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Review

The bacterial LexA transcriptional repressor

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Abstract. Bacteria respond to DNA damage by mounting a coordinated cellular response, governed by the RecA and LexA proteins. In *Escherichia coli*, RecA stimulates cleavage of the LexA repressor, inducing more than 40 genes that comprise the SOS global regulatory network. The SOS response is widespread among bacteria and exhibits considerable variation in its composition and regulation. In some well-characterised pathogens, induction of the SOS response modulates the evolution and dissemination of drug resistance, as well as synthesis, secretion and dissemination of the virulence. In this review, we discuss the structure of LexA protein, particularly with respect to distinct conformations that enable repression of SOS genes via specific DNA binding or repressor cleavage during the response to DNA damage. These may provide new starting points in the battle against the emergence of bacterial pathogens and the spread of drug resistance among them.

Keywords. LexA, transcription factor, DNA damage, SOS response, gene expression, antibiotic resistance.

The SOS transcriptional response

Regulation of transcription initiation is a major control point in bacterial gene expression. All RNA is synthesised by a single multisubunit RNA polymerase species [1] and regulation occurs by inhibiting or stimulating the recognition of promoters by RNA polymerase, thereby affecting the principal step in transcription initiation. In prokaryotes, many different molecules can enhance or reduce the stability of initiation complexes [2].

Regulation of the expression of genetic material in response to environmental change is essential for cell survival, as is the maintenance of the structural and functional integrity of the genome. The coordinated cellular response to DNA damage was first described in detail in *Escherichia coli* [3], having been proposed in the 1970s by Miroslav Radman [4] and named the SOS response. This response requires *lexA* (locus for X-ray sensitivity A [5]) and *recA* (recombinase A). The SOS response induces the expression of a set of genes in response to DNA damage, leading to the arrest of cell division and induction of DNA repair and prophages and concomitant mutagenesis [6]. The SOS system is a programmed DNA repair regulatory network, which results in mutations and genetic exchange [7], presumably to facilitate bacterial evolution in times of stress [8]. The SOS response in *E. coli* can be induced by stalled replication forks, unrepaired defects following recombination or chromosome segregation, and DNA damage caused by metabolic intermediates in well-fed [9] or starved cells [10]. The response can be triggered by diverse

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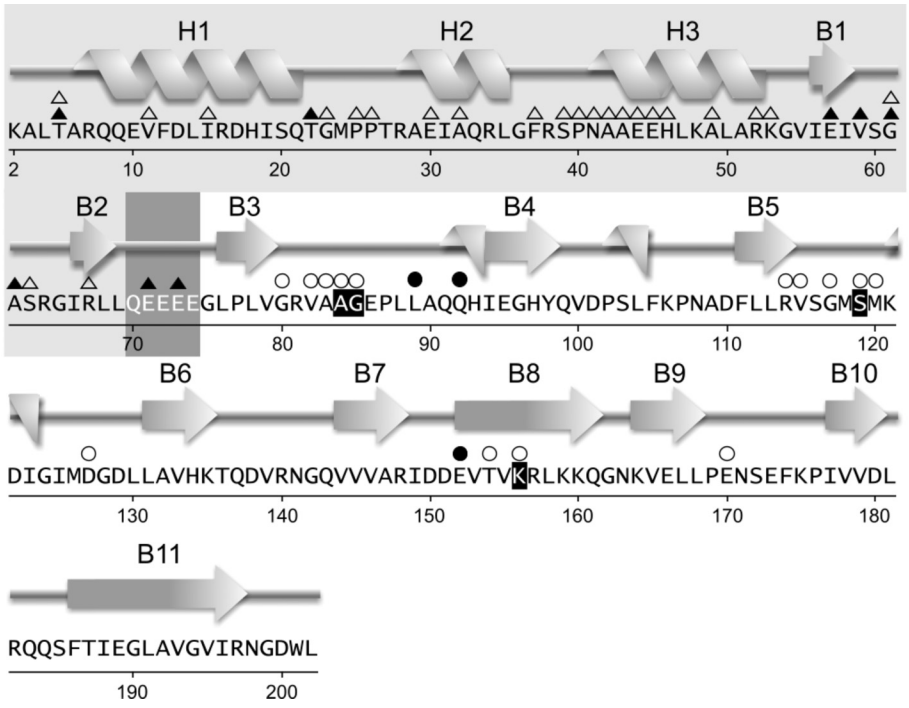

Figure 1. Sequence of LexA protein and its secondary structures. The DNA binding amino-terminal domain (NTD) (in light grey) consists of three α helices (H1 to H3) and two β strands (B1 to B2). The hinge region is shaded dark grey, followed by the carboxy terminal domain (CTD) composed of nine β strands (B3 to B11). Black shading highlights residues Ser119 and Lys156 (the catalytic dyad) and Ala84 and Gly85 (which flank the peptide bond that is cleaved by the catalytic dyad). The triangles above residues indicate positions of substitutions that enhance (solid symbols) or prevent (open symbols) DNA binding [38, 39, 41]. The circles indicate positions of substitutions in hypercleavable LexA mutants (solid symbols) or noncleavable mutants (open symbols) [36, 37, 39, 40]. The GenBank accession number is J01643.

exogenous treatments that elicit DNA damage [11, 12]. For example, physical stresses such as high pressure induce the response, by triggering the activity of Mrr, a type IV restriction endonuclease [13].

The SOS system is controlled by the interplay of two key regulatory proteins, a repressor and an inducer, which alternate between on and off states [14]. LexA protein is the repressor, which, during normal bacterial growth downregulates its own expression and, in *E. coli*, the expression of at least 43 unlinked genes [15, 16]. The RecA protein is the inducer, which, in response to DNA damage, binds to single-stranded DNA (ssDNA) to form a filament [17, 18]. The ssDNA-RecA filament interacts with LexA and activates a self-cleaving activity in LexA [14]. Upon self-cleavage (between residues Ala84 and Gly85), LexA dissociates from its DNA targets (SOS boxes), causing the induction of the SOS regulon. Subsequently, as DNA damage is repaired, the coprotease activity of the RecA filament disappears and this allows functional LexA to re-accumulate and to bind to target sites to prevent expression of the SOS genes [12].

Induction of SOS gene expression is synchronized with the DNA repair process [15, 19]. However, an additional level of regulation beyond repression by LexA exists, and the expression of SOS genes is not simply induced until DNA damage is repaired and then turned off. Thus, it was recently reported that the *E. coli* SOS network is turned on in a pattern of discrete activation pulses and the number of pulses, but not their amplitude, increases with the level of DNA damage [19]. The UmuC and UmuD proteins that are involved in translesion DNA synthesis, appear to be key factors in maintaining this pattern, by somehow modulating the activities of SOS gene promoters [19, 20]. Other proteins also play a role in regulating the SOS response. Hence, depending on the nature of the DNA damage, the RecBCD or RecFOR complexes facilitate RecA-filament assembly, and the DinI and RecX proteins respectively stabilise or destabilise active RecA filaments [17].

The importance of the SOS response is underscored by the fact that it is widespread among bacteria. However, its components, and the mode of its regulation, exhibit diversity among bacterial phyla [21]. A *lexA*-like gene is present in all the *Proteobacteria* subclasses except for *Epsilonproteobacteria* [12]. Similarly *lexA*-like genes are found in Gram-positive bacteria [12], green nonsulfur bacteria [22] and *Cyanobacteria* [23]. The LexA regulons from *E. coli* [15], *Bacillus subtilis* [24, 25], *Pseudomonas aeruginosa* [26] and *Staphylococcus aureus* [27] have been defined from genomic studies. In each case, the regulon consists of at least 15 genes including *recA*, *lexA*, and at least one error-prone DNA polymerase. In several *Pseudomonas* and *Xanthomonas* bacterial species, the *lexA* gene is duplicated [28]. The LexA1 product assures the conventional SOS response, whilst LexA2 binds to a different DNA SOS box and regulates the expression of two error-prone DNA polymerases. In *B. subtilis*, the *lexA*-like gene *dinR*

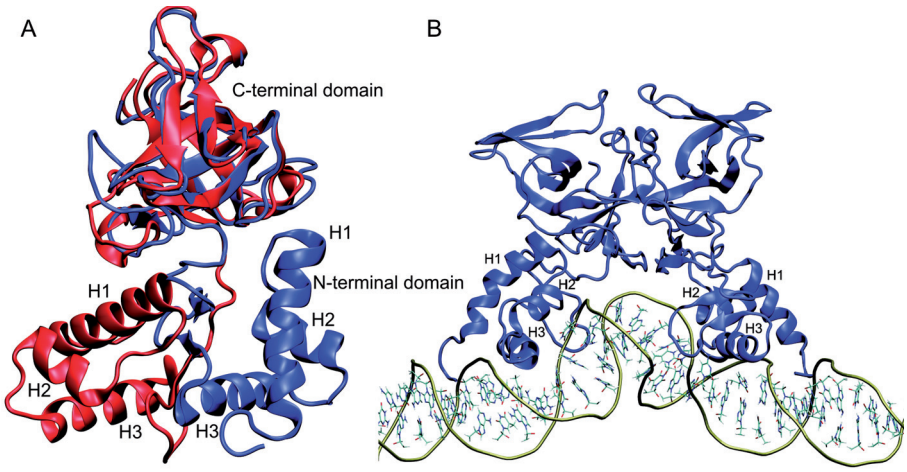

Figure 2. Predicted LexA conformational change needed for specific DNA binding and model of LexA repressor bound to target DNA. *(A)* Crystal structure of LexA monomer (pdb ID: 1jhh, chain A [37]; red) and its derived LexA monomer modelled in the bound conformation (blue) [46]. Structures are superimposed by the CTDs, the three α helices of the NTD are marked as H1 to H3 respectively. *(B)* Proposed model of the LexA repressor bound at the *E coli cka* gene regulatory region after 1.3 ns of molecular dynamics simulation [46]. The figure was generated by visual molecular dynamics (VMD) [85].

(damage-inducible gene) [29] controls a regulon of 63 genes [25]. Remarkably, only seven of these genes have homologues in *E. coli*, illustrating the diversity of SOS networks. For example, the *B. subtilis* SOS response includes a cell-wall hydrolase necessary for spore formation and dormancy [24, 30]. In all these cases, LexA acts as a transcriptional repressor, blocking access of RNA polymerase to target promoters [31, 32]. In contrast, in *Rhodobacter sphaeroides* [33] and the *Synechocystis sp.* cyanobacterium [34], the LexA paralogue can activate transcription.

Different regulatory circuits are controlled by the *lexA* products in different bacteria, but the most detailed studies have been conducted with *E. coli.* Hence, this review focuses on the *E. coli* LexA repressor. We outline how the LexA repressor discerns specific target DNA, operator sequences, and how DNA damage induces the SOS response. We discuss novel insights into the LexA biochemical processes in the context of regulation and the induction of mutagenesis in bacteria.

E. coli LexA monomer

The *E. coli lexA* gene encodes a 202 amino acid protein that folds into two structurally defined domains, which are linked by a flexible 'hinge' region [35, 37] (Fig. 1). Intact LexA dimerises by the carboxy-terminal domain (CTD) [37, 42], and binds to DNA sequences via a helix-turn-helix in its amino-terminal domain (NTD) [43].

The three-dimensional structure of the LexA-NTD has been solved by NMR spectroscopy (pdb ID: 1lea) [44] and, subsequently, the crystal structures of full-length mutant forms of LexA were reported [37]. LexA is a member of the 'winged helix' family of DNA binding proteins [45]. The NTD contains three regular α-helices, residues 6–21 (Helix I), 28–35 (Helix II) and 41–52 (Helix III), followed by two antiparallel β-strands (Fig. 1), with Helices II and III forming the helix-turn-helix DNA binding motif [44]. The NTD extends to residue Leu69 and is followed by linker residues that connect it to the CTD. In a LexA subunit, the NTD makes a small contact with the CTD, with 470 $Å^2$ of buried surface between the two domains [37].

The LexA linker region, from residues Gln70 to Glu74, is hydrophilic and is not just a simple connector, since its sequence is important for the formation of specific LexA-DNA contacts [46]. Studies of crystal structures of mutant LexA derivatives (Ser119Ala, Gly85Asp: pdb ID 1jhh and 1jhf), showed that the linker region is solvent exposed (Fig. 2). In the LexA dimer, the linker region of each subunit maintains the position of the two NTDs such that additional base-pairs between the two DNA binding motifs at an operator cannot be tolerated [35].

The LexA CTD, which is composed of β-strands, provides the determinants for oligomerisation and self-cleavage activity [37]. The key catalytic residues are Ser119 and Lys156, which act as a serine nucleophile and a general base, respectively [47]. The Ser-Lys dyad catalyzes the cleavage of the bond between residues Ala84 and Gly85. Ala84 and Gly85 are located in a loop that can switch between two conformations. In one conformation, the loop is adjacent to the catalytic dyad, whilst in the other conformation, it is further away [37]. Under physiological conditions, LexA is stable to intracellular degradation [48] but cleavage activity is triggered by the interaction of LexA with activated RecA protein [14]. At alkaline pH, self-cleavage can occur in a RecA-independent manner *in vitro* [49].

Functional repressor formation

A non-induced *E. coli* cell contains approximately 7200 molecules of RecA and 1300 molecules of LexA, with 20 % of the LexA free, and not bound to DNA. Upon formation of active RecA filaments and induction, the level of LexA falls 10-fold [50, 51]. LexA contains a single tryptophan residue at position 201, which is partially buried within the dimer interface, and, thus, pressure-dependent changes in LexA intrinsic tryptophan fluorescence can be measured. Using this, it was found that free LexA is predominantly a dimer and that monomer levels are very small [52]. This argues that LexA recognizes its targets as a dimer. Targets carry two symmetrically inverted DNA binding elements, each of which accommodates one LexA subunit [41], and binding increases LexA dimer stability by 1000-fold [52].

The determinants for LexA dimerisation fall entirely within the CTD [37, 42]. The dimerisation interface includes residues in two loops, Gln99 to Asp110 and Ser116 to Gly128, together with a portion of the β-11 strand (Fig. 2), and has a buried surface area of approximately 1380 $Å^2$ [37]. From *in vitro* studies, it was found that the formation of LexA dimers and their dissociation to monomers is a slow process, taking minutes rather than seconds [53]. However, when LexA is inactivated by self-cleavage, the C-terminal cleavage fragments dissociate faster from the heterodimer.

Higher LexA oligomeric forms

The oligomeric state of some transcriptional repressors is important for regulation of gene expression. For example, equilibrium between Lac repressor dimers and tetramers modulates repression of *lac* operon expression [54, 55]. *E. coli* LexA repressor dimer also forms higher aggregates, which affects its activity, and this is particularly important during adaptation to acid when lower pH turns on the SOS response independently of the RecA protein [56]. Presumably, this is part of a bacterial survival strategy for when the gastric acid barrier is crossed. This appears to be because, at lower pH values, LexA forms aggregates, which are less stable and less able specifically to repress transcription initiation at SOS gene target promoters. Note that intracellular acidification arrests protein synthesis [57], and thus, early phase SOS repair takes place only when cells resume metabolism and neutral cytoplasmic pH is restored.

In vitro studies show that LexA has the highest affinity for specific targets near neutral pH [58]. At pH 4.0, LexA repressor aggregates to tetramers and to larger oligomers, resulting in a drop in the concentration of dimers that can bind stably to DNA targets. At even lower pH (pH 2.5), a tetrameric state is adopted, and unfolding of the NTD DNA-binding domain causes a loss of specific DNA binding and an increase in non-specific DNA binding [56]. At pH values close to the LexA isoelectric point (6.5) and at low salt concentration, the LexA repressor precipitates from concentrated solutions and cannot be redissolved. This aggregation is due to the CTD and not the DNA-binding NTD [59].

Variable affinity for different operators

Although most of the *E. coli* SOS regulon genes possess a single LexA protein operator site [60], some carry two or three DNA sites for LexA. For example, the promoter region of the *lexA* gene itself carries separated tandem targets [31, 61], whilst promoter regions of colicin genes carry overlapping tandem DNA sites for LexA [62, 63]. The *recN* gene has three separated DNA sites for LexA [60].

The expression of LexA is autoregulated and thus it controls its own level in the cell by a feedback mechanism [64]. Autoregulation enables a rapid response of the system to even small amounts of inducing signal, and cooperative binding of LexA to the two operators makes the system very sensitive [65]. The level, timing and duration of induction of different LexA-regulated genes differs significantly [15, 19], depending on the strength of the different SOS boxes, their location relative to the target promoter and promoter strength [15, 60, 65]. Since LexA binds some operators more weakly than others, selective derepression of certain genes might occur in response to even minor endogenous DNA damage. In contrast, some genes may be expressed only upon drastic DNA damage and a persistent inducing signal. In certain conditions, where the SOS system is subinduced and the steady state level of RecA-induced LexA cleavage is counterbalanced by LexA synthesis, the physiological state of the cell is altered [66]. Differential induction of SOS genes is also affected by the internal pH, which can change the DNA binding selectivity of LexA [58]. In addition, specific cations and anions also affect recognition of specific DNA sequences by LexA and thus may play a role in fine-tuning expression of the SOS system.

Establishing specific DNA binding

It is supposed that gene regulatory proteins first bind to DNA non-specifically, mainly by electrostatic

interactions, and then 'slide', diffusing in one dimension over the DNA molecule [67]. This is an important step in the binding process, facilitating translocation of the protein to specific targets in the genome [68]. Although bacterial chromosomes are folded into a nucleoid, which might restrict transcriptional factors from reaching certain sites [69], a recent study, exploiting chromatin immunoprecipitation, showed that *E. coli* LexA can access binding targets in all parts of the genome [70].

The binding of a protein at specific DNA targets requires energetically favourable interactions with specific base pairs and this may be coupled to conformational changes in both protein and DNA [71–74]. Many transcriptional regulators switch between DNA binding and non-binding conformations and these are exploited in gene regulation. The consensus DNA target for *E. coli* LexA is CTGT-N_8-ACAG, which consists of a palindrome, known as the LexA box or SOS box [3]. This consensus is conserved in many Gram-negative bacteria, whilst in Gram-positive bacteria, the consensus is GAAC-N_4-GTTC [12], and is termed the Cheo box [75]. In *E. coli*, 30 LexA boxes have been identified, but LexA also appears to interact at 19 targets that lack a conventional site [70]. It is supposed that one or more additional factors are required for LexA binding at these targets *in vivo*, since no binding could be detected *in vitro*.

The full structure of a LexA-DNA complex has not yet been determined by either NMR or X-ray crystallography [76]. However, biochemical and biophysical methods have been used to investigate the specific contacts made by *E. coli* LexA NTD upon binding at targets [38, 39, 41, 62, 77–79] and a structural model has been generated [43] by docking the NTD NMR solution structure (average of pdb ID: 1leb) onto DNA (Fig. 2B). LexA NTD interacts with an operator half-site via a winged helix-turn-helix motif [44]. The N-terminal part of Helix III contains residues that insert into the major groove and contact specific bases, whilst the shorter Helix II lies along the major groove and makes mostly contacts with the DNA phosphate backbone [43, 76, 80]. Residues at the N-terminus of Helix I also interact with target bases, together with the loop region connecting the β-strands, which hydrogen bonds to sugars and phosphates located near the dyad axis of the operator.

E. coli LexA repressor can bind to operator half-sites, but with 1000-fold lower affinity [81]. Thus, tight binding at consensus targets with dyad symmetry requires LexA subunit-subunit interactions, which stabilize interactions with both halves of the DNA duplex [52, 82]. Models indicate that the free conformation of LexA dimer, observed in crystal structures [37], would not allow the NTD on both subunits to dock with both halves of a symmetric operator [83]. Molecular dynamic simulations of LexA repressor dimer binding to its operator using the CHARMM biomolecular simulation program [84] revealed that, for stable and specific operator binding, a reorientation of the LexA DNA binding NTD with respect to the CTD is essential (Fig. 2). This was confirmed with *in vitro* experiments that exploited cysteine cross-linking [46]. Interestingly, this reorientation appears to not to be unique to *E. coli* LexA, and a study of the *Bacillus subtilis* homologue indicated that, for specific binding, either substantial DNA bending or a conformational change in LexA must occur [86].

The symmetry of the complex between the LexA dimer and an operator appears to preclude the formation of tetramers. This is in contrast to the situation with the bacteriophage λ CI repressor, which binds to its operators on λ with an unusual overall architecture [87], resulting in asymmetry that enables the dimers to associate cooperatively.

The key step in the SOS response

The crucial point in turning on the global SOS response in *E. coli* is the inactivation of the LexA repressor, which is cleaved between Ala84 and Gly85. This cleavage is induced by LexA binding within the deep helical groove of active RecA filaments that form on single-stranded DNA at sites of DNA damage [88, 89]. Thus, by acting as a co-protease, RecA inactivates the repressor of its own gene, thereby inducing its expression together with more than 40 other SOS genes [15]. LexA cleavage can proceed spontaneously, independently of RecA at alkaline pH, but this does not appear to occur *in vivo* [51, 90].

Crystal structures of several LexA mutants revealed that the cleavage site can adopt two conformations, which appear to correspond to cleavable and non-cleavable states (Fig. 3A). In one state, the cleavage site is located adjacent to the Ser119-Lys156 dyad, within the catalytic centre binding pocket, whilst, in the other state, it is ~20 Å away from the active site [37]. The catalytic centre binding pocket is an extended hydrophobic cleft with the catalytic dyad residues at the end. The structures show that the cleavage site region can enter the binding pocket and that it forms a long, twisted hairpin that lies in the cleft. Its conformation is stabilized by series of parallel β sheet hydrogen bond interactions between B3 and B5, B8 of the catalytic core. This enables the peptide bond between Ala84 and Gly85 to be presented to the catalytic Ser119, with the unprotonated form of Lys156 [91] activating the nucleophilic activity of

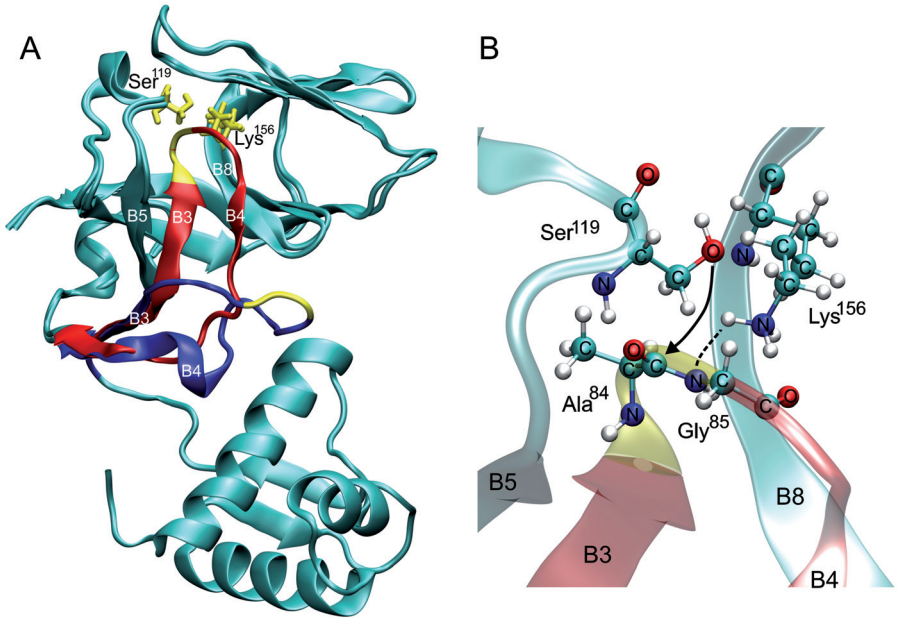

Figure 3. Two distinct conformations of the LexA cleavage site region and a closer view of the active site. *(A)* Crystal structure of LexA monomer in the noncleavable state (pdb ID: 1jhh, chain A [37]; cleavage site region in blue) superimposed on the CTD LexA structure in the cleavable state (pdb ID: 1jhe, chain A [37]; cleavage site region in red). The catalytic residues Ser119 and Lys156 are presented as a stick model and cleavage site Ala84 and Gly85 as a ribbon representation in yellow. *(B)* Proposed LexA self-cleavage mechanism in which Ser119 is activated by a neutral general base Lys156 [14]. The hydroxyl group of activated Ser119 is the nucleophile that attacks the carbonyl carbon of the scissile peptide bond (arrow), followed by the transfer of a proton to the newly generated amino group (dotted line). In pdb ID: 1jhh, Ala119 was modified to Ser119 and in pdb ID: 1jhe, Ala156 was modified to Lys156 as in the wild-type. Missing hydrogen atoms in pdb ID: 1jhe were added with the HBUILD tool from CHARMM [84]. The figure was generated by VMD [85].

Ser119 [92, 93]. This results in self-cleavage (Fig. 3B) [49] and separation of the DNA binding NTD from the CTD, and a 10-1000-fold weakening of DNA binding [81, 94, 95]. The affinity of truncated LexA1–84 for targets is similar to that of full-length repressor for operator half-sites.

The self-cleavage also triggers LexA degradation. After induction, otherwise dormant protease recognition signals are exposed in the cleaved LexA1–84 and C-terminal LexA85–202 fragments, resulting in degradation by the ClpXP protease [48]. This process is important, since accumulation of the LexA DNA-binding NTD, which retains some repressor function, might be deleterious after DNA damage [35, 81]. Thus, in most conditions, N- and C-terminal cleavage products are degraded rapidly with half-lives of approximately 4 minutes and 1 minute respectively [48]. Degradation of cleaved CTD is facilitated by Lon protease [66].

Structured conformations affect RecA-mediated cleavage

For unbound LexA, the conformational equilibrium strongly favours the noncleavable state. Hence, cleavage is extremely slow, but it increases when LexA binds to an activated RecA filament [96]. During normal growth, RecA activity is crucial in dealing with inactivated replication forks, but this is insufficient for substantial induction of the LexA regulon. However, after an inducing treatment, LexA is degraded within a few minutes and the self-cleavage rate increases by approximately 50-fold [66]. It has been suggested that interaction with RecA induces a conformational change in LexA and deprotonation of Lys156 [96]. The interaction accounts for the energetic cost of burying the terminal amino group of Lys156 and this controls the self-cleavage reaction [37]. It was also suggested that RecA may preferentially interact with and stabilize the LexA cleavable state [37], but recently it has been suggested that active RecA filaments can bind to LexA in both states [53]. RecA-induced self-cleavage of LexA is more rapid when LexA is dimeric, and it is hypothesized that the CTD in one subunit determines the conformation of other subunit's cleavage site region (Fig. 4). Thus, subunit-subunit interactions appear to drive one of the two subunits into the cleavable form [53].

The consequences of the fact that cleavage of each LexA dimer takes place while it is bound to specific DNA targets are not understood. For example, the

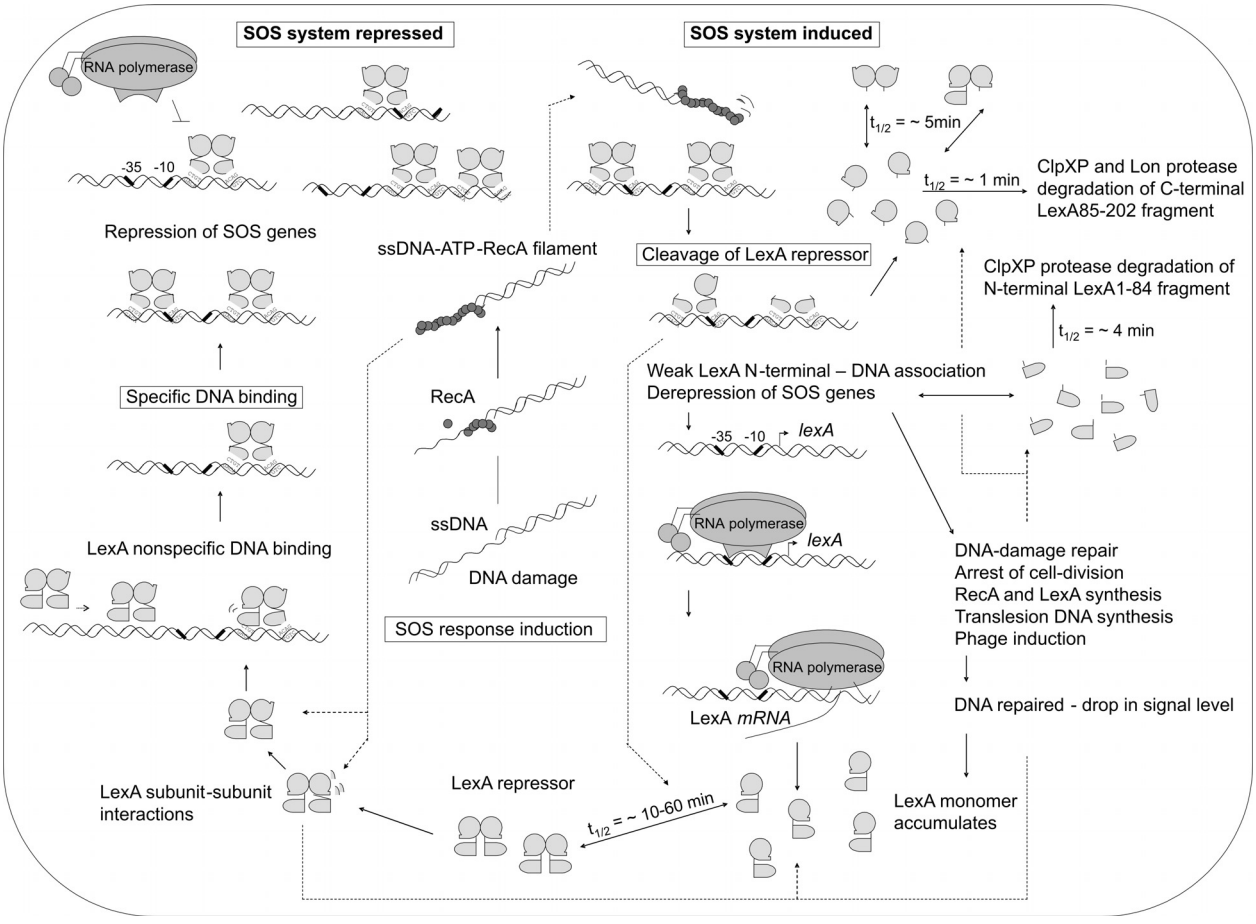

Figure 4. Model of regulation of the SOS response in *E. coli.* LexA repressor bound to operators of SOS genes hinders their transcription. Increased levels of DNA damage cause the formation of RecA filaments that induce the self-cleavage of LexA, thereby de-repressing the system. When DNA damage is repaired, SOS induction is reversed.

proposed re-orientation of the DNA binding NTD with respect to the CTD in one subunit may favour the adoption of the catalytically proficient conformation in the other subunit. This suggestion is based on the crystal structure of Ser119Ala mutant LexA dimer [37], where one subunit is well-ordered throughout and in the non-cleavable state, whereas the second subunit, whilst disordered in the NTD, adopts the cleavable state in the CTD.

Other LexA-type self cleaving domains

The LexA CTD shows extensive homology with the CTDs of several CI repressors from temperate bacteriophages, and with the UmuD SOS response protein [37, 97, 98]. The CTD homology is linked to the common property of these proteins to interact with RecA-ssDNA-ATP filaments, which induces self-cleavage. The differences between the non-cleavable and cleavable states of CI repressor had been thought to be less extensive than for LexA [99]. However this is contradicted by more recent crystal structures that show that the two states of bacteriophage λ CI resemble those of LexA [87].

Members of the LexA super-family have a structurally conserved catalytic core that performs the cleavage reaction via a conserved serine-lysine dyad [37, 99]. The dimer interfaces of LexA, bacteriophage λ CI and UmuD are similar (note that dimeric UmuD is converted to functionally active UmuD' by RecA-facilitated self-cleavage that is analogous to the inactivation of the LexA) [99–101]. Remarkably, the cleavage of UmuD appears to occur intermolecularly [102], in contrast to the intramolecular self-cleavage of LexA. The cleavage of UmuD, CI repressor and LexA takes place at different speeds. Thus, RecA mediates slow self-cleavage of CI repressor [103] and UmuD [104] compared to LexA and, hence, prophage induction and mutagenesis are triggered only when cells are severely damaged and may not survive [103]. These rate differences set the threshold of DNA damage tolerance below which the DNA damage is repaired

without phage induction. Below this threshold, DNA damage can be repaired and the system reset, whilst above the threshold, induction of bacteriophage lysis is irreversible. Similarly, in some strains, higher levels of DNA damage induce the synthesis of bacteriocins. These are released into the environment only after the production of a protein that causes lysis and death of the host cell [63]. SOS-induced production of bacteriocins thus resembles bacteriophage gene induction [105].

Variety in SOS induction and the LexA regulon

Many different factors can trigger induction of the SOS response. Thus, when strains of *E. coli* pass into warm-blooded animals, they encounter many factors, for example acidic pH, that can induce the SOS response [56]. Another example is the production of antimicrobial molecules such as hydrogen peroxide by neutrophils that result in DNA damage and contribute to pathogenesis, for example in enterohemorrhagic *E. coli* [106]. In addition, nitric oxide has been shown to induce SOS response [107]. Salmonella encounters DNA damaging nitric oxide inside macrophages, and in the gallbladder, its niche for chronic infections, bile is proposed to induce the SOS response, which is also the case for *E. coli* [108, 109].

The discovery that LexA directly regulates the expression of different colicins clearly shows that members of the LexA regulon are not solely concerned with the upkeep of the genome [110, 111]. For example, the SOS response also affects virulence factor synthesis in *Staphylococcus aureus* [112] and type III secretion in enteropathogenic *E. coli* [113]. Interestingly, colicin production by *E. coli* populations in the mammalian colon [114] has the potential to promote microbial diversity [115].

The SOS system can drive evolution

Induction of the *E. coli* SOS regulon involves three DNA polymerases, PolII (*polB*), PolIV (*dinB*) and PolV (*umuC*, *umuD*), that operate in a poorly processive and error-prone manner, permitting 'irreparable' DNA lesions that block replication to be repaired [116, 117]. As well as orchestrating repair of DNA damage, these polymerases enable bacteria to increase their mutation rate [3]. Remarkably, in *Mycobacterium tuberculosis*, an error-prone α subunit of DNA-polymerase III (*dnaE2*) was found to be required for persistence during infection and for the development of antibiotic resistance [118]. Note that the *dnaE2* gene is located on a DNA damage-inducible cassette that is also widely distributed among *Proteobacteria* [12].

Sub-lethal doses of some commonly used antibiotics induce the SOS response and the synthesis of error-prone DNA polymerases. These include ribonucleotide reductase inhibitors that arrest DNA replication, such as trimetoprim [119], DNA topoisomerase inhibitors, such as ciprofloxacin [120], RNA polymerase inhibitors, such as rifampicin [121], and, surprisingly, cell wall inhibitors such as β-lactams [122]. The effects of β-lactams are mediated by the DpiBA two-component system which, upon activation, causes interruption of DNA replication, thereby triggering RecA-mediated LexA cleavage. Hence, antibiotics can speed up mutagenesis, which accelerates evolution, for example by the acquisition of point mutations that result in the drug's inactivation or efflux.

SOS-inducing antibiotics can also trigger the self-catalytic cleavage of phage repressors, leading to the horizontal spread of temperate phage and associated pathogenicity islands. One example of this is the dissemination of genes encoding staphylococcal virulence factors [123]. Another important example is the lateral transfer in *Vibrio cholerae* of the filamentous bacteriophage CTXΦ, encoding cholera toxin [124], and the self-transmissible integrating conjugative element SXT that harbours antibiotic resistance genes [125]. In some cases, induction results in the expression of toxins, for example, the prophage-encoded *E. coli* Shiga toxin [126].

A frightening consequence of the ability of antibiotics to induce the SOS response and accelerate the spread of mobile DNA elements arises from the fact that some mobile elements are composed of cassettes encoding multiple antibiotic resistance determinants. Thus, because the SOS response does not discriminate between the coding regions that are being mobilised, one antibiotic can induce the spread of resistance to several other completely unrelated antibiotics and thus promote cross-resistance. The conclusion from these observations is that antibiotic therapy can be counteracted at many levels by the SOS response. A further simple example of this is found in *E. coli*, where the SOS response gene *sulA* inhibits septum formation and delays cell division until DNA damage has been repaired, thereby temporarily nullifying effects of antibiotics that interfere with cell wall synthesis [122].

Conclusions

The LexA repressor plays a key role in the induction of the SOS response and its importance in regulating responses to stress suggests that it should be exploited

as a drug target. Recent progress in understanding the molecular details of specific LexA binding at targets and how it is cleaved, together with genomic information on the LexA regulon in different organisms, now make this a possibility. Clearly, intervention at this level could assist in the battle against the evolution of antibiotic resistance, and prolong the efficacy of existing therapeutic antibiotics. For us to 'Save Our Souls', prudent solutions need to be found to inhibit the SOS response of bacterial pathogens.

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