INTRODUCTION

Bacterial cells respond to DNA damage by changing gene expression (Butala et al., 2009). In many organisms this response is governed by the LexA repressor protein, which, upon induction, releases expression of the SOS regulon (Kelley, 2006). The SOS regulated genes carry out diverse functions in response to DNA damage such as nucleotide excision repair, translesion DNA replication, homologous recombination and cell division arrest. Of importance to bacterial pathogens, the SOS response also modulates the dissemination of mobile genetic elements expressing antibiotic resistance and virulence factors (Beaber et al., 2004; Maiques et al., 2006; Ubeda et al., 2005). In addition, it is well known that several groups of antibiotics are in fact inducers of the SOS response such as the fluoroquinolones (Cirz et al., 2005; Mesak et al., 2008), trimethoprim (Lewin & Amyes, 1991) and β-lactams (Maiques et al., 2006; Miller et al., 2004). Thus, treatment of an infection may, via the SOS response, influence both acquisition and spread of antibiotic resistance.

The paradigm of SOS control is primarily based on studies of Escherichia coli (recent review by Butala et al., 2009). Repression of SOS-regulated genes in the absence of DNA damage is mediated by LexA binding to operator sequences (SOS boxes, or Cheo boxes in Bacillus subtilis) (Cheo et al., 1991; Little et al., 1981). LexA binds to the SOS boxes as a dimer with each monomer consisting of an N-terminal DNA-binding domain (NTD) and a C-terminal dimerization domain (CTD) separated by a hinge region (Flynn et al., 2001). Following DNA damage, LexA undergoes auto-cleavage in a process relying on the formation of RecA nucleoprotein filament along stretches of single-stranded DNA. This RecA filament promotes autodegradation of the LexA repressor, leading to induction of the response (Sassanfar & Roberts, 1990). In E. coli, the auto-cleavage occurs at Ala84–Gly85 and similar sequences are conserved in other LexA-like proteins as well as in phage repressors (Flynn et al., 2001). While auto-cleavage of LexA separates the two domains, the NTD is still able to bind to the SOS box and retains some LexA repressor function (Bertrand-Burggraf et al., 1987; Little & Hill, 1985). In fact, auto-cleavage of E. coli LexA exposes hidden proteolytic recognition signals promoting degradation of the CTD by the Lon protease (Little & Gellert, 1983) and the NTD by

Clp-dependent proteolysis of the LexA N-terminal domain in Staphylococcus aureus

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The SOS response is governed by the transcriptional regulator LexA and is elicited in many bacterial species in response to DNA damaging conditions. Induction of the SOS response is mediated by autocleavage of the LexA repressor resulting in a C-terminal dimerization domain (CTD) and an N-terminal DNA-binding domain (NTD) known to retain some DNA-binding activity. The proteases responsible for degrading the LexA domains have been identified in Escherichia coli as ClpXP and Lon. Here, we show that in the human and animal pathogen Staphylococcus aureus, the ClpXP and ClpCP proteases contribute to degradation of the NTD and to a lesser degree the CTD. In the absence of the proteolytic subunit, ClpP, or one or both of the Clp ATPases, ClpX and ClpC, the LexA domains were stabilized after autocleavage. Production of a stabilized variant of the NTD interfered with mitomycin-mediated induction of sosA expression while leaving lexA unaffected, and also significantly reduced SOS-induced mutagenesis. Our results show that sequential proteolysis of LexA is conserved in S. aureus and that the NTD may differentially regulate a subset of genes in the SOS regulon.
the ClpXP proteolytic complex (Neher et al., 2003). In E. coli, inactivation of clpX or overproduction of the NTD fragment results in UV sensitivity (Neher et al., 2003), suggesting that the NTD and its turnover is important in fine-tuning the DNA damage response. LexA-like regulators, such as HdiR and IvrR, which vary considerably in primary amino acid sequence but contain highly conserved domain structures, have been identified in the Gram-positive bacteria Lactococcus lactis and Streptococcus mutans, respectively. For these repressors, the importance of proteolysis appears to be conserved as the N-terminal cleavage products are also removed by Clp proteolytic complexes prior to de-repression of target gene expression (Niu et al., 2010; Savijoki et al., 2003).

Staphylococcus aureus is a Gram-positive pathogen that can cause a variety of diseases ranging from mild skin infections to sepsicaemia and endocarditis. Particularly disturbing is its ability to develop antibiotic resistance, as has been observed in the case of vancomycin, considered the 'last-resort' drug (Boneca & Chiosis, 2003). Genes encoding antibiotic resistance to vancomycin, considered the 'last-resort' drug (Boneca & Chiosis, 2003). Genes encoding antibiotic resistance and virulence are often carried by mobile genetic elements that in some instances may be mobilized in response to DNA damage. One such example is the mobile staphylococcal pathogenicity islands that occupy specific chromosomal sites but that are excised and replicated by temperate phages upon SOS induction (Novick, 2003; Ruzin et al., 2001; Ugeda et al., 2008). The induction of the SOS response in S. aureus has been studied in strain UAMS-1 where exposure to the DNA damaging agent mitomycin C (MMC) induced expression of 73 genes including lexA, recA, the umucC-like gene SACOL1400, and genes involved in the nucleotide excision repair (uvra, uvrB) and recombination repair (sbcD, sbcD) (Anderson et al., 2006). The staphylococcal SOS regulon may, however, be more limited as only 16 genes differed in expression between a wild-type strain (8325-4) and a mutant strain encoding a non-cleavable derivative of LexA that were exposed to the DNA damaging antibiotic ciprofloxacin (Cirz et al., 2007). One of the upregulated genes is fnbB encoding one of two fibronectin binding proteins, FnBPB, which is important for attachment of S. aureus to surfaces, a critical step in the early stage of infection. Thus, induction of the SOS response can result in virulence modulation in S. aureus through both mobilization of virulence genes and enhanced virulence factor expression (Bisognano et al., 2004; Goerke et al., 2006; Ubeda et al., 2005). In the present study, we have examined whether the S. aureus SOS response follows the E. coli paradigm with LexA autocleavage and domain degradation as important elements of SOS-induced gene expression. The biological role of the NTD has also been investigated and will be discussed.

**METHODS**

**Bacterial strains and growth conditions.** The bacterial strains and plasmids used in this study are listed in Table 1. E. coli strains were grown in Luria–Bertani medium (LB; Oxoid) or on LB agar, while S. aureus strains were grown in Brucella agar (BDA; Oxoid) or on BDA agar, as previously described (Novick, 1967). E. coli TOP10 and TOP10 F- were used as the parental strains for the plasmids used in this study. These were grown at 37°C in LB medium (Oxoid) supplemented with 1% (w/v) glucose. E. coli strains were grown with 50 μg/ml kanamycin (Invitrogen). E. coli TOP10 F- was transformed with plasmid DNA using the Qiagen (Valencia, CA) Maxi kit according to the manufacturer’s instructions.

### Table 1. Bacterial strains and plasmids used in the study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant characteristics</th>
<th>Source or reference</th>
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<tbody>
<tr>
<td><strong>Strains</strong></td>
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<tr>
<td>E. coli</td>
<td></td>
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<tr>
<td>TOP10</td>
<td>F− mcrA Δ(mrr-hsdRMS-mrcB) ϕ80lacZΔM15 ΔlacX74 recA1 araD139 Δ(ara-leu) 7697 galU galK rpsL (Strr) endA1 nupG Δ(λ)</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>BL21-A1</td>
<td>F− ampT hsdSB(rB mB) gal dcm araB:: T7rap-tetA</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>S. aureus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8325-4</td>
<td>Wild-type strain (rsbU mutant)</td>
<td>Novick (1967)</td>
</tr>
<tr>
<td>RN4220</td>
<td>Restriction-defective derivative of RN450</td>
<td>Kreiswirth et al. (1983)</td>
</tr>
<tr>
<td>RTC3002</td>
<td>8325 lexA1(S130A)::SpecΔ</td>
<td>Cirz et al. (2007)</td>
</tr>
<tr>
<td>8325-4ΔclpP</td>
<td>Deletion of the entire clpP gene</td>
<td>Frees et al. (2003)</td>
</tr>
<tr>
<td>8325-4ΔclpX</td>
<td>651 bp in-frame deletion in clpX</td>
<td>Frees et al. (2003)</td>
</tr>
<tr>
<td>8325-4ΔclpC</td>
<td>1122 bp in-frame deletion in clpC</td>
<td>Frees et al. (2004)</td>
</tr>
<tr>
<td>8325-4ΔclpX</td>
<td>8325-4ΔclpX clpC::ermR</td>
<td>Frees et al. (2004)</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
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<tr>
<td>pCN41</td>
<td>Shuttle vector with promoterless blaZ, ErmR&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Charpentier et al. (2004)</td>
</tr>
<tr>
<td>pLexA</td>
<td>Transcriptional plexA–blaZ fusion with SOS box 1-5 in pCN41 (Fig. 1)</td>
<td>This study</td>
</tr>
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<td>pLexA_NTD89</td>
<td>Transcriptional plexA–blaZ fusion with SOS box 1-5 and NTD89 in pCN41 (Fig. 1)</td>
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</tr>
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<td>Transcriptional psoA–blaZ fusion with SOS box 1-5 in pCN41 (Fig. 1)</td>
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<td>This study</td>
</tr>
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<td>pLexA1_NTD89</td>
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<td>This study</td>
</tr>
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<td>This study</td>
</tr>
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<td>pLexA3_NTD89</td>
<td>Transcriptional plexA–blaZ fusion with SOS box 1-3 and NTD89 in pCN41 (Fig. 1)</td>
<td>This study</td>
</tr>
<tr>
<td>pLexA_his&lt;sub&gt;6&lt;/sub&gt;</td>
<td>pDEST17 derivative with inducible his&lt;sub&gt;6&lt;/sub&gt;-lexA (kan&lt;sup&gt;R&lt;/sup&gt;)</td>
<td>This study</td>
</tr>
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</table>
**aureus** strains were grown in tryptic soy broth (TSB; Oxoid) or on tryptic soy agar (TSA; Oxoid). Ampicillin (100 μg ml⁻¹), kanamycin (50 μg ml⁻¹) or erythromycin (5 μg ml⁻¹) were added when required.

**Plasmid construction.** For construction of plasmids, PCR fragments comprising the promoter regions (see Fig. 1) were cloned into pCN41 in front of a promoterless β-lactamase gene with its own ribosome binding site using Sall and EcoR1. The constructs monitoring lexA (pLexA) and sosA (pSosA) also express the N-terminal 47 aa of sosA, which did not affect cell phenotype (data not shown). The reporter plasmids monitoring the lexA promoter activity in the presence of box 1, box 1-2 or box 1-2-3 also expressed the NTD of LexA, which does not affect lexA expression (Fig. 2a). E. coli TOP10 was used for cloning and all clones were transformed into RN4220, before being transferred by transformation to 8325-4.

**Nitrocefin assay.** The nitrocefin assay was modified from the method described by O’Callaghan et al. (1972). TSB was inoculated with an overnight culture to OD₆₀₀=0.05 and grown at 37 °C to mid-exponential phase (OD₆₀₀=0.5). An aliquot (1 ml) of culture was snap-frozen and stored at −80 °C. MMC was added (0.2 μg ml⁻¹) and the culture was allowed to continue to grow at 37 °C. At 20, 40, 60 and 80 min after MMC was added, 1 ml samples were removed and snap-frozen after OD₆₀₀ was measured. β-Lactamase enzyme activity was measured by mixing 50 μl thawed sample and diluting to OD₆₀₀=0.5, with 50 μl rehydrated nitrocefin from Oxoid (3 μg) in a 96-well plate. β-Lactamase activity was measured as units (mg cell dry weight)⁻¹, where one unit was defined as the hydrolysis of 1 μmol nitrocefin min⁻¹. Measurements were done by following OD₄₈₅ at 20 s intervals for 20 min at 25 °C using a PowerWave XS microplate reader from BioTek.

**Phage induction.** Phage induction, transduction and determination of titre were done as described previously (Lindsay et al., 1998; Úbeda et al., 2008). S. aureus 8325-4 containing plasmid pLexA or pLexA_NTD89 was first made lysogenic for bacteriophage 80x. Induction of 80x from these two strains was done by adding MMC (2 μg ml⁻¹) and incubating at 32 °C for 4 h. The lysate was then filtered through a 0.2 μm filter and the phage titre was determined.

**Mutagenesis.** Mutation frequencies were measured basically as described previously (Weel-Sneve et al., 2008). Bacteria were grown with shaking at 37 °C and samples were withdrawn at mid-exponential phase (OD₆₀₀=0.5). Cells were washed in physiological saline and a 5 ml suspension was irradiated with 50 J UV light m⁻². An aliquot (5 ml) of either irradiated or non-irradiated cells was mixed with 5 ml TSB and grown overnight with shaking at 37 °C. Appropriate dilutions of the culture were plated on TSB to determine the total number of bacteria and on rifampicin-containing TSB plates (0.1 μg ml⁻¹) to determine the number of rifampicin resistant mutants.

**Overexpression and purification of LexA protein and generation of anti-LexA antibodies.** A fragment containing the full-length lexA gene was generated and cloned into pENTR/D-TOPo (Invitrogen) and next pDEST17 (Invitrogen) according to the procedure recommended for the Gateway Technology system (Invitrogen). DNA sequence analysis was performed to verify the construct pLexA_his6. His₆-LexA was purified from E. coli BL21-A1 (Invitrogen) carrying pLexA_his6 as described by the manufacturer (Qiagen). The purified His₆-LexA was used for custom antibody production in rabbits (CovalAB).

**Western blot analysis.** S. aureus strains were grown as specified above. At an OD₆₀₀ of 0.5 the cells were challenged with MMC (1 μg ml⁻¹) to induce the SOS response and were subsequently incubated at 37 °C for 30 min. Following induction, chloramphenicol (150 μg ml⁻¹) was added to stop translation. Cells were harvested for isolation of intracellular protein when MMC was added and at 0, 10, 20 and 40 min after chloramphenicol treatment. Cell pellets were resuspended in 50 mM Tris/HCl (pH 8.0), lysed by the addition of 50 μg lysostaphin ml⁻¹ (Sigma) and incubated at 37 °C for 20 min. The protein concentration was determined by using a Bio-Rad protein assay (Bio-Rad Laboratories) and samples were separated on Novex 10% Tricine gels (Invitrogen) using Tris/glycine buffer (Invitrogen). The protein was transferred onto a polyvinylidene difluoride membrane (Invitrogen) using an XCell sure-lock mini-cell system (Invitrogen) as recommended by the supplier. LexA was probed with a 1:3000 dilution of LexA antibody and the WesternBreeze chemiluminescent anti-rabbit kit (Invitrogen).

**RESULTS**

**LexA is subjected to auto-cleavage and Clp-mediated degradation in S. aureus**

Homologues of LexA have been identified in a number of bacteria including *S. aureus*. Commonly they are able to undergo RecA-stimulated proteolytic self-cleavage during SOS-inducing conditions (Erill et al., 2007; Kelley, 2006). To confirm if this is also the case in *S. aureus*, we followed expression of LexA by Western blot analysis using *S. aureus* LexA-specific polyclonal antibodies raised as described in Methods. When treating wild-type (strain 8325-4) cells with MMC and blocking subsequent protein synthesis by chloramphenicol, we observed that full-length LexA was rapidly processed to disappear 10 min after addition of chloramphenicol leaving a small fragment of approximately 13 kDa to react with the LexA antibody (Fig. 3a). Further, these fragments were not visible in a stain carrying a LexA derivative (RTC3002) unable to undergo auto-proteolytic cleavage (Fig. 3a; Cirz et al., 2007). When comparing *S. aureus* LexA with those of other organisms, a conserved auto-proteolytic cleavage site is present at amino
acid sequence Ala93–Gly94 (Maiques et al., 2006) corresponding to a LexA NTD of 10.5 kDa and a CTD of 12.8 kDa. Thus, the appearance of a 13 kDa fragment after MMC treatment suggests that only the CTD is visible in wild-type cells (Fig. 3a).

Since a Clp proteolytic complex is responsible for the turnover of LexA cleavage products in E. coli (Neher et al., 2003), we examined the stability of the cleavage products in S. aureus strains lacking either the proteolytic component, ClpP, or one of the two Clp ATPases predicted to interact with ClpP, namely ClpC or ClpX (Frees et al., 2003, 2004). In the absence of clpP, clpX or clpCX, two LexA fragments were visible after MMC induction corresponding to the predicted NTD and CTD fragments (Fig. 3a). The smaller NTD was not apparent in wild-type or clpP mutant cells whereas in clpX mutant cells, the NTD was stabilized. Since the NTD LexA fragment accumulates to a greater extent in clpCX mutant cells than in the clpX mutant, our results indicate that while ClpXP is the main protease responsible for the turnover of NTD, ClpCP also contributes. In comparison, the LexA CTD was generally more stable than the NTD but appeared to be stabilized by the clp mutations, suggesting a minor role of the Clp proteins in CTD degradation.

**lexA and sosA expression is differentially induced by MMC**

With the aim of investigating whether the NTD influences the S. aureus SOS response, we examined two genes, namely lexA and sosA, previously shown to be controlled by LexA in S. aureus (Anderson et al., 2006). lexA and sosA are divergently transcribed from a common promoter region (S. aureus NCTC 8325, GenBank accession no. YP_499864) which shows 65% sequence identity to the lexA–yneA intergenic region of B. subtilis (http://genolist.pasteur.fr/SubtiList/). While sosA is predicted to encode a 77 aa product of unknown function, the relative location of sosA with respect to lexA suggests that it may encode a cell division inhibitor (Kawai et al., 2003; Ogino et al., 2008; van der Veen et al., 2010). In B. subtilis, LexA, also denoted DinR, binds to a consensus DNA-binding motif (the SOS box) CGAAC-N4- GTTCG (Hajjema et al., 1996). When allowing two mismatches relative to the B. subtilis SOS box, three putative LexA binding sites were identified in the S. aureus lexA–sosA intergenic region designated box 1-3 (Fig. 1) (Mesak et al., 2008). In addition, two putative SOS boxes, box 4 (CGAAC-N4-GTGC) and box 5 (GGAAC-N4-GTACG), are located within the coding region of sosA (Fig. 1). In B. subtilis, the central AA/TT of the SOS box is essential for LexA binding (Groban et al., 2005), but considering that the SOS boxes of the LexA-controlled fnbB gene in S. aureus all contain mismatches in one of the AA/TT pair (Bisognano et al., 2004), the S. aureus LexA recognition motif might deviate somewhat from the B. subtilis consensus.

In order to monitor lexA and sosA expression we constructed a series of blaZ reporter gene fusions (Fig. 1 and Table 1). Two of the constructs, designated pLexA for the lexA promoter and pSosA for the sosA promoter, encompass the lexA–sosA intergenic region in both orientations as well as part of the sosA coding region in order to include the putative box 4 and box 5. Also, reporter constructs measuring lexA promoter activity in the presence of box 1, box 1-2 or box 1-2-3 were created. The plasmids were introduced into 8325-4 and expression was followed after addition of MMC. Fig. 2 (dark bars) shows that MMC induced transcription of lexA by twofold (Fig. 2a) whereas expression of sosA was induced 22-fold 80 min
after induction (Fig. 2b). Thus, expression of both lexA and sosA are induced by MMC, but they differ substantially with respect to the magnitude of induction. When we investigated the impact of the SOS boxes on lexA expression, the number of SOS boxes did not influence the MMC-induced expression level of lexA (Fig. 4). Furthermore, the construction containing only the lexA proximal SOS box 1 was sufficient to show repression of

**Fig. 3.** *In vivo* stability of LexA under DNA damaging conditions (MMC) in *S. aureus* 8325-4 and ΔclpP, ΔclpC, ΔclpX and ΔclpCX mutant derivatives and *S. aureus* RTC3002. (a) Cells were treated with 1 μg MMC ml⁻¹ in mid-exponential phase (0') followed 30 min later by sample withdrawal at 0, 10, 20 and 40 min after the addition of chloramphenicol (150 μg ml⁻¹). (b) Cells were treated as in (a) and harvested in mid-exponential phase (0') and 0 and 10 min after addition of chloramphenicol. In (a) and (b), proteins were separated, transferred and hybridized as described in Methods.

**Fig. 4.** lexA promoter activity controlled by various numbers of SOS boxes. The promoter activities of reporter constructs pLexA1_NTD89, pLexA2_NTD89, pLexA3_NTD89 and pLexA_NTD89 (with 1, 2, 3 or full promoter, respectively) were monitored either before (dark grey bars) or 80 min after (light grey bars) induction with MMC (0.2 μg ml⁻¹). Promoter activity was monitored as β-lactamase activity relative to the β-lactamase activity of pLexA_NTD89 before MMC induction [0.063 units (mg dry weight)⁻¹]. Data are the mean±SD of a minimum of three independent measurements.
**Accumulation of stable NTD interferes with induction of sosA**

Since the N-terminal cleavage products of LexA and a LexA-like transcriptional regulator HdiR have previously been shown to retain DNA-binding activity, we aimed to investigate the possible regulatory role of the LexA NTD in *S. aureus* (Hurstel et al., 1986; Savijoki et al., 2003). To this end we exploited the fact that our reporter constructs express a LexA NTD fragment (named NTD89) that lacks the C-terminal 4 aa compared with the native NTD, that are predicted to be involved in turnover of the fragment (Fig. 1). When examining NTD89, we found that it appeared stable compared with the wild-type NTD resulting from auto-cleavage (Fig. 3b). Since the lexA and sosA promoters differ in the extent to which they are induced by MMC, we monitored their respective activities at various times after SOS induction in the presence of NTD89 (Fig. 2, light grey bars). Interestingly, expression of NTD89 barely affected the induction of lexA expression, whereas the sosA promoter was repressed in the presence of NTD89 with only a 7-fold induction by MMC in the presence of NTD89 compared with 22-fold in its absence. Thus, our experiments show that a stable NTD differentially modulates expression of two genes within the SOS regulon upon induction with MMC.

**Stable NTD expression prevents UV-induced mutagenesis**

To address the biological role of the NTD we examined the accumulation of mutations in cells expressing NTD89 after exposure to UV. In cells expressing NTD89, the UV-induced mutation frequency was reduced significantly compared with cells not producing NTD89 (Table 2). In fact, the expression of NTD89 repressed mutation frequency to the same level as observed for the *S. aureus* strain RTC30002 that carries a LexA variant unable to undergo proteolytic self-cleavage and thus is unable to induce the SOS response (Cirz et al., 2007). Another process depending on the SOS system is the excision of prophages. Consequently we examined whether expression of NTD89 affected the ability of the lysogenic phage 80 to excise but found that NTD89 did not affect this process (data not shown). Thus, our data demonstrate that the stable NTD89 fragment reduces UV-induced mutagenesis but not SOS-induced phage excision in *S. aureus*.

**DISCUSSION**

In order to study the fate of the LexA protein in *S. aureus* following DNA damage, we raised antibodies against the protein and followed turnover after MMC addition. *S. aureus* LexA appears to undergo auto-proteolytic cleavage when exposed to DNA damaging agents, resulting in two fragments of 10.5 and 12.8 kDa. In *E. coli*, the ClpXP protease degrades the LexA NTD in a process depending on the exposure of a C-terminal degradation signal revealed upon auto-cleavage (Neher et al., 2003). Our results show that in *S. aureus*, both ClpXP and ClpCP proteolytic complexes contribute to degradation of the NTD. Inspection of the C-terminal end of the NTD of *S. aureus* LexA further revealed a motif, Val 91–Thr 92–Ala 93–COOH, with similarity to the *E. coli* ClpXP degradation motif (Flynn et al., 2001). Removal of the C-terminal 4 aa (aa 90–93) of NTD (resulting in NTD89) led to stabilization of the fragment, thus indicating that this motif also directs degradation in *S. aureus*. When comparing the stability of the NTD and CTD of *S. aureus* LexA, we found that in contrast with *E. coli*, the *S. aureus* CTD is more stable than the NTD and is completely stabilized in the absence of the Clp proteins. The slow turnover of the *S. aureus* LexA CTD in wild-type cells may be due to the absence of the Lon protease that is responsible for the CTD turnover in *E. coli* (Neher et al., 2003).

When studying the impact of NTD accumulation we chose to focus on the divergently transcribed *lexA* and *sosA* genes for two reasons. First, it allowed us to construct reporter fusions that at the same time produce the stable NTD89 from its own promoter. Secondly, it enabled us to examine the effect of the NTD on expression of genes with different induction levels in response to MMC (Anderson et al., 2006). Using the reporter constructs, we found that MMC induced *lexA* 2-fold and *sosA* 22-fold 80 min after addition. These results agree well with the 5- and 31-fold induction reported previously (Anderson et al., 2006). Interestingly, expression of the stable NTD89 interfered differentially with MMC-mediated induction of *lexA* and *sosA* expression. While induction of *lexA* expression was barely affected by the presence of NTD89 following MMC addition, the induction of *sosA* expression was reduced from 22-fold to only 7-fold. Repressor activity of the *E. coli* LexA protein in the absence of MMC, as has also been observed in *B. subtilis* (Haijema et al., 1996). Studies of the *sosA* promoter were hampered by the fact that the SOS box furthest from *sosA* (SOS box 1, see Fig. 1) lies between the putative −10 and −35 promoter sequences of *sosA* and thus, manipulation eliminated promoter activity (data not shown).

**Table 2. Number of rifampicin-resistant (rifR) colonies in *S. aureus* following UV treatment**

Values are one representative of three independent experiments and show the mean (of three technical replicates) number of colonies per 10^6 viable cells. UV treatment was at 50 J m^{-2}.

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>No. of rifR colonies</th>
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<tr>
<td></td>
<td>No UV treatment</td>
</tr>
<tr>
<td>pLexA</td>
<td>4</td>
</tr>
<tr>
<td>pLexA_NTD89</td>
<td>5</td>
</tr>
<tr>
<td>RTC3002</td>
<td>2</td>
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</table>
NTD has been shown in several studies (Bertrand-Burggraf et al., 1987; Hurstel et al., 1986; Little & Hill, 1985). Neher et al. (2003) further proposed that the NTD may differentially affect induction of SOS genes and our data support this notion. In another study, Michel et al. (2006) reported that in the absence of SOS induction, expression of lexA is increased in a S. aureus strain lacking ClpP. This finding suggests that even in un-induced cells, Clp proteolytic complexes may influence LexA repression and it further stresses the significance of the Clp proteins in modulating the SOS response.

Lastly, we examined the effect of the stabilized NTD on several biological processes and, while it substantially reduced UV-induced mutagenesis, it did not interfere with induction of the 80x prophage. The latter result suggests that RecA activity is not affected by NTD98 since RecA is required for phage induction (Maïques et al., 2006). In conclusion, our data suggest that sequential proteolysis of LexA may allow a subset of the SOS regulon genes to be differentially controlled by the NTD following SOS induction. Future studies will be aimed at identifying conditions that stabilize NTD in the presence of ClpP and the biological processes differentially modulated by the NTD.

ACKNOWLEDGEMENTS


REFERENCES


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