Editor's Choice

Effects of conserved residues and naturally occurring mutations on Mycobacterium tuberculosis RecG helicase activity

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RecG is a helicase that is conserved in nearly all bacterial species. The prototypical Escherichia coli RecG promotes regression of stalled replication forks, participates in DNA recombination and DNA repair, and prevents aberrant replication. Mycobacterium tuberculosis RecG (RecGMtb) is a DNA-dependent ATPase that unwinds a variety of DNA substrates, although its preferred substrate is a Holliday junction. Here, we performed site-directed mutagenesis of selected residues in the wedge domain and motifs Q, I, Ib and VI of RecGMtb. Three of the 10 substitution mutations engineered were detected previously as naturally occurring SNPs in the gene encoding RecGMtb. Alanine substitution mutations at residues Q292, F286, K321 and R627 abolished the RecGMtb unwinding activity, whilst RecGMtb F99A, P285S and T408A mutants exhibited ~25–50 % lower unwinding activity than WT. We also found that RecGMtb bound ATP in the absence of a DNA cofactor.

INTRODUCTION

DNA and RNA helicases are motor proteins that drive nucleoside triphosphate-dependent unwinding of duplex nucleic acids, thereby facilitating many essential nucleic acid transactions (Lohman et al., 2008). Escherichia coli RecG is a superfamily 2 (SF2) DNA helicase thought to play important roles in the reversal of stalled replication forks, DNA recombination, DNA repair and prevention of aberrant DNA replication (McGlynn & Lloyd, 2002; Rudolph et al., 2009a, b, 2010). In addition to being present in bacteria, recG gene homologues have also been identified in most vascular plants and green algae, but not in other eukaryotes or archaea (Zegeye et al., 2012). The co-crystal structure of Thermotoga maritima RecG in complex with a partial DNA replication fork provided insight into how RecG recognizes stalled replication forks and catalyses fork regression/reversal (Singleton et al., 2001). It has been difficult to define fully the biological role(s) of RecG in vivo due to the fact that bacterial recG mutants have diverse phenotypes and RecG proteins interact with a wide variety of DNA substrates in vitro (Rudolph et al., 2010).

SF2 helicases share seven or more short, highly conserved motifs that are essential for catalytic activity (Gorbalenya & Koonin, 1993; Hall & Matson, 1999; Tuteja & Tuteja, 2004). RecG contains the seven helicase motifs (Gorbalenya & Koonin, 1993) as well as the Q-motif (Tanner et al., 2003) and the TRG (translocation in RecG) motif (Mahdi et al., 2003). Substitution of a conserved alanine in motif III with valine impaired the branch migration activity of E. coli RecG (Sharples et al., 1994), and a lysine to alanine substitution in motif I abolished helicase and ATPase activity of E. coli RecG (McGlynn et al., 2000). Mutations in the TRG motif revealed that it is required during translocation of RecG on duplex DNA (Mahdi et al., 2003), and aromatic residues in the N-terminal wedge domain region of E. coli RecG facilitate binding to branched DNA (Briggs et al., 2005).

Mycobacterium tuberculosis is the causative agent of tuberculosis, a deadly disease that claims ~1.5 million lives every year and the second most common cause of death from infectious disease after HIV/AIDS (WHO, 2011,
2012). In addition, the World Health Organization estimates that approximately one in three individuals worldwide are infected latently with *M. tuberculosis* (WHO, 2011). As *M. tuberculosis* cells enter and propagate intracellularly in human macrophages, the bacteria are exposed to abundant reactive oxygen and nitrogen intermediates (Ehrt & Schnappinger, 2009; Stallings & Glickman, 2010). However, this pathogen appears to endure and combat oxidative and nitrosative DNA damage efficiently (Dos Vultos et al., 2009; Ehrt & Schnappinger, 2009; Voskuil et al., 2011). Accordingly, *M. tuberculosis* isolated from mouse macrophages and human lung samples showed upregulation of genes including the *RecG* and *dinG* (Rachman et al., 2006; Schnappinger et al., 2003) that can attenuate the damaging effects of these reactive molecules. This suggested that RecG and DinG may stabilize the *M. tuberculosis* genome during infection, but more detailed understanding of this process in *M. tuberculosis* is needed.

Recent studies have revealed that the *M. tuberculosis* RecG (RecG*Mtb*) enzyme has a broad DNA substrate affinity, like other related SF2 helicases. Conserved residues in the catalytic activity of RecG and RecG*Mtb* samples showed upregulation of genes including the *RecG* gene. Therefore, engineered and naturally occurring mutations in selected conserved amino acid residues in the RecG*Mtb* were pooled and dialysed against storage buffer [20 mM Tris/HCl (pH 7.5), 300 mM NaCl, 1 mM DTT, 20 % glycerol] and stored at −80 °C until used. All the mutant proteins purified were as soluble as the WT (data not shown). RecG*Mtb* protein concentrations were determined by the Bradford method (Bio-Rad) using BSA as the standard.

Purification of His<sub>6</sub>-tagged WT and mutant RecG*Mtb* proteins. Overexpression and purification of the WT and mutant RecG*Mtb* proteins to homogeneity were performed as described previously (Zegeye et al., 2012) except for the following modifications. After nickel-nitritotriacetic acid column purification, the eluted fractions containing the RecG*Mtb* were pooled and dialysed against a buffer [20 mM Tris/HCl (pH 8.6), 100 mM NaCl, 1 mM DTT] overnight. Further purification was carried out using a RESOURCE Q column (Pharmacia Biotech). Fractions containing purified RecG*Mtb* protein were pooled and dialysed against storage buffer [20 mM Tris/HCl (pH 7.5), 300 mM NaCl, 1 mM DTT, 20 % glycerol] and stored at −80 °C until used. All the mutant proteins purified were as soluble as the WT (data not shown). RecG*Mtb* protein concentrations were determined by the Bradford method (Bio-Rad) using BSA as the standard.

Circular dichroism (CD). CD spectra were recorded using a J-810 spectropolarimeter (Jasco International) calibrated with d-camphor-10-sulfonate (Icatayama Chemical). The recordings were made in 20 mM phosphate buffer (pH 7.5) with final protein concentrations of 0.1 mg ml<sup>−1</sup> (150 μl). All measurements were done using a quartz cuvette (Starwa) with 0.1 cm path length. The ellipticity as a function of frequency was scanned five times between 245 and 190 nm at a scanning rate of 0.1 mg ml<sup>−1</sup> until used. All the mutant proteins purified were as soluble as the WT (data not shown). RecG*Mtb* protein concentrations were determined by the Bradford method (Bio-Rad) using BSA as the standard.
EMSA. EMSA was conducted as detailed previously (Zegeye et al., 2012). Briefly, the reactions were initiated by adding 1 µM WT or mutant RecGMtb protein into a DNA binding buffer [50 mM Tris/HCl (pH 8.0), 5 mM EDTA, 100 µg BSA ml⁻¹, 6 % glycerol, 2 mM DTT, 50 ng poly(dI–dC) µl⁻¹ (Thermo Scientific)] and 0.1 nM HJ substrate, in a 20 µl reaction volume. Reaction mixtures were incubated for 15 min on ice followed by adding 4 µl of 60 % glycerol and resolving on a 4 % (29 : 1) non-denaturing polyacrylamide gel using a low-ionic-strength buffer (Whitby & Lloyd, 1998) with a constant buffer recirculation. The gels were dried, exposed to a phosphorimaging screen, autoradiographed by Typhoon 9410 (Amersham Biosciences) and quantified by ImageQuant TLv (GE Healthcare).

ATPase assay. ATPase activity of WT and mutant RecGMtb proteins were determined by separating released γ-32Pi from [γ-32P]ATP using TLC as described by Kornberg et al. (1978). Briefly, 150 nM of WT or mutant RecGMtb protein was added to a reaction mixture (10 µl final volume) containing an ATPase buffer [20 mM Tris/HCl (pH 7.5), 2 mM MgCl₂, 2 mM ATP, 100 µg BSA ml⁻¹, 100 µM cold ATP, 0.025 nM [γ-32P]ATP, 2 mM DTT] and 100 nM of an unlabelled HJ DNA cofactor. The reaction mixtures were incubated at 37 °C for 30 min followed by quenching with 5 µl 0.5 M EDTA. A sample of 2 µl of the mixture was spotted onto TLC plates (Cellulose PEI F; Merck) and developed with a solution containing 1 M formic acid and 0.5 LiCl. The TLC plates were dried and exposed to a phosphorimaging screen and analysed as described for EMSA. The proportion of hydrolysed ATP (%) was calculated as [counts for γ-32Pi/counts for γ-32Pi+ counts for [γ-32P]ATP]×100. Background correction was performed by subtracting the signal obtained from a ‘no enzyme’ control.

Unwinding (helicase) activity assay. The unwinding activity reactions were initiated by adding 150 nM WT or mutant RecGMtb protein to a reaction mixture containing a helicase buffer [20 mM Tris/HCl (pH 7.5), 2 mM MgCl₂, 2 mM ATP, 100 µg BSA ml⁻¹, 2 mM DTT] and 0.5 nM HJ substrate. The reaction mixtures were incubated at 37 °C for 30 min and stopped with 10 µl of 3 x helicase stop solution [50 mM EDTA, 40 % (w/v) glycerol, 0.9 % SDS, 0.1 % bromophenol blue, 0.1 % xylene cyanol] containing tenfold excess of trap strand (unlabelled H1 oligonucleotide). The reaction products were resolved by electrophoresis on a 10 % non-denaturing polyacrylamide gel using 1 x Tris-Borate-EDTA buffer at 150 V for 2 h. The gels were dried and analysed as described above for EMSA. The proportion of helicase substrate unwound was calculated as described by Brosh et al. (2006) as: per cent DNA unwound = [P/(S + P)]×100, where P is unwound product and S is the residual substrate. P and S were determined by subtracting the background values in controls containing no enzyme or heat-denatured substrate, respectively.

ATP binding assay. The ATP binding activities of WT and mutant RecGMtb proteins were assayed by the UV-cross-linking method as described previously (Pause & Sonenberg, 1992), with some modifications. Briefly, WT or mutant RecGMtb protein (2 µM final concentration) was added to initiate a reaction (10 µl) containing ATP binding buffer [20 mM Tris/HCl (pH 5), 0.5 µCi [γ-32P]ATP (3000 Ci mmol⁻¹) (PerkinElmer), 2 mM MgCl₂, 100 µg BSA ml⁻¹, 2 mM DTT]. To prevent hydrolysis of the bound ATP, we omitted a DNA cofactor from the reaction mixture, as RecGMtb is a strictly DNA-dependent ATPase (Zegeye et al., 2012). After incubating at 37 °C for 15 min, the reaction mixtures were transferred into a pre-cooled 96-well microtitre plate (Sarstedt) and irradiated with UV light (CL-1000 UV cross-linker; UVP) for 20 min on ice at a distance of 9.5 cm from the lamp. After irradiation, LDS sample buffer (Invitrogen) was added, heated at 70 °C for 10 min and electrophoresed on a 10 % LDS-polyacrylamide gel (Invitrogen). The gel was then dried and autoradiographed, and quantified as described for EMSA. The ATP binding activity was calculated as percentage of WT activity.

Statistical analysis. An unpaired Student’s t-test was used to determine statistical significance.

RESULTS

Mapping conserved motifs in RecGMtb

The classical helicase motifs (Gorbalenya & Koonin, 1993; Tuteja & Tuteja, 2004), the Q-motif (Cordin et al., 2004), the C-terminal TRG motif (Mahdi et al., 2003) and the N-terminal wedge domain (Singleton et al., 2001) are all conserved throughout the RecG family of proteins (Fig. 1b). Whilst the wedge domain is unique to the RecG protein family, the TRG motif is also present in Mfd helicases (Mahdi et al., 2003). The conserved motifs and protein domains were mapped onto the 3D RecGMtb structure as illustrated in Fig. 2. The evolutionary conservation of the helicase motifs in the two RecA-like domains is clearly important for the biological function of RecG. In contrast, the wedge domain region is quite divergent, with poor conservation of the putative DNA binding surface in this domain (Fig. 1b).

Site-directed mutagenesis of RecGMtb residues

Previously, we reported that RecGMtb can bind and unwind branched DNA substrates with the highest affinity towards HJ (Zegeye et al., 2012), which corroborates the latest report by Thakur et al. (2013). In order to understand the role of selected conserved amino acid residues in RecGMtb, we introduced amino acid substitutions in its catalytic domain and also made amino acid substitutions corresponding to three nsSNPs (A52E, P285S and A723T) identified in the recG gene of clinical isolates of M. tuberculosis (Dos Vultos et al., 2008) (Fig. 1a, b). The purified proteins were subjected to CD spectroscopy measurements to determine whether the single-amino acid substitutions induced any major changes in the secondary structures. The results showed that all 10 RecGMtb mutant proteins displayed similar patterns of spectra to that of the WT (data not shown).

Biochemical activities of the purified RecGMtb proteins

RecGMtb wedge domain mutations: A52E, F99A and D203A. The wedge domain of E. coli RecG is thought to facilitate RecG processivity during strand separation (Briggs et al., 2005). Relatively few residues in this domain are highly conserved (Fig. 1b) and none of these appear to be interacting directly with DNA. The mutant RecGMtb A52E, a nsSNP identified in a clinical isolate of M. tuberculosis (Dos Vultos et al., 2008), exhibited nearly WT levels of DNA binding and ATPase activity, but had
**Fig. 1.** Site-directed mutations of selected amino acid residues of RecG<sub>Mtb</sub>. (a) Diagram showing the approximate positions of the 10 mutated residues introduced in RecG<sub>Mtb</sub>. The figure is not drawn to scale. (b) A multiple sequence alignment of RecG<sub>Mtb</sub> and orthologues shows the conservation of the classical helicase motifs, the Q-motif, the linker connecting the two RecA-like helicase domains and the TRG motif in *M. tuberculosis* H37Rv (GenBank accession no. NP_217489), *E. coli* (continued)...
even higher helicase activity than WT RecG<sub>Mtb</sub> (Fig. 3a). In the <i>T. maritima</i> RecG structure (Singleton <i>et al.</i>, 2001), F204 and Y208 are stacked with two different bases in the branched DNA structure. In the present study, RecG<sub>Mtb</sub> F99, the equivalent of F204 in <i>T. maritima</i> RecG, was substituted with alanine. The RecG<sub>Mtb</sub> F99A mutant protein had significantly lower affinity to DNA than WT RecG<sub>Mtb</sub> (<i>P</i>&lt;0.001), and exhibited around three- and eightfold reductions in unwinding and ATPase activities, respectively (Fig. 3a). The RecG<sub>Mtb</sub> D203A protein, predicted to be phenotypically equivalent to WT, was included as a control due to the fact that it is a poorly conserved residue and, as predicted, it had near WT levels of all activities tested here (Figs 3 and 4).

**RecG<sub>Mtb</sub> Q-motif mutations: P285S, F286A and Q292A.**

The Q-motif, usually located 15–22 residues upstream of motif I (Fig. 1b), contains an essential, absolutely conserved glutamine. Here, the RecG<sub>Mtb</sub> P285S, F286A and Q292A substitutions in the Q-motif were constructed individually, and the purified proteins were characterized. All the three mutant proteins had WT affinity for an HJ substrate (Fig. 3a). However, the ATPase activity of P285S was reduced by around fourfold, and it was severely diminished in F286A and Q292A (Fig. 3a). Consistent with the ATPase activity observed, the unwinding activity of P285S was reduced by around twofold, and it was completely abolished in F286A and Q292A (Fig. 3a–c).

**RecG<sub>Mtb</sub> motif I mutation: K321A.**

Motif I, also known as the Walker A or P-loop motif, contains the consensus sequence GXXXXGKT in SF2 helicases (Deyrup <i>et al.</i>, 1998; Tuteja & Tuteja, 2004). We substituted the highly conserved lysine residue (K321) of RecG<sub>Mtb</sub> with alanine. The resulting mutant protein, RecG<sub>Mtb</sub> K321A, lost its ATPase activity and exhibited much reduced ATP binding (Figs 3 and 4). Although RecG<sub>Mtb</sub> K321A bound the HJ substrate with near WT affinity (Fig. 3a), it exhibited no DNA unwinding activity (Fig. 3a, c).

**RecG<sub>Mtb</sub> motif Ib mutation: T408A.**

Motif Ib of RecG<sub>Mtb</sub> includes absolutely conserved T408 (Fig. 1b), which is thought to play a critical role during translocation in SF2 helicases (Myong <i>et al.</i>, 2007; Pyle, 2008). Substitution of RecG<sub>Mtb</sub> T408 with alanine reduced the helicase and ATPase activities by 4.5- and 1.5-fold, respectively (Fig. 3a); however, the DNA binding affinity was not affected (Fig. 3a).

**RecG<sub>Mtb</sub> motif VI mutation: R627A.**

Motif VI is suggested to mediate conformational changes in SF2 helicases upon ATP binding and hydrolysis (Tuteja & Tuteja, 2004). Critical RecG<sub>Mtb</sub> residues in motif VI include R624 and R627, highly conserved in all SF2 helicases. Based on the <i>T. maritima</i> RecG structure, R622 in RecG<sub>Mtb</sub> is likely to be packed in the core of the C-terminal RecA-like helicase domain (Singleton <i>et al.</i>, 2001). One or both of the two other arginines in motif VI form the ‘arginine finger’, located near the γ-phosphate of ATP. RecG<sub>Mtb</sub> R627A lost both its ATPase and unwinding activities (Fig. 3), presumably due to its defect in ATP binding (Fig. 4), but the DNA binding activity was not affected (Fig. 3a).

**Mutation of a RecG<sub>Mtb</sub> C-terminal residue: A723T.**

Five naturally occurring nsSNPs have been reported in RecG<sub>Mtb</sub> (Dos Vultos <i>et al.</i>, 2008), one of which is a threonine substitution of the C-terminal A723. In our studies, the RecG<sub>Mtb</sub> A723T showed activities that were more or less the same as that of WT RecG<sub>Mtb</sub> (Fig. 3a).

**DISCUSSION**

Nearly all bacteria, including the major human pathogen <i>M. tuberculosis</i>, express a homologue of recG (Sharple <i>et al.</i>, 1999). In order to understand the role of the conserved amino acid residues in the catalytic domain(s) of RecG<sub>Mtb</sub> we mutated selected conserved residues and analysed the biochemical activity of the corresponding mutant proteins. Similar analyses were also performed on the site-directed mutants corresponding to three individual nsSNPs identified in clinical isolates of <i>M. tuberculosis</i>. An alanine substitution mutation at the RecG<sub>Mtb</sub>-conserved residues F286, Q292, K321 or R627 impaired ATPase and helicase activities severely, but not DNA binding activity. The two Q-motif residues F286 and Q292 of RecG<sub>Mtb</sub> are thought to play roles in the adenine recognition and ATPase activity of DEAD-box proteins (Cordin <i>et al.</i>, 2004). In contrast to RecG<sub>Mtb</sub>, a Gln→Ala mutation in the Q-motif of the Fanconi anemia group J protein FANCJ, an SF2 helicase, disabled both the DNA binding activity and ATPase activity of the enzyme (Wu <i>et al.</i>, 2012), which suggested that the role of some conserved residues may be helicase-specific. K321 of RecG<sub>Mtb</sub> is a conserved residue in the Walker A motif (motif I) that is suggested to interact with β-phosphoryl oxygen moieties of ATP, thereby stabilizing the transition state during hydrolysis (Velankar <i>et al.</i>, 1999). The observed lack of helicase and ATPase activities of K321A in the present study is consistent with the mutagenesis studies of the corresponding residues in <i>E. coli</i> RecG (McGlynn <i>et al.</i>, 2000) and PcrA (Velankar <i>et al.</i>, 1999). The absence of significant ATPase activity in RecG<sub>Mtb</sub> F286A,
Q292A, K321A and R627A could be attributed to the highly reduced ability to bind ATP (Fig. 4a, b). The fact that F286A, Q292A, K321A and R627A lack substantial ability to bind ATP (Fig. 4a, b), while no significant secondary structure changes occurred upon mutation (as determined by CD spectra measurement), strongly suggests that these residues play pivotal roles in proper binding of ATP. Notably, the structural model shows that F286 stacks with the ADP base, whilst Q292 interacts with the N6 and N7 positions of adenine in the RecG\textsubscript{Mtb} model (Fig. 5a).
As indicated above, R627 is a critical moiety for the activity of RecGMtb, and RecGMtb R627A demonstrated severely impaired ATPase and helicase activities. Our modelling of RecGMtb using the *T. maritima* RecG structure suggests that R627 lies closer to ADP than the other two arginines in motif VI; therefore, we propose that R627 acts as the putative arginine finger for RecGMtb (Fig. 5a). Arginine fingers stabilize the transition state during ATP hydrolysis and are thought to couple ATP hydrolysis to unwinding activity (Elles & Uhlenbeck, 2008). The lack of helicase and ATPase activities observed in the putative arginine finger mutant, RecGMtb R627A, is consistent with studies of the analogous mutant of *E. coli* DbpA helicase (Elles & Uhlenbeck, 2008).

The present data also indicate that F99A, P285S and T408A mutants of RecGMtb exhibit a moderate reduction...
(~25–50 %) of helicase activity, while the control D203A and A723T had near WT levels of activity. F99 is a moderately conserved residue in bacterial RecG, that appears to stack with one of the DNA bases (Fig. 5b), an observation consistent with the fact that RecGMtb F99A has significantly reduced affinity for DNA and reduced ATPase activity (Fig. 3a). In contrast, RecGMtb T408A demonstrates reduced ATPase and helicase activities, without an apparent effect on DNA and ATP binding activity. The results imply that the Thr408→Ala mutation may loosen the grip of RecGMtb on DNA; this could allow the enzyme to slip, thus impairing its ability to translocate and/or promote the migration of HJ. The reduced ATP hydrolysis of T408A is thus attributed to its reduced translocation on DNA as RecGMtb is a DNA-dependent ATPase (Zegeye et al., 2012).

Our previous biochemical characterization of WT RecGMtb indicated that neither the binding nor the hydrolysis of ATP was required for its DNA binding activity (Zegeye et al., 2012). Notably, the present study confirms that RecGMtb binds ATP independently of DNA binding (Fig. 4a), suggesting independent ATP and DNA binding activities. However, ATP hydrolysis remains coupled to DNA binding, as expected (Zegeye et al., 2012).

The M. tuberculosis genome is conserved highly, with only limited numbers of SNPs compared with the genomes of other well-characterized bacterial pathogens (Bifani et al., 2002; Filliol et al., 2006). For example, lateral gene transfer is an extremely rare to absent phenomenon in M. tuberculosis (Gagneux, 2009). Therefore, unlike many other bacteria, adaptation in M. tuberculosis occurs essentially...
through chromosomal mutations that result in SNPs, insertions and deletions (Dos Vultos et al., 2009; Gorna et al., 2010). Twice as many SNPs have been reported in *M. tuberculosis* genes involved in DNA replication, repair and recombination (3R) than in other housekeeping genes, facilitating mutations in these and other genes (Dos Vultos et al., 2008). In fact, nearly all acquired drug resistance in *M. tuberculosis* strains is attributed to SNPs in anti-tuberculosis treatment target genes, such as *rpoB* (Telenti et al., 1993; Zaczek et al., 2009) and *katG* (Ramaswamy et al., 2003). Our analysis of the helicase activity of three of the five previously identified nsSNPs in recGMtb showed that, whilst RecGMtb A52E had enhanced activity, A723T had more or less WT activity (Fig. 3a). However, the P285S mutant showed approximately twofold reduction in unwinding activity (Fig. 3a). Our structural modelling suggests that P285 does not directly interact with ATP (Fig. 5a); nevertheless, the highly conserved P285 might have a role in stabilizing and orienting the surrounding protein loop region in the RecGMtb structure.

The nsSNP P285S was identified in the W-Beijing lineage of *M. tuberculosis* (Mestre et al., 2011), which are strains that have disseminated worldwide (Bifani et al., 2002) and are associated with a higher propensity for gaining multidrug resistance (European Concerted Action on New Generation Genetic Markers and Techniques for the Epidemiology and Control of Tuberculosis, 2006). nsSNPs in DNA repair genes such as *mutT2*, *mutT4* and *ogt* were also reported in some strains of this lineage, which could at least explain partly the frequent acquisition of antibiotic resistance (Ebrahimi-Rad et al., 2003). The presumed mutability of these strains could increase their adaptability, promoting survival in macrophages exposed to antibiotics in a clinical scenario. In *E. coli*, mutation in *recG* conferred an SOS constitutive phenotype that increases the mutation frequency (Asai & Kogoma, 1994; O’Reilly & Kreuzer, 2004). Similarly, in *Vibrionaceae*, *recG* is SOS regulated. In mycobacteria, however, no evidence to date exists indicating that *recG* expression is regulated by LexA/RecA or a ClpR-dependent mechanism (Gamulin et al., 2004; Smollett et al., 2012). Nevertheless, a recent report indicated that *M. tuberculosis* recG is DNA-damage inducible (Thakur et al., 2013). As the precise role of RecG in *M. tuberculosis* is not yet defined, the putative in vivo phenotype of a more potent RecGMtb A52E or the less potent P285S can only be speculated on. Provided that the main function of RecG Mtb is curbing pathological DNA replication at sites remote from *oriC* as its *E. coli* orthologue (Rudolph et al., 2010), thereby facilitating resolution of conflicts between replication and transcription (Mahdi et al., 2012), a mutation such as RecGMtb A52E with enhanced efficiency might favour a more stable genome. However, a mutation that partially inactivates a 3R component, such as the P285S mutation in RecGMtb, may reduce the fidelity of the 3R system of the genetically secluded *M. tuberculosis*. This might, in turn, provide a propensity for acquiring mutations through which *M. tuberculosis*...
tuberculosis drives exclusively its microevolution in the human host (Warner et al., 2013). Potentially, altered RecG<sub>Mtb</sub> fidelity may influence bacterial fitness in the long or short term.

CONCLUSIONS

Our investigations on the conserved RecG<sub>Mtb</sub> residues involved in ATP hydrolysis, i.e. F286, K321, Q292 and R627, demonstrated that these are more critical for enzymic activity than those residues interacting with DNA, such as F99 and T408. Overall, the conserved RecG<sub>Mtb</sub> residues studied here exhibited more or less similar functions to the corresponding residues in other SF2 helicases studied to date, suggesting that their roles are conserved evolutionarily. The three nsSNPs characterized in our study led to overall functional proteins and thus it appears that amino acid substitutions occur in parts of RecG<sub>Mtb</sub> that are permissible to change without affecting its catalytic activity too much, yielding viable mutants. To our knowledge, this is the first study that links a functional consequence to polymorphisms observed in <i>M. tuberculosis</i> clinical isolates. Since <i>M. tuberculosis</i> has profound clinical significance globally, it is important to understand the genome dynamics of this pathogen and the mechanisms that allow it to infect successfully and cause human disease. In particular, a better understanding of how <i>M. tuberculosis</i> survives and propagates inside human macrophages is critical. In turn, exploiting helicase functions could lead to alleviating drug resistance development, and potentially pave the way to improve therapeutic and preventive tools for fighting this pathogen.

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