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Eons of distress: an evolutionary perspective on the bacterial SOS response

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Abstract

The SOS response of bacteria is a global regulatory network targeted at addressing DNA-damage. Governed by the products of the *lexA* and *recA* genes, it coordinates a comprehensive response against DNA lesions and its description in *Escherichia coli* has stood for years as a textbook paradigm of stress-response systems in bacteria. In this paper we review the current state of research on the SOS response outside *E. coli*. By retracing research on the identification of multiple diverging LexA-binding motifs across the Bacteria Domain, we show how this work has led to the description of a minimum regulon core, but also of a heterogeneous collection of SOS regulatory networks that challenges many tenets of the *E. coli* model. We also review recent attempts at reconstructing the evolutionary history of the SOS network that have cast new light on the SOS response. Exploiting the newly gained knowledge on LexA-binding motifs and the tight association of LexA with a recently described mutagenesis cassette, these works put forward likely evolutionary scenarios for the SOS response, and we discuss their relevance on the ultimate nature of this stress-response system and the evolutionary pressures driving its evolution.

Keywords: SOS response; DNA-damage; LexA; regulon; evolution; binding site.

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Introduction

Ever since its identification and initial description in the late 1970s (first reviewed in (Witkin, 1976)), the SOS response quickly became a textbook paradigm of coordinated gene expression, following a model of autogenous negative regulation by induction. As with many other genetic pathways, the SOS response was first identified and then thoroughly studied in *Escherichia coli*, in which prophage induction (Hertman & Luria, 1967), cell filamentation (Green*, et al.*, 1969) and mutation (Weigle, 1953) were repeatedly reported in early work with UV-irradiated cells. These phenomena were later linked with susceptibility to irradiation in *lexA* and *recA* mutants (Gudas & Pardee, 1975), leading to the hypothesis of a global response against DNA damage (Radman, 1974; Radman, 1975). Subsequent work confirmed that this response, aptly termed the SOS response after the naval Save Our Souls distress signal, does indeed constitute a mechanism to address DNA lesions in *E. coli* and is regulated by the *lexA* and *recA* gene products, which act, respectively, as inducer and repressor of the system and are both members of the SOS regulatory network (Little & Mount, 1982). Even though later work has identified over a thousand genes that seem to be induced in *E. coli* DNA-damaged cells (Courcelle*, et al.*, 2001; Khil & Camerini-Otero, 2002; Quillardet*, et al.*, 2003), the SOS network has been traditionally defined as the set of nearly 40 genes directly regulated by *lexA* and *recA* (Fernandez De Henestrosa*, et al.*, 2000).

Even though the textbook model of the *E. coli* SOS response is still a valid reference for most experimental work, research in the last decade has complemented and corrected this model, providing an evolutionary perspective of the SOS response. Taken from an evolutionary point of view, a global regulatory network, or regulon, like the SOS response conveys various types of information that can be analyzed to gain some insight into its nature. The type, sequence and number of regulated genes, the specific sequence of regulatory motifs, the system inductors and the very presence or absence of the regulatory network are all sources of information that contribute to explain the evolutionary history of a regulon and its role and function in bacterial cells. In this review, we take stock of recent work in all these areas to present a broader view of the bacterial SOS response and its evolution.

The *E. coli* SOS response

In the classic, thoroughly studied model of the *E. coli* SOS response (adeptly reviewed in (d'Ari, 1985; Shinagawa, 1996; Walker, 1984)), the LexA protein represses a set of genes whose products are involved in a number of different cellular processes, such as inhibition of cell division, error-prone replication or excision repair. Control by the LexA protein is exerted by the specific binding of its N-terminal domain to 16 bp-long palindromic motifs in the promoter region of SOS genes. These motifs, with consensus sequence CTGTATATATATACAG and conventionally called SOS boxes, are typically located near or inside the RNA-polymerase binding-site. Therefore, binding of a LexA dimer to the SOS box physically interferes with RNA-polymerase activity, effectively blocking transcription initiation and repressing gene expression. On the other hand, the RecA protein acts as sensor of the SOS system (Fig. 1). Sensing is mediated by unspecific binding of RecA to single-stranded DNA fragments, generated either by DNA damage-mediated interruption of replication or by enzymatic processing of broken DNA ends (Sassanfar & Roberts, 1990), a process in which the recBC-encoded exonuclease plays a decisive role (Barbe*, et al.*, 1985). After binding, RecA acquires an active state that enables it to promote the autocatalytic cleavage of LexA and several other transcriptional repressors, such as the λ phage CI repressor (Sauer*, et al.*, 1982). Autocatalytic cleavage of LexA Ala^{84}-Gly^{85} bond, carried out by LexA residues Ser^{119} and Lys^{156} (Little, 1991), is similar to that mediated by serine proteases and effectively inhibits LexA from binding SOS boxes, thereby inducing the SOS response.

Derepression of SOS genes induces the programmed expression of a host of genes aimed at dealing with DNA damage and its repercussions inside the cell (reviewed in (Crowley & Courcelle, 2002)). Since replication fork arrest is a main trigger of SOS induction, several SOS genes, like *recA* and *ssb*, are rapidly induced to protect and stabilize the fork, while a second set of genes (including *uvrA*, *uvrB*, *ydjQ*, *uvrD*, *recN* and *ruvAB*) is expressed to deal with the offending lesions through nucleotide excision or recombination repair mechanisms (Walker, 1984). To circumvent those lesions that cannot be easily repaired and thus forestall the advance of the replication fork, the *E. coli* SOS system also regulates the induction of three DNA polymerases (*polB*, *dinB* and *umuDC*) that are able to perform translesion DNA synthesis (Jarosz*, et al.*, 2007; Napolitano*, et al.*, 2000). Even

though these polymerases have been shown to be error prone and poorly processive, their ability to operate through damaged DNA bases allows replication to proceed, thereby sacrificing long-term genetic fidelity for short-term viability. Lastly, the SOS response acts also on *E. coli* cell division by regulating several genes involved in septation. Most notably, induction of the *sulA* gene inhibits septum formation by interacting with the *ftsZ* gene product, leading to filamentation (Trusca*, et al.*, 1998), and the rationale for this process seems to lie in delaying cell division until DNA damage has been rightly addressed.

Once DNA lesions have been repaired or bypassed, RecA ceases to be activated by single-stranded DNA fragments. Since both *lexA* and *recA* are also de-repressed during the SOS response (Walker, 1984), levels of non-cleaved LexA protein rapidly increase as non-activated RecA levels raise, returning the system to its repressed state. In addition to this basic reinstatement mechanism, several SOS genes seem to be involved in the fine-tuning and temporal modulation of the SOS response. The products of *dinI* and *recX*, for instance, stabilize and destabilize, respectively, *recA*-ssDNA filaments, thereby modulating the response time and recovery rate of the system (Renzette*, et al.*, 2007). Likewise, the *umuDC* product, which also undergoes RecA-dependent self-cleavage, has been proposed as a key element in cell-cycle control following DNA damage (Opperman*, et al.*, 1999; Sutton*, et al.*, 2001), a fact that has been recently demonstrated by careful analysis of SOS expression in single cells (Friedman*, et al.*, 2005).

Universality of the SOS response

The early identification of a LexA homologue in the Firmicutes *Bacillus subtilis* (Wojciechowski*, et al.*, 1991), a phylum substantially removed from the Proteobacteria to which *E. coli* belongs, and the discovery that it also regulated a set of genes involved in DNA repair suggested initially that the SOS response might be a universal adaptation of bacteria to DNA damage. Indeed, later work in other bacterial species has mainly confirmed this idea. Functional LexA homologues regulating genes involved in DNA repair have been characterized for instance in the Actinobacterium *Mycobacterium tuberculosis* (Movahedzadeh*, et al.*, 1997), the Thermotogae *Thermotoga naepolitana* (Zverlov & Schwarz, 1999), the Alpha Proteobacterium *Rhodobacter sphaeroides* (Fernandez de Henestrosa*, et al.*, 1998) or in the Cyanobacterium *Anabaena sp.* (Mazon*, et al.*, 2004a). In addition, *lexA*

sequence homologues can be found in almost all the bacterial genomes sequenced to date, covering a large number of phyla, and thereby suggesting both an ancient origin and a widespread distribution of *lexA* and the SOS response.

In spite of this apparent universality, several exceptions to this trend have been identified (Fig. 2). Of particular interest is the apparent absence of *lexA* sequence homologues in whole bacterial classes and phyla. For instance, no *lexA* homologues have been detected in the Bacteroidetes-Green sulfur bacteria group or in the Epsilon Proteobacteria subclass, where this absence is all the more intriguing given that functional LexA homologues have been characterized in all the other Proteobacteria subclasses (Campoy*, et al.*, 2003; Campoy*, et al.*, 2005; Erill*, et al.*, 2003; Erill*, et al.*, 2004). The absence of LexA homologues in diverse bacterial groups points to a richer evolutionary history than that presumable for a universal response system and implicitly poses some intriguing questions. In some cases, such as in the Epsilon Proteobacteria, the absence of a LexA homologue may be partly explained by the evolutionary pressures imposed by genomic reduction that are plainly observed in other genera. It seems clear that intracellular parasites from classes in which a LexA-governed SOS response has otherwise been positively identified, such as the Rickettsiae and the Mycoplasmataceae (Fig. 2), have probably lost their respective *lexA* genes due to the selective pressure towards genomic reduction and the probable need to maintain a constitutive expression of repair genes in the adverse environment of their host cell (Mertens*, et al.*, 2005; Renesto*, et al.*, 2005).

Even though genomic reduction might be a reasonable explanation for the absence of a *lexA* gene and its accompanying regulon in the Epsilon Proteobacteria, this same rationale does not apply easily to the Bacteroidetes-Green sulfur bacteria group, the Aquifex class and other isolated instances of LexA loss. In this respect, it is interesting to note data on the Streptococci, which also lack a *lexA* gene and, in particular, on the major human pathogen *Streptococcus pneumoniae* (Claverys*, et al.*, 2006). In contrast to other Firmicutes with conventional SOS responses, *S. pneumoniae* seems to have co-opted its competence regulon, involved mainly in natural DNA transformation, to coordinate some of its response to DNA damage (Prudhomme*, et al.*, 2006). This suggests that part of the specific DNA-damage response system provided by the RecA-LexA tandem can be sometimes

substituted by adapting other stress-sensitive regulatory networks. Moreover, the case of *S. pneumoniae* also illustrates a positive evolutionary pressure towards either conservation or replacement of some DNA damage-response mechanism, a fact that had not been explicitly acknowledged before and which has implications on the evolution of the SOS response.

A horde of LexA-binding motifs

The characterization in *B. subtilis* of a LexA box (GAAC-N_4-GTTC) that was remarkably unrelated to the known *E. coli* one (Cheo*, et al.*, 1991) was the first hint that the history of the SOS network might be far more complicated that it could have been assumed. In fact, the complex nature of the LexA-binding motif is perhaps the most perplexing feature arising from the study of the SOS response across different phyla, setting it quite apart from many other regulons, such as *arg*, *bio* or *phoB*, in which motif conservation is often the rule (Khan*, et al.*, 2006; Makarova*, et al.*, 2001; Rodionov*, et al.*, 2002).

The consensus sequence of the *B. subtilis* LexA box, initially dubbed Cheo box, was later redefined as CGAACRNRYGTTCG (Winterling*, et al.*, 1998) and shares with the *E. coli* one a dyad-spacer-dyad palindromic structure. A variation of the *B. subtilis* LexA box was later described thoroughly in *Mycobacterium smegmatis* and *M. tuberculosis* (Movahedzadeh*, et al.*, 1997), with a consensus sequence TCGAACNNNNGTTCGA (Davis*, et al.*, 2002), and a GAAC-N_4-GTTC box was also positively identified in *Clostridium perfringens* (Johnston*, et al.*, 1997; Nuyts*, et al.*, 2001), *Staphylococcus aureus* (Bisognano*, et al.*, 2004) and in the Streptomycetes (Mikoc*, et al.*, 1997; Vierling*, et al.*, 2001), establishing the GAAC-N_4-GTTC motif as the monophyletic LexA box of Gram-positive bacteria. In a similar vein, early indications that the *E. coli* LexA box, abbreviated as CTGT-N_8-ACAG, was monophyletic in the Gamma Proteobacteria (Fernandez de Henestrosa*, et al.*, 1991; Zhao & McEntee, 1990) where soon experimentally confirmed (Garriga*, et al.*, 1992; Riera & Barbe, 1993) and later extended to the close Beta Proteobacteria subclass (Erill*, et al.*, 2003).

A turning point

The identification of unmistakably different LexA boxes in two divergent phylogenetic groups and the later discovery that these motifs are also

monophyletic in both groups should not be taken lightly, as they indicate a turning point in the evolution of the LexA regulon. A significant change in the LexA box introduces a point-of-no-return in the evolution of the LexA regulon since, as it was soon discovered (Lovett, *et al.*, 1994; Riera, *et al.*, 1994), a LexA protein recognizing a derived motif cannot take up its regulatory role in other species. In principle, thus, LexA boxes could be used as consistent landmarks of phylogenetic branching points and might therefore contribute to the unraveling of the complex evolutionary history of the LexA regulon. In the last decade, this goal has spurred research in the identification of new LexA-binding motifs in different phyla, yielding additional cues on the evolution of the LexA protein.

Besides *B. subtilis* and *E. coli*, the first novel LexA-binding motif was discovered in the Alpha Proteobacteria *Rhizobium etli* and *Rhodobacter sphaeroides* (Fernandez de Henestrosa, *et al.*, 1998; Tapias & Barbe, 1998; Tapias, *et al.*, 2000), and its identification constituted again a major surprise. The new motif, which later work has shown to be monophyletic for the Alpha Proteobacteria subclass (Erill, *et al.*, 2004), was characterized as GTTC-N_7-GTTC and presented two striking features: an odd spacer and a direct-repeat (instead of palindromic) structure, which set it substantially apart from those observed in either *B. subtilis* or *E. coli*. Leaving aside the huge evolutionary leap implied by a change from palindromic to direct-repeat structure, further work on *R. sphaeroides* LexA also demonstrated that, depending on its intracellular concentration, this organism LexA could act both as a transcriptional repressor or as an activator (Tapias, *et al.*, 2002). Even though many questions brought up by the identification of the Alpha Proteobacteria LexA-binding sequence remain yet to be elucidated, later work in other species has identified half a dozen additional SOS boxes in quite different phyla, providing a wide but patchy map of LexA-binding site evolution (Fig. 3).

The ever-changing motif

In 2002, a novel LexA-binding sequence with consensus sequence TTAG-N_6-TACTA was identified in the Xanthomonadaceae *Xanthomonas campestris* and *Xylella fastidiosa* (Campoy, *et al.*, 2002; Yang, *et al.*, 2002) and, a year later, the LexA box of the Delta Proteobacterium *Myxococcus xanthus* was described as CTA-N_6-GTTCAGG (Campoy, *et al.*, 2003). As in the case of the Alpha Proteobacteria, these two new LexA binding motifs, which have been later verified through *in silico*

methods (Erill, *et al.*, 2006), made it necessary to revise the LexA box paradigm by introducing the notion of highly asymmetrical and imperfectly palindromic dyads.

Meanwhile, the domains of the *B. subtilis* LexA box had been extended beyond the Gram-positive bacteria, with the identification of the same regulatory motif in the Gram-negative bacterium *Dehalococcoides ethenogenes*, (Fernandez de Henestrosa, *et al.*, 2002) and the description of a markedly similar motif in the Cyanobacteria (GTAC-N_4-GTWC), which was shown to readily bind *B. subtilis* LexA (Mazon, *et al.*, 2004a). Subsequent work identified a LexA box similar to that of *B. subtilis* and the Cyanobacteria (GTAC-N_4-GTRC) in a duplicated *lexA* gene that is present in some Pseudomonadaceae and Xanthomonadaceae (Abella, *et al.*, 2004), while a new LexA-binding motif, with consensus sequence TGCHC-N_4-GHYCA and relatively close to the *B. subtilis* one, was reported in *Fibrobacter succinogenes* (Fig. 3). Further work in the Delta Proteobacteria class singled out two additional LexA boxes in this group (GGTT-N_{10}-WACC for *Geobacter sulfurreducens* and TTAC-N_3-GTAA for *Bdellovibrio bacteriovorus*), revealing for the first time substantial variability in the LexA-binding motif of a single bacterial subclass (Campoy, *et al.*, 2005; Jara, *et al.*, 2003).

The repertoire of identified LexA-binding motifs ends with several phyla reported recently, which add a further degree of heterogeneity to the LexA box collection. A new palindromic LexA-binding motif (CCT-N_{10}-AGG), plainly divergent from other Proteobacteria motifs, has been described in the Proteobacteria *Magnetococcus sp.* MC-1 (Fernandez de Henestrosa, *et al.*, 2003). Besides, the LexA box of the Thermotogae *Petrotoga miotherma* has been reported as GANT-N_6-GANNAC (Mazon, *et al.*, 2006a), while the binding motif of the Spirochete *Leptospira interrogans* LexA has been described as TTTG-N_5-CAAA (Cune, *et al.*, 2005). More recently, the LexA-binding sequence of *Acidobacterium capsulatum*, from the Fibrobacteres-Acidobacteria group, has been shown to be far removed from the Fibrobacteres LexA box, with a consensus sequence (GTTC-N_7-GTTC) closely resembling instead the Alpha Proteobacteria one, and suggesting a possible event of lateral gene transfer between the later and the Acidobacteria (Mazon, *et al.*, 2006b).

The case for a core LexA regulon

In view of the identification of a multitude of LexA-binding motifs that were patently divergent from the *E. coli* one, the description in *B. subtilis* of the first LexA-regulated network outside *E. coli* was prone to yield some unexpected results. Even though the initial characterization of the *B. subtilis* SOS network (Cheo*, et al.*, 1991; Gillespie & Yasbin, 1987; Haijema*, et al.*, 1996) reported only five SOS-inducible genes (*recA*, *lexA*, *uvrB*, *dinB*, and *dinC*), the fact that three of them (*recA*, *lexA* and *uvrB*) were also regulated by LexA in *E. coli* suggested a significant overlap between both systems. In fact, recent work (Au*, et al.*, 2005) has further confirmed this hypothesis, enlarging the list of LexA-regulated genes in *B. subtilis* to 33, with a host of genes apparently involved in DNA repair and translesion synthesis, and reporting additional coincidences with the *E. coli* SOS system, like the *ruvAB* operon (Table 1). Moreover, and taking into account the aforementioned exception of the Streptococci, which lack a *lexA* gene, the SOS response network seems to be roughly consistent among Gram-positive bacteria. For instance, in the Actinobacterium *Mycobacterium tuberculosis*, the other Gram-positive species in which the SOS system has been analyzed substantially, 21 genes have been identified as members of the LexA regulon, which again encompasses *lexA*, *recA*, *uvrA* and the *ruvCAB* operon (Davis*, et al.*, 2002). Curiously enough, however, many of the DNA repair genes of *M. tuberculosis* have been shown to be DNA damage-inducible in a LexA/RecA-independent manner, revealing the existence of an overlapping stress-response system in the Actinobacteria that could act as a backup system in case of LexA loss (Gamulin*, et al.*, 2004; Rand*, et al.*, 2003).

The regulon core hypothesis

Even though there was a significant overlap between the *E. coli* and *B. subtilis* LexA networks, the thorough description of the *B. subtilis* and *M. tuberculosis* SOS responses also highlighted several noteworthy differences between the Gram-positive and *E. coli* LexA networks. For instance, the absence in the Gram-positive LexA network of some genes (*dinI*, *recX* or *umuDC*) that are involved in the precise modulation of the *E. coli* SOS response suggests that, beyond its basic induction mechanism, many details of the SOS response may have evolved to fit the specific needs of different species and groups. Nevertheless, the overlap between both systems is still quite remarkable in view of the differences in their respective LexA-binding motifs. This striking coincidence in regulated genes led to the hypothesis of a putative common set of genes, or regulon core, that might be conserved in all

LexA networks, and the validation of this hypothesis has been the subject of active study in later research. Owing to the identification of several LexA-binding sequences reported above, recent studies in several phyla have revealed that this initial picture of homogeneity quickly fades out as one moves away from *E. coli* or the Gram-positive bacteria, revealing a substantial heterogeneity in LexA regulon contents (Table 1).

Gram-positive-related LexA networks

Emerging from the Gram-positive bacteria, experimental analysis of *D. ethenogenes* reveals that, among the former coincidences between Gram-positive bacteria and *E. coli*, only the *uvrA* and *lexA* genes (but not *recA* or *ruvAB*) are LexA-regulated in *D. ethenogenes* (Fernandez de Henestrosa, *et al.*, 2002). Interestingly, a similar experimental result has also been reported in the Thermotogae *P. miotherma*, whose LexA protein regulates again the *uvrA* and *lexA* genes, but does not regulate *recA* or *ruvAB* (Mazon, *et al.*, 2006a). In this same vein, none of the two *lexA* homologues of *Deinococcus radiodurans* has been shown to regulate *recA* (Narumi, *et al.*, 2001; Sheng, *et al.*, 2004), and there is ample evidence that the DNA damage response of *D. radiodurans* is managed by an alternative regulatory network, a fact that has been attributed to the specific need of this organism to coordinate a comprehensive response against radiation (Satoh, *et al.*, 2006; Tanaka, *et al.*, 2004).

In contrast, LexA does regulate *lexA*, *recA*, *uvrA* and an additional homologue of a gene from the *E. coli* SOS regulon (*ssb*) in the Cyanobacteria *Anabaena sp.* and *Nostoc punctiforme*, even though it apparently does only regulate itself in the close Cyanobacterium *Synechocystis sp.* (Mazon, *et al.*, 2004a). Similarly, the LexA protein of *F. succinogenes* (Fibrobacteres-Acidobacteria group) has been shown to regulate also *recA*, *uvrA*, *ssb* and the *ruvAB* operon (Mazon, *et al.*, 2004b), while the *A. capsulatum lexA* gene does not regulate any of them (Mazon, *et al.*, 2006b). To further complicate matters, the *lexA* homologue of the Spirochaete *L. interrogans*, a phylum in which lack of a *lexA* gene seems to be the rule, has been shown to regulate *recA*, but not itself (Table 1). This constitutes a previously unreported phenomenon that has been attributed to the process of genomic reduction this species seems to be undergoing (Cune, *et al.*, 2005).

***E. coli*-related LexA networks**

The outline of the SOS response emerging from *E. coli* does also contravene partially the picture of regulon homogeneity. Even though many of the same genes regulated by LexA in *E. coli* are also LexA-regulated in close relatives like *Salmonella enterica* sv Typhimurium (Benson*, et al.*, 2000; Erill*, et al.*, 2003), experimental and comparative genomics analyses have shown that the number of coincident genes decreases rapidly in more distant relatives like *Pseudomonas aeruginosa*, whose LexA protein does not regulate *uvrA*, *uvrB* or the *ruvAB* operon (Cirz*, et al.*, 2006) (Rivera*, et al.*, 1996; Rivera*, et al.*, 1997), and is down to three (*lexA*, *recA* and *recN*) in the Beta Proteobacterium *Ralstonia solanacearum* (Erill*, et al.*, 2003) (see Table 1). In addition, it has been shown that the LexA protein of the Gamma Proteobacteria *Xylella fastidiosa* does only regulate itself and a DNA-modification methylase (Campoy*, et al.*, 2002), questioning to some extent the idea of a common set of LexA-regulated genes.

In contrast, comparative genomics and experimental evidence in the Alpha Proteobacteria reveal again a set of genes shared between their LexA regulon and that of *E. coli* and most Gamma Proteobacteria (Erill*, et al.*, 2004; Tapias & Barbe, 1999; Tapias*, et al.*, 2002). This set of LexA-regulated genes, maintained in all the Alpha Proteobacteria species but the aforementioned Rickettsiae (which lack a *lexA* gene), encompasses again *recA*, *ssb*, *uvrA* and the *ruvCAB* operon. Further out from *E. coli*, however, experimental results on the components of the LexA regulon become more scarce and difficult to interpret. It has been shown, for instance, that neither *recA* nor *ssb* or *recN* are regulated by LexA in the Proteobacterium *Magnetococcus sp.* MC-1 (Fernandez de Henestrosa et al., 2003). Similarly, LexA regulation in the Delta Proteobacteria *G. sulfurreducens* and *B. bacteriovorus* does not include *recA*, *ssb* and *uvrA* (Campoy*, et al.*, 2005; Jara*, et al.*, 2003). Likewise, in *M. xanthus*, where LexA-independent induction of conventional SOS genes (*recN* and *ssb*) has been experimentally assessed, only *lexA* and one of its two *recA* copies (but not *uvrA*, *recN* or *ssb*) are LexA-regulated (Campoy*, et al.*, 2003).

Flexible regulon, plastic core

Taken together with the diversity of LexA-binding motifs, the results presented above yield revealing facts on the evolutionary history of the LexA regulon that may seem somewhat contradictory: an apparent prevalence of a minimal core regulon and a remarkable plasticity in terms of regulon members (Table 1). On the

one hand, and even taking into account slight differences in the methodologies used to map each of the analyzed LexA networks, it remains a fact that the LexA regulon in many species and groups, like the Delta Proteobacteria, seems to be composed of, at the most, a handful of genes. This is in stark contrast with the well-known LexA regulons of *E. coli* and *B. subtilis*, encompassing more than 30 genes, and with other LexA regulons that have been thoroughly studied using microarray expression data and comparative genomics approaches (Table 1). Besides the already mentioned LexA regulon of *M. tuberculosis*, which contains 21 genes (Davis*, et al.*, 2002), the LexA regulon of Alpha Proteobacteria has been shown to control typically between 15 and 18 genes (Erill*, et al.*, 2004) and that of *P. aeruginosa* has been reported to control 15 genes (Cirz*, et al.*, 2006), with other Gamma Proteobacteria species having a LexA regulon that takes in between 13 and 30 genes (Erill*, et al.*, 2003). This apparent variability in the gene contents of the LexA regulon evidences a substantial degree of flexibility in the LexA network. Combined with the reported addition of several specialized genes in different organisms (Davis*, et al.*, 2002; Erill*, et al.*, 2003; Erill*, et al.*, 2004), this flexibility constitutes sound evidence of strong selection forces at work, adapting the SOS response to different ecological niches. Furthermore, the evidence of active selective pressures governing regulon contents does also put forward a reasonable evolutionary mechanism to explain the profusion of different LexA-binding motifs described earlier.

On the other hand, and in spite of the substantial list of exceptions outlined above, the fact that a similar set of LexA-regulated genes emerges repeatedly in such different groups as the Gram-positive bacteria, the Cyanobacteria or the Gamma and Alpha Proteobacteria is strong evidence in favor of postulating a core LexA regulon composed of *lexA*, *recA*, *uvrA*, *ssb* and the *ruvAB* operon. Then again, this leaves open the question of whether the thus defined core LexA regulon represents a vestigial LexA regulon that has subsequently been altered in several species and groups or, on the contrary, it is the result of convergent evolution. The notion of a common core that has later degenerated or adapted into several specializations may be intuitively more parsimonious than hypothesizing multiple events of convergent evolution. However, by postulating conservation, it sits relatively at odds with the evidence of extensive changes to the LexA-binding sequence between the same phylogenetic groups used to define this core. In

addition, the observed choice of core LexA regulon genes (involved mainly in DNA repair and fork stabilization) is quite sound from an evolutionary point of view and, thus, the possibility of convergent evolution should not be discarded hastily. In this sense, the LexA-independent induction of many standard core LexA regulon members (*recA*, *ruvB*, *uvrA*) in *D. radiodurans*, constitutes a strong advocate for the convergent evolution hypothesis, although more thorough analyses of substitute SOS-like networks in species lacking a *lexA* gene are required to provide definite answers regarding this issue.

Reaching beyond the chromosome

The ability of the LexA protein to regulate cis-acting binding motifs in genes that are not strictly chromosomal may not be an ordinary part of the textbook SOS paradigm, but it should not come as a surprise either, since it was discovered quite early to take place in naturally occurring plasmids (Elledge & Walker, 1983; Glazebrook*, et al.*, 1983). Similarly, it was also soon discovered that RecA activation, a prerequisite for SOS induction, was also the trigger for cleavage of several lytic-cycle CI repressors from temperate bacteriophages. As in the case of LexA, these repressors undergo RecA-mediated autocatalytic cleavage through their serine-protease domain (Roberts & Devoret, 1983; Roberts*, et al.*, 1982; Sauer*, et al.*, 1982), suggesting either a co-option of the RecA induction pathway by bacteriophages or a possible bacteriophage-related origin of the *lexA* gene.

In any case, the strategy from a temperate bacteriophage standpoint is clear enough: RecA activation signals trouble for the cell, and opting for lytic development and leaving the compromised host behind is a reasonable evolutionary policy. It thus came as no surprise when other SOS-dependent mechanisms for bacteriophage evasion were discovered. Some temperate bacteriophages with non RecA-cleavable repressors, such as the Coliphage 186 and the Salmonella prophages Fels-2, Gifsy-1 and Gifsy-2 (Bunny*, et al.*, 2002; Shearwin*, et al.*, 1998), encode an anti-repressor protein (Tum) that is capable of inducing their lytic cycle by interfering with CI repressor activity. In these phages, therefore, SOS-dependent evasion cannot be carried by direct RecA-cleavage of the lytic-cycle repressors and is instead mediated by LexA repression of the *tum* gene (Shearwin*, et al.*, 1998), providing further evidence of a positive selection for some kind of SOS-mediated evasion policy in temperate bacteriophages.

Virulence and the SOS response

At the time of the aforementioned discoveries, the implications of SOS-dependent phage evasion techniques did not raise many alarms. After all, even though bacteriophages are important vectors for gene dissemination, the SOS response was considered a moderately infrequent event, triggered mainly by UV irradiation and seldom used DNA-damaging antibiotics, like mitomycin C. Nonetheless, clinical repercussions of RecA-mediated induction of Shiga-like toxins encoded in enterohemorrhagic *E. coli* bacteriophages were soon reported in cancer patients treated with mitomycin C (Muhldorfer*, et al.*, 1996) and the later widespread use of quinolones, powerful SOS inductors, raised the issue of the convenience of using certain antibiotic families in Shiga toxin-producing *E. coli* infections (Kimmitt*, et al.*, 2000). The scenario became grimmer with the recent discovery that the *Vibrio cholerae* CTXphi prophage, which encodes the cholera toxin, is also SOS-inducible and that, in this particular case, induction is mediated by LexA through control of a specific LexA box in the CTXphi *rstA* promoter (Quinones*, et al.*, 2005; Waldor & Friedman, 2005). Moreover, the relationship between virulence and the SOS response was not exclusive to animal pathogens, as virulence genes of phytopathogenic bacteria, such as *Erwinia carotovora*, had long been known to be DNA-damage inducible through activation of a RecA-dependent pathway (Zink*, et al.*, 1985).

The spiral of virulent side effects of SOS induction reached its apex in 2004, with the discovery that SXT, an integrating conjugative element encoding resistance genes in *V. cholerae*, possessed a RecA-cleavable repressor (SetR) that induces SXT transfer during the SOS response (Beaber*, et al.*, 2004). Recent findings in another deadly pathogen, *S. aureus*, suggest that SXT is not an evolutionary spur-of-the-moment. Excision and replication of SaPIbov1, a *S. aureus* pathogenicity island, were shown to be induced after SOS induction of different temperate phages (Ubeda*, et al.*, 2005). Furthermore, SaPIbov1 was then packaged into phage-like particles and transferred efficiently (Ubeda*, et al.*, 2007). In both cases, the implications were clear enough: SXT carries genes that confer resistance to streptomycin, sulphamethoxazole, trimethoprim and chloramphenicol, while SaPIbov pathogenicity islands harbor multiple virulence genes. Suddenly, the SOS response was not only involved in virulence activation,

but also in the dissemination of antibiotic resistance genes (reviewed in (Kelley, 2006)).

Relaying SOS triggers

The evolutionary forces behind the SOS regulation of mobile pathogenicity elements become more apparent when one takes into consideration recent work on the triggers of the SOS response. As stated earlier, the SOS response was first identified in UV-irradiated *E. coli* cells and was soon also linked to other DNA-damaging agents, like mitomycin C (Costa de Oliveira*, et al.*, 1987), strengthening the case for a generalized DNA-damage response system. As it turned out with other aspects of the SOS response, however, this neat scheme of induction by external agents was quickly done away with as new evidence suggested that several internal and additional external processes could also trigger the SOS response (Fig. 4).

Intracellular induction of the SOS response was first reported in starved *E. coli* cells (Taddei*, et al.*, 1995), in which is dependent on cAMP levels, linking the SOS response to cellular metabolism and to adaptive mutation in starved cells (Bjedov*, et al.*, 2003; Janion*, et al.*, 2002; McKenzie*, et al.*, 2000), reviewed in (Bridges, 1998). Moreover, endogenous alkylating agents like nitrosated amines or S-adenosylmethionine, resulting from a variety of metabolic processes, have also been shown to be efficient inductors of the SOS response (Drablos*, et al.*, 2004; Mizrahi & Andersen, 1998; Volkert*, et al.*, 1989). Further work has identified up to 42 genes that, upon inactivation, lead to chronic SOS induction (O'Reilly & Kreuzer, 2004). These mutations are linked to repair or replication pathways, like the *dam* (Peterson & Mount, 1993) or *dnaQ* (Slater*, et al.*, 1994) mutants, and induce the SOS response as a consequence of the defects they present.

Exotic SOS induction

Besides internal triggers, the list of external inducers of the SOS response has also grown considerably in recent years (Fig. 4). In addition to the classic DNA-damaging agents, it was soon discovered that other environmental aggressions, like oxidative stress (Imlay & Linn, 1987), chromate shock (Ackerley*, et al.*, 2006) or acoustic cavitation (Vollmer, 1998; Vollmer, 1996), could result in DNA damage and thus indirectly induce the SOS system. In a similar setting, and even though it

was known that high pH could induce *in vitro* autodigestion of LexA in a RecA-independent manner (Smith*, et al.*, 1991), the description of *in vivo* SOS activation under acidic or alkaline stress yielded some surprises, as it reported for the first time a SOS activation mechanism that did not rely directly on DNA damage. In pH-mediated induction of the SOS response, the affinity of LexA for binding unspecific DNA rises significantly due a decrease in intracellular pH, leading to a gradual derepression of the SOS system in highly acidic or alkaline media. Thus, instead of RecA-mediated self-cleavage, induction of the SOS response by pH is mediated by conformation changes in LexA at low intracellular pH levels (Sousa*, et al.*, 2006), in what constitutes a completely novel method of induction.

Still, recent work has identified more indirect means of activating the SOS response (Fig. 4). The exact trigger of the *Salmonella enterica* SOS response during lytic cycle development by infecting bacteriophages has yet to be determined, but it has been shown to specifically involve the *kil* gene of these bacteriophages (Campoy*, et al.*, 2006). As a consequence of SOS induction, temperate bacteriophages residing in the infected cell activate their lytic routines, suggesting that their exploitation of the SOS system as an evasion warning sign may have evolved also as a defense mechanism against invasion by heteroimmune infecting phages. On another tack, the probable existence of specific genetic pathways designed to activate the SOS response in the apparent absence of a direct DNA-damaging agent was further confirmed by the analysis of SOS induction by high-pressure stress in *E. coli* (Aertsen*, et al.*, 2004). Instead of conformational changes in LexA, induction of the SOS response was found to be caused by high-pressure triggered activation of the cryptic type IV restriction endonuclease Mrr, which creates DNA double-strand breaks that subsequently lead to SOS induction (Aertsen & Michiels, 2005).

Looping the loop

The existence of indirect genetic pathways to activate the SOS response would probably be anecdotal if it were not for the recent discovery of one such pathway designed to activate the SOS system in response to cell wall stress induced by β-lactam antibiotics (Miller*, et al.*, 2004). As it turns out, the culprit of SOS induction in response to β-lactam-induced cell wall stress is the two-component signal transduction system DpiBA (Fig. 4). It has been postulated that, while DpiB senses

cell wall stress, induced DpiA is capable of binding AT-rich sequences in the replication origins of the *E. coli* chromosome. Binding of DpiA at these sites competes with binding of the replication proteins DnaA and DnaB, interrupting replication and leading to SOS induction (Miller*, et al.*, 2003). β-lactam induction of the SOS response has recently been reported also in *S. aureus*, and the positive effect of this induction on the dissemination of pathogenicity islands was demonstrated in the same study (Maiques*, et al.*, 2006). The ability of antibiotics based on mechanisms other than DNA damage to trigger the SOS response, and the reported direct linkage between SOS induction and dissemination of mobile elements carrying resistance genes, yields a clear picture of a powerful, reinforcing evolutionary mechanism. In the light of this, previous calls to arms, based on the connection between SOS and adaptive mutagenesis, to fight antibiotic resistance by inhibiting the SOS response in the clinic are being issued with renewed vigor (Avison, 2005; Cirz*, et al.*, 2005).

On top of the growing relevance of the SOS response in connection with antibiotics and mutagenesis, recent work has also identified several *lexA* and *recA* independent pathways involved in antibiotic-induced mutagenesis and recombination. It has been demonstrated, for instance, that several kinds of β-lactam antibiotics are able to induce in *E. coli* transcription of the *dinB* gene, and thus induce translesion synthesis and mutagenesis, through a *recA* and *lexA* independent pathway (Perez-Capilla*, et al.*, 2005). Similarly, it has also been shown that fluoroquinolones are able to stimulate intra- and inter-chromosomal recombinogenic activity in *E. coli* cells through a mechanism that does not require LexA cleavage (Lopez*, et al.*, 2007).

Rethinking the core and beyond

Determination of the precise link between the SOS response and mutagenesis dates back from the late 1980's, when the *umuD*, *polB* and *dinB* genes encoding error prone polymerases were identified as members of the LexA regulon in *E. coli* (Bagg*, et al.*, 1981; Bonner*, et al.*, 1988; Iwasaki*, et al.*, 1990; Reuven*, et al.*, 1999; Tang*, et al.*, 1999; Wagner*, et al.*, 1999) and were linked to adaptive mutagenesis (Hersh*, et al.*, 2004; Tompkins*, et al.*, 2003; Yeiser*, et al.*, 2002). Interest in the subject, however, has significantly increased due to two recent developments. On the one hand, experimental proof that SOS-induced mutagenesis is required for

the development of resistance to antibiotics ciprofloxacin and rifampicin in animal models (Cirz*, et al.*, 2005; Cirz*, et al.*, 2006) sets SOS-induced adaptive mutagenesis in the clinical arena, and shares with SOS-mediated dissemination of resistance genes significant implications in the clinical strategies required to keep in check the ever-growing problem of antibiotic resistance (Wise, 2004). On the other hand, recent work has identified a novel error-prone polymerase under control of LexA in several bacterial species (Abella*, et al.*, 2004; Galhardo*, et al.*, 2005) and its intimate relationship with the *lexA* gene has led to a profound rethinking of the nature and evolution of the LexA regulon.

A pervasive mutagenesis cassette

A gene encoding an error-prone subunit α of DNA-polymerase III (*dnaE2*), which had been previously shown to be LexA regulated (Davis*, et al.*, 2002), was first described in *M. tuberculosis*. The DnaE2 protein was postulated to be a translesion polymerase and its presence in *M. tuberculosis* was rapidly linked to the emergence of antibiotic resistant strains in this organism (Boshoff et al., 2003). Later work (Abella*, et al.*, 2004) detected the presence of *dnaE2* homologues in *P. putida* presenting a peculiar configuration: a *lexA2-imuA-imuB-dnaE2* operon, in which the *lexA2* gene was clearly divergent from the *lexA1* gene of this species and from that of *E. coli*, recognizing a LexA box (GTAC-N_4-GTRC) clearly divergent from that usually found (CTGT-N_8-ACAG) in the Gamma Proteobacteria (Table 3). The same configuration was observed in other Pseudomonadaceae, like *P. fluorescens* and *P. syringae*, and in some Xanthomonadaceae like *X. campestris*, involving always an operon governed by their respective *lexA2* gene. The *lexA2-imuA-imuB-dnaE2* operon was shown to be a self-regulated and DNA damage-inducible transcriptional unit and it was soon discovered that, in a number of different configurations (Table 3), the *lexA2-imuA-imuB-dnaE2* cassette was widely distributed among Proteobacteria (Abella*, et al.*, 2004).

In silico analysis of several genomes revealed the presence of three-gene cassettes (*imuA-imuB-dnaE2*) in other Gamma Proteobacteria, like *P. aeruginosa, Vibrio parahaemolyticus or Shewanella oneidensis*, and the same configuration could be also detected among almost all the Beta Proteobacteria and Alpha Proteobacteria analyzed (Abella*, et al.*, 2004) (Table 3). Experimental assays demonstrated that the three-gene cassette was explicitly regulated by LexA in *P.*

aeruginosa and in the Alpha Proteobacteria *Sinorhizobium meliloti* and *Agrobacterium tumefaciens*. Moreover, in all the other studied species high-scoring putative boxes for their respective LexA proteins were located upstream of the *imuA* gene through *in silico* methods (Abella*, et al.*, 2004). Later work on *Caulobacter crescentus* confirmed the role of the *dnaE2* gene in SOS-mediated mutagenesis, but linked its effects with the presence of the two additional cassette genes, termed *imuA* and *imuB* for inducible mutagenesis (Galhardo*, et al.*, 2005). Even though the precise role of ImuA has yet to be elucidated, ImuB was found to be similar to proteins of the Y-family of polymerases, and it was proposed that it cooperates with DnaE2 in lesion bypass, yielding an unusual, transversion-rich record of mutational activity. In addition, novel cassette configurations in which the *dnaE2* gene has split from the *imuA-imuB* tandem were identified through *in silico* analyses in *M. tuberculosis* and *Rhodopirellula baltica*, extending the domains of this DNA damage-inducible gene cassette well beyond the Proteobacteria (Galhardo*, et al.*, 2005).

Persistent regulation: towards a new core

Recently, extensive computer searches in newly sequenced genomes have revealed the true spread of the *imuA-imuB-dnaE2* gene cassette (Erill*, et al.*, 2006). Homologues of this same cassette structure, and nearly all possible combinations between its members, have been described in all the subdivisions of the Actinobacteria, in *Verrucomicrobium spinosum*, the Green non-sulfur bacterium *Thermomicrobium roseum*, the Acidobacterium *Solibacter usitatus* and the Delta Proteobacteria *Anaeromyxobacter dehalogenans* and *B. bacteriovorus* (Campoy*, et al.*, 2005; Erill*, et al.*, 2006; Mazon*, et al.*, 2006b). Even though there was huge variation in cassette numbers and configurations (in some species, like *S. usitatus*, multiple *imuA-imuB-dnaE2* cassettes coexisted, often in plasmids), the common denominator for cassette genes was explicit LexA regulation (Table 3). In fact, for all the phyla and groups in which the LexA-binding motif had been previously reported, a LexA box was found upstream of at least one of the cassette genes, and it was shown that presence of a LexA box was mandatory for either the *dnaE2* or *imuB* genes (or the first gene of their respective transcriptional units) (Erill*, et al.*, 2006). The Alpha Proteobacterium *Oceanicola batsensis*, in which the three cassette genes (*imuA*, *imuB* and *dnaE2*) reside at different genomic loci, is a paradigmatic example of this trend, since all three genes present a high-scoring

LexA box in their promoter region (Table 3). Moreover, in many species, like *A. capsulatum* or *B. bacteriovorus*, the *imuA-imuB-dnaE2* gene cassette seems to be the only transcriptional unit regulated by LexA (Campoy*, et al.*, 2005; Mazon*, et al.*, 2006b).

All these findings supported the hypothesis that *imuB* and *dnaE2* might cooperate in lesion bypass, but they also underscored the tight association between *lexA* and this multiple gene cassette. Besides the identification of additional *lexA-imuA-imuB-dnaE2* gene cassettes in other Proteobacteria, the persistent LexA-regulation of the cassette genes across phyla, in spite of drastic changes in LexA-binding sequence and cassette configurations, yielded the picture of a novel core regulon, far more supported than the formerly defined (*recA*, *uvrA*, *ruvAB* and *recN*), and composed of *lexA* and the members of the *imuA-imuB-dnaE2* gene cassette. The proposal of such a new core hypothesis is significant in several ways. For one, it situates the *E. coli* and *B. subtilis* genera, in which there is no trace of the multiple gene cassette, as exceptions rather than paradigms of the LexA regulon. In addition, by defining a smaller but more conserved regulon core, it opens a window into the evolutionary history of the LexA regulon by tracing its core constituent elements. Finally, but perhaps most significantly, the definition of such a new regulon core has implications on the primary function and origins of the LexA-governed SOS response, shifting the weight of evidence away from a precise and orchestrated mechanism of DNA repair and back towards a simple and handy DNA damage-induced translesion synthesis system.

A history of distress

Reconstructing the evolutionary history of the SOS response is not an easy task, since much data is still missing on many aspects of this genetic network in different phyla. In addition, the sequence of the system's regulatory protein, a typically powerful source of information on regulon history, is too short to support reliable phylogenetic inferences over domain spans. Given these constrains, it is not surprising that the two recent attempts at deciphering the evolutionary history of the *lexA* gene and its accompanying regulon have based their efforts on the tracing of two complementary pieces of information: the LexA-binding sequence (Mazon*, et al.*, 2004b) and the evolution of the core LexA regulon (Erill*, et al.*, 2006).

Putting box upon box

The first of these two works approached the subject of LexA evolution by experimentally validating the ability of diverged LexA proteins to bind their counterparts' regulatory motifs (Mazon, *et al.*, 2004b; Mazon, *et al.*, 2004a). By analyzing the number of changes required in, for instance, a *M. xanthus* LexA-binding motif to be bound by *E. coli* LexA, a probable history of the LexA box, and henceforth of the LexA protein, was extrapolated. The results showed that, as one would expect from other phylogenetic evidence, the LexA boxes of *F. succinogenes* and *M. xanthus* are probable intermediates between that of *B. subtilis* and *E. coli*. Conversely, the highly divergent Alpha Proteobacteria LexA box seemingly arose from a different evolutionary path and evolved its capacity to recognize direct-repeat motifs through the Cyanobacteria, which recognize a motif (GTAC-N_4-GTWC) that can be interpreted either as a direct or as an inverted repeat (Fig. 5). Furthermore, these results suggested for the first time there might be a direct equivalence between the *E. coli* and *B. subtilis* LexA-binding motifs, as the third dyad position in the *E. coli* LexA-binding motif (ctGt) seems to correspond to the first one in the *B. subtilis* LexA box (Gttc). Since no insertions were found in the aligned residues of the LexA recognition helices between these species, it was suggested that the LexA protein had evolved the capacity to recognize different spacer lengths by modifying its hinge dimerization angle, a fact that has also been advocated by modeling the binding of *B. subtilis* LexA to this organism LexA box (Groban, *et al.*, 2005).

Revealing as they were, though, the results from cross-regulation assays present some difficulties. Since they rely on ad-hoc point mutations in relatively short sequence elements, the ability of several LexA proteins to cross-regulate mutated operators might be simply hinting at common origins and binding mechanisms, instead at a straight, enumerable phylogenetic relationship. Taking stock of these results, a second study tackled the evolutionary history of *lexA* through a completely different route. This second analysis exploited the widespread distribution of the above described *imuA-imuB-dnaE2* gene cassette, to track down *in silico* the evolution of the LexA regulon (Erill, *et al.*, 2006).

Retracing the core

Taking advantage of its persistent regulation by *lexA*, phylogenetic analyses were conducted on the DnaE2 protein sequence, which is markedly larger than the LexA one and thus a good candidate for domain-wide phylogenetic inference. The results positively demonstrated (Fig. 6) that the history of the *lexA* gene is intimately linked with that of the *imuA-imuB-dnaE2* gene cassette and pointed to several outstanding events during the evolutionary history of the LexA regulon. On the one hand, for instance, possible lateral gene transfer instances of the *imuA-imuB-dnaE2* were identified in the Planctomycetes *R. baltica* and in several Alpha Proteobacteria species, in which plasmid dissemination was clearly established. On the other hand, and regarding *lexA*, the distribution of several *lexA-imuA-imuB-dnaE2* cassette instances with markedly different *lexA* genes suggested a duplication of this four-gene cassette that could explain the emergence of duplicated *lexA* genes with diverging LexA-binding motifs previously reported in the Gamma Proteobacteria (Comas*, et al.*, 2006) (Fig. 6). Furthermore, loss of this gene cassette in the Gamma Proteobacteria correlated well with the emergence in the Proteobacteria of the *umuDC* operon, also LexA-regulated in all its know instances, suggesting that the later might have compensated the loss of the mutagenic *imuA-imuB-dnaE2* cassette in the Enterobacteriaceae.

The evidence also suggested a role of the cell-division inhibitor protein SulA in the reconfiguration process that led to the split of the four-gene cassette and its later disappearance in *E. coli*. In this respect, it is interesting to note that the largest LexA regulons identified to date correspond to two species (*B. subtilis* and *E. coli*) lacking the *imuA-imuB-dnaE2* gene cassette, and that both contain cell-division inhibitor analogues regulated by LexA: *sulA* in *E. coli* (Huisman*, et al.*, 1984) and *yneA* in *B. subtilis* (Kawai*, et al.*, 2003). It has previously been shown that the LexA-binding sequence of *E. coli sulA* has evolved far less than its protein coding sequence (Freudl*, et al.*, 1987) and it has been suggested that *sulA*–mediated lethality in *lexA* mutants (Huisman*, et al.*, 1980) imposes a sort of bottleneck effect in the evolution of the LexA binding motifs (Erill*, et al.*, 2003). In this sense, it is tempting to speculate that the integration of a cell-division inhibitor in the LexA regulon sets it in an evolutionary dead-end with respect to its LexA box. By stabilizing the sequence of its LexA-binding motif, the presence of a cell-division inhibitor like SulA may promote the inclusion of additional genes to the regulon, leading to the large numbers of LexA-regulated genes in *E. coli* and *B.*

subtilis and, conversely, explaining the much meager regulons observed in other phyla.

The role of *sulA*, the behavior of multiple *lexA* genes in a same organism and many other interesting questions are still open concerning the nature and evolution of the LexA regulon, and addressing them will probably cast further light on the selective forces that for eons have shaped the SOS response. Even though the true origin of the *lexA* gene may probably remain forever unknown shrouded behind the veils of evolution, identifying new LexA-binding motifs, SOS networks and inducing signals in still unexplored phyla will surely put forward new testable theories on the nature and purpose of this particular regulatory network, which probably originated as a simple translesion synthesis system, but that has culminated in a fearsome antibiotic resistance machine deep within our guts.

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TABLES

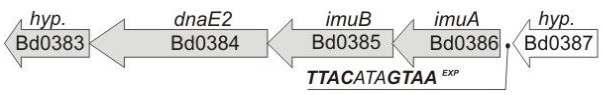
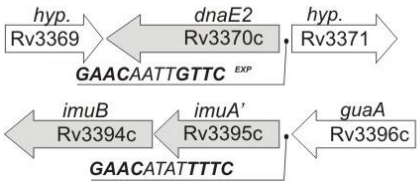
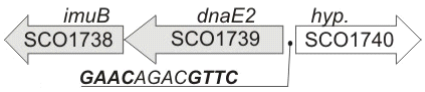
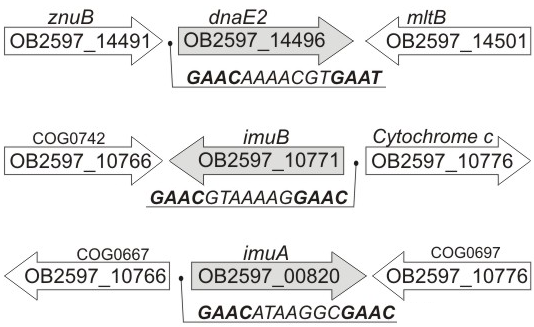
Table 1 – Regulon size and presence of regulon core genes in representative species of different phyla. The *in silico* predicted (PRED) or experimentally determined (EXP) number of LexA-regulated transcriptional units (TU) are shown if available.

Phylum	**Representative species**	**TU**		**Core genes**					**Reference**
		EXP	**PRED**						
Firmicutes (Gram +)	*Bacillus subtilis*	18	-	*lexA*	*recA*		*ruvCAB*		Au et al., 2005
Actinobacteria (Gram +)	*Mycobacterium tuberculosis*	15	-	*lexA*	*recA*	*uvrA*	*ruvCAB*		Davis et al., 2002
Green non-sulfur bacteria	*Dehalococcoides ethenogenes*	2	-	*lexA*		*uvrA*			Fernández de Henestrosa et al., 2002
Thermotogae	*Petrotoga miotherma*	2	2	*lexA*		*uvrA*			Mazón et al., 2006a
Cyanobacteria	*Anabaena sp.*	6	6	*lexA*	*recA*	*uvrA*		*ssb*	Mazón et al., 2004a
Fibrobacteres/Acido bacteria	*Fibrobacter succinogenes*	5	5	*lexA*	*recA*	*uvrA*	*ruvAB*	*ssb*	Mazón et al., 2004b
Fibrobacteres/Acido bacteria	*Acidobacterium capsulatum*	4		*lexA*					Mazón et al., 2006b
Spirochaetes	*Leptospira interrogans*	1	1		*recA*				Cuñé et al., 2005
Unclassified Proteobacteria	*Magnetococcus sp. MC-1*	2	-	*lexA*					Fernández de Henestrosa et al., 2003
Delta Proteobacteria	*Myxococcus xanthus*	2	-	*lexA*	*recA1*				Campoy et al., 2003
Alpha Proteobacteria	*Sinorhizobium meliloti*	7	15	*lexA*	*recA*	*uvrA*	*ruvCAB*	*ssb*	Erill et al., 2004
Beta Proteobacteria	*Ralstonia solanacearum*	-	3	*lexA*	*recA*				Erill et al., 2003
Gamma Proteobacteria	*Escherichia coli*	25	27	*lexA*	*recA*	*uvrA*	*ruvAB*	*ssb*	Fernández de Henestrosa et al., 2000

Table 2 – Representative instances of genes in mobile genetic elements that are known to be inducible through a RecA-dependent pathway.

Gene	Function	Inductor used	Induction effect	Genetic element	Host species	Induction type	Reference
CI	Repressor of lytic growth	Nalidixic acid	Prophage induction	Enterobacteria phage λ	*E. coli*	LexA-independent	(Sauer, *et al.*, 1982)
		Norfloxacin	Prophage induction and production of Shiga toxin	Stx1 converting phage	*E. coli*	LexA-independent	(Matsushiro, *et al.*, 1999)
		Ciprofloxacin and trimethoprim	Prophage induction and virulence genes (*sak*) expression	Staphylococcus phage phi13	*S. aureus*	LexA-independent	(Goerke, *et al.*, 2006)
SetR	CI-like repressor	Ciprofloxacin and mitomycin C	SXT transfer	STX Integrating conjugative element	*V. cholerae*	LexA-independent	(Beaber, *et al.*, 2004)
tum	Lysogenic cycle maintenance	UV radiation	Prophage induction	Coliphage 186	*E. coli*	LexA-dependent	(Shearwin, *et al.*, 1998)
caa	Colicin A	Mitomycin C	Colicin A production and release	Plasmid ColA	*E. coli*	LexA-dependent	(Lloubes, *et al.*, 1986)
cka	Colicin K	Ciprofloxacin	Colicin K production and release	Plasmid pColK-K235	*E. coli*	LexA-dependent	(Jerman, *et al.*, 2005)
rstA	Replication initiation factor	UV radiation	Prophage induction and production of Cholera toxin	Vibrio phage CTX	*V. cholerae*	LexA-dependent	(Quinones, *et al.*, 2005)
orf5	Small subunit of terminase	Ciprofloxacin, ampicilin, penicillin, ceftriaxone and cloxacillin	SaPIbov1 replication and transfer	SaPIbov1 pathogenicity island	*S. aureus*	LexA-dependent	(Maiques, *et al.*, 2006; Ubeda, *et al.*, 2005)

Table 3 – Schematic representation of the main cassette configurations found in complete and incomplete genome sequences. Locus or draft annotation names are provided for all cassette instances, together with their respective LexA-binding sequences. EXP stands for experimentally verified LexA-binding motifs.

Illustrative example	Configuration	Distribution
P. putida KT2440 	*lexA-imuA-imuB-dnaE2*	γ-Proteobacteria
B. bacteriovorus 	*imuA-imuB-dnaE2-hyp*	δ-Proteobacteria
A. tumefaciens 	*imuA-imuB-dnaE2*	α-Proteobacteria β-Proteobacteria γ-Proteobacteria Actinobacteria Acidobacteria Verrucomicrobia Planctomycetes Green non-sulfur bacteria
M.tuberculosis 	*imuA-imuB* *dnaE2*	Actinobacteria
S. coelicolor A3(2) 	*dnaE2-imuB*	Actinobacteria
Oceanicola batsensis 	*imuA* *imuB* *dnaE2*	α-Proteobacteria

FIGURES & LEGENDS

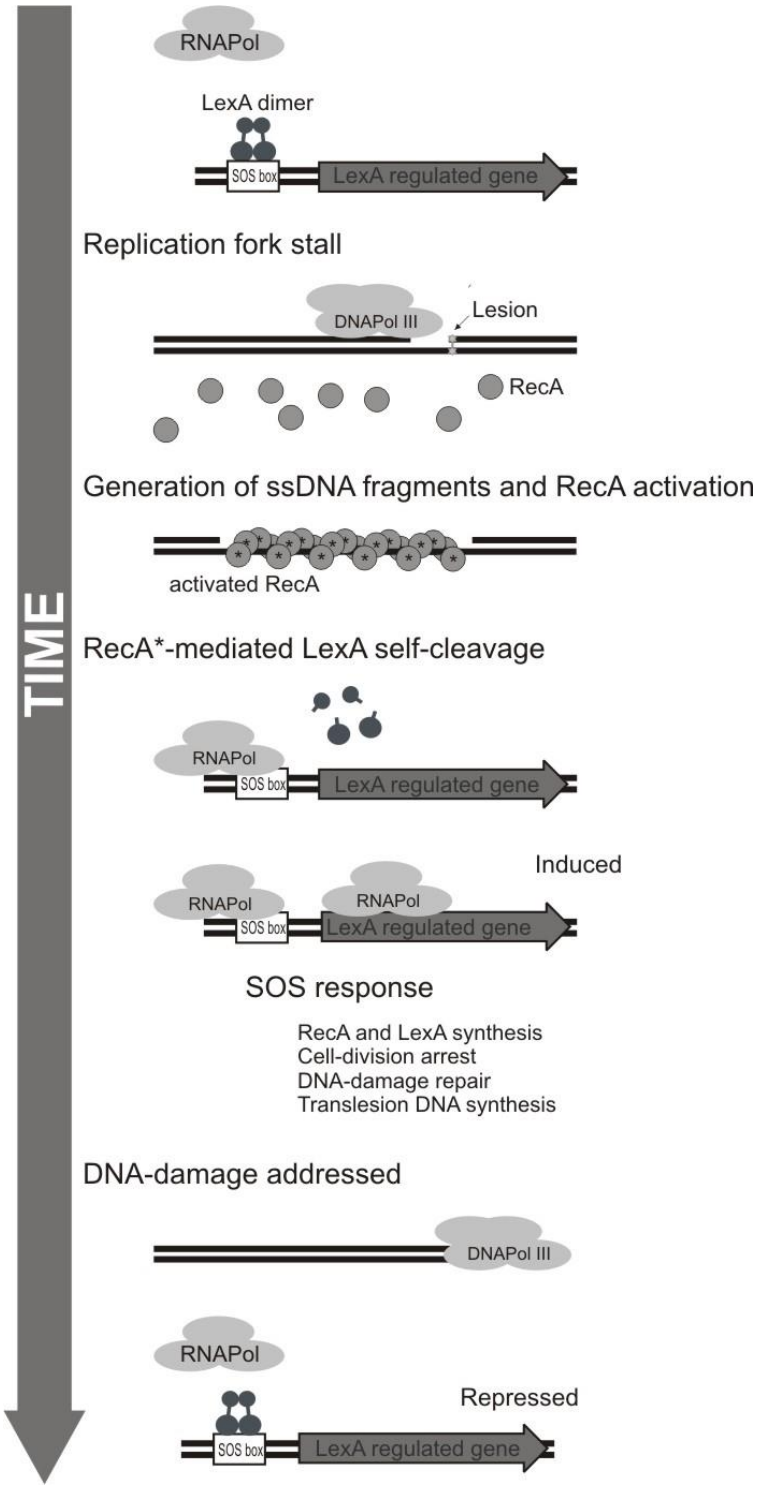


Fig. 1 – Schematic representation of the *E. coli* SOS induction process. LexA is initially bound to its binding sites (SOS boxes) upstream of SOS genes, hindering their transcription by blocking RNA-polymerase activity. DNA lesions lead to RecA activation, which in turn induces LexA self-cleavage. Cleaved LexA cannot form dimers and cannot bind to its binding sites, thereby de-repressing the system.

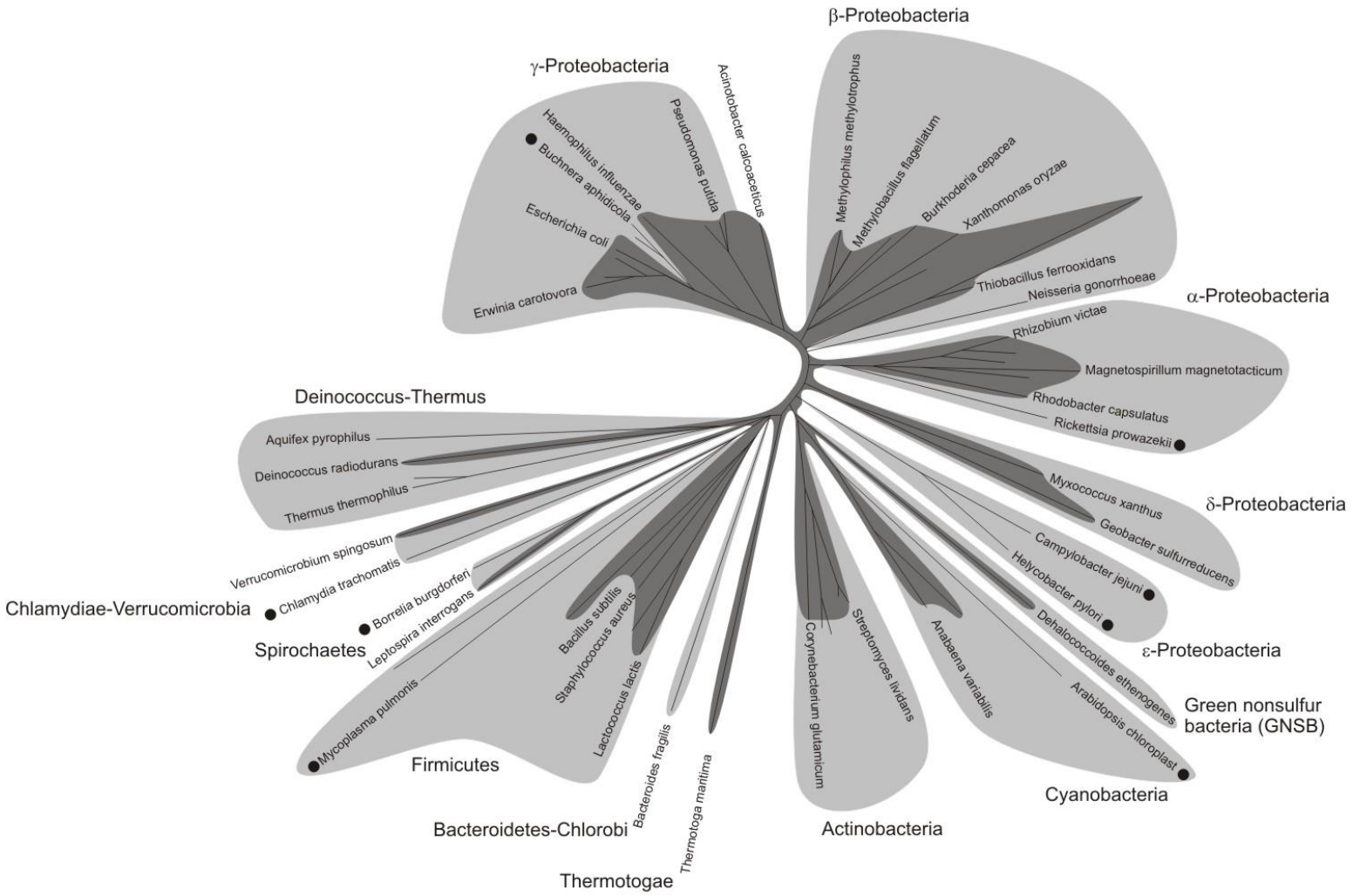


Fig. 2 – Distribution of the *lexA* gene across the bacterial domain, based on the phylogenetic distribution derived from RecA protein sequences (Eisen, 1995). Light grey areas enclose phylogenetic groups, while dark grey areas indicate presence of *lexA*. Filled circles denote species that have undergone substantial genomic reduction.

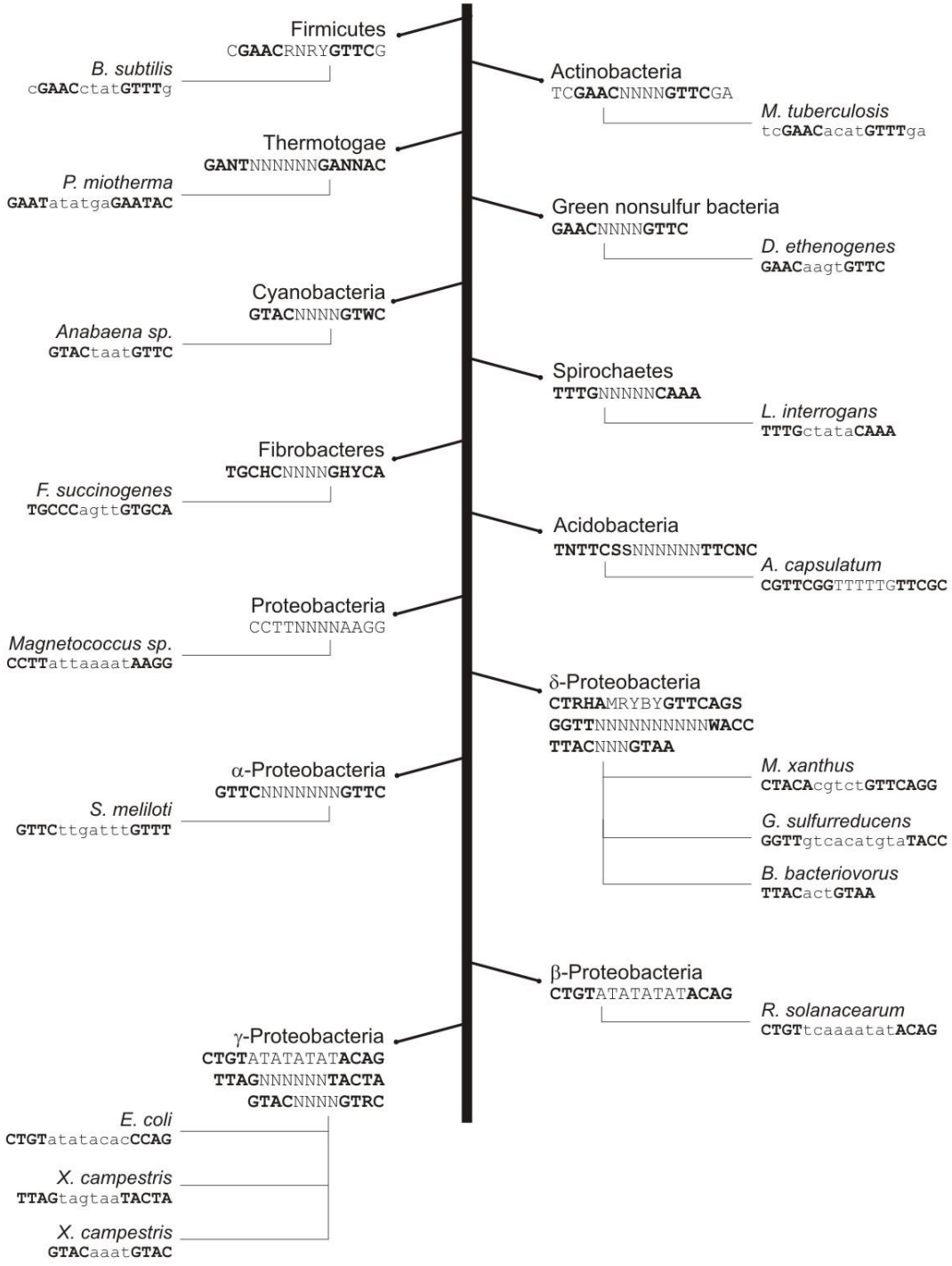


Fig. 3 – Distribution of LexA-biding sequences across the Bacteria Domain, following the branching points derived from phylogenetic signature analysis (Gupta, 2001). The figure shows both the phylum/class consensus motifs and the sequence of real motifs in representative species. Motif sequences correspond to the closest LexA-binding motif in the *lexA* promoter of the species, except for *L. interrogans* (in which the motif is located in the *recA* promoter). Bases in bold denote the conserved dyads of the different LexA binding motifs.

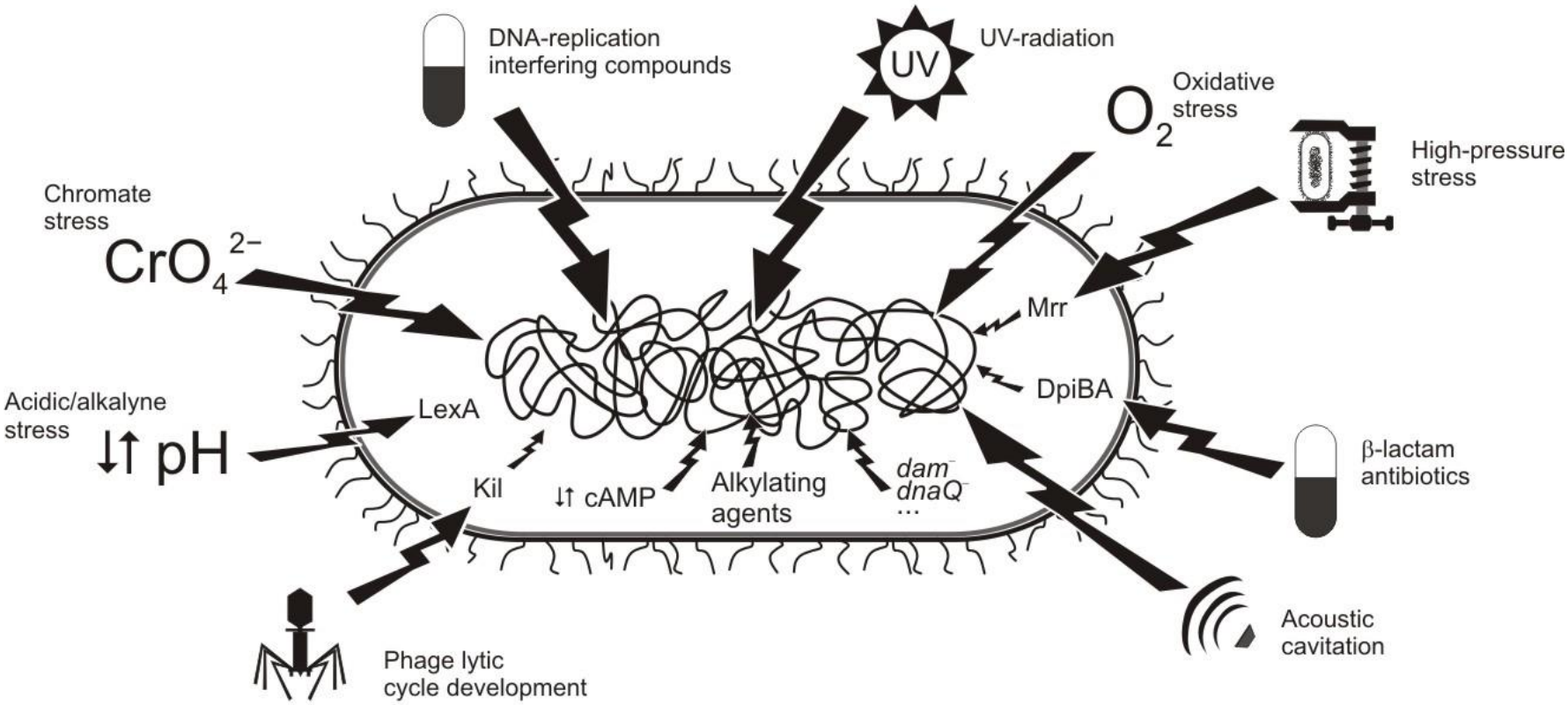


Fig. 4 – Direct and indirect triggers of the SOS response. Intermediary molecules involved in SOS induction are shown within their respective experimentally verified pathways. Endogenous activation mechanisms are designated by molecule/mutation names without external arrows.

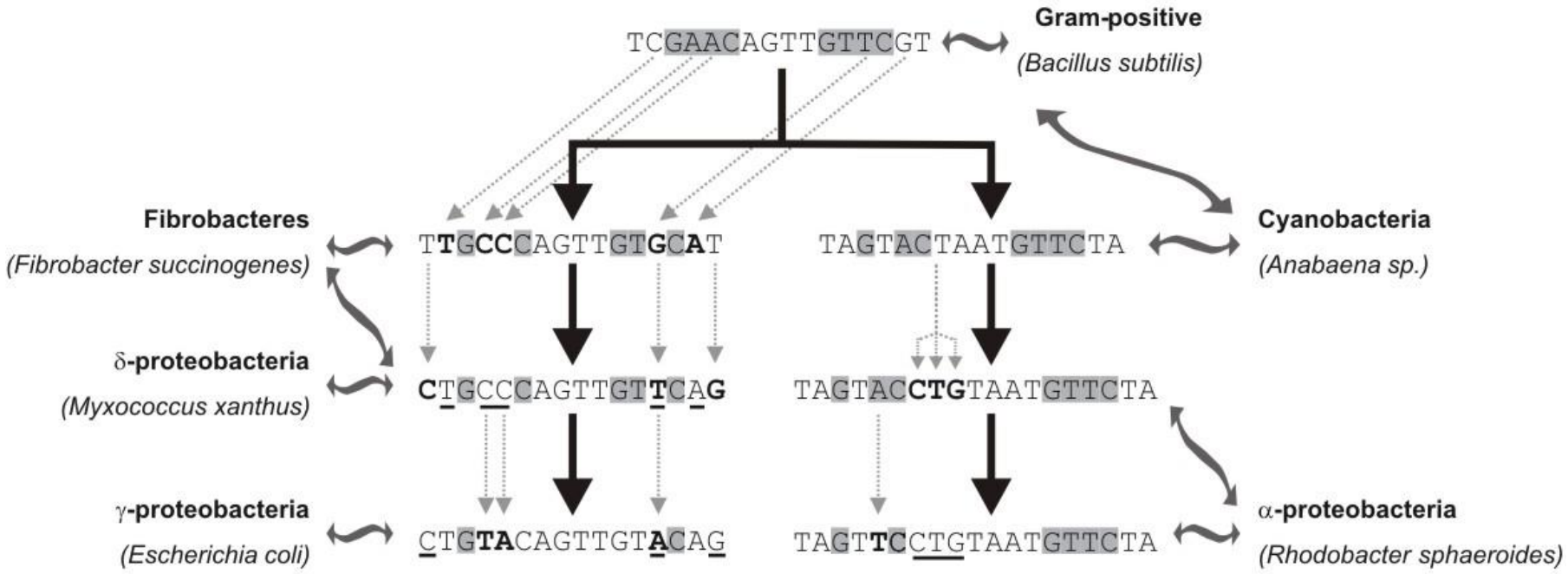


Fig. 5 – Reconstruction of the evolutionary history of LexA-binding motifs through directed mutagenesis and evaluation of LexA cross-binding ability (Mazon, *et al.*, 2004b). Solid arrows indicate the proposed evolutionary history, with two divergent pathways emerging from the Gram-positive bacteria. Dotted arrows point out the changes introduced by directed mutagenesis. Changes to the LexA-binding sequence are highlighted in bold at the step in which they were introduced, and remain underlined in the subsequent step. Two-sided arrows reveal the ability of different LexA proteins to cross-bind diverged LexA-binding motifs. Shaded bases reveal conserved positions from the dyads of the ancestral Gram-positive motif (GAAC-N_4-GTTC).

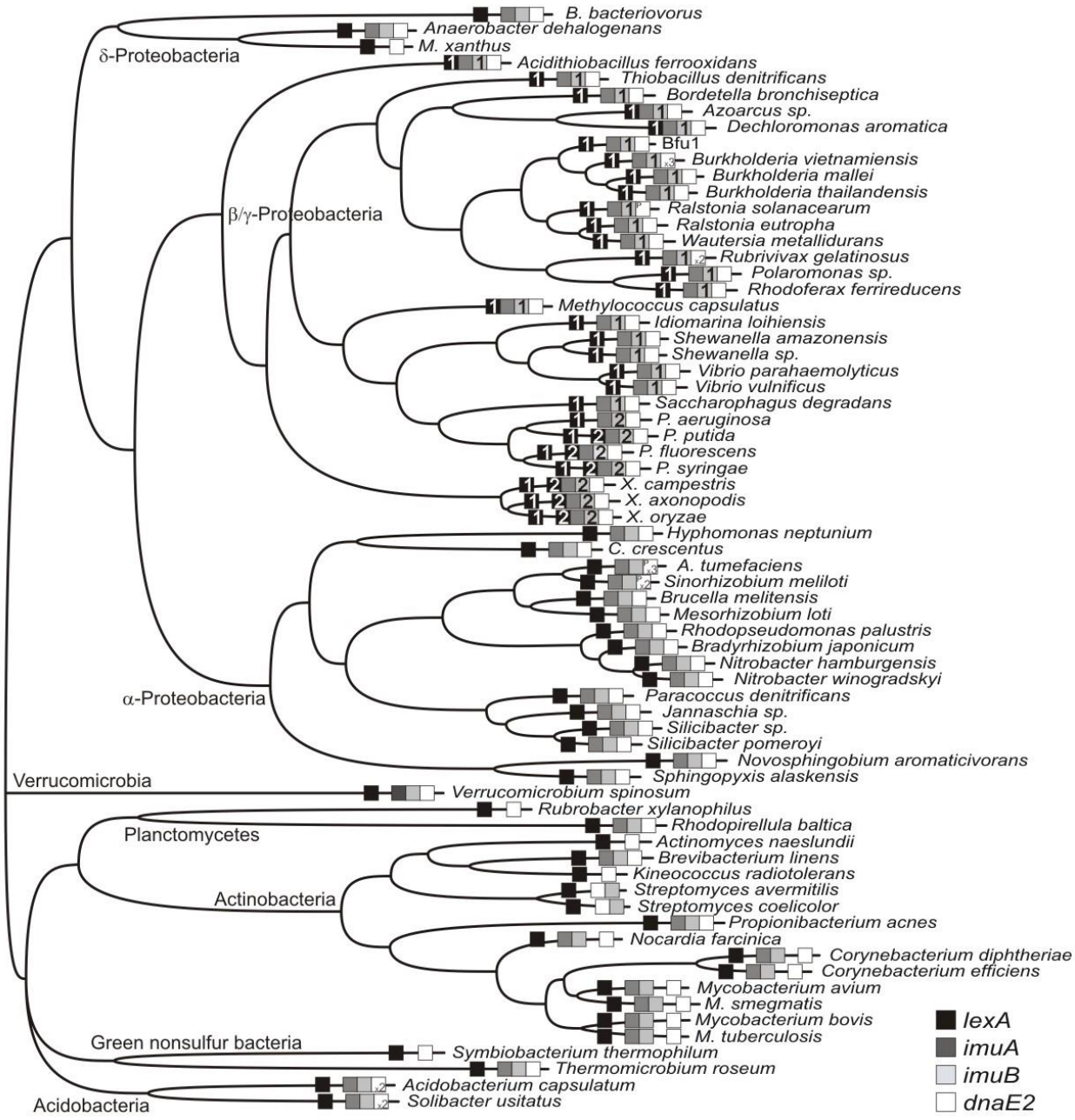


Fig. 6 – Distribution of *imuA-imuB-dnaE2* cassette variants across the Bacteria domain, following the phylogenetic distribution inferred from DnaE sequences (Erill*, et al.*, 2006). Cassette configurations are represented by filled squares designating their constituent genes. A x2 or x3 symbol in the *dnaE2* gene square indicates presence of, respectively, two or three cassettes in the same organism. A P in the *dnaE2* gene square indicates that cassettes are borne in plasmids in the corresponding organism. 1 and 2 symbols in the *lexA* and *imuB* gene squares denote the two copies of the *lexA-imuA-imuB-dnaE2* cassette in the β- and γ-Proteobacteria, after the duplication hypothesis previously proposed in this group (Erill*, et al.*, 2006).

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