ISSN 0006-2979, Biochemistry (Moscow), 2012, Vol. 77, No. 4, pp. 378-387. © Pleiades Publishing, Ltd., 2012.
Published in Russian in Biokhimiya, 2012, Vol. 77, No. 4, pp. 481-492.

Physical and Functional Interactions between 3-Methyladenine DNA Glycosylase and Topoisomerase I in Mycobacteria

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Received September 13, 2011
Revision received November 15, 2011

Abstract—DNA glycosylases play important roles in DNA repair in a variety of organisms, including humans. However, the function and regulation of these enzymes in the pathogenic bacterium *Mycobacterium tuberculosis* and related species are poorly understood. In the present study, the physical and functional interactions between 3-methyladenine DNA glycosylase (MAG) and topoisomerase I (TopA) in *M. tuberculosis* and *M. smegmatis* were characterized. MAG was found to inhibit the function of TopA in relaxing supercoiled DNA. In contrast, TopA stimulated the cleavage function of MAG on a damaged DNA substrate that contains hypoxanthine. The interaction between the two proteins was conserved between the two mycobacterial species. Several mutations in MAG that led to the loss of its interaction with and activity regulation of TopA were also characterized. The results of this study further elucidate glycosylase regulation in both *M. smegmatis* and *M. tuberculosis*.

DOI: 10.1134/S0006297912040098

Key words: *Mycobacterium tuberculosis*, DNA glycosylase, topoisomerase I, DNA repair

DNA can be damaged by various exogenous and endogenous factors such as replication errors, radiation, and alkylating agents. DNA damage can then lead to gene mutation and cell death if not repaired [1-4]. Several DNA repair systems have evolved to correct alkylated nucleobases. Among such systems, base excision repair is widely studied [5-7]. The excision of a variety of alkylated bases can be catalyzed by DNA glycosylases via the hydrolysis of N-glycosidic bonds [6, 8]. 3-Methyladenine DNA lesions can be processed and removed by DNA glycosylases, which are found in bacteria, yeast, plants, rodents, and humans [9, 10]. Other DNA glycosylases with different substrates act on multiple types of damaged DNA bases [9, 11].

Mammalian cells have only one 3-methyladenine DNA glycosylase (MAG), which is also called 3-alkyladenine DNA glycosylase [9]. Intriguingly, a gene similar to eukaryotic MAGs exists in several mycobacterial species, such as *Mycobacterium tuberculosis*, *M. bovis*, and *M. smegmatis*. The gene is annotated as *mpg* (Rv1688) in *M. tuberculosis* [12]. *Mycobacterium tuberculosis* is an intracellular pathogen that faces multiple environmental and endogenous factors. These factors include reactive oxygen and nitrogen species that can induce DNA damage during the infection processes [13, 14]. The genome of *M. tuberculosis* has an unusually high mutation frequency and drug resistance when in an infected host. These features pose serious challenges in prevention of tuberculosis [15]. The conservation of the MAG gene in many mycobacterial species suggests that it may play a significant role. However, the characteristics and regulation of MAGs in *M. tuberculosis* are not yet fully understood [16].

In a recent screen [17], the MAG (Rv1688) of *M. tuberculosis* was linked to the topoisomerase I (TopA) (Rv3646) of *M. tuberculosis*. In the current study, the activities of MAGs in *M. tuberculosis* and *M. smegmatis* were characterized. The physical and functional interactions of these MAGs with TopA were also examined. Physical interaction between the two proteins was found to enhance MAG activity in cleaving DNA substrates that contain hypoxanthine. In contrast, MAG inhibited TopA ability to relax supercoiled DNA.

Abbreviations: 3-AT, 3-amino-1,2,4-triazole; MAG, 3-methyladenine DNA glycosylase; SPR, surface plasmon resonance; TopA, topoisomerase I.

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MATERIALS AND METHODS

DNA and oligonucleotides. The oligonucleotides were synthesized by Invitrogen (USA) (Table 1). The lesion-containing DNA strand (containing hypoxanthine) 5′-CGATAGCATCCTICCTTCT CT CCAT-3′ and the normal DNA strand 5′-CGATAGCATCCTAC-CTTCTCTCCAT-3′ were labeled on their 5′ ends with [γ-^{32}P]ATP using polynucleotide kinase. The labeled strand was then annealed with the complementary strand 5′-ATGGAGAGAAGGTAGGATGCTATCG-3′ [10].

Cloning, expression, and purification of recombinant proteins. The mycobacterial genes *Ms3759* (a putative MAG in *M. smegmatis*), *MsTopA*, *Rv1688*, and *MtbTopA*, as well as their mutant variants were amplified by polymerase chain reaction. Each gene was cloned into a modified overexpression vector, pET28a or pGEX (Novagen, USA), to produce recombinant vectors [18] (Table 2).

Escherichia coli BL21(DE3) cells were transformed with the recombinant plasmid. Protein expression was induced by the addition of 0.3 mM isopropyl β-D-1-thiogalactopyranoside. Harvested cells were sonicated in binding buffer (100 mM Tris-HCl, pH 8.0, 500 mM NaCl, and 10 mM imidazole for His-tagged proteins or 1× PBS buffer for glutathione-S-transferase (GST)-tagged proteins) and the lysate was centrifuged and the cleared supernatant was loaded onto an affinity column for purification. The protein was eluted using elution buffer (100 mM Tris-HCl, pH 8.0, 500 mM NaCl, and 250 mM imidazole for His-tagged proteins or PBS buffer containing 10 mM reduced glutathione (GSH) for GST-tagged proteins). The eluate was then dialyzed overnight and stored at −80°C. Protein concentrations were determined according to the method of Gill and Hippel [19].

Gene mutations and bacterial two-hybrid analysis. Random mutations in *Ms3759* were created using a previously published procedure [20]. The mutant library produced was subsequently screened using a bacterial two-hybrid technique using a recombinant pBT-MsTopA as the bait plasmid. Bacterial two-hybrid analysis was carried out according to the procedure supplied with the commercial kit and as described in a previous report [21]. A pair of pBT/pTRG plasmids was co-transformed into the reporter strain, and spotted onto a screening medium plate containing 5 mM 3-amino-1,2,4-triazole (3-AT) (Stratagene, USA), 8 μg/ml streptomycin, 15 μg/ml tetracycline, 34 μg/ml chloramphenicol, and 50 μg/ml kanamycin. The plate was incubated at 30°C for 3-4 days. A co-transformant containing pBT-LGF2 and pTRG-Gal11P (Stratagene) was used as a positive control (CK+) for expected growth on the screening medium. A co-transformant containing the empty vectors pBT and pTRG was used as a negative control (CK−).

Surface plasmon resonance (SPR) analysis. The physical interaction of MAG with TopA was analyzed using a BIAcore 3000 instrument (GE Healthcare, UK) according to our previously published procedures [21, 22]. The His-tagged TopA protein was immobilized onto nitrilotri-

Table 1. Primers used for PCR amplification

Gene	Primer	Sequence* (5′–3′)
MtbRv1688	Rv1688**	ATGCGAATTCGCATGAACGCTGAGGAACTGGC
	Rv1688***	ATATATTCTAGACTAGTCGCTGGCTCCCCGGG
MtbRv3646c	Rv3646c**	GACCGAATTCAATTGGCTGACCCGAAAACGAA
	Rv3646c***	AATTAATCTAGACTAGTCGCGCTTGGCTGCCT
Ms3759	3759**	ATGCGAATTCGCATGAGCGTCGACCTGCTG
	3759***	TCGACTTCTAGATCAGTCACTGCTGCCGGG
MsTopA	topA**	GATCGAATTCATAGTGGAGCGTTGGCACAGTTGGCT
	topA***	GGTATCTCTAGATTCGGCGGAAACCTAGGCCTTCTT
Damaged substrate	substrate**	CGATAGCATCCTICCTTCTCTCCAT
	substrate***	GCTATCGTAGGATGGAAGAGAGGTA
Undamaged substrate	substrate**	CGATAGCATCCTACCTTCTCTCCAT
	substrate***	GCTATCGTAGGATGGAAGAGAGGTA

* The sites for restriction enzymes are underlined.
** Forward primer.
*** Reverse primer.

Table 2. Plasmids used for protein expression and interaction assays

Plasmid	Description	Source
pET28a-MtbRv1688	pET28a derivative for expression 6×His-Rv1688	*
pET28a-tbRv3646c	pET28a derivative for expression 6×His-Rv3646c	*
pET28a-Ms3759	pET28a derivative for expression 6×His-Ms3759	*
pET28a-Ms3759 G57S/M137I	pET28a derivative for expression 6×His-Ms3759G57S/M137I	*
pET28a-Ms3759 A92T	pET28a derivative for expression 6×His-Ms3759 A92T	*
pET28a-MsTopA	pET28a derivative for expression 6×His-MstopA	*
pGEX	pGEX derivative for expression GST	Stratagene
pGEX-MtbRv1688	pGEX derivative for expression GST-Rv1688	*
pGEX-Ms3759	pGEX derivative for expression GST-Ms3759	*
pGEX-Ms3759 G57S/M137I	pGEX derivative for expression GST-Ms3759G57S/M137I	*
pGEX-Ms3759 A92T	pGEX derivative for expression GST-Ms3759A92T	*
pGEX-MtbRv1246c	pGEX derivative for expression GST-Rv1246c	*
pBT	bacterial two-hybrid assay bait vector	Stratagene
pBT-MtbRv3646c	bacterial two-hybrid assay target vector	*
pBT-MsTopA	pBT derivative for bacterial two-hybrid assay	*
pBT-MtbRv1246c	pBT derivative for bacterial two-hybrid assay	*
pBT-MtbRv1247c	pBT derivative for bacterial two-hybrid assay	*
pTRG	pTRG derivative for bacterial two-hybrid assay	Stratagene
pTRG-MtbRv1688	pTRG derivative for bacterial two-hybrid assay	*
pTRG-Ms3759	pTRG derivative for bacterial two-hybrid assay	*
pTRG-Ms3759 G57S/M137I	pTRG derivative for bacterial two-hybrid assay	*
pTRG-Ms3759 A92T	pTRG derivative for bacterial two-hybrid assay	*
pTRG-MtbRv1246c	pTRG derivative for bacterial two-hybrid assay	*

* This study.

acetic acid (NTA) chips. The purified GST-MAG protein, to be used as a ligand, was diluted in HBS buffer (10 mM Hepes, pH 7.4, 150 mM NaCl, 50 μM EDTA, 5 mM ATP, and 0.005% surfactant P20 (BIAcore, Sweden)). For the negative control, the GST protein was substituted for the GST-DNA glycosylase protein. An overlay plot was produced to depict the interaction between the two proteins.

DNA glycosylase assay. The DNA glycosylase assays were carried out according to procedures published previously [23]. The reaction was stopped by the addition of 1.2 μl of 1 M NaOH, and heating at 70°C for 30 min. About 11.2 μl of formamide dye was then added to this mixture. The products were resolved on 20% denaturing urea-polyacrylamide gel using 1× Tris-borate-EDTA buffer at 200 V for 3 h at room temperature. The extent of the substrate cleavage was analyzed by phosphorimaging.

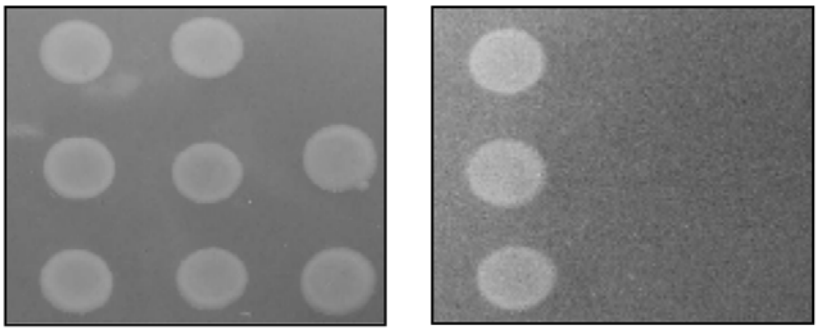
Topoisomerase relaxation assay. The topoisomerase relaxation was assayed according to procedures published previously [24-26]. The supercoiled pBluescript plasmid was incubated in the presence of TopA with or without

various amounts of DNA glycosylase protein or its mutant variants and incubated at 37°C for 45 min. Reaction was stopped by the addition of 1 μl of 20 mg/ml proteinase K and the proteinase K buffer (100 mM Tris-HCl, pH 7.8, 5 mM EDTA, 0.5% SDS) at 37°C for 15 min. Products were then loaded onto a 1% agarose gel for analysis.

RESULTS

Interaction of *M. smegmatis* MAG (Ms3759) with *M. smegmatis* topoisomerase I (MsTopA). To determine the regulation mechanism of Ms3759, the potential association of the glycosylase with MsTopA [17] was further examined in *M. smegmatis*. The bacterial two-hybrid technique was used. Figure 1a shows that the co-transformant containing Ms3759/MsTopA grew well on the screening medium. No growth was observed for the self-activation controls. An unrelated mycobacterial protein, MtbRv1246c (a RelE-like toxin), was unable to interact with Ms3759. This inability was evidenced by the absence of growth in the strains co-transformed with Ms3759 and MtbRv1246c on the same screening medium. However, Rv1246 and Rv1247 (a RelB-like antitoxin) clearly interacted, in accordance with a previous finding [27]. These results suggested that a specific interaction exists between Ms3759 and MsTopA.

a



CK$^+$	CK$^-$	
pBT-Mtb Rv1247c pTRG-Mtb Rv1246c	pBT-Mtb Rv1246c pTRG-Ms3759	pBT-Mtb Rv1246c pTRG
pBT-MsTopA pTRG-Ms3759	pBT pTRG-Ms3759	pBT-MsTopA pTRG

b

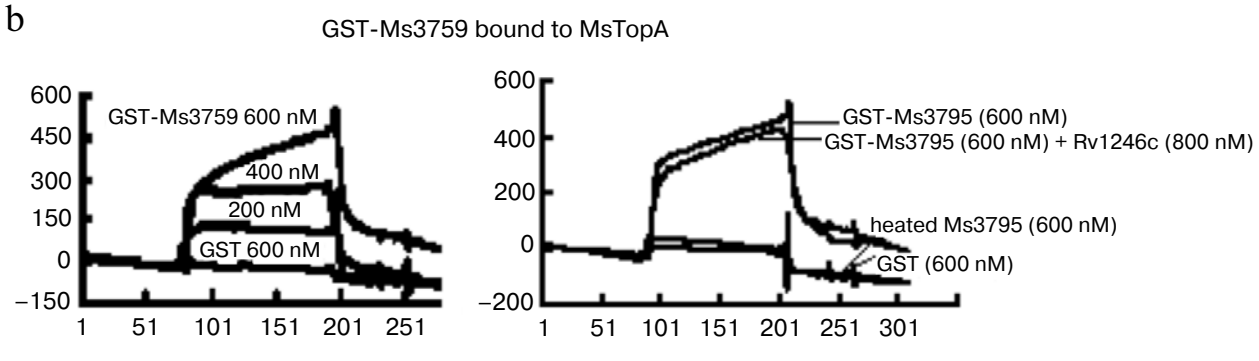


Fig. 1. Assays for the interaction between Ms3759 and MsTopA. a) Bacterial two-hybrid assay for the interaction between Ms3759 and MsTopA, performed as described in "Materials and Methods". b) SPR assays. The interaction between Ms3759 and MsTopA was monitored using surface plasmon resonance on a BIAcore 3000.

An SPR assay was then conducted to scrutinize this interaction. Figure 1b shows that a 6× His-tagged MsTopA protein was immobilized on an NTA chip. With increased amounts of GST-tagged Ms3759 protein (200, 400, and 600 nM) passed over the chip, a substantial response of about 500 RU was observed (Fig. 1b, left panel). No significant response was observed for GST alone. In another assay, an unrelated protein, Rv1246, did not compete with the association between Ms3759 and MsTopA; no significant change was observed in the binding curve even at a high Rev1246 concentration (800 nM) (Fig. 1b, right panel). No interaction was observed when a heat-denatured Ms3759 protein was used in the same assay (Fig. 1b, right panel). All these results demonstrated that Ms3759 specifically interacts with MsTopA.

Mutual regulation of Ms3759 DNA glycosylase and MsTopA activities. The physical interaction between Ms3759 and MsTopA suggests a functional correlation between these two proteins. The effects of this interaction on the activity of MsTopA were first examined using the pBluescript plasmid as a DNA substrate. Figure 2a shows that upon MsTopA (300 nM) addition to the reaction mixture, the supercoiled species of plasmids disappeared. This finding was concomitant with the appearance of a band corresponding to a relaxed plasmid of a slower mobility in the gel (Fig. 2a, lane *2*), and confirmed that MsTopA could uncoil the DNA substrate. With increased amounts of Ms3759 (200-1500 nM) added to 300 nM MsTopA, the coiled species of plasmids reappeared. This result indicated that Ms3759 relieved the activity of MsTopA in inhibiting the degree of plasmid supercoiling (Fig. 2a, left panel, lanes *3-8*). No significant effect was observed when either GST protein alone was added with MsTopA (Fig. 2a, right panel, lanes *4-7*). This finding suggested that the functional interaction between MsTopA and Ms3759 is specific.

The effect of MsTopA on the DNA glycosylase activity of Ms3759 was then examined using a DNA substrate containing a damaged purine, hypoxanthine (Table 1). Figure 2b (left upper panel) shows that Ms3759 (20 nM) alone was able to cleave the damaged DNA. Consequently, 12 bp products appeared on the gel (compare lanes *1* and *2*), as expected. The heat-inactivated MsTopA, even at a high concentration (400 nM), did not inhibit the cleavage activity (lanes *3-5*). On the other hand, with increased amounts of active MsTopA (100-400 nM) mixed with 20 nM Ms3759, more DNA substrates were cleaved, as revealed by the steady increase in 12 bp products (Fig. 2b, left lower panel, lanes *3-7*). This finding indicated that MsTopA significantly stimulated the cleavage activity of Ms3759. The stimulation of the cleavage activity of Ms3759 was caused neither by the unrelated protein GST (Fig. 2b, right upper panel, lanes *5-7*) nor by the mycobacterial protein Rv1246c (Fig. 2b, right lower panel, lanes *5-7*). No cleavage activity was observed for Ms3759 on the undamaged DNA substrate (Fig. 2b, right panels, lanes *1*).

These results showed that Ms3759 inhibits the function of MsTopA in relaxing supercoiled DNA, whereas MsTopA stimulates the cleavage activity of Ms3759 on damaged DNA.

Non-interaction between two Ms3759 mutant proteins and MsTopA. To determine the specificity of the interaction between Ms3759 and MsTopA, two mutant variants of Ms3759 incapable of interacting with MsTopA were characterized. A gene mutagenesis method previously reported [20] was used in combination with a bacterial two-hybrid screen. As shown in Fig. 3a, in contrast with the wild-type Ms3759 expressing cells, the co-transformant strain (containing either the Ms3759-G57S/M137I or Ms3759-A92T mutant gene and the MsTopA gene) did not grow well on the screening medium in the two-hybrid experiment. No growth was observed for the self-activation controls of the two mutant Ms3759 variants. Interestingly, the two MAG residues, A92 and M137, are conserved in two mycobacterial species (Fig. 4).

Another SPR assay was conducted to investigate the interaction of the two Ms3759 mutants with MsTopA. Figure 3b shows that when wild-type or mutant GST-Ms3759 protein (500 nM) was passed over a chip immobilized with His-tagged MsTopA, the two mutant proteins had very low responses (about 100 RU). In contrast, the response of wild-type Ms3759 was about 500 RU. These observations are in conformity with those obtained from the bacterial two-hybrid assay described above.

The effects of protein interaction on the activity of MsTopA were then examined using the pBluescript plasmid as a DNA substrate. Figure 3c shows that upon MsTopA (300 nM) addition to the reaction mixture, the supercoiled (S) species of plasmids disappeared. This finding was concomitant with the appearance of a band corresponding to a relaxed (R) plasmid of a slower mobility in the gel (Fig. 3c, lane *2*), and confirmed that MsTopA could uncoil the DNA substrate. With increased amounts of Ms3759 (600-1500 nM) mixed with 300 nM MsTopA, the supercoiled (S) species of plasmids reappeared. The degree of plasmid supercoiling steadily increased with increased amounts of Ms3759, indicating that Ms3759 relieved the activity of MsTopA in inhibiting the degree of plasmid supercoiling (Fig. 3c, lanes *3-5*). Compared with wild-type Ms3759, the two mutant proteins (Fig. 3c, lanes *6-11*) only partially retained their abilities to inhibit the activity of MsTopA even at a high concentration (1500 nM).

On the other hand, as shown in Fig. 3d, in the absence of topoisomerase MsTopA, the two Ms3759 mutant proteins (lanes *5-7* and *9-11*) had cleavage activities comparable to that of wild-type Ms3759 (lanes *4* and *8*). When increasing amounts of MsTopA (200-400 nM) were added together with wild-type or mutant Ms3759 protein (20 nM) into the reaction mixture, MsTopA did not significantly stimulate the cleavage activity of the

mutant proteins (lanes *5-7* and *9-11*); in contrast, it had an obviously stimulatory effect on the activity of the wild-type Ms3759 protein (lanes *1-3*). Therefore, two Ms3759 mutant proteins lost the interaction with MsTopA and thus did not inhibit its activity in relaxation of supercoiling. Meanwhile, MsTopA lost the regulatory effect on the cleavage activity of the two Ms3759 mutant proteins.

Conserved interaction between MAG and TopA in *M. tuberculosis* and *M. smegmatis*. *Mycobacterium tuberculosis* also contains a potential MAG, Rv1688, and a topoisomerase I, MtbTopA (Rv3646). By a bacterial two-hybrid assay, an interaction between Rv1688 and MtbTopA was found. Cross-interactions between Rv1688 and MsTopA, as well as between Ms3759 and MthTopA were also confirmed by our observation that all co-trans-

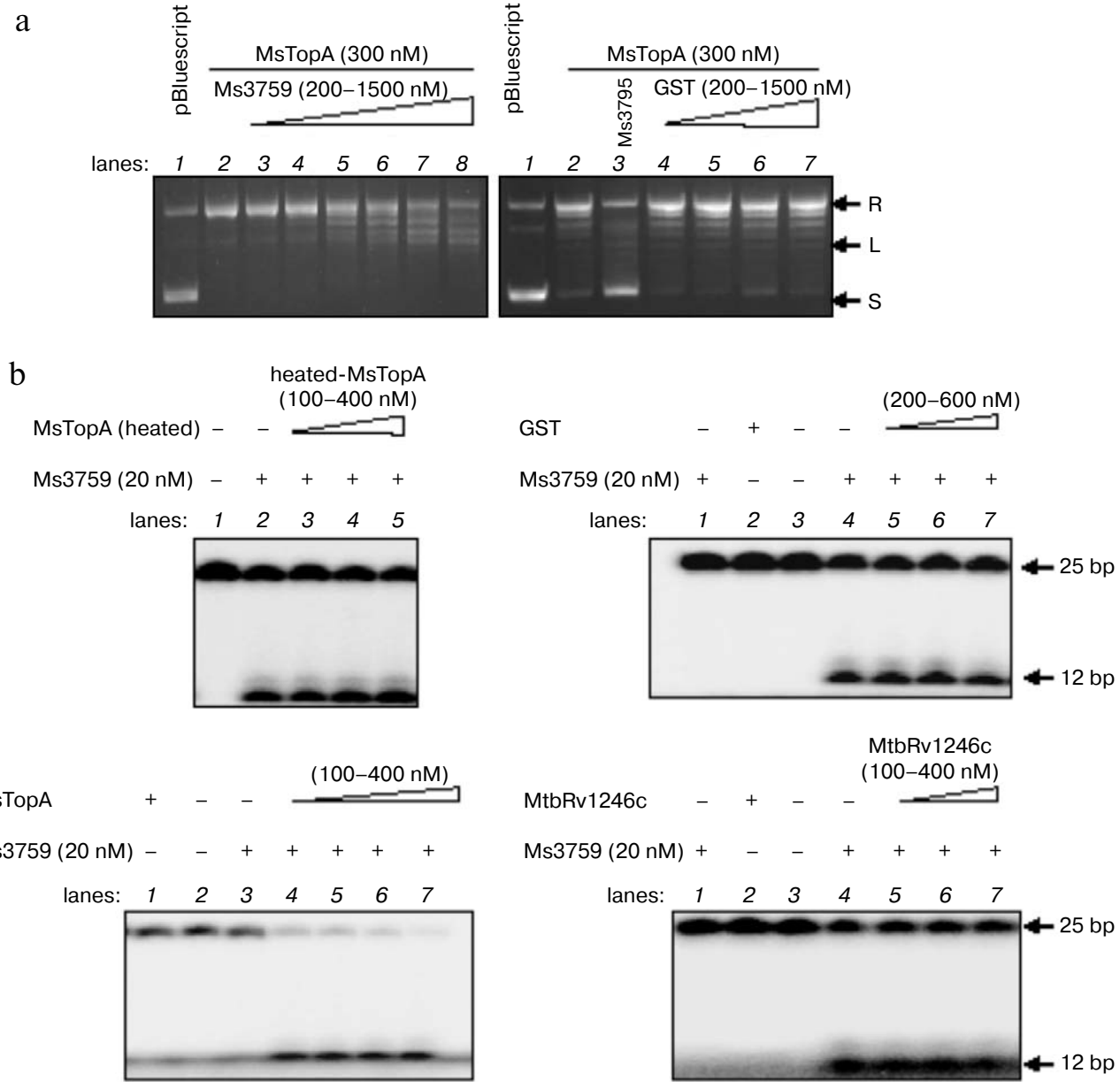


Fig. 2. Assays for the mutual effects of Ms3759 and MsTopA on their respective functions. a) Topoisomerase activity assays were performed as described in "Materials and Methods". The plasmid pBluescript was used as a DNA substrate in all reaction mixtures. Lanes: *1*) negative control (no topoisomerase); *2*) positive control (+ 300 nM topoisomerase). L, linearized plasmid; R, relaxed plasmid; S, supercoiled plasmid. Various amounts (200-1500 nM) of Ms3759 (*3-8*) or GST (*4-7*) were mixed with MsTopA (300 nM) in the reaction mixture to analyze their effects. b) Assays for the effect of MsTopA on the cleavage activity of Ms3759 on the DNA substrate containing hypoxanthine. An undamaged DNA substrate was also used a control (right panels, lanes *1*). The protein species and their concentrations are indicated on top of the panel. GST, heated MsTopA, and MtbRv1246 were used as negative controls to determine the effect of MsTopA on the cleavage activity of Ms3759. The sizes of DNA-oligonucleotide substrate and product of hydrolase reaction were 25 and 12 bp, respectively.

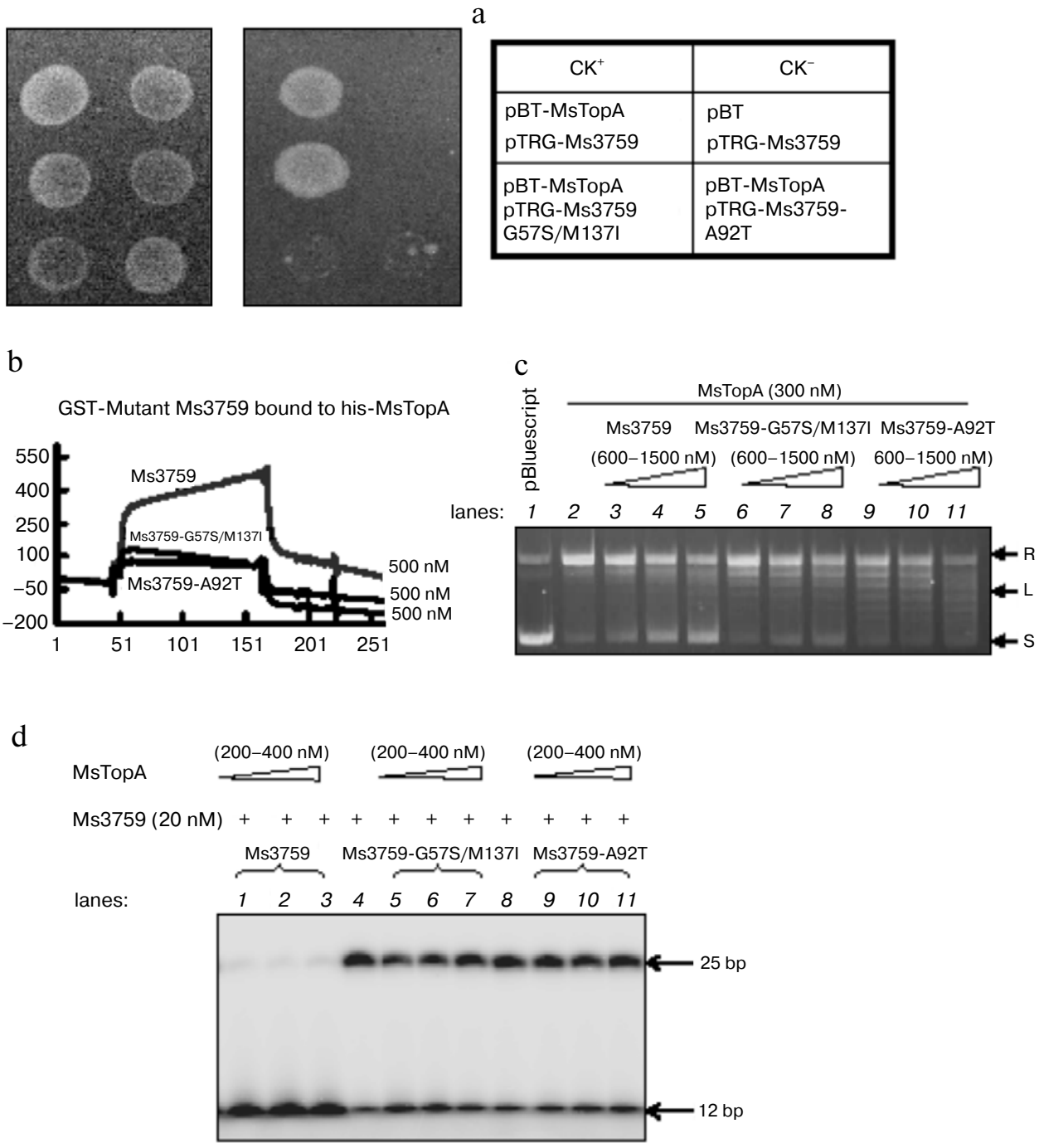


Fig. 3. Characterizations of two Ms3759 mutants and their effects on the interaction of Ms3759 with MsTopA. a) Bacterial two-hybrid assay (Stratagene) for the interaction between two Ms3759 mutants and MsTopA, performed as described in "Materials and Methods". b) SPR assays for the interactions between the two mutants and MsTopA. c) Topoisomerase activity assays were performed as described in "Materials and Methods". Lanes: *1*) negative control (no topoisomerase); *2*) positive control (+ 300 nM topoisomerase). L, linearized plasmid; R, relaxed plasmid; S, supercoiled plasmid. Various amounts of Ms3759 or mutant proteins (600-1500 nM) were mixed with MsTopA in the reaction mixture to analyze their effects. d) Assays for the effect of MsTopA on the cleavage activity of two Ms3759 mutants on the DNA substrate containing hypoxanthine. The protein species and their concentrations are indicated on top of the panel. The 25-bp DNA substrate and 12-bp products are indicated on the right of the panel.

formant strains grew well on the screening medium (Fig. 5). This finding suggested that the two proteins, MAG and TopA, as well as their interaction are conserved between the two mycobacterial strains. Figure 6a shows that both cross-interactions were confirmed by SPR analysis. The His_6-tagged MtbTopA protein was immobilized onto an NTA chip. With increased amounts of GST-tagged Rv1688 protein (150, 300, and 750 nM) passed

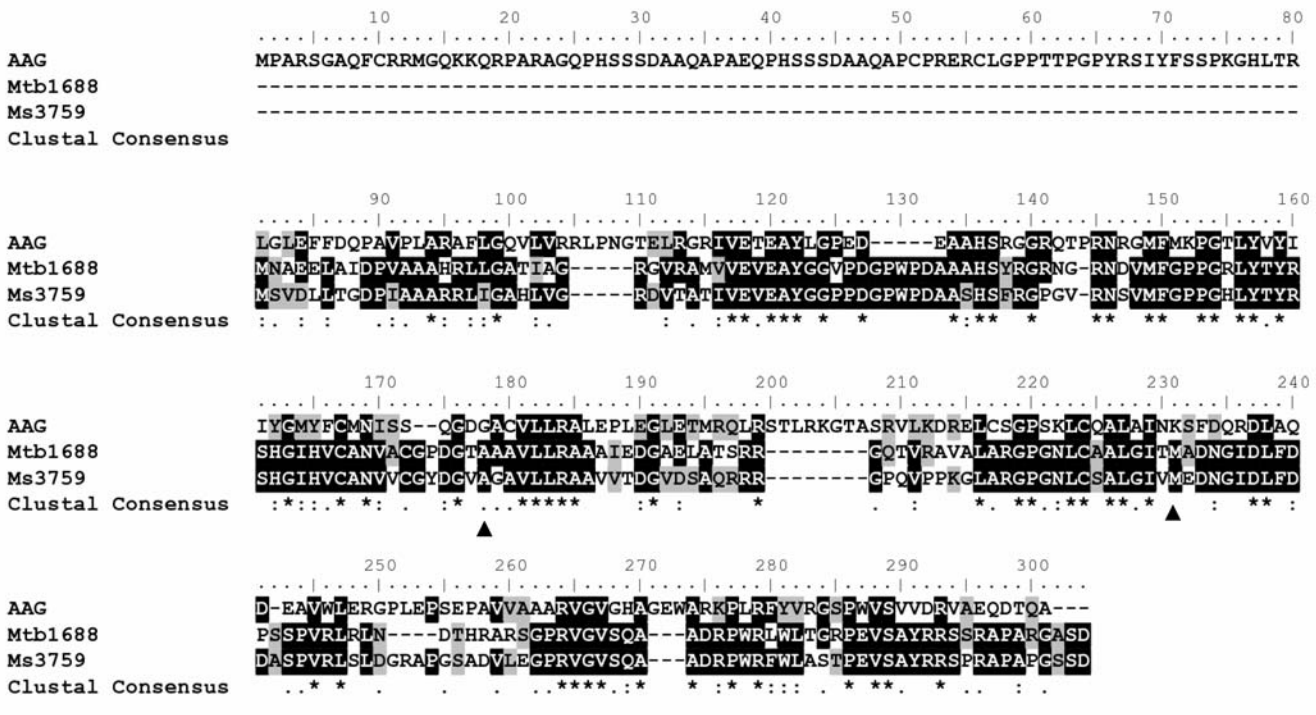
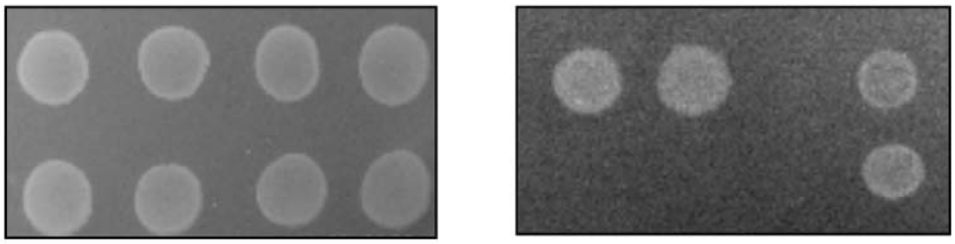


Fig. 4. Alignment of the amino acid sequences of 3-methyladenine DNA glycosylase of *M. tuberculosis* (Rv1688), *M. smegmatis* (Ms3759), and human (AAG). Two residues, A92 and M137, are conserved in two mycobacterial species and indicated by arrow.

a



b

CK^+	pBT-MtbTopA pTRG-MtRv1688	pBT-MtbTopA pTRG	pBT-MsTopA pTRG-MtbRv1688
CK^-	pBT pTRG-MtbRv1688	pBT pTRG-Ms3759	pBT-MtbTopA pTRG-Ms3759

Fig. 5. Bacterial two-hybrid assay for the cross-interaction between MtbRv1688 and MsTopA as well as between Ms3759 and MtbTopA. a) Left panel: plate minus streptomycin (str) and 5 mM 3-amino-1,2,4-triazole (3-AT); right panel: plate plus str and 5 mM 3-AT. b) An outline of the plates. CK^+, co-transformant containing pBT-LGF2 and pTRG-Gal11P was used as a positive control; CK^-, co-transformant containing pBT and pTRG used as a negative control.

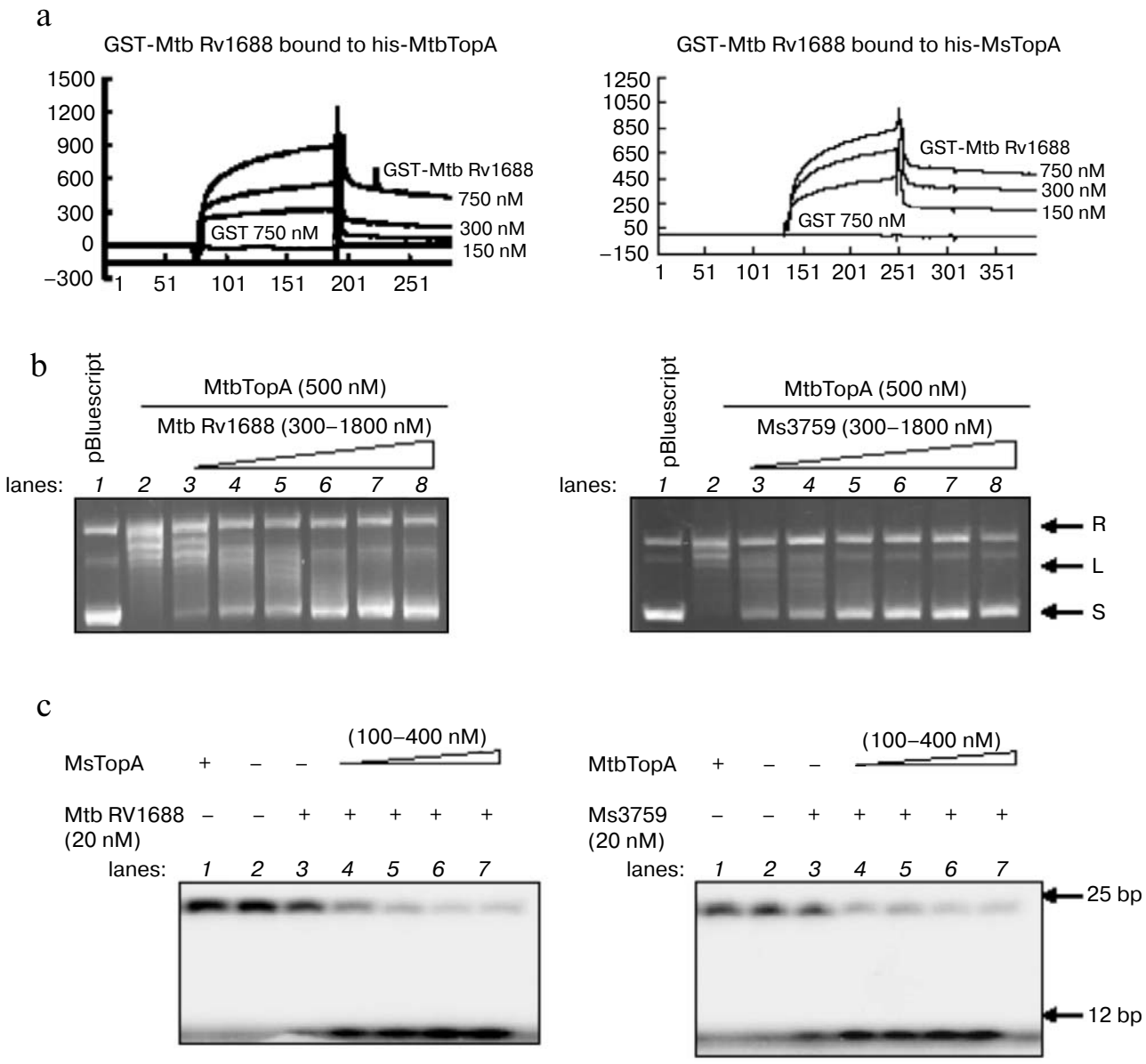


Fig. 6. Cross-interactions between two mycobacterial MAGs and TopA. a) SPR assays for the interactions between Rv1688 and MtbTopA or MsTopA. The assays were performed as described in "Materials and Methods". b) The effects of Rv1688 and Ms3759 proteins on the topoisomerase activity of MtbTopA. The assays were performed as described in "Materials and Methods". Lanes: *1*) negative control (no topoisomerase); *2*) positive control (+ 500 nM topoisomerase). L, linearized plasmid; R, relaxed plasmid; S, supercoiled plasmid. Various amounts (300-1800 nM) of Rv1688 (left panel) or Ms3759 (right panel) were mixed with MtbTopA in the reaction mixture to analyze their effects. c) Assays for the effect of MtbTopA on the cleavage activity of Rv1688 or Ms3759 on the DNA substrate containing hypoxanthine. The protein species and their concentrations are indicated on top of the panel.

over the chip, a substantial response of about 900 RU was observed (Fig. 6a, left panel). No significant response was observed for GST alone. Similarly, when the His_6-tagged MsTopA protein was immobilized, a substantial response of about 850 RU was observed with increased amounts of GST tagged-Rv1688 protein passed over the chip (Fig. 6a, right panel).

With increased amounts of Rv1688 (300-1800 nM) mixed with MtbTopA in the reaction mixture, the degree of plasmid supercoiling (S) steadily increased (Fig. 6b, left panel). This result indicated that Rv1688 inhibited the activity of MtbTopA. Similarly, Ms3759 had an inhibitory effect on the activity of MtbTopA (Fig. 6b, right panel). On the other hand, MtbTopA stimulated the cleavage activity of both Rv1688 and Ms3759 (Fig. 6c). These observations indicated that both MAG and TopA, as well as their interaction, are conserved between the two mycobacterial species.

DISCUSSION

Genome sequencing has revealed that the genome of *M. tuberculosis* encodes a variety of MAGs. However, their functions and regulations are still unknown. In the current study, the physical and functional interaction between MAGs and TopA in *M. tuberculosis* and *M. smegmatis* were characterized. Several MAGs have previously been reported to repair some deamination product of adenine, such as hypoxanthine in *Escherichia coli*, yeast, rat, and human [28-30]. Genome sequencing has revealed that the genome of *M. tuberculosis* encodes a variety of MAGs, but their functions are still unknown. In the current study, we have successfully confirmed that the MAGs of two mycobacterial species had cleavage activities on the DNA substrates that contain hypoxanthine. Therefore, the MAGs might be involved in the repair of hypoxanthine in mycobacteria. Interestingly, the expression of *M. tuberculosis* Rv1688 (Mpg) was previously reported to be elevated in clinical lung samples [31]. Therefore, our current results raise the interesting possibility that the *M. tuberculosis* MAG might play critical roles in response to DNA damage.

DNA topoisomerases are ubiquitous enzymes that play vital roles in numerous cellular processes by maintaining the superhelical density of DNA [32]. An important finding in the current study was the existence of a conserved interaction between MAG and TopA in *M. tuberculosis* and *M. smegmatis*. TopA stimulated the activity of MAG in repairing damaged bases, whereas MAG inhibited the ability of TopA in relaxing supercoiled DNA. Two random mutants of MAG that had defective interactions with TopA were also created. In contrast with the wild-type protein, the mutant protein lacked the ability of inhibiting TopA activity. Therefore, the functional interaction between DNA glycosylase and TopA is based on the direct physical interaction between the two proteins in the mycobacteria.

In summary, the activity of MAG and its interaction TopA in *M. tuberculosis* and *M. smegmatis* were characterized. DNA glycosylase inhibited the function of TopA in relaxing supercoiled DNA. In contrast, TopA stimulated the cleavage function of DNA glycosylases on a damaged DNA substrate containing hypoxanthine. The present findings further elucidate the regulation of glycosylase in both *M. smegmatis* and *M. tuberculosis*.

This work was supported by the National Natural Science Foundation of China (30930003, 31025002), the Fundamental Research Funds for the Central Universities (2011PY140), and Hubei Chutian Scholar Program (to Z.-G. He).

REFERENCES

1. Lindahl, T. (1993) *Nature*, **362**, 709-715.
2. Allan, J. M., Engelward, B. P., Dreslin, A. J., Wyatt, M. D., Tomasz, M., and Samson, L. D. (1998) *Cancer Res.*, **58**, 3965-3973.
3. Engelward, B. P., Allan, J. M., Dreslin, A. J., Kelly, J. D., Wu, M. M., Gold, B., and Samson, L. D. (1998) *J. Biol. Chem.*, **273**, 5412-5418.
4. Paulovich, A. G., and Hartwell, L. H. (1995) *Cell*, **8**, 841-847.
5. Lindahl, T., and Wood, R. D. (1999) *Science*, **286**, 1897-1905.
6. Scharer, O. D., and Jiricny, J. (2001) *Bioessays*, **23**, 270-281.
7. Hitomi, K., Iwai, S., and Tainer, J. A. (2007) *DNA Repair* (Amst.), **6**, 410-428.
8. Lindahl, T. (1986) *Basic Life Sci.*, **38**, 335-340.
9. Lau, A. Y., Scharer, O. D., Samson, L., Verdine, G. L., and Ellenberger, T. (1998) *Cell*, **9**, 249-258.
10. O'Brien, P. J., and Ellenberger, T. (2004) *J. Biol. Chem.*, **279**, 26876-26884.
11. O'Brien, P. J., and Ellenberger, T. (2004) *J. Biol. Chem.*, **279**, 9750-9757.
12. Cole, S. T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S. V., Eiglmeirer, K., Gas, S., Barry, III, C. E., et al. (1998) *Nature*, **393**, 537-544.
13. Nathan, C., and Shiloh, M. U. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 8841-8848.
14. Warner, D. F., and Mizrahi, V. (2006) *Clin. Microbiol. Rev.*, **19**, 558-570.
15. Boshoff, H. I., Reed, M. B., Barry, C. E., 3rd, and Mizrahi, V. (2003) *Cell*, **113**, 183-193.
16. Parish, T., and Brown, A. (2009) *Mycobacterium*: *Genomics and Molecular Biology*, Caister Academic Press, pp. 79-89.
17. Wang, Y., Cui, T., Zhang, C., Yang, M., Huang, Y., Li, W., Zhang, L., Gao, C., He, Y., Li, Y., Huang, F., Zeng, J., Huang, C., Yang, Q., Tian, Y., Zhao, C., Chen, H., Zhang, H., and He, Z. G. (2010) *J. Proteome Res.*, **9**, 6665-6677.
18. Zeng, J., Zhang, L., Li, Y., Wang, Y., Wang, M., Duan, X., and He, Z. G. (2010) *Protein Exp. Purif.*, **69**, 47-53.
19. Gill, S. C., and von Hippel, P. H. (1989) *Anal. Biochem.*, **182**, 319-326.
20. Zhang, H., Tian, Y., Liu, Z., Huang, F., Hu, L., and He, Z. G. (2011) *Biochemistry* (Moscow), **76**, 658-665.
21. Zhang, L., Zhang, L., Liu, Y., Yang, S., Gao, C., Gong, H., Feng, Y., and He, Z. G. (2009) *Proc. Natl. Acad. Sci. USA*, **106**, 7792-7797.
22. He, Z. G., Rezende, L. F., Willcox, S., Griffith, J. D., and Richardson, C. C. (2003) *J. Biol. Chem.*, **278**, 29538-29545.
23. Lingaraju, G. M., Kartalou, M., Meira, L. B., and Samson, L. D. (2008) *DNA Repair*, **7**, 970-982.
24. Colangeli, R., Helb, D., Vilcheze, C., Hazbon, M. H., Lee, C. G., et al. (2007) *PLoS Pathog.*, **3**, e87.
25. Grasser, K. D., Ritt, C., Krieg, M., Fernandez, S., Alonso, J. C., et al. (1997) *Eur. J. Biochem.*, **249**, 70-76.
26. Huang, F., and He, Z. G. (2010) *Nucleic Acids Res.*, **38**, 8219-8230.
27. Yang, M., Gao, C., Wang, Y., Zhang, H., and He, Z. G. (2010) *PLoS One*, **5**, e10672.
28. Singer, B., Antoccia, A., Basu, A. K., Dosanjh, M. K., Fraenkel-Conrat, H., Gallagher, P. E., Kusmierek, J. T., Qiu, Z. H., and Rydberg, B. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 9386-9390.
29. Saparbaev, M., and Laval, J. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 5873-5877.
30. Saparbaev, M., Kleibl, K., and Laval, J. (1995) *Nucleic Acids Res.*, **23**, 3750-3755.
31. Rachman, H., Strong, M., et al. (2006) *Infect. Immun.*, **74**, 1233-1242.
32. Wang, J. C. (1996) *Annu. Rev. Biochem.*, **65**, 635-692.