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Phosphoprotein phosphatase of *Mycobacterium tuberculosis* dephosphorylates serine–threonine kinases PknA and PknB

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Abstract

The regulation of cellular processes by the modulation of protein phosphorylation/dephosphorylation is fundamental to a large number of processes in living organisms. These processes are carried out by specific protein kinases and phosphatases. In this study, a previously uncharacterized gene (Rv0018c) of *Mycobacterium tuberculosis*, designated as mycobacterial Ser/Thr phosphatase (*mstp*), was cloned, expressed in *Escherichia coli*, and purified as a histidine-tagged protein. Purified protein (Mstp) dephosphorylated the phosphorylated Ser/Thr residues of myelin basic protein (MBP), histone, and casein but failed to dephosphorylate phospho-tyrosine residue of these substrates, suggesting that this phosphatase is specific for Ser/Thr residues. It has been suggested that *mstp* is a part of a gene cluster that also includes two Ser/Thr kinases *pknA* and *pknB*. We show that Mstp is a trans-membrane protein that dephosphorylates phosphorylated PknA and PknB. Southern blot analysis revealed that *mstp* is absent in the fast growing saprophytes *Mycobacterium smegmatis* and *Mycobacterium fortuitum*. PknA has been shown, whereas PknB has been proposed to play a role in cell division. The presence of *mstp* in slow growing mycobacterial species, its trans-membrane localization, and ability to dephosphorylate phosphorylated PknA and PknB implicates that Mstp may play a role in regulating cell division in *M. tuberculosis*.

Keywords: Phosphoprotein phosphatase; Serine–threonine kinase; Serine–threonine phosphatase; PP2C family; Protein phosphorylation

Bacterial pathogens facilitate their survival in the host by adaptive regulation of gene expression in response to environmental alterations. These pathogens are fully equipped to transduce these environmental signals into cellular responses by employing signal transduction systems utilizing protein phosphorylation/dephosphorylation as a molecular switch. Protein kinases and phosphatases control a multitude of cellular processes such as cell differentiation, regulation of metabolic processes, and responses to environmental stresses. Moreover, protein kinases and phosphatases have been identified as virulence determinants in several bacterial pathogens [1,2].

The *Mycobacterium tuberculosis* genome has 11 putative eukaryotic-type serine/threonine kinases and a putative phosphoprotein phosphatase [3]. Five kinases (PknA, PknB, PknD, PknF, and PknG) have been biochemically characterized [4–7]. Ser/Thr kinase, PknA, of *M. tuberculosis* has been shown to regulate the morphological changes associated with cell division [4]. Similarly, PknB has been proposed to have a role in cell division and elongation [8,9]. However, significance of such phosphorylation/dephosphorylation events in the survival/pathogenicity of *M. tuberculosis* is poorly understood.

Protein phosphatases are classified into three families; PTP, PPP, and PPM, based on their substrate specificity, metal ion requirement, and sensitivity to inhibitors

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[10,11]. The PTPases specifically dephosphorylate phospho-tyrosine residues of substrates, whereas proteins belonging to PPP and PPM families dephosphorylate phospho-serine and phospho-threonine residues.

In this study, we characterized Rv0018c gene from *M. tuberculosis* which occur in the same gene cluster as Ser/Thr kinases *pknA* and *pknB* that have been proposed to play a critical role in cell division and growth of *M. tuberculosis*. This paper addresses cloning, expression, and characterization of *mstp* gene of *M. tuberculosis*. Mstp is a trans-membrane protein belonging to the PP2C subfamily of Ser/Thr phosphatases. In addition, we also show that phosphorylated PknA and PknB are dephosphorylated by Mstp.

Experimental procedures

Bacterial culture and growth conditions. Mycobacterial strains (*M. tuberculosis* Erdman, *M. tuberculosis* H37Ra, *M. bovis* BCG, *M. smegmatis*, and *M. fortuitum* obtained from Dr. J.S. Tyagi, AIIMS, N. Delhi) were grown in Middlebrook 7H9 broth supplemented with 0.5% glycerol and 10% ADC at 37 °C with shaking at 220 rpm for 3–4 weeks. The *Escherichia coli* strains DH5α and BL-21 were used for transformations and grown in Luria–Bertani (LB) broth or on LB agar plate at 37 °C with shaking at 220 rpm.

Bioinformatic analysis. Multiple sequence alignment of Mstp of *M. tuberculosis* with different families of Ser/Thr phosphatase from various organisms was carried out using ClustalW software (available at http://www.ebi.ac.uk/clustalw). Similarly, various bioinformatic tools (such as Das, TMHMM, and Tmpred available at http://us.expasy.org) were used to predict the location of Mstp in mycobacterial cells.

Cloning of mstp, pknA, and pknB of M. tuberculosis. Mycobacterium tuberculosis genomic DNA was used as template for the amplification of genes coding for Mstp (Rv0018c), PknA (Rv0015c), and PknB (Rv0014c). The *mstp* gene was amplified in two fragments. Finally, complete gene was amplified using an equimolar concentration of these two fragments as templates in an overlapping PCR. Nucleotide sequences of primers were; 5′-CCG CTT GCG GAT CCG AGT GGC GCG carrying *Bam*HI site at the 5′end (forward primer) and 5′-CTT GCA GTC GAA TTC TCA TGC CGC carrying an *Eco*RI site at 3′ end (reverse primer). Internal primers were 5′-ATC GTT AAA CGC GTT CCG CCA CAG and 5′-CTG TGG CGG AAC GCG TTT AAC GAT. The amplified fragment was digested with *Bam*HI–*Eco*RI and ligated to *Bam*HI–*Eco*RI digested pPROEx-HTc plasmid (Invitrogen, Germany). The resulting plasmid carrying *mstp* was designated as pPRO-*mstp*.

Similarly, PCR products of *pknA* and *pknB* were digested with *Bam*H1–*Eco*R1 and *Bam*H1–*Xho*1, respectively, and cloned into *Bam*H1–*Eco*R1 and *Bam*H1–*Xho*I digested pGEX-5X-3 plasmid (Amersham–Pharmacia Biotech, India). The resulting plasmids were designated as pGEX-*pknA* and pGEX-*pknB*. Nucleotide sequences of primers for amplification of *pknA* were; 5′-GCA CTG CAG GGA TCC CCA TGA GC carrying *Bam*HI site at the 5′-end (forward primer) and 5′-GGT GGG AAG GAA TTC TCA TTG CGC carrying *Eco*RI site at 3′ end (reverse primer). Nucleotide sequences of primers for amplification of *pknB* were; 5′-TAC GAG GGA TTC CAA TGA CCA CC carrying *Bam*HI site at the 5′ end (forward primer) and 5′-CTA GCG CGG CTC GAG CTA CTG GCC which carried a *Xho*I site at 3′ end (reverse primer).

The nucleotide sequences of all clones were confirmed by Automated Sequence Analyzer (Applied Biosystem Genetic Analyzer, Model 3100).

Expression and purification of proteins. BL-21 competent cells were transformed with plasmid pPRO-*mstp*, pGEX-*pknA*, and pGEX-*pknB* separately and the transformants were selected on LB media plates supplemented with ampicillin (100 μg/ml). Mstp was expressed as a His-tagged fusion protein and purified using Ni–nitrilotriacetic acid (Ni-NTA) resin according to manufacturer's instructions. PknA and PknB were expressed as GST-fusion proteins and purified using glutathione–Sepharose 4B resin according to manufacturer's instructions.

Production of polyclonal anti-Mstp. For primary immunization, purified Mstp (500 μg) was solubilized in 500 μl of Freund's incomplete adjuvant (Sigma Chemicals) and injected subcutaneously into rabbit. Subsequently, three booster doses of 200 μg of Mstp in 200 μl of Freund's incomplete adjuvant were administered at an interval of 14 days. Ten days after the final injection, animals were bled and the titer of anti-Mstp antibody in serum was determined by enzyme-linked immunosorbent assay.

Biochemical characterization of Mstp. The phosphatase activity of purified Mstp was determined using *p*-nitrophenyl phosphate (pNPP) [2] and phosphorylated myelin basic protein (MBP), histone, and casein as substrates [12]. The effect of different cations (4 mM each) on the activity of Mstp was examined by substituting Mn^{2+} with other cations (Mg^{2+}, Ca^{2+}, Zn^{2+}, Ba^{2+}, and Li^{+}) in the assay system. The optimal concentration of Mn^{2+} was determined by varying the concentration of Mn^{2+} (0–10 mM) in the reaction buffer. A standard curve to determine the relationship between absorbance (A_{405}) of *p*-nitrophenol (pNP) to its molar concentration was plotted. The phosphatase activity of Mstp on pNPP was calculated as the number of micromoles of pNP liberated/μg protein.

Effect of inhibitors of specific families of phosphatases on the activity of Mstp was examined by carrying out phosphatase activity in the presence of these inhibitors. The various inhibitors and their concentrations used were; sodium orthovanadate (2, 20, and 200 μM), sodium fluoride (5, 50, and 500 mM), okadaic acid (1, 10, and 100 μm), cyclosporine (50, 500 μg, and 5 mM), and calyculinA (0.1 and 1 μM).

In the second method, enzymatic activity of Mstp was examined using labeled myelin basic protein (MBP), histone, and casein. These substrates were phosphorylated either at tyrosine residues by tyrosine kinase (Abl tyrosine kinase, CST) or at serine–threonine residues by Erk kinase in separate reactions as described earlier [12]. The phosphatase activity of Mstp was determined by measuring the release of Pi from ^{32}P-labeled MBP, histone, and casein. In brief, Mstp was incubated with phosphorylated MBP, histone, and casein (2.5 μg each) in imidazole buffer (25 mM, pH 7.0, containing 0.05% β-mercaptoethanol, and 0.1 mg of bovine serum albumin per ml) at 37 °C. After incubation for a specific period, the reaction was terminated by the addition of 200 μl of 20% trichloroacetic acid (TCA) and the supernatant was used to determine released ^{32}P. Counts were taken in a scintillation counter (Beckman, USA).

Auto-phosphorylation of PknA and PknB. Purified PknA and PknB were autophosphorylated using a modified protocol as described previously [4,5]. In brief, GST-PknA and B (2.5 μg each) were bound to glutathione–Sepharose 4B and autophosphorylated by incubating with 2 μCi of [γ-^{32}P]ATP in kinase buffer (50 mM Tris–HCl, pH 7.6, 50 mM NaCl, and 10 mM $MnCl_2$) for 30 min at room temperature. After incubation, resin-bound phosphorylated PknA and PknB were washed three times with wash buffer (50 mM Tris, pH 7.4, 1 mM $MnCl_2$, 1 mM DTT, and 1 mM BSA) to remove free ATP. PknA and PknB were eluted from Sepharose beads using elution buffer (1% SDS and 50 mM EDTA), run on 10% SDS–PAGE, and autoradiographed.

Effect of Mstp on autophosphorylated PknA and PknB. Dephosphorylation of phosphorylated PknA and PknB by Mstp was examined by measuring release of ^{32}Pi. Glutathione–Sepharose 4B beads bound phosphorylated GST-PknA and GST-PknB (2.5 μg each) were incubated with Mstp (2 μg) for different time intervals. After incubation, beads were washed twice with wash buffer to remove liberated ^{32}Pi and proteins were eluted using elution buffer at 65 °C for 10 min as reported earlier [13]. Radioactivity was measured with a scintillation counter. Decrease in counts of phosphorylated PknA/PknB in the presence of Mstp is a measure of dephosphorylation activity of Mstp.

Immunoelectron microscopy. Immunogold labeling was used to localize Mstp in mycobacterial cells. *M. tuberculosis* cells were prepared for immunogold electron microscopy as described earlier [14]. The sections were viewed at an acceleration voltage of 80 kV under a Philip CM-10 transmission electron microscope.

Analysis of mstp and pknB in other mycobacterial strains. Southern blot analysis was carried out to reveal the presence of homologues of *mstp* and *pknB* in other mycobacterial species. Genomic DNA was isolated from various mycobacterial species. Genomic DNAs (1 μg each) from *M. tuberculosis* Erdman, *M. tuberculosis* H_{37}Ra, *M. bovis* BCG, *M. smegmatis* LR222, and *M. fortuitum* were digested with restriction enzyme (*Bam*HI). Digested products were separated by electrophoresis on 1% agarose gel at 25–30 V for 16 h. The DNA fragments were transferred onto Hybond-N membrane (Amersham), cross-linked by UV irradiation, and hybridized with ^{32}P-labeled fragment containing the complete coding region of *mstp* in 50% formamide at 42 °C for 16 h. Blots were washed once with 2× SSC-0.1% SDS at room temperature for 30 min followed by two washes with 0.1× SSC, 0.5% SDS at 65 °C for 30 min and subjected to autoradiography.

Blot was deprobed using standard protocol and then reprobed with ^{32}P-labeled fragment containing the complete coding region of *pknB*. Finally, blot was developed as described above.

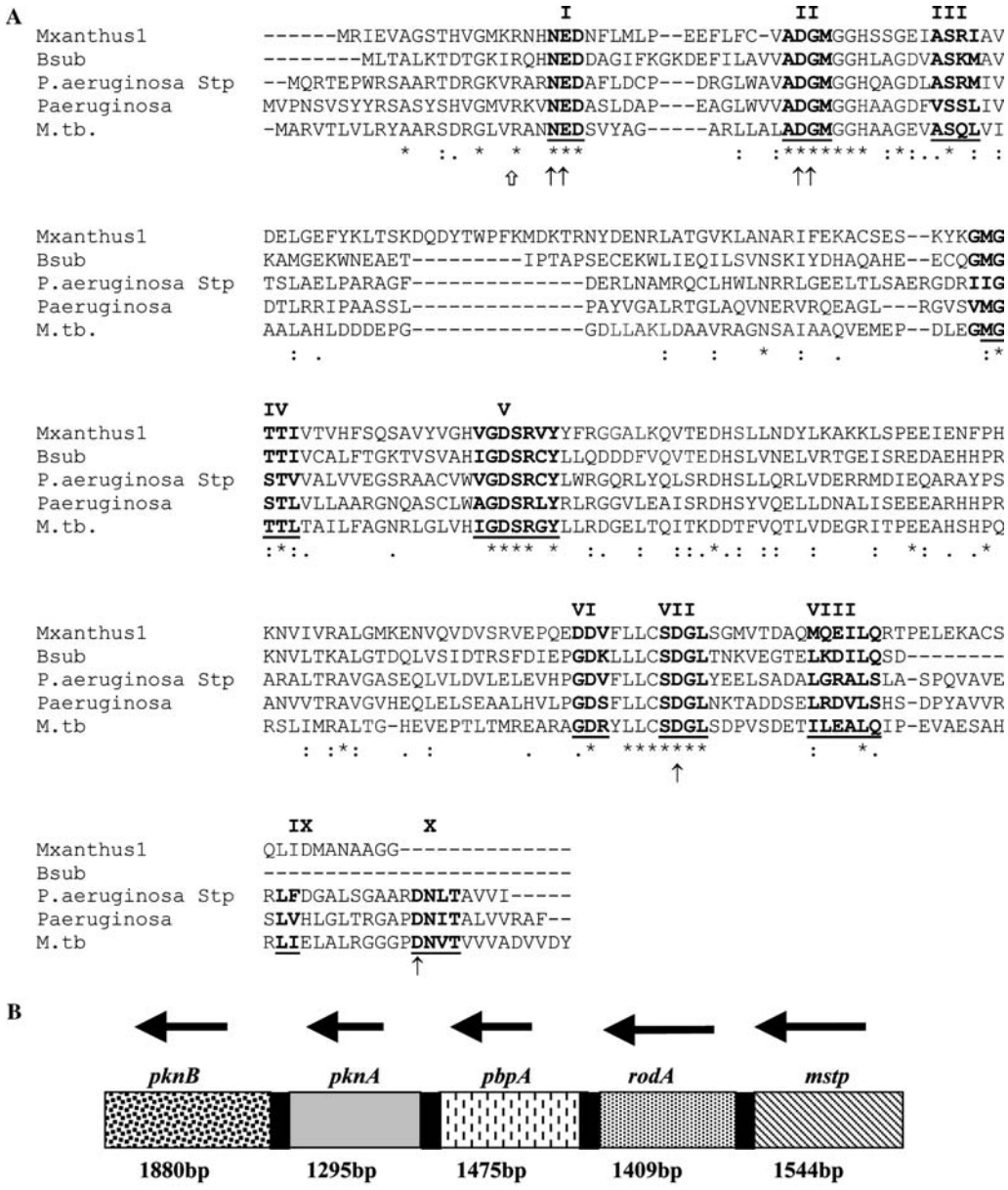

Fig. 1. Sequence analysis of Mstp. (A) Comparison of Mstp with Ser/Thr phosphatases of PP2C family of other organisms. Mstp of *M. tuberculosis* was aligned with Stp of *P. aeruginosa*, Pph1 of *M. xanthus*, SpoE II of *B. subtilis*, and PPP of *P. aeruginosa* using ClustalW software. Identical amino acids are indicated by asterisks and high similarity is indicated by double dots. The gaps are introduced to optimize the alignment and are indicated by the dashes. Various motifs described in the text are marked and shown in bold. Residues involved in binding metal and phosphate ions are indicated by small filled and open arrows, respectively. (B) Genetic organization of serine threonine kinases (*pknA*, *pknB*) and mycobacterial Ser/Thr phosphatase (*mstp*). A 7.60 kb region of *M. tuberculosis* genome encodes five overlapping ORFs. Arrows indicate the orientation of ORFs. Overlapping sequences between the ORFs are indicated by black region.

Results

Sequence analysis of mycobacterial Ser/Thr phosphatase

Bacterial Ser/Thr phosphatases characterized so far have been shown to regulate a complex array of processes. To relate Mstp of *M. tuberculosis* with other functionally characterized Ser/Thr phosphatases, comparative sequence analysis was performed using bioinformatic approaches. Members of the PPM family (which also include PP2C subfamily) of Ser/Thr phosphatases are characterized by the presence of eleven motifs, of which eight are highly conserved in all members [15,16]. Multiple sequence alignment of the amino acid sequence of Mstp with other bacterial PP2C family phosphatases revealed that Mstp shares significant homology with these phosphatases. Moreover, Mstp has ten out of eleven motifs, including all the eight motifs that are universally conserved and characteristic of PP2C phosphatases. The positions of residues involved in binding metal ions and the phosphate group of the substrates were conserved in Mstp (Fig. 1A).

Genomic organization of *mstp* in *M. tuberculosis* revealed that this gene is a part of 7.60 kb region that encodes five overlapping ORFs. This gene cluster consists of two Ser/Thr kinases (*pknA* and *pknB*) and other genes involved in cell wall synthesis(*pbpA* and *rodA*) (Fig. 1B).

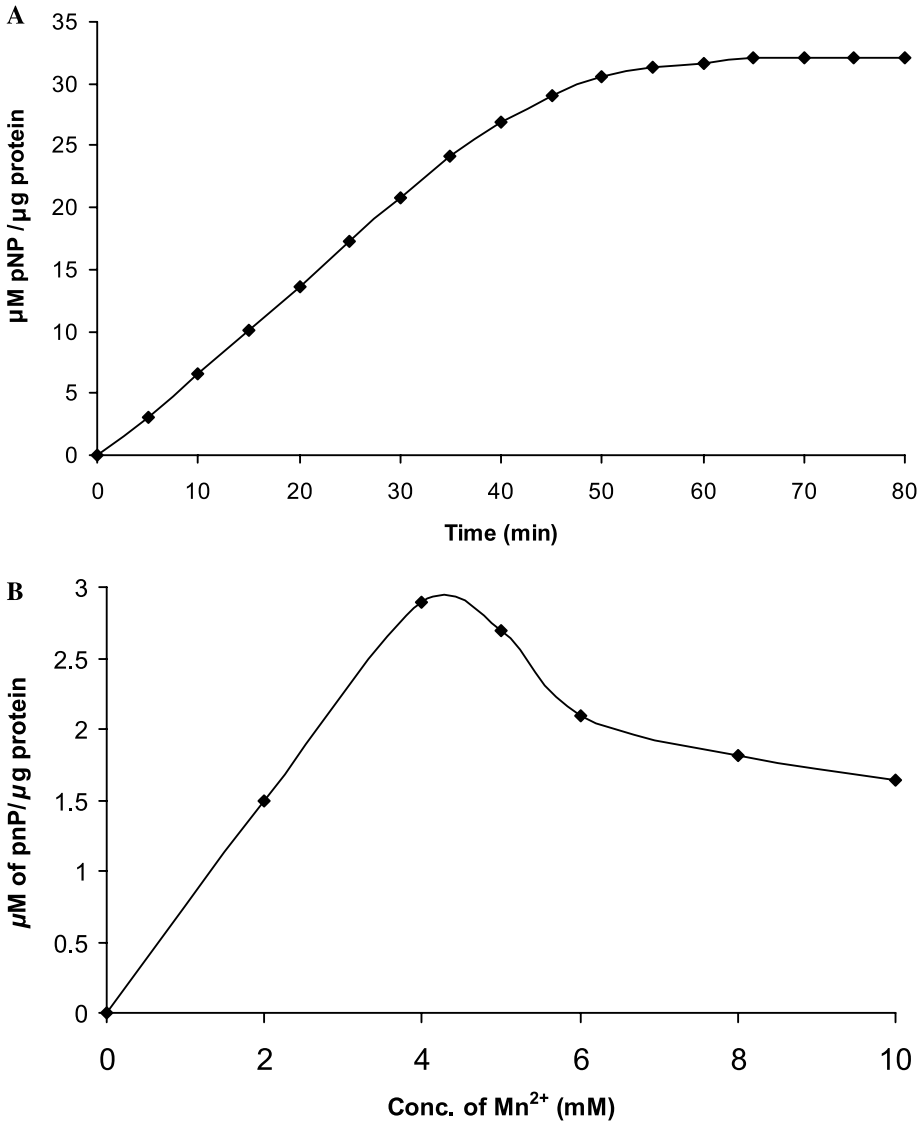

Fig. 2. Biochemical characterization of Mstp. Enzymatic activity of purified Mstp was determined by the hydrolysis of a low molecular weight substrate, pNPP. Purified Mstp (2 µg) was incubated with pNPP (10 mM) in a phosphatase assay buffer at room temperature and at the end of incubation; absorbance was recorded at 405 nm. (A) Time dependent activity of Mstp. (B) Effect of various concentrations of Mn^{2+}. The micromole of pNP liberated/µg of protein was calculated as described in the Materials and methods.

Expression and purification of Mstp, PknA, and PknB

Mstp protein was purified using Ni-NTA affinity resin as histidine-tagged protein. Similarly, PknA and PknB were purified as GST-fusion protein by using glutathione–Sepharose 4B beads. All the three proteins migrated on 10% SDS–PAGE concordant with their predicted molecular weights (data not shown).

Biochemical characterization of Mstp

The phosphatase activity of purified Mstp was examined by hydrolysis of *p*-nitrophenyl phosphate (pNPP). The phosphatase activity of the protein was directly proportional to the amount of *p*-nitrophenol formed and detected as yellow colored *p*-nitrophenolate ion by measuring absorbance at 405 nm (Fig. 2A). Activity of Mstp was Mn^{2+} dependent and maximal activity was obtained at 4 mM Mn^{2+} (Fig. 2B). The requirement of Mn^{2+} was very specific as other cations such as Mg^{2+}, Ca^{2+}, Ba^{2+}, Zn^{2+}, and Li^{+} (4 mM each) failed to substitute Mn^{+2} (data not shown).

Effect of various inhibitors on the activity of Mstp was also studied. Sodium orthovanadate, a potent inhibitor of tyrosine phosphatases, and calyculinA, a specific inhibitor of PP1 and PP2A family of Ser/Thr phosphatases, had no effect on the phosphatase activity of Mstp. Sodium fluoride, a non-specific inhibitor of Ser/Thr phosphatases and cyclosporine, a specific inhibitor of PP2B family of Ser/Thr phosphatase, inhibited around 25% of the activity of Mstp. The activity of Mstp was not affected by okadaic acid, a potent inhibitor of PP2A and PP2B family of phosphatases (data not shown). Insensitivity to okadaic acid is one of the unique characteristics of the PP2C subfamily [11].

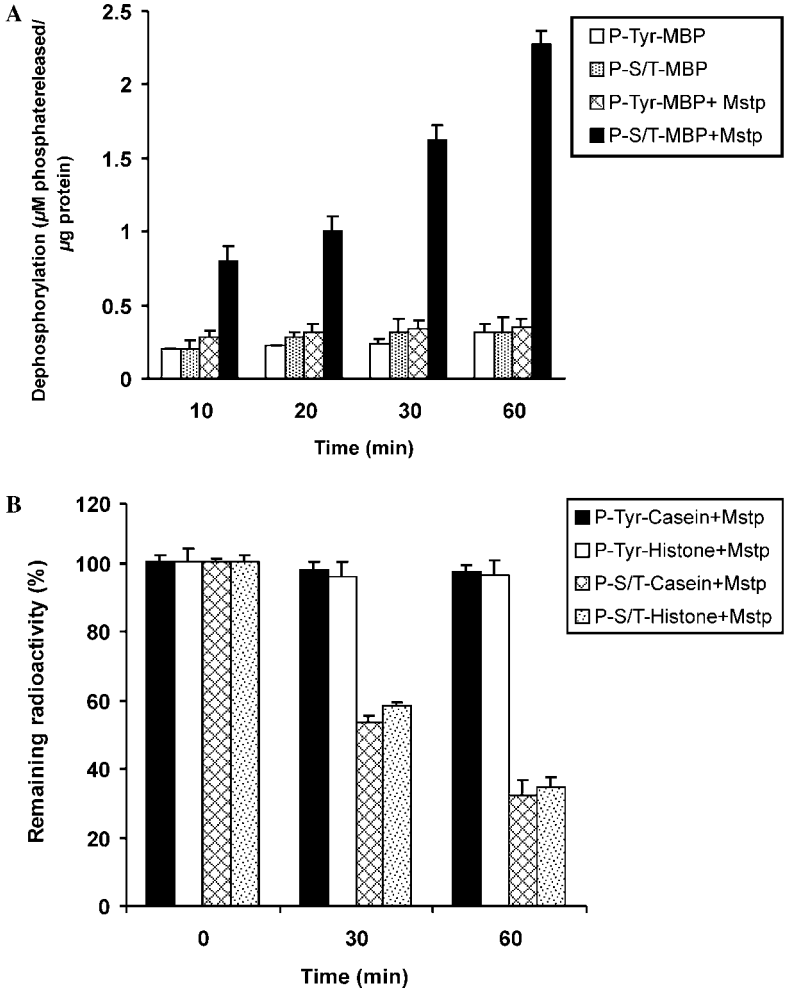

Fig. 3. Phosphatase activity of Mstp using phosphorylated MBP, histone, and casein as substrates. MBP, histone, and casein (2.5 μg each) phosphorylated at Ser/Thr or Tyr residues were incubated with purified Mstp (2 μg) for various time periods at 37 °C. The reactions were terminated by the addition of 20% TCA and supernatant was counted in a scintillation counter to determine released ^{32}P. (A) The phosphatase activity was displayed as μM phosphate released/μg protein and calculated from the labeled MBP. (B) Shown is the remaining phosphorylated casein and histone after incubation with Mstp for different time periods. Each value is the average of two individual reactions and representative of three experiments.

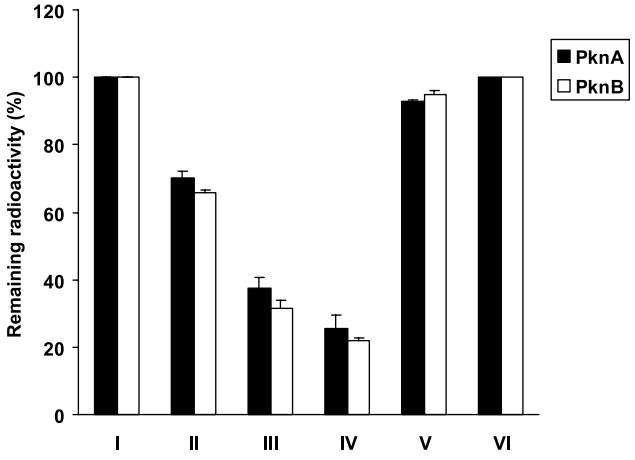

Fig. 4. Mstp dephosphorylates the phosphorylated form of Ser/thr kinases (PknA and PknB). The Mstp-mediated dephosphorylation of phosphorylated PknA and PknB was examined by measuring the decrease in the PknA and PknB bound radioactivity after incubation with Mstp. Shown is the remaining PknA and PknB bound radioactivity after incubation with Mstp. I–IV, 0, 10, 30, and 60 min incubation with Mstp, respectively; V, 60 min incubation without Mstp; and VI, 60 min incubation with heat inactivated Mstp. Each value is the average of two individual reactions and representative of three experiments.

Mstp is a Ser/Thr specific phosphatase

Substrate specificity of Mstp was determined by examining its ability to dephosphorylate substrates such as MBP, histone, and casein, phosphorylated either at tyrosine or Ser/Thr residues. Purified Mstp dephosphorylated MBP, histone, and casein, phosphorylated at Ser/Thr residues (Figs. 3A and B). However, Mstp failed to dephosphorylate MBP, histone, and casein phosphorylated at tyrosine residues (Figs. 3A and B).

The phosphorylated PknA and PknB are substrates for Mstp

In an attempt to identify the natural substrate of Mstp, the effect of Mstp on the autophosphorylated PknA and PknB was studied. Incubation of phosphorylated PknA and PknB with Mstp resulted in 75% and 79% dephosphorylation, respectively, in 60 min. Heat inactivated Mstp (80 °C, 10 min) failed to dephosphorylate phosphorylated PknA and PknB (Fig. 4).

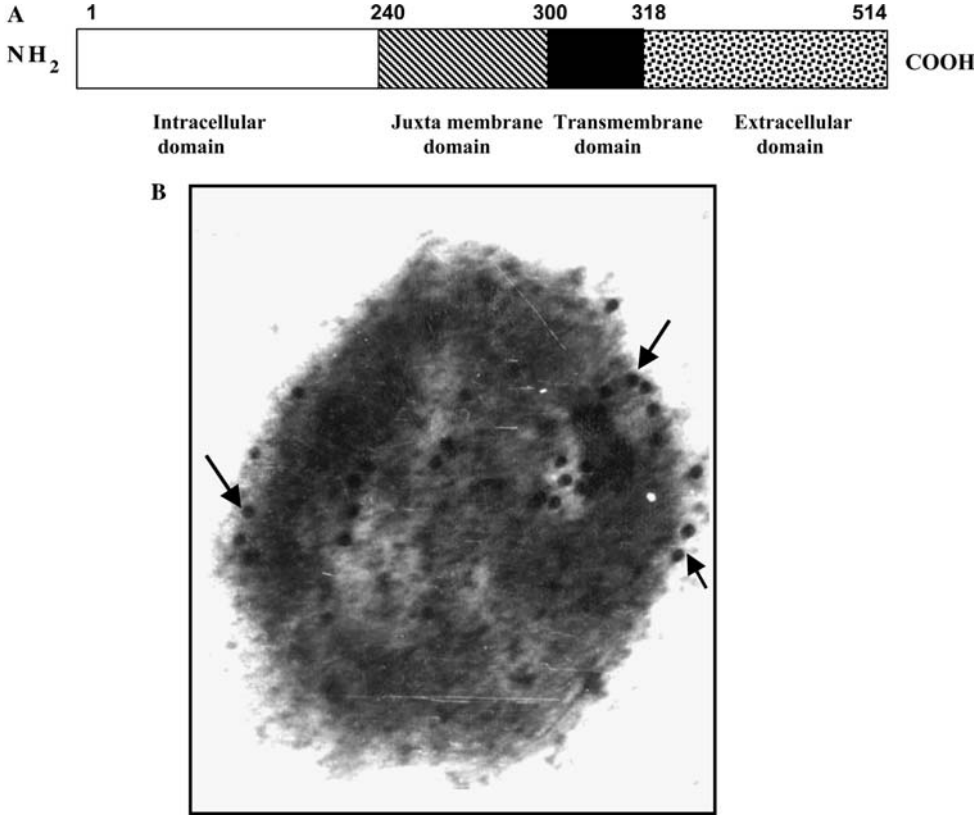

Fig. 5. Localization of Mstp in mycobacteria by immunogold electron microscopy. (A) Structural components of Mstp. Shown are the positions of different domains in the Mstp. (B) Localization of Mstp by immunogold electron microscopy. Cross section of *M. tuberculosis* H37Rv cells that were labeled with anti-Mstp antiserum and anti-rabbit antibody loaded with 1 nm colloidal gold particles (black dots). Sections were visualized by transmission electron microscope. Arrows indicate the deposition of gold particles.

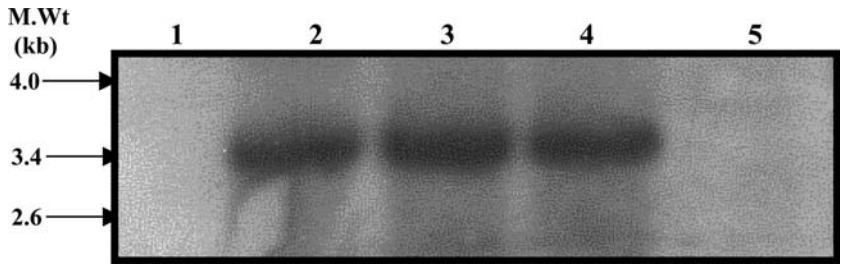

Fig. 6. Presence of *mstp* in other mycobacterial species. Genomic DNA (1 μg) from various strains of Mycobacteria was digested by *Bam*HI, resolved by 1% agarose gel at 20–30 V for 16 h, and transferred to Hybond-N membrane. Hybridization was performed using ^{32}P-labeled *mstp* as probe and blot was developed by autoradiography. Lane 1, *M. fortuitum*; lane 2, *M. bovis* BCG; lane 3, *M. tuberculosis H37Ra*; lane 4, *M. tuberculosis* Erdman; and lane 5, *M. smegmatis*.

Localization of Mstp in mycobacterial cells

Bioinformatic analysis suggested that Mstp is a trans-membrane protein. As shown in Fig. 5A, Mstp has a trans-membrane domain of 18 amino acids (amino acids 300–318).

Immunogold electron microscopy has been used to localize MspA, a highly stable oligomeric porin, in the cell wall of *M. smegmatis* [17], a similar technique was used to localize Mstp in *M. tuberculosis*. Cross-sections of *M. tuberculosis* H37Rv treated with anti-Mstp and gold loaded anti-rabbit antibodies revealed the labeling at and close to the cell wall (Fig. 5B). Immunogold labeling showed that gold particles were also deposited in the cytoplasm of *M. tuberculosis* (Fig. 5B).

Analysis of presence of mstp and pknB in other species of mycobacteria

Southern blot analysis revealed that gene homologous to *mstp* was present in slow growing species *M. tuberculosis* H_{37}Ra and *M. bovis* BCG and absent in the saprophytic fast growing organisms, *M. smegmatis* and *M. fortuitum* (Fig. 6). Similar distribution of *pknB* was also observed in various pathogenic and saprophytic mycobacterial species (data not shown).

Discussion

The conspicuous presence of kinases and phosphatases in *M. tuberculosis* suggests that these signaling molecules may have a specific relevance in the events related to survival and/or pathogenesis of this bacterium. However, the significance of phosphorylation/dephosphorylation events mediated by these kinases and phosphatases has not been elucidated. Understanding the role of these protein kinases and phosphatases would give us an important insight into the regulation of the signaling network involving host–pathogen interaction.

Genome sequence of *M. tuberculosis* has shown the presence of a putative phosphoprotein phosphatase, now designated as Mstp. In an attempt to characterize Mstp and identify its possible substrates in mycobacteria, *mstp* was cloned, expressed in *E. coli*, and purified as histidine-tagged protein. Mstp was found to be a Mn^{2+} dependent, Ser/Thr specific phosphatase. Studies with specific inhibitors of phosphatases of various families revealed that Mstp belongs to the PP2C subfamily of Ser/Thr phosphatases. Bioinformatic analysis also supported this observation (Fig. 1A).

Genome sequence analysis of *M. tuberculosis* indicated that *mstp* is present in a gene cluster with two Ser/Thr kinases (*pknA* and *pknB*) and two genes involved in cell wall synthesis (*pbpA* and *rodA*) [9,18,19]. It has also been proposed that *pknA*, *pbpA*, and *rodA* encode putative morphogenic proteins and belong to the SEDS (shape, elongation, division, and sporulation) protein family [4,20]. Members of this family of genes have been shown to be involved in controlling cell shape and peptidoglycan synthesis in *Bacillus subtilis* [20] and *E. coli* [21]. Therefore, presence of these kinases and Mstp in the same gene cluster of *M. tuberculosis* suggested a possible regulatory role of Mstp in mycobacterial cell division. Similar genetic organization of cognate Ser/Thr kinases and phosphatases has also been observed in the case of *B. subtilis* [16], *Mycoplasma genitalium* [22], *Pseudomonas aeruginosa* [23], and *Streptococcus agalactiae* [2]. Recently, it has been shown that mutants defective for Ser/Thr kinase (Stk1) or both Stk1 and its cognate phosphatases (Stp1) in *S. agalactiae* exhibited pleiotropic effects on cell growth, cell segregation, and virulence [2]. In the present study, an attempt was made to determine if PknA and PknB could serve as substrates for Mstp. Mstp dephosphorylated the phosphorylated form of both PknA and PknB (Fig. 4). These observations suggest that Mstp acts as a regulator of these kinases and hence may play a role in the regulation of cell division and growth of *M. tuberculosis*.

Bioinformatic tools suggested that Mstp is a trans-membrane protein (Fig. 5A). This observation was confirmed by Immunogold labeling experiments that showed the deposition of gold particles on the surface of *M. tuberculosis*, suggesting that Mstp is a trans-membrane protein (Fig. 5B). Southern blot analysis showed that *mstp* and *pknB* were restricted to slow growing mycobacterial species (Fig. 6). In an earlier report, *pknA* has already been shown to be absent in *M. smegmatis* [4].

Trans-membrane localization of Mstp prompts us to speculate that this protein after sensing the environment may control the timing of cell division or septation by regulating the phosphorylation state of PknA and PknB. Similar hypothesis has also been proposed earlier [19]. This hypothesis is further supported by the observations that members of the PP2C family of proteins are reported to be specifically expressed during the decisive stages of the cell's life cycle. These stages include responses to various stresses [24], reactivation from dormancy in pathogenic organisms [25], and cell growth and development [26].

Based on these findings, it can be suggested that Mstp may play a crucial role in cell division of *M. tuberculosis*. To elucidate the detailed functional aspect of this protein, experiments are in progress to create a knockout of *mstp* in *M. tuberculosis*

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