Chapter

The *Escherichia coli* SOS Response: Much More than DNA Damage Repair

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Abstract

The *Escherichia coli* SOS response is an inducible DNA damage repair pathway controlled by two key regulators, LexA, a repressor and RecA, an inducer. Upon DNA damage RecA is activated and stimulates self cleavage of LexA, leading to, in *E. coli,* derepresion of approximately 50 SOS genes. The response is triggered by exogenous and endogenous signals that bacteria encounter at a number of sites within the host. Nevertheless, besides regulating DNA damage repair the SOS response plays a much broader role. Thus, SOS error prone polymerases promote elevated mutation rates significant for genetic adaptation and diversity, including antibiotic resistance. Here we review the *E. coli* SOS response in relation to recalcitrance to antimicrobials, including persister and biofilm formation, horizontal gene tranfer, gene mobility, bacterial pathogenicity, as well SOS induced bacteriocins that drive diversification. Phenotypic heterogeneity in expression of the SOS regulator genes, *recA* and *lexA* as well as colicin activity genes is also discussed.

Keywords: SOS response, *Escherichia coli*, DNA damage, antibiotic resistance, persisters, horizontal gene transfer, virulence, biofilms, bacteriocins, phentypic heterogeneity

1. Introduction

Bacteria are constantly exposed to a changing and stressful environment. Coordinated responses by bacterial global regulatory systems enable their survival and adaptation [1].

In all organisms genome integrity is constantly threatened by endogenous and exogenous agents. Exogenous DNA damaging agents are physical (UV and ionizing irradiation, oxidants, drugs) and chemical (oxidizing, crosslinking, alkylating). Endogenous triggers are the result of cellular metabolism such as reactive oxygen species (ROS), stalled replication forks and defects following recombination or chromosome segregation [2, 3]. To cope with DNA damage organisms possess a number of error free and error prone mechanisms [4, 5]. Most bacteria seem to have evolved a coordinated response to DNA damage. In *Escherichia coli* the inducible DNA repair pathway is designated the SOS response and is controlled by two regulators, LexA, a repressor and RecA, an inducer. DNA damage generates an increase in single stranded DNA (ssDNA) as DNA polymerase stalls at a lesion while helicase continues unwinding DNA. RecA is activated (RecA*) by binding to single

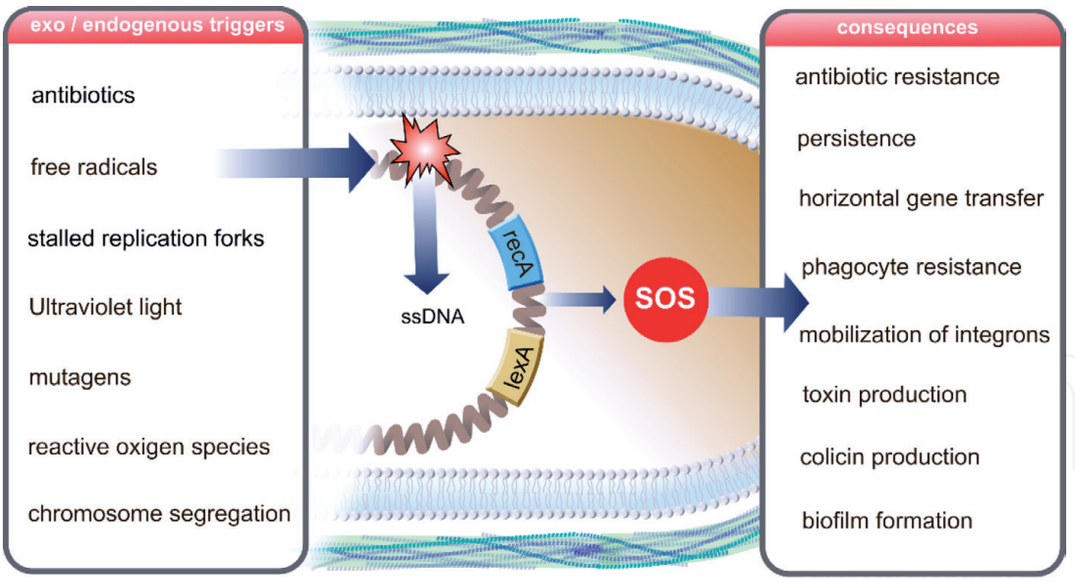

Figure 1.
Exo and endogenous triggers induce the E. coli SOS response leading to antibiotic resistance, persistence, horizontal gene transfer, expression of virulence factors, intraspecies competition and biofilms.

stranded DNA forming a nucleoprotein filament that stimulates self cleavage of LexA and in *E. coli* de-repression of more than 50 SOS genes. A hallmark of the SOS response is its temporal control. High-fidelity repair mechanisms are induced first followed by low fidelity, damage tolerance pathways involving error prone translesion DNA polymerases PolII (*polB*), PolIV (*dinB*) and PolV (*umuC, umuD*). These are active only following extensive and persistent DNA damage. While the error prone/last resort polymerases enable repair of lesions that block DNA replication by the primary replicative DNA polymerase PolIII [4, 5], they also promote an increase in mutation rate.

Even though RecA and LexA are the key SOS regulators, induction/SOS factors may also be governed by other stress response pathways namely, alternative sigma factors RpoS and RpoH of the general stress responses, the stringent response, cAMP and reactive oxgen species (ROS) [6–10].

Whilst the SOS response was initially recognized as regulating DNA damage repair, it is now well established that it plays' a much broader role. Thus, SOS error prone polymerases by promoting elevated mutation rates generate genetic diversity and adaptation. The SOS response is also involved in horizontal gene transfer, virulence factor expression, biofilms, persistence, sustained colonization of the mammalian gut, controls toxin-antitoxin systems as well as intraspecies competition and phenotypic variation (**Figure 1**) [5, 11, 12].

2. Mutagenesis and antibiotic resistance

Antibiotic resistance is one of the most serious global health threats. Resistance occurs by mutation of resident genes or/and by uptake of resistance genes. Antibiotic resistance mechanisms belong to one of several classes with resistance genes encoded on chromosomes and mobile genetic elements such as plasmids, transposons and integrons. As stated above, the error prone translesion DNA polymerases PolII, PolIV and PolV allow translesion DNA replication but also promote an increased level of mutation, significant for evolution of antibiotic resistance.

Exposure of bacteria to antibiotics, even at subinhibitory concentrations, has been shown to increase mutation and recombination frequencies via the SOS

response [13, 14]. In *E. coli* as well as a number of other clinically significant bacterial species, some of the most common antibiotics have been shown to induce the SOS response and mutagenesis [15, 16]. Exposure of environmental bacteria to antibiotics, even subinhibitory levels, could thus generate variants with higher rates of genetic modifications and select for resistance.

2.1 Persisters

In addition to antibiotic resistance other mechanisms allow bacterial growth in the presence of antibiotics; (i) population wide tolerance, (ii) persisters, subpopulations characterized by a transient dormant state and transient tolerance [17] and (iii) shielding that protects and enables survival in the presence of antibiotics [18].

Persisters and antimicrobial tolerance have been extensively studied in *E. coli*. One of the first and most thoroughly investigated examples of persister cell formation involving the SOS system, is activation via the toxin-antitoxin TisB/IstR module. TisB is a small membrane-acting peptide that decreases the proton motive force and ATP levels, shutting down cell metabolism and inducing dormancy [19]. The *tisB* gene is repressed by the SOS repressor LexA, while the IstR-1 antitoxin is constitutively expressed. Following DNA damage and SOS induction, *tisB* transcription strongly increases and exceeds that of the antitoxin IstR-1 [20].

Nevertheless, in *E. coli,* the SOS response in persisters also accelerates antibiotic resistance [21, 22]. Thus, from fluoroquinolone (FQ) persisters, the SOS response promotes resistance to unrelated antibiotics following a single FQ exposure [23].

Recently, sub-inhibitory concentrations of ciprofloxacin were shown to, in *E. coli*, induce transient differentiation of a small gambler subpopulation that, generates cross-resistant mutants. Gamblers are characterized by high levels of ROS and a σ^S general stress-response. In gamblers, ROS activate the σ^S response, which allows mutagenic repair of antibiotic-triggered DNA double strand breaks. Further required is SOS induced inhibition of cell division, provoking the presence of multiple chromosomes. Thus, in gamblers, a highly regulated, transient differentiation process with within-cell chromosome cooperation drives evolution of resistance to new antibiotics [24].

3. Mobile genetic elements

Horizontal gene transfer is a significant driving force of bacterial genome evolution, including the emergence and dissemination of antibiotic resistance and virulence genes. The SOS response has been shown to play an important role in gene transfer in a number of bacterial species.

One of the first reports of the involvement of the SOS response in horizontal gene transfer was SOS induction of transfer of antibiotic resistances encoded by the *Vibrio cholerae* integrating conjugative element, SXT [13]. The SOS response has also been shown to induce lambdoid prophages due to SOS induced self cleavage of the CI phage repressor [25].

In turn, as conjugative plasmid DNA transfer and transformation, involve uptake of ssDNA, these mechanisms induce the SOS response [26, 27].

An important class of SOS controlled mobile genetic elements are the wide spread integrons. They are associated particularly with transposons and conjugative plasmids and have played an important role in the evolution of antibiotic resistance among pathogenic bacteria [28]. Integrons encode a site specific recombination system that promotes integration and expression of gene cassettes with antibiotic resistance and metabolism associated functions.

On the basis of integrase sequences, five classes of integrons are distinguished. Class 1 integrons are by far the most prevalent and clinically relevant. Recently, the class 1 integrons were found in a considerable fraction of *E. coli* isolates [29].

Integrons are composed of an *int*I gene encoding an integrase, followed by a recombination site, attI and a variable array of gene cassettes each ending in a recombination attC site [30]. Integron cassette expression is driven by the Pc promoter situated upstream of the array. Cassettes closest to the promoter are expressed at highest levels [31]. The integron integrases are frequently controlled by the LexA protein [11].

Integrons enable bacteria to evolve in response to new antibiotic challenges via rapid optimization of cassette expression. Activity of the integrase allows: (i) modulation of cassette expression, (ii) rapid gain of additional copies of selected cassettes and (iii) elimination of redundant cassettes. Integrase activity does not compromise genome integrity due to the high specificity of integrase-mediated recombination [32–34]. Thus, integrase-mediated cassette re-shuffling in stressful environments could accelerate bacterial evolution allowing bacteria to optimize cassette expression and maximize fitness. Relavant cassettes could be positioned near the Pc promoter for maximal expression, while unnecessary cassettes could be kept at the end of the array and be moved forward when required [33].

DNA acquired by HGT, including pathogenicity islands (PAIs) with virulence factor genes, must either replicate autonomously or be integrated into the bacterial chromose or plasmid. Integration is mediated by recombinases/integrases that are often encoded on PAIs and perform either integration or excision from the chromosome. It was recently shown that SOS inducing antibiotics, including clinically relevant for treatment of UTI, led to in a subpopulation, increased promoter activity as well as increased loss of PAIs [35].

4. *E. coli* virulence

The species *E. coli* encompasses commensals of the gut, pathogens and probiotics. Conditions conducive to SOS induction are encountered by *E. coli* at various host anatomical sites. Recently, the SOS response has been shown to play a vital role in maintaining colonization of the murine gut by commensal *E. coli*. Competing commensal organisms could be a source of genotoxic stress [12].

Pathogenic *E. coli* strains producing virulence factors such as adhesins, iron uptake systems, capsules, toxins and invasins, can provoke infections [36]. Pathogenic strains are broadly classified into two major groups, with regard to their virulence factors and diseases they provoke, the nondiarrheagenic and diarrheagenic. The nondiarrheagenic are designated extraintestinal pathogenic *E. coli* (ExPEC) while diarrheagenic provoke diarrhea and include the Shiga toxin (Stx) producing *E. coli* (STEC) as well as enterohemorrhagic *E. coli* (EHEC) [37].

Among ExPEC infections, the most common are urinary tract infections (UTI) followed by septicaemia and meningitis [36, 38].

UTIs represent a serious worldwide health problem [39] with uropathogenic *E. coli* strains (UPEC) responsible for 75–95% of community-acquired UTIs [40].

To provoke UTI, UPEC undergo a complex intracellular cycle [41] and the SOS response plays an important role in bacterial dissemination and persistence within the urinary tract. UPEC enter the urinary tract through an ascending route and travel up the urethra to colonize the bladder via internalization by the umbrella cells. Infected cells produce nitric oxide that attacks bacterial DNA, inducing the SOS response with inhibition of cell division and UPEC filamentation. Filamentous UPEC successfully resist phagocyte killing, allowing dissemination and persistence

within the urinary tract. UTI frequently lead to chronic infection and a persister subpopulation could be responsible for generating relapsing infections [42].

In the intestinal tract, DNA damage and subsequent SOS induction, can be provoked by host factors, e.g. bile salts, and by competing microbes. Intestinal inflammation triggered by infection or the gut immune system involving ROS, also induces the SOS response.

All EHEC strains, including the notorius serotype O157;H7, produce Stx the main virulence factor associated with hemorrhagic colitis [37].

Production of Stx, by O157:H7 is mediated by quorum sensing [43] however, it is also well documented that the SOS response amplifies Shiga toxin production in enterohemorrhagic *E. coli* (EHEC). Stx is encoded on a lambdoid prophage. Induction of the prophage, via repressor autocleavage, and the subsequent upregulation of *stx* expression are controlled by the SOS response [25]. Therefore, DNA-damaging agents, including certain antibiotics, increase Stx synthesis and are counterindicated during treatment of infection [44]. In addition to SOS inducing antibiotics, bacteriocins and microcins secreted by members of the gut microbiota have been shown to amplify Stx synthesis. In the complex intestinal environment, survival involves competition for space and nutrients [45, 46]. Bacteria have therefore evolved mechanisms to counteract competitors [47] such as, production of bacteriocins, proteinaceous toxins, that inhibit growth and survival of usually closely related bacteria competing for similar resources [48]. A subtype of bacteriocins, known as colicins, are produced by *Enterobacteriaceae* while microcins are bacteriocins that are generally smaller than 10 kDa [49].

Thus, a strain producing the nuclease colicin E9 (ColE9) as well as extracted DNase colicins were shown to induce Stx [50]. Recently, microcin B17 (MccB17), a DNA gyrase inhibitor, as well as a putative microcin, were also shown to amplify Stx2a production [51, 52]. Thus in the gut, nonpathogenic *E. coli* strains could, via secretion of DNA damaging colicins and microcins, increase Stx production by O157:H7.

In addition to DNA damage induced by host factors, e.g. bile salts, and by competing microbes, intestinal inflammation triggered by infection or the gut immune system involving ROS, also provokes the SOS response and dysbiosis, suppressing anaerobes and inciting *Enterobacteriaceae* overgrowth with competition for nutrients [53].

5. Biofilms

Biofilms are surface attached structured bacterial communities that create a protective environment for bacterial cells [54]. Biofilm formation is a highly regulated process and is controlled by a number of environmental and genetic factors [55–57]. Biofilms are also induced by antimicrobial stress/SOS response. While biofilm formation is an integral part of the prokaryotic life cycle, biofilms also cause biofilm associated diseases that are difficult to treat, e.g. urinary tract infections (UTI), chronic infections in cystic fibrosis (CF) patients, colonization of medical devices and periodontal diseases [58].

A number of factors allow bacteria in biofilms to survive high dose antibiotic treatment [58, 59]. Antibiotic diffusion is prevented by a mechanical barrier formed by the extracellular matrix. Further, low oxygen and nutrient concentrations within biofilms create niches with low bacterial metabolic activity. In addtion, up to 1% of bacterial cells in biofilms may be dormant persister cells not affected by antimicrobials [60]. Furthermore, high cell density within biofilms enhances horizontal gene transfer and competition, that together with accumulation of metabolic products,

microaerobic areas and oxidative stress, incite DNA damage and provoke the SOS response. Starvation stress in biofilm bacteria was shown to increase the level of tolerance to the fluoroquinolone ofloxacin in *E. coli* biofilms and was dependent on the presence of a functional bacterial SOS response [59].

In biofilms, phenotypic variants e.g. small colony variants (SCV), that are slow growing and very tolerant to host defenses and antimicrobials have also been described. SCVs exhibit increased production of exopolysaccharides, can autoaggregate and attach stronly to surfaces [61–63]. They are potentially responsible for difficult to treat persistent infections, wherein bacteria persist in the host for prolonged periods of time despite antimicrobial therapy. Thus, recalcitrance of biofilms to antimicrobials can be due to tolerance, when dispersed biofilm cells exhibit antibiotic sensitivity and low MIC, as well as resistance, characterized by increased MICs and a resistant phenotype of dispersed biofilm bacteria. The SOS response plays a significant role in biofilm formation but in turn, in the dynamic biofilm environment, SOS inducing factors are generated that promote mutagenesis and diversification.

6. Bacteriocins and phenotypic heterogeneity

Colicins are bacteriocins, toxic proteins that are produced by and act against *E. coli* and its close relatives. Sensitive cells are killed by targeting DNA, RNA, cell membranes or by inhibition of peptidoglycan and lipopolysaccharide (LPS) O-antigen. Colicin genes are found within genomic clusters on colicinogenic plasmids. These clusters typically contain the colicin activity gene for the toxin, an immunity gene for a protein that confers self-resistance by binding to and inactivating the toxin protein, and a lysis gene for a protein that aids in colicin release by lysis of the producer cell [64]. Colicin production is found with high frequency among natural *E. coli* isolates [65].

Colicins are expressed from strong promoters whose activity is tightly repressed by the LexA protein. Nutrient limitation and DNA damage are major signals that control colicin production [64, 66]. Nevertheless, additional regulators, in conjunction with LexA, have been found to regulate/delay colicin expression. Thus, the global transcriptional factor, IscR, in response to the nutritional status of the cell and, co-dependently with LexA, delays induction of pore-forming colicin genes following SOS induction [67]. On the other hand, temporal induction of DNA and RNA targeting colicins is co-regulated by the AsnC repressor. At the colicin E8, *cea8* promoter, AsnC repression reflects L-asparagine levels and presumably serves as an indicator of general amino acid abundance and availability [68]. Thus, promoters of nuclease and pore-forming colicins have adopted different transcription regulators and specific metabolic inputs to regulate transcription in conjunction with the LexA repressor.

Colicins have an *in vivo* antagonistic role promoting microbial diversity within *E. coli* populations in the mammalian colon [69] and the potential to promote microbial genetic diversity [70]. Sublethal concentrations of ciprofloxacin have been shown to induce colicin expression in an SOS-dependent manner and imply that SOS-inducing antibiotics could thus affect microbial strain diversification, as well as promote the acquisition and dissemination of antibiotic resistance [71].

Furthermore, monitoring the transcriptional response of *E. coli* to colicins E9, an endonuclease, and E3, an RNase, has shown that the former induces the SOS response while the latter upregulates expression of DNA integrases, invertases, and recombinases. Colicins thus also have the potential to, through the induction of error-prone DNA polymerases, promote microbial diversity, gene transfer, DNA

rearrangements affect horizontal gene transfer as well expression of virulence factor genes.

Colicin production has also been found to be a specialized function within a population of genetically identical cells, an example of phenotypic heterogeneity. The colicin K activity gene was shown to be expressed in only a small fraction of a population, while the immunity gene is expressed in the large majority of the cells [72]. A number of colicins are released semispecifically, by cell lysis. Differential expression of the activity and lysis genes prevents excessive lysis. Alternatively, upon DNA damage and induction of the SOS response, all cells express the activity gene. Lysis of the producer releasing colicin as well as lysis of the sensitive target cell, provides material for bacterial shielding or biofilm matrix as well as resources for growth for nonexpressing insensitive cells. A recent study showed ampicillin induced bacterial cell lysis provides a matrix of cell debris that shields viable cells from antimicrobial activity [18]. Further, lysed cells release molecules that could sequester antibiotics.

Subsequently, additional LexA regulated genes, including *lexA* and recA, were also shown to exhibit phenotypic heterogeneity with high level expression, in the absence of DNA damaging agents, in a small subpopulation of cells [73]. Heterogenous expression was found to be established primarily by stochastic factors and the binding affinity of LexA to SOS boxes. Heterogenous expression of *recA* and *lexA* genes could affect a number of phenomenon e. g., subpopulations with higher proficiency in recombination, antibiotic tolerance/persistence, horizontal gene transfer, prophage induction and virulence among pathogenic *E. coli* strains.

7. Conclusions

Given the mounting threat posed by antibiotic resistance, a better understanding of the mechanisms bacteria employ to evolve resistance, persistence as well as pathogenesis is urgently needed. Conditions conducive to SOS induction are encountered by *E. coli* at various host anatomical sites and drive bacterial adaptation to stress, including antibiotic resistance and amplified toxin production. Numerous interdependent mechanisms involving the SOS response are evident, including amplification of the inducing signal in the bacterial population, e.g. (i) SOS induction of horizontal gene transfer which in turn, via ssDNA transfer, induces the SOS response in recipients, (ii) promotion of biofilm formation that generates a dynamic environment with DNA damaging agents and high cell density, conducive to HGT, all in turn inducing the SOS response, (iii) induction of bacteriocins targeting DNA which induce the SOS response in sensitve cells. Nevertheless, our understnding of the modes and the levels of the SOS response, including its connections with other stress response pathways is still lacking. Novel antimicrobial treatment approaches should seek to target the SOS response, possibly the inducer RecA.

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Conflict of interest

The authors declare no conflict of interest.

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