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Expression of canonical SOS genes is not under LexA repression in *Bdellovibrio bacteriovorus*

by

Susana Campoy[1], Noelia Salvador[2], Pilar Cortés[2], Ivan Erill[3], and Jordi Barbé[*1,2]

[1] Centre de Recerca en Sanitat Animal (CReSA), 08193 Bellaterra, Spain

[2] Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona 08193 Bellaterra, Spain

[3] Biomedical Applications Group, Centro Nacional de Microelectrónica, 08193 Bellaterra, Spain

[*] To whom reprint requests should be addressed.

E-mail: jordi.barbe@uab.es

Phone: 34 - 93 – 581 1837

Fax: 34 – 93 – 581 2387

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ABSTRACT

The here reported identification of the LexA-binding sequence of *Bdellovibrio bacteriovorus*, a bacterial predator belonging to Delta Proteobacteria, has made possible a detailed study of its LexA regulatory network. Surprisingly, only the own *lexA* gene and a multiple gene cassette including *dinP* and *dnaE* homologues are regulated by the LexA protein in this bacterium. *In vivo* expression analyses have confirmed that this gene cassette forms indeed a polycistronic unit that, like the *lexA* gene, is DNA damage inducible in *B. bacteriovorus.* Conversely, genes such as *recA*, *uvrA*, *ruvCAB* and *ssb*, which constitute the canonical core of the Proteobacteria SOS system, are not repressed by the LexA protein in this organism, hinting at a persistent selective pressure to maintain both the *lexA* gene and its regulation on the reported multiple gene cassette. In turn, *in vitro* experiments show that the *B. bacteriovorus* LexA-binding sequence is not recognized by other Delta Proteobacteria LexA proteins, but binds to the cyanobacterial LexA repressor. This places *B. bacteriovorus* LexA at the base of the Delta Proteobacteria LexA family, revealing a high degree of conservation in the LexA regulatory sequence prior to the diversification and specialization seen in deeper groups of the Proteobacteria phylum.

INTRODUCTION

Bacterial cells contain several pathways targeted at the repair of DNA damage and, among these, one of the most extensively studied is the SOS system. Initially described in *Escherichia coli* (1), the SOS response regulates in this organism the expression of up to 40 genes under direct control of the RecA and LexA proteins, which are also members of this regulon (12, 21). The products of *E. coli* SOS genes target a number of different cellular processes, such as inhibition of cell division, error-prone replication or excision repair (34). The LexA protein is the repressor of the system, and mediates its repression through the specific binding of its N-terminal domain to the regulatory motifs present in the promoter region of SOS genes. This regulatory motif, commonly dubbed the LexA box, has in *E. coli* a consensus sequence $CTGTN_8ACAG$ (34) that has been reported also in many other members of the Gamma and Beta Proteobacteria (9). The RecA protein acts as sensor and inducer of the SOS system. Sensing is mediated by unspecific binding of RecA to single-stranded DNA fragments, generated by DNA damage-mediated interruption of replication, the enzymatic processing of broken DNA ends (29) or the inactivation of chromosome replication involved genes (24). After binding, RecA acquires an active state that enables it to promote the autocatalytic cleavage of LexA Ala^{84}-Gly^{85} bond (20). This cleavage, carried out by LexA C-terminal residues Ser^{119} and Lys^{156}, is similar to that mediated by serine proteases (20, 22) and effectively inhibits LexA from binding its target recognition sequences, thereby inducing the SOS response and activating the expression of DNA repair genes. Once DNA lesions have been repaired, RecA ceases to be activated and non-cleaved LexA protein returns to its normal levels, repressing again the transcription of SOS genes. The *lexA* gene is widespread among bacteria, and is present in most phylogenetic groups, in which different monophyletic LexA-binding motifs have been described (9, 11, 35). Moreover, comparative analyses of the SOS system in different Proteobacteria classes (Alpha, Beta and Gamma) indicate that a common set of genes (*lexA*, *recA*, *ssb*, *uvrA* and *ruvCAB*) is directly repressed by LexA in all these species and, therefore, constitutes the canonical gene composition of the SOS regulon in this phylum (9, 10).

Bdellovibrio bacteriovorus is a Gram-negative, vibrio-shaped bacterium belonging to the Delta Proteobacteria class that preys on other Gram-negative bacteria. Its typical life cycle consists of an obligate alternation between two distinct morphological stages: an attack and a growth phase (31). The first is initiated by a flagellated, notoriously fast free-swimming cell that is incapable of independent proliferation. In this phase, *B. bacteriovorus* recognizes, binds, attacks and enters the periplasmic space of its prey. Following penetration, a variety of morphological and physiological changes take place in both *B. bacteriovorus* and its host, enabling *B. bacteriovorus* to grow efficiently into a septate filament at the expense of its host cellular material. Afterwards, the filamented *B. bacteriovorus* cell typically fragments into flagellated attack phase cells, although it may form a bdellocyst that is able to linger in the ghost prey cell until harsh conditions or polluted environments die away. In spite of the fact that *B. bacteriovorus* wild-type strains are host-dependent predators (HD), host-independent (HI) mutants have been isolated and have been used to study several aspects of this organism (30).

Since its parasitic lifestyle requires it to undergo regular and extensive contact with host populations, *B. bacteriovorus* is frequently exposed to a host of bacteriocins, microcins and antibiotics that target DNA, and it is to be expected that such an environmental pressure should reflect on its DNA repair systems. Strict parasites such as the *Rickettsiae*, for instance, have adapted to similar conditions by adopting a constitutive expression of DNA repair genes that goes in conjunction with the loss of their *lexA* gene due to drastic genome reduction (2). No *in vivo* data is available on the *B. bacteriovorus* repair systems, but the sole information on this subject, coming mainly from the *B. bacteriovorus* genome sequence (25), indicates that *B. bacteriovorus* presents homologues of all the genes that constitute the canonical core of the Proteobacteria SOS system, including *lexA*. In this context, the LexA-binding sequence of this organism has been identified here to determine which genes constitute its LexA regulon and to analyze its response against DNA damage.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *Bdellovibrio bacteriovorus* HI100 host-independent strain used in this work was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) and it was grown at 30°C in PPYE medium (30). All plasmid constructions and cloning experiments were performed in *E. coli* DH5α using the pGEM-T® vector (Promega). Plasmid DNA was transformed into competent *E. coli* cells as described previously (27).

Nucleic acids techniques. RNA and DNA total extraction was carried out by standard methods (27). Genes and promoter fragments for electrophoretic mobility shift assays (EMSAs) were isolated by PCR from total DNA extraction, using suitable oligonucleotide primers designed in accordance to the *B. bacteriovorus* published sequence. Mutants in the *B. bacteriovorus lexA* promoter were obtained by PCR mutagenesis using oligonucleotides that carried the desired substitutions. The DNA sequence of all PCR-mutagenized fragments was determined by the dideoxy method (28) on an ALF Sequencer (Pharmacia Biotech). RT-PCR assays were done using Titan One Tube RT-PCR System (Roche) following the manufacturer instructions. Real time RT-PCR analysis of gene expression was performed for all genes as reported (5) and using specific internal oligonucleotide primers for each one (Table 1) (25). In all cases, the absence of DNA in RNA samples was tested by PCR without reverse transcriptase addition. The specificity of primers was checked by cloning and sequencing each amplification product by the dideoxy method (28) on an ALF Sequencer (Pharmacia Biotech). The RNA concentration of the gene to be analyzed was always normalized to that of the *B. bacteriovorus* total RNA as previously described (8). *In silico* identification of *B. bacteriovorus* LexA-regulated genes was carried out using RCGScanner, a consensus-building software for the prediction of regulatory motifs that has been previously described (9).

Purification of LexA protein. The *B. bacteriovorus lexA* gene was cloned by PCR using specific primers designed from its published sequence (25). The PCR fragment containing the *B. bacteriovorus lexA* gene was cloned into a pGEM-T® vector and inserted into a pET15b expression vector. The pET15b-derivative containing the *B. bacteriovorus lexA* gene was then transformed into the *E. coli lexA* (Def) BL21 (DE3) codon plus strain (12) for over-expression of its encoding LexA protein, which was subsequently purified using the Talon ™ Metal Affinity Resin Kit (Clontech) as reported (23). The *B. bacteriovorus* LexA protein thus obtained was above 95 % purity as determined with Coomassie Blue staining of SDS-PAGE (15 %) polyacrylamide gels (data not shown) following standard methodology (9). The *Bacillus subtilis* purified LexA protein was kindly provided by Dr. Roger Woodgate. LexA protein from *Anabaena* PCC7120 also used in this work had been previously purified (23).

Electrophoresis mobility shift and footprinting assays. LexA-DNA binding was analyzed for each gene promoter by electrophoresis mobility shift assays (EMSAs) using purified *B. bacteriovorus* LexA protein. DNA probes were prepared by PCR amplification with one of the primers labeled at its 5' end with digoxigenin (DIG) and purifying each product in a 2%–3% low-melting-point agarose gel. DNA-protein reactions (20 μl) typically containing 20 ng of the DIG-DNA-labeled probe and 80 nM of purified LexA protein were incubated in binding buffer: 10 mM N-2-Hydroxyethyl-piperazine-N' 2- ethanesulphonic acid (HEPES), NaOH (pH 8), 10 mM Tris-HCl (pH 8), 5% glycerol, 50 mM KCl, 1 mM EDTA, 1 mM DTT, 1mg ml-1 of salmon DNA and 50 μg/ml BSA. After 30 minutes at 30ºC, the mixture was loaded onto a 6% non-denaturing Tris-glycine polyacrylamide gel (pre-run for 30 minutes at 10 V/cm in 25 mM Tris-HCl (pH 8.5), 250 mM glycine, 1 mM EDTA). DNA-protein complexes were separated at 150 V for 80 min, followed by transfer to a Biodine B nylon membrane (Pall Gelman Laboratory). DIG-labeled DNA-protein complexes were detected following the manufacturer protocol (Roche). For the binding-competition experiments, a 300-fold molar excess of either specific or non-specific unlabelled competitor DNA was also included in the mixture. All EMSAs were repeated a minimum of three times to ensure reproducibility of results. The DNAse I footprinting assay carried out on the Alf Sequencer (Pharmacia Biotech) was performed as described before (6). *B. bacteriovorus* LexA protein was added to the union reaction at a 160 nM final concentration.

RESULTS

Identification of the *B. bacteriovorus* LexA recognition sequence. The first necessary step in the analysis of the *B. bacteriovorus* LexA network was the elucidation of the LexA binding sequence in this species. Taking advantage of the fact that self-regulation is to date a defining property of the *lexA* gene, electrophoresis mobility shift assays (EMSAs) were carried out with purified *B. bacteriovorus* LexA protein, using as a probe a DNA fragment extending from –201 to +73 (with respect to its translation start point) of the *lexA* gene. As expected, the addition of LexA protein specifically decreased the mobility of the *lexA* promoter fragment, while an excess of unlabelled *lexA* promoter abolished the delay, thereby confirming specific binding of LexA to the *lexA* promoter (Fig. 1). To further determine the position of the *B. bacteriovorus* LexA binding sequence, serial deletions of the *lexA* upstream promoter region were generated and analyzed in EMSAs with the purified LexA protein. EMSAs results suggested that the *B. bacteriovorus* LexA box is located between the –35 and –15 positions upstream of the *lexA* translation start codon (data not shown).

After honing in the approximate location of the *B. bacteriovorus* LexA box, the specific sequence recognized by LexA was identified through footprinting experiments with a 172 bp fragment extending from positions –99 to +73 of the *lexA* promoter. The data thus obtained shows that a core region of 35 nucleotides was protected by LexA-binding when both *lexA* coding and non-coding strands were analyzed (Fig. 2), and a visual inspection of this protected sequence revealed the presence of the ATTTACACTGTAAGT imperfect palindrome. To further confirm that this palindrome was indeed the LexA-binding motif of *B. bacteriovorus*, and taking into account that the *recA* gene has been shown to be a robust candidate for LexA regulation, additional EMSAs were carried out with purified LexA protein and the promoter regions of the two annotated copies of *recA* (Bd0386 and Bd0512) in this organism.

The results revealed that only one of the two annotated *recA* genes (Bd0386) did bind LexA (Fig. 3), whereas the mobility of Bd0512 was not affected by LexA (data not shown). In this respect, it should be noted that, while the product of Bd0512 is an obvious homolog of *E. coli* RecA (64% identity using BLAST), the same does not hold true for the product of the Bd0386 gene (25% identity). To further elucidate whether the product of Bd0386 was a functional RecA protein, both Bd0386 and Bd0512 genes were cloned into pGEM-T® vectors and used to trans-complement a *recA* defective *E. coli* strain. Comparative analyses of survival rates following UV irradiation (data not shown) revealed that Bd0512 is able to complement a *recA E. coli* mutant, whereas complementation with Bd0386 does not increase survival rates when compared to non-complemented *E. coli recA* strain (data not shown).

The above data is in agreement with the distinct placement by phylogenetic inference algorithms of Bd0386 as a natural out-group to a tree of bacterial RecA sequence (data not shown) and, together, both results give convincing support to the hypothesis that Bd0386 is not a *recA* gene. Moreover, the three ORFs (Bd0385, Bd0384, Bd0383) immediately downstream of Bd0386 have been annotated, respectively, as a hypothetical protein with homology to COG0389 (*dinP*), a DNA polymerase III α subunit (*dnaE*) and a conserved hypothetical protein. Recently, a *lexA*-dependent DNA damage-inducible gene cassette consisting of a single polycistronic transcriptional unit that encompasses a *sulA*-like gene, *dinP* and *dnaE* genes has been shown to be widespread among Proteobacteria (1). In the light of this, and taking into account that some internal regions of the RecA protein present a strong structural similarity with those of SulA (7), it seems likely that the product encoded by the Bd0386 ORF may be the SulA-like protein described in the aforementioned gene-cassette.

In agreement with the above described results, a close examination of the Bd0386 promoter region revealed the presence of a palindromic sequence very similar to that present in the *lexA* promoter (ATTTACATAGTAAGT), whilst no similar sequence could be found in the promoter region of its own *recA* gene (Bd0512). Comparison of the Bd0386 and the *lexA* motifs yielded the consensus sequence ATTTAC-AYW-GTAAGT, hinting at the dyad-spacer-dyad structure that is typical of LexA-binding motifs. To further elucidate which nucleotides of the observed motif were directly involved in LexA binding and thus constituted the LexA box of *B.*

bacteriovorus, the LexA-binding motif present in the *lexA* promoter was analyzed through site-directed mutagenesis. Point mutations were introduced into the left (ATTTAC) and right (GTAAGT) halves of the *lexA* promoter motif and into the variable spacer region (ACT), and their effect on the electrophoretic mobility of the *lexA* promoter was analyzed through EMSAs.

EMSA results revealed that only the four internal bases of each dyad (TTAC and GTAA, respectively) were strictly required for binding, and that changes in the spacer region did not affect binding (Fig. 4). Taken together, these data demonstrate that the presence of a TTACN$_3$GTAA palindromic sequence is required for the binding of the *B. bacteriovorus* LexA protein to its own promoter, indicating that the *B. bacteriovorus* LexA box is substantially different from that of other Delta Proteobacteria, such as *G. sulfurreducens* or *M. xanthus* (6, 16).

Presence of the *B. bacteriovorus* LexA recognition sequence upstream of additional genes. After identification of the *B. bacteriovorus* LexA box, a search against its complete genome sequence was carried out using the consensus-building software RCGScanner (9) and the identified LexA-binding sequence (TTACN$_3$GTAA) as a template. Surprisingly, besides the previously identified sequences in the promoters of *lexA* and Bd0386, only a low-scoring putative motif was detected in the vicinity of one of the annotated *B. bacteriovorus uvrA* genes (Bd2442). To analyze the binding affinity of this putative *uvrA2* motif, and to determine whether the lack of additional positive results for other canonical SOS genes was due to an excessively astringent motif search, EMSA analyses were carried out on the promoter region of canonical SOS genes besides *recA* (i.e. *uvrA1, uvrA2*, *ssb* and *ruvCAB*). The results (data not shown) revealed that none of these genes is able to bind *B. bacteriovorus* LexA protein, and demonstrated that the low-scoring motif in the promoter of *uvrA2* was not functional. Moreover, even though real time RT-PCR experiments confirmed that both *lexA* and Bd0386 are induced in the presence of mitomycin C (Table 2), they also demonstrated that the expression of *recA* (Bd0512), *uvrA1, uvrA2* and *ssb* is constitutive and not DNA-damage inducible (Table 2). Conversely, it is worth noting that expression of the *ruvCAB* operon is positively triggered by DNA injuries (Table 2), even though no LexA binding motifs, nor experimental LexA-binding activity, can be detected in its promoter.

Regarding the Bd0386 gene, and its putative relation with the *sulA*-like gene of described *sulA-dinP-dnaE* multiple gene cassettes, RT-PCR analyses consistently demonstrated that Bd0386, Bd0385, Bd0384 and Bd0383 ORFs are encoded in a single polycistronic mRNA (Fig. 5). This result, together with the presence of a functional LexA-binding motif in the promoter of Bd0386, indicates that the Bd0386-Bd0385-Bd0384-Bd0383 operon is probably another instance of the aforementioned *sulA-dinP-dnaE* cassette and that, in accordance with that observed in other Proteobacteria, expression of this gene cassette is directly regulated by LexA in *B. bacteriovorus*.

***B. bacteriovorus* LexA-binding motif binds the Cyanobacteria LexA repressor.** The here identified LexA-binding sequence of *B. bacteriovorus* and the atypical conformation (lacking all the canonical SOS genes) of the *B. bacteriovorus* LexA-regulon both raise interesting questions with regard to the particular evolution of the *lexA* gene in this species. In particular, and in view of the monophyletic character of LexA-binding sequences in many clades, the presence of a LexA-binding motif that is substantially divergent with respect to other previously reported Delta Proteobacteria LexA-binding sequences (*G. sulfurreducens* and *M.*

xanthus) is remarkable, as it hints at a period of heightened evolution of the LexA protein, which ultimately might have given rise to the prototypical LexA proteins and binding sequences observed in the Gamma or Alpha Proteobacteria subclasses (9, 10). To further pin down the location of the *B. bacteriovorus* LexA protein in the aforementioned evolutionary thread, the relationship between the LexA protein of this organism and that of other species was explored here by an experimental cross-binding analysis of its LexA-binding sequence.

In an initial analysis, the binding capability of the LexA protein from closely related species to the *B. bacteriovorus lexA* promoter was analyzed through EMSAs. The results (data not shown) indicate that none of the available Delta Proteobacteria LexA proteins (*G. sulfurreducens* and *M. xanthus*) is able to bind the *B. bacteriovorus lexA* promoter, and that the same holds true for both Alpha (*R. sphaeroides*) and Gamma (*E. coli*) Proteobacteria LexA proteins, as should be expected if these proteins are the result of a further specialization from their common ancestor with *B. bacteriovorus* LexA.

Since the *lexA* gene is absent from several phylogenetic groups (e.g., *Bacteroides*, Green Sulfur bacteria) immediately preceding the Delta Proteobacteria appearance in all accepted phylogenies (13, 14), the most probable common ancestor of *B. bacteriovorus* LexA should be with the Cyanobacteria, whose LexA-binding sequence has recently been shown to be strongly related to the Gram-positive one (23). To check whether this hypothesis held true at the binding sequence level, a cross-binding assay was carried out with *Anabaena* and *B. subtilis* LexA proteins. As shown in Fig. 6, the *B. subtilis* LexA protein is unable to bind *B. bacteriovorus lexA* promoter, but the Cyanobacteria LexA protein is manifestly able to bind it, as a LexA-DNA complex can be detected with the wild-type promoter but not with a mutant derivative unable to bind *B. bacteriovorus* LexA. This suggests that *B.*

bacteriovorus LexA represents a primordial Delta Proteobacteria LexA protein, prior to the further specialization seen in the rest of species of this and other Proteobacteria Classes.

DISCUSSION

The results reported in this work convey conclusive evidence that the canonical SOS genes (*recA*, *uvrA*, *ssb* and *ruvCAB*) are not repressed by LexA in *B. bacteriovorus*. It has also been established that the *B. bacteriovorus lexA* gene presents self-regulation and that its LexA protein binds and directly regulates a multiple-gene cassette consisting of four ORFs (Bd0386- Bd0385- Bd0384- Bd0383) whose homologues have been previously associated to DNA-repair activity (1) In concordance with the above data, it has been shown that the *lexA* gene and the aforementioned multiple-gene cassette are both DNA-damage inducible. Moreover, it has also been demonstrated that the expression of most Proteobacteria canonical SOS genes (*recA*, *uvrA* and *ssb*) is constitutive and does not respond to DNA damage.

Taking into account the host-dependent lifestyle of *B. bacteriovorus*, which exposes it regularly to a collection of antagonist compounds (e.g. colicins, microcins), the fact that most canonical SOS genes are not DNA-damage inducible should not constitute an unexpected result. Under such an environmental pressure, it is reasonable to expect a constitutive expression of DNA-repair pathways, such as those encompassed within the LexA regulon, to neutralize the deleterious effects of antagonist compounds. In fact, many of the obligate parasitic bacteria that thrive inside eukaryote cells, in which endogenous DNA-damaging agents abound, have lost their *lexA* gene and maintain most of their DNA repair genes under constitutive expression (2, 15, 32). In the light of this, the here reported DNA-damage mediated induction of the *ruvCAB* operon independent of LexA and the own presence of a functional *lexA* in *B. bacteriovorus* constitute surprising results. In this respect, it should be noted that LexA-independent DNA-damage induction of DNA repair genes has been previously reported in other bacteria (5, 6, 24) and it seems evident that, at least in some bacterial species, LexA might not be the sole regulator of the global response against DNA injuries.

In the above framework, however, both the presence of a functional *lexA* gene in *B. bacteriovorus* and the particular organization of its LexA regulon pose an intriguing question. If the environmental pressure stemming from *B. bacteriovorus* host-dependent lifestyle is towards a progressive loss of SOS regulation, a counter-selective factor must be invoked to explain the persistence of a functional *lexA* gene. Apart from itself, *B. bacteriovorus* LexA only regulates a multiple-gene cassette with homologues in several Proteobacteria subclasses. Interestingly, in all the instances reported to date this multiple-gene cassette is explicitly regulated by LexA, either via a LexA-binding motif or through the constitution of a larger operon in which *lexA* is the leading gene (1, 9, 10). Taking into account that, despite the existence of a minimal core of LexA regulated genes in all bacteria studied so far which present a *lexA* gene, many conventional SOS genes are not under direct LexA control in some species, the fact that this multiple-gene cassette is overtly regulated by LexA in all its known occurrences suggests that regulation by LexA of one or more of its encoded products is either mandatory or extremely beneficial to the bacterial cell. Having established that the first cassette gene (Bd0386) is not a *recA* homolog, two complementary hypothesis arise to explain the regulation of this multiple gene cassette (typically composed of *sulA*, *dinP* and *dnaE* homologues) in *B. bacteriovorus* and, hence, the conservation of a functional copy of LexA in this organism. On the one hand, the *E. coli sulA* product has been shown to be a cell division inhibitor that blocks FtsZ ring formation, leading to filamentation and, eventually, cell death (3). Hence, if Bd0386 is indeed a functional *sulA* homolog, its regulation by LexA should be mandatory, as has been observed in all known instances of *E. coli sulA* homologues (9). On the other hand, the *E. coli* DNA polymerase IV encoded by *dinP*, has been shown to yield mutator phenotypes when deregulated (18), leading to a lower adaptative fitness (33). The second gene in *B. bacteriovorus* multiple gene cassette (Bd0385) is precisely a *dinP* homolog and, therefore, the functional presence of LexA could be equivalently explained by the adaptative advantage of explicitly repressing the Bd0385 product until it is strictly necessary for survival (i.e. the SOS response). Following this line of thought, the third gene in the aforementioned gene cassette is a homolog of the *dnaE* protein. *B. bacteriovorus* presents two *dnaE* genes (Bd0384 and Bd2078), but the one included in the multiple gene cassette (Bd0384) shares the highest identity with the alpha subunit of the DnaE2 polymerase described in *Mycobacterium tuberculosis*, while Bd2078 is most probably the catalytic unit of its replicative polymerase. In this respect, the product of the *M. tuberculosis dnaE2* gene has been shown to participate in error-prone DNA repair synthesis (4). Therefore, its is also possible that it is the presence of this *dnaE2* gene in the in *B. bacteriovorus* multiple gene cassette which has led to its explicit regulation by LexA, a fact that has been also observed in other species harboring the *dnaE2* gene in the same or different genetic arrangements, such as *M. tuberculosis*, *Pseudomonas putida* or *Agrobacterium tumefaciens* (1,4).

Finally, the fact that the cyanobacterial LexA protein that was able to recognize the *B. bacteriovorus* LexA binding box indicates that the sequence of this motif has been significantly preserved during bacterial evolution, at least until the divergence process which generated more recent Proteobacteria Classes took place.

This line of reasoning is further reinforced by the fact that the *B. bacteriovorus* LexA network is markedly different from that described in the Cyanobacteria. The Anabaena LexA protein, for instance, has been shown to regulate the expression of canonical SOS genes such as *recA*, *lexA*, *uvrA* or *ssb* (23) that, but for *lexA*, are not regulated in *B. bacteriovorus*. Taken together, the proximity of LexA-binding motifs and the differences in LexA regulon composition between *B. bacteriovorus* and the Cyanobacteria support the idea that intense environmental pressure has led *B. bacteriovorus* to deregulate most of its canonical SOS genes in a relatively short evolutionary span and prior to the extensive diversification in LexA-binding motifs seen in ulterior Proteobacteria lineages.

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LEGENDS OF FIGURES

Fig. 1. Electrophoretic mobility shift assay (EMSA) of the *B. bacteriovorus lexA* promoter in absence (lane 1) or presence (lane 2) of 80 nM of purified *B. bacteriovorus* LexA protein. To determine the specificity of LexA binding, a 300-fold molar excess of either unlabelled *lexA* promoter (lane 3) or pGEM-T plasmid DNA (lane 4) were used as a specific or non-specific competitor fragment, respectively.

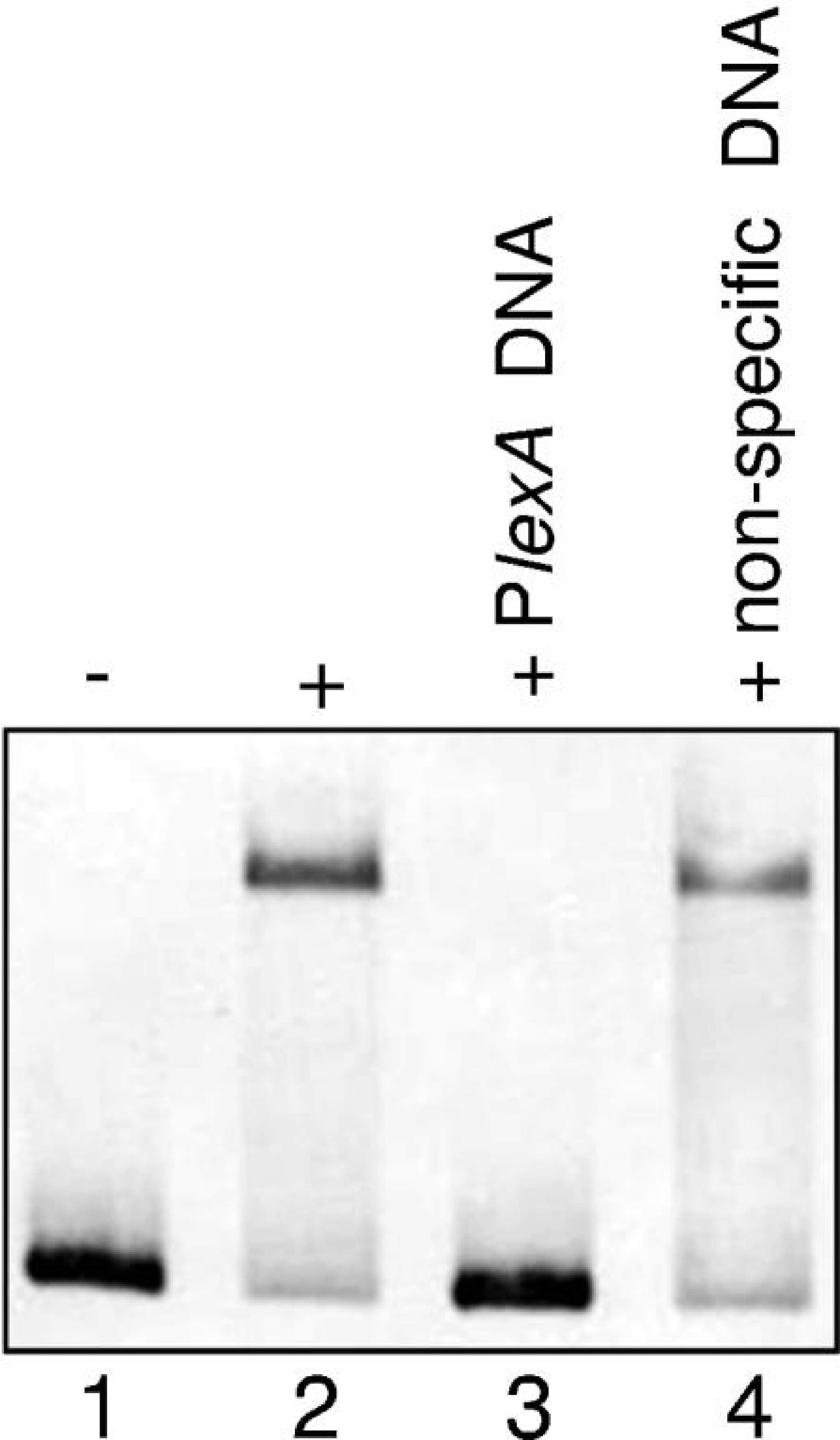
Fig. 2. DNase I footprinting assays with coding and non-coding Cy5-labelled strands of the DNA fragment containing the *B. bacteriovorus lexA* promoter in the absence or presence of 80 nM of purified *B. bacteriovorus* LexA protein. The arrows indicate the transcriptional direction of each strand.

Fig. 3. Effect of 300-fold molar excess of either *B. bacteriovorus* Bd0386 promoter (lane 3), *lexA* promoter (lane 4) or pGEM-T plasmid DNA (lane 5), used as non-specific DNA, in the electrophoretic mobility of Bd0386 DIG-labeled promoter in presence of purified LexA protein. The migration of this fragment without any additional DNA (lane 2) or in absence of LexA protein (lane 1) is also presented as positive and negative controls, respectively.

Fig. 4. Single-nucleotide substitutions in the ATTTACACTGTAAGT imperfect palindrome and their effect on the electrophoretic mobility of the *B. bacteriovorus lexA* promoter in presence of 80 nM of its own purified LexA protein. The mobility of the wild-type *B. bacteriovorus lexA* promoter in the absence (-) or presence (+) of LexA protein is shown as a control.

Fig. 5. A) Structural arrangement of *B. bacterovorus* Bd0386-*dinP*-*dnaE* genes and their surroundings. The position of primers used to determine the transcriptional organization is indicated. B) RT-PCR analysis of Bd0387-Bd0386-*dinP*-*dnaE*-Bd0383 transcripts present in total RNA from *B. bacteriovorus* (*RNA RT-PCR*). As controls, PCR experiments in the absence of reverse transcriptase were also carried out employing either DNA (*DNA-PCR*) or RNA (*RNA-PCR*) as a template. The *Hinf*I-digested DNA of ϕx174 was used as molecular mass marker.

Fig. 6. EMSAs showing the binding ability of either *Anabaena* (lane 3) or *B. subtilis* (lane 4) LexA proteins to the wild type *B. bacteriovorus lexA* promoter. As a control, in lane 5, a mutant derivative of *lexA* promoter, at which the A of the LexA binding motif-left half (TTAC) is substituted by a G, was assayed in the presence of *Anabaena* LexA protein. The mobility of the *lexA* promoter with (lane 2) or without (lane 1) *B. bacteriovorus* LexA is also shown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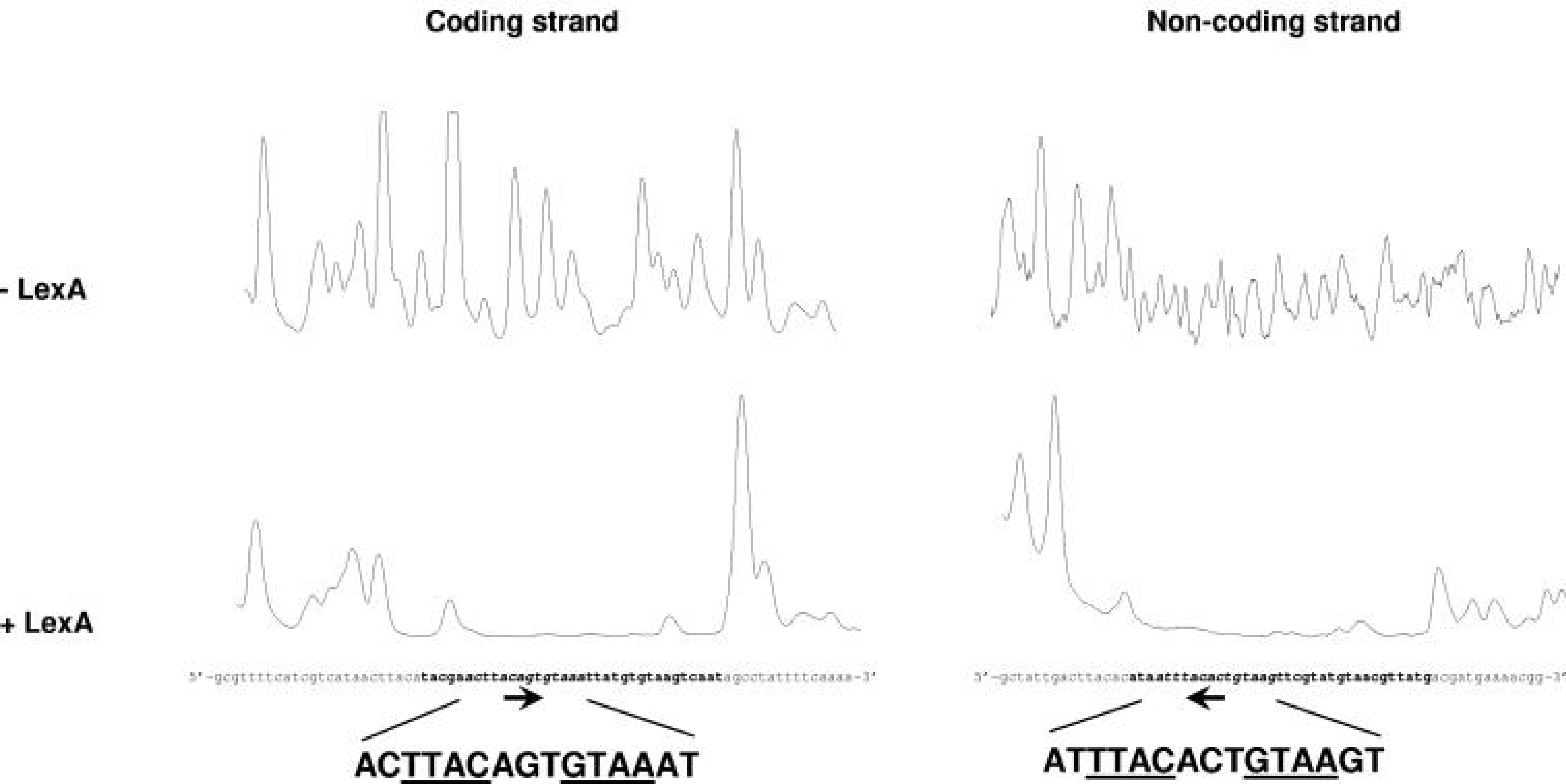
+
+ P*lexA* DNA
+ non-specific DNA
1
2
3
4



Coding strand
Non-coding strand
- LexA
+ LexA
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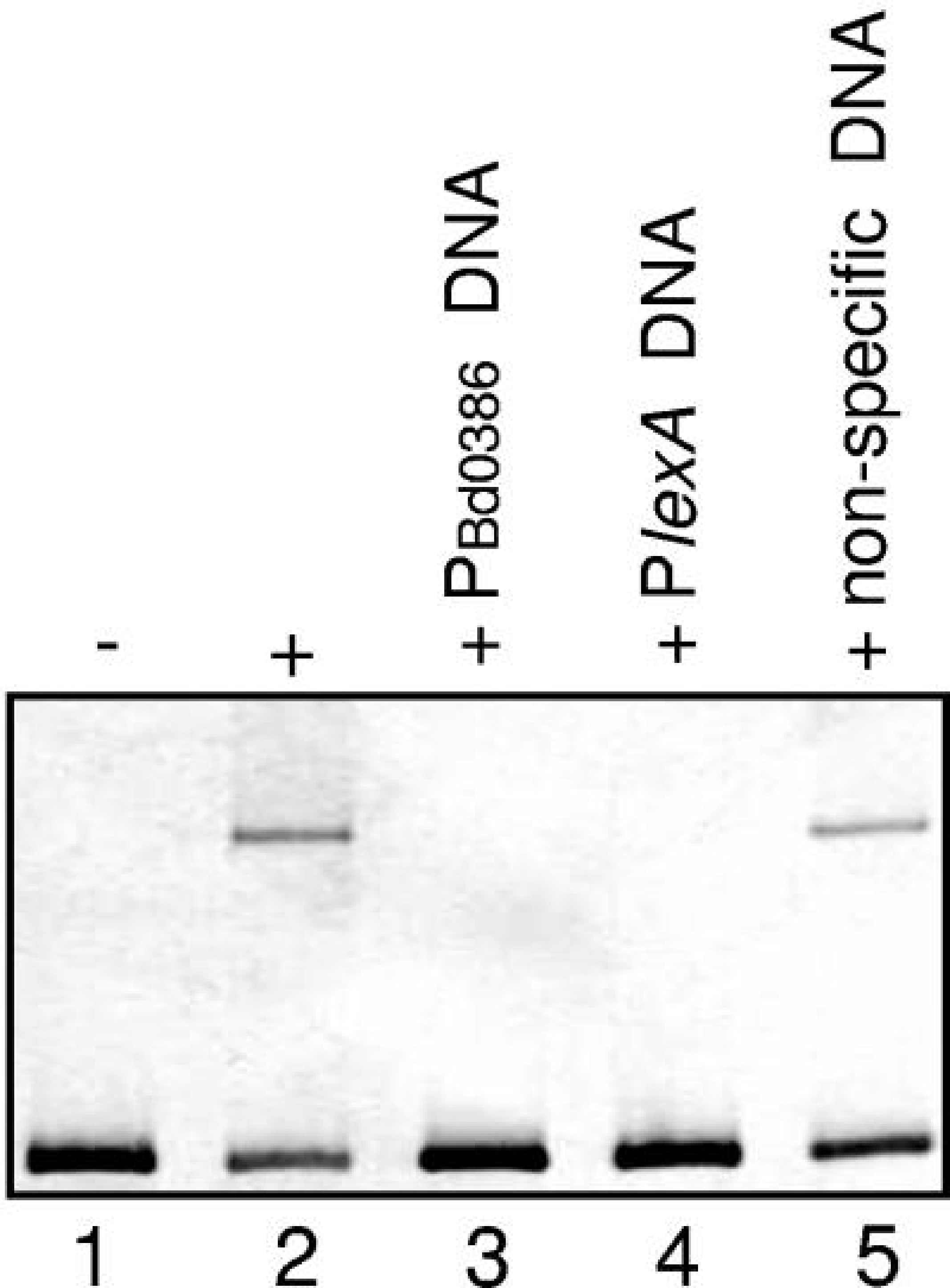
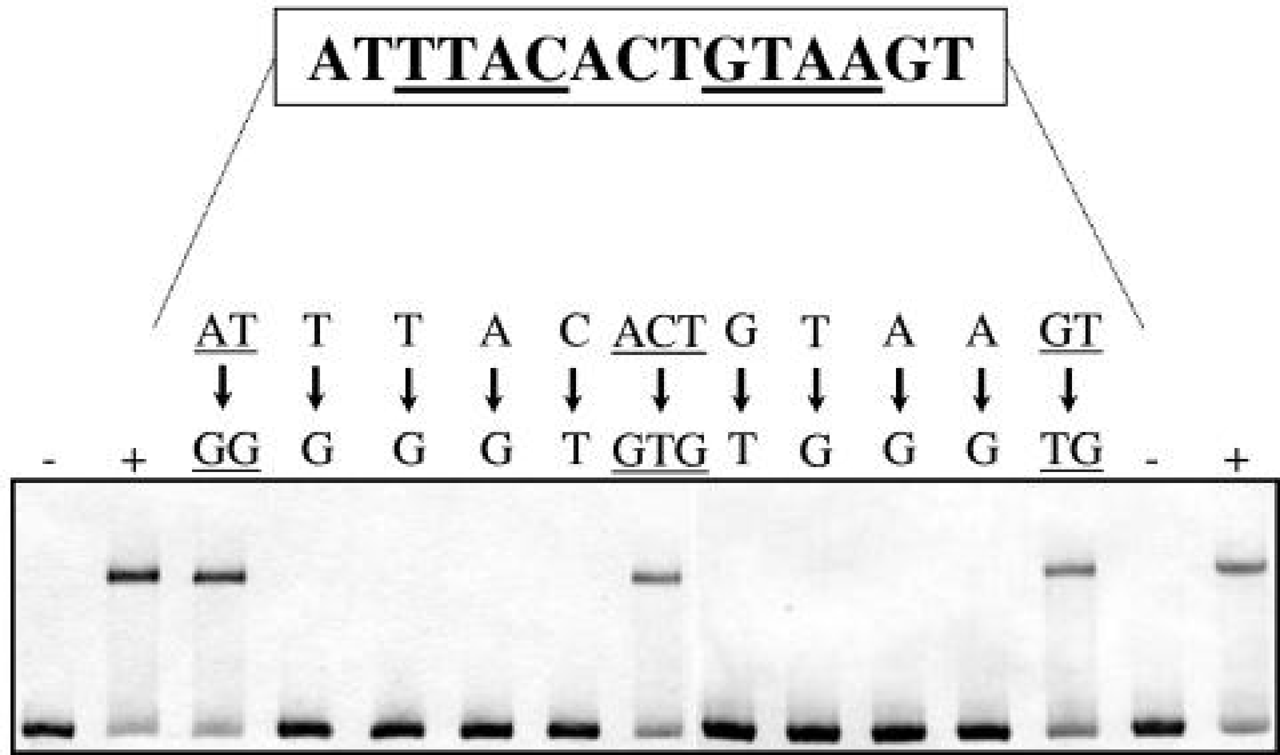
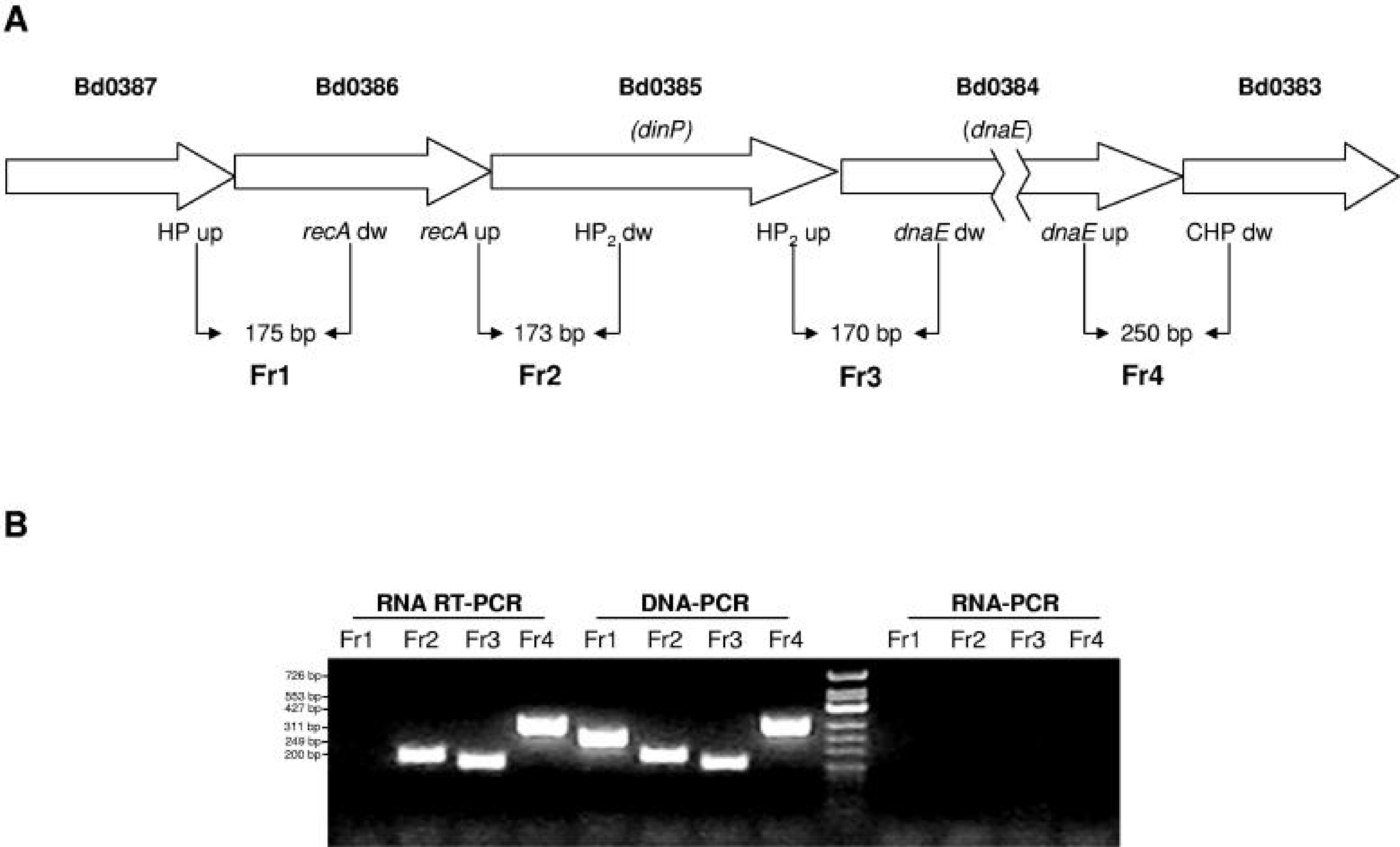
5'-gctattgacttacacataatttacactgtaagttcgtatgtaacgttatgacgatgaaaacgg-3'
ACTTACAGTGTAAAT
ATTTACACTGTAAGT


-
+
+ PBd0386 DNA
+ PlexA DNA
+ non-specific DNA
1
2
3
4
5


ATTTACACTGTAAGT
AT T T A C ACT G T A A GT
- + GG G G G T GTG T G G G TG - +


A
Bd0387
Bd0386
Bd0385
(dinP)
Bd0384
(dnaE)
Bd0383
HP up
recA dw
recA up
HP2 dw
HP2 up
dnaE dw
dnaE up
CHP dw
175 bp
173 bp
170 bp
250 bp
Fr1
Fr2
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B
RNA RT-PCR
DNA-PCR
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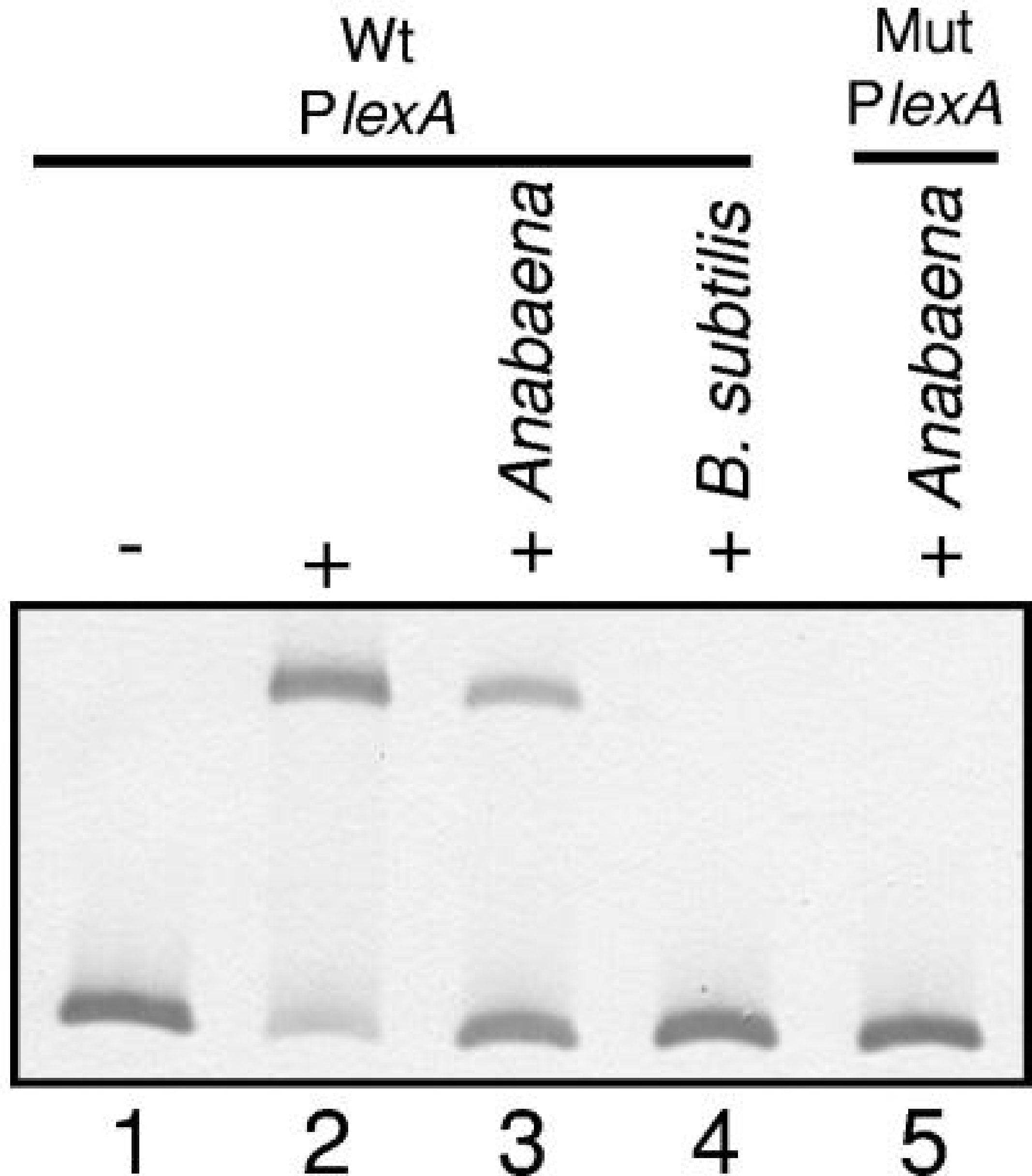
427 bp
311 bp
249 bp
200 bp


Wt
P lexA
Mut
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Anabaena
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-
+
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5