Slow induction of RecA by DNA damage in *Mycobacterium tuberculosis*

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In mycobacteria, as in most bacterial species, the expression of RecA is induced by DNA damage. However, the authors show here that the kinetics of recA induction in *Mycobacterium smegmatis* and in *Mycobacterium tuberculosis* are quite different: whilst maximum expression in *M. smegmatis* occurred 3–6 h after addition of a DNA-damaging agent, incubation for 18–36 h was required to reach peak levels in *M. tuberculosis*. This is despite the fact that the *M. tuberculosis* promoter can be activated more rapidly when transferred to *M. smegmatis*. In addition, it is demonstrated that in both species the DNA is sufficiently damaged to give maximum induction within the first hour of incubation with mitomycin C. The difference in the induction kinetics of recA between the two species was mirrored by a difference in the levels of DNA-binding-competent LexA following DNA damage. A decrease in the ability of LexA to bind to the SOS box was readily detected by 2 h in *M. smegmatis*, whilst a decrease was not apparent until 18–24 h in *M. tuberculosis* and then only a very small decrease was observed.

Keywords: LexA, SOS induction, mycobacteria

INTRODUCTION

RecA is a central component of the bacterial response to DNA damage. Not only does it directly participate in DNA repair, but it also regulates the expression of other genes whose functions promote increased survival following DNA damage (Walker, 1984). The system studied in most detail is that of *Escherichia coli*, where the so-called SOS response, the induction of around 30 genes in response to DNA damage (Fernandez de Henestrosa *et al.*, 2000; Friedberg *et al.*, 1995), is regulated by RecA in conjunction with LexA (Little & Mount, 1982). The recA and lexA genes are themselves part of the SOS regulon. LexA is a repressor protein which under normal conditions binds to a specific sequence, termed the SOS box, upstream of the SOS genes to restrict their expression (Brent & Ptashne, 1981; Little *et al.*, 1981). When DNA damage occurs RecA protein binds to regions of single-stranded DNA resulting from processing of that damage or replication blockage (Sassanfar & Roberts, 1990) to form nucleoprotein filaments. In this form RecA stimulates the autocatalytic cleavage of LexA (Little, 1991). The resulting fragments of LexA do not bind to the SOS boxes (Bertrand-Burggraf *et al.*, 1987); therefore, repression by LexA is alleviated and expression of the SOS genes is induced.

Although the details of this system have been worked out from studies of *E. coli*, the key elements appear to hold in other bacteria. Amongst Gram-positive bacteria the most information is available for *Bacillus subtilis*, where the functional homologue of LexA is called DinR (Hajjema *et al.*, 1996; Miller *et al.*, 1996; Winterling *et al.*, 1997). As with the LexA protein in *E. coli*, the DinR protein of *B. subtilis* binds to specific sites upstream of various DNA-damage-inducible genes including dinR and recA, although the sequence recognized is quite different from that bound by *E. coli* LexA (Cheo *et al.*, 1991, 1993; Winterling *et al.*, 1998). In addition, DNA-damage induction is dependent on the presence of an intact recA gene (Gassel & Alonso, 1989; Lovett *et al.*, 1988) and the cellular levels of DinR decrease following DNA damage (Lovett *et al.*, 1993; Miller *et al.*, 1996),
indicating that the mechanism deduced in E. coli is also valid in B. subtilis.

In mycobacteria the recA and lexA genes have been identified and the LexA protein has been shown to bind to a specific site similar to the SOS box of B. subtilis upstream of each of these genes (Durbach et al., 1997; Movahedzadeh et al., 1997a, b; Papavinasasundaram et al., 1997). In addition, the expression of recA has been shown to be inducible by DNA-damaging agents in both M. tuberculosis and M. smegmatis (Durbach et al., 1997; Movahedzadeh et al., 1997b; Papavinasasundaram et al., 1997). Preliminary experiments had indicated that a longer period of induction was required for M. tuberculosis as compared with M. smegmatis to see similar levels of recA induction. In this study we have analysed the kinetics of recA induction in M. smegmatis and M. tuberculosis in detail.

METHODS

Bacterial strains, media, transformation and DNA damage induction. The media for growing Escherichia coli DH5α (Sambrook et al., 1989), and the mycobacterial strains Mycobacterium smegmatis mc²155 (Snapper et al., 1990) and Mycobacterium tuberculosis H37Rv have been described previously (Movahedzadeh et al., 1997b). M. tuberculosis was grown in tissue culture flasks laid flat in a 37°C incubator. Published protocols were followed for preparing electrocompetent cells of mycobacteria (Papavinasasundaram et al., 1998) and for electroporation (Jacobs et al., 1991). To induce DNA damage, mitomycin C (0-2 µg ml⁻¹) or ofloxacin (1 µg ml⁻¹) was added to growing cultures (at an OD₆₅₀ of 0.6) and incubated for the time indicated. For pulse damage, the cultures were incubated with mitomycin C for a specified period and then the bacteria were harvested, washed and incubated in drug-free medium for expression. The total period of incubation (pulse treatment and expression) was 5 h for M. smegmatis and 24 h for M. tuberculosis. To compare the effects of the mitomycin C treatments on the viability of M. smegmatis and M. tuberculosis, colony-forming units were determined by serial dilution of mitomycin-C-treated cultures in 0.9% NaCl/0.1% Tween 80 and plating on 7H11 agar. The treatments assessed were exposure for 1 h or 5 h for M. smegmatis and for 1 h or 24 h for M. tuberculosis.

Recombinant DNA techniques and construction of plasmids. Plasmid DNA was prepared using SNAP miniprep kits (Invitrogen). For other DNA manipulations, standard DNA protocols were followed (Sambrook et al., 1989). Through a series of plasmid manipulations, a lacZ transcriptional reporter plasmid (pEJ14) based on the mycobacterial integrating vector pMV306 (Stover et al., 1991) containing a promoterless E. coli lacZ gene from pMC1871 (Casadaban et al., 1983) was constructed. The plasmid pEJ14 (sequence available on request) had five copies of the trp terminator cloned at the beginning of a polylinker sequence to block readthrough from any vector promoters, as this number of copies had been shown to be effective in M. smegmatis. The M. tuberculosis recA promoter was cloned as a 0.35 kb PvuII–HindIII fragment from pFM6 (Movahedzadeh et al., 1997b) into the NruI–HindIII sites of the polylinker in pEJ14 to make pEJ147. The same fragment had been shown previously to have promoter activity (Movahedzadeh et al., 1997b). Nucleic acid sequences of the clones at the cloning junctions, and the promoter and the terminator sequences, were determined on an ABI PRISM 377 DNA sequencer using the ABI PRISM dRhodamine dye terminator cycle sequencing kit (PE Applied Biosystems). The promoter region was also recovered from the mycobacterial strains into which the clone was introduced by PCR of genomic DNA and the PCR products were sequenced to confirm no changes had occurred.

RNA extraction and real-time quantitative Taqman PCR assay. Commercially available kits were used for the isolation of total RNA (Hybaid Ribolysis Blue kit) from bacterial cultures (100 ml), to digest contaminating DNA from the RNA preparations using RNase-free DNase (Roche), and subsequent cleanup procedures (RNeasy Mini Kit; Qiagen). First-strand cDNA synthesis was carried out using Superscript II (Life Technologies) following the published protocol (Papavinasasundaram et al., 1997). Real-time quantitative PCR was carried out on the ABI Prism 7700 Sequence Detection system using the Taqman Universal PCR Master Mix (PE Applied Biosystems). The primers and the Taqman probes (carrying both a fluorophore and a quencher) were designed using the Primer Express software and obtained from PE Applied Biosystems. The sequences of the primers and the probes are listed in Table 1.

Preparation of cell-free extracts. Untreated and mitomycin-C-treated bacteria were harvested, washed three times in Z buffer without β-mercaptoethanol (Z⁻) and resuspended in 0.5 ml Z⁺ buffer. Bacteria were lysed in the presence of glass beads (150–212 µm, Sigma) in a Ribolysor (Hybaid) at a speed setting of 6.5 for 2 × 25 s. The supernatant was collected by centrifugation, and in the case of M. tuberculosis filtered through a low-binding Durapore 0.22 µm membrane filter (Ultrafree-MC; Millipore). An aliquot of the cell extract was used to determine its protein concentration using a BCA protein assay kit (Pierce). To the remaining extract, β-mercaptoethanol was added to a final concentration of 50 mM and used to estimate β-galactosidase activity as described by Miller (1972) but using half-size reactions and reading the absorbance of 300 µ reaction mix in a flat-bottom microtitre plate reader. The specific activity in units (mg protein)⁻¹ was calculated using the formula defined by Miller (1972).

Western blotting. Cell-free extracts corresponding to 20 µg protein were used in RecA Western blots and 5 µg in LexA Western blots. Following electrophoresis, the proteins were electroblotted onto a PVDF membrane using a semi-dry blotter (Hybaid) at 60 V for 1 h. Equal loading of the proteins

<p>| Table 1. Sequences of the primer and probes (5’ → 3’) used in the real-time quantitative Taqman PCR assay |</p>
<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Taqman probe</th>
</tr>
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<tbody>
<tr>
<td>recA</td>
<td>ACCGGGCAGCGCTGAATA</td>
<td>CGCGGAGGCTGGTGTGATG</td>
<td>TTCGGGCACACGGGCATC</td>
</tr>
<tr>
<td>gls</td>
<td>ACATCGGCGGTTGATCATCAGAT</td>
<td>GCTTGCAAGACGGTTTGTG</td>
<td>CGAATTCCTCGCACCCAC</td>
</tr>
<tr>
<td>gnd</td>
<td>GTCCACAAACGGCATCGAGTA</td>
<td>GCTGTTACCGGTGCTTGATG</td>
<td>TCCGACATGCAGCTCATCGTGA</td>
</tr>
</tbody>
</table>
RecA induction in *M. tuberculosis* and *M. smegmatis*

was confirmed by Coomassie staining of an identical gel and the efficiency of transfer was verified by staining the blots with a solution of 0.1% Ponceau S in 1% acetic acid. Published protocols were followed for blocking non-specific sites and subsequent washing steps (Papavinasasundaram *et al*., 1998). The primary antisera, anti-RecA and anti-LexA raised in mice against recombinant *M. tuberculosis* RecA and LexA proteins respectively, were used at 1:1000 dilutions. LexA was purified as described previously (Movahedzadeh *et al*., 1997a) and purified RecA was kindly provided by K. Muniyappa. Mouse antibody conjugated to horseradish peroxidase (Dako) was used as the second antibody. The blots were washed and developed with diaminobenzidine reagent solution as described previously (Davis *et al*., 1992).

**Gel retardation analysis.** Oligonucleotides containing either the wild-type or mutated *M. tuberculosis* recA SOS box (Movahedzadeh *et al*., 1997b) were designed such that following annealing, the double-stranded oligonucleotides had ‘AATT’ overhangs on both ends that were filled in with [α-32P]dATP, dTTP and Klenow enzyme (Promega). This method of fill-in labelling helped to prevent non-specific binding of proteins such as single-strand-binding proteins in the cell-free extracts to single-stranded DNA. Approx. 0.4 pmol of the labelled oligonucleotide was incubated with cell-free extracts and 1 µg poly[d(I-C)] nonspecific competitor DNA in a 20 µl binding reaction [1 × binding buffer contained 20 mM HEPES (pH 7.6), 30 mM KCl, 10 mM (NH₄)₂SO₄, 1 mM EDTA, 1 mM DTT and 0.2% (w/v) Tween 20] for 15 min at room temperature. Protein–DNA complexes were resolved from free DNA on a 10% non-denaturing polyacrylamide gel by electrophoresis in 0.5 × TBE buffer (Sambrook *et al*., 1989) at 180 V for 5 h at 4 °C. Gels were dried and the radioactive bands were visualized by autoradiography. Alternatively, gels were blotted onto a double layer of membranes, one being nitrocellulose and the other DE81 paper, following a published Shift-Western protocol (Demczuk *et al*., 1993). Proteins were retained on the nitrocellulose, which was developed using anti-LexA antibodies as described above, and DNA was retained on the DE81 paper and visualized by autoradiography.

**RESULTS**

**Induction of RecA is delayed in *M. tuberculosis* compared with *M. smegmatis***

Expression of the recA gene in mycobacteria has been shown previously to be inducible by DNA-damaging agents (Durbach *et al*., 1997; Movahedzadeh *et al*., 1997b; Papavinasasundaram *et al*., 1997). In this study we examined the kinetics of this response. Samples were taken at 1 h intervals following the addition of the DNA-damaging agent mitomycin C (0.2 µg ml⁻¹) to an exponential phase (OD₆₀₀ 0.6) culture of *M. smegmatis* and analysed for RecA expression by Western blotting with an antibody raised to *M. tuberculosis* RecA protein (Fig. 1a). Induction of RecA expression was apparent...
We also examined the response of (0 mRNA before and after exposure to mitomycin C (0.5 gm l\(^{-1}\)) for 5 h, again there was a further increase in expression at 24 h, then the expression level remained constant to 36 h. As a control the expression of the gltS gene encoding glutamyl-tRNA synthase was measured in the same samples and as expected there was no induction in its expression following exposure to mitomycin C (Fig. 2). This analysis of the recA mRNA levels supports the results obtained by Western analysis of the RecA protein levels described above, and additionally indicates that the response is complete at 24 h.

The slow induction of RecA in M. tuberculosis is not intrinsic to the M. tuberculosis recA promoter

An integrating lacZ transcriptional reporter vector (pEJ414) was constructed and a 0.35 kb fragment from upstream of the M. tuberculosis recA gene, which had previously been demonstrated to have promoter activity (Movahedzadeh et al., 1997b), was cloned into it to yield PEJ417. This clone was then introduced into both M. smegmatis mc\(^{155}\) and M. tuberculosis H37Rv, and the expression of the lacZ reporter gene was determined by assaying \(\beta\)-galactosidase activity at various time points following exposure to mitomycin C (0.2 \(\mu\)g ml\(^{-1}\)) for both species. The time-course in M. tuberculosis (Fig. 3b) confirmed the earlier results obtained by Western analysis. There was no induction at 3 h, then a slight increase at 6 h followed by increasing levels of expression with time to 36 h, the last time point taken. The continued increase in expression at very late time points may reflect the stability of the reporter mRNA and protein, but again there was no clear response until 12 h. In contrast, the same clone containing the M. tuberculosis recA promoter exhibited a much more rapid response to DNA damage when present in M. smegmatis (Fig. 3a). Although there was no induction at 30 min, an increase in expression was detectable after 1 h and expression reached maximal levels between 4 and 7 h, mirroring the results obtained for expression of RecA protein from the M. smegmatis recA promoter described above. Thus, the M. tuberculosis recA promoter is capable of responding to an inducing signal quickly, and the slow induction of RecA expression in M. tuberculosis is not intrinsic to the M. tuberculosis recA promoter.

DNA damage has occurred long before full induction

A trivial explanation for the delayed response to DNA-damaging agents in M. tuberculosis would be that the thick cell wall hinders the entry of these agents and so the DNA is not damaged until a longer period of time has elapsed. To test this possibility we exposed both M. smegmatis and M. tuberculosis carrying the reporter plasmid pEJ417 to a pulse of mitomycin C (0.2 \(\mu\)g ml\(^{-1}\)), followed by incubation in medium free of DNA-damaging agents such that the total incubation period was 5 h for M. smegmatis and 24 h for M. tuberculosis. The expression of the lacZ reporter gene was determined following pulse exposures of 1 h and 3 h for both
species, with an additional pulse exposure of 6 h for M. tuberculosis. The level of expression arising from continuous exposure to mitomycin C for various times was compared with that from the pulsed exposure. It was apparent that exposure of M. smegmatis to mitomycin C for 1 h with a 4 h expression step in the absence of DNA-damaging agents was sufficient to induce an equivalent level of expression to that seen following continuous exposure for 5 h (Fig. 4a). This was in marked contrast to cells harvested immediately after a 1 h exposure. Similarly, exposure of M. tuberculosis to mitomycin C for 1 h with a 23 h expression step in the absence of DNA-damaging agents was sufficient to induce an equivalent level of expression to that seen following continuous exposure for 24 h (Fig. 4b). Again, this result was in sharp contrast to those obtained when cells were harvested immediately after short time periods such as 3 h, when no induction was apparent. Whilst a 1 h exposure to mitomycin C resulted in a small decrease in viable counts the effect in the two species was comparable (82% for M. smegmatis and 83% for M. tuberculosis). These experiments established that DNA damage occurs within 1 h in both M. smegmatis and M. tuberculosis and thus that a delay in the appearance of damaged DNA cannot be the reason for the slow induction of recA expression.

The amount of DNA-binding competent LexA declines only slowly and to a small extent in M. tuberculosis compared with M. smegmatis

In E. coli the SOS genes, which include recA, become induced when the repressor protein LexA is cleaved into two fragments; these fragments no longer bind to the LexA binding sites upstream of the genes regulated by LexA (Friedberg et al., 1995; Little & Mount, 1982). There is evidence for an analogous system in mycobacteria (Durbach et al., 1997; Movahedzadeh et al., 1997a, b; Papavinasadunaram et al., 1997). Of particular significance to this work is the fact that the LexA homologue from M. tuberculosis has been shown to bind to a specific site upstream of the recA gene. It was possible that the slow induction of recA expression seen in M. tuberculosis was due to a slow rate of cleavage of LexA in this species.

![Fig. 3. Kinetics of PrecA-lacZ induction in (a) M. smegmatis and (b) M. tuberculosis strains bearing the plasmid pEJ417. Cell-free extracts were prepared from cultures at the indicated time points after the addition of mitomycin C and assayed for β-galactosidase activity. The plot was drawn from the mean values obtained from duplicate assays of three independent experiments; the error bars indicate standard deviation. Background expression from the vector pEJ414 (uninduced and induced) was 2–4 U (mg protein)⁻¹ in both M. smegmatis and M. tuberculosis.](image1)

![Fig. 4. Induction of PrecA-lacZ following pulse exposure to mitomycin C: β-galactosidase expression in (a) M. smegmatis and (b) M. tuberculosis cultures harvested either immediately after incubation with mitomycin C for the duration indicated (plain bars), or after further incubation in drug-free medium (pulse exposure; hatched bars). The height of the bars indicates the mean from duplicate assays of three independent experiments; the error bars indicate standard deviation.](image2)
To investigate this, the amount of LexA protein present in *M. smegmatis* and in *M. tuberculosis* after various times of DNA damage was assessed by Western blotting with an antibody raised to *M. tuberculosis* LexA protein, using the same samples as had been used above in the Western analysis of RecA expression levels. Under the conditions used the intensity of the signal from the antibody directly correlated with the amount of LexA protein applied to the blot as determined using varying amounts of purified LexA (data not shown). In *M. smegmatis* a gradual decline in LexA levels occurred with time up to about 3 h, after which there was no further change (Fig. 5a). At no time point examined did the LexA become undetectable, unlike the response of *B. subtilis* to mitomycin C (Miller et al., 1996). Surprisingly, in *M. tuberculosis* there was very little decrease in the amount of LexA present throughout the time course to 36 h (Fig. 5b).

It remained possible that these small changes in the amount of LexA were sufficient to affect binding to the LexA binding site. Alternatively, some modification other than cleavage might occur which affected the ability of the LexA protein to bind to its regulatory sites. To investigate these possibilities we tested the ability of cell extracts from uninduced and induced cultures to bind to an oligonucleotide containing the mycobacterial LexA binding site by a gel retardation assay. When extracts from *M. smegmatis* were used a retarded band indicating LexA binding was clearly observed for the uninduced sample, whereas in the induced samples binding progressively decreased with time of exposure, reaching a very low level by 2 h (Fig. 6a). In contrast the *M. tuberculosis* samples, both uninduced and induced, showed a relatively small variation in the degree of binding of LexA to the probe DNA (Fig. 6b). The use of different amounts of the uninduced extract in this assay established that it was sufficiently sensitive to detect a twofold reduction in the amount of LexA, and indicated that the amount of LexA in the induced samples changed by a maximum of a factor of two. This small decrease in the amount of LexA capable of DNA binding was not apparent until 12–18 h after treatment in accord with the timing of *recA* induction. These extracts had all been induced with 0–2 µg mitomycin C ml$^{-1}$, so, in case the *M. tuberculosis* LexA needed higher levels of DNA damage for a clear response, we repeated the 24 h time point using 1 and 2 µg mitomycin C ml$^{-1}$; the result was unchanged (data not shown).

To confirm that the protein responsible for the retarded band was indeed LexA, the gel was blotted and probed with anti-LexA antibodies. A positive signal was obtained for each of the retarded bands (data not shown), identifying the presence of LexA in the complexes. In an alternative procedure, the addition of anti-
LexA was required for the complex formation. In addition, when an oligonucleotide mutated at the LexA binding site was used no retardation was observed (data not shown). Thus, the reduction in repression by LexA following exposure to mitomycin C in *M. tuberculosis* is slower and to a lesser degree than that in *M. smegmatis*.

**DISCUSSION**

In this study we have demonstrated that whilst *recA* expression is DNA-damage inducible in both *M. smegmatis* and *M. tuberculosis* the kinetics of this induction are quite different in the two species, with optimal induction times being in the region of 5 h for *M. smegmatis* and 24 h for *M. tuberculosis*. In comparison, analysis by immunoblotting has indicated that *B. subtilis* RecA is fully induced between 60 and 90 min after the addition of mitomycin C (Lovett et al., 1988, 1993). However, the activity of a *recA–xylE* reporter was greater 3 h following treatment than at earlier time points (Raymond-Denise & Guillen, 1992). Thus, the kinetics of RecA induction in *M. smegmatis* bear some similarity to the response in *B. subtilis*, another Gram-positive bacterium. In contrast, the induction of RecA by DNA damage in *M. tuberculosis* is markedly slower. This is despite the fact that the organism is capable of responding much more rapidly to other stresses. For example the heat-shock genes *hsp60* and *hsp70* are induced to maximum levels in less than 1 h following a shift to 45 °C (Patel et al., 1991), suggesting that the rate of mRNA synthesis is not limiting. The disparity in the response to DNA damage between the two mycobacterial species is reflected in their differential sensitivities to DNA damage, with *M. tuberculosis* being more sensitive to UV irradiation (David, 1973).

In each mycobacterial species there is a similar relationship between the induction time and the generation time of that species (about 3 h for *M. smegmatis* in the medium used here and about 20–24 h for *M. tuberculosis*). Thus, one possible explanation for the difference in kinetics between the species is that the time required for induction is related to the rate of replication of the chromosome, which has been reported to be 11 times slower in *M. tuberculosis* than in *M. smegmatis* (Hiriyanna & Ramakrishnan, 1986). In this scenario, the ssDNA-inducing signal would arise from replication blockage by the damaged DNA. Whilst this remains an attractive explanation it is perhaps noteworthy that neither mitomycin C nor ofloxacin depends on replication to generate the SOS-inducing signal in *E. coli* (Sassanfar & Roberts, 1990).

We established that the DNA-damaging agent is taken up and, therefore, as only a chemical reaction is then required between mitomycin C and DNA, presumably that the DNA is also damaged, within 1 h in both *M. smegmatis* and *M. tuberculosis*. Thus differential rates of damage cannot explain the different rates of RecA induction. In both species a period of further incubation, during which the damaging agent does not need to be present, is required for maximal levels of induction to be
obtained. This time must be necessary for recognition and/or processing of the damage to generate the inducing signal and translation of this signal into increased expression. The mechanism responsible for this and the rate-limiting step in this pathway remain to be determined, but the process is evidently slower in M. tuberculosis than in M. smegmatis.

When using the reporter plasmid pEJ417 containing the M. tuberculosis recA promoter region we noticed that the basal level of expression we obtained from uninduced cultures was quite different in the two mycobacterial species. The β-galactosidase activity in M. smegmatis was around a quarter to a third of that in M. tuberculosis. One potential explanation for this could be that there is a higher occupancy of the LexA binding site by repressor molecules in M. smegmatis, either because the LexA protein binds to the SOS box with higher affinity or because there is a higher intracellular concentration of LexA. Alternatively, it could be that the transcriptional machinery of M. smegmatis works less efficiently on the M. tuberculosis recA promoter than that of M. tuberculosis itself. It is to be noted that when the basal expression level of the native RecA protein in the two species is compared it is actually greater in M. smegmatis. This suggests that the primary reason for the lower β-galactosidase activity from pEJ417 in M. smegmatis is reduced transcriptional efficiency of the heterologous promoter.

In E. coli, DNA damage is processed into regions of ssDNA by one of various mechanisms, depending on the nature of the damage. When RecA binds to such regions of ssDNA it becomes ‘activated’ and stimulates the auto-catalytic cleavage of LexA. The LexA cleavage products no longer bind to the SOS boxes upstream of the LexA-regulated genes, resulting in an increase in the expression of those genes (Friedberg et al., 1995; Little & Mount, 1982). In B. subtilis cleavage of the LexA homologue DinR following DNA damage by mitomycin C is clearly seen both by Western analysis (Miller et al., 1996) and by gel retardation assay (Lovett et al., 1993), with intact DinR declining to undetectable levels in less than 1 h. Whilst we detected a similar decrease in the ability of LexA to bind to a mycobacterial SOS box in induced extracts of M. smegmatis, we saw only a small change in this property in M. tuberculosis extracts following DNA damage and this only at extended time periods. The timing of this change in LexA binding coincides with that of recA induction in the two species of mycobacteria. Nevertheless, the relatively slight change seen in M. tuberculosis raises the possibility that other factors might be involved in recA induction in this species. A second mechanism for regulating gene expression in response to DNA damage might be beneficial to M. tuberculosis if it experienced conditions in which only a subset of the genes normally induced were required. The slow response to DNA damage in M. tuberculosis might be an adaptation permitting a more sustained response over a longer period of time, which could be advantageous for withstanding the defences of the macrophage on infection.

ACKNOWLEDGEMENTS

We thank K. Muniyappa for providing the purified M. tuberculosis RecA protein used for raising antibodies.

REFERENCES


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Received 27 April 2001; revised 9 August 2001; accepted 17 August 2001.