The *Mycobacterium tuberculosis phoPR* Operon Is Positively Autoregulated in the Virulent Strain H37Rv†

Jesús Gonzalo-Asensio,1,6 Carlos Y. Soto,2 Ainhoa Arbués,1,6 Javier Sancho,3 María del Carmen Menéndez,4 María J. García,4 Brigitte Gicquel,5 and Carlos Martín1,6,*

Departamento de Microbiología, Medicina Preventiva y Salud Pública, Universidad de Zaragoza, Zaragoza, Spain; Departamento de Química, Facultad de Ciencias, Universidad Nacional de Colombia, Bogotá, Colombia; Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Ciencias, y Instituto for Biocomputacion and Physics of Complex Systems (BIFI), Universidad de Zaragoza, Zaragoza, Spain; Departamento de Medicina Preventiva, Universidad Autónoma de Madrid, Madrid, Spain; Unité Génétique Mycobactérienne, Institut Pasteur, Paris, France; and CIBER Enfermedades Respiratorias, Mallorca, Spain.

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The attenuated *Mycobacterium tuberculosis* H37Ra strain is an isogenic counterpart of the virulent paradigm strain H37Rv. Recently, a link between a point mutation in the PhoP transcriptional regulator and avirulence of H37Ra was established. Remarkably, a previous study demonstrated negative autoregulation of the phoP gene in H37Ra. These findings led us to study the transcriptional autoregulation of PhoP in the virulent H37Rv strain. In contrast to the negative autoregulation of PhoP previously published for H37Ra, our experiments using a phoP promoter-lacZ fusion showed that PhoP is positively autoregulated in both H37Rv and H37Ra compared with an H37Rv phoP deletion mutant constructed in this study. Using quantitative reverse transcription-PCR (RT-PCR) analysis, we showed that the phoP gene is transcribed at similar levels in H37Rv and H37Ra. Gel mobility shift and DNase I footprinting assays allowed us to identify the precise binding region of PhoP from H37Rv to the phoP promoter. We also carried out RT-PCR studies to demonstrate that phoP is transcribed together with the adjacent gene phoR, which codes for the cognate histidine kinase of the phoP two-component system. In addition, quantitative RT-PCR studies showed that phoR is independently transcribed from a promoter possibly regulated by PhoP. Finally, we discuss the possible role in virulence of a single point mutation found in the phoP gene from the attenuated H37Ra strain but not in virulent members of the *M. tuberculosis* complex.

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The role of the phoP gene in *Mycobacterium tuberculosis* virulence has been characterized extensively in various in vitro cultures and animal models. The inactivation of phoP in a fully virulent *M. tuberculosis* clinical isolate results in impaired growth in cultured macrophages and no bacillary multiplication in mouse organs (32). Vaccination with the phoP mutant can be accounted for between H37Ra and *M. tuberculo-

...domain. It was clearly demonstrated that this point mutation is in the synthesis of these complex lipids along with several...
TABLE 1. Plasmids and primers used in this study

<table>
<thead>
<tr>
<th>Plasmid or primer</th>
<th>Description or sequence (5’–3’ [position with respect to phoP start codon])</th>
<th>Reference</th>
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<td>Promega</td>
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<td>pMT1</td>
<td><em>phoP</em> gene in pGEM-T Easy vector</td>
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<td>pET15b</td>
<td><em>E. coli</em> expression vector; Ap’</td>
<td>Novagen</td>
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<td><em>phoP</em> gene in pET15b</td>
<td>This study</td>
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<td>pNBV1</td>
<td><em>E. coli</em>-mycobacterial shuttle plasmid; Hyg’</td>
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<td>pSO5</td>
<td><em>phoP</em> gene in pNBV1</td>
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<td>pSO5K</td>
<td>pSO5 derivative carrying a Km’ marker</td>
<td>19</td>
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<td>pIJUZ1</td>
<td><em>phoPR</em> genes in pNBV1</td>
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<td>pTBLAC</td>
<td><em>phoP</em> promoter in pGEM-T Easy vector</td>
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<td>pJEM14</td>
<td><em>E. coli</em>-mycobacterial shuttle vector for promoter-lacZ fusions; Km’</td>
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<td>pTBGAL</td>
<td><em>phoP</em> promoter in pJEM14</td>
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**Primers**

<table>
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<td>AATCTAGATACAGCATACGCC (-1000)</td>
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<td>PhoPR</td>
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**Primers for qRT-PCR**

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<td>RT sigA fw</td>
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</tr>
<tr>
<td>RT sigA rv</td>
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**Construction of plasmids.** The vectors and oligonucleotides used are shown in Table 1. For the overproduction of PhoP in *E. coli*, the *phoP* gene was amplified from *M. tuberculosis* genomic DNA by PCR, using the primers PhoPexp and PhoPR, and the PCR fragment was inserted into the pGEM-T Easy vector to obtain pMT1. The insert was then excised by digestion with NdeI and XhoI and ligated into pET15b to give pTEX1. This recombinant plasmid contains the codons for six histidine residues at the 5’ end of *phoP* and therefore generates a protein with a His6 motif at the N terminus (His6-PhoP). The insert in pTEX1 was sequenced and confirmed to be identical to the *phoP* gene from the H37Rv strain. To construct the *phoP* promoter-lacZ fusion plasmid, a 238-bp fragment from positions +226 to +14 with respect to the *phoP* start codon was PCR amplified from *M. tuberculosis* MT103 genomic DNA by use of the primers BCG2B and PhoPR. The PCR product was cloned into pGEM-T Easy to obtain pTBLAC. The *phoP* promoter region was released from pTBLAC by digestion with Apal and KpnI and then inserted into the pJEM14-*E. coli*-mycobacterial shuttle plasmid to give pTBGAL. The sequence of the *phoP* promoter cloned in pTBGAL was identical to that in the H37Rv strain.

**Quantification of β-galactosidase activity.** Mycobacterial strains grown to stationary phase (optical density at 600 mm [OD600] = 2) were used to inoculate 50 ml of fresh medium at a ratio of 1:50. Aliquots were taken from this culture at the indicated times and centrifuged, and the pellet was washed with phosphate-buffered saline and stored at ~80°C. Cells were resuspended in 1 ml Z buffer (0.06 M Na2HPO4, 0.04 M NaH2PO4, 0.01 M KCl, 1 mM MgSO4, and 0.05 M β-mercaptoethanol [pH 7 at 25°C]) and sonicated to generate a cell extract. The chromogenic substrate o-nitrophenyl-β-galactoside was added to cell ex-
tracts at a final concentration of 0.66 mg/ml. The mixtures were incubated at 28°C for 1 h, and the enzymatic reaction was stopped by adding 0.29 M Na2CO3. The A405 of the supernatant was determined, and β-galactosidase activity = (1,000 × A405)/(time [min] × aliquot volume [ml] × A405).

Overproduction and purification of PhoP. The PhoP protein from H37Rv was overproduced from pTEX1 in E. coli BL21(DE3)/pLysS and purified using a HiTrap affinity column (Amersham Pharmacia Biotech) as previously described (10).

RESULTS

phoP promoter activity in the presence of PhoP from M. tuberculosis H37Rv. It has been reported that PhoP from the H37Ra strain represses its own transcription if M. smegmatis is used as a surrogate host (21). We investigated whether this autoregulation was affected by the Ser219Leu mutation in PhoP by carrying out a similar experiment with PhoP from the H37Rv strain. A transcriptional fusion between the phoP promoter and a promoterless lacZ gene was constructed and inserted into pJEM14 (47), an E. coli-mycobacterial shuttle plasmid, yielding pTBGAL (Table 1). This plasmid was used to transform M. smegmatis mc2155. The resulting strain was co-transformed with pSO5 (32), which contains both the promoter region and the entire phoP coding region in the multicopy plasmid pNBV1 (24). The cotransformed strain was grown at 37°C in 7H9 medium supplemented with hygromycin and kanamycin. Aliquots were collected at the indicated times, and β-galactosidase activity was elevated to determine levels of transcription from the phoP promoter in the presence of PhoP. β-Galactosidase activity levels were two to three times higher in M. smegmatis mc2155 cotransformed with pTBGAL and pSO5 than in the control strain cotransformed with pTBGAL and pNBV1 (Fig. 1). Thus, phoP expression seems to be positively autoregulated by the H37Rv version of PhoP when M. smegmatis is used as a surrogate host.

Positive autoregulation of PhoP in M. tuberculosis H37Rv and H37Ra. In order to corroborate the previous finding and to rule out differences between the transcriptional machineries of the fast-growing M. smegmatis strain and the slow-growing M. tuberculosis strain, we attempted to study the expression of the phoP promoter in H37Rv, H37Ra, and a defective phoP mutant. Firstly, we constructed and characterized an H37Rv mutant. Firstly, we constructed and characterized an H37Rv

www.ncbi.nlm.nih.gov/sites/entrez). We also sequenced the cod-
remained uncolored in the presence of X-Gal (data not shown). Taken together, these results indicate that \textit{phoP} could be positively autoregulated in both \textit{M. tuberculosis} H37Rv and H37Ra, and this effect was more noticeable at the time points described above.

**EMSA with the \textit{phoP} promoter and PhoP from H37Rv.** Previous works have demonstrated that PhoP from H37Ra binds to its own promoter (21, 38). Here we studied whether PhoP from \textit{M. tuberculosis} H37Rv specifically binds to its own promoter sequence, possibly mediating autoregulation of \textit{phoP} expression. The same 238-bp fragment from the \textit{phoP} promoter previously used in \textit{\beta}-galactosidase experiments was used for gel shift assays. This fragment was amplified by PCR using the BCG2B and PhoPro primers (Table 1) and then labeled for use as a probe in mobility shift experiments. The labeled probe displayed a clear shift in electrophoretic mobility in the presence of PhoP, and larger amounts of protein resulted in a gradual decrease in probe mobility (Fig. 3, lanes 2 to 6). We confirmed the specificity of binding by using an unlabeled fragment as a competitor. PhoP binding to the labeled \textit{phoP} promoter was reduced by adding a 100-fold excess of unlabeled probe (Fig. 3, lane 7). Further support for the specificity of the interaction with DNA was provided by experiments in which a nonspecific fragment corresponding to the \textit{sigA} coding sequence was used, and as expected, no shift in DNA mobility was detected (data not shown). Similar experiments were performed in the presence of PhoP previously incubated with the phosphorylation reagent acetyl phosphate. Even if phosphorylation of PhoP did not appear to affect binding specificity, we cannot rule out differences in binding affinity due to phosphorylation (data not shown).

**DNase I protection of the \textit{phoP} promoter with PhoP.** We attempted to compare the PhoP binding region in H37Rv with that previously reported for H37Ra (21). The sequence to which PhoP binds was mapped by DNase I footprinting assays with a 463-bp fragment from positions −226 to +237 relative to the \textit{phop} start codon. The regions protected by PhoP from degradation by DNase I were located between positions −66 and −18 on the noncoding strand and −38 and +1 on the coding strand, resulting in a 67-bp binding region, from positions −66 to +1 (Fig. 4). The degree of protection was higher for the coding strand than for the noncoding strand (Fig. 4). Similar results were obtained in other footprinting assays using radiolabeled primers (data not shown). Our results indicate that the PhoP binding site described here, although similar, is shorter to that previously described for the H37Ra strain,

![FIG. 1. \textit{phoP} promoter activity in \textit{M. smegmatis} expressing the H37Rv variant of PhoP.](http://jb.asm.org/)

![FIG. 2. \textit{phoP} promoter activity in H37Rv and H37Ra. White, black, and hatched bars represent \textit{\beta}-galactosidase activities from the H37Rv \textit{\DeltaphoP::hyg}, H37Rv, and H37Ra strains transformed with pTBGAL and cultured until the indicated OD\textsubscript{600}. Miller units for the aforementioned strains transformed with the empty vector (pJEM14) were below 10 and have been omitted for clarity. Results shown are the means and standard errors from two independent experiments.](http://jb.asm.org/)
which is located between positions −79 and +9 relative to the phoP start codon (21).

Transcriptional organization of the M. tuberculosis phoPR operon. In the M. tuberculosis H37Rv chromosome, the phoP gene maps upstream of the phoR gene, coding for the HK of the 2CS PhoPR, and the stop codon of phoP is separated from the start codon of phoR by a 47-bp intergenic region (Fig. 5), suggesting that both genes may be transcribed in an operon. RT-PCR was used to determine whether phoP and phoR were transcribed to give a single mRNA. We obtained amplification products for the 5′ end of phoP and the 3′ end of phoR and a 393-bp amplification product for the phoPR intergenic region (Fig. 5), indicating that these two genes are cotranscribed. For confirmation of the cotranscription of phoPR, a larger region, from positions −52 to +1900 with respect to the phoP start codon, was amplified and the expected cotranscribed fragment obtained (Fig. 5). We checked that the RNA samples were not contaminated with genomic DNA by carrying out RT-PCRs without reverse transcriptase. The lack of amplification products for all of the fragments described above demonstrates clearly that the RT-PCR products obtained were amplified from RNA that had been reverse transcribed into cDNA (Fig. 5). Our results indicate that the two genes are cotranscribed in an operon, as described for other 2CSs (22), but these results do not exclude the possibility of independent promoters for phoP and phoR.

Quantification of phoP and phoR expression by qRT-PCR. M. tuberculosis H37Rv and H37Ra were cultured to mid-exponential growth phase. RNAs were extracted and reverse transcribed to generate cDNAs, which were then amplified in qRT-PCR experiments. The expression of the phoP gene was measured by qRT-PCR and normalized to sigA expression levels. Transcription rates for the phoP gene were similar in H37Rv and H37Ra (Fig. 6A), in concordance with our previous results from the β-galactosidase activity assays. Once we demonstrated that phoP is similarly transcribed in H37Rv and H37Ra, we sought to compare phoR expression levels in both strains. qRT-PCR experiments using phoR-specific primers (Table 1) indicated that phoR is transcribed about 10-fold more in H37Rv than in H37Ra (Fig. 6A), suggesting that the phoP mutation could affect phoR transcription. To assess this possibility, we compared phoR expression between an M. tuberculosis clinical isolate MT103 wild-type strain and an MT103 defective phoP mutant (19). Similar to that observed in H37Rv, phoR expression was highly reduced in the phoP mutant of M. tuberculosis (Fig. 6B). To rule out the possibility that phoR transcription was reduced as a consequence of a polar effect of the phoP mutation on the transcription of the entire phoPR operon, we studied phoR expression in the phoP mutant complemented with pSO5K (19), a multicopy plasmid carrying the phoP gene from H37Rv. We observed that phoR transcription was restored in the complemented strain (Fig. 6B). Moreover, the levels of phoR transcripts seemed to be correlated directly with PhoP production, since overexpression of PhoP from the multicopy plasmid resulted in a significant increase in the level of phoR with respect to that in the wild-type strain (Fig. 6B). Altogether, these results indicate that the phoR gene could be transcribed from a PhoP-regulated promoter.

Characterization of the Ser219Leu mutation in PhoP. The M. tuberculosis PhoPR system is highly conserved among slow-growing mycobacteria. Sequence alignment indicated full conservation of the phoP gene in all eight M. tuberculosis complex genomes annotated to date, with the remarkable exception of the H37Rv strain (Fig. 7A). The phoP gene from M. tuberculosis H37Rv has acquired a point mutation in codon 219 (TCC→TTG), resulting in the replacement of a serine by a leucine residue (Fig. 7A). Bioinformatic approaches based on sequence alignment led to the identification of two putative distinct domains in PhoP. The PhoP N-terminal domain is involved in the phosphotransfer reaction through the conserved residue Asp71, whereas the C-terminal domain is involved in DNA binding (data not shown). The recent structure of the PhoP DNA-binding domain (50) situates the above-mentioned missense mutation in the DNA recognition helix (Fig. 7B), potentially affecting DNA interactions and consequently the role of this protein in transcriptional regulation.

A model of the three-dimensional structure of the PhoP Ser219Leu mutant was constructed, and both the wild-type and mutant structures were superimposed on the structure of the PhoB-DNA complex from E. coli (Fig. 7B). The mutation is located in the central part of the C-terminal α-helix (α3) of the three-helix bundle of the effector domain (Fig. 7B). The helix is amphipathic, with the wild-type serine residue facing the solvent. Replacement of a solvent-exposed serine residue by a leucine residue is unlikely to significantly reduce the conformational stability of the protein. On the other hand, the mutant leucine residue cannot easily favor any aggregation of the protein because the region is highly charged. Apparently, the Ser219Leu mutation should give rise to a protein with stability and solubility similar to those of the wild-type protein. In contrast, the mutation is clearly expected to reduce the affinity and/or specificity of PhoP for its DNA-binding region. As the superposition of the model structure of PhoP with the PhoB-DNA complex indicates, the helix bearing the mutation (α3) is the DNA recognition helix of a modified helix-turn-helix DNA-binding motif where loop α2-α3 replaces the turn (4). The mutation therefore takes places right at the DNA-binding site (Fig. 7B). Given the capability of serine residues to estab-
FIG. 4. DNase I footprinting assay of the phoP promoter. (A) Fluorograms indicate fluorescence intensities after DNase I digestion of fragments containing Cy5-labeled coding and noncoding strands in the absence (−PhoP) and presence (+PhoP) of recombinant PhoP. Sequencing reaction mixtures with Cy5-labeled primers were included in the gel (data not shown). Sites protected by PhoP are indicated by boxes. The ATG translation initiation triplet is indicated by asterisks. (B) PhoP binding region. The nucleotides to which PhoP binds are indicated in a box. The ATG translation initiation triplet is indicated by asterisks.
lish hydrogen bonds, Ser219 could be important for the specificity of the binding to DNA. But even if the contribution of Ser219 to specificity and/or binding affinity is small, its replacement by a bulky leucine residue is expected to interfere with a tight protein-DNA interaction and therefore to reduce the affinity of the complex.

**DISCUSSION**

In 1934, Steenken et al. observed that subculture of the H37 strain by serial passages resulted in two different colony morphologies, with one of them being highly attenuated for guinea pigs. The passages continued until two stable strains were obtained, the virulent H37Rv strain and the attenuated H37Ra strain (28, 43–45). Since then, both strains have been used extensively worldwide; indeed, H37Rv is the most widely used virulent *M. tuberculosis* laboratory reference strain today.

The H37Rv genome was the first mycobacterial genome to be sequenced (11) and provided general insight into the biology of *M. tuberculosis*. However, the genetic basis for H37Ra attenuation has remained unclear. H37Ra is likely to have acquired multiple point mutations, deletions, and/or genomic rearrangements during in vitro passage. Somewhat analogously to the attenuation process for BCG, the current antituberculosis vaccine was derived by serial passage of *M. bovis* in the laboratory over a period of 13 years (18), resulting in the loss of more than 100 genes (3).

Even if the presence of genomic variations between H37Rv and H37Ra has been confirmed, their role in H37Ra attenuation remains unclear (5). Pioneering studies in mycobacterial genetics sought to restore virulence to the H37Ra strain by in vivo complementation with an H37Rv cosmid library in an attempt to identify genomic fragments associated with virulence. Despite allowing the identification of a genomic fragment which conferred an enhanced in vivo growth rate, this study failed to completely restore virulence to the H37Ra strain (31).

The recent release of the H37Ra genome sequence available from the NCBI website (NC_009525) should increase our understanding of the mechanism of attenuation of H37Ra. Recent studies have identified a number of nucleotide polymorphisms between H37Rv and H37Ra (16, 25), making it possible to evaluate the role of discrete regions of the genome in *M. tuberculosis* virulence.

In this study, we focused on the *phoP* gene, which has been shown to play an important role in virulence (19, 27, 32). PhoP is fully conserved in all of the annotated genomes of the *M. tuberculosis* complex except that of H37Ra. The *phoP* gene from H37Ra contains a point mutation that results in the replacement of the polar residue Ser219 by the nonpolar residue Leu in the DNA-binding domain of PhoP.

*FIG. 5. RT-PCR analysis of phoPR from* *M. tuberculosis.* (A) Schematic diagram of *M. tuberculosis* phoPR and the gene-specific primers used for RT-PCR (Table 1). The direction of transcription for *phoP* and *phoR* is indicated by arrows. Primers used for RT-PCR and the sizes of the fragments obtained with each pair of primers are indicated. (B) RT-PCR of phoPR. The combination of primers is indicated above each set of reactions. Amplification products for the 5′ end of *phoP* (436 bp), the intergenic region (393 bp), and the 5′ end of *phoR* (464 bp) are shown. A larger fragment, from the *phoP* promoter region to the 3′ end of *phoR* (1,952 bp), was also amplified. The positions of the standard DNA size markers are indicated on the right. Each set of three reactions consists of a positive control PCR assay with genomic DNA as the template (+), an RT-PCR (+), and a negative control assay without reverse transcriptase (−).
used a 238-bp fragment containing the promoter region of \( \text{phoP} \) to demonstrate that the point mutation in H37Ra appears to diminish the DNA-binding affinity of PhoP (data not shown). The results presented in this work are in agreement with a recent study which demonstrated that the H37Ra version of PhoP displays a decreased ability for DNA binding to the PhoP binding motif (25).

The PhoP binding site in the \( \text{phoP} \) promoter extends from nucleotides –79 to +9 relative to the \( \text{phoP} \) start codon in H37Ra (21) and from nucleotides –66 to +1 in the H37Rv strain (Fig. 4). Even if the Ser219Leu substitution may be responsible for these subtle variations, the differences in the methods used should also be taken into account.

PhoP negative autoregulation has been characterized using the protein from \( M. \text{tuberculosis} \) H37Ra (21). Here we carried out similar experiments with PhoP from H37Rv and found that unlike that reported for H37Ra, the \( \text{phoP} \) gene from H37Rv is positively autoregulated with respect to \( \text{phoR} \) expression in the MT103 wild-type strain. Results are the means for two independent experiments; error bars indicate the standard deviations of the means.

Results for the PhoP regulon in \( M. \text{tuberculosis} \) H37Rv and the transcriptome comparison between H37Rv and H37Ra further support the implications of the PhoP mutation in H37Ra avirulence. The \( \text{Rv1184c}, \text{fadD21}, \) and \( \text{papA1} \) genes, encoding proteins involved in the biosynthesis of the virulence-associated lipids SL, DAT, and PAT (12, 39), are much less expressed in H37Ra than in H37Rv (17), which probably accounts for the absence of these lipids in H37Ra. Other genes downregulated in H37Ra (\( \text{Rv1639c}, \text{cdh}, \text{nirK1}, \text{Rv2376c}, \text{nirA}, \text{fadD9}, \text{Rv3312A}, \text{Rv3479}, \text{lsp}, \text{Rv3686c}, \) and \( \text{Rv3822} \) (17) have also been shown to belong to the PhoP regulon identified in H37Rv (49). Altogether, these observations indicate that 14 of the 22 genes differentially expressed between H37Rv and H37Ra are under the control of PhoP. This strongly suggests that the mutated version of PhoP contributes to \( M. \text{tuberculosis} \) H37Ra attenuation by leading to major global changes in the gene expression profile of this avirulent strain. Downregulation of PhoP-regulated genes in H37Ra could be a consequence of the decreased ability of PhoP for DNA binding and/or the inadequate phosphorylation of the protein as a result of decreased expression of \( \text{phoR} \) in H37Ra.

Despite these important contributions of the PhoP mutation to the attenuation process of H37Ra, complementation of this strain with the H37Rv version of PhoP only partially restored the virulence of H37Ra (16, 25). This is probably due to the existence of other polymorphisms affecting the expression of virulence factors. Supporting this hypothesis, it has been found that the production of phthiocerol dimycocerosates—a family of lipids implicated in virulence (6, 35)—is abrogated in the
H37Ra strain and that their synthesis is independent of the PhoP mutation. Alternatively, given that phoR is expressed much less in H37Ra than in H37Rv, which may be a consequence of the phoP mutation in the former strain (Fig. 6), another plausible explanation for the partial virulence complementation of H37Ra is that decreased expression of PhoR in this strain could result in inadequate phosphorylation-mediated activation of PhoP. Accordingly, complementation of H37Ra with the whole H37Rv phoPR operon could restore virulence more proficiently than complementation with only the phoP gene.

Naturally occurring mutations causing attenuation of bacterial pathogens have already been described. Sequencing of low-virulence field strains of Listeria monocytogenes recently showed multiple point mutations affecting the virulence of this intracellular pathogen (46). Remarkably, like the Ser219Leu mutation in PhoP, the naturally occurring mutation Lys220Thr in the transcriptional regulator PrfA from L. monocytogenes results in abrogated DNA binding, no expression of PrfA-regulated proteins, and attenuated virulence (48).

Our study also suggests that differences between M. tuberculosis strains should be taken into account in genetic studies. Continuous passages in synthetic laboratory media may well result in genetic polymorphisms and, consequently, in substrain variability. H37Rv is a highly pathogenic strain and can be manipulated only in biosafety level 3 containment laboratories. For this reason, many genetic studies have made use of the attenuated counterpart, H37Ra, based on the assumption that the results obtained could be extrapolated to all M. tuberculosis complex strains. However, different works, including this one, clearly demonstrate the important implications of a single mutation in virulence regulation.

ACKNOWLEDGMENTS

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FIG. 7. Characterization of the point mutation in PhoP from H37Ra. (A) Domain organization of PhoP. The N-terminal domain of PhoP (blue) contains the Asp71 residue involved in the phosphotransfer reaction. The C-terminal domain (red) interacts with target DNA molecules, modulating gene expression. Alignment of the phoP gene sequences from the annotated genomes of the M. tuberculosis complex shows a point mutation responsible for the Ser219Leu substitution in the H37Ra strain, indicated by an asterisk. (B) DNA-binding domain of PhoP (blue) (see text for details) superimposed on the structure of a PhoB-DNA complex (gray ribbon, protein; sticks in CPK color, DNA). Solid spheres show the wild-type serine 219 residue (left) or the leucine residue that appears in the mutant protein (right). The mutant leucine residue is expected to interfere with DNA binding and/or recognition.


