JOURNAL OF BACTERIOLOGY, Mar. 2006, p. 2134–2143 Vol. 188, No. 6
0021-9193/06/$08.00+0 doi:10.1128/JB.188.6.2134–2143.2006

MprAB Is a Stress-Responsive Two-Component System That Directly Regulates Expression of Sigma Factors SigB and SigE in *Mycobacterium tuberculosis*§

Hongjun He,[1] Raymond Hovey,[1]† Jason Kane,[2] Vineet Singh,[1]‡ and Thomas C. Zahrt[1]*

Department of Microbiology and Molecular Genetics[1] and Department of Pediatrics,[2] Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Received 22 August 2005/Accepted 18 December 2005

The genetic mechanisms mediating the adaptation of *Mycobacterium tuberculosis* within the host are poorly understood. The best-characterized regulatory systems in this organism include sigma factors and two-component signal transduction systems. *mprAB* is a two-component system required by *M. tuberculosis* for growth in vivo during the persistent stage of infection. In this report, we demonstrate that MprAB is stress responsive and regulates the expression of numerous stress-responsive genes in *M. tuberculosis*. With DNA microarrays and quantitative real-time reverse transcription-PCR, genes regulated by MprA in *M. tuberculosis* that included two stress-responsive sigma factors were identified. Response regulator MprA bound to conserved motifs in the upstream regions of both *sigB* and *sigE* in vitro and regulated the in vivo expression of *sigB* and *sigE* in *M. tuberculosis*. In addition, *mprA* itself was induced following exposure to stress, establishing a direct role for this regulatory system in stress response pathways of *M. tuberculosis*. Induction of *mprA* and *sigE* by MprA in response to stress was mediated through the cognate sensor kinase MprB and required expression of the extracytoplasmic loop domain. These results provide the first evidence that recognition of and adaptation to specific stress in *M. tuberculosis* are mediated through activation of a two-component signal transduction system that directly regulates the expression of stress-responsive determinants.

Tuberculosis remains a serious health concern worldwide, with nearly 8 million new cases documented each year. According to the World Health Organization, it is estimated that approximately 25 million individuals will succumb to the disease in the coming decade (34). Infection with the causative agent, *Mycobacterium tuberculosis*, can result in several disease scenarios depending on the immune state of the host. Whereas infection of an immunocompromised host frequently leads to acute tuberculosis, infection of an immunocompetent host by *M. tuberculosis* frequently results in establishment of latent infection (5). Despite the fact that 1.8 billion people are latent carriers of *M. tuberculosis*, current antituberculosis drugs are largely ineffective against bacilli in this stage of infection. Thus, development of new therapeutic agents to treat latent infections requires a better understanding of the molecular mechanisms mediating long-term infection of *M. tuberculosis* within the host.

The ability of *M. tuberculosis* to survive within the host requires resistance to various physiological and environmental stresses. Recent studies provide evidence that long-term infection by the tubercle bacillus requires expression of a complex array of genetic determinants, including those involved in intermediary and secondary metabolism, cell wall processes, stress responses, and signal transduction pathways (3, 28). Sigma factors and two-component signal transduction systems encode two major families of transcriptional regulators necessary for adaptation processes. Sigma factors regulate gene expression by binding to the RNA polymerase core and conferring promoter specificity. In *M. tuberculosis*, a total of 13 putative sigma factors have been annotated in the genomic sequence (4), several of which belong to the extracellular function (ECF) family, whose members frequently mediate resistance to external stress (17). Expression of sigma factors, including *sigB*, *sigE*, and *sigH*, has been shown to be required for the resistance of *M. tuberculosis* to various environmental stresses likely to be encountered in vivo (17–19, 26). Furthermore, a subset of these sigma factors is induced during the growth of *M. tuberculosis* within macrophages and is required for aspects of virulence in animal model systems (1, 8, 12, 16, 17, 19).

Bacterial adaptation also requires genes from the two-component signal transduction family (32). Two-component systems are comprised of a histidine kinase sensor and a cognate cytoplasmic response regulator. Collectively, these protein pairs sense environmental stimuli and initiate adaptive transcriptional programs by means of phosphotransfer reactions. Eleven complete and several orphaned two-component signal transduction members are present in the *M. tuberculosis* genome (4), many of which are important for aspects of virulence in the tubercle bacillus (14, 22, 23, 25, 27, 35). One of these systems, *mprAB*, is required by *M. tuberculosis* for persistent infection (35), is part of a 41 gene genomic island whose expression is induced during in vivo growth of *M. tuberculosis* (30), and is induced in *M. tuberculosis* during growth within an artificial granuloma

* Corresponding author. Mailing address: Department of Microbiology and Molecular Genetics, Medical College of Wisconsin, Milwaukee, WI 53226. Phone: (414) 456-7429. Fax: (414) 456-6535. E-mail: tzahrt@mcw.edu.

§ Supplemental material for this article may be found at http://jb.asm.org./

† Present address: Department of Biological Sciences, University of Wisconsin at Milwaukee, Milwaukee, WI 53201.

‡ Present address: Department of Microbiology and Immunology, Kirksville School of Osteopathic Medicine, Kirksville, MO 63501.

TABLE 1. Primers and oligonucleotides used in this study[a]

Primer or oligonucleotide	Sequence (5′→3′)	Application
sigB-GShiftF	ACAGCCGGCAGAGCGGTCA	Gel shift *sigB* upstream region
sigB-GShiftR	CCGATCGGCTTGCACTGTCAT	Gel shift *sigB* upstream region
sigEGS-F	CGTCCATGCCCGCAGCGTATGT	Gel shift *sigE* upstream region
sigEGS-R	AGTTCCATGGGAATTACCGTCGCGT	Gel shift *sigE* upstream region
sigHGS-F	TGCGATTCAGCGCTGGAAATAACC	Gel shift *sigH* upstream region
sigHGS-R	GTCGGCCATCTTGATTAACTGGGT	Gel shift *sigH* upstream region
sigEupts1	AGGTAACGGTATGGTTTCTAAGCCAAAGCTCAGATTGCTCATATA	Gel shift *sigE* wt distal site
sigEupbs1	TATATGAGCAATCTGAGCTTTGGCTTAGAAACCATACCGTTACCT	Gel shift *sigE* wt distal site
sigEupts2	TGCTCATATATGGCCCATACGCCGGTACGCGACGGTAATTCCCAT	Gel shift *sigE* wt proximal site
sigEupbs2	ATGGGAATTACCGTCGCGTACCGGCGTATGGGCCATATATGAGCA	Gel shift *sigE* wt proximal site
sigEupts3	AGGTAACGGTATGGTTAAAAAGCCAAAGCTCAGATTGCTCATATA	Gel shift *sigE* mutant distal site
sigEupbs3	TATATGAGCAATCTGAGCTTTGGCTTTTTAACCATACCGTTACCT	Gel shift *sigE* mutant distal site
sigE80F	GGACAGCGCAGGTAACGGTAT	Gel shift *sigE* dist. and prox.
sigE80R	ACCGTCGCGTACCGGCGTAT	Gel shift *sigE* dist. and prox.
sigE60mF	ATGGTTAAAAAGCCAAAGCTCAGATTG	Gel shift *sigE* dist. and prox.
sigBGS-R-MluI2	CCACGCGTAAGCCGGACTTTGAGTTCCCACCC (RP for *sigB* upstream region)	DNase I *sigB* antisense
sigBGS-RS2	CGCGTAAGCCGGACTTTGAGTTCCCACCC (primer for sequencing ladder)	Sequencing *sigB*
sigE-R-MluI3	CCACGCGTCAACCCGGGGTCCGCCG (RP for *sigE* upstream region)	DNase I *sigE* antisense
sigE-RS3	CGCGTCAACCCGGGGTCCGCCG (primer for sequencing ladder)	Sequencing *sigE*
Rv0981NotI	GCGGCCGCTCGGCCGCGCGGCTGTGGACACTAACT	Cloning of *mprAB*
Rv0983NotI	GCGGCCGCCTAGGTTGCTCTTCCTGTACTAGG	Cloning of *mprAB*
mprB(del39aa)F1	GATGTCAACGCGATGCTGGTCAACCGGGTGCTTGCCATCCGT	Site-directed mutagenesis of *mprB*
mprB(del39aa)R1	ACGGATGGCAAGCACCCGGTTGACCAGCATCGCGTTGACATC	Site-directed mutagenesis of *mprB*
sigARTsense	GCCGATGACGACGAGGAG	qRT-PCR
sigARTantisense	GGCGGATGCGGTGAGTTC	qRT-PCR
sigBRTsense	GGTCTGATCCGAGCGATGG	qRT-PCR
sigBRTantisense	GATGGTGCGGCTCTGGTC	qRT-PCR
mprARTsense	CATTGCTGGAGATGCCTGATCG	qRT-PCR
mprARTantisense	CTCGGTCTTGCGGCGTAG	qRT-PCR
sigERTsense	AATCGCAACTTTGCGTTGCCG	qRT-PCR
sigERTantisense	ATGAGACATGCTGGTCGGACTCAA	qRT-PCR
Rv1057RTsense	GACGGTGCCATCGCCTACG	qRT-PCR
Rv1057RTantisense	GACCAACCCGCCGATCTCG	qRT-PCR
Rv1813cRTsense	GGTTGCCTATCCCTCCGATTATCC	qRT-PCR
Rv1813cRTantisense	TCTAGTGCGACTTGCTCTGCTC	qRT-PCR
Rv2053cRTsense	CTGCTGGTGCCGCCGATC	qRT-PCR
Rv2053cRTantisense	GTGCCGCTCTCGCCGAAG	qRT-PCR
Rv2626cRTsense	GAGATGCTCAACGTCATGGAAGAAC	qRT-PCR
Rv2626cRTantisense	GCAGGTGTCGGGCGATGTC	qRT-PCR
Rv2627cRTsense	AGGAGGAGGAGTCGCTGATCG	qRT-PCR
Rv2627cRTantisense	GCCACTGGGACACCGAGAATC	qRT-PCR
Rv2628RTsense	ACCAGGAGGCGATGATGAATCTAG	qRT-PCR
Rv2628RTantisense	CGGACACGGTGCTAGTGATCTC	qRT-PCR

[a] RP, reverse primer; wt, wild type; dist., distal; prox., proximal.

model system (11). While the biological contribution of MprAB to *M. tuberculosis* virulence is unknown, *mprAB* is induced in *M. tuberculosis* in response to nutrient starvation (2) and by low concentrations of sodium dodecyl sulfate (SDS) in a SigE-dependent manner (19). In addition, MprA directly regulates its own expression and that of the downstream gene *pepD*, a putative trypsin-like serine protease, through cooperative recognition of a 19-bp direct repeat motif, an activity that requires phosphorylation of MprA at active-site Asp48 (9). Here we report that *mprA* is induced in response to several environmental stresses and provide evidence that MprA functions in the stress response network in *M. tuberculosis* through direct regulation of stress-responsive genetic determinants.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and antibiotics. *M. tuberculosis* strains are derivatives of H37Rv and were grown aerated (150 rpm) at 37°C in Middlebrook 7H9 broth or on 7H10 agar medium (Becton Dickinson) supplemented with 0.5% glycerol, 10% (vol/vol) oleic acid-albumin-dextrose-catalase (Becton Dickinson), and 0.05% (vol/vol) Tween 80 (Sigma). When required, kanamycin sulfate and hygromycin B were added to the medium at a final concentration of 25 and 50 μg/ml, respectively. For DNA microarray or real-time quantitative reverse transcription-PCR (qRT-PCR) experiments, individual *M. tuberculosis* strains were grown to mid-exponential phase ($A_{600} \approx 0.6$ to 0.8), divided into three separate cultures, and either left untreated or exposed to stress for 90 min with shaking at 37°C.

Construction of *M. tuberculosis* mutants. The *M. tuberculosis mprA*::Km^r mutant has been described previously (35). This strain is unable to make MprA and expresses reduced levels of MprB due to polarity imparted from the Km^r insertion. To construct strains TB1, TB2, and TB4, the *mprA*::Km^r mutant was transformed with derivatives of integrating plasmid pTZ189 (35) expressing various alleles of *mprA* and *mprB* under the control of its endogenous promoter. pTZ189 is a derivative of pMV306 that carries a Hyg^r cassette in place of the Km^r cassette. TB1 carries the pTZ189 vector alone. TB2 carries pTZ189 expressing wild-type MprA and a deletion derivative of MprB lacking 39 amino acids from the extracytoplasmic loop domain (amino acids 95 to 133). TB4 contains pTZ189 expressing wild-type *mprA* and *mprB*. Primers used to construct *M. tuberculosis* mutants are described in Table 1.

RNA extraction and purification. RNA was extracted essentially as described previously (10) except that cells were first treated with RNA*later* (Ambion) at 4°C for 24 h prior to lysis to inactivate RNases. Resulting RNA was treated twice with TurboDNase (Ambion) at 37°C for 2 h and purified by affinity chromatography using RNeasy purification columns (QIAGEN). For microarray studies, mRNA was enriched from total RNA (10 μg) using the MICROB*Express* bacterial mRNA purification kit as recommended by the manufacturer (Ambion).

Microarray studies. *M. tuberculosis* microarrays were constructed using the *M. tuberculosis* array-ready oligonucleotide set (version 1.0). Oligonucleotides were printed in duplicate on each slide as previously described (10). mRNA-enriched samples obtained from three independent cultures of wild-type *M. tuberculosis* H37Rv or the *mprA*::Km^r mutant were used to generate labeled cDNA by reverse transcription using SuperScript III reverse transcriptase (Invitrogen), a cocktail of synthetically generated decamers (7), and Cy3- or Cy5-dCTP (Am-

ersham). Dye flip control experiments were also conducted, resulting in a total of six heterotypic hybridizations for each experiment. For all experiments, hybridization reactions were carried out between H37Rv and the *mprA*::Km^r mutant. Labeled cDNAs were purified, mixed, hybridized, and analyzed as described previously (10). Cy-3 and Cy-5 intensities for each spot were quantified using ScanAlyze (Stanford), the median background values subtracted, and the values normalized based on the total intensity of good-quality spots over background values (11). Signals were considered of good quality if the intensity of >60% of the pixels in at least one channel was >1.5-fold higher than the average background. Data were analyzed using SAM (significance analysis of microarrays) (31) to determine significant expression changes. *M. tuberculosis* genes exhibiting a statistical significance (one class response; k, nearest-neighbor imputer; false-discovery rate, <10%; $q < 0.05$; and a 1.8-fold or greater difference in fluorescence from baseline) in at least four out of six dye swap experiments were considered biologically significant and thereby regulated by MprA.

qRT-PCR assays. Specific primer pair sets (Table 1) for target genes were generated using Beacon Designer 2.0 (Biosoft International). The *mprA* primer set amplifies a fragment upstream of the Km^r insertion, allowing detection of *mprA* transcripts in the *mprA*::Km^r mutant. cDNAs were synthesized using 0.5 μg of DNase-treated RNA in reaction buffer containing arbitrary decamers and SuperScript III. Control reaction mixtures lacking reverse transcriptase were also used to confirm the absence of contaminating genomic DNA in all samples. All PCRs were performed using the Bio-Rad iCycler iQ system, and melt curve analysis was conducted to ensure amplification of a single product. Transcript levels of each putative MprA-regulated gene were normalized to that of *sigA* (15) and expression value calculated using the $2^{-\Delta\Delta c_T}$ method (13). For each condition, RNA from three independent cultures was utilized and the mean and standard deviation determined. For experiments measuring gene expression between treated and untreated cultures of *M. tuberculosis* H37Rv or the *mprA*::Km^r mutant, gene expression values from stressed cultures were normalized to expression values from a common untreated culture.

EMSAs and DNase I protection assays. His-MprA and the phosphorylation-deficient His-MprA(D48A) mutant were used in DNA binding reactions (9). For electrophoretic mobility shift assays (EMSAs), DNA fragments derived from the upstream regions of *sigB*, *sigE*, and *sigH* were amplified from the *M. tuberculosis* H37Rv genome or generated by annealing complementary single-stranded DNA oligonucleotides, end-labeled with [γ-^{32}P]ATP (MP Biomedicals) using T4 polynucleotide kinase, and incubated at room temperature for 20 min with His-MprA that had been previously phosphorylated in vitro with acetyl phosphate (9). Reaction mixtures were electrophoresed on a 5% nondenaturing polyacrylamide gel for 2.5 to 3.0 h at 120 V at 4°C. Gels were dried and exposed to Biomax MR (Kodak) X-ray film overnight. For DNase I footprinting, DNA fragments encompassing the *sigB* and *sigE* upstream regions were PCR amplified, cloned into pCR2.1-TOPO (Invitrogen), digested with MluI and EcoRI, separated on an agarose gel, purified, and end labeled with [α-^{32}P]dCTP (MP Biomedicals) using Klenow enzyme. Thirty-five nanograms of labeled DNA was then incubated with phosphorylated His-MprA at room temperature for 20 min and treated with 0.2 units of DNase I at room temperature for 2 min, and reactions were stopped by the addition of 2× stop solution. Reaction products were phenol-chloroform extracted and precipitated with 3 volumes of 100% ethanol. DNA was suspended in 4 μl loading solution, loaded on a 6% denaturing polyacrylamide gel alongside a DNA sequencing ladder, and separated for 2 h at 60 W before the gel was dried and exposed to Biomax MR X-ray film at −80°C. The DNA sequencing ladder was prepared using the Sequenase version 2.0 DNA sequencing kit (USB). Primers and DNA oligonucleotides used to generate DNA fragments are described in Table 1.

Statistical analysis. Statistical analyses (analysis of variance and Fisher's protected least-significant difference analysis) of qRT-PCR values were performed with superANOVA (version 1.11; Abicus Software). Values were determined to be statistically significant at a P of <0.05.

Microarray accession numbers. Microarray hybrdization data have been deposited with the ArrayExpress public database (http://www.ebi.ac.uk/arrayexpress) with accession numbers E=MExP-552 and A-MExP-315.

RESULTS

Identification of MprA-regulated genes by DNA microarray analysis. To determine genes regulated by response regulator MprA, global gene expression profiling by DNA microarray analysis was conducted using wild-type *M. tuberculosis* H37Rv and the isogenic *mprA*::Km^r mutant. Individual genes were represented twice on each microarray, allowing gene expression profiles to be investigated in duplicate for each hybridization reaction. During the growth of wild-type *M. tuberculosis* H37Rv and the *mprA*::Km^r mutant under physiological conditions, a total of 141 genes in H37Rv were statistically determined to be regulated by MprA at levels that were ≥1.8-fold above the mutant levels (Table S1 in the supplemental material). Approximately 67% of these genes were expressed at higher levels in *M. tuberculosis* H37Rv than in the *mprA* mutant, suggesting that MprA functions predominantly as a transcriptional activator. MprA-regulated genes fell into multiple functional categories, including virulence, detoxification and adaptation, lipid metabolism, information pathways, cell wall processes, intermediary metabolism and respiration, and regulatory functions (4). Multiple genes from the PE/PPE gene family and many hypothetical and conserved hypothetical proteins were also regulated. When the complete panel of genes positively or negatively regulated by MprA were examined closer, a large subset of these genes were previously reported to be responsive to various stress conditions (2, 7, 11, 19, 24, 29, 33). Interestingly, two of these genes included the stress-responsive sigma factors *sigB* and *sigE*.

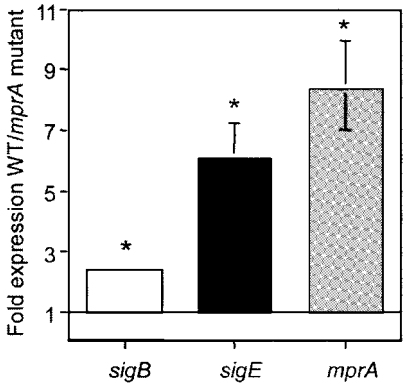


FIG. 1. MprA regulates the in vivo expression of *sigB* and *sigE*. Real-time qRT-PCR was used to measure the regulation of *sigB*, *sigE*, and *mprA* by MprA during the growth of *M. tuberculosis* under physiological conditions. Values represent mean differences (*n*-fold [±standard deviations]) between wild-type *M. tuberculosis* H37Rv and the *mprA*::Km^r mutant from experiments performed in triplicate. All values were determined to be statistically significant (*) ($P < 0.05$).

MprA regulates the in vivo expression of *sigB* and *sigE* in *M. tuberculosis*. Microarray analyses indicated that *sigB* and *sigE* were expressed at levels approximately threefold and approximately twofold higher, respectively, in wild-type *M. tuberculosis* H37Rv than in the *mprA*::Km^r mutant (Table S1 in the supplemental material). To confirm these microarray results, RNA from wild-type *M. tuberculosis* H37Rv and *mprA*::Km^r cultures were subjected to qRT-PCR analyses and *sigB* and *sigE* expression levels determined. Expression levels of *mprA* were also determined, as MprA was previously shown to autogenously regulate its own expression (9). For all genes, expression levels were normalized to that of *sigA*, a sigma factor whose expression has been shown to be constitutive under a variety of conditions (15). Expression of *sigB*, *sigE*, and *mprA* was confirmed to be higher in H37Rv than in the *mprA* mutant (Fig. 1). In particular, *sigB* and *sigE* expression was 2.4- ± 0.1-fold and 6.1- ±

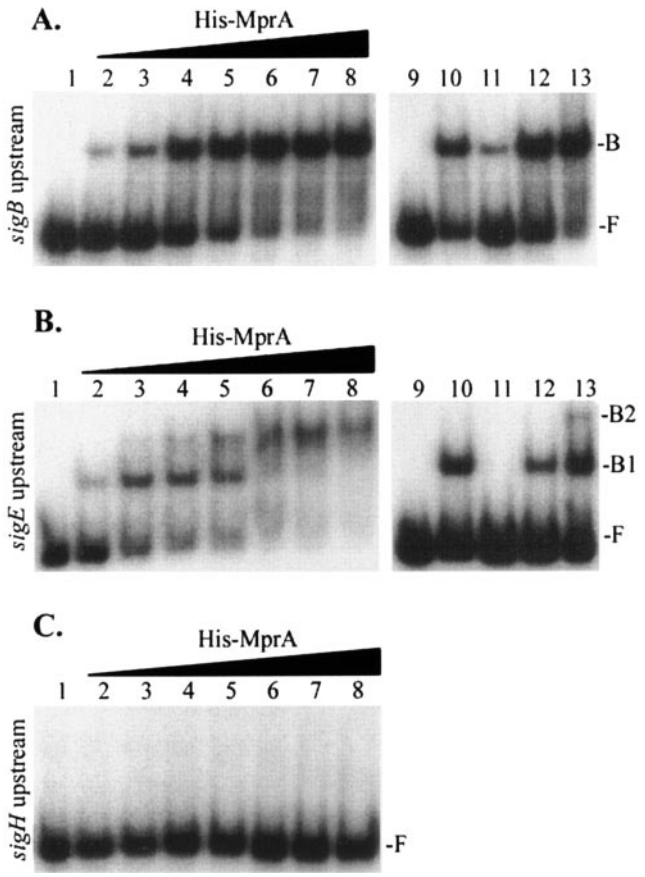


FIG. 2. MprA directly binds upstream regions of stress-responsive sigma factors. ^{32}P-labeled DNA from the *sigB* (A), *sigE* (B), and *sigH* (C) upstream regions were incubated with His-MprA~P and used in EMSAs. Seven nanograms of labeled DNA was used in all binding reaction mixtures. Labeled DNA was incubated in the absence (lane 1) or presence (lanes 2 to 8) of 0.44 to 35.0 pmol wild-type His-MprA~P. Binding specificity was determined by incubation of labeled DNA (lane 9) with 6 pmol His-MprA~P in the absence (lane 10) or presence of a 150-fold excess of cold DNA from either the *mprA* upstream region (lane 11) or *PPE19* upstream region (lane 12). Labeled DNA was also incubated with 14 pmol of the phosphorylation-deficient His-MprA(D48A) mutant (lane 13). F, free DNA; B, bound DNA.

1.2-fold higher, respectively, in *M. tuberculosis* H37Rv than in the *mprA* mutant ($P < 0.05$). In addition, expression of *mprA* was 8.3- ± 1.6-fold higher ($P < 0.05$) in H37Rv than in the *mprA*::Km^r mutant. Thus, MprA positively regulates *sigB*, *sigE*, and *mprA* expression in *M. tuberculosis* during growth under physiological conditions.

MprA interacts directly with the *sigB* and *sigE* upstream regions. MprA autogenously regulates its own expression and that of downstream gene *pepD* by binding to 8-bp direct repeat motifs located upstream of these coding sequences (9). To determine if the differences in *sigB* and *sigE* expression detected by microarray analysis and qRT-PCR were a result of direct regulation by MprA, EMSAs and DNase I footprinting assays were conducted. His-MprA was labeled in vitro with acetyl phosphate and used in these reaction mixtures to enhance binding of this protein to its target sequences (9). His-MprA~P retarded the mobility of DNA fragments comprising the upstream regions of both *sigB* and *sigE* (Fig. 2A and B, lanes 2 to 8) but failed to retard the DNA fragment encompassing the *sigH* upstream region (Fig. 2C, lanes 2 to 8), a stress-responsive sigma factor implicated in *sigB* and *sigE* regulation (18, 26). A single protein-DNA complex was observed with the *sigB* upstream region upon increasing concentrations of His-MprA~P, while two complexes were observed with the *sigE* upstream region under similar conditions, suggesting that MprA may bind two sites in this region. Addition of cold competitor DNA from the *mprA* upstream region significantly reduced binding by His-MprA~P to the *sigB* upstream region (Fig. 2A, lane 11), while binding by His-MprA~P to the *sigE* upstream region was completely abrogated following the addition of similar amounts of cold competitor DNA from this region (Fig. 2B, lane 11). These results suggest that the *sigB* upstream region may represent a higher-affinity binding site for MprA~P relative to those present upstream of either *mprA* or *sigE*. Importantly, binding by His-MprA~P to either the *sigB* or *sigE* upstream region was not affected by addition of noncompetitor DNA from the *PPE19* upstream region (Fig. 2A and B, lane 12), a region previously shown not to interact with His-MprA~P (9).

Response regulators must be phosphorylated to regulate downstream effector gene expression in vivo. However, binding of many response regulators to their recognition sequence in vitro occurs independently of protein phosphorylation. To determine whether the interaction of His-MprA~P with the *sigB* and *sigE* upstream regions in vitro was also independent of protein phosphorylation, binding reactions were repeated with His-MprA(D48A), a mutant protein that is unable to be phosphorylated in vitro or in vivo (9, 36). His-MprA(D48A) bound efficiently to both the *sigB* and *sigE* upstream regions in these reactions (Fig. 2A and B, lane 13). These results are consistent with those previously observed for MprA binding to the *mprA* and *pepD* upstream regions (9).

Finally, to confirm direct binding by His-MprA and determine the specific site(s) of interaction, DNase I footprinting of the *sigB* and *sigE* upstream regions was also performed. His-MprA~P protected a region of ~27 bp beginning 108 nucleotides upstream of the *sigB* coding sequence (Fig. 3A and B). Binding by His-MprA~P to this region was efficient, as the addition of as little as 44 pmol protein was sufficient to provide complete protection from DNase I digestion. Similarly, His-MprA~P protected two regions totaling 51 bp beginning 15 nucleotides upstream of the *sigE* translational start site (Fig. 3A and C). His-MprA~P bound to the region distal to the *sigE* translational start site with high affinity, while a lower-affinity binding site was apparent in the region proximal to the translational start site. Additionally, binding by His-MprA~P to both the *sigB* and *sigE* upstream regions induced DNase I hypersensitivity at sites adjacent to protected regions, suggesting that His-MprA~P binding induces conformational changes in the DNA. Thus, in vitro binding by His-MprA to the *sigB* and *sigE* regions is specific, is concentration dependent, and proceeds independently of protein phosphorylation.

MprA binds cooperatively to distal and proximal direct repeat motifs upstream of *sigE*. When protected regions from the *sigB* and *sigE* upstream regions were subjected to MEME (multiple expectation maximization for motif elicitation) analyses using Genetics Computer Group (GCG) software, se-

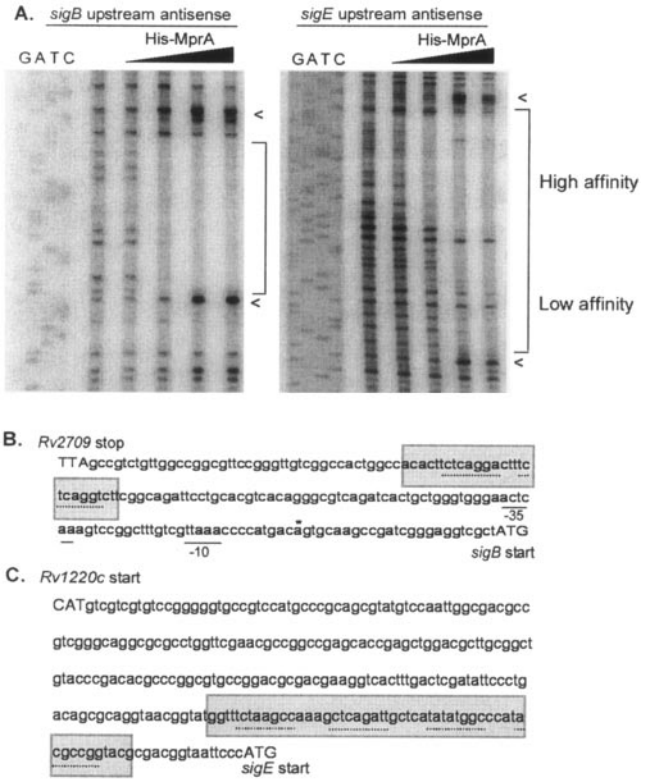


FIG. 3. DNase I footprints of *sigB* and *sigE* upstream regions. (A) ^{32}P-labeled DNA from the *sigB* and *sigE* upstream regions was incubated in the absence or presence of 1.3 to 260 pmol His-MprA~P and treated with 0.2 units DNase I; arrowheads denote sites of DNase I hypersensitivity. (B) Protected regions (shaded areas) are depicted for both *sigB* (B) and *sigE* (C) upstream regions along with the known transcriptional start site (*) and −10 and −35 sites (solid lines). Putative 8-bp direct repeat motifs (dotted line) were determined by GCG analyses and were similar to those found upstream of *mprA* and *pepD*. Start and stop codons are depicted in capital letters.

quence motifs similar to those bound by His-MprA~P in the *mprA* and *pepD* upstream regions were identified (Fig. 4A) (9). Relative to the MprA recognition sequence upstream of *mprA*, a single nucleotide substitution was observed in each putative repeat subunit upstream of *sigB*, suggesting that this motif likely represents a bone fide site of MprA recognition. In contrast, numerous nucleotide substitutions were observed in the putative distal and proximal direct repeat motifs upstream of *sigE* (Fig. 4A). To determine whether these variant repeat motifs represented legitimate MprA binding sites, short DNA fragments corresponding to the distal, proximal, or distal and proximal repeat motifs were generated and utilized in EMSAs. While His-MprA~P bound efficiently to the probe containing the distal repeat motif alone (Fig. 4C), it was unable to bind the probe containing the proximal repeat motif alone (Fig. 4D). Furthermore, replacement of the first three nucleotides comprising the first subunit of the distal repeat motif abrogated binding by His-MprA~P (Fig. 4E), demonstrating that this motif represents a legitimate MprA binding site. To determine whether the inability to observe binding by His-MprA~P to the proximal site was due to a requirement for cooperative binding with the distal site, EMSAs were repeated

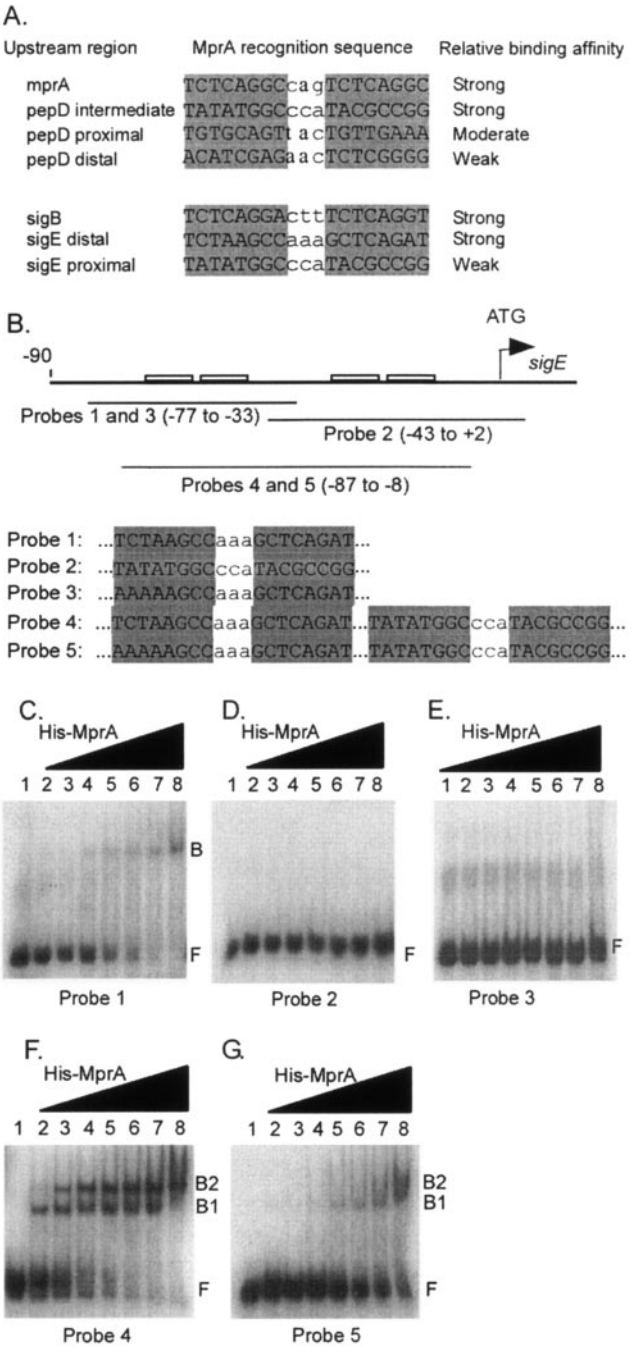


FIG. 4. Binding to *sigE* distal and proximal repeat sites requires cooperative binding by MprA. (A) Alignment of 8-bp direct repeat motifs (shaded boxes) upstream of genes directly regulated by MprA. Relative binding affinity was determined from EMSAs or DNase I footprinting experiments (9). (B) Probes were generated to distal (probes 1 and 3), proximal (probe 2), or distal and proximal (probes 4 and 5) repeats and used in EMSAs. Probes either contained wild-type sequences or carried nucleotide substitutions in the first subunit of the distal repeat motif. (C to G) Probe DNA (2.5 ng) was labeled with ^{32}P and used in all binding reaction mixtures. Labeled DNA was incubated in the absence (lane 1) or presence (lanes 2 to 8) of 0.44 to 35.0 pmol wild-type His-MprA~P. F, free DNA; B, bound DNA.

using short DNA probes that carried both distal and proximal repeat motif sequences. Two protein-DNA complexes were observed following incubation of His-MprA~P with this probe (Fig. 4F). However, the ability of His-MprA~P to bind this probe was significantly reduced when nucleotides comprising the distal repeat motif were mutated (Fig. 4G). When taken together, these binding patterns suggest that recognition of the lower-affinity proximal repeat motif by MprA requires cooperative binding with protein bound at the higher-affinity distal repeat motif.

***mprA* expression is induced in *M. tuberculosis* in response to stress.** Because MprA directly regulated two stress-responsive sigma factors during growth under physiological conditions, we next investigated whether *mprA* itself was regulated by stress. Previous studies have demonstrated that *mprA* is induced following exposure of *Mycobacterium* to subinhibitory concentrations of SDS in a manner partially dependent on SigE (19). Therefore, wild-type or *mprA*::Km^r mutant cultures of *M. tuberculosis* were treated for 90 min with various stimuli, RNA stabilized, and extracted, and qRT-PCR was performed. Exposure of wild-type *M. tuberculosis* H37Rv to 0.05% SDS, 0.2% Triton X-100, and alkaline pH (7H9 medium adjusted to pH 8.5) resulted in induction of *mprA* expression relative to that in untreated samples (Fig. 5A). This observed upregulation was dependent on MprA, as little or no increase in *mprA* expression levels relative to those in untreated controls were observed in the *mprA*::Km^r mutant under these conditions (Fig. 5A). For example, induction of *mprA* in the *mprA*::Km^r mutant was partially reduced relative to H37Rv following exposure to SDS and alkaline pH, while *mprA* induction was completely abolished following exposure to Triton X-100. In contrast, treatment with 10 μg/ml amikacin, an aminoglycoside that targets protein synthesis machinery, did not induce *mprA* expression in either H37Rv or the *mprA*::Km^r mutant. Thus, *mprA* expression is differentially regulated in *M. tuberculosis* in response to various stresses, and this regulation is partially or completely dependent on MprA.

Induction of *sigE* expression in *M. tuberculosis* in response to stress requires MprA. Because MprA regulated the expression of *sigB* and *sigE* in *M. tuberculosis* during growth under physiological conditions, qRT-PCR expression assays were repeated to determine whether MprA also regulated expression of these genes in response to stress. Treatment with SDS, alkaline pH, or Triton X-100 induced *sigE* expression in *M. tuberculosis* H37Rv relative to that in untreated cultures (Fig. 5B). This observed upregulation was completely dependent on MprA, as no induction was observed in the *mprA*::Km^r mutant following exposure to these stresses (Fig. 5B). However, no significant differences in *sigE* expression were observed in either wild-type H37Rv or the *mprA*::Km^r mutant following exposure to amikacin, suggesting that *sigE* is not regulated in response to this stress (Fig. 5B). In contrast, regulation of *sigB* expression by MprA was not readily observed in *M. tuberculosis* following exposure to the various stresses. For example, *sigB* expression was only slightly higher in wild-type *M. tuberculosis* than in the *mprA*::Km^r mutant following exposure to alkaline pH, Triton X-100, or amikacin (Fig. 5C). Furthermore, *sigB* expression was not regulated by MprA in response to SDS exposure. Thus, *sigE* but not *sigB* expression is significantly regulated by MprA in *M. tuberculosis* in response to the various stresses examined.

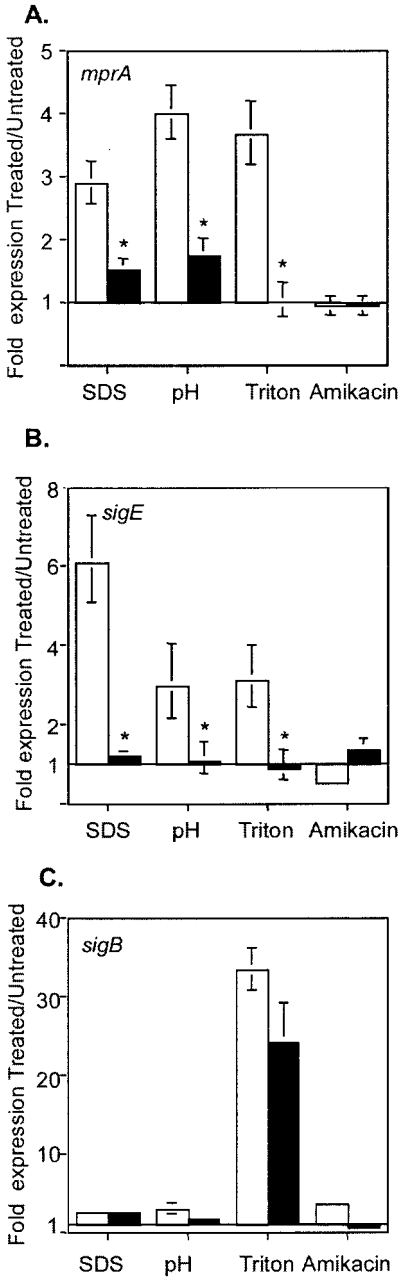


FIG. 5. Regulation of *mprA* and *sigE* is dependent on MprA in response to stress. Wild-type *M. tuberculosis* H37Rv (open bars) or the *mprA*::Km^r mutant (filled bars) were incubated in the absence of stress or with 0.05% SDS, alkaline pH 8.5, 0.2% Triton X-100, or 10 μg/ml amikacin for 90 min with shaking at 37°C. RNA was extracted, DNase treated, and subjected to qRT-PCR. Expression values were determined for *mprA* (A), *sigE* (B), and *sigB* (C) and are presented as the mean differences (*n*-fold [±standard deviations]) in treated versus untreated cultures from experiments performed in triplicate. Note that a common set of untreated cultures was used for each strain. *, $P < 0.05$ between H37Rv and *mprA*::Km^r.

Induction of *mprA* and *sigE* expression by MprA is mediated through the extracytoplasmic domain of MprB. *mprB* encodes

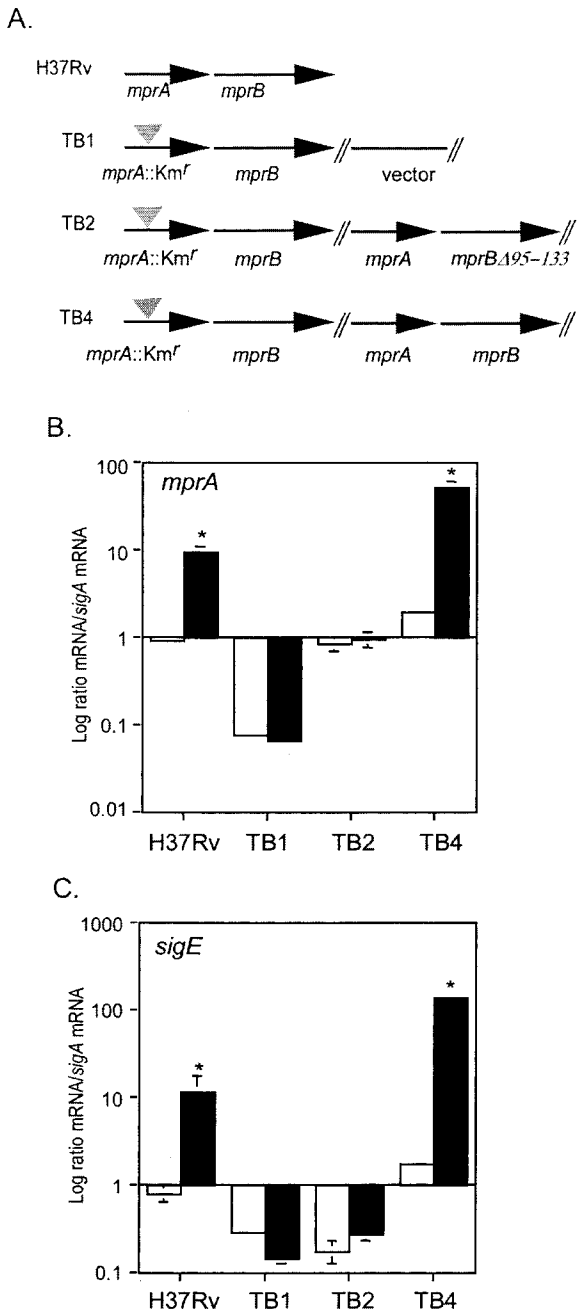
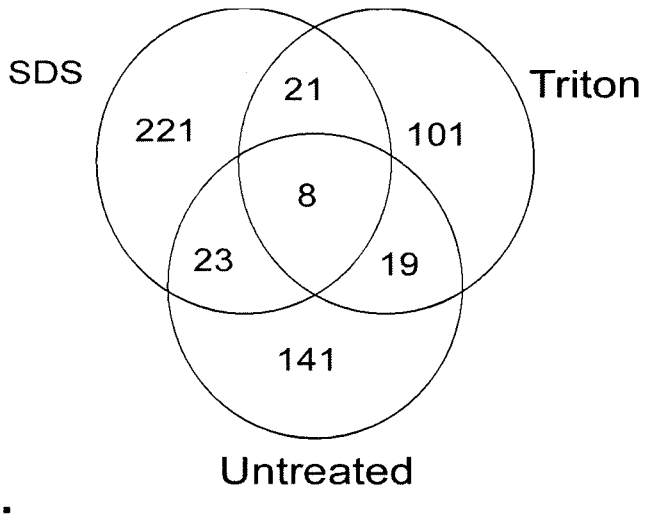


FIG. 6. MprA-dependent induction of *mprA* and *sigE* expression requires the extracytoplasmic loop domain of MprB. Wild-type or *mprA*::Km^r mutants of *M. tuberculosis* expressing various alleles of *mprA* and *mprB* from an integrative plasmid (A) were examined for the expression of *mprA* (B) or *sigE* (C) in the absence of stress (open bars) or following exposure to 0.2% Triton X-100 (filled bars). Values are presented as mean differences (*n*-fold [±standard deviations]) of *mprA* or *sigE* mRNA levels from that of *sigA* mRNA and were determined from experiments performed in triplicate. *, $P < 0.05$ between untreated and treated cultures.

an integral membrane sensor kinase that contains two transmembrane-spanning regions and an intervening 113-amino-acid extracytoplasmic loop domain that is exposed to the external environment. To determine whether MprA-mediated induction of *mprA* and *sigE* expression occurred through MprB and required this extracytoplasmic domain, qRT-PCR expression studies were conducted on *M. tuberculosis* derivatives expressing wild-type *mprAB* or wild-type *mprA* and an *mprB* allele in which 39 amino acids had been deleted from the extracytoplasmic domain (Fig. 6A). Alleles were expressed from their endogenous promoter in *trans* using a site-specific integration vector (9). The *mprA*::Km^r mutant served as the genetic background for these mutations, as the Km^r insertion is predicted to be polar on endogenous *mprB* expression. For these experiments, *M. tuberculosis* cultures were grown to mid-log phase and left unexposed or exposed to 0.2% Triton X-100 to facilitate MprA-mediated induction of *mprA* and *sigE* expression. Expression values were measured by determining the mean ratio of *mprA* or *sigE* mRNA levels to *sigA* levels (Fig. 6B and C). As observed previously, exposure of *M. tuberculosis* H37Rv to Triton X-100 induced the expression of *mprA* and *sigE* relative to levels in untreated cultures. In contrast, *mprA* and *sigE* expression levels were not induced over background levels following Triton X-100 exposure in strains unable to make MprA (TB1) or in strains expressing an MprB derivative lacking the extracytoplasmic domain (TB2). However, induction of *mprA* and *sigE* following Triton X-100 exposure was fully restored when wild-type *mprAB* was expressed in *trans* (TB4). The inability to induce *mprA* and *sigE* expression in TB2 following Triton X-100 exposure was not due to an inability to make MprB(Δ95-133), as *mprB*(Δ*95-133*) expression was detected by qRT-PCR and MprB(Δ95-133) was detectable in cell extracts by Western blotting (data not shown). Rather, lack of induction of *mprA* and *sigE* is likely due to an inability of *M. tuberculosis* to recognize stressful stimuli through MprB. The increased levels of *mprA* and *sigE* observed in TB4 relative to wild-type *M. tuberculosis* H37Rv may be attributed to enhanced expression of MprAB from its site in *trans*. Alternatively, the Km^r insertion may impart only partial polarity on expression of the endogenous *mprB* gene. Regardless, these results demonstrate that MprA-mediated induction of *mprA* and *sigE* occurs through MprB and requires the extracytoplasmic loop domain.

MprA regulates diverse gene sets in response to different stresses. Global gene expression profiling between *M. tuberculosis* H37Rv and the *mprA*::Km^r mutant indicated that a large group of genes were regulated by MprA during growth under physiological conditions. To determine whether MprA regulated a similar set of genes following exposure to stress, DNA microarray analyses were repeated using RNA extracted from cultures of H37Rv or the *mprA*::Km^r mutant treated with 0.05% SDS or 0.2% Triton X-100 for 90 min. With the same statistical criteria and threshold cutoff as those used for untreated cultures, a total of 221 genes were found to be differentially expressed between H37Rv and the *mprA*::Km^r mutant following SDS exposure (Table S2 in the supplemental material), while 101 genes were differentially expressed in these strains in response to Triton X-100 exposure (Table S3 in the supplemental material and Fig. 7A). The majority of genes regulated by MprA under each stress condition were distinct and were different from those regulated by MprA during growth under physiological conditions. However, several of these genes were found to be commonly regulated under more than one condition. In particular, 23 genes were

A.



B.

H37Rv#	Gene	Untreated	SDS	Triton X-100
Rv0981	*mprA*	10.96±3.18	15.9±4.45	16.34±3.35
Rv1057		1.95±0.48	4.30±1.05	54.57±32.25
Rv1221	*sigE*	3.57±0.28	7.15±2.21	82.71±6.55
Rv1813c		2.87±0.61	2.61±0.77	2.01±0.12
Rv2053c		3.00±0.45	1.65±0.34	4.82±1.12
Rv2626c		8.48±1.52	14.85±4.60	1.60±0.15
Rv2627c		7.11±0.78	5.17±1.56	2.89±0.71
Rv2628		8.18±2.44	4.85±1.70	6.19±0.92

FIG. 7. Synopsis of MprA-regulated genes as determined with DNA microarrays (A) and qRT-PCR (B). (A) Genes were regulated by MprA either under a single condition (values encompassed by one circle) or under multiple conditions (values encompassed by more than one circle). (B) Genes regulated under all conditions were validated by qRT-PCR. Cultures were untreated or exposed to 0.05% SDS or 0.2% Triton X-100 for 90 min before RNA was extracted, DNase treated, and subjected to qRT-PCR. Values for individual genes in H37Rv are expressed as mean differences (*n*-fold [±standard deviations]) in expression levels from those in the *M. tuberculosis mprA*::Km^r mutant during growth under each condition. Values were obtained from experiments performed in triplicate. All values were statistically significant ($P < 0.05$).

regulated by MprA under physiological conditions and SDS exposure, 19 genes were regulated by MprA under physiological conditions and Triton X-100 exposure, and a common set of 21 genes was regulated by MprA in *M. tuberculosis* in response to either SDS or Triton X-100. Finally, eight genes, including *mprA* and *sigE*, were regulated by MprA under all conditions tested. When this panel of commonly regulated genes was subjected to expression analyses by qRT-PCR, all in *M. tuberculosis* H37Rv were differentially regulated from those in the *mprA* mutant under each condition (Fig. 7B) ($P < 0.05$), validating results from DNA microarray experiments. Thus, in response to different stresses, MprA regulates a complex set of genetic determinants in *M. tuberculosis*, including a small subset which is regulated by multiple stimuli.

DISCUSSION

M. tuberculosis is exposed to various environmental and/or physiological stresses during growth in the host. Within a granulomatous lesion, where *M. tuberculosis* is likely to reside during persistent stages of infection, a tubercle bacillus may encounter stresses that include hypoxia, nutrient limitation, reactive oxygen and nitrogen intermediates, low pH, alveolar surfactants, and toxic proteins and fatty acids (17). To adapt to these conditions, the tubercle bacillus encodes a variety of two-component regulatory systems and stress-responsive sigma factor genes. Here we present evidence that the MprAB two-component regulatory system participates in stress response pathways of *M. tuberculosis*. In par-

ticular, MprAB recognizes and responds to various environmental stimuli, including those that induce membrane stress. Importantly, MprA directly regulates two key stress-responsive sigma factors, *sigB* and *sigE*.

sigB encodes a σ^A-like sigma factor that is dispensable for the growth of *M. tuberculosis* and *Mycobacterium smegmatis* (17), while *sigE* is one of 10 ECF sigma factors present in the *M. tuberculosis* genome (4). The mechanisms regulating *sigB* and *sigE* expression are complex and appear to be multifactorial. Transcription of *sigB* is dependent on SigE under physiological conditions and following exposure to SDS (19). However, the ECF sigma factor SigH regulates *sigB* expression in response to heat shock and oxidative stress (18, 26). The regulation of *sigE* is less well characterized and may or may not be dependent on SigH and SigE itself (18, 19, 26). The observations that MprA regulates *sigE* and *sigB* expression and that several of the genes regulated by MprA (including *mprAB*) are also regulated by SigE (19) indicate that external stimuli can be processed by *M. tuberculosis* in hierarchical transcriptional networks. Interestingly, while MprA regulated *sigE* expression in *M. tuberculosis* in the absence and presence of specific stressful stimuli, MprA-mediated regulation of *sigB* was observed only during the growth of *M. tuberculosis* under physiological conditions. The reasons for this discrepancy are currently unclear but may be related to the specific stress conditions utilized in these assays. Additional tests utilizing other stimuli are under way to identify environmental conditions that stimulate *sigB* expression through MprA.

mprA and *sigE* expression is induced in an MprA-dependent manner following the exposure of *M. tuberculosis* to several stresses, including ionic and nonionic detergents and alkaline pH. These results indicate that MprAB may respond to stress that is manifested at the cell membrane. Although the exact molecular mechanisms mediating the activation of MprAB are currently unclear, the induction of *mprA* and *sigE* by MprA requires the extracytoplasmic domain of sensor kinase MprB. Although our data cannot rule out the possibility that deletion of 39 amino acids from the extracytoplasmic loop domain abrogates the ability of MprB to mediate autophosphorylation or transphosphorylation reactions with MprA, a mutant MprB lacking the first 195 amino acids, including both transmembrane domains and the intervening extracytoplasmic domain, can still be autophosphorylated and transfer this phosphate to MprA (36). Thus, we speculate that the extracytoplasmic domain is required for the recognition of stress and initiation of subsequent signal transduction events.

While the regulation of two-component systems by sigma factors has been extensively described, to our knowledge this is the first report of direct and sequence-specific regulation of stress-responsive sigma factors by a response regulator. However, this regulatory mechanism is likely to exist in other organisms. For example, the well-characterized CpxR response regulator has been reported to bind upstream of and negatively regulate the expression of stress-responsive sigma factor *rpoE* in K-12 strains of *Escherichia coli*. However, direct binding by CpxR~P to the *rpoE* promoter region occurs only at high protein concentrations (>150 pmol) in vitro and the sequence specificity of CpxR binding within the *rpoE* promoter region has not been investigated (6). Similarly, the *cseBC* two-component system regulates expression of stress-responsive sigma factor *sigE* in *Streptomyces coelicolor* A3(2) (21). Although *cseB* and *sigE* mutants exhibit similar phenotypes, including hypersensitivity to lysozyme and a requirement for high Mg^{2+} concentrations during the growth of *S. coelicolor* in vitro (20, 21), direct regulation of *sigE* by CseB has not yet been demonstrated. Regardless, regulation of stress-responsive sigma factor genes by two-component systems may represent an efficient and cooperative mechanism utilized by bacteria to rapidly sense and respond to a variety of stress conditions in vitro or in vivo.

The ability of MprAB to regulate multiple sigma factors, two-component systems, and general transcription factors may explain, in part, the relatively diverse gene panel that is induced or repressed by this system under physiological conditions or in response to stress. Surprisingly, only eight genes are regulated by MprAB under all conditions examined. These genes include *mprA*, Rv1057, *sigE*, Rv1813c, Rv2053c, Rv2626c, Rv2627c, and Rv2628. Rv1057, Rv1813c, Rv2626c, Rv2627c, and Rv2628 encode hypothetical or conserved hypothetical proteins, while Rv2053c encodes a putative transmembrane protein. *mprB* is also regulated by MprA under these conditions but was not represented on our microarrays due to a printing problem during the microarray fabrication. Interestingly, the extent of upregulation mediated by MprA for many of these genes varies between the different conditions examined. This indicates that several MprA-regulated genes apart from *mprA* and *sigE* possess multiple regulatory inputs and therefore are differentially regulated. Consistent with this idea, the expression of Rv1813c, Rv2626c, Rv2627c, and Rv2628 is highly induced during the growth of *M. tuberculosis* under hypoxia, and all are under the control of the two-component response regulator DosR (24). The coregulation of these genes by both DosR and MprA suggests that exposure to low oxygen environments may also initiate stress response pathways in *M. tuberculosis*.

While at least eight genes are regulated by MprAB under all conditions examined, the majority of genes regulated by MprAB are induced or repressed in response to only a single condition. However, many of these genes have also been shown by DNA microarray analysis and/or qRT-PCR to be regulated in response to other environmental stresses, including hypoxia, nutrient starvation, heat shock, antibiotic treatment, low pH, SDS, and growth in human macrophages in vitro and artificial granulomas in vivo (2, 7, 11, 19, 24, 29, 33), supporting our hypothesis that MprAB participates in a multifaceted stress response network in the tubercle bacillus. Because MprAB regulates a heterologous gene panel that differs depending on the environmental conditions and because the mechanism and specificity of regulation by MprA have not yet been investigated for the majority of these genes, the composition of the complete MprAB regulon remains unclear. MprA recognizes conserved 8-bp direct repeat motifs that are separated by a 3-nucleotide spacer region; however, some variation in these repeat motifs can be tolerated by MprA and likely contributes to the overall differences in levels of gene expression mediated by this transcription factor. Consequently, further studies are required to better define the genetic targets regulated by MprAB during the growth of *M. tuberculosis* under physiological conditions or in response to stress.

In summary, MprAB is a two-component system that is

responsive to stress in *M. tuberculosis* and regulates the expression of sigma factors and other stress-responsive genetic determinants during growth under a variety of conditions. Continued study of these systems is essential to understanding the complex and interconnected regulatory networks utilized by *M. tuberculosis* to survive environmental stress and to adapt during infection within the host.

ACKNOWLEDGMENTS

We are grateful to Martin J. Hessner and Lisa Meyer of the Human and Molecular Genetics Center at the MCW for oligoarray fabrication and Dara Frank for critical review of the manuscript.

This work is supported by NIH grant AI51669 to T.C.Z.

REFERENCES

1. **Ando, M., T. Yoshimatsu, C. Ko, P. J. Converse, and W. R. Bishai.** 2003. Deletion of *Mycobacterium tuberculosis* sigma factor E results in delayed time to death with bacterial persistence in the lungs of aerosol-infected mice. Infect. Immun. **71:**7170–7172.
2. **Betts, J. C., P. T. Lukey, L. C. Robb, R. A. McAdam, and K. Duncan.** 2002. Evaluation of a nutrient starvation model of *Mycobacterium tuberculosis* persistence by gene and protein expression profiling. Mol. Microbiol. **43:** 717–731.
3. **Clark-Curtiss, J. E., and S. E. Haydel.** 2003. Molecular genetics of *Mycobacterium tuberculosis* pathogenesis. Annu. Rev. Microbiol. **57:**517–549.
4. **Cole, S. T., R. Brosch, J. Parkhill, T. Garnier, C. Churcher, D. Harris, S. V. Gordon, K. Eiglmeier, S. Gas, C. E. Barry III, F. Tekaia, K. Badcock, D. Basham, D. Brown, T. Chillingworth, R. Connor, R. Davies, K. Devlin, T. Feltwell, S. Gentles, N. Hamlin, S. Holroyd, T. Hornsby, K. Jagels, B. G. Barrell, et al.** 1998. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature **393:**537–544.
5. **Cosma, C. L., D. R. Sherman, and L. Ramakrishnan.** 2003. The secret lives of the pathogenic mycobacteria. Annu. Rev. Microbiol. **57:**641–676.
6. **De Wulf, P., A. M. McGuire, X. Liu, and E. C. Lin.** 2002. Genome-wide profiling of promoter recognition by the two-component response regulator CpxR-P in *Escherichia coli*. J. Biol. Chem. **277:**26652–26661.
7. **Fisher, M. A., B. B. Plikaytis, and T. M. Shinnick.** 2002. Microarray analysis of the *Mycobacterium tuberculosis* transcriptional response to the acidic conditions found in phagosomes. J. Bacteriol. **184:**4025–4032.
8. **Graham, J. E., and J. E. Clark-Curtiss.** 1999. Identification of *Mycobacterium tuberculosis* RNAs synthesized in response to phagocytosis by human macrophages by selective capture of transcribed sequences (SCOTS). Proc. Natl. Acad. Sci. USA **96:**11554–11559.
9. **He, H., and T. C. Zahrt.** 2005. Identification and characterization of a regulatory sequence recognized by *Mycobacterium tuberculosis* persistence regulator MprA. J. Bacteriol. **187:**202–212.
10. **Hessner, M. J., V. K. Singh, X. Wang, S. Khan, M. R. Tschannen, and T. C. Zahrt.** 2004. Utilization of a labeled tracking oligonucleotide for visualization and quality control of spotted 70-mer arrays. BMC Genomics **5:**12.
11. **Karakousis, P. C., T. Yoshimatsu, G. Lamichhane, S. C. Woolwine, E. L. Nuermberger, J. Grosset, and W. R. Bishai.** 2004. Dormancy phenotype displayed by extracellular *Mycobacterium tuberculosis* within artificial granulomas in mice. J. Exp. Med. **200:**647–657.
12. **Kaushal, D., B. G. Schroeder, S. Tyagi, T. Yoshimatsu, C. Scott, C. Ko, L. Carpenter, J. Mehrotra, Y. C. Manabe, R. D. Fleischmann, and W. R. Bishai.** 2002. Reduced immunopathology and mortality despite tissue persistence in a *Mycobacterium tuberculosis* mutant lacking alternative sigma factor, SigH. Proc. Natl. Acad. Sci. USA **99:**8330–8335.
13. **Livak, K. J., and T. D. Schmittgen.** 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta c_T}$ method. Methods **25:**402–408.
14. **Malhotra, V., D. Sharma, V. D. Ramanathan, H. Shakila, D. K. Saini, S. Chakravorty, T. K. Das, Q. Li, R. F. Silver, P. R. Narayanan, and J. S. Tyagi.** 2004. Disruption of response regulator gene, devR, leads to attenuation in virulence of *Mycobacterium tuberculosis*. FEMS Microbiol. Lett. **231:**237–245.
15. **Manganelli, R., E. Dubnau, S. Tyagi, F. R. Kramer, and I. Smith.** 1999. Differential expression of 10 sigma factor genes in *Mycobacterium tuberculosis*. Mol. Microbiol. **31:**715–724.
16. **Manganelli, R., L. Fattorini, D. Tan, E. Iona, G. Orefici, G. Altavilla, P. Cusatelli, and I. Smith.** 2004. The extra cytoplasmic function sigma factor σ^E is essential for *Mycobacterium tuberculosis* virulence in mice. Infect. Immun. **72:**3038–3041.
17. **Manganelli, R., R. Provvedi, S. Rodrigue, J. Beaucher, L. Gaudreau, and I. Smith.** 2004. Sigma factors and global gene regulation in *Mycobacterium tuberculosis*. J. Bacteriol. **186:**895–902.
18. **Manganelli, R., M. I. Voskuil, G. K. Schoolnik, E. Dubnau, M. Gomez, and I. Smith.** 2002. Role of the extracytoplasmic-function sigma factor sigma(H) in *Mycobacterium tuberculosis* global gene expression. Mol. Microbiol. **45:** 365–374.
19. **Manganelli, R., M. I. Voskuil, G. K. Schoolnik, and I. Smith.** 2001. The *Mycobacterium tuberculosis* ECF sigma factor sigmaE: role in global gene expression and survival in macrophages. Mol. Microbiol. **41:**423–437.
20. **Paget, M. S., L. Chamberlin, A. Atrih, S. J. Foster, and M. J. Buttner.** 1999. Evidence that the extracytoplasmic function sigma factor σ^E is required for normal cell wall structure in *Streptomyces coelicolor* A3(2). J. Bacteriol. **181:**204–211.
21. **Paget, M. S., E. Leibovitz, and M. J. Buttner.** 1999. A putative two-component signal transduction system regulates sigmaE, a sigma factor required for normal cell wall integrity in *Streptomyces coelicolor* A3(2). Mol. Microbiol. **33:**97–107.
22. **Parish, T., D. A. Smith, S. Kendall, N. Casali, G. J. Bancroft, and N. G. Stoker.** 2003. Deletion of two-component regulatory systems increases the virulence of *Mycobacterium tuberculosis*. Infect. Immun. **71:**1134–1140.
23. **Parish, T., D. A. Smith, G. Roberts, J. Betts, and N. G. Stoker.** 2003. The *senX3-regX3* two-component regulatory system of *Mycobacterium tuberculosis* is required for virulence. Microbiology **149:**1423–1435.
24. **Park, H. D., K. M. Guinn, M. I. Harrell, R. Liao, M. I. Voskuil, M. Tompa, G. K. Schoolnik, and D. R. Sherman.** 2003. *Rv3133c/dosR* is a transcription factor that mediates the hypoxic response of *Mycobacterium tuberculosis*. Mol. Microbiol. **48:**833–843.
25. **Perez, E., S. Samper, Y. Bordas, C. Guilhot, B. Gicquel, and C. Martin.** 2001. An essential role for *phoP* in *Mycobacterium tuberculosis* virulence. Mol. Microbiol. **41:**179–187.
26. **Raman, S., T. Song, X. Puyang, S. Bardarov, W. R. Jacobs, Jr., and R. N. Husson.** 2001. The alternative sigma factor SigH regulates major components of oxidative and heat stress responses in *Mycobacterium tuberculosis*. J. Bacteriol. **183:**6119–6125.
27. **Rickman, L., J. W. Saldanha, D. M. Hunt, D. N. Hoar, M. J. Colston, J. B. Millar, and R. S. Buxton.** 2004. A two-component signal transduction system with a PAS domain-containing sensor is required for virulence of *Mycobacterium tuberculosis* in mice. Biochem. Biophys. Res. Commun. **314:**259–267.
28. **Smith, I.** 2003. *Mycobacterium tuberculosis* pathogenesis and molecular determinants of virulence. Clin. Microbiol. Rev. **16:**463–496.
29. **Stewart, G. R., L. Wernisch, R. Stabler, J. A. Mangan, J. Hinds, K. G. Laing, D. B. Young, and P. D. Butcher.** 2002. Dissection of the heat-shock response in *Mycobacterium tuberculosis* using mutants and microarrays. Microbiology **148:**3129–3138.
30. **Talaat, A. M., R. Lyons, S. T. Howard, and S. A. Johnston.** 2004. The temporal expression profile of *Mycobacterium tuberculosis* infection in mice. Proc. Natl. Acad. Sci. USA **101:**4602–4607.
31. **Tusher, V. G., R. Tibshirani, and G. Chu.** 2001. Significance analysis of microarrays applied to the ionizing radiation response. Proc. Natl. Acad. Sci. USA **98:**5116–5121.
32. **West, A. H., and A. M. Stock.** 2001. Histidine kinases and response regulator proteins in two-component signaling systems. Trends Biochem. Sci. **26:**369–376.
33. **Wilson, M., J. DeRisi, H. H. Kristensen, P. Imboden, S. Rane, P. O. Brown, and G. K. Schoolnik.** 1999. Exploring drug-induced alterations in gene expression in *Mycobacterium tuberculosis* by microarray hybridization. Proc. Natl. Acad. Sci. USA **96:**12833–12838.
34. **World Health Organization.** 2004. Fact sheet no. 104. World Health Organization, Geneva, Switzerland.
35. **Zahrt, T. C., and V. Deretic.** 2001. *Mycobacterium tuberculosis* signal transduction system required for persistent infections. Proc. Natl. Acad. Sci. USA **98:**12706–12711.
36. **Zahrt, T. C., C. Wozniak, D. Jones, and A. Trevett.** 2003. Functional analysis of the *Mycobacterium tuberculosis* MprAB two-component signal transduction system. Infect. Immun. **71:**6962–6970.