Role of Stress Response Sigma Factor SigG in *Mycobacterium tuberculosis*\(^\dagger\)†

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*Myobacterium tuberculosis* causes disease in humans and has been one of the most important human pathogens since it was first identified 125 years ago. It is estimated that approximately 9 million new cases of tuberculosis and 2 million deaths due to this disease occur each year (6). A unique feature of *M. tuberculosis* is its ability to persist as a latent infection, where it lies dormant and escapes detection by the host immune system. The environmental stress response in bacteria is controlled by the extracytoplasmic function (ECF) sigma factors, which contribute to the resistance to stress conditions, such as high and low temperature, oxidative stress, carbon starvation, and low pH. The number of ECF sigma factors varies in different bacterial species. In this study, we focused on the ECF sigma factor genes under normal in vitro growth conditions (9). However, a previous report showed that *sigG* was one of the most highly induced genes during macrophase infection and that its expression was increased 9.7-fold (2). The promoter region of *sigG* is similar to that of the *recA* gene, which has a key role in the SOS response (5). These data suggested that SigG is unlikely to play a significant role in bacterial physiology during in vitro growth in rich medium but that it may be conditionally up-regulated in response to certain environmental signals. In this study, we constructed a *sigG* deletion mutant and characterized its expression patterns and phenotypes compared to wild-type *M. tuberculosis*.

**Construction of ΔsigG mutant.** *sigG* mutant construction was carried out as described previously (21) using the suicide vector pCK0686 (7). The left flanking region of *sigG* was amplified with primers sigGP1 and sigGP2, and the right flanking region was amplified with primers sigGP3 and sigGP4 (see Table S1 in the supplemental material). After amplification by PCR, the two flanks were ligated into pCK0686. To construct the complemented strain, approximately 5.5 kb of the region containing the *sigG* gene was amplified by using primers gcomp1 and gcomp2 and then cloned into the pMH94 vector (8) at the XbaI sites. Putative mutant candidates were screened by PCR with the RT-sigG primer set (Fig. 1B) and by Southern blotting (Fig. 1C and D). The ΔsigG mutant strain was confirmed by the absence of a 17.5-kb band using the *sigG* gene probe (Fig. 1C) and the appearance of a 12-kb band using the hygromycin gene probe (Fig. 1D). Complementation was achieved by cloning a 5.5-kb *sigG*-containing fragment into pMH94 and introducing this plasmid into the mutant strain (Fig. 1C).

Recently, a sequence similar to the *recA* promoter of the *recA* gene was identified in the upstream region of *sigG* (5), which suggested that *sigG* may be involved in the SOS response mechanism. However, using the TubercuList database (http://genolist.pasteur.fr/TubercuList/), this promoter region was located in the open reading frame of the *sigG* gene. Therefore, we determined the exact transcription start point of the *sigG* gene by the random amplification of cDNA ends technique (see Fig. S1 in the supplemental material). The transcription start point of the *sigG* gene was identified as an A residue 5 bp from the putative start site, GTG (see Fig. S1 in the supplemental material). While a 5-nucleotide region is a relatively short 5′ untranslated region (UTR), we did observe an ATG codon 102 bp distal to the currently annotated start site of translation. Hence, it is possible that SigG protein translation initiates at this alternative site.

**Gene expression profiles of a ΔsigG strain.** We monitored changes in *sigG* expression during the growth cycle, comparing log-phase (optical density [OD], 0.5), early-stationary-phase (OD, 1.0), and late-stationary-phase (OD, 2.0) levels by real-time reverse transcription PCR (RT-PCR) with the RT-sigG primer set (see Table S1 in the supplemental material). The threshold cycle (C\(_T\)) value was normalized to the value for *sig4*, and the expression level of the wild type was compared with that of the ΔsigG strain. As shown in Fig. 2, the ΔsigG mutant was con-
confirmed not to express \textit{sigG} mRNA. For the other sigma factors, \textit{sigH} and \textit{sigF} gene expression was decreased, while the expression of \textit{sigD} was increased. It is known that the expression of the \textit{M. tuberculosis} sigma factors is interdependent. \textit{SigH} regulates \textit{sigE} and \textit{sigB}, and \textit{SigL} and \textit{SigF} regulate \textit{sigB} (3, 10, 16), while other sigma factors, such as \textit{sigD}, \textit{sigK}, and \textit{sigM}, have autotranscription properties (18). The effect of deletion of the \textit{sigG} gene on the expression of \textit{sigH} and \textit{sigD} represents another example of cascade gene regulation among sigma factors in \textit{M. tuberculosis}.

To identify the \textit{sigG}-dependent genes, a microarray study was performed as described previously (21). The slides were scanned using an Axon 400B scanner, and the image data were quantified using GenePix pro 4.0 software. Each data point was then normalized to the total intensity of the spots, and the ratio of Cy5 to Cy3 was calculated. Our microarray expression profile of the \textit{\textDelta sigG} mutant compared to that of the wild-type CDC1551 strain revealed over 100 genes that were down-regulated in the \textit{\textDelta sigG} mutant relative to the wild type (see Table S2 in the supplemental material). Several genes which may be involved in fatty acid metabolism were found to be down-regulated, including \textit{aceA}, the isocitrate lyase gene (Rv0467), \textit{fadE5}, the acyl-coenzyme A dehydrogenase gene (Rv0244), and \textit{SCO}, the succinyl-coenzyme A gene (Rv2504c). Other genes that were down-regulated have been shown to be induced in the murine macrophage model; these genes include the heat shock protein gene, Rv0251c (which is induced during exposure to heat, H$_2$O$_2$, and sodium dodecyl sulfate), the isocitrate lyase gene, \textit{aceA} (12, 20), and \textit{lat}, the l-lysine-epsilon aminotransferase gene (Rv3290c) (which is induced in a star-
The SOS box showed LexA binding in a gel shift assay (1). The relative expression value of each gene of interest (GOI) was normalized to the housekeeping gene (HKG). Regulation of individual genes was calculated using the following term:

\[ \frac{C_{\text{mutant}} - C_{\text{wild type}}}{C_{\text{mutant}} + C_{\text{wild type}}} \]

where \( S \) is the \( \Delta \text{sigG} \) strain and \( C \) is the wild-type strain. Open bars, OD of 1; shaded bars, OD of 2.

![FIG. 2. Expression profiles of \( M. \) tuberculosis \( \Delta \text{sigG} \) mutant strain. The relative expression profile of each sigma factor in the \( \Delta \text{sigG} \)-deficient mutant was determined by real-time RT-PCR at OD of 1 and 2. The \( C_{\text{T}} \) value of each gene of interest (GOI) was normalized with the housekeeping gene (HKG), \( \text{sigG} \). The relative expression value was obtained by the \( \Delta \Delta C_{\text{T}} \) method: \( \Delta C_{\text{T}} = \Delta C_{\text{GOI}} - \Delta C_{\text{HKG}} \). Regulation of individual genes was calculated using the following term: \( 2^{-\Delta \Delta C_{\text{T}}} \), where \( S \) is the \( \Delta \text{sigG} \) strain and \( C \) is the wild-type strain.

Two interesting findings were the reduction in the repressor \( \text{lexA} \) (Rv2720) transcription and the increase in \( \text{recA} \) (Rv2737c) transcription in the \( \Delta \text{sigG} \) mutant. \( \text{recA} \) is a gene involved in the SOS response and contains a LexA binding motif (SOS box) in the promoter region. We also found that \( \text{lexA} \) contained a putative SigG consensus binding site in its 5' UTR 262 bp from the start codon, which was confirmed to be regulated by SigG by in vitro transcription (Fig. 4) and real-time RT-PCR (Fig. 5). For the in vitro transcription assay, the entire open reading frame of the \( \text{sigG} \) gene was amplified with primers petsigG1 (see Table S1 in the supplemental material) and petsigG2 and cloned into the pET22b(+) vector (Novagen). The construct was transformed into \( \text{Escherichia coli} \) BL21(DE3), and protein expression was induced with 1 mM isopropyl-\( \beta \)-D-thiogalactopyranoside (IPTG), and the protein was purified by Ni-nitriilotriacetic (Ni-NTA) acid affinity chromatography. In vitro transcription assays were performed as described in a

![TABLE 1. Microarray analysis of sigma factor- and SOS box-related genes in the \( M. \) tuberculosis \( \Delta \text{sigG} \) mutant compared to the wild type\(^a\)](http://genolist.pasteur.fr/TubercuList/)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Code</th>
<th>Rv no.</th>
<th>Relative signal (mutant/wild type)</th>
<th>( q ) value (^b)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{sigH} )-related genes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{sigH} )</td>
<td>2</td>
<td>Rv3223c</td>
<td>0.4 ± 0.0</td>
<td>0.00</td>
<td>RNA polymerase sigma factor</td>
</tr>
<tr>
<td>( \text{clpB} )</td>
<td>0</td>
<td>Rv0384c</td>
<td>0.2 ± 0.0</td>
<td>0.00</td>
<td>Endopeptidase ATP binding protein</td>
</tr>
<tr>
<td>( \text{dnaK} )</td>
<td>0</td>
<td>Rv0350</td>
<td>0.3 ± 0.0</td>
<td>0.00</td>
<td>Chaperone protein</td>
</tr>
<tr>
<td>( \text{Rv2466c} )</td>
<td>10</td>
<td>Rv2466c</td>
<td>0.2 ± 0.0</td>
<td>0.00</td>
<td>Conserved hypothetical protein</td>
</tr>
<tr>
<td>( \text{trxB2} )</td>
<td>7</td>
<td>Rv3913</td>
<td>0.8 ± 0.0</td>
<td>0.05</td>
<td>Thioredoxin reductase</td>
</tr>
<tr>
<td>( \text{sigD} )-related genes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{sigD} )</td>
<td>2</td>
<td>Rv3414c</td>
<td>1.6 ± 0.5</td>
<td>0.07</td>
<td>RNA polymerase sigma factor</td>
</tr>
<tr>
<td>( \text{Rv1815} )</td>
<td>10</td>
<td>Rv1815</td>
<td>5.2 ± 0.7</td>
<td>0.07</td>
<td>Conserved hypothetical protein</td>
</tr>
<tr>
<td>( \text{Rv1884c} )</td>
<td>3</td>
<td>Rv1884c</td>
<td>11.0 ± 0.3</td>
<td>0.07</td>
<td>Resuscitation-promoting factor</td>
</tr>
<tr>
<td>SOS box</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{sab} )</td>
<td>2</td>
<td>Rv0054</td>
<td>0.7 ± 0.1</td>
<td>0.01</td>
<td>Single-strand binding protein</td>
</tr>
<tr>
<td>( \text{dnaB} )</td>
<td>2</td>
<td>Rv0058</td>
<td>1.4 ± 0.1</td>
<td>0.07</td>
<td>Replicative DNA helicase</td>
</tr>
<tr>
<td>( \text{ding} )</td>
<td>2</td>
<td>Rv1329e</td>
<td>0.6 ± 0.0</td>
<td>0.07</td>
<td>ATP-dependent helicase</td>
</tr>
<tr>
<td>( \text{recN} )</td>
<td>2</td>
<td>Rv1696</td>
<td>1.0 ± 0.1</td>
<td>0.07</td>
<td>DNA repair protein</td>
</tr>
<tr>
<td>( \text{ravB} )</td>
<td>2</td>
<td>Rv2592c</td>
<td>0.6 ± 0.1</td>
<td>0.06</td>
<td>Holliday junction DNA helicase</td>
</tr>
<tr>
<td>( \text{ravB} )</td>
<td>2</td>
<td>Rv2593c</td>
<td>0.8 ± 0.1</td>
<td>0.07</td>
<td>Holliday junction DNA helicase</td>
</tr>
<tr>
<td>( \text{ravC} )</td>
<td>2</td>
<td>Rv2594c</td>
<td>1.6 ± 0.0</td>
<td>0.07</td>
<td>Crossover junction endodeoxyribonuclease</td>
</tr>
<tr>
<td>( \text{lexF} )</td>
<td>9</td>
<td>Rv2720</td>
<td>0.6 ± 0.1</td>
<td>0.01</td>
<td>LexA</td>
</tr>
<tr>
<td>( \text{recA} )</td>
<td>2</td>
<td>Rv2737c</td>
<td>1.5 ± 0.1</td>
<td>0.07</td>
<td>RecA</td>
</tr>
<tr>
<td>( \text{dnaF} )</td>
<td>2</td>
<td>Rv3056</td>
<td>0.7 ± 0.0</td>
<td>0.07</td>
<td>DNA damage-inducible protein</td>
</tr>
<tr>
<td>( \text{dnaE2} )</td>
<td>2</td>
<td>Rv3370c</td>
<td>0.6 ± 0.1</td>
<td>0.07</td>
<td>DNA polymerase</td>
</tr>
</tbody>
</table>

\(^a\) Wild-type and mutant strains were grown to an OD of 1.0. Codes (functional categories) and gene annotation data were obtained from the TubercuList database (http://genolist.pasteur.fr/TubercuList/). The function codes indicate the following functions: 0, virulence, detoxification, and adaptation; 2, information pathways; 3, cell wall and cell processes; 7, intermediary metabolism and respiration; 9, regulatory proteins; 10, conserved hypothetical proteins.

\(^b\) The \( q \) value was calculated using SAM (http://wwwstat.stanford.edu/~tibs/SAM/) with 1% false discovery rate.

\(^c\) The SOS box showed LexA binding in a gel shift assay (1).
previous study (21). The lexA gene promoter was amplified by PCR using primers lex1 (see Table S1 in the supplemental material) and lex2, the 400-bp PCR product was purified by gel extraction (Qiagen), and 0.09 µg was used as a DNA template. As shown in Fig. 4, we detected the anticipated in vitro transcript with a length of 300 nucleotides.

**Role of sigG in SOS response and mitomycin C (MMC) susceptibility.** Upon DNA damage, the SOS response is induced through the RecA-LexA regulon. The LexA protein binds to the SOS box upstream of genes and acts as a repressor of SOS genes. Following DNA damage LexA is cleaved in an autocatalytic reaction stimulated by RecA. The resulting fragment of LexA cannot bind efficiently to the SOS box and thereby cannot repress the transcription of SOS genes (17). This RecA-LexA regulon has also been identified in *M. tuberculosis*. The *M. tuberculosis* recA gene also has two promoter sequences. One sequence is a typical sigma 70-like promoter sequence, and the other is a sigma 32-like sequence. In these two promoter sequences, the SOS box is located between the −35 and −10 regions of the sigma 32-like promoter sequence (13). The promoter sequence upstream of sigG shares close similarity to this sigma 32-like sequence, implying that recA and sigG may be transcribed by the same sigma factor in response to SOS stress conditions.

In a previous study lexA was found to contain an SOS box with the sequence TCAGAACYCATGTTGGA upstream of its promoter region (1). The SigG consensus binding site identified in this study was located upstream of this SOS box. This suggests that lexA transcription by SigG is limited to the SOS response and is controlled by sigG rather than the principal sigma factor. Many *M. tuberculosis* genes, such as dnaB (Rv0058), ruvA (Rv2593c), ruvC (Rv2594c), lexA (Rv2720), and recA (Rv2737c), have a putative LexA binding site which showed positive binding in a gel shift assay. However, the LexA protein does not bind the same consensus binding site in the promoter region of ssb (Rv0054), dinG (Rv1329c), recN (Rv1696), ruvB (Rv2592c), dinP (Rv3056), or dnaE2 (Rv3370c) (1). These results are consistent with our microarray data (Table 1) since many of the same genes were underexpressed in the ΔsigG mutant.

**The M. tuberculosis ΔsigG mutant is resistant to mitomycin C.** MMC is a potent SOS response inducer and has antibacterial activity against *Mycobacterium bovis* (14). MMC causes cell death during DNA damage, but there is also rescue due to DNA repair and the SOS response (error-prone response system). However, in the absence of LexA repression, the SOS system is constitutively expressed, and therefore lexA mutants have been demonstrated to display relative MMC resistance compared to the wild type (24). Therefore, we tested the MMC resistance of the *M. tuberculosis* ΔsigG strain. The ΔsigG mutant strain was treated with 0.2 and 1 µg/ml MMC. Using *A*500 to monitor growth, we found that the wild-type and complemented strains were more susceptible than the mutant to both 0.2 and 1.0 µg/ml MMC (Fig. 6). This study was repeated two times.
times, and a similar pattern was observed. This indicates that deletion of sigG confers resistance to MMC. The CFU of bacteria were also counted after serial dilution (10^3- to 10^6-fold). For the ΔsigG strain the bacterial counts were 10^7 CFU/ml without MMC and 10^6 CFU/ml in the presence of 0.2 μg/ml MMC after 2 and 3 days. We did not detect the wild-type and complemented strains at this concentration. With a higher concentration of MMC, we did not detect any bacteria on the plates with the same dilution ratios. These data indicate that deletion of sigG confers resistance to MMC in M. tuberculosis.

Macrophage infection. To assess the role of sigG in macrophage infection, we compared the ability of the ΔsigG mutant to invade and survive within J774A.1 murine macrophages with that of its parental wild-type strain (CDC1551). The initial intracellular inoculum after 2 h of infection was about 10^5 CFU/ml without MMC and 10^4 CFU/ml in the presence of 0.2 μg/ml MMC after 2 and 3 days. We did not detect the wild-type and complemented strains at this concentration. With a higher concentration of MMC, we did not detect any bacteria on the plates with the same dilution ratios. These data indicate that deletion of sigG confers resistance to MMC in M. tuberculosis.

M. tuberculosis causes attenuation in monocyte-derived macrophages (23). The hsp gene (Rv0251c) was reported to belong to the sigE regulon, and its expression was reduced by sigE deletion. However, we identified the sigG consensus promoter recognition sequence in the upstream region of the hsp gene, whose expression was also reduced by deletion of sigG. As determined by real-time RT-PCR, deletion of sigG led to a reduction in sigH expression, but it did not change the expression of sigE. These observations support the hypothesis that hsp is also regulated by sigG. Another sigG regulon gene, aceA, was induced in macrophages, and the deletion mutant showed attenuation in the macrophage infection model.

Previous studies with M. tuberculosis indicated that sigG may play a role in the stress response during macrophage infection and in the SOS response. sigG is one of the most highly induced genes during macrophage infection (2). The promoter region of the sigG gene showed similarity with a known recA P1 promoter sequence upstream of the M. tuberculosis recA gene, which has been shown to be a key factor in the SOS response (5). This suggests that sigG is cotranscribed with the recA gene by the same RNA polymerase-sigma factor holoenzyme and that sigG may be a key regulator in the stress response in M. tuberculosis.

In this study, we identified the significance of sigG during macrophage infection and in the SOS response. Genes regulated by the SigG sigma factor are important for M. tuberculosis survival inside macrophages and for SOS stress response-related functions.
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