RecA proteins (Figure 5), we performed a converse experiment in which MtUvrD1 was titrated into reactions containing a fixed amount of MtUvrA. The extent of inhibition on the reaction catalyzed by MtRecA increased with increasing amounts of MtUvrD1, reaching maximal inhibition at 75 nM (Figure 6C,D). MtUvrD1 elicited a similar concentration-dependent inhibition on strand exchange promoted by MsRecA (Figure 6E,F). On the other hand, higher concentrations of MtUvrD1 were required to exert significant inhibition on the reaction promoted by EcRecA (Figure 6A,B). These results suggest a functional synergistic interaction between MtUvrA and MtUvrD1, presumably reflecting an underlying physical interaction between cognate proteins.

Next, for *M. tuberculosis* UvrA, we performed multiple sequence alignment by ClustalW2. A closer inspection of the sequence of *M. tuberculosis* UvrA with *M. smegmatis* UvrA and *E. coli* UvrA shows that it shares 86% and 55% overall amino acid identity and 92% and 71% similarity with its homologues from *M. smegmatis* and *E. coli*, respectively (Supporting Information Table 1). As expected, *M. tuberculosis* UvrA is more closely related to the *M. smegmatis* homologue than to its *E. coli* counterpart. The most conserved regions are found at the N- and C-terminal regions of the UvrA proteins, which include the nucleotide binding region (P-loop).

Physical Interaction between RecA and UvrD1 or RecA and UvrA in Vitro. One possible mechanism by which UvrD1 or UvrA might repress DNA strand exchange promoted by RecA is through direct protein−protein interactions. Specific interaction between RecA and UvrD1 or between RecA and UvrA proteins was examined by using purified proteins and *in vitro* pulldown and coimmunoprecipitation assays (co-IPs). Following incubation of MtUvrD1 with RecA, from the indicated source, the immunoprecipitates were analyzed on SDS−polyacrylamide gels, followed by Western blot analysis. Physical association between MtUvrD1 and RecA was ascertained by two parallel experiments. In one experiment, the complexes were immunoprecipitated with anti-UvrD1 antibodies, and the immunoprecipitates were analyzed by blotting with anti-RecA antibodies. In parallel experiments, immunoprecipitation was performed with anti-RecA antibodies and then blotted with anti-UvrD1 antibodies. Figure 7A, panel i, shows that MtUvrD1 was able to pull

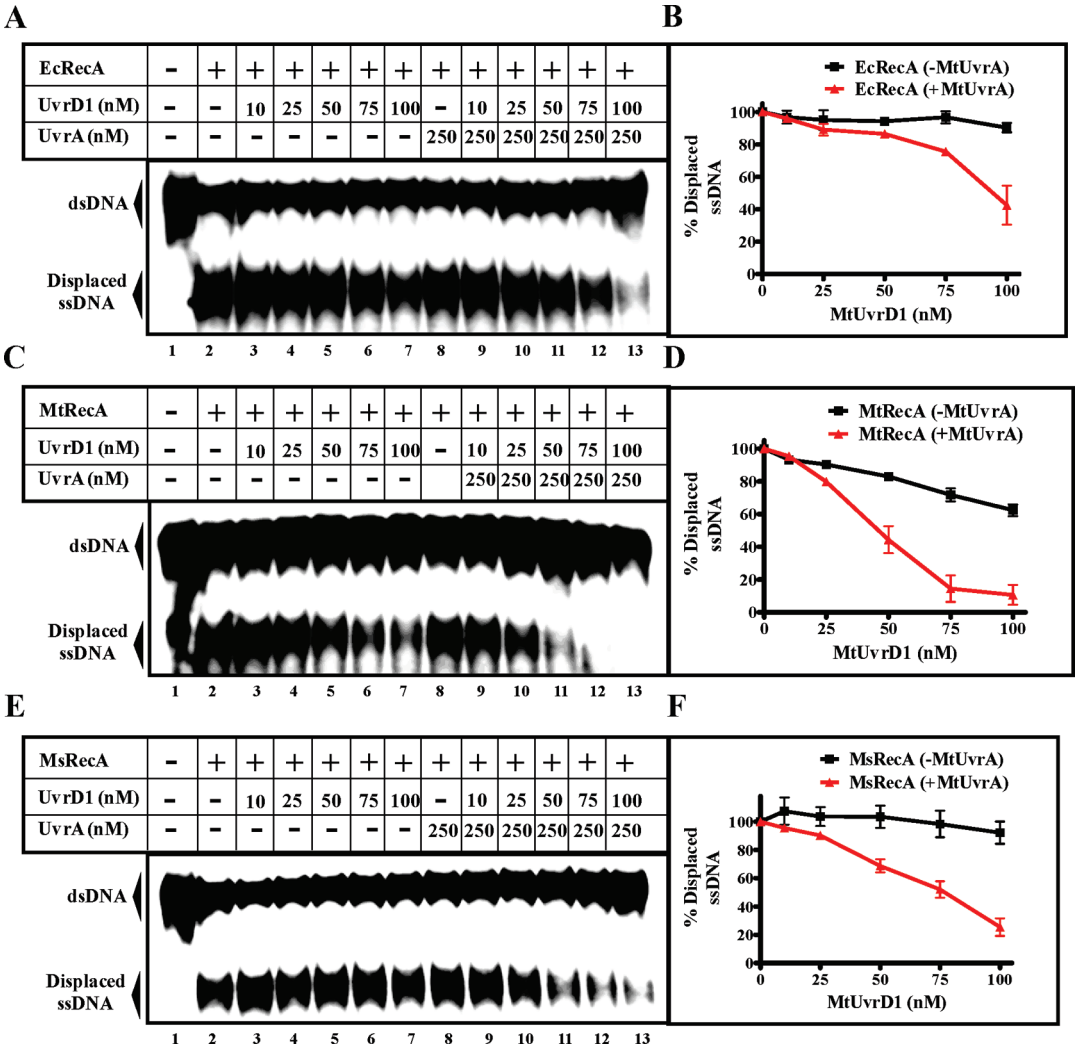

FIGURE 6: Effect of UvrD1 and UvrA on strand exchange reactions promoted by RecA proteins. Assay was performed as described in the legend of Figure 1. Strand exchange reactions performed with EcRecA (panel A), MtRecA (panel C), and MsRecA (panel E) in the absence (lanes 2−7) or in the presence (lanes 8−13) of UvrA (250 nM) and in the presence of 10, 25, 50, 75, and 100 nM MtUvrD1 (lanes 3−7 and 9−13), respectively. The positions of double-stranded DNA and displaced ssDNA are indicated on the left-hand side. Panels B, D, and F show the graphical representation of the extent of inhibition of strand exchange promoted by EcRecA, MtRecA, and MsRecA as a function of increasing concentrations of MtUvrD1 in the presence of a fixed amount of MtUvrA. The percent activity (shown as % displaced ssDNA) in the absence of MtUvrD1 or MtUvrA has been normalized to 100%. The data points represent the mean ± SE of four independent experiments.

down its cognate RecA, indicating physical association between these two proteins. Additional co-IP experiments were performed to test whether the noncognate RecA proteins interact with MtUvrD1. Figure 7A, panels ii and iii, shows the results of complex formation between MtUvrD1 and noncognate RecA proteins. The co-IP experiments were performed in a reciprocal manner (i.e., IP with anti-RecA and immunoblotting with anti-MtUvrD1 antibodies) to corroborate interaction between RecA and MtUvrD1. Figure 7A, panels iv−vi, confirm complex formation between MtUvrD1 and cognate and noncognate RecA proteins. The interaction was specific, because neither MtUvrD1 nor RecA were present in the control samples, i.e., RecA with protein A−Sepharose (lane 3) or MtUvrD1 incubated with protein A−Sepharose (lane 4) or incubated with purified protein alone or nonspecific antibody (lane 5).

Having shown that UvrD1 interacts with cognate and noncognate RecA proteins, we carried out co-IPs to further assess the interaction between MtUvrA and its cognate RecA. Here, we coimmunoprecipitated purified MtUvrA using an monoclonal antibody directed against the $(His)_4$ epitope followed by blotting with anti-RecA antibody. Figure 7B shows specific interaction between MtRecA and MtUvrA. However, the interaction seems much weaker compared to the interaction of MtRecA with its cognate UvrD1.

DISCUSSION

In this study, we show that *M. tuberculosis* UvrD1 exerts a strong inhibitory effect on strand exchange promoted by its cognate MtRecA, compared to noncognate MsRecA or EcRecA. A second observation of interest is that MtUvrA also modulates the MtRecA reaction although it has no discernible effect on strand exchange promoted by EcRecA and only a limited effect on MsRecA.

These findings provide the first evidence that MtUvrA and MtUvrD1 can act together to inhibit the MtRecA-catalyzed strand exchange, compared to the same reaction promoted by MsRecA or EcRecA. This inhibitory effect raises the possibility that MtUvrD1 and MtUvrA might function together to counteract the negative effects of RecA nucleoprotein filaments *in vivo*.

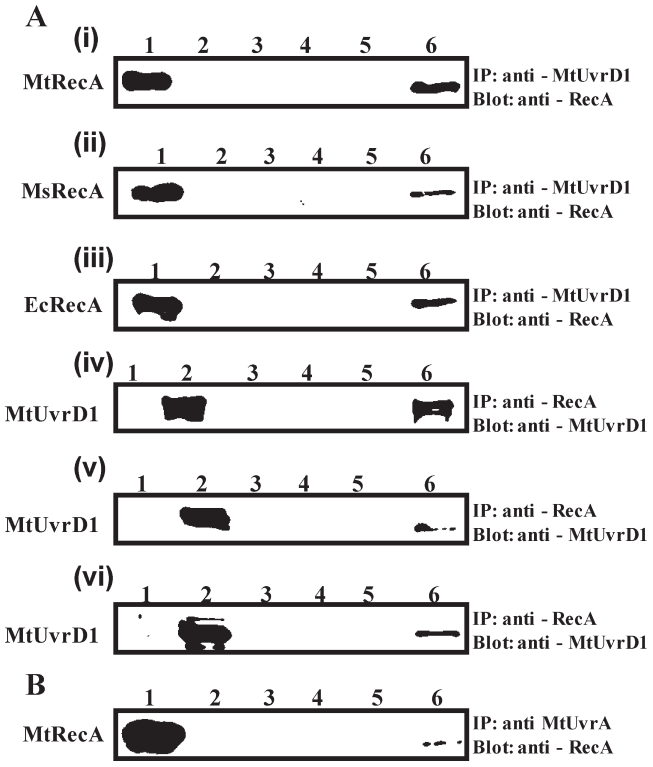

FIGURE 7: Physical association of MtUvrD1 with cognate and noncognate RecA proteins and MtUvrA with its cognate RecA *in vitro*. (A) MtUvrD1 associates with cognate and noncognate RecA proteins. Panels i−iii: MtRecA, MsRecA, or EcRecA was immunoprecipitated (IP) with anti-MtUvrD1 antibodies and immunoblotted (Blot) with anti-RecA antibodies. MtUvrD1 was incubated with MtRecA, MsRecA, or EcRecA and immunoprecipitated as described under Experimental Procedures. Lane 1, MtRecA, MsRecA, or EcRecA; lane 2, MtUvrD1; lane 3, MtRecA, MsRecA, or EcRecA incubated with protein A−Sepharose CL-4B beads; lane 4, MtUvrD1 incubated with protein A−Sepharose CL-4B beads; lane 5, RecA alone incubated with nonspecific antibody; lane 6, MtUvrD1 and RecA incubated with anti-MtUvrD1 antibody. Panels iv−vi: IP and immunoblot (Blot) were performed as described in panels i−iii except that pulldown and co-IP experiments were performed in a reciprocal manner (i.e., IP with anti-RecA and immunoblotting with anti-MtUvrD1 antibodies). Lane 1, MtRecA, MsRecA, or EcRecA; lane 2, MtUvrD1; lane 3, MtRecA, MsRecA, or EcRecA incubated with protein A−Sepharose CL-4B beads; lane 4, MtUvrD1 incubated with protein A−Sepharose CL-4B beads; lane 5, MtUvrD1 alone incubated with nonspecific antibody; lane 6, RecA and MtUvrD1 incubated with anti-RecA antibody. (B) MtUvrA interacts with its cognate RecA *in vitro*. MtRecA was immunoprecipitated (IP) with anti-MtUvrA antibodies and immunoblotted (Blot) with anti-RecA antibodies. Lane 1, MtRecA; lane 2, MtUvrA; lane 3, MtRecA incubated with protein A−Sepharose CL-4B beads; lane 4, MtUvrA incubated with protein A−Sepharose CL-4B beads; lane 5, MtRecA alone incubated with nonspecific antibody; lane 6, MtRecA and MtUvrA incubated with anti-UvrA antibody.

These observations are consistent with a molecular mechanism, whereby RecA promoted strand exchange is negatively regulated jointly by UvrA and UvrD1 proteins.

DNA helicases are ubiquitous and play crucial roles in the processes of DNA metabolism such as replication, repair, recombination, and transcription (*2*, *3*, *12*, *14*, *15*, *57*). Their DNA unwinding activities are well studied at the biochemical and structural levels (*58*−*62*). In particular, characterized examples include *E. coli* UvrD and Rep as well as PcrA from *B. stearothermophilus* and *Staphylococcus aureus*. UvrD belongs to superfamily I(SFI)DNA helicases with well-documented roles in nucleotide excision repair and methyl-directed mismatch repair in addition to poorly understood roles in replication and recombination (*58*−*62*). In *E. coli*, the primary components of the NER pathway include the excinuclease ABC (UvrA, UvrB, and UvrC) and DNA helicase II UvrD (*1*, *6*, *10*, *14*, *15*). Mycobacteria possess all of the genes necessary to encode the proteins involved in this pathway (*32*, *36*). Typically, NER entails recognition of DNA damage by UvrA and UvrB, incision of the strand containing the damage by UvrB and UvrC (*2*), and removal of the damaged region by UvrD helicase, followed by repair synthesis, which fills the gap and is completed by ligation of the repaired section to the undamaged DNA. However, the functional relationship between NER, MMR and HR is poorly understood.

In *E. coli*, genetic studies suggest that *uvrD* mutants are hyperrecombinogenic (*16*−*21*). The frequency of Hfr conjugation was increased 8-fold in a *uvrD* null mutant, suggesting that *uvrD* antagonizes Hfr conjugation in wild-type cells (*63*). As in *E. coli*, *Helicobacter pylori uvrD* plays an important role in limiting homologous intergenomic recombination (*64*). In light of these observations, attempts have been made to understand its biochemical role during HR. Under *in vitro* conditions, *E. coli* UvrD can both promote (*22*) and inhibit (*22*, *23*) strand exchange catalyzed by its cognate RecA. In this study, we have tested whether the action of MtUvrD1 and MtUvrA on RecA-promoted strand exchange is conserved in other bacterial species. We studied for their effect on strand exchange promoted by *E. coli* and mycobacterial RecA proteins. In addition, we also wanted to determine the role of helicase/ATPase activities of MtUvrD1 in the inhibition of strand exchange promoted by RecA proteins. The amino acid sequence of MtRecA is 90% identical to MsRecA and 59% identical to EcRecA, respectively, when compared over their entire length. Consistent with this, the crystal structure of EcRecA exhibits extensive similarity to MtRecA and MsRecA proteins (*65*). Our results show that, in the presence of dATP, $MtUvrD1^{WT}$ was able to inhibit strand exchange promoted by cognate RecA more efficiently, compared with noncognate RecA proteins (Figure 1). Interestingly, $MtUvrD1^{Q276R}$, which is devoid of both DNA helicase and ATPase activities, was able to inhibit strand exchange catalyzed by mycobacterial RecA proteins but not of EcRecA (Figure 3). We note that the effect of $MtUvrD1^{Q276R}$ on strand exchange is very reminiscent of that observed with the helicase/ATPase PcrA mutant of *S. aureus* (*66*). This mutant, which was severely defective in helicase/ATPase activities, was able to inhibit *E. coli* RecA promoted strand exchange with efficiencies comparable to that of the wild-type PcrA (*67*).

The current understanding of the role of the SFI family of DNA helicases in processes related to DNA transactions is that they simply dislodge or remove DNA-bound proteins in their path. One of its members, UvrD, has been shown to disrupt the active RecA-ssDNA nucleoprotein filament (*26*). Similarly, Srs2, the eukaryotic orthologue of UvrD1 displaces Rad51 from DNA (*28*). Taken together, these results suggest that UvrD1 or Srs2 suppress strand exchange because of their ability to disassemble the RecA or Rad51 nucleoprotein filaments, respectively. The results from this study are consistent with the antirecombinogenic activity of UvrD1 and further establish the pertinent parameters, including a role for UvrA in DNA strand exchange.

Previously, we have shown that *M. smegmatis* RecA, not *M. tuberculosis* RecA, was able to couple dATP hydrolysis to strand transfer in a pH-dependent manner (*45*). The assays in the aforementioned study were performed with long DNA substrates

(6.4-kb linear duplex DNA and homologous circular single-stranded DNA). With the shorter DNA substrates used in this study (83-bp linear duplex DNA and homologous single-stranded DNA), RecA proteins from *E. coli*, *M. tuberculosis*, and *M. smegmatis* catalyze strand exchange in the presence dATP at pH 7.5 to a similar extent (data not shown). Furthermore, dATP has been shown to increase the stability of RecA nucleoprotein filaments (*68*). In light of these observations, we have used dATP in strand exchange reactions catalyzed by all three RecA proteins. In addition, we took advantage of the known effect of ATPγS on RecA promoted reactions. *In vitro*, RecA proteins bind ssDNA almost irreversibly in the presence of ATPγS (*2*). In the presence of ATPγS, strand exchange promoted by MtRecA was inhibited by MtUvrD1, albeit less efficiently, indicating that the mechanism of inhibition might not be entirely due to its ability to disassemble the RecA nucleoprotein filaments. On the other hand, strand exchange promoted by MsRecA or EcRecA was not significantly affected by MtUvrD1 under similar conditions (Figure 2). It has been proposed that the mechanism by which DNA helicases displace RecA-like proteins from DNA involves their helicase/ATPase coupled translocation on DNA (*26*−*28*). Consistent with these results, the helicase mutant of Srs2 failed to inhibit strand exchange promoted by the Rad51 protein (*26*). In contrast to the helicase mutant Srs2, our results show that MtUvrD1^{Q276R} was inhibitory to the strand exchange promoted by mycobacterial RecA proteins but not of EcRecA. These findings disclose a lack of commonality in the mechanism of action of DNA helicases from widely divergent sources. Therefore, it is possible that MtUvrD1^{Q276R} may display its antirecombinogenic function independently of its ATPase/helicase activities. Neverthless, these results suggest functional interaction between MtUvrD1 with its cognate and noncognate RecA proteins.

E. coli UvrD has been shown to dislodge proteins from DNA, which is coupled to unwinding of DNA or DNA-RNA hybrids (*12*, *14*, *57*). During NER, UvrD helicase removes both the 12-mer containing the lesion and UvrC protein (*69*). Likewise, *E. coli* UvrD also displaces the Tus protein bound to its *ter* site *in vitro* (*70*), the topoisomerase IV from its cleaved DNA−substrate intermediate (*71*), and LacI from its DNA operator site (*72*). The action of UvrD during recombination and replication seems to be conserved in other species. For example, UvrD action on RecA is conserved in evolution as it can be performed by UvrD homologues (*57*, *60*, *73*, *74*).

To our knowledge this is the first report examining the roles of UvrA and UvrD on DNA strand exchange *in vitro*. It is clear from a number of studies that UvrA and UvrD proteins are functionally distinct and also function in the same pathway. In *E. coli*, *uvrA* by itself suppresses illegitimate recombination (*53*). Additionally, overproduction of the UvrA protein suppressed the hyperillegitimate recombination phenotype of the *recQ* or *uvrB* mutant (*53*). On the other hand, UV-stimulated HR of nonreplicating λ phage has been shown to be *uvrABC*-dependent (*52*). These findings led us to examine the effect of MtUvrA by itself, and together with MtUvrD1, on strand exchange promoted by *E. coli* and *mycobacterial* RecA proteins. The results presented here suggest that MtUvrA alone and in concert with MtUvrD1 can exert antirecombination activities. Since UvrA protein has DNA-binding activity (*14*, *15*), it is reasonable to suppose that inhibition of strand exchange may be caused by preventing strand passage, with UvrA acting either directly as a roadblock or indirectly by impeding the unwinding of duplex DNA during the search for homologous sequences (*75*). The co-IP experiments reported here (Figure 7) further provide evidence that interaction between UvrD1 and RecA on one hand and RecA and UvrA on the other is likely to be functionally significant for the mechanism of homologous recombination *in vivo*.

In summary, the mechanism underlying the inhibition of RecA promoted strand exchange by UvrA and UvrD proteins appears to be complex. Despite this complexity, *E. coli and mycobacterial* RecA proteins share common and mechanistically important features. It is likely that species specialization has led to key differences between and even within UvrD and UvrA proteins. Further studies are clearly needed for a more complete understanding of the biological function of UvrA and UvrD in recombination, but the identification here of their roles in strand exchange provides fascinating new insights into their antirecombination activity.

SUPPORTING INFORMATION AVAILABLE

Figure 1, purification of RecA, UvrD1, and UvrA proteins; Figure 2, DNA unwinding activity of *M. tuberculosis* UvrD1^{WT} and UvrD1^{Q276R} mutant proteins; Figure 3, ATPase activity of *M. tuberculosis* UvrD1^{WT} and UvrD1^{Q276R} mutant proteins; Table 1, percent of amino acid sequence identity/similarity between *E. coli* and *mycobacterial* UvrD1 and UvrA proteins. This material is available free of charge via the Internet at http://pubs.acs.org.

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