The role of multiple SOS boxes upstream of the *Mycobacterium tuberculosis* *lexA* gene – identification of a novel DNA-damage-inducible gene

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Four potential binding sites for LexA were identified upstream of the *Mycobacterium tuberculosis* *lexA* gene. A mutational analysis of these sites in a *lexA-lacZ* reporter construct revealed that only one of these SOS boxes was required for DNA-damage-mediated regulation of *lexA* expression. A novel DNA-damage-inducible gene, *Rv2719c*, was identified that was divergently transcribed relative to *lexA*; the other three SOS boxes were found to be involved in regulating expression of this novel mycobacterial-specific gene. The SOS boxes lay in the respective promoter regions of the genes that they regulated.

Keywords: LexA, SOS box, mycobacteria

INTRODUCTION

Bacteria respond to DNA damage by inducing various genes that are required for DNA repair or for DNA damage tolerance. The best characterized of these systems is the SOS response of *Escherichia coli*, in which over 40 genes are regulated by a common mechanism (Courcelle *et al.*, 2001; Friedberg *et al.*, 1995; Little & Mount, 1982). At the heart of this regulatory mechanism is the repressor protein LexA, which binds to a particular sequence (CTGTatatatACAG) (Friedberg *et al.*, 1995), called the SOS box, upstream of the genes whose transcription it regulates (Brent & Ptashne, 1981; Little *et al.*, 1981). When DNA damage occurs, the recombination protein RecA binds to regions of single-stranded DNA resulting from processing of the damaged DNA or blockage of replication and forms nucleoprotein filaments (Sassanfar & Roberts, 1990). In this activated form RecA stimulates the autocatalytic cleavage of LexA (Little, 1991), the fragments of which no longer bind to the SOS boxes (Bertrand-Burggraf *et al.*, 1987), thus increasing transcription of the SOS genes.

The basic principles of this regulatory mechanism are found in many other species of bacteria, although the

DNA sequence of the LexA-binding site, or SOS box, varies. For example, in *Bacillus subtilis* the sequence CGAACRNRYGTTCG (Winterling *et al.*, 1998) is recognized by the LexA homologue known as DinR. A related motif is used in mycobacteria; originally the motif GAACnnnnGTTC was shown to bind *Mycobacterium tuberculosis* LexA (Durbach *et al.*, 1997; Movahedzadeh *et al.*, 1997), whilst a more recent analysis of the requirements for LexA binding *in vivo* have revealed that the mycobacterial SOS box has the consensus sequence TCGAACnnnnGTTCGA (Davis *et al.*, 2002).

The SOS box is generally positioned close to, and often overlaps, the promoter of the gene regulated by LexA binding to it. In *E. coli* there are examples of genes whose SOS boxes overlap the –35 sequence, are between the –35 and –10 sequences, overlap the –10 sequence or are downstream of the –10 sequence (Schnarr *et al.*, 1991). Many genes have a single SOS box that is sufficient to confer regulation, and there are even some instances of divergently oriented genes each being regulated by a common SOS box (Courcelle *et al.*, 2001). However, there are also a few examples of genes which possess two or even three SOS boxes. In *E. coli* the *lexA* gene has two SOS boxes separated by just 5 bp, one overlapping the –10 sequence and the other lying just downstream of the transcription start site (Brent & Ptashne, 1981; Little *et al.*, 1981). The *recN* gene of *E. coli* has three SOS boxes, one overlapping the –35 sequence, a second overlapping the –10 sequence and a

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Abbreviation: RACE, rapid amplification of cDNA ends.

third downstream of the transcription start site (Rostas *et al.*, 1987).

In *B. subtilis* two motifs with homology to the then defined bacillus SOS box were initially identified upstream of *recA*, but a functional analysis of these sites revealed that only one of these is actually required for regulation of *recA* expression (Cheo *et al.*, 1993). Similarly, there are three sites which bind the LexA homologue DinR upstream of *dinR* in *B. subtilis* (Hajjema *et al.*, 1996; Winterling *et al.*, 1998), one of which overlaps the -35 sequence whilst the other two lie further upstream. However, just the site nearest to the gene is sufficient to confer DNA damage induction by the same factor as seen when all three are present (Hajjema *et al.*, 1996). There are also two SOS boxes upstream of *dinC* in *B. subtilis*, one being between the -10 and -35 sequences whilst the other lies a few bases upstream of the -35 site. Although both of these sites bind DinR *in vitro* (Lovett *et al.*, 1993; Miller *et al.*, 1996; Winterling *et al.*, 1998), their requirement for induction *in vivo* has not been reported.

Examination of the DNA sequence upstream of the *M. tuberculosis* *lexA* gene revealed several motifs with similarity to the mycobacterial SOS box. Here we investigate the roles they each play in regulating expression of *lexA* and of a divergent ORF, *Rv2719c*, using transcriptional fusions to a *lacZ* reporter gene.

METHODS

Bacterial strains and media. *E. coli* DH5 α was used for general cloning, whilst *E. coli* XL1Blue was used for site-directed mutagenesis (Sambrook *et al.*, 1989). The mycobacterial strains used in this study were *Mycobacterium smegmatis* mc²155 (Snapper *et al.*, 1990) and *M. tuberculosis* H37Rv. *E. coli* was grown in L broth (Sambrook *et al.*, 1989) and *M. smegmatis* and *M. tuberculosis* were grown in modified Dubos medium supplemented with albumen and 0.2% (w/v) glycerol. *E. coli* and *M. smegmatis* were grown at 37 °C with shaking and *M. tuberculosis* was grown at 37 °C in a rolling incubator at 2 r.p.m. All procedures with live *M. tuberculosis* were carried out under Containment level 3 conditions. Antibiotics were added to the growth media as required: 100 μ g ampicillin ml⁻¹ for *E. coli*; 50 μ g kanamycin ml⁻¹ for *E. coli*; 20 μ g kanamycin ml⁻¹ for mycobacteria.

Recombinant DNA techniques. Plasmid DNA was prepared using SNAP miniprep kits (Invitrogen) according to the manufacturer's instructions. Site-directed mutagenesis was performed as described in the instructions for the Quick-Change Site-Directed Mutagenesis Kit (Stratagene). For other DNA manipulations, standard DNA protocols were followed (Sambrook *et al.*, 1989). For each clone or mutant made, the sequences of the promoter region and the junctions to the vector were determined on an ABI PRISM 377 DNA sequencer using the ABI PRISM dRhodamine dye terminator cycle sequencing kit (PE Applied Biosystems).

Plasmid construction. A 587 bp PCR fragment containing the entire intergenic sequence upstream of *lexA* and extending into the coding sequence of a divergent ORF (*Rv2719c*) as well as that of *lexA* itself was generated using the primers GCTCTAGACGGCGCCAGCGAGGTCCATC and GCAAGCTTCGGGGGCGGTTCGGGGTCAC. The bases in bold indicate restriction sites for *Xba*I and *Hind*III, respectively,

which were added to facilitate cloning. Following its digestion with these restriction enzymes, the PCR product was cloned into the mycobacterial promoter-probe vector pEJ414 (Papavinasundaram *et al.*, 2001) to form a transcriptional fusion of the *lexA* promoter with the *lacZ* reporter gene (pEMD8). Subsequently, a 315 bp PCR fragment was amplified using the primers CGAAGCTTATTGAGCGGATCGGGGGTAT and CGTCTAGAGAGGTGTCGTTGCTGTCGTTCA and similarly cloned into pEJ414 to form a transcriptional fusion of the *Rv2719c* promoter with the *lacZ* reporter gene (pEJ544). This fragment included the entire intergenic sequence between the *lexA* and *Rv2719c* genes as well as extending into the coding sequence of *Rv2719c*, but only overlapped the *lexA* mRNA rather than its coding sequence.

Introduction of clones into mycobacteria, and verification of insertion by PCR and sequencing. Published protocols were followed for preparing electrocompetent cells of *M. smegmatis* or *M. tuberculosis* (Papavinasundaram *et al.*, 1998) and for electroporation (Jacobs *et al.*, 1991). After streaking out the resulting transformants, a preparation of total DNA suitable for PCR was isolated using InstaGene matrix (Bio-Rad) according to the manufacturer's instructions, except that we used more bacteria. The insert and junctions of each clone were isolated as a PCR product as described previously (Davis *et al.*, 2002) and sequenced on an ABI PRISM 377 DNA sequencer using the ABI PRISM dRhodamine dye terminator cycle sequencing kit.

Induction conditions. To induce DNA damage in *M. smegmatis* transformants, mitomycin C was added to one aliquot of a culture at an OD₆₀₀ value of between 0.5 and 0.6 to a final concentration of 0.2 μ g ml⁻¹; the culture was then incubated for 5 h. An equal volume of the same culture was also incubated for the same period of time without any addition, to provide an uninduced control. For *M. tuberculosis*, a similar procedure was followed except that cultures were induced at an OD₆₀₀ value of between 0.4 and 0.5, and induction was for 24 h. Following incubation in the presence or absence of the inducer, the bacteria were harvested, washed three times in Z buffer (Miller, 1972) without β -mercaptoethanol (Z*) and stored as a pellet at -20 °C.

Preparation of cell-free extracts and β -galactosidase assays.

Cell lysates were prepared, and protein and β -galactosidase assays were performed as described previously (Davis *et al.*, 2002). In the case of *M. tuberculosis* lysates, the supernatants were filtered through a low-binding Durapore 0.22 μ m membrane filter (Ultrafree-MC; Millipore) to eliminate any residual intact bacteria prior to assay. The specific activity, in units (mg protein)⁻¹, was calculated using the formula defined by Miller (1972).

Gel-shift assays. Gel-shift assays to analyse LexA binding to individual SOS boxes were performed as described previously (Brooks *et al.*, 2001). The sequence of the oligonucleotide containing Box 3 was gtagTCactCatgtGTTTCGAtatc, in which the bases matching the consensus SOS box sequence are shown in upper case. The complementary oligonucleotide was annealed with the aforementioned sequence and the resulting double-stranded probe was used in the assay. Binding to Box 3 was assayed alongside the other SOS boxes, which acted as controls.

Rapid amplification of cDNA ends (RACE) analysis. This procedure was carried out using a FirstChoice RLM-RACE kit (Ambion) according to the manufacturer's instructions but omitting the steps prior to ligation of the RACE adapter, which only apply to eukaryotic RNA. For RNA isolated from *M. tuberculosis*, two primers specific for *Rv2719c* were used for the nested PCR – an outer primer, RaceRv2719c (GACA-

TCCCGACCCCGGTGCGGTGGTAGC), and an inner primer, XbaRv2719c (GGTCTAGAATTGAGCGGATCGGGGTATGC), which included an *Xba*I site for cloning (shown in bold). For RNA isolated from *M. smegmatis* containing pEJ544 only the inner primer could be used, as the outer primer bound outside of the fragment cloned in pEJ544, so a semi-nested PCR was performed using XbaRv2719c and the two supplied primers that were complementary to the adapter. The cycle conditions for PCR were 94 °C for 2 min, followed by 35 cycles at 94 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s, with a final step at 72 °C for 7 min. The final PCR product was cloned into pBluescriptKS⁺ following its digestion with *Bam*HI and *Xba*I, and individual transformants were sequenced.

RESULTS AND DISCUSSION

Identification of four SOS boxes upstream of *lexA*

Examination of the sequence upstream of the *M. tuberculosis* *lexA* gene revealed the presence of four motifs with similarity to the recently defined mycobacterial SOS box (TCGAACnnnnGTTCGA). We have numbered these sites starting with that closest to the *lexA* coding region (Fig. 1). The individual motifs have different bases, and different numbers of bases, which differ from the consensus sequence. Thus, Box 1 (TCGAACacatGTTTGA) has a single mismatch, Box 2 (CCGAACatttGATCGA) and Box 4 (TCGCTCgcgtG-

TTCGA) each have two mismatches and Box 3 (TCAC-TCatgtGTTCTGA) has three mismatches from the consensus (the mismatches being highlighted in bold). Intriguingly, inspection of the *Mycobacterium leprae* sequence (Cole *et al.*, 2001) reveals the presence of a motif with only a single mismatch (TCGATCcatgGTTCTGA) in a position identical to Box 3. Box 2 and Box 3 are separated by only 3 bp, there are just 8 bp between Box 3 and Box 4, and Box 1 and Box 2 are separated by 117 bp (Fig. 1). Thus, the first bases of Boxes 2, 3 and 4 are located 181, 200 and 224 bp, respectively, upstream of the transcription start point of *lexA*, whilst Box 1 lies in the region of the *lexA* promoter -35 element.

Three of the sites are the same as three of those identified using the earlier, shorter SOS consensus sequence (Brooks *et al.*, 2001) whilst the remaining site (Box 3) differs. In the study of Brooks *et al.* (2001) it was shown that two of these sites (Boxes 1 and 4) bound LexA under physiologically relevant conditions, but no binding to Box 2 was detected. Interestingly, LexA also bound to Box 3 as assessed by gel shift under the same conditions (data not shown), although Box 3 contained more mismatches than the other sites. Therefore, it remained possible that LexA would bind to Box 2 in its native context when Boxes 3 and 4 were present very close to Box 2.

The roles of each of the four SOS boxes in regulating *lexA* expression

The roles of each of the four SOS boxes in regulating *lexA* expression were investigated by a mutagenic approach using a fusion of the *lexA* promoter, which included the upstream region containing all of the SOS boxes, to the reporter gene *lacZ* in an integrating plasmid (pEMD8). Mutations were made which eliminated LexA binding to each SOS box individually, by changing the core of the sequence to TCCAnnnnTGGA. Box 2 was included in this analysis, even though it had failed to bind LexA in the gel-shift assay, to investigate whether it played a role when in the presence of the other SOS boxes.

The wild-type and mutated constructs were introduced into *M. smegmatis* mc²155 and expression of the *lacZ* reporter gene was assessed following growth of the strains in both the absence and presence of the DNA-damaging agent mitomycin C (0.2 µg ml⁻¹). As expected, the wild-type sequence containing the *lexA* promoter and all four SOS boxes conferred DNA-damage-inducible expression, with an induction ratio of 5.4 (Fig. 2a). Interestingly, when Box 1 alone was eliminated expression was no longer DNA-damage-inducible, with the induction ratio falling to 0.5 (Fig. 2a). Normally when a repressor protein is prevented from binding to its regulatory site the result is constitutive expression at around the level seen for the induced wild-type. However, the level of expression seen for this Box 1 mutation was substantially lower than the induced wild-type level; this is presumably because the mutation introduced also has an effect on promoter activity.

Fig. 1. Sequence upstream of the *M. tuberculosis* *lexA* gene. The sequence is shown in the opposite direction to *lexA*, beginning at the predicted start codon of LexA and continuing to that of *Rv2719c*, which is transcribed divergently from *lexA*. Motifs resembling SOS boxes are boxed and numbered 1–4; the predicted initiation codons for each gene are underlined. The transcriptional start site of *lexA*, determined previously (Movahedzadeh *et al.*, 1997), is indicated by the left-facing arrow. Two possible transcriptional start sites for *Rv2719c*, determined here by RACE analysis, are indicated by the right-facing arrows; the majority of clones were consistent with the solid arrow, whilst a longer product observed in two clones suggested the possibility of initiation at the dotted arrow.

Fig. 2. DNA damage induction of the *lexA* promoter with and without mutations in the SOS boxes in (a) *M. smegmatis* and (b) *M. tuberculosis*. The *lexA* promoter was transcriptionally fused to a *lacZ* reporter gene and β -galactosidase activity for each construct was determined following incubation in the presence (hatched bars) or absence (solid bars) of mitomycin C (0.2 μ g ml⁻¹). The graph shows the mean values obtained from duplicate assays of at least three independent inductions, with the error bars showing the s.d. The SOS boxes that were mutated are shown as solid blocks in the schematic, where B1 refers to Box 1, B2 refers to Box 2, etc.

In contrast, constructs containing a Box 2, Box 3 or Box 4 knockout mutation individually showed similar patterns of expression to the wild-type (Fig. 2a). To ascertain whether this might be because one of these boxes could compensate for a mutation in another, we introduced mutations into each of Boxes 2, 3 and 4 simultaneously and assessed the effect of these changes on expression. Once again, expression remained clearly inducible. A small increase in the uninduced level of expression was observed, suggesting that the presence of LexA at these sites may play a minor role in down-regulating expression from the *lexA* promoter. However, taken together the results from these experiments clearly show that induction is determined by binding of LexA to Box 1.

To confirm that this was also the case in *M. tuberculosis* itself, the constructs containing the wild-type sequence, the Box 1 mutation and the combined Boxes 2, 3 and 4 mutations were introduced into *M. tuberculosis* H37Rv and expression was assessed following growth of the recombinant strains in the absence and presence of mitomycin C. The absolute expression levels in these strains were about twofold higher than that seen in *M. smegmatis*, but the pattern of expression from the mutated constructs was the same. Thus, the mutation in Box 1 eliminated induction, whilst the strain carrying the combined mutations in Boxes 2, 3 and 4 showed expression equivalent to that of the wild-type (Fig. 2b).

Could the other SOS boxes be regulating expression of a divergent gene?

Upstream from the *M. tuberculosis* *lexA* gene there is a divergent gene of unknown function termed *Rv2719c* (Cole *et al.*, 1998). All four of the SOS boxes under study here are also upstream of this gene. As Boxes 2, 3 and 4 do not appear to have a role in regulating the expression of *lexA*, it was conceivable that they might regulate the expression of *Rv2719c* instead. To investigate this possibility, we first determined whether the expression

of *Rv2719c* was DNA-damage-inducible. A transcriptional *lacZ* fusion of the *Rv2719c* promoter and upstream region, containing all four SOS boxes, was made by cloning part of the fragment used above for the *lexA* reporter construct in the opposite orientation. Assays of *lacZ* reporter gene expression in *M. smegmatis* with and without treatment with the DNA-damaging agent mitomycin C (0.2 μ g ml⁻¹) revealed that the expression of this ORF was indeed DNA-damage-inducible, with an induction ratio of 4.0 (Fig. 3a). When the corresponding constructs containing mutations which eliminated LexA binding to each SOS box were examined individually, the greatest reduction in the induction ratio, to 1.4, was seen with the Box 2 mutation, with smaller changes seen with the Box 3 or Box 4 mutations and very little effect seen with the Box 1 mutation. As no single SOS box knockout resulted in constitutive expression, we went on to examine various combinations of mutations. When any two of Boxes 2, 3 and 4 were mutated simultaneously the induction ratio was reduced to ca. 1.3, whilst the triple knockout of Boxes 2, 3 and 4 resulted in completely constitutive expression, with an uninduced level similar to that of the wild-type induced level (Fig. 3a). Thus, Boxes 2, 3 and 4 are all involved in regulating the expression of *Rv2719c*.

Again, this conclusion was confirmed in *M. tuberculosis* using the constructs containing the wild-type sequence, the Box 1 mutation and the combined Boxes 2, 3 and 4 mutations. In the first place, this analysis confirmed that expression of *Rv2719c* was indeed DNA-damage-inducible in *M. tuberculosis*. Although the induction ratio was lower than that seen in *M. smegmatis*, the absolute expression level was higher than that of *M. smegmatis*, as seen with the *lexA* promoter. Significantly, the pattern of expression from the mutated constructs was the same as in *M. smegmatis*. Thus, the mutation in Box 1 remained inducible, whilst the combined mutations in Boxes 2, 3 and 4 resulted in constitutive expression (Fig. 3b).

Fig. 3. DNA-damage induction of the *Rv2719c* promoter with and without mutations in the SOS boxes in (a) *M. smegmatis* and (b) *M. tuberculosis*. The *Rv2719c* promoter was transcriptionally fused to a *lacZ* reporter gene and β -galactosidase activity for each construct was determined following incubation in the presence (hatched bars) or absence (solid bars) of mitomycin C (0.2 μ g ml⁻¹). The graph shows the mean values obtained from duplicate assays of at least three independent inductions, with the error bars showing the SD. The SOS boxes that were mutated are shown as solid blocks in the schematic, where B1 refers to Box 1, B2 refers to Box 2, etc.

It has previously been reported that some genes in *M. tuberculosis* are DNA-damage-inducible even though they lack binding sites for LexA (Brooks *et al.*, 2001), suggesting that an alternative mechanism of induction exists. Nevertheless, mutation of the SOS boxes to prevent LexA binding eliminated DNA damage induction of *lexA* and *Rv2719c*, indicating that these genes are regulated by LexA in *M. tuberculosis*.

The SOS boxes lie in the respective promoter regions of the genes that they regulate

The specific effects of mutating Box 1 on *lexA* expression and of mutating Boxes 2, 3 and 4 on *Rv2719c* expression could be explained by the locations of these boxes relative to the promoters for both of these genes. The transcriptional start site of *lexA* has been determined previously (Movahedzadeh *et al.*, 1997), and indicates that Box 1 overlaps or lies close to the -35 site of the *lexA* promoter. We would predict that the promoter elements for *Rv2719c* would lie around or between Boxes 2, 3 and 4. Such an arrangement would explain why Box 1 regulates expression of *lexA* only and Boxes 2, 3 and 4 regulate expression of *Rv2719c* only.

To test the hypothesis that the promoter elements for *Rv2719c* lie around or between Boxes 2, 3 and 4, we attempted to determine the transcriptional start site of *Rv2719c*. This was done by RACE using RNA extracted from *M. tuberculosis* and from *M. smegmatis* bearing the *Rv2719c-lacZ* fusion. The majority of clones analysed were consistent with the transcriptional start site being an A located 36 bp upstream of the predicted initiation codon (Fig. 1) in *M. tuberculosis* itself and when the *M. tuberculosis* promoter was being expressed

in *M. smegmatis*. This A is situated within Box 4, and inspection of the sequence upstream from this point revealed a motif (TACAGT) with similarity to the *E. coli* σ^{70} -10 consensus sequence positioned between Box 3 and Box 4. A further motif (CGGATA) bearing some resemblance to the corresponding -35 consensus is located upstream of this, with a spacing of 17 bp from the putative -10 motif; this putative -35 motif partially overlaps Box 2. However, the possibility of transcription in *M. tuberculosis* starting further upstream than this was suggested by a longer product observed in two instances, which would correlate with initiation at a G 97 bp upstream of the coding sequence. This G is located upstream of all three of the SOS boxes that regulate the expression of *Rv2719c*. In either case, Boxes 2, 3 and 4 are appropriately positioned to regulate *Rv2719c* whereas Box 1 is not, in accordance with the results described above.

Analogous to our findings for *M. tuberculosis*, it may be that the two SOS boxes further upstream of *dinR* in *B. subtilis* are regulating a divergent ORF. Indeed, a divergent ORF termed *yneA* is found upstream of *dinR*. There are good matches to -35 and -10 motifs a similar distance upstream of *yneA* to those reported for *dinR* and the central SOS box of the three is located between these motifs, a typical position for a LexA-binding site.

Rv2719c homologues appear to be regulated similarly in other mycobacteria

No homologues of *Rv2719c* were found by searching the general sequence databases, but homologues of this gene were found in other mycobacterial species for which

Fig. 4. Alignment of the intergenic regions between *lexA* and *Rv2719c* from *M. tuberculosis*, *M. leprae*, *M. avium* and *M. smegmatis*. The sequence is shown from two bases beyond the *lexA* transcriptional start site, which is 57 bp before the predicted start codon (see Fig. 1), to the predicted initiation codon of *Rv2719c*. Identical bases are highlighted and the four SOS boxes are marked.

genomic sequence information is available in a readily searchable form, namely *M. leprae*, *Mycobacterium avium* and *M. smegmatis* (TBLASTN scores of 8×10^{-34} , 1.6×10^{-39} and 1×10^{-31} , respectively). Thus, this ORF appears to represent a mycobacterial-specific gene. Not only was a homologue present in each of these mycobacterial species but it was also located in the same relative position in the genome, i.e. upstream of *lexA* and in the opposite orientation to it. Significantly, there are blocks of sequence identity amongst *M. tuberculosis*, *M. leprae*, *M. avium* and *M. smegmatis* in the intergenic region between *lexA* and *Rv2719c*, and all four SOS boxes are conserved in all four species (Fig. 4). Thus, the regulation of *lexA* by a single SOS box and of *Rv2719c* homologues by three SOS boxes is common amongst mycobacteria. It would appear that having multiple copies of the SOS box can compensate for the presence of multiple mismatches in the individual motifs.

The function of *Rv2719c* is unknown. However, the database generated by a recent novel approach to predicting protein functional classes from sequence data (King *et al.*, 2000) suggests that the product of *Rv2719c* is involved in cell-envelope macromolecule metabolism. The DNA-damage-inducible gene *dinC* of *B. subtilis* is also known as *tagC* as it was thought to play a role in

polyglycerol phosphate teichoic acid biosynthesis, although whether this is the case remains unresolved (Mauel *et al.*, 1994).

In conclusion, the results of the present study suggest that some DNA-damage-inducible genes in *M. tuberculosis* may be regulated by LexA binding to multiple sites with two or more mismatches from the consensus sequence, rather than to a single site with up to one mismatch. This may allow a more graded response, depending on the degree of DNA damage. However, it also complicates the identification of further LexA-regulated genes by motif identification.

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