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.

Received 16 July 2010
Revised 9 December 2010
Accepted 22 December 2010

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; Úbeda *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 autocleavage of the LexA repressor, leading to induction of the response (Sassanfar & Roberts, 1990). In *E. coli*, the autocleavage 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 autocleavage 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, autocleavage 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

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Abbreviations: CTD, C-terminal dimerization domain; MMC, mitomycin C; NTD, N-terminal DNA-binding domain.

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 septicemia and endocarditis. Particularly disturbing is its ability to develop antibiotic resistance, as has been observed by the spread of meticillin resistance and the incidences of 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; Úbeda *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 *umuC*-like gene SACOL1400, and genes involved in the nucleotide excision repair (*uvrA*, *uvrB*) and recombination repair (*sbcC*, *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; Úbeda *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.*

Table 1. Bacterial strains and plasmids used in the study

Strain or plasmid	Relevant characteristics	Source or reference
Strains		
<i>E. coli</i>		
TOP10	F ⁻ <i>mcrA</i> Δ(<i>mrr-hsdRMS-mcrBC</i>) φ80 <i>lacZ</i> ΔM15 Δ <i>lacX</i> 74 <i>recA1 araD</i> 139 Δ(<i>ara-leu</i>) 7697 <i>galU galK rpsL</i> (Str ^R) <i>endA1 nupG λ</i> ⁻	Invitrogen
BL21-A1	F ⁻ <i>ompT hsdS_B</i> (r _B ⁻ m _B ⁻) <i>gal dcm araB::T7rnsp-tetA</i>	Invitrogen
<i>S. aureus</i>		
8325-4	Wild-type strain (<i>rsbU</i> mutant)	Novick (1967)
RN4220	Restriction-defective derivative of RN450	Kreiswirth <i>et al.</i> (1983)
RTC3002	8325 <i>lexA</i> (S130A)::Spec ^R	Cirz <i>et al.</i> (2007)
8325-4 Δ <i>clpP</i>	Deletion of the entire <i>clpP</i> gene	Frees <i>et al.</i> (2003)
8325-4 Δ <i>clpX</i>	651 bp in-frame deletion in <i>clpX</i>	Frees <i>et al.</i> (2003)
8325-4 Δ <i>clpC</i>	1122 bp in-frame deletion in <i>clpC</i>	Frees <i>et al.</i> (2004)
8325-4 Δ <i>clpXC</i>	8325-4 Δ <i>clpX clpC::ermR</i>	Frees <i>et al.</i> (2004)
Plasmids		
pCN41	Shuttle vector with promoterless <i>blaZ</i> , Erm ^R	Charpentier <i>et al.</i> (2004)
pLexA	Transcriptional <i>plexA-blaZ</i> fusion with SOS box 1-5 in pCN41 (Fig. 1)	This study
pLexA_NTD89	Transcriptional <i>plexA-blaZ</i> fusion with SOS box 1-5 and NTD89 in pCN41 (Fig. 1)	This study
pSosA_NTD89	Transcriptional <i>psosA-blaZ</i> fusion with SOS box 1-5 and NTD89 in pCN41 (Fig. 1)	This study
pSosA	Transcriptional <i>psosA-blaZ</i> fusion with SOS box 1-5 in pCN41 (Fig. 1)	This study
pLexA1_NTD89	Transcriptional <i>plexA-blaZ</i> fusion with SOS box 1 and NTD89 in pCN41 (Fig. 1)	This study
pLexA2_NTD89	Transcriptional <i>plexA-blaZ</i> fusion with SOS box 1-2 and NTD89 in pCN41 (Fig. 1)	This study
pLexA3_NTD89	Transcriptional <i>plexA-blaZ</i> fusion with SOS box 1-3 and NTD89 in pCN41 (Fig. 1)	This study
pLexA_his ₆	pDEST17 derivative with inducible <i>his₆-lexA</i> (Kan ^R)	This study

aureus strains were grown in tryptic soy broth (TSB; Oxoid) or on tryptic soy agar (TSA; Oxoid). Ampicillin ($100 \mu\text{g ml}^{-1}$), kanamycin ($50 \mu\text{g ml}^{-1}$) or erythromycin ($5 \mu\text{g ml}^{-1}$) 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 *EcoRI*. 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 $\text{OD}_{600}=0.05$ and grown at 37°C to mid-exponential phase ($\text{OD}_{600}=0.5$). An aliquot (1 ml) of culture was snap-frozen and stored at -80°C . MMC was added ($0.2 \mu\text{g ml}^{-1}$) 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 OD_{600} was measured. β -Lactamase enzyme activity was measured by mixing $50 \mu\text{l}$ thawed sample and diluting to $\text{OD}_{600}=0.5$, with $50 \mu\text{l}$ rehydrated nitrocefin from Oxoid ($3 \mu\text{g}$) in a 96-well plate. β -Lactamase activity was measured as units ($\text{mg cell dry weight}^{-1}$), where one unit was defined as the hydrolysis of $1 \mu\text{mol}$ nitrocefin min^{-1} . Measurements were done by following OD_{482} 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 80 α . Induction of 80 α from these two strains was done by adding MMC ($2 \mu\text{g ml}^{-1}$) and incubating at 32°C for 4 h. The lysate was then filtered through a $0.2 \mu\text{m}$ 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 ($\text{OD}_{600}\sim 0.5$). Cells were washed in physiological saline and a 5 ml suspension was irradiated with $50 \text{ J UV light m}^{-2}$. 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 ($0.1 \mu\text{g ml}^{-1}$) 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 OD_{600} of 0.5 the cells were challenged with MMC ($1 \mu\text{g ml}^{-1}$) to induce the SOS response and were subsequently incubated at 37°C for 30 min. Following induction, chloramphenicol ($150 \mu\text{g ml}^{-1}$) 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 $50 \mu\text{g}$ 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 strain 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

Fig. 1. Depiction of the 139 bp intergenic region separating the *sosA* and *lexA* open reading frames with the predicted SOS boxes (1–3) and putative promoters for the two genes. The grey arrows indicate the genes *lexA* and *sosA* with the two putative SOS boxes (4 and 5) in *sosA*. The thin arrows indicate the size and orientation of the fragment inserted to direct *blaZ* reporter gene expression in pCN41. Not drawn to scale.

Fig. 2. *lexA* and *sosA* promoter activity in the presence or absence of NTD89 before (0 min) and 20, 40, 60 and 80 min after addition of MMC (0.2 $\mu\text{g ml}^{-1}$). Promoter activity was measured as β -lactamase activity expressed by (a) pLexA (dark grey bars) and pLexA_NTD89 (light grey bars) and (b) pSosA (dark grey bars) and pSosA_NTD89 (light grey bars). The β -lactamase activity of each bar is relative to the β -lactamase activity of pLexA prior to induction with MMC [0.063 units (mg dry weight) $^{-1}$]; data are the mean $\pm$ SD from a minimum of three independent measurements.

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 *clpC* mutant cells whereas in *clpP*, *clpCX* and, to a somewhat lesser extent, *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 (Haijema *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-GTGCT) 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

Fig. 3. *In vivo* stability of LexA under DNA damaging conditions (MMC) in *S. aureus* 8325-4 and $\Delta clpP$, $\Delta clpC$, $\Delta clpX$ and $\Delta clpCX$ mutant derivatives and *S. aureus* RTC3002. (a) Cells were treated with $1 \mu\text{g MMC ml}^{-1}$ in mid-exponential phase (0') followed 30 min later by sample withdrawal at 0, 10, 20 and 40 min after the addition of chloramphenicol ($150 \mu\text{g ml}^{-1}$). (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.

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. 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 ($0.2 \mu\text{g ml}^{-1}$). Promoter activity was monitored as β -lactamase activity relative to the β -lactamase activity of pLexA_NTD89 before MMC induction [$0.063 \text{ units (mg dry weight)}^{-1}$]. Data are the mean $\pm$ SD of a minimum of three independent measurements.

lexA promoter activity 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).

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 α 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*

Table 2. Number of rifampicin-resistant (rif^R) 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 10⁸ viable cells. UV treatment was at 50 J m⁻².

Strain or plasmid	No. of rif ^R colonies	
	No UV treatment	UV treatment
pLexA	4	343
pLexA_NTD89	5	7
RTC3002	2	5

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 80% prophage. The latter result suggests that RecA activity is not affected by NTD89 since RecA is required for phage induction (Maiques *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

We acknowledge the Danish Council for Independent Research, Technology and Production, and the Danish Council for Independent Research, Nature and Universe, for supporting P. K. and M. T. C. We thank F. Romesberg and R. Novick for kindly supplying strains and plasmids, and C. G. Buerholt and L. S. Hansen for expert technical assistance.

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Edited by: C. W. Price