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SOS, the formidable strategy of bacteria against aggressions

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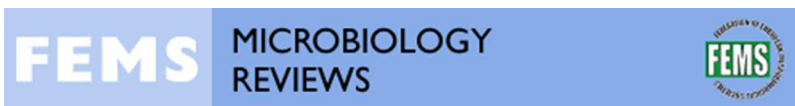
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SOS, the formidable strategy of bacteria against aggressions.

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Review

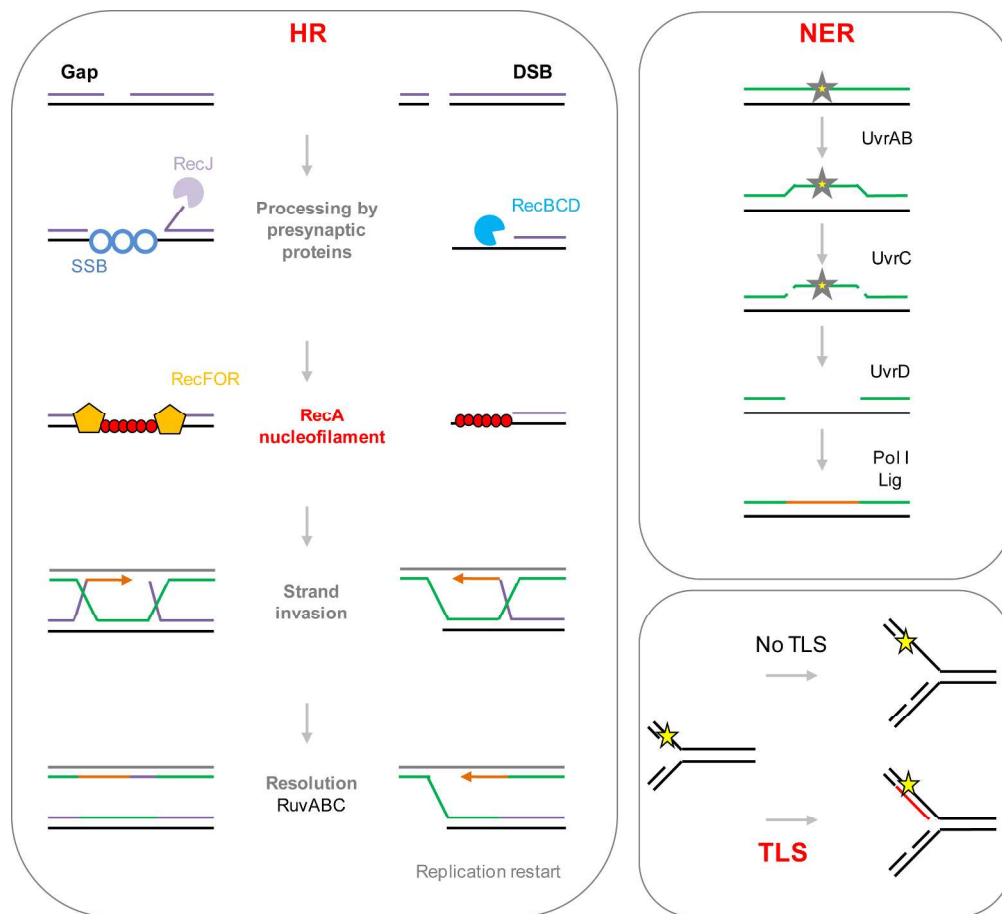


Figure 1. Simplified representations of DNA repair pathways induced by SOS (described in the introduction section). HR, NER and TLS are induced during the SOS response. HR: Homologous Recombination. Single strand nicks (gaps) are transformed into larger gaps by the RecJ exonuclease. Gaps are recognized by RecFOR presynaptic proteins. Double strand breaks (DSB) are recognized by the presynaptic RecBCD exonuclease/helicase complex. RecFOR/RecBCD recruit RecA to initiate homologous recombination through strand invasion with the sister chromosome, usually resulting in mutation free repair. DSB repair results in the formation of a replication fork. NER: Nucleotide Excision Repair. The UvrAB complex recognizes the DNA lesion; UvrC proceeds with a double incision on both sides of the lesion and the ssDNA carrying the lesion is removed by the UvrD helicase. DNA polymerase I (Pol I) resynthesizes the missing DNA and the ligase ligates the newly synthesized DNA to the template, usually resulting in mutation free repair. If the SOS inducing signal persists, translesion synthesis (TLS) polymerases are induced. In the absence of TLS, the replicative DNA polymerase (Pol III) cannot replicate damaged DNA, leading to fork stalling. TLS polymerases (Pol IV, Pol V) can replicate damaged DNA in a mutagenic manner. Yellow stars represent DNA lesions.

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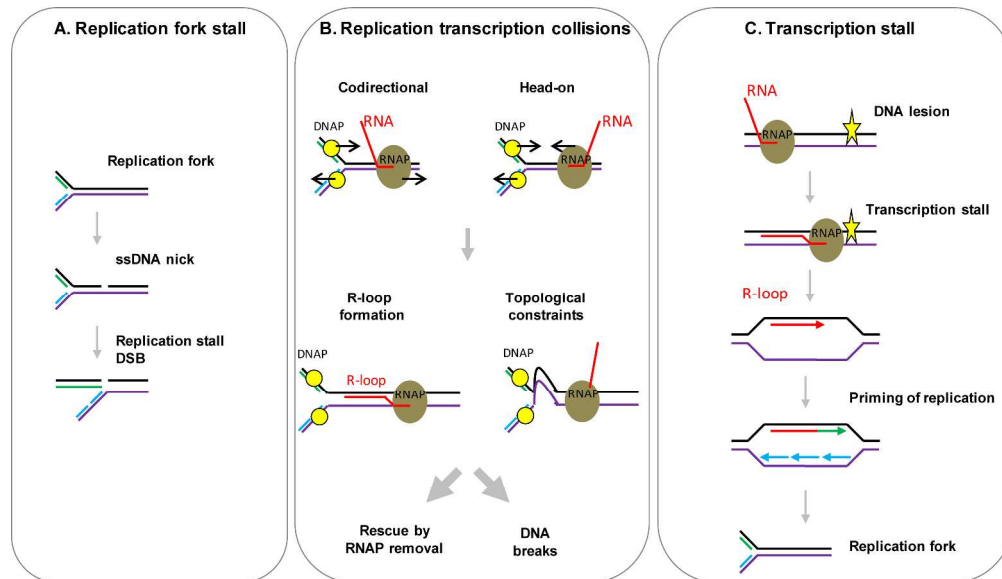
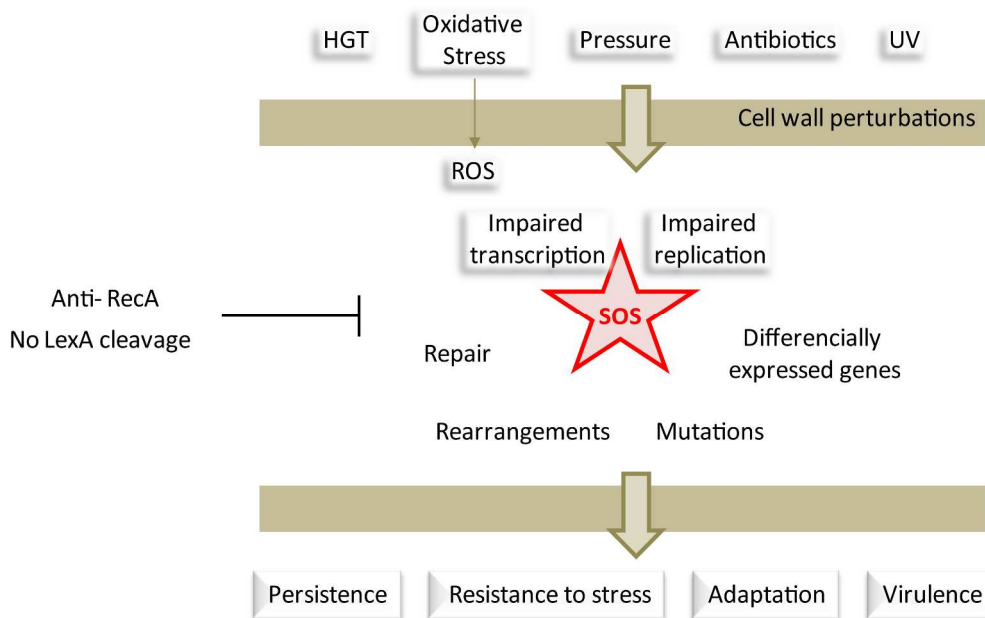


Figure 2. Origins of ssDNA inducing SOS. A. Replication fork stalling often leads to spontaneous ssDNA nicks. These breaks are eventually transformed into double strand breaks (DSBs) when replication restarts, and DSBs are potent inducers of SOS as described in section I. B. Replication transcription collisions. Adapted from (Helmrich et al., 2013). Co-directional collisions may happen because of the difference of velocity of the replisome and the transcription complex, and lead to DSB formation (Dutta et al., 2011). Head-on collisions occur when transcription and replication progress in opposite directions. Both types of collisions cause DSB formation through either R-loop formation or topological stress, if rescue pathways are insufficient. C. Transcription stalling. Adapted from (Wimberly et al., 2013). Elongating RNA polymerase (RNAP) can stall upon encounter with a DNA lesion or bulky protein complexes. In this case, transcribed RNA can anneal to the template DNA forming a structure called R-loop. R-loops are another type of SOS inducers as described in section I. R-loops can lead to re-priming of a replication fork. If the re-primed replication fork encounters a nick, it can stall and lead to DSBs (as in panel A).

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Causes and consequences of the induction of the SOS response.
254x162mm (300 x 300 DPI)

Review

SOS, the formidable strategy of bacteria against aggressions.

Abstract

The presence of an abnormal amount of single stranded DNA in the bacterial cell constitutes a genotoxic alarm signal that induces the SOS response, a broad regulatory network found in most bacterial species to address DNA damage. The aim of this review is to point out that beyond being a repair process, SOS induction leads to a very strong but transient response to genotoxic stress, during which bacteria can rearrange and mutate their genome, induce several phenotypic changes through differential regulation of genes, and sometimes acquire characteristics that potentiate bacterial survival and adaptation to changing environments. We review here the causes and consequences of SOS induction, but also how this response can be modulated under various circumstances and how it is connected to the network of other important stress responses. In the first section, we review articles describing the induction of the SOS response at the molecular level. The second section discusses consequences of this induction in terms of DNA repair, changes in the genome and gene expression and sharing of genomic information, with their effects on the bacteria's life and evolution. The third section is about the fine-tuning of this response in order to fit with the bacteria's "needs". Finally, we discuss recent findings linking the SOS response to other stress responses. Under these perspectives, SOS can be perceived as a powerful bacterial strategy against aggressions.

Introduction

SOS induction was first observed in cells where the replication fork encounters DNA lesions which it tries to replicate (Radman, 1975). Since these early investigations, a multitude of different studies have been carried out to decipher the triggers and components of the SOS response. We know now that other mechanisms, through which ssDNA is formed in the cell, exist and induce SOS.

The mechanism of induction of SOS by single stranded DNA (ssDNA) is well understood (Walker, 1996, Michel, 2005, Kelley, 2006). Briefly, RecA is recruited on ssDNA by presynaptic complexes RecBCD or RecFOR. RecBCD recognizes double strand DNA breaks (DSB) or double strand ends (DSE). Its helicase and nuclease activities result in the

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3 formation of an ssDNA substrate for RecA. RecFOR recognizes DNA nicks and gaps, and
4 recruits RecA to this ssDNA patch. RecA binds ssDNA in the form of a nucleofilament which
5 catalyzes the auto-proteolysis of the repressor LexA. LexA represses the genes belonging to
6 the SOS regulon by binding to its cognate LexA box sequence on their promoters. LexA
7 proteolysis thus leads to de-repression of this regulon, comprised of about 40 genes in *E. coli*
8 (Courcelle *et al.*, 2001). The affinity of LexA for these genes is variable and depends on the
9 LexA box sequence itself and on the number of LexA boxes present at the promoters of these
10 genes. The LexA box consensus sequence was proposed to be 5'-CTGTN₈ACAG-3' (Butala *et*
11 *al.*, 2009) and can be extended to 5'-TACTGT(AT)₄ACAGTA-3' in *E. coli* (Fernandez De
12 Henestrosa *et al.*, 2000, Wade *et al.*, 2005). LexA binding seems to be tolerant for variations
13 in the sequence of 8 base spacer region but its length is invariant, as suggested by structural
14 studies where the LexA-DNA complex was crystallized (Zhang *et al.*, 2010). This leads to
15 differential de-repression (Courcelle *et al.*, 2001)/re-repression of genes belonging to the SOS
16 regulon as shown *in vivo* and *in silico* using an algorithm based approach by Ronen and
17 colleagues (Ronen *et al.*, 2002). On the other hand, the LexA box consensus sequence varies
18 among bacterial species (cyanobacteria, gram positives, alphaproteobacteria,
19 gammaproteobacteria). The phylogeny of LexA boxes and the ability of LexA proteins from
20 various bacterial species to bind various LexA boxes was studied *in vitro* and *in silico* (Mazon
21 *et al.*, 2004) and shows that these sequences are related and in most cases follow the
22 phylogenetic relationships of those bacterial species. Examples of different LexA boxes are
23 given in Table 1 - the list is non-exhaustive, but gives insight in the similarity of these
24 sequences in different bacterial species.

25
26 The SOS-induced genes favor the repair of DNA lesions. Figure 1 shows simplified
27 representations of DNA repair pathways induced by SOS.

28 **Homologous recombination (HR):** RecA itself is induced by SOS, together with other
29 homologous recombination proteins such as RuvAB and RecFOR, to allow the repair of
30 single stranded lesions. Induction of HR can lead to rearrangements in the chromosome (see
31 section II-2-1).

32 Nucleotide Excision Repair (NER) by UvrABC (Sancar and Rupp, 1983): The *uvr*
33 genes involved in nucleotide excision repair (NER) allow the repair of lesions where the DNA
34 is double stranded (like a mismatch). Briefly, UvrABC endonuclease recognizes the lesion,
35 nicks the DNA and UvrD helicase removes the DNA patch carrying the lesion (Kumura *et al.*,
36 1985). DNA polymerase Pol I then fills the gap (Husain *et al.*, 1985).

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3 Translesion synthesis (TLS): the more numerous the initial amount of lesions, the
4 stronger the genotoxic signal and the more genes from the SOS regulon are induced. *umuD*
5 and *umuC* are induced later. Similarly to LexA, UmuD undergoes proteolytic cleavage
6 catalyzed by the RecA nucleofilament (Nohmi *et al.*, 1988). Cleaved UmuD (named UmuD')
7 becomes activated and, together with UmuC, forms the translesion synthesis DNA
8 polymerase UmuD'2C, or Pol V. PolV proceeds with DNA replication on damaged DNA by
9 incorporating any base accross from the DNA lesion that the proof-reading proficient Pol III
10 cannot replicate. Other TLS polymerases exist and, like Pol V, allow the replication of
11 damaged DNA in a mutagenic manner: these are the DNA polymerases Pol II (*polB*) (Pages
12 & Fuchs, 2003) and Pol IV (*dinB*) (Napolitano *et al.*, 2000). TLS can be highly mutagenic
13 because translesion polymerases can incorporate a correct or incorrect base in front of the
14 lesion on the template strand (Friedberg *et al.*, 2002). Unlike Pol III, TLS polymerases do not
15 have a proofreading activity, except for Pol II that has a high level of fidelity on undamaged
16 DNA. Induction of TLS leads to an increased frequency of spontaneous mutations (see
17 section II-2).
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28 LexA is a late-induced gene, and can stop the SOS induction when the genotoxic
29 signal disappears and LexA cleavage is not favored anymore. Ongoing biochemical and
30 sequence analysis studies aim at shedding light into the dynamics of SOS induction through
31 the study of RecA-LexA interactions (Kovacic *et al.*, 2013) or biochemical and structural
32 bases of auto-proteolysis and damage sensing (Butala *et al.*, 2009, Aravind *et al.*, 2013). In an
33 *in vitro* report about the timing of ssDNA-RecA-mediated self-cleavage of LexA, Butala and
34 colleagues showed that a LexA repressor which is bound to its target *lexA-box* is insensitive to
35 auto-proteolysis (Butala *et al.*, 2011). This observation explains how the binding affinity of
36 LexA for different promoters correlates with the timing of the gene's expression.
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43 SOS induction is a well-described cellular response. A comprehensive review about
44 the mechanism of SOS induction and the phylogenetic conservation of this cellular stress
45 response was published in 2007 by Erill, Campoy and Barbé (Erill *et al.*, 2007). In the light of
46 subsequent studies, more knowledge has been accumulated on the conditions leading to the
47 induction of the SOS response and its outcomes, such as virulence, persistence, emergence of
48 multiple resistances and, more generally, adaptation. We focus in this review on new data
49 uncovering the SOS-related strategies developed by bacteria in response to various
50 aggressions.
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I - Origin of SOS-inducing single stranded DNA (ssDNA)

1 - Origin of ssDNA in the absence of external damaging agents

Most ssDNA originates from double strand breaks (DSBs). As described in the introduction, ssDNA is the sole inducer of the SOS response. Experiments measuring the formation of DNA lesions capable of inducing SOS in *E. coli* using flow cytometry led to the observation that most of the chromosomal ssDNA (two thirds according to the authors) comes from the action of RecBCD on DSBs. The remaining third are ssDNA stretches formed independently of RecBCD (Pennington & Rosenberg, 2007). Such DSBs mostly originate from spontaneous DNA breakage during replication, most probably after fork stalling (Figure 2A). Several conditions leading to replication arrest are described below.

Replication fork problems can lead to DSB formation. It is known that replication arrest or stalling leads to formation of ssDNA on the lagging strand template, which constitutes a substrate for the formation of the RecA nucleofilament and SOS induction in the absence of DNA lesions (Walker, 1996). Such an event can happen when replication is impaired, as is the case for *E. coli* conditional replication mutants harboring thermo-sensitive alleles of genes coding for different sub-units of the replicative DNA polymerase Pol III (Flores *et al.*, 2005, Lestini & Michel, 2007). Figure 2A shows a synthetic representation of how ssDNA that induces SOS can be formed during replication. Replication arrests may also occur upon transcription replication collisions (Figure 2B), such as in conditions where bulky factors block the DNA polymerase complex. Such an example is described in an *E. coli* strain where the highly transcribed ribosomal *rrn* operons were inverted in order to be transcribed in the opposite direction of replication (Boubakri *et al.*, 2010). In this case, head-on collisions of the replisome with the bulky transcription complexes hinder the progression of the replication fork and lead to SOS induction. In the absence of stalled RNA polymerase (RNAP) removal systems, the DNA replication is impaired and SOS is induced (Boubakri *et al.*, 2010, McGlynn *et al.*, 2012).

R-loop formation in non-replicating bacteria also leads to DSB formation (Figure 2C). The conditions described above involve replication fork stalling, which implies active replication of the bacterial chromosome. However, DSBs are also formed in non-replicating conditions. The origin of these DSBs was found to lie in R-loop formation during transcription in *E. coli* (Wimberly *et al.*, 2013). R-loops are RNA-DNA hybrids that are formed when the RNAP is stalled on DNA and the RNA in synthesis anneals to the

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3 homologous transcribed ssDNA template in *E. coli* (reviewed in (McGlynn *et al.*, 2012)) and
4 eukaryotes (Helmrich *et al.*, 2011). This can happen, for example, when the RNA polymerase
5 encounters a roadblock and backtracks (Dutta *et al.*, 2011). In growing cells, this can lead to
6 replication-transcription conflicts as described above (for a review, see (Merrikh *et al.*,
7 2012)). R-loops were shown to lead to genomic instability in *E. coli*, as in eukaryotes (Gan *et*
8 *al.*, 2011), by impairing DNA replication.

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13 Formation of such R-loop structures was very recently shown to also lead to stress-
14 induced mutations in non-replicating *E. coli* (Wimberly *et al.*, 2013), suggesting that
15 problems at transcribed regions leading to RNAP stalling can constitute a genotoxic signal.
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18 19 **2 - ssDNA formation due to external damaging agents**

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21 DNA damage can be formed upon physical insults and ssDNA can also originate from
22 DNA damage provoked by external agents. For instance, UV irradiation causes DNA lesions
23 that need to be repaired in order for the cell to survive. The repair of damaged DNA by HR or
24 TLS requires the initial recruitment of RecA to the site of lesion (Fujii *et al.*, 2006). Hence,
25 inactivating the recruitment of RecA, for instance in a mutant deleted for *recA*, leads to cell
26 death because of lack of repair and possibly because of inefficient TLS in the absence of SOS
27 induction. As mentioned in the introduction, SOS induction leads to the expression of TLS
28 polymerases; hence, the repair of UV lesions is highly mutagenic.
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35 Another damaging factor described by Aertsen and colleagues is pressure. High
36 pressure indeed induces SOS through DSB formation through the Mrr nuclease (Aertsen &
37 Michiels, 2005), in *E. coli* strains carrying the *mrr* gene (Aertsen *et al.*, 2008). In turn, SOS
38 can induce high pressure resistance through spontaneous mutations inactivating the *mrr* gene
39 (Aertsen & Michiels, 2005). What is remarkable in this example is how the induction of the
40 SOS response is a subtle way for the cell to turn an external aggression into an advantage by
41 becoming better adapted to its environment.
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47 Another study on the effect of gamma radiation on *E. coli* reports a link between the
48 SOS response and radiation-induced cell death, a bacterial mechanism similar to the
49 eukaryotic programmed cell death (Wadhawan *et al.*, 2013). Moreover, SOS-dependent DNA
50 repair by HR and TLS were found to be necessary for cell recovery after gamma irradiation.
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52
53 Reactive oxygen species (ROS) can also lead to DSB formation. Recently, reactive
54 oxygen species and their various effects on cellular mechanisms have drawn a lot of attention.
55 ROS-like superoxide or hydroxyl radicals (OH⁻) are potent bacterial stressors, which can
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3 directly damage DNA. For instance, superoxide leads to the release of free iron ions from
4 proteins containing iron (Fe-S cluster proteins) (Keyer & Imlay, 1996, Imlay, 2003). Iron can
5 also localize along the phosphodiester backbone of nucleic acids. Moreover, high
6 concentrations of free ferrous iron lead to the production of hydroxyl radicals (OH⁻) through
7 the Fenton reaction (Henle *et al.*, 1999, Rai *et al.*, 2001) (Daly, 2009). OH⁻ attacks the DNA
8 backbone and ultimately causes DSBs (Jena, 2012). Moreover, superoxide and OH⁻ are also
9 formed after poisoning of the DNA gyrase (by fluoroquinolones or proteic inhibitors like the
10 CcdB toxin), an essential protein for chromosome replication (Dwyer *et al.*, 2007).

11
12 Another type of DNA damage caused by oxidative attack is the incorporation of
13 oxidized guanine residues during replication (7,8-Dihydro-8-oxo-guanine or 8-oxo-G)
14 (Sussenbach & Berends, 1964). Bacteria possess a defense system against such damage, first
15 by hydrolyzing the oxidized guanine present in the nucleotide pool and second, by affecting
16 the incorporated G through the base excision repair pathway (BER) (Lu *et al.*, 2001), by
17 limiting its incorporation and the formation of mismatches (Sobol, 2012). Incomplete action
18 of the BER system possibly leads to DSBs that are cytotoxic if unrepaired (Foti *et al.*, 2012).

19
20 ROS can also indirectly cause DNA damage (Farr & Kogoma, 1991). OH⁻ radicals can
21 damage proteins (Ide *et al.*, 1985) and lipids (Schaich & Borg, 1988) as shown in *E. coli* and
22 *Salmonella enterica* (Paez *et al.*, 2011) (also reviewed in (Dwyer *et al.*, 2009)). Additionally,
23 it is known that mistranslated and misfolded proteins are more susceptible to oxidation
24 (Dukan *et al.*, 2000). Mistranslational corruption of proteins may lead to replication fork
25 collapse and induction of the SOS response in *E. coli* (Balashov & Humayun, 2002, Al
26 Mamun *et al.*, 2006). Interestingly, in *Deinococcus radiodurans*, cell death by radiation is not
27 caused by direct DNA damage but primarily by oxidative damage of proteins, which
28 eventually results in the abolition of DNA repair (Krisko & Radman, 2010). The legendary
29 resistance of *D. radiodurans* to ionizing radiations is actually a consequence of a more
30 efficient proteome protection against ROS rather than a more efficient genome protection and
31 cell death correlates with protein carbonylation due to oxidative damage. Preserving cell
32 integrity in times of oxidation seems to depend as much on proteome protection against ROS
33 then on DNA protection. Furthermore, affecting the integrity of the proteome can result in the
34 induction of the SOS response through impaired DNA replication and repair.

35
36 Antibiotics can trigger bacterial stress at both lethal and sub-lethal concentrations.
37 Antibiotics may induce SOS through DNA damage or through replication arrest. The
38 fluoroquinolone (FQ) family of antibiotics which causes replication arrest by blocking DNA
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3 gyrase (Pohlhaus & Kreuzer, 2005), and trimethoprim (Lewin & Amyes, 1991) which affects
4 purine and pyrimidine synthesis, do not directly generate lesions but rather perturb DNA
5 replication. Mitomycin C (MMC) leads to T-T crosslinks that lead to DNA break formation. β
6 -lactams were also reported to induce SOS in some conditions by an unusual mechanism. In
7 *E. coli*, β -lactams cause cell wall damage, which induces the two-component system proteins
8 DpiBA. DpiA can bind the origin of replication of the chromosome and, together with DpiB,
9 impair replication, which results in the induction of the SOS response (Miller *et al.*, 2003,
10 Miller *et al.*, 2004).

11
12 Interestingly, SOS is also induced by low doses of several antibiotics, termed sub-MIC
13 for sub-minimal inhibitory concentration. The evidence that sub-MICs of antibiotics play an
14 important role in the appearance and dissemination of antibiotic resistance has emerged in
15 recent years (Kohanski *et al.*, 2010, Gullberg *et al.*, 2011). This is especially relevant
16 considering that a large proportion of ingested antibiotics are released intact in the
17 environment (Liu *et al.*, 1999, Liu *et al.*, 1999) and that low levels of antibiotics are found in
18 wastewater plants, hospitals and soil (Fick *et al.*, 2009, Haggard & Bartsch, 2009, Kummerer,
19 2009, Kummerer, 2009).

21
22 First, sub-MICs FQs have been shown to induce SOS in *Salmonella enterica*, even if
23 bacterial growth is not affected by these low concentrations (Yim *et al.*, 2011). Ciprofloxacin
24 at sub-MIC induces SOS and increases the frequency of point mutations in *S. aureus* (Mesak
25 *et al.*, 2008, Mesak & Davies, 2009). Moreover, ampicillin at sub-MIC was found to down-
26 regulate mismatch repair in *E. coli*, *P. aeruginosa* and *V. cholerae*, hence increasing mutation
27 frequencies due to SOS (Gutierrez *et al.*, 2013). This brings along a different problem than SOS
28 induction by the same antibiotics at high concentrations: in fact, at high concentration,
29 bacteria either acquire mutations that help them to survive or they die, the latter possibility
30 being the most likely. However, at low antibiotic concentrations, bacteria do not die and SOS
31 induction leads to an increase in bacterial genome variability. Mutations acquired in such a
32 way can then be fixed in populations if they provide a fitness advantage. More and more
33 reports now suggest that these low concentrations of antibiotics and the consequent induction
34 of SOS are one of the factors contributing to the acquisition of multiple resistances and
35 adaptation factors by bacteria.

36
37 Moreover, the effects of sub-MICs of antibiotics from different families - such as
38 antibiotics that target translation and not DNA (aminoglycosides, chloramphenicol and
39 tetracycline) - on the SOS response was further addressed. These antibiotics are not expected
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3 to induce SOS. Interestingly *V. cholerae*, but not *E. coli*, was found to induce SOS in
4 response to these antibiotics, which favors the appearance of resistant derivatives (Baharoglu
5 *et al.*, 2010). Moreover, aminoglycoside (AG) mediated SOS induction is conserved among
6 distantly related Gram negative pathogens (*Klebsiella pneumoniae* and *Photobacterium*
7 *luminescens*), suggesting that *E. coli* is more of an exception than a paradigm for the
8 physiological response to sub-MICs of antibiotics (Baharoglu *et al.*, 2013). It is also
9 interesting to mention here that AGs block translation. In fact, translating ribosomes control
10 the transcription elongation rate: active ribosomes can prevent RNAP from backtracking
11 (Proshkin *et al.*, 2010, Potenski & Klein, 2011). Consequently, inhibiting protein synthesis
12 increases levels of transcription-generated R-loops. Formation of transcriptional R-loops in
13 bacteria is indeed shown to be inhibited by ribosomes on the nascent transcript (Masse &
14 Drolet, 1999). As described in section I-1, R-loops have the potential to induce SOS.
15 Aminoglycosides target protein translation, but they clearly also have a negative effect on
16 transcription and replication at sub-MIC, which leads to SOS induction in several bacterial
17 species.

18
19 It can sound puzzling that sub-MICs of AGs induce SOS in several bacteria for they
20 do not directly target DNA synthesis or the DNA molecule. These observations point to a role
21 for intermediate factors that cause DNA damage. One of the reasons for this SOS induction
22 was actually found to be that sub-MIC AG treatment leads to the formation of ROS
23 (Baharoglu *et al.*, 2013). Further studies suggest a role for RNA polymerase stalling at AG-
24 mediated DNA lesions and formation of R-loops with the consequences on SOS as described
25 in the previous paragraph (Baharoglu *et al.*, 2013).

26
27 Several points are striking in these latter findings. First, low concentrations of
28 antibiotics that do not affect cell growth - even those that do not target DNA or its replication
29 - have the power of inducing the bacterial SOS response and as such increasing mutation
30 frequencies of bacteria (Baharoglu & Mazel, 2011). Second, formation of ROS is the factor
31 leading to the actual DNA damage, as discussed above. Finally, and once again, a tight
32 connection between transcription blocks and triggering of the SOS response is seen. Induction
33 of SOS by low levels of AGs is important for growth under these conditions. This aspect of
34 SOS induction needs further work in order to be fully understood.

3 - ssDNA formation during ssDNA uptake

Transformation is a mechanism of HGT that relies on ssDNA uptake and processing (Dubnau, 1999). Transformation occurs when a bacterial cell reaches a competent state rendering it capable of taking up DNA present in its environment and, in some cases, of integrating the acquired DNA in its genome by recombination (Claverys *et al.*, 2009). It was observed long ago in *Bacillus subtilis* that when lysogenic strains were rendered competent, the induction of a prophage led to the reduction in the frequency of transformation (Yasbin *et al.*, 1975, McVeigh & Yasbin, 1996). This was not due to reduced transformation efficiency but rather to the induction of a prophage in the recipient cells leading to the lysis of the cell and thus a drop in the number of viable transformants. Prophage induction is in many cases SOS-dependent. It is likely that the reduced transformation efficiency of lysogenic cells compared to non-lysogenic cells is the result of induction of the SOS response by the ssDNA acquired during transformation. Furthermore, it was shown in naturally competent *V. cholerae* that ssDNA uptake during competence induces the SOS response (Baharoglu *et al.*, 2012).

In addition to transformation, conjugation is another HGT mechanism where plasmid or chromosomal DNA from a donor strain enters the recipient cell in a single stranded fashion. Like transformation, conjugation also induces SOS in *E. coli* and *V. cholerae* (Baharoglu *et al.*, 2010).

II - Consequences of SOS induction.

1 - Repair

As mentioned earlier, the SOS response triggers homologous recombination, required especially for the repair of double strand ends/breaks (DSB/DSE). A recent study in *E. coli* proposed that instead of a genome wide search for homology, genotoxic stress-induced and RecA-dependent condensation of sister chromosomes, which are otherwise segregated at cell poles, allow repair of DSBs (Shechter *et al.*, 2013). Such genome packaging is also observed in *E. coli* in response to oxidative stress (Ko *et al.*, 2012). Moreover, the importance of RecN was recently discovered for the repair of DSBs after treatment with MMC, a DNA-damaging agent that leads to DSB formation (Keyamura *et al.*, 2013) and induction of the SOS response. RecN is an SMC (structural maintenance of the chromosome) family protein induced by SOS, underlining the significance of the SOS response in the repair of lethal chromosomal DSBs.

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3 Moreover, the importance of SOS induction in the case of replication-transcription
4 conflicts described above is notable. Indeed, in *E. coli* strains in which the transcription of *rrn*
5 operons leads to replication arrest after stalling of the RNAP, preventing induction of the SOS
6 response results in a decrease in fitness (or even absence of growth) (Boubakri *et al.*, 2010).
7 RNAP stalling can be an obstacle to the progression of the replication fork and needs to be
8 dislodged (McGlynn *et al.*, 2012). Intermediate DNA structures, R-loops, capable of inducing
9 SOS are formed at the stalled RNA polymerase. By inducing expression of genes coding for
10 helicases such as DinG, SOS also allows the processing and removal of bulky protein
11 complexes that prevent DNA polymerase action (Baharoglu *et al.*, 2010). Additionally, DinG
12 was proposed to inhibit the formation and accumulation of R-loops *in vivo* in *E. coli* cells
13 (Boubakri *et al.*, 2010, De Septenville *et al.*, 2012).

14
15 Recent data also shows that transcription stalling is involved in SOS induction in
16 response to DNA damage by sub-MIC AGs in *V. cholerae* (Baharoglu *et al.*, 2013), with a
17 role for the RNAP-binding protein Mfd. In fact, the helicase Mfd can recognize R-loops at
18 sites where the RNAP is stalled on damaged DNA and releases the transcription complex
19 (Roberts & Park, 2004, Deaconescu *et al.*, 2006, Savery, 2007). Interestingly, Mfd is also
20 required for the repair of DSBs caused by MMC in *Helicobacter pylori*, where an *mfd* mutant
21 is susceptible to antibiotics and has a DNA repair defect (Lee *et al.*, 2009). Mfd is involved in
22 increased mutation frequencies after FQ treatment in *Campylobacter jejuni* as well (Han *et*
23 *al.*, 2008). All of these observations point to Mfd as a possible partner of the SOS response.
24 There is also evidence that the NusA protein, a component of the elongating RNA
25 polymerase, is involved in stress-induced mutagenesis by interacting with the TLS
26 polymerase Pol IV in *E. coli* (Cohen & Walker, 2010). This is once again evidence
27 highlighting an undeniable connection between transcription and induction of the SOS
28 response.

2 - Change: outcome of SOS induction in terms of genome plasticity and gene expression

29
30 As described in the introduction, SOS induction increases the levels of proteins
31 involved in HR. Hence, one of the consequences of this SOS induction is to increase intra-
32 chromosomal recombination of homologous DNA sequences after DSB formation. This was
33 observed for instance in *E. coli* through facilitated reconstitution of a functional *lacZ* gene
34 from the fragments *lacZ'* and *lacZ* flanked by homologous regions in conditions inducing
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3 SOS (after treatment with sub-MIC of FQ) (Lopez & Blazquez, 2009). Interestingly, it was
4 found in *V. cholerae* that homologous recombination plays an unsuspected role in
5 chromosome fusions. *V. cholerae* has two circular chromosomes with different origins of
6 replication. Under genetic conditions leading simultaneously to impaired replication from the
7 origin of the second chromosome and to induction of the SOS response, it was found that
8 homologous recombination proceeds to the fusion of the two chromosomes through
9 homologous IS sequences present in both chromosomes, giving rise to changes in gene
10 dosage (Val *et al.*, 2013). In fact, the closer a gene is to the origin of replication, the more its
11 copy number in a given cell increases due to multiple firings of replication. When two
12 chromosomes are fused, only one origin is active, thus the gene dosage is subject to change.
13 This suggests that in the wild type strain, such fusions can happen even if they are not easily
14 detected and that SOS induction can favor their occurrence, which can influence gene
15 expression and cell physiology in general.

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18 Interestingly, DSB formation and subsequent SOS induction can also constitute an
19 advantage in harsh conditions: it was shown in pathogenic *Pseudomonas aeruginosa* biofilm
20 communities that ROS-mediated formation of DSBs were beneficial to bacteria in the
21 presence of antibiotics as they allowed genome rearrangements and resistance (Boles &
22 Singh, 2008).

23
24 In addition to HR, SOS leads to increased mutation frequencies through TLS.
25 Induction of SOS leads to a high state of mutability in the bacterial cells, for example after
26 antibiotic treatment in *E. coli* (Ysern *et al.*, 1990). This allows them to increase their chances
27 of generating mutations that will permit them to survive under the stress conditions they are
28 submitted to. Indeed, point mutations can result in antibiotic resistance acquisition. For
29 instance, resistance to ciprofloxacin (FQ) and to rifampicin is due to mutations caused during
30 the induction of the SOS response through the action of the error-prone polymerases IV/V
31 (Cirz & Romesberg, 2006). Sub-MICs of FQs were clearly shown to cause the development
32 of resistance in *S. aureus* (Didier *et al.*, 2011) and *S. enterica* (Hughes & Andersson, 2012).
33 The increased mutation frequency in the presence of FQs was proposed to lead to the overload
34 of MutS-dependent mismatch repair, causing the accumulation of unrepaired DNA and the
35 appearance of point mutations that lead to antibiotic resistance.

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38 A recent study achieved mathematical modeling of the link between DNA damage
39 after UV irradiation, SOS induction and mutation frequency by measuring the quantity of the
40 TLS polymerase UmuD₂C (Pol V) and corresponding mutation frequencies in *E. coli* (Ni *et*

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3 *al.*, 2008). The authors conclude that there is a close correlation between UmuD₂C levels and
4 mutation frequency (for a review on UV-mediated DNA damage and repair, see (Rastogi *et*
5 *al.*, 2010)). (DSB)-dependent stress-induced mutagenesis described in starving *E. coli* also
6 requires the SOS response and DinB (Pol IV) (Shee *et al.*, 2011). Interestingly, even in
7 mismatch repair deficient hyper-mutator strains, inactivation of the SOS response leads to
8 reduced development of antibiotic resistant bacteria (Cirz & Romesberg, 2006), showing the
9 importance of SOS induction in the appearance of point mutations.
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15 It was found that SOS induction mediated by sub-MIC antibiotics is also coupled with
16 an increased frequencies of spontaneous mutation in *E. coli* and *V. cholerae* (the appearance
17 of rifampicin resistance increased from 10⁻⁹ to around 10⁻⁸) (Baharoglu & Mazel, 2011).
18 Such a modest increase in mutation frequency is nonetheless of high importance since it was
19 shown to influence the evolution of multidrug resistance in *E. coli* (Denamur *et al.*, 2005).
20 Strains characterized by low or high mutation rates actually have a lower resistance to
21 antibiotics than strains that have an intermediate rate of mutation (around 10⁻⁸), regardless
22 of the antibiotic tested (Denamur *et al.*, 2005). In fact, high mutation frequencies probably more
23 often lead to deleterious mutations, demonstrating that SOS induction levels can be subtly
24 fine-tuned in order to achieve adapted levels of mutability.
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31 SOS induction in response to HGT also leads to genome rearrangements. As
32 mentioned in section I-3, during conjugation and transformation, plasmid or chromosomal
33 DNA enters the recipient cell in a single stranded fashion. The fact that HGT (i.e. incoming
34 DNA) induces SOS in the recipient is not trivial: by doing so, incoming DNA may induce its
35 own integration in the recipient bacterium's genome, highlighting once more the role of the
36 SOS response as an initiator of genome plasticity and in the acquisition of adaptation factors.
37 Interestingly, even narrow host range conjugative plasmids which cannot replicate or be
38 maintained in the recipient bacteria induce SOS upon entry into the cell (Baharoglu *et al.*,
39 2010). This means that even though the incoming plasmid is not maintained and the incoming
40 DNA is eventually degraded, conjugation still leaves an imprint in the recipient by inducing
41 SOS-dependent genome plasticity such as point mutations or integron rearrangement, as
42 discussed in the following paragraph.
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51 As depicted above, SOS is a stress response inducing the expression of recombination
52 and repair genes. But other genes are also part of the SOS regulon and induced following
53 genotoxic stress. For example, apart from inducing mutation frequency, another way for the
54 SOS response to confer antibiotic resistance and adaptive responses is through increasing the
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3 expression of resistance and adaptation genes. It was described for instance that SOS
4 induction after quinolone treatment could in turn induce the expression of plasmid-borne
5 quinolone resistance determinants in *Enterobacteria* (Da Re *et al.*, 2009). Resistance to
6 quinolones is here triggered by the presence of quinolones themselves, at sub-inhibitory
7 concentrations, or by other antibiotics that induce the SOS response. This latter point is
8 important. It means that bacteria not only become resistant to the factor triggering the
9 genotoxic stress, but also modulate their gene expression and modify their genome
10 (spontaneous mutations, genome rearrangements) so that they become able to resist to other
11 stresses when they come along. In this way, SOS is a powerful strategy of bacteria to turn
12 external aggressions in their favor. SOS is also involved in other bacterial processes such as
13 persistence, virulence and biofilm formation.

14
15 Persistence is a non-hereditary and reversible state. Persisters are antibiotic tolerant
16 cells that are not killed during treatment and resume growth when antibiotics are removed (for
17 a review (Lewis, 2010)). Dorr *et al.* showed that *E. coli* persisters are not pre-existing dormant
18 cells but that their formation is rather induced by the SOS response after FQ treatment (Dorr
19 *et al.*, 2009). Interestingly, the appearance of persister cells was shown to be much higher
20 during treatment with a sub-MIC of FQ than when higher antibiotic concentrations were used
21 (stronger SOS induction). SOS induction was thus necessary, but a low level of induction was
22 found to be more favorable to the development of persistence. Interestingly, in a report about
23 the dynamics of SOS induction, Butala and colleagues observed that the dissociation rate of
24 LexA from the promoters it regulates – which correlates with the levels of SOS induction -
25 directly influences the formation of *E. coli* persisters (Butala *et al.*, 2011).

26
27 SOS-induced dormancy was shown to be due to TisB toxin induction by SOS (Dorr *et al.*
28 *et al.*, 2010). Indeed, persister cell formation can occur through the induction of toxins from the
29 toxin-antitoxin family, such as TisB from the SOS regulon, which decreases the growth rate
30 (drop of ATP levels, no active peptidoglycan synthesis, no ribosome formation, no
31 replication), causing tolerance to multiple antibiotics. Interestingly, toxin-antitoxin modules
32 are found in many bacterial genomes (Pandey & Gerdes, 2005, Leplae *et al.*, 2011), and TisB
33 may not be the only toxin that leads to persistence. Therefore, the use of SOS-inducing
34 antibiotics at sub-MICs may lead to persistence and eventually contribute to the development
35 of multidrug resistance. In fact, other examples of SOS-related TA modules exist, like the *E.*
36 *coli* YafNO system which is induced by SOS (Singletary *et al.*, 2009), or CcdAB from the F
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3 plasmid which induces SOS through poisoning of the gyrase (Karoui *et al.*, 1983, Bernard &
4 Couturier, 1992).

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6 In addition to resistance to stress and adaptation, SOS induction also triggers virulence
7 in some bacteria. Indeed, SOS can directly regulate virulence factors: for example, LexA
8 regulates the expression of the *V. cholerae* prophage CTX genes leading to production of the
9 cholerae toxin (Kimsey & Waldor, 2009) (Quinones *et al.*, 2005). SOS induced in response to
10 sub-MIC of antibiotics also triggers the production of the shiga-toxin (Stx) in pathogenic *E.*
11 *coli* (Nassar *et al.*, 2013). Furthermore, SOS induces horizontal dissemination of virulence
12 factors present in pathogenicity islands in *S. aureus* (Ubeda *et al.*, 2005), which can happen
13 for instance in response to antibiotic treatment (Maiques *et al.*, 2006).

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16 Biofilm formation and quorum sensing can also be linked to the SOS response and
17 DNA damage in some cases. For instance, in *V. harveyi*, induction of bioluminescence was
18 shown to be important for repair of DNA damage caused by UV irradiation (Czyz *et al.*,
19 2000). Bioluminescence is regulated by quorum sensing and Czyz *et al* found that UV
20 irradiation induces bioluminescence. In subsequent studies, the authors showed that while
21 bioluminescence has a fitness cost on growing *V. harveyi* it confers a selective advantage
22 under conditions of DNA damage (caused by low doses of UV irradiation) (Czyz *et al.*,
23 2003). Although these studies do not directly demonstrate a link between the SOS response
24 and quorum sensing in the repair of damaged DNA, together with data suggesting that
25 quorum sensing is involved in SOS induction by sub-MIC of antibiotics (Baharoglu & Mazel,
26 2011), such a link seems plausible. Quorum sensing also regulates biofilm formation in *V.*
27 *cholerae* (Hammer & Bassler, 2003, Zhu & Mekalanos, 2003). On the basis that replication-
28 inhibiting antibiotics induce biofilm formation in various bacterial species (Hoffman *et al.*,
29 2005, Kaplan, 2011), Gotoh and colleagues tested and found that the SOS response is
30 involved in biofilm formation by *P. aeruginosa* (Gotoh *et al.*, 2010). Moreover, taking into
31 account reports showing that natural conjugation in *E. coli* (Ghigo, 2001) and extracellular
32 DNA uptake in *Staphylococcus epidermidis*, which induce SOS, also induce biofilm
33 formation (Kaplan *et al.*, 2011), and that the pathways regulating biofilm formation and
34 quorum sensing are intertwined, it is appealing to address in the future the possible links
35 between quorum sensing, biofilm formation and the SOS response.

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38 It was mentioned earlier how SOS can be used by bacteria to turn external aggressions
39 in their favor. The examples given were that of resistance to high pressure or to antibiotics
40 conferred by mutations acquired after SOS induction. In the same line of thought, increased

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3 resistance to one antibiotic can lead to the development of resistance to other classes of
4 antibiotics through SOS-mediated integron rearrangements. Hocquet and colleagues show that
5 metronidazole treatment of a patient infected with *P. aeruginosa* induces the SOS response
6 and integron recombination leading to β -lactam and ceftazidime resistance (Hocquet *et al.*,
7 2012). Moreover, in their recent study, they report the action of metronidazole on antibiotic
8 resistance acquisition in *P. aeruginosa*. They show that metronidazole, which is used against
9 anaerobic bacteria and not against *P. aeruginosa* triggers nevertheless the SOS stress response
10 in this bacterium. In turn SOS can trigger the appearance of resistances to multiple antibiotics
11 in *P. aeruginosa*. The authors clearly show the induction of SOS by metronidazole and
12 correlate this with increased resistance to aminoglycosides and fluoroquinolones *in vitro*
13 (Hocquet & Bertrand, 2013).
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16 An integron is a genetic platform of promoterless open reading frames called gene
17 cassettes, separated by recombination sites. The integron integrase, which encodes a site-
18 specific recombinase, catalyzes excision and integration of these cassettes in a single stranded
19 circular form (for a review, see (Mazel, 2006). Other than integron rearrangements, the
20 integrase also allows the capture of exogenous circular promoterless gene cassettes. Gene
21 cassettes placed at the first positions of the integron can be expressed from a constitutive
22 promoter (Levesque *et al.*, 1994, Jove *et al.*, 2010). Importantly, all known integron integrases
23 are regulated by the SOS response (Guerin *et al.*, 2009, Cambray *et al.*, 2011), which
24 highlights the integron as an evolutionary tool containing a pool of unexpressed genes able to
25 be recombined and expressed when needed, i.e. in response to stress. The SOS control of
26 integrase expression not only means that numerous rearrangements may take place within the
27 chromosomal integrons when they are present, but also that plasmid-borne integrases are
28 likely to be induced when their host's SOS response is triggered. To date, hundreds of
29 cassettes encoding resistance to antibiotics have been characterized in integrons (Partridge *et al.*,
30 2009). The induction of the integrase by SOS also explains how resistance and adaptation
31 genes can be recruited in such structures during stressful growth, and shows the power of this
32 structure coupled to SOS in the defense of bacteria harboring integrons.
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49 **3 –Share.**

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51 We saw earlier that HGT induces SOS. In turn, SOS induces HGT and consequently
52 induces the transfer of genetic information, as it was mentioned for the dissemination of
53 virulence determinants (section II-2-4). It was found for instance that SOS induction triggers
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3 the transfer of integrating conjugative elements (ICEs) from a donor to a recipient cell. An
4 example of this is the transfer of the *V. cholerae* ICE SXT, in which the two main ICE-
5 encoded transcriptional activators SetC and SetD are de-repressed when the host's SOS
6 response is induced (Beaber *et al.*, 2004). It is thought that the ICE-encoded repressor, SetR,
7 undergoes autoprolysis in the presence of the ssDNA-RecA nucleofilament similarly to
8 LexA. ICEs such as SXT are self-transmissible bacterial mobile elements that play a major
9 role in the dissemination of antibiotic resistance genes in bacterial populations. They transfer
10 by conjugation in a process similar to that of many conjugative plasmids, and their transfer
11 was shown to induce the SOS response in recipients to the same extent as was observed for
12 conjugative plasmids (Baharoglu *et al.*, 2010). In those terms, the SOS response is a mean for
13 bacteria not only to survive or change, but also to share information with neighboring cells.
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23 III – Fine tuning of SOS induction: attaining balance

24 1 - Bacteria can temper their SOS induction

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26 Not all ssDNA induces SOS. In an original recent study, it was observed in growing *E.*
27 *coli* using immunofluorescence microscopy that not all ssDNA formed after antibiotic
28 treatment induces SOS (Kohiyama *et al.*, 2013). This means that bacteria have a way of
29 insuring genome stability by neutralizing chromosomal ssDNA formed after fluoroquinolone
30 treatment, preventing SOS induction as long as its induction is not required.
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36 Mechanisms actively preventing RecA nucleofilament formation on ssDNA exist. One
37 of them is through the action of RecX. The *recX* gene is located downstream of *recA* in the *E.*
38 *coli* chromosome and was proposed by Fuchs and colleagues to regulate RecA (Pages *et al.*,
39 2003). RecX actually possesses an affinity for RecA and inhibits both its recombinase and
40 coprotease functions without interacting with ssDNA or double-stranded DNA (Stohl *et al.*,
41 2003). RecX was found to destabilize ssDNA-RecA nucleofilaments in log-phase cells in a
42 genetic context where RecA was over-expressed (Massoni *et al.*, 2012). Another protein that
43 turns off the SOS responses through inhibition of the RecA coprotease is DinI (Yasuda *et al.*,
44 1998). A higher mutation frequency was observed in an *E. coli* strain deleted for the *dinI* gene
45 after treatment with the SOS-inducing agent MMC. *In vitro* studies suggest that, conversely to
46 RecX, DinI does not prevent RecA nucleofilament assembly but stabilizes it, thus “hiding”
47 ssDNA (Lusetti *et al.*, 2004). DinI and RecX are themselves under the control of the SOS
48 response and affect the stability of the RecA filament and may thus participate in regulating
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3 the SOS response. Another protein able to antagonize RecA is UvrD, which is also induced by
4 SOS and involved in the last steps of NER (Lestini & Michel, 2007, Centore *et al.*, 2008).
5 Furthermore, when DinB (Pol IV) is over-produced, the active TLS polymerase Pol IV can
6 impede replication fork progression in such a way that fork arrest does not lead to additional
7 SOS induction (Mori *et al.*, 2012).
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11 Finally, “Trashing”, which consists in a sequestration of genotoxic ssDNA observed in
12 *vivo* in *E. coli* cells may represent a new facet of the regulation of the SOS response
13 (Kohiyama *et al.*, 2013). In this case, ssDNA is somewhat “hidden” from RecA and no
14 induction of the SOS response takes place. Bacteria have thus developed strategies to
15 attenuate SOS to decrease fitness cost and more importantly to mutate less in order to survive.
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20 21 **2 - Decreasing the fitness cost of SOS induction**

22 Apart from the evolution of bacterial resistance described in section II-2, moderate
23 SOS induction is also involved in the conservation of already existent multiple resistances by
24 the bacteria through the reduction of the fitness cost. For instance, bacteria that show slightly
25 increased frequencies of mutation and harbor antibiotic resistances are found in greater
26 proportions in the commensal flora of cystic fibrosis patients subjected to prolonged antibiotic
27 treatment (Gustafsson *et al.*, 2003). A recent report even proposes that upon acquisition of
28 resistance (to a β -lactam in this case) *E. coli* can reorganize its entire metabolic network in
29 order to reduce the fitness costs associated with the acquisition of this resistance (Handel *et*
30 *al.*, 2013). The authors observed there that the SOS response itself was down-regulated in
31 response to the acquisition of the resistance. SOS induction thus has a fitness cost better to
32 avoid if the induction is unnecessary.
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40 It also was mentioned in section I-3 that the entry of conjugative plasmids into a
41 recipient cell induces the SOS response. The exceptions to this are the plasmids carrying the
42 *psiB* (protein for SOS inhibition) gene. In fact, some narrow host range conjugative plasmids
43 harbor this gene which is transferred early during conjugation and which inhibits induction of
44 the SOS response in the recipient cell (Bagdasarian *et al.*, 1992), probably by binding to RecA
45 (Petrova *et al.*, 2009). The advantage of this could be to avoid inducing a stress response in
46 the host: as narrow host range plasmid can replicate only in a small number of hosts they may
47 have evolved to prevent unnecessary mutations and fitness costs.
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3 - Attenuate SOS induction to mutate less and survive

In several reports, intermediate levels of SOS induction (for instance in response to sub-MICs of antibiotics) were found to be a potent strategy of bacteria in order to become persistent (see section II-2) or acquire spontaneous mutations that allow them to survive (see section II-2). It was also observed in *E. coli* that the mismatch repair system tends to attenuate the appearance of mutations during UV-induced SOS by removing those introduced during by the UmuD₂C (PolV) translesion polymerase (Belov *et al.*, 2013).

We can speculate that bacteria use the SOS response and its mutagenic influence as a “last resort”, avoiding its induction when other mechanisms are sufficient to repair the damaged DNA (Delmas & Matic, 2005).

IV – SOS and other stress responses

We described how SOS is linked with transcription-related insults to DNA. SOS is actually connected with various stress responses, highlighting that responses to these various stresses are integrated into a larger network ensuring survival of the bacterial cells.

1 – Cell wall stress

The most straightforward example of a connection between cell wall stress and SOS is the effect of β -lactams on bacteria: β -lactams induce the SOS response and prophage induction in *S. aureus* (Maiques *et al.*, 2006) and *E. coli* (Wadhawan *et al.*, 2013). β -lactams actually induce the SOS response by triggering a cell wall stress through the DpiAB two-component system (Miller *et al.*, 2004).

In the absence of β -lactams, SOS induction by cell wall stress through the DpiAB system can also be observed by inactivating the genes coding for components of the cell membrane (such as penicillin binding proteins or PBP) (Miller *et al.*, 2004). Furthermore, the RpoE envelope stress response sigma factor of *E. coli* is also required for the increase in frequency of stress-induced mutations through formation of DSBs (Gibson *et al.*, 2010). All of these observations point to a link between cell wall stress and SOS induction.

One consequence of this is that SOS, in conjunction with the DpiAB system, confers β -lactam resistance by transiently halting cell division (Miller *et al.*, 2004). Once again, we observe that antibiotics trigger the bacterial stress response, which in turns allows the bacterial cell to resist to this external stress. According to recent reports, the resistance to β -

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3 lactams conferred by SOS induction is synergistic with alterations in the cell wall and in the
4 TCA cycle in *S. aureus* (Keaton *et al.*, 2013, Plata *et al.*, 2013), which implies a link with
5 oxidative stress.
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8 9 **2 – Oxidative stress.**

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11 In fact, reactive oxygen species (O_2^- , H_2O_2 , OH^\cdot) formed during oxidative stress have
12 the potential to damage DNA, and damaged DNA is a potent inducer of the SOS response.
13 Our group very recently showed that sub-MICs of AGs lead to the incorporation of oxidized
14 guanine in DNA, suggesting the occurrence of oxidative stress at concentrations 100-fold
15 below the MIC, a concentration at which no killing is observed. Subsequently, sub-MICs AGs
16 mediate induction of the SOS response mostly through oxidized guanine incorporation in
17 DNA (Baharoglu *et al.*, 2013). Since ROS can damage DNA and proteins and induce
18 mutagenesis (Mcbride *et al.*, 1991, Nunoshiba *et al.*, 1999), we proposed these as the missing
19 links between sub-MIC antibiotic treatment and guanine oxidation.
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23 The link between bactericidal antibiotics, ROS formation and cell death has lately
24 been subject to debate. In order to relieve possible confusion, it may be worth mentioning
25 here that the above-described connection between ROS and SOS happens in conditions where
26 ROS are created in the bacterial cell. For instance, we know that in the presence of sub-MICs
27 of antibiotics ROS are formed in *V. cholerae* and not in *E. coli* (Baharoglu *et al.*, 2013). On
28 the other hand, striking studies presented ROS formation as the key step leading to cell death
29 by β -lactams, FQs and AGs (Kohanski *et al.*, 2007, Kohanski *et al.*, 2010). This study by
30 Kohanski *et al.* suggests that all bactericidal antibiotics, regardless of their cellular target,
31 have the potential to induce ROS formation, which kills bacteria. Subsequent studies
32 challenged this hypothesis as no cell death due to ROS formation occurred using these
33 antibiotics in different experimental conditions (Keren *et al.*, 2013, Liu & Imlay, 2013).
34 Finally, a recent report elegantly demonstrates that in *E. coli* it is not ROS that kill bacteria
35 upon AG treatment but rather increased AG uptake due to differential intracellular iron levels
36 and synthesis of iron-sulfur clusters (Ezraty *et al.*, 2013). In these latter studies (Kohanski *et al.*,
37 2007, Ezraty *et al.*, 2013, Keren *et al.*, 2013, Liu & Imlay, 2013), antibiotics were used at
38 lethal concentrations because the lethality of antibiotics was what the authors wanted to
39 address. Conversely, in the study about ROS formation and SOS (Baharoglu *et al.*, 2013)
40 concentrations 100-fold below the MIC were used. Hence, these studies differ in the fact that
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3 they either address lethality or only SOS induction by antibiotics through ROS formation in
4 non-lethal conditions.

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6 The fact that low doses of antibiotics can induce SOS through ROS formation in
7 certain species like *V. cholerae*, *Klebsiella pneumoniae* and *Photobacterium luminescens*, might
8 indicate that some species overcome their poorly efficient protection system against oxidative
9 stress by being more easily capable of modifying their gene expression patterns (Mcbride *et*
10 *al.*, 1991, Nunoshiwa *et al.*, 1999).

11
12 In the same line of thought, the DinF protein belonging to the SOS regulon was found
13 to be involved in response to oxidative stress in *E. coli* (Rodriguez-Beltran *et al.*, 2012): in
14 fact, expression of DinF protects against bile salts and H₂O₂ by decreasing the effects of ROS
15 and protein carbonylation, showing that SOS and the oxidative stress response can be
16 complementary mechanisms in bacterial survival.

23 24 **3 – General stress.**

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26 SOS and RpoS were suggested to be complementary mechanisms in response to
27 certain stresses. RpoS, the stationary phase sigma factor, is induced in response to various
28 stresses during the exponential growth phase and increases resistance to stress in *E. coli*
29 (Merrick *et al.*, 2009, Merrick *et al.*, 2009). For instance, and similarly to the SOS response,
30 the RpoS regulon can also be induced during oxidative stress as well as cell wall stress (Allen
31 & Griffiths, 2012, Mika *et al.*, 2012). Genes expressed following the induction of the RpoS
32 regulon, namely catalases (KatE, KatG) and iron chelators, protect cells from ROS-related
33 DNA damage such as DSBs, which are SOS inducers. The link between the SOS and RpoS
34 responses lies in the fact that both can be considered as mechanisms providing bacteria with
35 means to survive DNA damage (Shee *et al.*, 2011). Other facts linking SOS and RpoS exist:
36 for example both regulate the *dinB* gene, which encodes the TLS polymerase Pol IV (Kim *et*
37 *al.*, 2001, Layton & Foster, 2003). Antibiotic-induced mutagenesis involves SOS-dependent
38 *dinB* induction (Kohiyama *et al.*, 2013) but also induction mediated by RpoS (Henle & Linn,
39 1997) or NusA (Cohen & Walker, 2010), a component of the RNA polymerase complex that
40 interacts with DinB. In this sense, RpoS and SOS can also be thought of as complementary
41 mechanisms that protect bacteria from DNA damage: when RpoS is not sufficient, SOS takes
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4 – Nutrient stress

A connection between nutrient stress and SOS is also conceivable. For instance, cAMP-dependent SOS induction and mutagenesis was first observed in resting *E. coli* populations (Taddei *et al.*, 1995). cAMP in complex with the CRP protein regulates a set of genes involved in the response to carbon source stress, called carbon catabolite regulation. More recently, it was found that the cAMP-CRP complex represses SOS-mediated mutations in stationary cell cultures (Macphee & Ambrose, 2010). However the mechanism of such a regulation remains to be elucidated. cAMP-CRP-dependent induction of SOS has also been observed in *E. coli* cells starved for arginine, but only when these cells resume growth (Janion *et al.*, 2002). The authors propose here that these cells accumulate DNA damage that induces SOS upon replication in a yet undiscovered mechanism implicating carbon catabolite control.

V – SOS-like responses

As described above, transformation is a mechanism of HGT that relies on ssDNA uptake and processing (Dubnau, 1999). Transformation occurs when a bacterial cell reaches a competent state rendering it capable of taking up external DNA present in its environment. Several mechanisms, such as special growth conditions or stress, lead to the induction of competence for natural transformation in *B. subtilis* and *Streptococcus pneumoniae* (Claverys *et al.*, 2009). Competence has been suggested to be a stress response that could substitute for the SOS response in some bacterial species that lack an SOS regulon but in which the DNA repair genes are part of the competence regulon (for a review see (Charpentier *et al.*, 2012)). For instance in *S. pneumoniae* which lacks LexA, it was found that AGs, FQs and MMC induce competence and RecA, as well as the rate of genetic exchange in response to antibiotics, which is reminiscent of the *E. coli* SOS response (Prudhomme *et al.*, 2006). However, recent data tone down this view: in *S. thermophilus* which carries the LexA-like repressor HdiR, competence and SOS were shown to be antagonistic mechanisms (Boutry *et al.*, 2013). In this bacterium, SOS-inducing agents induce the SOS regulon genes such as RecA but decrease cellular transformability. As a corollary, induction of competence negatively affects DNA repair under these circumstances. In *V. cholerae*, SOS-inducing agents do not induce competence either (Baharoglu *et al.*, 2012). However, DNA uptake during competence does induce SOS. Nevertheless, transformation seems to be induced in response to stress in many bacteria (Charpentier *et al.*, 2012), suggesting it as an alternative to SOS induction in several cases.

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3 An SOS-like response was also reported in *Acinetobacter baumannii*, where DNA
4 damage induces genes involved in response to DNA damage, namely *recA*, TLS polymerases
5 and NER genes (Norton *et al.*, 2013). However, the regulation of this SOS response does not
6 involve LexA, which is absent from the *A. baumannii* genome. Nevertheless, induction of this
7 stress response is responsible for damage repair and increase of mutation frequencies leading
8 to antibiotic resistance acquisition, as it happens for *E. coli* with SOS induction. The increased
9 mutation frequency was recently found to be the result of the *A. baumannii* TLS polymerase
10 UmuDAb which recognizes palindromic sequences at the promoters of 8 different DNA
11 repair genes and TLS polymerases and regulates their expression in a RecA-dependent
12 manner (Aranda *et al.*, 2013).
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20 **VI – Strategies to inhibit SOS in the battle against bacterial** 21 **resistance** 22 23

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25 In the search for compounds that can potentiate the effect of antibiotics on bacteria, an
26 engineered bacteriophage that suppresses the SOS response by over-expressing a non-
27 cleavable LexA repressor has also been reported to enhance killing by quinolones, AGs and
28 β -lactams in *E. coli*, to reduce the number of resistant bacteria that arise from antibiotic
29 treatment and to increase survival of infected mice (Lu & Collins, 2009). According to the
30 authors, these observations would be the result of disabling DNA damage repair. Such phages
31 could be used in combination with antibiotics as adjuvants that suppress SOS induction and
32 sensitize the bacterial cell to DNA damage. Another type of antimicrobial molecule was
33 recently identified as a repressor of RecA expression: the aminocoumarins. The use of these
34 gyrase inhibitors prevented SOS induction and decreased mutation frequency and
35 recombination even in the presence of FQs in *S. aureus* (Schroder *et al.*, 2013). Another
36 approach is based on inhibition of RecA expression by engineered artificial small RNAs that
37 are complementary to the *recA* mRNA (Sharma *et al.*, 2013). The use of such sRNAs resulted
38 in an increased sensitivity of *E. coli* to FQs in laboratory conditions. Finally, RNase E was
39 proposed as a possible target in order to prevent SOS induction because inactivation of RNase
40 E expression in *E. coli* impedes SOS induction by MMC (Manasherob *et al.*, 2012).
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42 A recent review discusses various steps in food processing which trigger SOS induction in
43 bacteria and the impact on food spoilage (van der Veen & Abee, 2011).
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As depicted in this review, SOS is a transient but very strong stress response used by bacteria to increase their chances to adapt to changing environments and to survive. This is synthesized in the graphical abstract. The stress signal can be a genotoxic one, such as during direct exposure to DNA-damaging agents, but it appears here that many stresses causing changes in a given bacterial lifestyle can be the origin of such a stress. SOS induction can thus be seen as a bacterial adaptation mechanism, which has to be tightly controlled by bacteria in order to prevent a decrease of fitness in conditions where it is not needed. It can be conceived to use SOS modulation as a weapon against bacteria, through the adoption of better policies of antibiotic usage and food processing to avoid SOS-dependent adaptation and resistance of bacteria.

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Table 1: LexA box sequences identified across different bacterial species.

Organism	LexA box consensus 5'-3'	Method of determination	References
GRAM negatives			
Beta- and Gamma-proteobacteria	CTGTN ₈ ACAG	<i>in silico</i>	(Erill et al., 2003)
<i>E. coli</i>	TACTGTATATATATACAGTA	<i>in vitro, in vivo</i>	(Fernandez De Henestrosa et al., 2000) (Wade et al., 2005)
Alpha-proteobacteria			
<i>Rhodobacter sphaeroides</i>	GTTCN ₇ GTTC	<i>in vitro</i>	(Fernandez de Henestrosa et al., 1998)
<i>Rhizobium</i> and <i>Agrobacterium</i>	GAACN ₇ GTAC	<i>in vitro, in vivo</i>	(Tapias & Barbe, 1998)
Cyanobacteria	RGTACN ₃ DGTWCB	<i>in vitro</i>	(Mazon et al., 2004)
Spirocheate			

<i>Leptospira interrogans</i>	TTTGN ₅ CAAA	<i>in vitro</i>	(Fonseca et al., 2013)
Acidobacteria			
<i>Acidobacterium capsulatum</i>	GTTCN ₇ GTTC	<i>in vitro, in vivo</i>	(Mazon et al., 2006)
GRAM positives			
Firmicutes			
<i>Bacillus subtilis</i>	CGAACRNRYGTTYC	<i>in vitro, in silico</i>	(Winterling et al., 1998)
Chloroflexi			
<i>Dehalococcoides ethenogenes</i>	GAACN ₄ GTTC	<i>in vitro</i>	(Fernandez de Henestrosa et al., 2002)
Actinobacteria			
<i>Mycobacterium</i>	TCGAACN ₄ GTTCGA	<i>in vivo</i>	(Davis et al., 2002)
Deinococcus			
<i>Deinococcus radiodurans*</i>	CGAACRNRYGTTTCG	<i>algorithm</i>	(Khan et al., 2008)
INTEGRONS			
	ACTGTW ₈ ACAGT	<i>in silico</i>	(Cambray et al., 2011)

*: even though the two LexA paralogues do not appear to play a role in the radioresistance of *D. radiodurans* (Narumi et al., 2001) (Jolivet et al., 2006), several studies point to a role of RecA recruitment at DNA lesions and intracellular LexA levels (Satoh et al., 2006, Satoh et al., 2012). Algorithm-based studies identified a conserved sequence related to the *Bacillus subtilis* LexA box upstream of the known SOS-regulated genes (Khan et al., 2008). Y: C or T; R: A or G; W: A or T; D: not C; B: not A; N: any base.

References

- Aertsen A & Michiels CW (2005) Mrr instigates the SOS response after high pressure stress in *Escherichia coli*. *Mol Microbiol* **58**: 1381-1391.
- Aertsen A, Tesfazgi Mebrhatu M & Michiels CW (2008) Activation of the *Salmonella typhimurium* Mrr protein. *Biochem Biophys Res Commun* **367**: 435-439.
- Al Mamun AA, Gautam S & Humayun MZ (2006) Hypermutagenesis in *mutA* cells is mediated by mistranslational corruption of polymerase, and is accompanied by replication fork collapse. *Mol Microbiol* **62**: 1752-1763.
- Allen KJ & Griffiths MW (2012) Impact of hydroxyl- and superoxide anion-based oxidative stress on logarithmic and stationary phase *Escherichia coli* O157:H7 stress and virulence gene expression. *Food Microbiol* **29**: 141-147.
- Aranda J, Poza M, Shingu-Vazquez M, Cortes P, Boyce JD, Adler B, Barbe J & Bou G (2013) Identification of a DNA-damage-inducible regulon in *Acinetobacter baumannii*. *J Bacteriol*.
- Aravind L, Anand S & Iyer LM (2013) Novel autoproteolytic and DNA-damage sensing components in the bacterial SOS response and oxidized methylcytosine-induced eukaryotic DNA demethylation systems. *Biology direct* **8**: 20.
- Bagdasarian M, Bailone A, Angulo JF, Scholz P, Bagdasarian M & Devoret R (1992) PsiB, an anti-SOS protein, is transiently expressed by the F sex factor during its transmission to an *Escherichia coli* K-12 recipient. *Mol Microbiol* **6**: 885-893.
- Baharoglu Z & Mazel D (2011) *Vibrio cholerae* triggers SOS and mutagenesis in response to a wide range of antibiotics: a route towards multiresistance. *Antimicrob Agents Chemother* **55**: 2438-2441.

- 1
2
3 Baharoglu Z, Bikard D & Mazel D (2010) Conjugative DNA transfer induces the bacterial
4 SOS response and promotes antibiotic resistance development through integron activation.
5 *PLoS Genet* **6**: e1001165.
- 6 Baharoglu Z, Krin E & Mazel D (2012) Transformation-induced SOS regulation and carbon
7 catabolite control of the *V. cholerae* integron integrase: connecting environment and genome
8 plasticity. *J Bacteriol.*
- 9 Baharoglu Z, Krin E & Mazel D (2013) RpoS Plays a Central Role in the SOS Induction by
10 Sub-Lethal Aminoglycoside Concentrations in *Vibrio cholerae*. *PLoS Genetics* **9(4)**:
11 **e1003421. doi:10.1371/journal.pgen.1003421.**
- 12 Baharoglu Z, Babosan A & Mazel D (2013) Identification of genes involved in low
13 aminoglycoside-induced SOS response in *Vibrio cholerae*: a role for transcription stalling and
14 Mfd helicase. *Nucleic Acids Res.*
- 15 Baharoglu Z, Lestini R, Duigou S & Michel B (2010) RNA polymerase mutations that
16 facilitate replication progression in the rep uvrD recF mutant lacking two accessory
17 replicative helicases. *Mol Microbiol.*
- 18 Balashov S & Humayun MZ (2002) Mistranslation induced by streptomycin provokes a
19 RecABC/RuvABC-dependent mutator phenotype in *Escherichia coli* cells. *J Mol Biol* **315**:
20 513-527.
- 21 Beaber JW, Hochhut B & Waldor MK (2004) SOS response promotes horizontal
22 dissemination of antibiotic resistance genes. *Nature* **427**: 72-74.
- 23 Belov OV, Chuluunbaatar O, Kapralov MI & Sweilam NH (2013) The role of the bacterial
24 mismatch repair system in SOS-induced mutagenesis: a theoretical background. *Journal of*
25 *theoretical biology* **332**: 30-41.
- 26 Bernard P & Couturier M (1992) Cell killing by the F plasmid CcdB protein involves
27 poisoning of DNA-topoisomerase II complexes. *J Mol Biol* **226**: 735-745.
- 28 Boles BR & Singh PK (2008) Endogenous oxidative stress produces diversity and
29 adaptability in biofilm communities. *Proc Natl Acad Sci U S A* **105**: 12503-12508.
- 30 Boubakri H, de Septenville AL, Viguera E & Michel B (2010) The helicases DinG, Rep and
31 UvrD cooperate to promote replication across transcription units in vivo. *EMBO J* **29**: 145-
32 157.
- 33 Boutry C, Delplace B, Clippe A, Fontaine L & Hols P (2013) SOS response activation and
34 competence development are antagonistic mechanisms in *Streptococcus thermophilus*. *J*
35 *Bacteriol* **195**: 696-707.
- 36 Butala M, Zgur-Bertok D & Busby SJ (2009) The bacterial LexA transcriptional repressor.
37 *Cellular and molecular life sciences : CMLS* **66**: 82-93.
- 38 Butala M, Klose D, Hodnik V, Rems A, Podlesek Z, Klare JP, Anderluh G, Busby SJ,
39 Steinhoff HJ & Zgur-Bertok D (2011) Interconversion between bound and free conformations
40 of LexA orchestrates the bacterial SOS response. *Nucleic Acids Res* **39**: 6546-6557.
- 41 Cambray G, Sanchez-Alberola N, Campoy S, Guerin E, Da Re S, Gonzalez-Zorn B, Ploy MC,
42 Barbe J, Mazel D & Erill I (2011) Prevalence of SOS-mediated control of integron integrase
43 expression as an adaptive trait of chromosomal and mobile integrons. *Mob DNA* **2**: 6.
- 44 Centore RC, Leeson MC & Sandler SJ (2008) UvrD303, a hyper-helicase mutant that
45 antagonizes RecA-dependent SOS expression by a mechanism that depends on its C-terminus.
46 *J Bacteriol.*
- 47 Charpentier X, Polard P & Claverys JP (2012) Induction of competence for genetic
48 transformation by antibiotics: convergent evolution of stress responses in distant bacterial
49 species lacking SOS? *Curr Opin Microbiol* **15**: 570-576.
- 50 Cirz RT & Romesberg FE (2006) Induction and inhibition of ciprofloxacin resistance-
51 conferring mutations in hypermutator bacteria. *Antimicrob Agents Chemother* **50**: 220-225.

- 1
2
3 Claverys JP, Martin B & Polard P (2009) The genetic transformation machinery: composition,
4 localization, and mechanism. *FEMS Microbiol Rev* **33**: 643-656.
- 5 Cohen SE & Walker GC (2010) The transcription elongation factor NusA is required for
6 stress-induced mutagenesis in *Escherichia coli*. *Curr Biol* **20**: 80-85.
- 7 Courcelle J, Khodursky A, Peter B, Brown PO & Hanawalt PC (2001) Comparative gene
8 expression profiles following UV exposure in wild-type and SOS-deficient *Escherichia coli*.
9 *Genetics* **158**: 41-64.
- 10 Czyz A, Wrobel B & Wegrzyn G (2000) *Vibrio harveyi* bioluminescence plays a role in
11 stimulation of DNA repair. *Microbiology* **146 (Pt 2)**: 283-288.
- 12 Czyz A, Plata K & Wegrzyn G (2003) Stimulation of DNA repair as an evolutionary drive for
13 bacterial luminescence. *Luminescence* **18**: 140-144.
- 14 Da Re S, Garnier F, Guerin E, Campoy S, Denis F & Ploy MC (2009) The SOS response
15 promotes qnrB quinolone-resistance determinant expression. *EMBO Rep* **10**: 929-933.
- 16 Daly MJ (2009) A new perspective on radiation resistance based on *Deinococcus radiodurans*.
17 *Nat Rev Microbiol* **7**: 237-245.
- 18 Davis EO, Dullaghan EM & Rand L (2002) Definition of the mycobacterial SOS box and use
19 to identify LexA-regulated genes in *Mycobacterium tuberculosis*. *J Bacteriol* **184**: 3287-3295.
- 20 De Septenville AL, Duigou S, Boubakri H & Michel B (2012) Replication fork reversal after
21 replication-transcription collision. *PLoS Genet* **8**: e1002622.
- 22 Deaconescu AM, Chambers AL, Smith AJ, Nickels BE, Hochschild A, Savery NJ & Darst
23 SA (2006) Structural basis for bacterial transcription-coupled DNA repair. *Cell* **124**: 507-520.
- 24 Delmas S & Matic I (2005) Cellular response to horizontally transferred DNA in *Escherichia*
25 *coli* is tuned by DNA repair systems. *DNA Repair (Amst)* **4**: 221-229.
- 26 Denamur E, Tenaillon O, Deschamps C, Skurnik D, Ronco E, Gaillard JL, Picard B, Branger
27 C & Matic I (2005) Intermediate mutation frequencies favor evolution of multidrug resistance
28 in *Escherichia coli*. *Genetics* **171**: 825-827.
- 29 Didier JP, Villet R, Huggler E, Lew DP, Hooper DC, Kelley WL & Vaudaux P (2011) Impact
30 of ciprofloxacin exposure on *Staphylococcus aureus* genomic alterations linked with
31 emergence of rifampin resistance. *Antimicrob Agents Chemother* **55**: 1946-1952.
- 32 Dorr T, Lewis K & Vulic M (2009) SOS response induces persistence to fluoroquinolones in
33 *Escherichia coli*. *PLoS Genet* **5**: e1000760.
- 34 Dorr T, Vulic M & Lewis K (2010) Ciprofloxacin causes persister formation by inducing the
35 TisB toxin in *Escherichia coli*. *PLoS biology* **8**: e1000317.
- 36 Dubnau D (1999) DNA uptake in bacteria. *Annu Rev Microbiol* **53**: 217-244.
- 37 Dukan S, Farewell A, Ballesteros M, Taddei F, Radman M & Nystrom T (2000) Protein
38 oxidation in response to increased transcriptional or translational errors. *Proc Natl Acad Sci U*
39 *S A* **97**: 5746-5749.
- 40 Dutta D, Shatalin K, Epshtein V, Gottesman ME & Nudler E (2011) Linking RNA
41 polymerase backtracking to genome instability in *E. coli*. *Cell* **146**: 533-543.
- 42 Dwyer DJ, Kohanski MA & Collins JJ (2009) Role of reactive oxygen species in antibiotic
43 action and resistance. *Curr Opin Microbiol* **12**: 482-489.
- 44 Dwyer DJ, Kohanski MA, Hayete B & Collins JJ (2007) Gyrase inhibitors induce an
45 oxidative damage cellular death pathway in *Escherichia coli*. *Mol Syst Biol* **3**: 91.
- 46 Erill I, Campoy S & Barbe J (2007) Aeons of distress: an evolutionary perspective on the
47 bacterial SOS response. *FEMS Microbiol Rev* **31**: 637-656.
- 48 Erill I, Escribano M, Campoy S & Barbe J (2003) In silico analysis reveals substantial
49 variability in the gene contents of the gamma proteobacteria LexA-regulon. *Bioinformatics*
50 **19**: 2225-2236.
- 51
52
53
54
55
56
57
58
59
60

- 1
2
3 Ezraty B, Vergnes A, Banzhaf M, *et al.* (2013) Fe-S cluster biosynthesis controls uptake of
4 aminoglycosides in a ROS-less death pathway. *Science* **340**: 1583-1587.
- 5 Farr SB & Kogoma T (1991) Oxidative Stress Responses in Escherichia-Coli and Salmonella-
6 Typhimurium. *Microbiological Reviews* **55**: 561-585.
- 7 Fernandez de Henestrosa AR, Rivera E, Tapias A & Barbe J (1998) Identification of the
8 Rhodobacter sphaeroides SOS box. *Mol Microbiol* **28**: 991-1003.
- 9 Fernandez de Henestrosa AR, Cune J, Erill I, Magnuson JK & Barbe J (2002) A green
10 nonsulfur bacterium, Dehalococcoides ethenogenes, with the LexA binding sequence found in
11 gram-positive organisms. *J Bacteriol* **184**: 6073-6080.
- 12 Fernandez De Henestrosa AR, Ogi T, Aoyagi S, Chafin D, Hayes JJ, Ohmori H & Woodgate
13 R (2000) Identification of additional genes belonging to the LexA regulon in Escherichia coli.
14 *Mol Microbiol* **35**: 1560-1572.
- 15 Fick J, Soderstrom H, Lindberg RH, Phan C, Tysklind M & Larsson DG (2009)
16 Contamination of surface, ground, and drinking water from pharmaceutical production.
17 *Environ Toxicol Chem* **28**: 2522-2527.
- 18 Flores MJ, Sanchez N & Michel B (2005) A fork-clearing role for UvrD. *Mol Microbiol* **57**:
19 1664-1675.
- 20 Fonseca LS, da Silva JB, Milanez JS, Monteiro-Vitorello CB, Momo L, de Moraes ZM,
21 Vasconcellos SA, Marques MV, Ho PL & da Costa RM (2013) Leptospira interrogans serovar
22 copenhageni harbors two lexA genes involved in SOS response. *PLoS One* **8**: e76419.
- 23 Foti JJ, Devadoss B, Winkler JA, Collins JJ & Walker GC (2012) Oxidation of the guanine
24 nucleotide pool underlies cell death by bactericidal antibiotics. *Science* **336**: 315-319.
- 25 Friedberg EC, Wagner R & Radman M (2002) Specialized DNA polymerases, cellular
26 survival, and the genesis of mutations. *Science* **296**: 1627-1630.
- 27 Fujii S, Isogawa A & Fuchs RP (2006) RecFOR proteins are essential for Pol V-mediated
28 translesion synthesis and mutagenesis. *EMBO J* **25**: 5754-5763.
- 29 Gan W, Guan Z, Liu J, Gui T, Shen K, Manley JL & Li X (2011) R-loop-mediated genomic
30 instability is caused by impairment of replication fork progression. *Genes Dev* **25**: 2041-2056.
- 31 Ghigo JM (2001) Natural conjugative plasmids induce bacterial biofilm development. *Nature*
32 **412**: 442-445.
- 33 Gibson JL, Lombardo MJ, Thornton PC, *et al.* (2010) The sigma(E) stress response is
34 required for stress-induced mutation and amplification in Escherichia coli. *Mol Microbiol* **77**:
35 415-430.
- 36 Gotoh H, Kasaraneni N, Devineni N, Dallo SF & Weitao T (2010) SOS involvement in stress-
37 inducible biofilm formation. *Biofouling* **26**: 603-611.
- 38 Guerin E, Cambray G, Sanchez-Alberola N, Campoy S, Erill I, Da Re S, Gonzalez-Zorn B,
39 Barbe J, Ploy MC & Mazel D (2009) The SOS response controls integron recombination.
40 *Science* **324**: 1034.
- 41 Gullberg E, Cao S, Berg OG, Ilback C, Sandegren L, Hughes D & Andersson DI (2011)
42 Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathog* **7**:
43 e1002158.
- 44 Gustafsson I, Sjolund M, Torell E, Johannesson M, Engstrand L, Cars O & Andersson DI
45 (2003) Bacteria with increased mutation frequency and antibiotic resistance are enriched in
46 the commensal flora of patients with high antibiotic usage. *J Antimicrob Chemother* **52**: 645-
47 650.
- 48 Gutierrez A, Laureti L, Crussard S, *et al.* (2013) beta-lactam antibiotics promote bacterial
49 mutagenesis via an RpoS-mediated reduction in replication fidelity. *Nature communications*
50 **4**: 1610.
- 51
52
53
54
55
56
57
58
59
60

- 1
2
3 Haggard BE & Bartsch LD (2009) Net changes in antibiotic concentrations downstream from
4 an effluent discharge. *J Environ Qual* **38**: 343-352.
- 5 Hammer BK & Bassler BL (2003) Quorum sensing controls biofilm formation in *Vibrio*
6 *cholerae*. *Mol Microbiol* **50**: 101-104.
- 7 Han J, Sahin O, Barton YW & Zhang Q (2008) Key role of Mfd in the development of
8 fluoroquinolone resistance in *Campylobacter jejuni*. *PLoS Pathog* **4**: e1000083.
- 9 Handel N, Schuurmans JM, Brul S & Ter Kuile BH (2013) Compensation of the Metabolic
10 Costs of Antibiotic Resistance by Physiological Adaptation in *Escherichia coli*. *Antimicrob*
11 *Agents Chemother* **57**: 3752-3762.
- 12 Helmrich A, Ballarino M & Tora L (2011) Collisions between replication and transcription
13 complexes cause common fragile site instability at the longest human genes. *Mol Cell* **44**:
14 966-977.
- 15 Helmrich A, Ballarino M, Nudler E & Tora L (2013) Transcription-replication encounters,
16 consequences and genomic instability. *Nature structural & molecular biology* **20**: 412-418.
- 17 Henle ES & Linn S (1997) Formation, prevention, and repair of DNA damage by
18 iron/hydrogen peroxide. *J Biol Chem* **272**: 19095-19098.
- 19 Henle ES, Han Z, Tang N, Rai P, Luo Y & Linn S (1999) Sequence-specific DNA cleavage
20 by Fe²⁺-mediated fenton reactions has possible biological implications. *J Biol Chem* **274**:
21 962-971.
- 22 Hocquet D & Bertrand X (2013) Metronidazole increases the emergence of ciprofloxacin- and
23 amikacin-resistant *Pseudomonas aeruginosa* by inducing the SOS response. *J Antimicrob*
24 *Chemother*.
- 25 Hocquet D, Llanes C, Thouverez M, Kulasekara HD, Bertrand X, Plesiat P, Mazel D & Miller
26 SI (2012) Evidence for induction of integron-based antibiotic resistance by the SOS response
27 in a clinical setting. *PLoS Pathog* **8**: e1002778.
- 28 Hoffman LR, D'Argenio DA, MacCoss MJ, Zhang Z, Jones RA & Miller SI (2005)
29 Aminoglycoside antibiotics induce bacterial biofilm formation. *Nature* **436**: 1171-1175.
- 30 Hughes D & Andersson DI (2012) Selection of resistance at lethal and non-lethal antibiotic
31 concentrations. *Curr Opin Microbiol* **15**: 555-560.
- 32 Husain I, Chaney SG & Sancar A (1985) Repair of cis-platinum-DNA adducts by ABC
33 excinuclease in vivo and in vitro. *J Bacteriol* **163**: 817-823.
- 34 Ide H, Kow YW & Wallace SS (1985) Thymine glycols and urea residues in M13 DNA
35 constitute replicative blocks in vitro. *Nucleic Acids Res* **13**: 8035-8052.
- 36 Imlay JA (2003) Pathways of oxidative damage. *Annu Rev Microbiol* **57**: 395-418.
- 37 Janion C, Sikora A, Nowosielska A & Grzesiuk E (2002) Induction of the SOS response in
38 starved *Escherichia coli*. *Environ Mol Mutagen* **40**: 129-133.
- 39 Jena NR (2012) DNA damage by reactive species: Mechanisms, mutation and repair. *J Biosci*
40 **37**: 503-517.
- 41 Jolivet E, Lecointe F, Coste G, Satoh K, Narumi I, Bailone A & Sommer S (2006) Limited
42 concentration of RecA delays DNA double-strand break repair in *Deinococcus radiodurans*
43 R1. *Mol Microbiol* **59**: 338-349.
- 44 Jove T, Da Re S, Denis F, Mazel D & Ploy MC (2010) Inverse correlation between promoter
45 strength and excision activity in class 1 integrons. *PLoS Genet* **6**: e1000793.
- 46 Kaplan JB (2011) Antibiotic-induced biofilm formation. *Int J Artif Organs* **34**: 737-751.
- 47 Kaplan JB, Jabbouri S & Sadovskaya I (2011) Extracellular DNA-dependent biofilm
48 formation by *Staphylococcus epidermidis* RP62A in response to subminimal inhibitory
49 concentrations of antibiotics. *Res Microbiol* **162**: 535-541.
- 50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 Karoui H, Bex F, Dreze P & Couturier M (1983) Ham22, a mini-F mutation which is lethal to
4 host cell and promotes recA-dependent induction of lambdoid prophage. *EMBO J* **2**: 1863-
5 1868.
- 6 Keaton MA, Rosato RR, Plata KB, Singh CR & Rosato AE (2013) Exposure of Clinical
7 MRSA Heterogeneous Strains to beta-Lactams Redirects Metabolism to Optimize Energy
8 Production through the TCA Cycle. *PLoS One* **8**: e71025.
- 9 Kelley WL (2006) Lex marks the spot: the virulent side of SOS and a closer look at the LexA
10 regulon. *Mol Microbiol* **62**: 1228-1238.
- 11 Keren I, Wu Y, Inocencio J, Mulcahy LR & Lewis K (2013) Killing by bactericidal
12 antibiotics does not depend on reactive oxygen species. *Science* **339**: 1213-1216.
- 13 Keyamura K, Sakaguchi C, Kubota Y, Niki H & Hishida T (2013) RecA recruits SMC-like
14 RecN to DNA double-strand breaks. *J Biol Chem*.
- 15 Keyer K & Imlay JA (1996) Superoxide accelerates DNA damage by elevating free-iron
16 levels. *Proc Natl Acad Sci U S A* **93**: 13635-13640.
- 17 Khan F, Singh SP & Mishra BN (2008) Conservation of the LexA repressor binding site in
18 *Deinococcus radiodurans*. *Journal of integrative bioinformatics* **5**.
- 19 Kim SR, Matsui K, Yamada M, Gruz P & Nohmi T (2001) Roles of chromosomal and
20 episomal dinB genes encoding DNA pol IV in targeted and untargeted mutagenesis in
21 *Escherichia coli*. *Mol Genet Genomics* **266**: 207-215.
- 22 Kimsey HH & Waldor MK (2009) *Vibrio cholerae* LexA coordinates CTX prophage gene
23 expression. *J Bacteriol* **191**: 6788-6795.
- 24 Ko KC, Tai PC & Derby CD (2012) Mechanisms of action of escapin, a bactericidal agent in
25 the ink secretion of the sea hare *Aplysia californica*: rapid and long-lasting DNA condensation
26 and involvement of the OxyR-regulated oxidative stress pathway. *Antimicrob Agents*
27 *Chemother* **56**: 1725-1734.
- 28 Kohanski MA, Dwyer DJ & Collins JJ (2010) How antibiotics kill bacteria: from targets to
29 networks. *Nat Rev Microbiol* **8**: 423-435.
- 30 Kohanski MA, DePristo MA & Collins JJ (2010) Sublethal antibiotic treatment leads to
31 multidrug resistance via radical-induced mutagenesis. *Mol Cell* **37**: 311-320.
- 32 Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA & Collins JJ (2007) A common
33 mechanism of cellular death induced by bactericidal antibiotics. *Cell* **130**: 797-810.
- 34 Kohiyama M, Contremoulins V & Baudin X (2013) Trashing of single-stranded DNA
35 generated during processing of arrested replication fork in *E. coli*. *J Mol Biol*.
- 36 Kovacic L, Paulic N, Leonardi A, Hodnik V, Anderluh G, Podlesek Z, Zgur-Bertok D, Krizaj
37 I & Butala M (2013) Structural insight into LexA-RecA* interaction. *Nucleic acids research*.
- 38 Krisko A & Radman M (2010) Protein damage and death by radiation in *Escherichia coli* and
39 *Deinococcus radiodurans*. *Proc Natl Acad Sci U S A* **107**: 14373-14377.
- 40 Kummerer K (2009) Antibiotics in the aquatic environment--a review--part II. *Chemosphere*
41 **75**: 435-441.
- 42 Kummerer K (2009) Antibiotics in the aquatic environment--a review--part I. *Chemosphere*
43 **75**: 417-434.
- 44 Kumura K, Sekiguchi M, Steinum AL & Seeberg E (1985) Stimulation of the UvrABC
45 enzyme-catalyzed repair reactions by the UvrD protein (DNA helicase II). *Nucleic Acids Res*
46 **13**: 1483-1492.
- 47 Layton JC & Foster PL (2003) Error-prone DNA polymerase IV is controlled by the stress-
48 response sigma factor, RpoS, in *Escherichia coli*. *Mol Microbiol* **50**: 549-561.
- 49 Lee GH, Jeong JY, Chung JW, Nam WH, Lee SM, Pak JH, Choi KD, Song HJ, Jung HY &
50 Kim JH (2009) The *Helicobacter pylori* Mfd protein is important for antibiotic resistance and
51 DNA repair. *Diagn Microbiol Infect Dis* **65**: 454-456.
- 52
53
54
55
56
57
58
59
60

- 1
2
3 Lepplae R, Geeraerts D, Hallez R, Guglielmini J, Dreze P & Van Melderen L (2011) Diversity
4 of bacterial type II toxin-antitoxin systems: a comprehensive search and functional analysis of
5 novel families. *Nucleic Acids Res* **39**: 5513-5525.
- 6 Lestini R & Michel B (2007) UvrD controls the access of recombination proteins to blocked
7 replication forks. *EMBO J* **26**: 3804-3814.
- 8 Levesque C, Brassard S, Lapointe J & Roy PH (1994) Diversity and relative strength of
9 tandem promoters for the antibiotic-resistance genes of several integrons. *Gene* **142**: 49-54.
- 10 Lewin CS & Amyes SG (1991) The role of the SOS response in bacteria exposed to
11 zidovudine or trimethoprim. *Journal of medical microbiology* **34**: 329-332.
- 12 Lewis K (2010) Persister Cells. *Annu Rev Microbiol*.
- 13 Liu Y & Imlay JA (2013) Cell death from antibiotics without the involvement of reactive
14 oxygen species. *Science* **339**: 1210-1213.
- 15 Liu YC, Huang WK, Huang TS & Kunin CM (1999) Extent of antibiotic use in Taiwan
16 shown by antimicrobial activity in urine. *Lancet* **354**: 1360.
- 17 Liu YC, Huang WK, Huang TS & Kunin CM (1999) Detection of antimicrobial activity in
18 urine for epidemiologic studies of antibiotic use. *J Clin Epidemiol* **52**: 539-545.
- 19 Lopez E & Blazquez J (2009) Effect of subinhibitory concentrations of antibiotics on
20 intrachromosomal homologous recombination in *Escherichia coli*. *Antimicrob Agents*
21 *Chemother* **53**: 3411-3415.
- 22 Lu AL, Li X, Gu Y, Wright PM & Chang DY (2001) Repair of oxidative DNA damage:
23 mechanisms and functions. *Cell Biochem Biophys* **35**: 141-170.
- 24 Lu TK & Collins JJ (2009) Engineered bacteriophage targeting gene networks as adjuvants
25 for antibiotic therapy. *Proc Natl Acad Sci U S A* **106**: 4629-4634.
- 26 Lusetti SL, Drees JC, Stohl EA, Seifert HS & Cox MM (2004) The DinI and RecX proteins
27 are competing modulators of RecA function. *J Biol Chem* **279**: 55073-55079.
- 28 Macphee DG & Ambrose M (2010) Catabolite repression of SOS-dependent and SOS-
29 independent spontaneous mutagenesis in stationary-phase *Escherichia coli*. *Mutat Res* **686**:
30 84-89.
- 31 Maiques E, Ubeda C, Campoy S, Salvador N, Lasa I, Novick RP, Barbe J & Penades JR
32 (2006) beta-lactam antibiotics induce the SOS response and horizontal transfer of virulence
33 factors in *Staphylococcus aureus*. *J Bacteriol* **188**: 2726-2729.
- 34 Manasherob R, Miller C, Kim KS & Cohen SN (2012) Ribonuclease E modulation of the
35 bacterial SOS response. *PLoS One* **7**: e38426.
- 36 Masse E & Drolet M (1999) *Escherichia coli* DNA topoisomerase I inhibits R-loop formation
37 by relaxing transcription-induced negative supercoiling. *J Biol Chem* **274**: 16659-16664.
- 38 Massoni SC, Leeson MC, Long JE, Gemme K, Mui A & Sandler SJ (2012) Factors limiting
39 SOS expression in log-phase cells of *Escherichia coli*. *J Bacteriol* **194**: 5325-5333.
- 40 Mazel D (2006) Integrons: agents of bacterial evolution. *Nat Rev Microbiol* **4**: 608-620.
- 41 Mazon G, Campoy S, Erill I & Barbe J (2006) Identification of the *Acidobacterium*
42 capsulatum LexA box reveals a lateral acquisition of the Alphaproteobacteria *lexA* gene.
43 *Microbiology* **152**: 1109-1118.
- 44 Mazon G, Lucena JM, Campoy S, Fernandez de Henestrosa AR, Candau P & Barbe J (2004)
45 LexA-binding sequences in Gram-positive and cyanobacteria are closely related. *Mol Genet*
46 *Genomics* **271**: 40-49.
- 47 Mazon G, Erill I, Campoy S, Cortes P, Forano E & Barbe J (2004) Reconstruction of the
48 evolutionary history of the LexA-binding sequence. *Microbiology* **150**: 3783-3795.
- 49 McBride TJ, Preston BD & Loeb LA (1991) Mutagenic Spectrum Resulting from DNA
50 Damage by Oxygen Radicals. *Biochemistry* **30**: 207-213.
- 51
52
53
54
55
56
57
58
59
60

- 1
2
3 McGlynn P, Savery NJ & Dillingham MS (2012) The conflict between DNA replication and
4 transcription. *Mol Microbiol* **85**: 12-20.
- 5 McVeigh RR & Yasbin RE (1996) Phenotypic differentiation of "smart" versus "naive"
6 bacteriophages of *Bacillus subtilis*. *J Bacteriol* **178**: 3399-3401.
- 7 Merrikh H, Ferrazzoli AE & Lovett ST (2009) Growth phase and (p)ppGpp control of IraD, a
8 regulator of RpoS stability, in *Escherichia coli*. *J Bacteriol* **191**: 7436-7446.
- 9 Merrikh H, Zhang Y, Grossman AD & Wang JD (2012) Replication-transcription conflicts in
10 bacteria. *Nat Rev Microbiol* **10**: 449-458.
- 11 Merrikh H, Ferrazzoli AE, Bougdour A, Olivier-Mason A & Lovett ST (2009) A DNA
12 damage response in *Escherichia coli* involving the alternative sigma factor, RpoS. *Proc Natl*
13 *Acad Sci U S A* **106**: 611-616.
- 14 Mesak LR & Davies J (2009) Phenotypic changes in ciprofloxacin-resistant *Staphylococcus*
15 *aureus*. *Res Microbiol* **160**: 785-791.
- 16 Mesak LR, Miao V & Davies J (2008) Effects of subinhibitory concentrations of antibiotics
17 on SOS and DNA repair gene expression in *Staphylococcus aureus*. *Antimicrob Agents*
18 *Chemother* **52**: 3394-3397.
- 19 Michel B (2005) After 30 years of study, the bacterial SOS response still surprises us. *PLoS*
20 *biology* **3**: e255.
- 21 Mika F, Busse S, Possling A, Berkholz J, Tschowri N, Sommerfeldt N, Pruteanu M &
22 Hengge R (2012) Targeting of *csgD* by the small regulatory RNA RprA links stationary
23 phase, biofilm formation and cell envelope stress in *Escherichia coli*. *Mol Microbiol*.
24 Miller C, Ingmer H, Thomsen LE, Skarstad K & Cohen SN (2003) DpiA binding to the
25 replication origin of *Escherichia coli* plasmids and chromosomes destabilizes plasmid
26 inheritance and induces the bacterial SOS response. *J Bacteriol* **185**: 6025-6031.
- 27 Miller C, Thomsen LE, Gaggero C, Mosseri R, Ingmer H & Cohen SN (2004) SOS response
28 induction by beta-lactams and bacterial defense against antibiotic lethality. *Science* **305**:
29 1629-1631.
- 30 Mori T, Nakamura T, Okazaki N, Furukohri A, Maki H & Akiyama MT (2012) *Escherichia*
31 *coli* DinB inhibits replication fork progression without significantly inducing the SOS
32 response. *Genes & genetic systems* **87**: 75-87.
- 33 Napolitano R, Janel-Bintz R, Wagner J & Fuchs RP (2000) All three SOS-inducible DNA
34 polymerases (Pol II, Pol IV and Pol V) are involved in induced mutagenesis. *EMBO J* **19**:
35 6259-6265.
- 36 Narumi I, Satoh K, Kikuchi M, Funayama T, Yanagisawa T, Kobayashi Y, Watanabe H &
37 Yamamoto K (2001) The LexA protein from *Deinococcus radiodurans* is not involved in
38 RecA induction following gamma irradiation. *J Bacteriol* **183**: 6951-6956.
- 39 Nassar FJ, Rahal EA, Sabra A & Matar GM (2013) Effects of Subinhibitory Concentrations
40 of Antimicrobial Agents on *Escherichia coli* O157:H7 Shiga Toxin Release and Role of the
41 SOS Response. *Foodborne pathogens and disease*.
- 42 Ni M, Yang L, Liu XL & Qi O (2008) Fluence-response dynamics of the UV-induced SOS
43 response in *Escherichia coli*. *Curr Microbiol* **57**: 521-526.
- 44 Nohmi T, Battista JR, Dodson LA & Walker GC (1988) RecA-mediated cleavage activates
45 UmuD for mutagenesis: mechanistic relationship between transcriptional derepression and
46 posttranslational activation. *Proc Natl Acad Sci U S A* **85**: 1816-1820.
- 47 Norton MD, Spilka AJ & Godoy VG (2013) Antibiotic resistance acquired through a DNA
48 damage-inducible response in *Acinetobacter baumannii*. *J Bacteriol* **195**: 1335-1345.
- 49 Nunoshiro T, Obata F, Boss AC, Oikawa S, Mori T, Kawanishi S & Yamamoto E (1999)
50 Role of iron and superoxide for generation of hydroxyl radical, oxidative DNA lesions, and
51 mutagenesis in *Escherichia coli*. *Journal of Biological Chemistry* **274**: 34832-34837.
- 52
53
54
55
56
57
58
59
60

- 1
2
3 Paez PL, Becerra MC & Albesa I (2011) Comparison of macromolecular oxidation by
4 reactive oxygen species in three bacterial genera exposed to different antibiotics. *Cell*
5 *Biochem Biophys* **61**: 467-472.
- 6 Pages V & Fuchs RP (2003) Uncoupling of leading- and lagging-strand DNA replication
7 during lesion bypass in vivo. *Science* **300**: 1300-1303.
- 8 Pages V, Koffel-Schwartz N & Fuchs RP (2003) recX, a new SOS gene that is co-transcribed
9 with the recA gene in Escherichia coli. *DNA Repair (Amst)* **2**: 273-284.
- 10 Pandey DP & Gerdes K (2005) Toxin-antitoxin loci are highly abundant in free-living but lost
11 from host-associated prokaryotes. *Nucleic Acids Res* **33**: 966-976.
- 12 Partridge SR, Tsafnat G, Coiera E & Iredell JR (2009) Gene cassettes and cassette arrays in
13 mobile resistance integrons. *FEMS Microbiol Rev* **33**: 757-784.
- 14 Pennington JM & Rosenberg SM (2007) Spontaneous DNA breakage in single living
15 Escherichia coli cells. *Nature genetics* **39**: 797-802.
- 16 Petrova V, Chitteni-Pattu S, Drees JC, Inman RB & Cox MM (2009) An SOS Inhibitor that
17 Binds to Free RecA Protein: The PsiB Protein. *Mol Cell* **36**: 121-130.
- 18 Plata KB, Riosa S, Singh CR, Rosato RR & Rosato AE (2013) Targeting of PBP1 by beta-
19 lactams determines recA/SOS response activation in heterogeneous MRSA clinical strains.
20 *PLoS One* **8**: e61083.
- 21 Pohlhaus JR & Kreuzer KN (2005) Norfloxacin-induced DNA gyrase cleavage complexes
22 block Escherichia coli replication forks, causing double-stranded breaks in vivo. *Mol*
23 *Microbiol* **56**: 1416-1429.
- 24 Potenski CJ & Klein HL (2011) R we there yet? R-loop hazards to finishing the journey. *Mol*
25 *Cell* **44**: 848-850.
- 26 Proshkin S, Rahmouni AR, Mironov A & Nudler E (2010) Cooperation between translating
27 ribosomes and RNA polymerase in transcription elongation. *Science* **328**: 504-508.
- 28 Prudhomme M, Attaiech L, Sanchez G, Martin B & Claverys JP (2006) Antibiotic stress
29 induces genetic transformability in the human pathogen Streptococcus pneumoniae. *Science*
30 **313**: 89-92.
- 31 Quinones M, Kimsey HH & Waldor MK (2005) LexA cleavage is required for CTX prophage
32 induction. *Mol Cell* **17**: 291-300.
- 33 Radman M (1975) SOS repair hypothesis: phenomenology of an inducible DNA repair which
34 is accompanied by mutagenesis. *Basic life sciences* **5A**: 355-367.
- 35 Rai P, Cole TD, Wemmer DE & Linn S (2001) Localization of Fe(2+) at an RTGR sequence
36 within a DNA duplex explains preferential cleavage by Fe(2+) and H2O2. *J Mol Biol* **312**:
37 1089-1101.
- 38 Rastogi RP, Richa, Kumar A, Tyagi MB & Sinha RP (2010) Molecular mechanisms of
39 ultraviolet radiation-induced DNA damage and repair. *Journal of nucleic acids* **2010**: 592980.
- 40 Roberts J & Park JS (2004) Mfd, the bacterial transcription repair coupling factor:
41 translocation, repair and termination. *Curr Opin Microbiol* **7**: 120-125.
- 42 Rodriguez-Beltran J, Rodriguez-Rojas A, Guelfo JR, Couce A & Blazquez J (2012) The
43 Escherichia coli SOS gene dinF protects against oxidative stress and bile salts. *PLoS One* **7**:
44 e34791.
- 45 Ronen M, Rosenberg R, Shraiman BI & Alon U (2002) Assigning numbers to the arrows:
46 parameterizing a gene regulation network by using accurate expression kinetics. *Proc Natl*
47 *Acad Sci U S A* **99**: 10555-10560.
- 48 Satoh K, Ohba H, Sghaier H & Narumi I (2006) Down-regulation of radioresistance by
49 LexA2 in Deinococcus radiodurans. *Microbiology* **152**: 3217-3226.
- 50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 Satoh K, Kikuchi M, Ishaque AM, Ohba H, Yamada M, Tejima K, Onodera T & Narumi I
4 (2012) The role of *Deinococcus radiodurans* RecFOR proteins in homologous recombination.
5 *DNA Repair (Amst)* **11**: 410-418.
- 6 Savery NJ (2007) The molecular mechanism of transcription-coupled DNA repair. *Trends*
7 *Microbiol* **15**: 326-333.
- 8 Schaich KM & Borg DC (1988) Fenton reactions in lipid phases. *Lipids* **23**: 570-579.
- 9 Schroder W, Goerke C & Wolz C (2013) Opposing effects of aminocoumarins and
10 fluoroquinolones on the SOS response and adaptability in *Staphylococcus aureus*. *J*
11 *Antimicrob Chemother* **68**: 529-538.
- 12 Sharma V, Sakai Y, Smythe KA & Yokobayashi Y (2013) Knockdown of *recA* gene
13 expression by artificial small RNAs in *Escherichia coli*. *Biochem Biophys Res Commun* **430**:
14 256-259.
- 15 Shechter N, Zaltzman L, Weiner A, Brumfeld V, Shimoni E, Fridmann-Sirkis Y & Minsky A
16 (2013) Stress-induced Condensation of Bacterial Genomes Results in Re-Pairing of Sister
17 Chromosomes: Implications for Double-Strand DNA Break Repair. *J Biol Chem*.
- 18 Shee C, Ponder R, Gibson JL & Rosenberg SM (2011) What limits the efficiency of double-
19 strand break-dependent stress-induced mutation in *Escherichia coli*? *J Mol Microbiol*
20 *Biotechnol* **21**: 8-19.
- 21 Singletary LA, Gibson JL, Tanner EJ, McKenzie GJ, Lee PL, Gonzalez C & Rosenberg SM
22 (2009) An SOS-regulated type 2 toxin-antitoxin system. *J Bacteriol* **191**: 7456-7465.
- 23 Sobol RW (2012) For MutY, it's all about the OG. *Chem Biol* **19**: 313-314.
- 24 Stohl EA, Brockman JP, Burkle KL, Morimatsu K, Kowalczykowski SC & Seifert HS (2003)
25 *Escherichia coli* RecX inhibits RecA recombinase and coprotease activities in vitro and in
26 vivo. *J Biol Chem* **278**: 2278-2285.
- 27 Sussenbach JS & Berends W (1964) Photodynamic degradation of guanine. *Biochem Biophys*
28 *Res Commun* **16**: 263-266.
- 29 Taddei F, Matic I & Radman M (1995) cAMP-dependent SOS induction and mutagenesis in
30 resting bacterial populations. *Proc Natl Acad Sci U S A* **92**: 11736-11740.
- 31 Tapias A & Barbe J (1998) Mutational analysis of the *Rhizobium etli* *recA* operator. *J*
32 *Bacteriol* **180**: 6325-6331.
- 33 Ubeda C, Maiques E, Knecht E, Lasa I, Novick RP & Penades JR (2005) Antibiotic-induced
34 SOS response promotes horizontal dissemination of pathogenicity island-encoded virulence
35 factors in staphylococci. *Mol Microbiol* **56**: 836-844.
- 36 Val ME, Kennedy SP, Soler-Bistue AJ, Barbe V, Bouchier C, Ducos-Galand M, Skovgaard O
37 & Mazel D (2013) Fuse or die: how to survive the loss of Dam in *Vibrio cholerae*. *Mol*
38 *Microbiol*.
- 39 van der Veen S & Abee T (2011) Bacterial SOS response: a food safety perspective. *Curr*
40 *Opin Biotechnol* **22**: 136-142.
- 41 Wade JT, Reppas NB, Church GM & Struhl K (2005) Genomic analysis of LexA binding
42 reveals the permissive nature of the *Escherichia coli* genome and identifies unconventional
43 target sites. *Genes Dev* **19**: 2619-2630.
- 44 Wadhawan S, Gautam S & Sharma A (2013) A component of gamma-radiation-induced cell
45 death in *E. coli* is programmed and interlinked with activation of caspase-3 and SOS
46 response. *Arch Microbiol*.
- 47 Walker GC (1996) The SOS Response of *Escherichia coli*. *Escherichia coli and Salmonella*.
48 *Neidhardt, FC Washington DC American Society of Microbiology* **1**: 1400-1416.
- 49 Wimberly H, Shee C, Thornton PC, Sivaramakrishnan P, Rosenberg SM & Hastings PJ
50 (2013) R-loops and nicks initiate DNA breakage and genome instability in non-growing
51 *Escherichia coli*. *Nature communications* **4**: 2115.
- 52
53
54
55
56
57
58
59
60

- 1
2
3 Winterling KW, Chafin D, Hayes JJ, Sun J, Levine AS, Yasbin RE & Woodgate R (1998)
4 The *Bacillus subtilis* DinR binding site: redefinition of the consensus sequence. *J Bacteriol*
5 **180**: 2201-2211.
6 Yasbin RE, Wilson GA & Young FE (1975) Transformation and transfection in lysogenic
7 strains of *Bacillus subtilis*: evidence for selective induction of prophage in competent cells. *J*
8 *Bacteriol* **121**: 296-304.
9 Yasuda T, Morimatsu K, Horii T, Nagata T & Ohmori H (1998) Inhibition of *Escherichia coli*
10 RecA coprotease activities by DinI. *EMBO J* **17**: 3207-3216.
11 Yim G, McClure J, Surette MG & Davies JE (2011) Modulation of *Salmonella* gene
12 expression by subinhibitory concentrations of quinolones. *J Antibiot (Tokyo)* **64**: 73-78.
13 Ysern P, Clerch B, Castano M, Gibert I, Barbe J & Llagostera M (1990) Induction of SOS
14 genes in *Escherichia coli* and mutagenesis in *Salmonella typhimurium* by fluoroquinolones.
15 *Mutagenesis* **5**: 63-66.
16 Zhang AP, Pigli YZ & Rice PA (2010) Structure of the LexA-DNA complex and implications
17 for SOS box measurement. *Nature* **466**: 883-886.
18 Zhu J & Mekalanos JJ (2003) Quorum sensing-dependent biofilms enhance colonization in
19 *Vibrio cholerae*. *Dev Cell* **5**: 647-656.
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24 Figure legends

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27 **Figure 1. Simplified representations of DNA repair pathways induced by SOS** (described
28 in the introduction section). HR, NER and TLS are induced during the SOS response. HR:
29 Homologous Recombination. Single strand nicks (gaps) are transformed into larger gaps by
30 the RecJ exonuclease. Gaps are recognized by RecFOR presynaptic proteins. Double strand
31 breaks (DSB) are recognized by the presynaptic RecBCD exonuclease/helicase complex.
32 RecFOR/RecBCD recruit RecA to initiate homologous recombination through strand invasion
33 with the sister chromosome, usually resulting in mutation free repair. DSB repair results in the
34 formation of a replication fork. NER: Nucleotide Excision Repair. The UvrAB complex
35 recognizes the DNA lesion; UvrC proceeds with a double incision on both sides of the lesion
36 and the ssDNA carrying the lesion is removed by the UvrD helicase. DNA polymerase I (Pol
37 I) resynthesizes the missing DNA and the ligase ligates the newly synthesized DNA to the
38 template, usually resulting in mutation free repair. If the SOS inducing signal persists,
39 translesion synthesis (TLS) polymerases are induced. In the absence of TLS, the replicative
40 DNA polymerase (Pol III) cannot replicate damaged DNA, leading to fork stalling. TLS
41 polymerases (Pol IV, Pol V) can replicate damaged DNA in a mutagenic manner. Yellow
42 stars represent DNA lesions.
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48 **Figure 2. Origins of ssDNA inducing SOS. A. Replication fork stalling** often leads to
49 spontaneous ssDNA nicks. These breaks are eventually transformed into double strand breaks
50 (DSBs) when replication restarts, and DSBs are potent inducers of SOS as described in
51 section I. **B. Replication transcription collisions.** Adapted from (Helmrich *et al.*, 2013). Co-
52 directional collisions may happen because of the difference of velocity of the replisome and
53 the transcription complex, and lead to DSB formation (Dutta *et al.*, 2011). Head-on collisions
54 occur when transcription and replication progress in opposite directions. Both types of
55 collisions cause DSB formation through either R-loop formation or topological stress, if
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3 rescue pathways are insufficient. **C. Transcription stalling.** Adapted from (Wimberly *et al.*,
4 2013). Elongating RNA polymerase (RNAP) can stall upon encounter with a DNA lesion or
5 bulky protein complexes. In this case, transcribed RNA can anneal to the template DNA
6 forming a structure called R-loop. R-loops are another type of SOS inducers as described in
7 section I. R-loops can lead to re-priming of a replication fork. If the re-primed replication fork
8 encounters a nick, it can stall and lead to DSBs (as in panel A).
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