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Characterization of a functional C-terminus of the *Mycobacterium tuberculosis* MtrA responsible for both DNA binding and interaction with its two-component partner protein, MtrB

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Virulence in pathogenic bacteria is due in part to the action of two-component systems. However, in the human pathogen *Mycobacterium tuberculosis*, the molecular mechanisms underlying these systems are as yet unclear. In this study, MtrA was shown to contain a functional C-terminus and also to have Ca^{2+} as its preferred cofactor for DNA binding. Further mutation experiments demonstrated that the C-terminus of MtrA was responsible for specific interactions with the target DNA motif and also with its partner protein, MtrB. The physical interaction between MtrA and MtrB inhibited DNA binding by MtrA. These findings yield critical information about the unique regulatory mechanisms of the essential MtrAB two-component system in this pathogen.

Keywords: *Mycobacterium tuberculosis*/MtrAB/ two-component system.

Abbreviations: 3-AT, 3-amino-1, 2, 4-triazole; EMSA, electrophoretic mobility shift assay; ICP–OES, inductively coupled plasma-optical emission spectrometry; NTA-chip, Nitilotriacetic acid chip; SA chips, Streptavidin chip; SDS–PAGE, SDS-poly-acrylamide gel; SPR, surface plasmon resonance; TCRs, two-component systems.

Mycobacterium tuberculosis, the causative microbe of tuberculosis (TB), has a unique ability to persist for long periods of time within its host tissue (*1*). This dormancy is part of its resilience as an infective agent, and its threat to human health has only become worse with the emergence of multidrug-resistant TB and the prevalence of global co-infection with HIV (*2*). The enduring pathogenicity of *M. tuberculosis* suggests that this pathogen must possess unique regulation mechanisms, as indicated by a number of transcription-related genes encoded by its genome (*3*). Of particular note are 11 paired two-component systems (TCRs) and several orphan kinases and regulators (*3*).

TCRs, which are widely distributed among bacteria and plants, enable organisms to regulate gene expression in response to a variety of environmental stimuli (*4*, *5*). Some TCRs are clearly involved in the regulation of virulence in pathogenic bacteria (*6*). In *M. tuberculosis*, several TCRs are apparently required for its growth under specific conditions, including *mprA-mprB* for maintenance of persistence (*7*), *prrA-prrB* for intramacrophage growth (*8*), and *devR-devS* for regulation of hypoxic responses (*9*). Among the 11 known *M. tuberculosis* TCRs, only the *mtrA-mtrB* system has been confirmed as essential for survival, both *in vitro* and *in vivo* (*10–12*). The regulator MtrA modulates *M. tuberculosis* proliferation, possibly by regulating the expression of *dnaA*, a gene necessary for the initiation of chromosome replication (*13*). A recent report shows that the *M. tuberculosis* origin of replication and the promoter for immunodominant secreted antigen 85B are the targets of MtrA (*14*). Therefore, the two-component system MtrAB may play a variety of important roles in the survival of the pathogen *M. tuberculosis* within macrophages.

The crystal structure of MtbMtrA has recently been solved (*15*). The N-terminal fragment apparently encodes a regulatory domain, while the C-terminus encodes a winged helix-turn-helix DNA-binding domain (*15*). The conserved Asp56 within the regulatory domain is a phosphorylation residue, which regulates binding of a metal ion (Mg^{2+} or Ca^{2+}) (*15*). The DNA-binding domain is presumed to inhibit the phosphotransferase activity of the regulatory domain. Thus, the contacts between the N- and C-terminal domains of MtrA essentially lock the regulatory domain into an inactive conformation, lowering its rate of phosphorylation (*15*). However, the functional roles of the domains of MtrA and the effects of ions on the DNA binding of MtrA remain to be characterized.

The MtrAB system appears to be essential for the survival and for the infectivity of *M. tuberculosis*. However, the impact of the cognate partner protein, MtrB, on the DNA–protein interactions remains unknown. In the current study, we have examined the physical interactions between MtrA and its cognate partner MtrB, focusing on effects on the DNA-binding activities of MtrA. The data presented here add important new information to our understanding of the regulatory mechanisms of this essential two-component system MtrAB in *M. tuberculosis*.

Material and Methods

Bacterial strains, plasmids, enzymes and chemicals

Escherichia coli BL21(DE3) cells and pET28a, purchased from Novagen, were used as the host strain and vector to express *M. tuberculosis* proteins. pBT and pTRG vectors and *E. coli* host strains were acquired from Stratagene. Biological enzymes and deoxynucleoside triphosphates (dNTPs) were obtained from TaKaRa Biotech. DNA purification kits were from Watson Biotechnologies. Antibiotics were purchased from TianGen. The Ni-NTA (Ni^{2+}-nitrilotriacetate) agarose column was obtained from Qiagen. [γ-^{32}P] ATP was purchased from FuRui Company.

Primers and oligonucleotides

All DNA primers and oligonucleotides (Supplementary Table S1) were synthesized by Invitrogen. Oligonucleotides were labelled using T4 polynucleotide kinase and [γ-^{32}P] ATP. To prepare the double-stranded substrates, the labelled oligonucleotide was annealed to a 1.5-fold molar excess of a cold complementary strand, according to our previously published procedure (*16–18*). Duplex DNA substrates for electrophoretic mobility shift assays were constructed by annealing the appropriate combinations of a pair of oligos.

Cloning, expression and purification of recombinant proteins

Mycobacterium tuberculosis genes, including *mtrA*, *mtrB,* and their mutants, were amplified using their primers (Supplementary Table S1) from genomic DNA. The MtrAB genes were cloned into overexpression vector pET28a or pGEX-4T-1 to produce recombinant plasmids (Supplementary Table S2). *Escherichia coli* BL21(DE3) cells transformed with the recombinant plasmid were grown at 37°C in 1 l of LB medium containing 30 μg/ml kanamycin or 100 μg/ml ampicillin, respectively. Protein purification was carried out as described in earlier reports (*16–19*). Protein concentrations were determined by spectrophotometric absorbance at 280 nm, based on a previously published procedure (*20*).

Bacterial two-hybrid analysis

The BacterioMatch II Two-Hybrid System Library Construction Kit (Stratagene) was used to detect protein–protein interactions between mycobacterial proteins. The pBT and pTRG vectors containing mycobacterial genes were generated (Supplementary Table S2). Bacterial two-hybrid analysis was carried out as described in earlier work (*21–23*). Positive co-transformants were selected on a Screening Medium plate containing 5 mM 3-AT (Stratagene), 8 μg/ml streptomycin, 15 μg/ml tetracycline, 34 μg/ml chloramphenicol and 50 μg/ml kanamycin. Co-transformant containing pBT-LGF2 and pTRG-Gal11P as a positive control and co-transformant containing pBT and pTRG as a negative control (*21–23*).

Electrophoretic mobility shift assay

DNA-binding assays of *M. tuberculosis* MtrA and its mutant proteins were performed using a modification of an *electrophoretic mobility shift assay* (EMSA), as described earlier (*16–18*) but with several changes. The DNA substrate used in this study was a short oligonucleotide (5′-GCGGTG TAGTTATCACGCCGTTT CA GCGTGGAAACGGCACTC-3′) from the promoter region of *dnaA*, which contains a previously reported MtrA-binding site (underlined) (*14*). The reactions (10 μl) for measuring the mobility shift contained 200 fmol ^{32}P-labelled DNA and various amounts of MtrA diluted in a buffer containing 20 mM Tris–HCl (pH 7.5), 100 mM NaCl, 0.5 mM $MgCl_2$, 10 μg/ml sonicated salmon sperm DNA, 0.7 mM 2-mercaptoethanol and 5% glycerol. Reactions were performed at room temperature for 20 min, loaded onto 6% polyacrylamide/bis (37.5 : 1) gels in 0.5× TBE buffer, and run at a constant voltage of 150 V for 40 min. Gels were exposed to a storage-phosphor screen overnight at room temperature. The images were acquired by a Typhoon Scanner (GE Healthcare).

Surface Plasmon Resonance analysis

The interaction between MtrA and the regulatory region of the *M. tuberculosis dnaA* gene was assayed by surface plasmon resonance (SPR). Biotin-labelled promoter DNA was immobilized onto the SA chip (Streptavidin chip) (BIAcore) based on a previously published procedure (*19*). The purified MtrA protein was passed over the chip. DNA–protein interaction assays were performed at 25°C. Each analysis was performed in triplicate. An overlay plot was generated to show the interactions. To detect the physical interaction between MtrA and MtrB, 6× his-tagged proteins were immobilized onto NTA chips (Nitrilotriacetic acid chip) for further SPR assays. The GST-tagged proteins were then passed over the chip. Experiments were performed in a running buffer consisting of 100 mM HBS buffer [10 mM HEPES (pH 7.4), 150 mM NaCl, 50 μM EDTA and 0.005% Biacore surfactant P20) at a flow rate of 10 ml per min at 25°C. All proteins were diluted in the running buffer supplemented with 500 mg/ml bovine serum albumin. Each analysis was performed three times. An overlay plot was generated for depicting the interactions.

Determinations of metal ion content

The metal ion content of the wild-type MtrA, as expressed and purified from an *E. coli* host, was determined using inductively coupled plasma–optical emission spectrometry (ICP–OES) (Optima 2000, Perkin-Elmer, Waltham, MA, USA), as described in previous work (*24*). Purified proteins (800 μl, 2.0 mg/ml) were digested with nitric acid (200 μl) and diluted to 4 ml. The metal ion content in the purified protein was determined by ICP–OES against a metal ion standard solution. To remove ions from the wild-type MtrA and to obtain apo-MtrA (calcium free form of MtrA), the purified wild-type MtrA was dialysed for 1 day against the MES buffer (20 mM, pH 8.0), containing 2 mM EDTA and 2 mM 1,10-phenanthroline, and then against the MES buffer

alone to remove the remaining EDTA and the 1,10-phenanthroline. Each analysis was performed three times. Representative data are shown.

Phosphorylation assay

The phosphorylation assay was carried out as described earlier (*25*) with several changes: MtrB-ΔN protein (0.5 μM) was first self-phosphorylated for 10 min at 30°C in buffer (50 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 1 mM Dithiothreitol and 100 mM NaCl). Afterwards, the MtrA and its mutant proteins (1 μM) were added to the reaction mixture for an additional 30 min at 30°C. The reaction was stopped with 2× SDS–PAGE sample buffer, resolved in 12% SDS–PAGE, and further analysed by Coomassie blue staining and autoradiography.

Results

MtrA contained a functional C-terminus

The functions of the domains of MtrA were established with mutants in which the C-terminus (MtrA-ΔC) or N-terminus (MtrA-ΔN) was deleted (Fig. 1A). Their DNA-binding activities were characterized using EMSA assays, as shown in Fig. 1C. The wild-type MtrA demonstrated a DNA-binding activity. A DNA-binding band was also observed for MtrA-ΔN, but no specific band was observed for MtrA-ΔC and MtrB-ΔN (Fig. 1C). Therefore, it was the C-terminus, not the N-terminus, that was responsible for the DNA-binding activity of MtrA.

The importance of the N-terminus and C-terminus of MtrA for DNA-binding was confirmed by SPR experiments using the *dnaA* gene promoter associated with the chip. As shown in Fig. 1D, MtrA-ΔN bound to the DNA on the chip (250 RU) with a similar, although somewhat less pronounced, association with the wild-type MtrA (300 RU). In contrast, no binding was observed for MtrA-ΔC. In addition, no association was found with MtrB-ΔN, the C-terminal protein kinase domain (N-terminal transmembrane domain was deleted). The interaction of MtrA and MtrA-ΔN with the *dnaA* promoter (300 nM) was also further confirmed by a detailed SPR assay (Fig. 2). The binding activity improved with stepwise increases in the protein concentration of MtrA and MtrA-ΔN (100–500 nM) (Fig. 2). Thus, the C-terminal domain of MtrA appeared to be responsible for its DNA-binding function.

MtrA had Ca^{2+} as its preferred cofactor for DNA-binding activity

Specific metal ion species were essential for the function of MtrA, as determined by ICP–OES, EMSA and SPR assays. As shown in Fig. 3A, using ICP–OES, we detected a significantly high amount of Ca^{2+}, but not Mg^{2+} or K^+, associated with the purified MtrA protein. No other ion showed a similar enrichment to

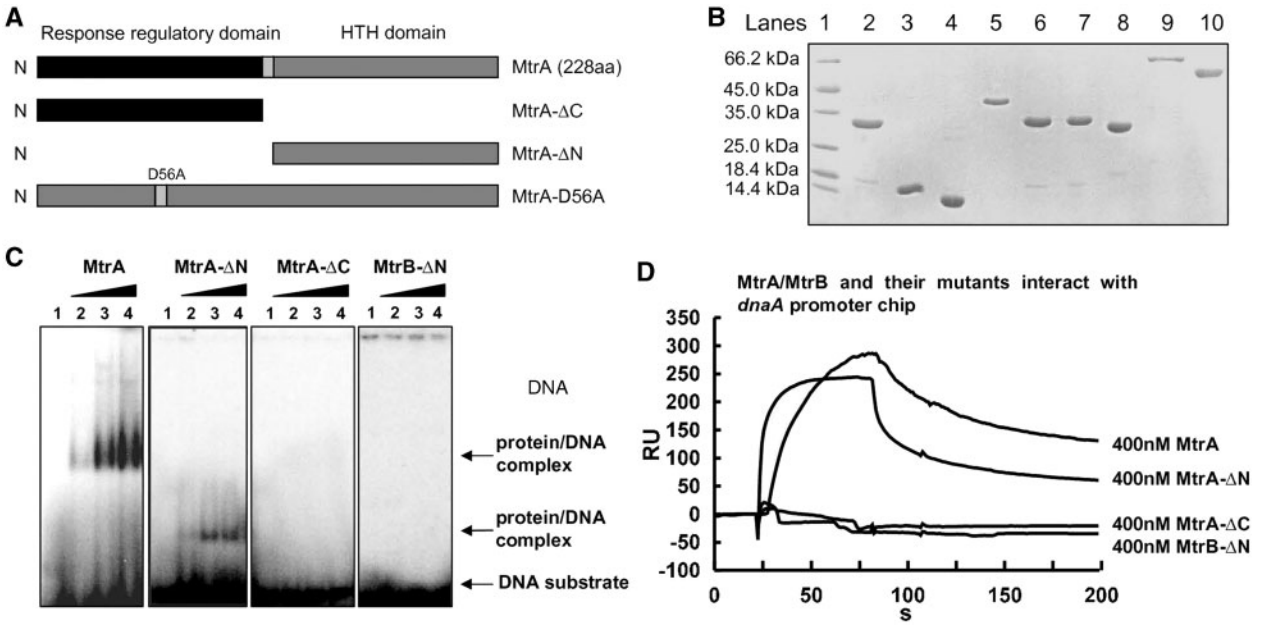

Fig. 1 DNA-binding activities of MtrA, MtrB and its mutant proteins. (A) Schematic representation of *M. tuberculosis* MtrA domains and several of its mutants. (B) MtrA, MtrB and their mutant proteins used in this study were resolved in 12% SDS–PAGE and the gel was coomassie blue stained. Lanes 1, protein marker; 2, His-MtrA; 3, His-MtrA-ΔN; 4, His-MtrA-ΔC; 5, His-MtrB-ΔN; 6, apo-His-MtrA; 7, His-MtrA-D56A; 8, GST; 9, GST-MtrB-ΔN; 10, GST-MtrA. Molecular weight of each protein band of marker was indicated. (C) EMSA assays for the DNA-binding activities of MtrA, MtrB and their mutant proteins. The reactions (10 μl) for measuring the mobility shift contained 200 fmol ^{32}P-labelled DNA substrate (5′-GCGGTG TAGTTATCACGCCGTTTCA GCGTGGAAACGGCACTC-3′) as described in the 'Materials and Methods' and increasing amounts of MtrA, MtrB or their mutant proteins (100, 200, 400 and 600 nM). Reactions were performed at room temperature for 20 min, loaded onto 6% polyacrylamide/bis (37.5 : 1) native gels, and run at a constant voltage of 150 V for 40 min. Gels were exposed to a storage-phosphor screen. The images were acquired by a Typhoon Scanner (GE Healthcare). The protein/DNA complex was indicated by the arrows on the right of the panels. (D) SPR assays for the DNA-binding activities of MtrA, MtrB and their mutant proteins. Biotin-labelled promoter DNA was immobilized onto the SA chips (Streptavidin chip) (BIAcore). The purified 6× his-tagged proteins (400 nM) were passed over the chip. An overlay plot was generated to show the interactions.

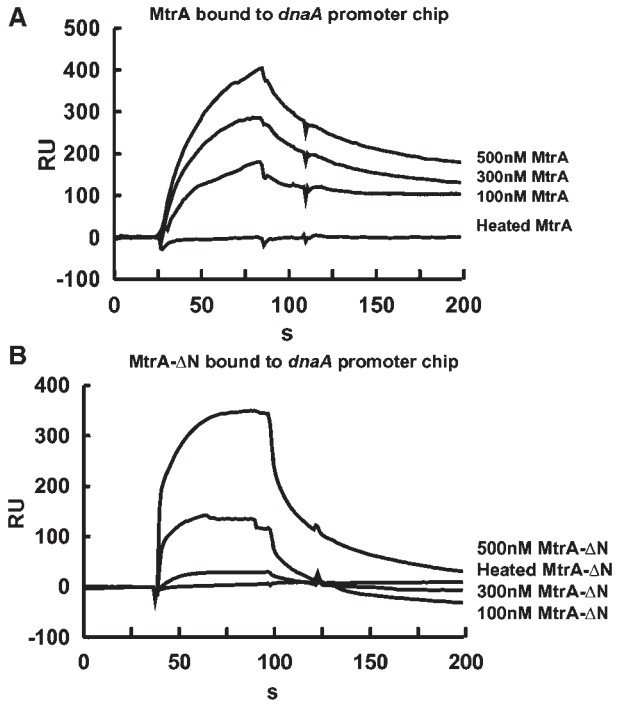

Fig. 2 SPR assay for the DNA-binding activities of MtrA and MtrA-ΔN. SPR experiments were carried out as described in the 'Materials and Methods' section. (A) SPR assay for the DNA-binding activities of MtrA. The protein concentration was indicated on the right of the panels. (B) SPR assay for the DNA-binding activities of MtrA-ΔN.

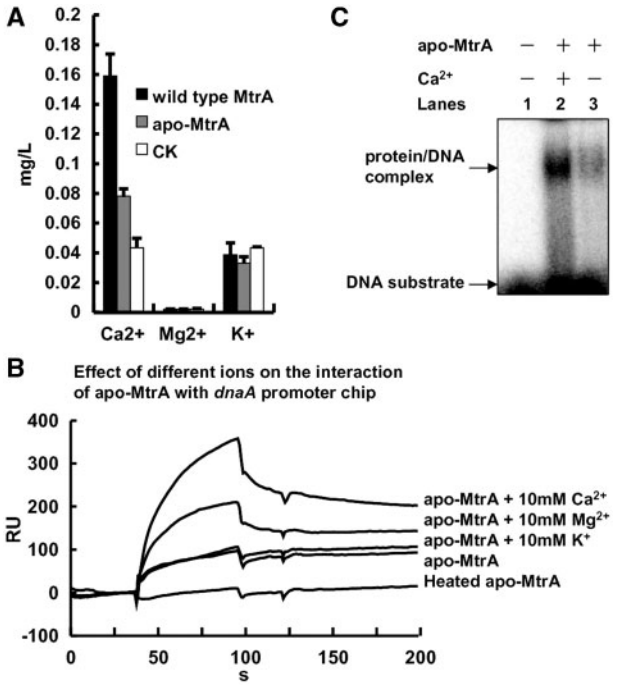

Fig. 3 Effects of metal ions on the DNA-binding activity of apo-MtrA. Assays for metal ion content and their effects on the DNA-binding activity of MtrA. (A) The wild-type MtrA was expressed and purified from *E. coli* host. The purified wild-type MtrA was dialysed for 1 day against the MES buffer (20 mM, pH 8.0), containing 2 mM EDTA and 2 mM 1,10-phenanthroline, and then against the MES buffer alone to remove the remaining EDTA and the 1,10-phenanthroline to obtain apo-MtrA. Metal ion contents were determined using ICP–OES assays. Each analysis was performed in thrice. CK represents the MES buffer. (B) SPR assays for effect of different ions (10 mM) on the interaction of MtrA with *dnaA* promoter. Apo-MtrA (400 nM) was pre-incubated with different ions for 10 min at room temperature and then passed over the *dnaA* promoter chip. An overlay plot was generated to show the interactions. (C) Represents Ca^{2+}-dependent DNA-binding activity assays by EMSA. DNA-binding activities of apo-MtrA (600 nM) were assayed in the presence or absence of Ca^{2+}. The protein/DNA complex is indicated by the arrows on the left of the panels.

that seen with Ca^{2+} (data not shown). This indicated that the recombinant MtrA protein had contained some amount of Ca^{2+} ion when expressed and purified from *E. coli*. In a SPR assay, the DNA-binding activity of apo-MtrA was significantly stimulated by 10 mM Ca^{2+} (response value increase from 100 RU up to 350 RU) (Fig. 3B). In comparison, no stimulation by K^+ was observed. Mg^{2+} also promoted some stimulation but to a much lower degree than that achieved with Ca^{2+} (Fig. 3B). In a further EMSA assay, apo-MtrA showed weak DNA-binding activity in the absence of additional Ca^{2+} (Fig. 3C, lane 3) while Ca^{2+} could clearly stimulate the activity (Fig. 3C, lane 2). Considering all of these observations, Ca^{2+} was the preferred cofactor for DNA-binding activity.

The C-terminal fragment of MtrA was responsible for its physical interaction with MtrB

A bacterial two-hybrid experiment characterized the physical interaction between MtrA and its cognate partner MtrB. As shown in Fig. 4A, an interaction between wild-type MtrA and MtrB was observed. In contrast to MtrA-ΔC, the mutants MtrA-ΔN and MtrA-D56A interacted with MtrB, as their co-transformants grew well on the screening medium (Fig. 4A). In a further SPR assay, both wild-type MtrA (Fig. 4B) and MtrA-ΔN (Fig. 4C) interacted with the kinase domain of MtrB (MtrB-ΔN). Therefore, these results indicate that the C-terminal domain of MtrA was also responsible for the interaction with its two-component kinase, MtrB.

SPR and EMSA experiments clearly demonstrated an inhibition of the DNA-binding activity of MtrA by MtrB-ΔN. As shown in Fig. 5A and B (left panel), pre-incubation of MtrA or MtrA-ΔN with an increasing concentration of MtrB-ΔN (100–300 nM) clearly decreased the DNA-binding ability of MtrA or MtrA-ΔN. Pre-incubation with GST protein (300 nM) did not have this effect (Fig. 5A and B, left panel). Physical interaction of two proteins competitively inhibited the DNA-binding activity of MtrA or MtrA-ΔN. The effects of these interactions on the DNA-binding activity of MtrA were examined with further EMSA assays. As shown in Fig. 5A and B (right panel), a specific band corresponding to the protein/DNA complex was observed in the presence of MtrA or MtrA-ΔN alone. However, when an increasing amount of MtrB-ΔN (200–800 nM) was added stepwise to the reactions, this unique band slowly disappeared, indicating that the MtrB-ΔN inhibited the DNA-binding ability of MtrA and MtrA-ΔN (Fig. 5A and B, right panel).

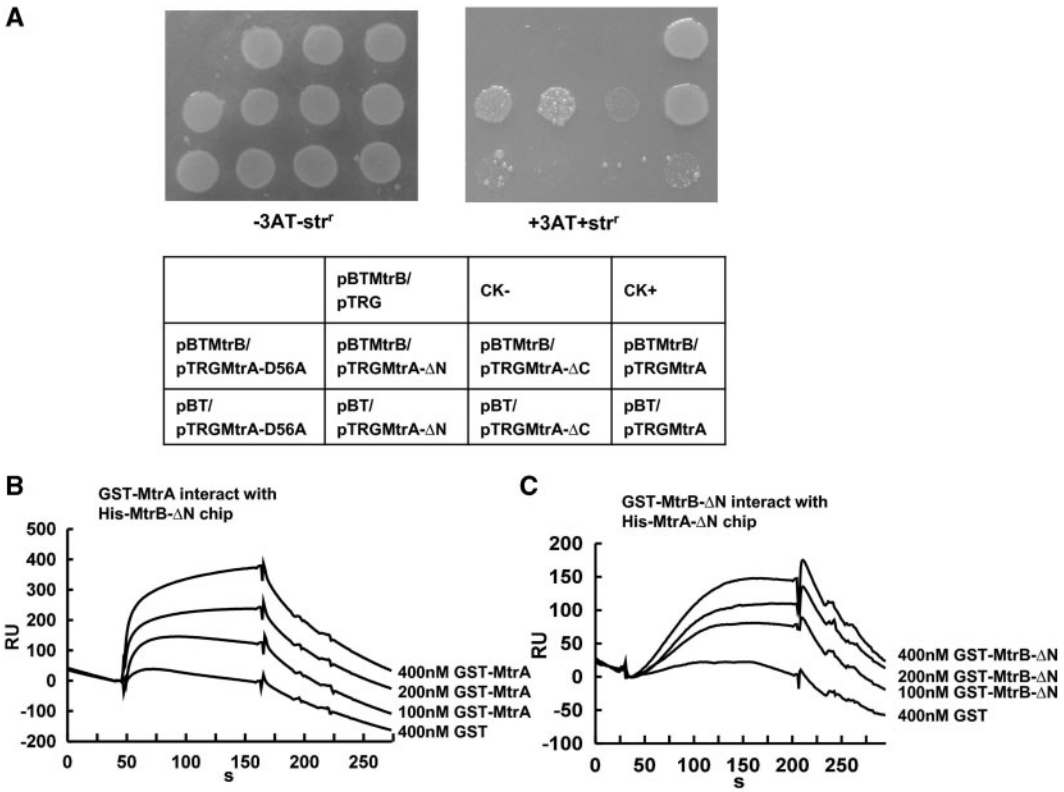

Fig. 4 The physical interaction between MtrB and MtrA. (A) The bacterial two-hybrid technique (Stratagene) was used to detect protein–protein interactions between MtrA and MtrB. Positive co-transformants were selected on the Screening Medium plate containing 5 mM 3-AT (Stratagene), 8 μg/ml streptomycin, 15 μg/ml tetracycline, 34 μg/ml chloramphenicol and 50 μg/ml kanamycin. The lower panel presents an outline of the plates. Each unit represents the corresponding co-transformant in the plates. CK^+, co-transformant containing pBT-LGF2 and pTRG-Gal11P was used as a positive control. CK^-, co-transformant containing pBT and pTRG was used as a negative control. (B) SPR assays for the interaction between MtrA or MtrA-ΔN and MtrB-ΔN. For the detection of interaction between MtrA and MtrB-ΔN, 6× his-tagged MtrB-ΔN proteins were immobilized onto the NTA chips. Different amounts of GST-MtrA (left panel) proteins were passed over the chip. (C) For the detection of interaction between MtrA-ΔN and MtrB-ΔN, 6× his-tagged MtrA-ΔN proteins were immobilized onto the NTA chips. Different amounts of GST-MtrB-ΔN (right panel) proteins were passed over the chip. The GST-tag (400 nM) was used as a negative control for the specific interactions. An overlay plot was produced to show the interactions.

In summary, the C-terminal fragment of MtrA was responsible for the physical interaction of MtrA with MtrB. Direct physical interaction inhibited the DNA-binding activity of MtrA.

The N-terminal fragment of MtrA was responsible for its phosphorylation by MtrB

Attempts to detect the *in vitro* phosphorylation of the full-length MtrA by MtrB kinase of *M. tuberculosis* were unsuccessful. No interaction between MtrB and the N-terminal regulatory domain of MtrA was detected by bacterial two-hybrid experiments (Fig. 4A) or SPR assay (data not shown). This may reflect a steric hindrance effect of the GST-tag or the limitations of these methods in detecting transient interactions. Interestingly, we confirmed by a phosphorylation assay that MtrB-ΔN could phosphorylate itself and also the C-terminus deleted mutant, MtrA-ΔC (Fig. 6). In comparison, MtrB was unable to phosphorylate the DNA-binding domain of MtrA, MtrA-ΔN, the full length MtrA or a phosphorylation-defective mutant MtrA-D56A (Fig. 6). This is consistent with the idea that the full length MtrA was locked by its C-terminal fragment into an inactive conformation (*15*). In *Corynebacterium glutamicum*, MtrA could be phosphorylated by the full length MtrB that was reconstituted into liposomes (*26*). Therefore, we assume that the intact structure of MtrB is necessary for the activation of MtrA. The N-terminus deleted mutant of MtrB lost the ability to change the inactive conformation of MtrA, although it could phosphorylate the C-terminus deleted mutant of MtrA. This will require further experiments to confirm. The impact of phosphorylation on the DNA-binding activity of the full length MtrA should also be further addressed. In summary, we found that MtrB phosphorylated the N-terminal fragment of MtrA.

Discussion

The two-component systems of *M. tuberculosis* are clearly involved in the regulation of virulence, resistance and persistence following infection (*8–13*). However, the mechanisms by which this involvement is established remain unclear. MtrAB regulates the expression of the replication initiator gene *dnaA* of *M. tuberculosis* and affects pathogenic proliferation (*13*). However, the effects of MtrB on its DNA recognition are unknown.

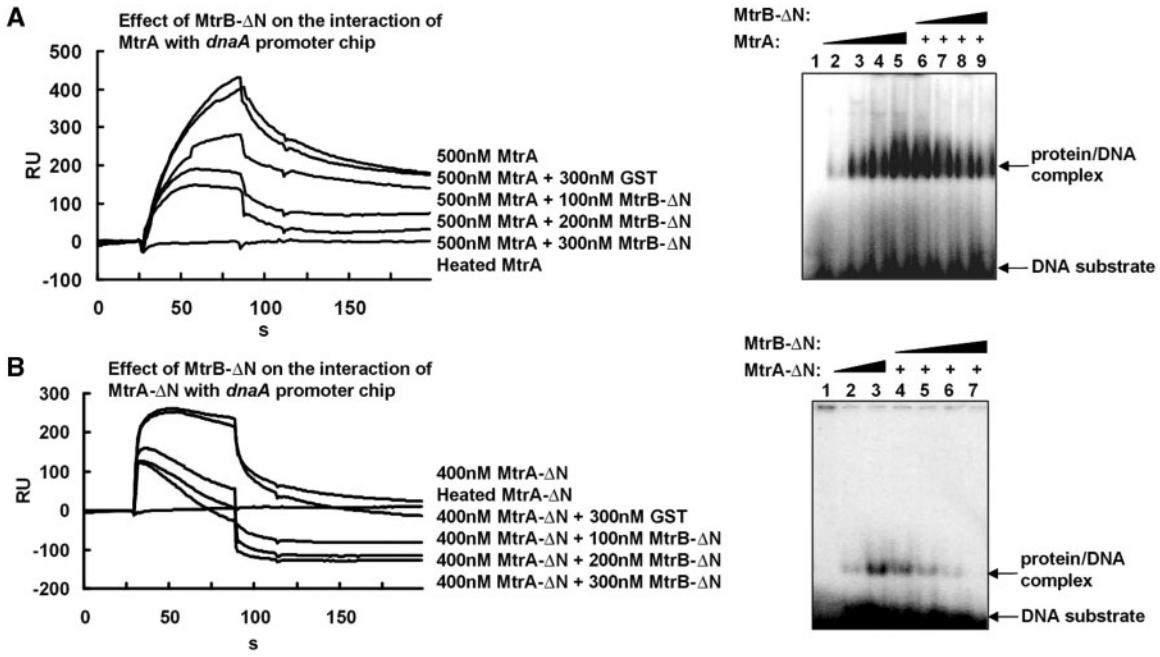

Fig. 5 Effect of the physical interaction of MtrB with MtrA on the DNA-binding activity of MtrA and MtrA-ΔN. (A) SPR and EMSA assays for the effects of MtrB-ΔN on the DNA-binding activity of MtrA. Left panel presents a representative regulation of MtrB-ΔN on the DNA-binding activity of MtrA measured by SPR assays. GST (300 nM) was used as a negative control for competition experiments. An overlay plot was produced to show the interactions. The protein/DNA complex in EMSA assays is indicated by the arrows on the right of the panels. The EMSA assays were carried out using increasing amounts of MtrB-ΔN (200, 400, 600 and 800 nM) in the presence of MtrA (500 nM). (B) SPR and EMSA assays for the effects of MtrB-ΔN on the DNA-binding activity of MtrA-ΔN. Left panel presents a representative regulation of MtrB-ΔN on the DNA-binding activity of MtrA-ΔN measured by SPR assays. GST (300 nM) was used a negative control for competition experiments. An overlay plot was produced to show the interactions. The protein/DNA complex in EMSA assays is indicated by the arrows on the right of the panels. The EMSA assays were carried out using increasing amounts of MtrB-ΔN (200, 400, 600 and 800 nM) in the presence of MtrA-ΔN (400 nM).

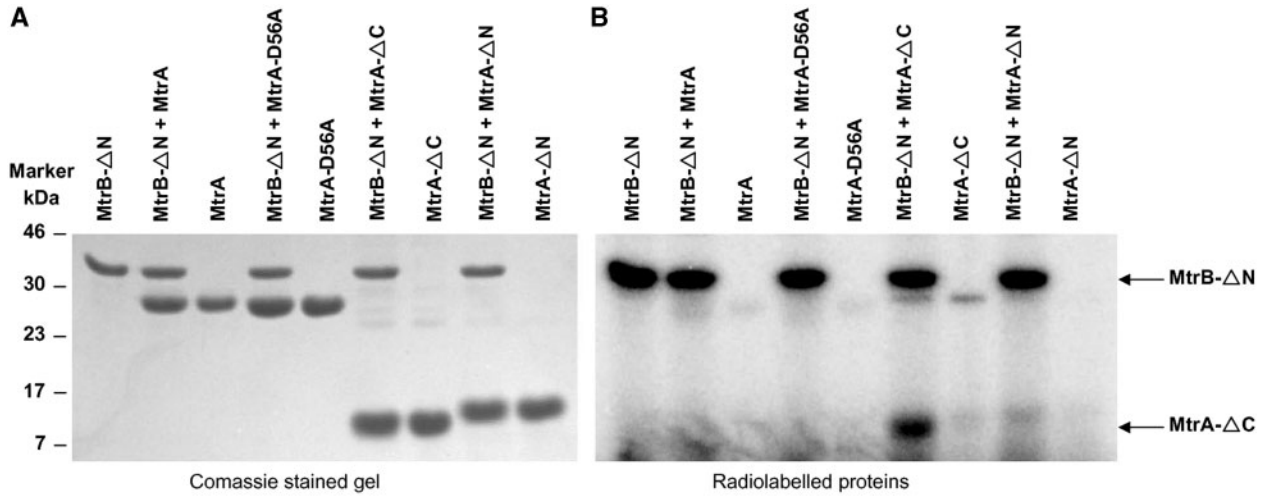

Fig. 6 Phosphorylation assay. The experiment was carried out as described in the 'Materials and Methods' section. (A) Coomassie blue stained gel. (B) Radiolabelled proteins. The phosphorylated proteins are indicated by the arrows.

The current study shows that the C-terminal fragment of MtrA is apparently responsible for specific interactions with both the target DNA as well as its partner protein, MtrB. The direct physical interaction between MtrA and MtrB negatively modulated the DNA-binding ability of MtrA. The regulator MtrA was a Ca^{2+}-dependent DNA-binding protein. Finally, we proposed a regulatory model of the MtrAB two-component system of *M. tuberculosis*.

MtrB is the cognate partner kinase of the two-component regulator MtrA (*3*, *13*). The present study represents the first characterization of the effect of MtrB on the DNA binding of MtrA. The finding (Fig. 5) that the direct physical interaction of MtrB inhibited the DNA-binding activity of MtrA is significant. MtrB interacted with the C-terminal fragment of MtrA; this domain was also responsible for its DNA-binding ability. Thus, the inhibitory effect of

MtrB was associated with the C-terminus of MtrA which was responsible for the DNA-binding activity. On the other hand, the N-terminal domain of MtrA was also found to regulate the DNA-binding ability of MtrA. The kinetic binding properties of MtrA and MtrA-ΔN on the *dnaA* promoter chip look different (Fig. 1D). The MtrA-ΔN demonstrated a stronger DNA-binding activity than the full-length MtrA. The crystal structure of MtrA (*15*) has suggested that the interaction between the N- and C-terminal domains of MtrA may also impact the DNA-binding activity of MtrA. Importantly, the MtrB-ΔN was shown to strongly reduce the DNA-binding activity of MtrA-ΔN (Fig. 5B), suggesting that MtrB could interact more effectively with the C-terminal domain of MtrA than with the full-length protein. Therefore, our data were consistent with the structural study of the MtrA (*15*).

We confirmed that MtrB kinase could phosphorylate itself and also the C-terminus deleted mutant, MtrA-ΔC (Fig. 6). A promising model can therefore be proposed. Regarding the negative regulation of MtrA on cell proliferation and initiation of DNA replication, it is possible that the function of MtrA is inhibited by the physical interaction between its C-terminal domain and MtrB under free growth conditions. Alternatively, during infection, environmental signals might regulate MtrB and promote it to phosphorylate the N-terminal domain of MtrA. This would result in a conformation change and the activation of the regulator MtrA. Thus, the target gene would be extensively regulated to promote the cell to enter into a persistent state [Note: The signal response by MtrB may be mediated by LpqB, a lipoprotein that has recently been reported to interact with the extracellular domain of MtrB (*27*)].

In conclusion, we have documented the interaction of MtrA with its partner protein, MtrB, and proposed a regulatory model of the MtrAB two-component system of *M. tuberculosis*. Our findings provide important novel information for understanding the unique regulatory mechanisms used by the pathogen to adapt the multiple stresses within the macrophage during its infection process.

Supplementary Data

Supplementary Data are available at *JB* Online.

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Conflict of interest
None declared.

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