JOURNAL OF BACTERIOLOGY, Feb. 2011, p. 1007–1011 Vol. 193, No. 4
0021-9193/11/$12.00 doi:10.1128/JB.01241-10

SigG Does Not Control Gene Expression in Response to DNA Damage in *Mycobacterium tuberculosis* H37Rv▽§

Katherine L. Smollett,† Lisa F. Dawson,†‡ and Elaine O. Davis*

Division of Mycobacterial Research, MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, United Kingdom

Received 15 October 2010/Accepted 5 December 2010

Expression of the *Mycobacterium tuberculosis sigG* sigma factor was induced by a variety of DNA-damaging agents, but inactivation of *sigG* did not affect induction of gene expression or bacterial survival under these conditions. Therefore, SigG does not control the DNA repair response of *M. tuberculosis* H37Rv.

Mycobacterium tuberculosis has at least two mechanisms regulating gene expression following DNA damage (6): the SOS response mediated by LexA and RecA (5, 9, 17) and an alternative mechanism independent of both RecA and LexA (6, 21). It is not known what controls the RecA-independent response, although a potential promoter motif common to a number of genes in this regulon that suggests the possible involvement of an alternative sigma factor has been identified (11). Of the 13 sigma factors in *M. tuberculosis* (2), SigG was the most highly induced following DNA damage and macrophage infection (1, 21). Moreover, a recent study suggested that SigG was responsible for transcription of *lexA* in a clinical isolate, CDC1551 (14).

Here, we addressed the role of SigG in *M. tuberculosis* H37Rv in response to DNA damage by comparing the global transcriptional profile of a *sigG* mutant strain with that of the wild type. Our results indicate that the absence of SigG does not affect the induction of known DNA damage response genes. In addition, we could find no evidence for the involvement of SigG in the transcription of *lexA in vivo*. Furthermore, the *sigG* mutant was no more susceptible to DNA damage than the parental strain. Therefore, we conclude that SigG does not control expression of either the RecA-dependent or the RecA-independent genes in response to DNA damage in *M. tuberculosis* H37Rv.

A potential role for SigG in regulating the response to DNA damage was initially proposed based on its induction following exposure to mitomycin C (21), which causes interstrand cross-links and alkylation damage. Basing our analysis on the pelleting properties of the bacteria, we suspect mitomycin C also affects the mycobacterial cell surface. Therefore, we measured the levels of *sigG* mRNA following other treatments predicted to damage DNA, namely, bleomycin exposure, ofloxacin exposure, and UV irradiation, resulting in breaks in DNA, inhibition of DNA gyrase, and induction of the formation of thymine dimers, respectively. The effect of cell surface stress on *sigG* expression was also assessed by treatment with ethambutol and exposure to sodium dodecyl sulfate (SDS). *M. tuberculosis* H37Rv cultures (See Table 1 for strains and plasmids used) were grown under Advisory Committee on Dangerous Pathogens (ACDP) containment level 3 conditions as described previously (6). Cultures were divided into aliquots at an optical density at 600 nm (OD_{600}) of 0.3 to 0.4; samples were induced with mitomycin C (0.2 μg ml^{-1}), ofloxacin (7.5 μg ml^{-1}), or bleomycin (1.5 μg ml^{-1}) for 5 h at 37°C, exposed to 23.5 J m^{-2} UV irradiation followed by 5 h of recovery at 37°C, or induced with SDS (0.05% for 5 h at 37°C) or ethambutol (15 μg ml^{-1} for 24 h at 37°C). Another sample was incubated in parallel without treatment to provide an uninduced control. RNA was extracted and purified as described previously (10), and cDNA synthesis was performed using Superscript II reverse transcriptase (RT) (Invitrogen). Quantitative PCR was carried out using Fast SYBR green master mix (Applied Biosystems) on an Applied Biosystems 7500 Fast instrument and analyzed with 7500 Fast SDS software version 1.4. Gene-specific primers (Table 2) were designed using Primer Express version 3.0 (Applied Biosystems). cDNA samples (and their RT-negative controls) were run alongside genomic DNA standards and relative expression levels calculated as described previously (10) except that normalization was based on the *rrs* gene encoding 16S rRNA. These values were divided by the corresponding values of the untreated sample to give induction ratios. Expression of *sigG* was induced by each DNA-damaging agent at a level similar to that seen with mitomycin C but was not increased by cell surface stress (Fig. 1). Induction resulting from DNA damage was also shown to occur in a Δ*recA* strain, indicating that DNA-damage-specific induction of *sigG* was due to the alternative RecA-independent DNA damage response and not the SOS response.

We constructed a strain in which *sigG* was inactivated by replacing region 2.4, which is responsible for interactions with the −10 promoter element, and region 4.2, which interacts with the −35 element, with a hygromycin cassette (Fig. 2). Potential mutants were screened by Southern blot analysis as described previously (10), and isolates confirmed to have the correct genotype were selected for further study. The mutation

* Corresponding author. Mailing address: Division of Mycobacterial Research, MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, United Kingdom. Phone: 44 (0)20 8816 2358. Fax: 44 (0)20 8816 2564. E-mail: edavis@nimr.mrc.ac.uk.

† K.L.S. and L.F.D. contributed equally to this paper.

‡ Present address: Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London WC1E 7HT, United Kingdom.

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

▽ Published ahead of print on 17 December 2010.

TABLE 1. Strains and plasmids used in the study

Strain or plasmid	Description	Reference or source
Strains		
DH5α	*Escherichia coli* strain used for general cloning	Invitrogen
XL1-Blue	*E. coli* strain used for site-directed mutagenesis (SDM)	Stratagene
H37Rv	*M. tuberculosis* wild-type strain	18
Δ*sigG*1	H37Rv with mutation of *sigG* constructed by homologous recombination with targeting construct pLD1; contains secondary mutation of *ppsE*	This study
Δ*sigG*2	H37Rv with mutation of *sigG* constructed by homologous recombination with targeting construct pLD1	This study
Plasmids		
pBackbone	Modified pBluescript KS(−) with 160-bp deletion in *lacZ′*, PacI site, and Kan^r cassette	12
pUC-Hyg	Plasmid carrying Hyg^r cassette	15
pGoal17	Plasmid carrying *lacZ-sacB* cassette	20
pLD1	*sigG* targeting construct; made by cloning of a 4.3-kb DNA fragment containing *sigG* and surrounding sequence amplified with primers sigG1 and sigG2 into pBackbone, removal of 691 bp of the coding region of *sigG* by inverse PCR using primers i-SigGF and i-SigGR, and replacement with a 1.6-kb hygromycin cassette from pUC-Hyg; a *sacB-lacZ* cassette from pGOAL17 was introduced for counterselection and screening	This study
pEJ414	Integrating mycobacterial *lacZ* transcriptional reporter vector	19
pEMD8	587-bp *lexA* upstream region in pEJ414	8
pEJ554	TAC-to-GCG mutation in SigA −10 motif of pEMD8, made by SDM with primers lexpr-10bf and lexpr-10br	This study

was shown by quantitative RT-PCR (qRT-PCR) to cause a partial polar effect, resulting in a decrease in expression of *Rv0181c*, the gene locus immediately downstream of *sigG*, but unchanged levels of expression of *Rv0180c* (data not shown). Isolate Δ*sigG*1 was used for microarray analysis, which revealed a defect in expression of the *ppsE* gene in the mutant strain which was not reversed on complementation (data not shown). PpsE is a polyketide synthase involved in the production of the cell wall lipid phthiocerol dimycocerosate (PDIM) (22); loss of PDIM has been shown to occur spontaneously *in vitro* (7). Expression of *ppsE* was shown not to have been affected in the independently isolated Δ*sigG*2 isolate, which was then used for all subsequent experiments.

The global transcriptional response to DNA damage in the Δ*sigG*1 strain was investigated and compared with that of wild-

TABLE 2. Primers used in the study

Primer	Sequence
sigG1	AAAGATCTAAGCCCGCCAATAGCAGCAAGAGG
sigG2	GGATGCATAGCGCAAGCACGTCGTCCACATAA
i-SigGF	TACCTAGGGCCATCGTCACCCTCATTCACCAA
i-SigGR	TACCTAGGTCGGTGTGGGCGGAGAAGT
lexpr-10bf	GGTGCGAATGCGACGCGATTCATTGCCATG
lexpr-10br	CATGGCAATGAATCGCGTCGCATTCGCACC
qRT-PCR primers	
rrs-qRTF	AAGAAGCACCGGCCAACTAC
rrs-qRTR	TCGCTCCTCAGCGTCAGTTA
sigG-qRTF	TGAACTGCTCGCACACTGCTA
sigG-qRTR	AGCGTCTCCTGAACAAGGTCTT
lexA-qRTF	GGAGCGCAAGGGCTACCT
lexA-qRTR	GCACCGCGCACATTGAC
recA-qRTF	ATCGAGAAGAGTTACGGCAAAGG
recA-qRTR	GCCCAGGGCCACGTCTA
ruvC-qRTF	CAACGGTTCCGCAGACAAG
ruvC-qRTR	GCCGGTGTCGGTTTAGCTT
radA-qRTF	GGACCGCGTTCGCTAGAG
radA-qRTR	TTGTCGTGCAGGAGGAAACA
uvrA-qRTF	CGCGAGCAGCGGTTCT
uvrA-qRTR	CGCCGTAGGGCGAGTTG
ung-qRTF	ACTTTCCCGTTCGACAACGT
ung-qRTR	AGCATGTCCTGGAGTCGGATA
Rv2191-qRTF	TTCGCCACCCTGGTAAACC
Rv2191-qRTR	CCACCATCGCCGTAGTGATA
Rv3202-qRTF	AGAGCAGGTCATGGTCCTTAGC
Rv3202-qRTR	GGCGATAACTACCAGATCCCATT

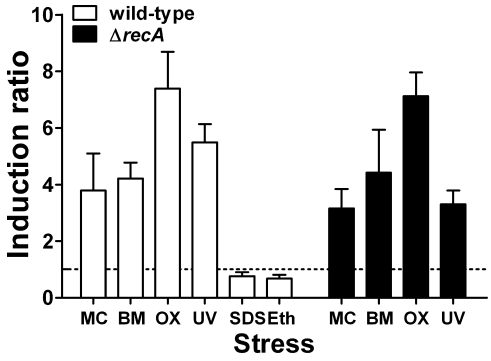

FIG. 1. Induction of *sigG* in response to different DNA-damaging agents. qRT-PCR was used to determine expression of *sigG* in response to exposure to the following DNA-damaging agents under the indicated conditions: mitomycin C (MC), 0.2 μg ml^{-1} for 5 h at 37°C; bleomycin (BM), 1.5 μg ml^{-1} for 5 h at 37°C; ofloxacin (OX), 7.5 μg ml^{-1} for 5 h at 37°C; and UV irradiation, 23.5 J m^{-2} followed by 5 h of recovery at 37°C. Each data set represents the means + standard deviations of the results determined with three biological replicates. Alternatively, the response to cell surface stress was determined by exposure to SDS (0.05% for 5 h at room temperature) or ethambutol (Eth) (15 μg ml^{-1} for 24 h at 37°C); for these determinations, each data set represents the means + standard deviations of the results determined with two biological replicates. Expression levels were determined and normalized to *rrs* expression, and induction ratios were calculated relative to untreated control results. The dotted line indicates an induction ratio of 1 (signifying lack of induction). *sigG* expression was induced under DNA-damaging conditions in both wild-type and Δ*recA* strains of *M. tuberculosis* but was not induced by the cell surface stresses caused by the presence of SDS or ethambutol.

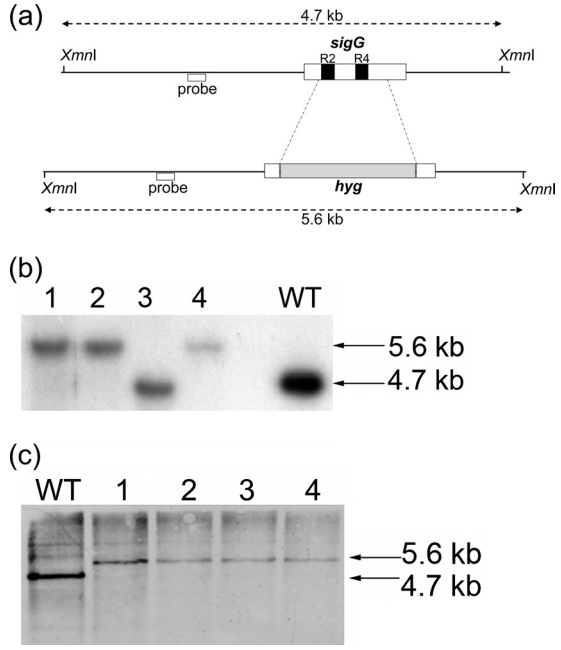

FIG. 2. Construction of *sigG* mutant strains. (a) A total of 691 bp internal to *sigG* was deleted by allelic recombination and replaced with a 1.6-kb hygromycin cassette. The schematic shows the locations of the regions replaced within *sigG* (dotted lines) and the DNA binding regions 2.4 (R2) and 4.2 (R4) (black boxes). The positions of the probes (white boxes below lines) and XmnI restriction sites used in Southern blot analysis are indicated, along with the expected fragment sizes detected in the wild-type and mutant strains (double-headed arrows). (b and c) Genomic DNA was extracted from potential Δ*sigG* mutant colonies (lanes 1 to 4) and Southern blot analysis of the genomic DNA performed alongside wild-type DNA analysis (lanes WT) after digestion was performed with XmnI and either a radiolabeled probe (b) or a horseradish peroxidase-labeled probe (c) used to detect potential double crossovers. (b) Δ*sigG*1 mutant construction; colonies 1, 2, and 4 showed the correct genotype. (c) Δ*sigG*2 mutant construction; all 4 colonies showed the correct genotype.

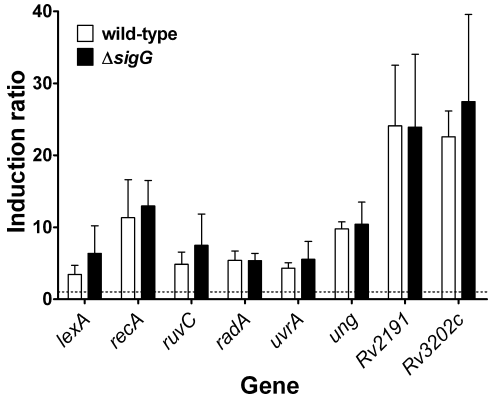

FIG. 3. Expression of selected DNA repair genes in Δ*sigG* compared to wild-type *M. tuberculosis* strains in response to mitomycin C induction. Expression levels were determined by qRT-PCR after treatment with 0.2 μg ml^{-1} mitomycin C for 24 h and normalized to *rrs* expression, and induction ratios were calculated relative to untreated control results. The dotted line indicates an induction ratio of 1 (signifying no induction). Data represent means + standard deviations of the results obtained with three biological replicates. There were no significant differences in the levels of induction of any of these genes in the two strains.

type H37Rv by microarray analysis. Whole-genome *M. tuberculosis* microarray slides were obtained from the Bacterial Microarray Group at St. George's, London, United Kingdom. The array design is available at BμG@Sbase (accession no. A-BUGS-1; http://bugs.sgul.ac.uk/A-BUGS-1) and also at ArrayExpress (accession no. A-BUGS-1). Cy5-labeled RNA versus Cy3-labeled DNA hybridizations were performed, using control DNA obtained from Colorado State University, as described previously (10). As the sets of RNA samples were competitively hybridized against genomic DNA, rather than against each other, dye-swap studies were not necessary (24). The microarray slides were scanned using a GenePix Axon 4000A scanner (Axon Instruments), and image data were processed using Bluefuse for Microarrays 3.6 (BlueGnome). The data were normalized as described previously (10) but using GeneSpring GX 11 (Agilent Technologies) analysis software. Significant differences were determined using two-way analysis of variance (ANOVA) with the Benjamini and Hochberg False Discovery Rate correction. Fully annotated microarray data have been deposited in BμG@Sbase (accession number E-BUGS-111; http://bugs.sgul.ac.uk/E-BUGS-111) and ArrayExpress (accession number E-BUGS-111). The expression levels of 94 genes were increased at least 1.5-fold with statistical significance ($P \leq 0.05$) in the wild-type or Δ*sigG*1 strains following exposure to 0.02 μg ml^{-1} mitomycin C for 24 h (see Table S1 in the supplemental material); this list of genes corresponded well to that reported previously from RNA-versus-RNA hybridizations and exposure to 0.2 μg ml^{-1} mitomycin C (21). When the expression levels of these 94 genes were compared between the two strains, none were found to differ significantly ($P > 0.05$). In fact, only five genes were found to differ significantly between the wild-type and Δ*sigG*1 strains under either set of conditions; further analysis found that these differences were most likely due to a secondary mutation and not to the absence of *sigG* (see above). We tested a subset of genes by quantitative RT-PCR using the Δ*sigG*2 mutant (Fig. 3). No differences in induction were observed between wild-type and Δ*sigG*2 strains for genes known to show RecA-dependent (*lexA*), partially RecA-dependent (*recA*, *ruvC*, *radA*), or RecA-independent (*uvrA*, *ung*, *Rv2191*, *Rv3202c*) DNA damage induction. Taken together, these data demonstrate that SigG does not regulate gene expression following DNA damage in *M. tuberculosis* H37Rv.

Transcription and translation start sites for *lexA* have previously been shown to coincide (17, 23), and appropriately located motifs resembling mycobacterial SigA promoter elements (25) have been identified, suggesting that *lexA* was transcribed by SigA in *M. tuberculosis* (Fig. 4a). A putative SigG promoter has also been identified upstream of *lexA* but is at too great a distance to correspond to transcription initiation at the mapped start site. A transcriptional fusion of the region upstream of *lexA* with the reporter gene *lacZ* was assayed for β-galactosidase activity as described previously (5). The wild-type *lexA* promoter sequence in pEMD8 directed high-level expression of β-galactosidase in the wild-type strain that increased approximately 4-fold upon mitomycin C induction, as shown previously (8). Changing the first three bases of the putative SigA −10 motif from TAC to GCG in pEJ554 dra-

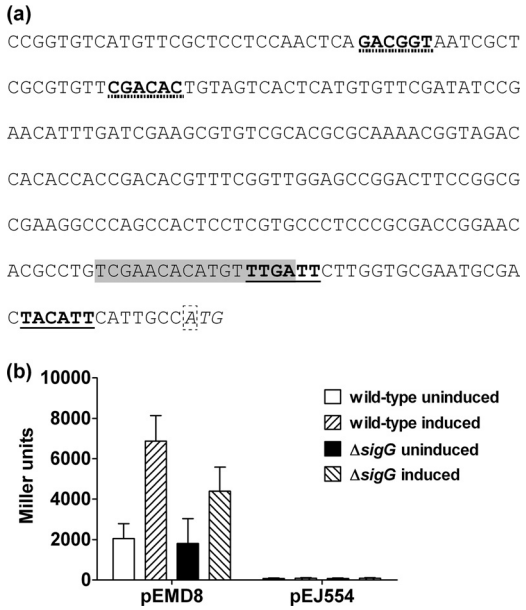

FIG. 4. SigG does not effect expression of LexA. (a) The upstream region of *lexA*, showing the LexA start codon (italics), the transcriptional start site (dashed box), the SigA promoter motif (bold, solid underline), the SigG promoter motif (bold, dotted underline), and the SOS box (shaded). (b) β-Galactosidase activity of a *lacZ* transcriptional fusion to the wild-type *lexA* promoter region (pEMD8) or of that seen with a mutation in the *sigA* consensus promoter (pEJ554). Activity of the wild-type *lexA* promoter was increased 3- to 4-fold following induction by 0.02 μg ml^{-1} mitomycin C for 24 h in both the wild-type (WT) and Δ*sigG* strains of *M. tuberculosis*, with no significant difference between the two strains in induced expression levels (*t* test; $P > 0.05$). Mutation of the potential SigA promoter resulted in a dramatic reduction in activity of the *lexA* promoter and no induction by mitomycin C. Data represent means + standard deviations of the results determined with at least three different biological replicates.

matically reduced expression to 1 to 4% (depending on whether the samples were induced) of that observed with pEMD8 (Fig. 4b). Expression from pEMD8 did not differ significantly from wild-type expression in the Δ*sigG*2 strain; the apparent reduction in activity following induction by mitomycin C was not statistically significant ($P > 0.05$). Importantly, there was also no decrease in the residual expression seen with pEJ554 in the Δ*sigG*2 strain, showing that this expression was not due to a second SigG-dependent promoter. Thus, a promoter matching the consensus for SigA is responsible for at least 95% of the expression of *lexA* and the remaining 5% of expression is not due to SigG.

The sensitivity of the wild-type and Δ*sigG*2 strains to DNA damage caused by UV irradiation and different DNA-damaging drugs was assessed as described previously (3, 13). Survival rates after exposure to different doses of UV irradiation were similar for the Δ*sigG* and the wild-type strains; in contrast, enhanced susceptibility was seen with a *uvrB* mutant used as a control (Fig. 5), as shown previously (4). Viability in response to different concentrations of DNA-damaging drugs was examined by the use of a microplate Alamar blue assay, and again there was no difference between the wild-type and Δ*sigG* strains in the MIC or the 50% inhibitory concentration (IC_{50}) for mitomycin C, bleomycin, or ofloxacin (Table 3). As we did

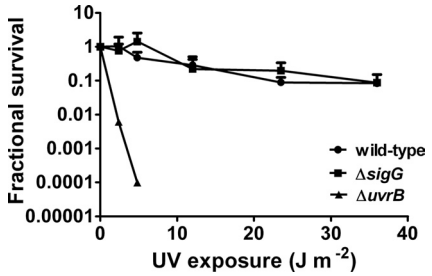

FIG. 5. Susceptibility of the *sigG* mutant to UV irradiation. CFUs for wild-type and mutant strains were determined after exposure to different doses of UV and compared to untreated control results. The UV-sensitive Δ*uvrB* strain is shown as a control. Data represent means + standard deviations of the results obtained with three different biological replicates.

not identify any phenotype caused by inactivation of *sigG*, complementation experiments were not necessary.

The work presented here contradicts a recent report in which SigG was predicted to control the RecA-dependent DNA damage response (14). The differences between these two findings may reflect differences between *M. tuberculosis* strains H37Rv and CDC1551, although the nucleotide sequences for *sigG* and *lexA* and their surrounding genes are identical in the two strains. Alternatively, the differences may reflect the different growth phases studied. *sigG* was reported to exhibit the lowest expression level of all the sigma factors during exponential growth, and this expression level was shown to be reduced in the stationary phase (16). We also found that *sigG* expression declined with increasing OD (data not shown), and so we examined the effects of SigG under DNA-damaging conditions in which *sigG* is induced and likely to be active. Lee et al. (14) performed their gene expression analyses at ODs of 1 and 2, as they found the *sigG* expression level to be increased under those conditions. However, they normalized mRNA levels to that of *sigA*, expression of which is reduced in the stationary phase (16).

In conclusion, SigG, despite being induced by DNA-damaging agents, does not control either the RecA-independent or SOS responses of *M. tuberculosis*. Instead, SigG forms part of the RecA-independent regulon. The rest of the regulon con-

TABLE 3. MIC and IC_{50} values for the wild-type and *sigG*2 mutant strains in response to DNA-damaging agents assessed by microplate Alamar blue assay[a]

Drug	Result for indicated strain			
	Wild type		Δ*sigG*	
	MIC (μg ml^{-1})	IC_{50} (μg ml^{-1})	MIC (μg ml^{-1})	IC_{50} (μg ml^{-1})
Mitomycin C	0.036 ± 0.009	0.008 ± 0.001	0.036 ± 0.009	0.010 ± 0.001
Bleomycin	0.013 ± 0.004	0.004 ± 0.001	0.011 ± 0.006	0.003 ± 0.002
Ofloxacin	0.843 ± 0.178	0.405 ± 0.044	0.980 ± 0.264	0.432 ± 0.067

[a] The MIC was defined as the lowest drug concentration to cause at least 90% inhibition. As the MIC value for each experiment represents an actual tested concentration, which differed between experiments and was not calculated from the dose response curve, the values can be identical. Values represent means ± standard deviations of the results of at least three separate experiments. The IC_{50} was defined as the midpoint of a dose response curve determined using GraphPad Prism 5.00 (GraphPad). Values represent means ± standard deviations of the results of at least three separate experiments.

tains many genes known to be involved in DNA repair and essential for survival. SigG possibly controls a second wave of gene expression that, although related to DNA damage, is not responsible for repair of the damaged DNA.

The research was supported by the United Kingdom Medical Research Council (program number U1175 32056).

We thank Krishna Gopaul for contributing to the construction of the *sigG* targeting plasmid, Kathryn Lougheed and Alison Gaudion for help with assay design, Joanna Dillury for the Δ*uvrB* UV susceptibility data, and Heran Darwin and Carl Nathan for kindly providing us with the *M. tuberculosis uvrB* mutant strain. We thank Colorado State University for providing control *M. tuberculosis* H37Rv DNA for use in microarray analysis and BμG@S for the supply of the *M. tuberculosis* microarrays and for help with uploading our microarray data into public databases.

REFERENCES

1. **Cappelli, G., et al.** 2006. Profiling of *Mycobacterium tuberculosis* gene expression during human macrophage infection: upregulation of the alternative sigma factor G, a group of transcriptional regulators, and proteins with unknown function. Res. Microbiol. **157:**445–455.
2. **Cole, S. T., et al.** 1998. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature **393:**537–544.
3. **Collins, L., and S. G. Franzblau.** 1997. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. Antimicrob. Agents Chemother. **41:**1004–1009.
4. **Darwin, K. H., and C. F. Nathan.** 2005. Role for nucleotide excision repair in virulence of *Mycobacterium tuberculosis*. Infect. Immun. **73:**4581–4587.
5. **Davis, E. O., E. M. Dullaghan, and L. Rand.** 2002. Definition of the mycobacterial SOS box and use to identify LexA-regulated genes in *Mycobacterium tuberculosis*. J. Bacteriol. **184:**3287–3295.
6. **Davis, E. O., et al.** 2002. DNA damage induction of *recA* in *Mycobacterium tuberculosis* independently of RecA and LexA. Mol. Microbiol. **46:**791–800.
7. **Domenech, P., and M. B. Reed.** 2009. Rapid and spontaneous loss of phthiocerol dimycocerosate (PDIM) from *Mycobacterium tuberculosis* grown in vitro: implications for virulence studies. Microbiology **155:**3532–3543.
8. **Dullaghan, E. M., P. C. Brooks, and E. O. Davis.** 2002. The role of multiple SOS boxes upstream of the *Mycobacterium tuberculosis lexA* gene—identification of a novel DNA-damage-inducible gene. Microbiology **148:**3609–3615.
9. **Durbach, S. I., S. J. Andersen, and V. Mizrahi.** 1997. SOS induction in mycobacteria: analysis of the DNA-binding activity of a LexA-like repressor and its role in DNA damage induction of the *recA* gene from *Mycobacterium smegmatis*. Mol. Microbiol. **26:**643–653.
10. **Fivian-Hughes, A. S., and E. O. Davis.** 2010. Analyzing the regulatory role of the HigA antitoxin within *Mycobacterium tuberculosis*. J. Bacteriol. **192:**4348–4356.
11. **Gamulin, V., H. Cetkovic, and I. Ahel.** 2004. Identification of a promoter motif regulating the major DNA damage response mechanism of *Mycobacterium tuberculosis*. FEMS Microbiol. Lett. **238:**57–63.
12. **Gopaul, K. K.** 2002. Transcription of the *Mycobacterium tuberculosis recA* gene. University College London, London, United Kingdom.
13. **Güthlein, C., et al.** 2009. Characterization of the mycobacterial NER system reveals novel functions of the *uvrD1* helicase. J. Bacteriol. **191:**555–562.
14. **Lee, J. H., D. E. Geiman, and W. R. Bishai.** 2008. Role of stress response sigma factor SigG in *Mycobacterium tuberculosis*. J. Bacteriol. **190:**1128–1133.
15. **Mahenthiralingam, E., et al.** 1998. Site-directed mutagenesis of the 19-kilodalton lipoprotein antigen reveals no essential role for the protein in the growth and virulence of *Mycobacterium intracellulare*. Infect. Immun. **66:**3626–3634.
16. **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.
17. **Movahedzadeh, F., M. J. Colston, and E. O. Davis.** 1997. Characterization of *Mycobacterium tuberculosis* LexA: recognition of a Cheo (*Bacillus*-type SOS) box. Microbiology **143:**929–936.
18. **Oatway, W. H. J., and W. J. Steenken.** 1936. The pathogenesis and fate of tubercle produced by dissociated variants of tubercle bacilli. J. Infect. Dis. **59:**306–325.
19. **Papavinasasundaram, K. G., et al.** 2001. Slow induction of RecA by DNA damage in *Mycobacterium tuberculosis*. Microbiology **147:**3271–3279.
20. **Parish, T., and N. G. Stoker.** 2000. Use of a flexible cassette method to generate a double unmarked *Mycobacterium tuberculosis tlyA plcABC* mutant by gene replacement. Microbiology **146:**1969–1975.
21. **Rand, L., et al.** 2003. The majority of inducible DNA repair genes in *Mycobacterium tuberculosis* are induced independently of RecA. Mol. Microbiol. **50:**1031–1042.
22. **Rao, A., and A. Ranganathan.** 2004. Interaction studies on proteins encoded by the phthiocerol dimycocerosate locus of *Mycobacterium tuberculosis*. Mol. Genet. Genomics **272:**571–579.
23. **Smollett, K. L., et al.** 2009. Experimental determination of translational start sites resolves uncertainties in genomic open reading frame predictions—application to *Mycobacterium tuberculosis*. Microbiology **155:**186–197.
24. **Talaat, A. M., S. T. Howard, W. Hale IV, R. Lyons, H. Garner, and S. A. Johnston.** 2002. Genomic DNA standards for gene expression profiling in *Mycobacterium tuberculosis*. Nucleic Acids Res. **30:**e104.
25. **Unniraman, S., M. Chatterji, and V. Nagaraja.** 2002. DNA gyrase genes in *Mycobacterium tuberculosis*: a single operon driven by multiple promoters. J. Bacteriol. **184:**5449–5456.