Cohabitation of Two Different lexA Regulons in Pseudomonas putida

Marc Abella,1‡ Susana Campoy,2‡ Ivan Erill,3 Fernando Rojo,4 and Jordi Barbé1,2*

Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain1; Centre de Recerca en Sanitat Animal (CReSA), 08193 Bellaterra, Spain2; Biomedical Applications Group, Centro Nacional de Microelectrónica, CNM-IMB (CSIC), 08193 Bellaterra, Spain3; and Departamento de Biotecnología Microbiana, Centro Nacional de Biotecnología, CSIC, Campus de la Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain4

Received 27 July 2007/Accepted 1 October 2007

In contrast to the vast majority of the members of the domain Bacteria, several Pseudomonas and Xanthomonas species have two lexA genes, whose products have been shown to recognize different LexA binding motifs, making them an interesting target for studying the interplay between cohabiting LexA regulons in a single species. Here we report an analysis of the genetic composition of the two LexA regulons of Pseudomonas putida KT2440 performed with a genomic microarray. The data obtained indicate that one of the two LexA proteins (LexA1) seems to be in control of the conventional Escherichia coli-like SOS response, while the other LexA protein (LexA2) regulates only its own transcriptional unit, which includes the imuA, imuB, and dnaE2 genes, and a gene (PP_3901) from a resident P. putida prophage. Furthermore, PP_3901 is also regulated by LexA1 and is required for DNA damage-mediated induction of several P. putida resident prophage genes. In silico searches suggested that this marked asymmetry in regulon contents also occurs in other Pseudomonas species with two lexA genes, and the implications of this asymmetry in the evolution of the SOS network are discussed.

Bacterial cells are able to display a range of responses against the wide spectrum of DNA-damaging agents to which they are frequently exposed. Among these, one of the most studied is the SOS response. First described in Escherichia coli (38), the SOS response comprises a network of more than 40 genes involved in DNA repair, error-prone DNA replication, and cell division regulation (10, 16, 19). Typically, SOS genes are negatively regulated by the LexA repressor protein, which in E. coli recognizes specifically a CTGTN8ACAG regulatory motif present in the promoter region of its target genes. This motif, usually called the SOS or LexA box, has been shown to be different in different phyla, such as the gram-positive bacteria or the α-, β-, γ-, and δ-Proteobacteria (22), and has been shown to be monophyletic in some of them (22). Induction of the SOS genes is triggered by activation of the RecA protein after binding to single-stranded DNA fragments generated either by stalling of the replication fork or enzymatic processing of broken DNA ends (33). Activated RecA is able to induce the autocatalytic cleavage of LexA, thus preventing it from binding its target sites and activating the expression of SOS genes (21). Once DNA damage has been properly addressed, the activated RecA concentration drops and newly synthesized LexA is able to bind again to its target sites, thus switching off the coordinated response. It is worth noting that not all LexA-regulated genes are induced at the same level or at the same time. Instead, the timing, duration, and level of the induction may vary significantly among different SOS genes (10, 30).

Even though the SOS response has been thoroughly characterized in several microorganisms, such as E. coli (10), Pseudomonas aeruginosa (9), and the gram-positive organisms Bacillus subtilis (2) and Staphylococcus aureus (8), both of which have an SOS box (GAACN4GTTC) (8, 40) markedly different from that of E. coli (38), recent work has offered a glimpse of the composition of the LexA regulon in several additional bacterial species (20). In contrast to the examples cited above, the genetic composition of the LexA network has been found to vary widely among phyla and even among species belonging to the same class (14, 22). In this respect, it is interesting that although the majority of bacterial genomes sequenced so far have only one or no LexA homologue, duplication of LexA has been reported previously. The δ-proteobacterium Geobacter sulfurreducens, for instance, has two lexA genes whose products recognize the same regulatory motif and which seem to be the result of a recent duplication event (18). In contrast, the two LexA proteins encoded by several Pseudomonas and Xanthomonas species have been shown to recognize markedly different LexA binding motifs (1, 41). In Pseudomonas putida, one of the two LexA proteins (LexA1) recognizes an E. coli-like SOS box (CTGTN5ACAG), while the other LexA protein (LexA2) binds to a manifestly divergent motif (GTACN4GTGC) (1). In Xanthomonas (41), however, while LexA2 recognizes the same motif that P. putida LexA2 does, the LexA1 protein has been shown to bind a motif (TTAGN6TACTA) previously also found in Xylella fastidiosa, which belongs to the same class (6).

Besides the coincidence in LexA binding motifs, the relationship between Xanthomonas and Pseudomonas LexA proteins has been established through comprehensive phylogenetic analyses (13). Interestingly, the results of these studies also indicate that the lexA gene is always cotranscribed with three other DNA damage-inducible genes (PP_3117, PP_3118, and PP_3119, designated here, following the recently proposed nomenclature, imuA, imuB, and dnaE2) (13, 17, 20), which
make up a cassette that has been shown to be widespread in the domain Bacteria and to be involved in DNA damage-mediated mutagenesis (1, 13, 17). During its evolutionary history, this multiple-gene cassette has undergone a number of reorganizations, with three-gene, two-gene, and completely split cassettes reported in different phyla but always showing apparent regulation by LexA (13). Phylogenetic reconstruction of the cassette history suggests that the reorganization leading to the lexA2-imuA-imuB-dnaE2 gene cassette seen in Xanthomonas and Pseudomonas took place soon before the split of γ- and β-Proteobacteria and was soon followed by duplication of the cassette and its accompanying lexA gene, at a time when the E. coli LexA binding sequence or a closely related motif had probably already evolved (13).

The presence of two different LexA proteins that do not recognize the same binding site is an uncommon feature in the domain Bacteria that can provide clues concerning the evolution of complex regulatory networks like the SOS response, addressing such issues as the behavior of coexisting lexA genes or the evolution of novel LexA binding sequences. For this reason, the aim of the present work was to analyze the genetic composition of the P. putida lexA1 and lexA2 regulons in order to determine the possible relationships and overlaps between both networks. Gene expression patterns in wild-type cells of P. putida with damaged DNA, as well as in lexA1 and lexA2 mutant derivatives, were determined and examined to reveal the extent of the interplay between the networks.

MATERIALS AND METHODS

Bacterial strains and growth conditions. All bacterial strains and plasmids used in this work are listed in Table S1 in the supplementary material. P. putida KT2440 (25) was grown in LB medium (32) at 30°C. The antibiotic concentrations used for each microorganism were the concentrations previously reported (1, 24). All plasmid construction and cloning experiments were performed using E. coli DH5α (32). Plasmid DNA was transformed into competent E. coli cells as described previously (32). All P. putida mutants were constructed by marker exchange using plasmid pUA68 (Gm') (see Table S1 in the supplementary material) with the gene of interest interrupted by insertion of an ßKm cassette into a Smal restriction target generated by inverted PCR using suitable oligonucleotides (see Table S2 in the supplementary material), as described previously (1). The presence of a transcriptional terminator in the ßKm cassette eliminated the expression of genes downstream of the truncated gene. In all cases, after serial passages, all the mutant derivatives were genetically stable. The growth ratio in LB media and the cell survival after mitomycin C treatment were determined for each of the constructed mutant derivatives, and the values were not different from the values for the isogenic wild-type P. putida KT2440 strain. Likewise, microscale observation of the mutant strains did not reveal any phe-

protein binding reactions were performed as previously described (1), and the mixtures were loaded onto a 6% nondenaturing Tris-glycine polyacrylamide gel that was prerun for 30 min at 10 V/cm in 25 mM Tris-HCl (pH 8.5)-250 mM glycine-1 mM EDTA. DNA-protein complexes were separated at 100 V for 90 min, and this was followed by transfer to a Biodine B nylon membrane (Pall Gelman Laboratory). DIGoxigenin-labeled DNA-protein complexes were detected using the manufacturer’s protocol (Roche). When necessary, either lexA1 or lexA2 promoter fragments, obtained by PCR performed with the suitable oligonucleotides, were used as an unlabeled DNA competitor (1).

Hybridization and processing of microarrays. A previously described genomewide oligonucleotide-based DNA microarray for P. putida KT2440 was used (42). Twenty micrograms of total RNA was transformed to cDNA, and after this Cy3 or Cy5 fluorescent dyes (Amersham Biosciences) were coupled to the amine-modified first-strand cDNA. The labeling efficiency was assessed using a NanoDrop ND1000 spectrophotometer (NanoDrop Technologies). Prehybridization and hybridization microarray analyses were performed as described previously (42). Slides were scanned with a ScanArray 4000 fluorescence scanner. For each experiment, three independent RNA extractions were performed, the extracts were pooled to obtain single samples, and each sample was analyzed with at least two microarrays. The statistical analysis was based on an empirical Bayes method implemented in the software package LIMMA for the R-computing environment (www.r-project.org) (37). A gene was considered to be induced when either the ratio of the treated preparation to the nontreated preparation or the ratio of the wild-type preparation to the mutant preparation was greater than 2.0 and the P value was <0.01.

In silico searches for LexA binding sites. In silico searches for LexA binding sites were performed with Fimo, a general search program for detection of DNA binding sites based on position-specific weight matrices (PSWM) of known sites that implements several reported methods for scoring putative binding sites. Searches were carried out using the information content-averaged Berg-von Hippel heterology index as the scoring function (3, 27, 28, 35). In order to ensure that the results obtained had low false-positive rates and thus were a reasonable, although necessarily abridged, approximation of data for the true regulon, a restrictive threshold (a score better than one-half the PSWM sites) was applied to putative binding sites when full genomes were analyzed. A more relaxed threshold (a score better than 85% of the original PSWM sites), was applied to search for LexA binding sites in the promoter regions of genes that had been shown to be LexA regulated in P. putida. Collections of binding sites to construct both PSWM were obtained from previous publications. For lexA1, 23 experimentally verified binding sites from E. coli (15, 23, 29, 31) were used to construct the PSWM, while seven LexA2 binding sites from several Pseudomonas and Xanthomonas species were used to construct the LexA2 PSWM (1, 41). Genomic sequences were all downloaded from the NCBI GenBank repository through its FTP server (ftp.ncbi.nlm.nih.gov).

Microarray accession number. All the array data have been deposited in the Arrayexpress database (http://www.ebi.ac.uk/arrayexpress) under accession number E-MEXP-1187.

RESULTS AND DISCUSSION

Analysis of gene expression in P. putida lexA1 and lexA2 mutant derivatives. To determine the genetic composition of the LexA1 and LexA2 regulons and their possible overlap, P. putida KT2440 lexA1 and lexA2 mutant derivatives were obtained by marker exchange by inserting a construct in which either lexA1 or lexA2 was interrupted by a ßKm cassette insertion. Both P. putida mutant derivatives were completely viable and did not have observable phenotypic differences from the wild-type strain. Using a previously described P. putida KT240 oligonucleotide-based genomic array (42), the gene expression patterns of both mutant strains were compared to that of the wild type before and after treatment of the latter with mitomycin C. The results of the array experiments, summarized in Fig. 1, revealed that the two mutant derivatives and the nontreated wild-type strain had different expression profiles, indicating that different sets of genes were under control of the LexA1 and LexA2 proteins, as inferred from their increased expres-
sion in the mutant derivatives. Moreover, for all genes whose expression was different in the nontreated wild-type strain and the mutant derivatives, LexA binding sequences of the corresponding LexA proteins could be located in their promoter regions, suggesting that there was direct regulation by either LexA1 or LexA2 protein. Microarrays were also used to analyze the effect of mitomycin C on the transcriptome profile of the wild-type strain. As expected, all genes exhibiting increased expression in the mutant derivatives were also induced in the wild-type strain treated with mitomycin C (Fig. 1), confirming their regulation by either LexA1 or LexA2. Data obtained by microarray analysis were confirmed for all the genes using quantitative RT-PCR (see Materials and Methods). Filled and open circles indicate the presence of LexA1 and LexA2 binding sites, respectively, in the corresponding promoter region. For the genes identified in the genome annotation, the gene designations are shown. Open reading frames without designations are indicated by their locus names. Genes belonging to *P. putida* genomic island 79 (from position 4363000 to position 4428000 of *P. putida* genome sequence and annotated as a bacteriophage [39]) are indicated by dark gray arrows. Connected arrows indicate polycistronic transcriptional units.

As shown in Fig. 1, there are 18 transcriptional units under direct control of LexA1, while only 2 transcriptional units are regulated by LexA2. A more detailed analysis of the LexA1 regulon revealed that, as expected, most of the regulated genes are involved in recombination and DNA repair. Furthermore, the LexA1 regulon of *P. putida* has a significant overlap with the reported LexA regulons of both *E. coli* and *P. aeruginosa* (Table 1), in which there is only one LexA protein that recognizes the same regulatory motif (CTGTN₈ACAG) as *P. putida* LexA1 (9, 16). In contrast, the *P. putida* LexA2 protein controls only its own transcriptional unit and a gene (PP_3901) that is also regulated by LexA1. To ascertain whether the observed disparity in regulon contents was the general trend in other *Pseudomonas* species having two LexA proteins, restrictive in silico searches with both regulatory motifs were conducted for several *Pseudomonas* genomes to gauge the approximate extents of their LexA1 and LexA2 networks.

The results, summarized in Table 1, suggest that the imbalance in regulon contents between the two LexA proteins is present in other *Pseudomonas* species and give further credence to previous phylogenetic analyses (13) which suggested that the lexA2 gene arose as a duplication of an early ancestor. The only gene directly controlled by both LexA proteins belongs to a prophage. Apart from the 18 transcriptional units regulated by LexA1 and LexA2 shown in Fig. 1, there are a number of genes that were induced in the mitomycin C-treated
TABLE 1. Comparison of \( P. \) \textit{putida} \( \text{lexA1} \) and \( \text{lexA2} \) regulons for both experimentally validated \( \text{lexA} \) networks of \( \text{E. coli} \) and \( P. \) \textit{aeruginosa} and \( P. \) \textit{fluorescens} and in silico searches in other \( P. \) \textit{putida} species encoding both \( \text{LexA} \) proteins

<table>
<thead>
<tr>
<th>( P. ) \textit{putida} transcriptional units (accession no. \textit{NC}_002947)(^a)</th>
<th>( P. ) \textit{aeruginosa} PA01 (accession no. \textit{NC}_002516)</th>
<th>( \text{Escherichia coli} ) K-12 (accession no. \textit{U}00096)</th>
<th>( P. ) \textit{entomophila} L48 (accession no. \textit{NC}_008027)</th>
<th>( P. ) \textit{fluorescens} P-5 (accession no. \textit{NC}_004129)</th>
<th>( P. ) \textit{syringae} pv. \textit{syringae} B728a (accession no. \textit{NC}_007005)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{PP}_1629 ) (( \text{recA} ))</td>
<td>+(^c)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>( \text{PP}_1630 ) (( \text{recX} ))</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>( \text{PP}_1125 ) (( \text{divG} ))</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>( \text{PP}_2143 ) (( \text{lexA1} ))</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>( \text{PP}_2142 ) (( \text{soa} ))</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>( \text{PP}_4616 ) (( \text{yebG} ))</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>( \text{PP}_4729 ) (( \text{recN} ))</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>( \text{PP}_1203 ) (( \text{dinP} ))</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>( \text{PP}_1974 ) (( \text{uvrB} ))</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>( \text{PP}_5352 ) (( \text{uvrD} ))</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>( \text{PP}_2109 ) (hp)(^d)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>NP</td>
</tr>
<tr>
<td>( \text{PP}_2452 ) (hp)</td>
<td>NP</td>
<td>NP</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>( \text{PP}_2451 ) (( \text{endA} ))</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>( \text{PP}_4019 ) (( \text{topB} ))</td>
<td>NP</td>
<td>-</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>( \text{PP}_5393 ) (hp)</td>
<td>NP</td>
<td>NP</td>
<td>+</td>
<td>+</td>
<td>NP</td>
</tr>
<tr>
<td>( \text{PP}_2924 ) (hp)</td>
<td>NP</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>( \text{PP}_2923 ) (hp)</td>
<td>NP</td>
<td>NP</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>( \text{PP}_1110 ) (( \text{cysE} ))</td>
<td>-</td>
<td>NP</td>
<td>NP</td>
<td>-</td>
<td>NP</td>
</tr>
<tr>
<td>( \text{PP}_1111 ) (hp)</td>
<td>-</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>( \text{PP}_1112 ) (hp)</td>
<td>-</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>( \text{PP}_1113 ) (( \text{cysK} ))</td>
<td>NP</td>
<td>-</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
</tr>
</tbody>
</table>

\( P. \) \textit{putida} transcriptional units regulated by LexA2 in other species

| \( \text{PP}_3116 \) (\( \text{lexA2} \)) | NP\(^e\) | NP | + | + | + |
| \( \text{PP}_3117 \) (\( \text{imaX} \)) | -\(^e\) | NP | + | + | + |
| \( \text{PP}_3118 \) (\( \text{imaB} \)) | -\(^e\) | NP | + | + | + |
| \( \text{PP}_3119 \) (\( \text{dnaE2} \)) | -\(^e\) | NP | + | + | + |
| \( \text{PP}_3901 \) (hp) | NP | NP | P | P | P |

\(^a\) In \( P. \) \textit{putida} KT2440, \( P. \) \textit{aeruginosa} PA01, \( E. \) \textit{coli} K-12, \( P. \) \textit{entomophila} L48, \( P. \) \textit{fluorescens} P-5, and \( P. \) \textit{syringae} pv. \textit{syringae} B728a, 18, 11, 27, 15, 21, and 25 transcriptional units, respectively, belong to the LexA1 regulon. In \( P. \) \textit{putida} KT2440, \( P. \) \textit{entomophila} L48, \( P. \) \textit{fluorescens} P-5, and \( P. \) \textit{syringae} pv. \textit{syringae} B728a, 2, 1, and 1 transcriptional units, respectively, belong to the LexA2 regulon. The LexA2 regulon is not present in \( P. \) \textit{aeruginosa} PA01 and \( E. \) \textit{coli} K-12. The data for \( P. \) \textit{putida} KT2440, \( P. \) \textit{aeruginosa} PA01, and \( E. \) \textit{coli} K-12 were obtained in this study and from references 9 and 19, respectively. The data for \( P. \) \textit{entomophila} L48, \( P. \) \textit{fluorescens} P-5, and \( P. \) \textit{syringae} pv. \textit{syringae} B728a were obtained by in silico searches performed as described in Materials and Methods.

\(^b\) Each of the following sets of genes constitutes a single transcriptional unit: \( \text{PP}_1629 \) and \( \text{PP}_1630 \); \( \text{PP}_2143 \) and \( \text{PP}_2142 \); \( \text{PP}_2924 \) and \( \text{PP}_2923 \); \( \text{PP}_1110 \); \( \text{PP}_1111 \); \( \text{PP}_1112 \); and \( \text{PP}_3116 \), \( \text{PP}_3117 \), \( \text{PP}_3118 \), and \( \text{PP}_3119 \).

\(^c\) +, gene present in the genome and \( \text{LexA} \) regulated; --, gene present in the genome and not \( \text{LexA} \) regulated; NP, gene not present in the genome; P, \( \text{PP}_3901 \) paralogous gene is controlled by the \( E. \) \textit{coli}-like \( \text{LexA} \) protein but not by the \( P. \) \textit{putida} LexA2-like repressor.

\(^d\) hp, hypothetical protein.

\(^e\) Gene controlled by the \( E. \) \textit{coli}-like \( \text{LexA} \) protein.

Wild-type strain but were not deregulated in either \( \text{lexA} \) mutant derivative. All these genes correspond to the coding region of a bacteriophage that has previously been described as \( P. \) \textit{putida} KT2440 genomic island 79 of atypical sequences composed of elements annotated with phage-like gene signatures (39), and their DNA damage-mediated induction profile was initially attributed to a \( \text{LexA} \)-independent pathway, like that seen in many bacteriophages in which activated \( \text{RecA} \) promotes self-cleavage of the lytic cycle repressors, thus inducing the bacteriophage (34). However, the fact that the only gene upregulated in both \( \text{lexA1} \) and \( \text{lexA2} \) mutant derivatives (\( \text{PP}_3901 \)) was in the same bacteriophage coding region suggested that some kind of \( \text{LexA} \) coregulation might cause the observed induction of phage genes. \( \text{PP}_3901 \) has regulatory motifs for both \( \text{LexA1} \) and \( \text{LexA2} \) in its promoter region, and the ability of both \( \text{LexA} \) proteins to specifically bind the \( \text{PP}_3901 \) promoter was determined by EMSA (Fig. 2). Moreover, a quantitative RT-PCR experiment was performed (Fig. 3), which validated the array data and confirmed not only that \( \text{PP}_3901 \) is simultaneously and directly regulated by \( \text{LexA1} \) and \( \text{LexA2} \) but also that the \( \text{LexA1} \) protein plays the leading role in the transcriptional control of \( \text{PP}_3901 \) expression (Fig. 3). BLAST-P searches with the 86-amino-acid protein coding sequence of \( \text{PP}_3901 \) resulted in significant matches with paralo-
gous proteins in *P. putida* (PP_2452), in other *Pseudomonas* species, like *P. syringae* (PSPPH_2123), and even in the *P. aeruginosa* F116 bacteriophage (F116928). In all these cases, however, LexA binding sequences corresponding to the LexA1 motif, but not LexA binding sequences corresponding to LexA2, could be identified in the promoter region of the PP_3901 homologues.

To better understand the possible role of PP_3901 in the induction of phage genes and of its coregulation by the two LexA proteins of *P. putida*, a PP_3901 mutant derivative was constructed after ensuring, through RT-PCR analysis, that PP_3901 was not part of a transcriptional unit and that its inactivation would not result in polar effects on downstream genes (data not shown). As observed for both LexA mutant derivatives, the morphology and growth of the PP_3901 mutant derivative did not differ from the morphology and growth of the wild-type strain (data not shown). Since PP_3901 is in the coding region of a genome-encoded bacteriophage, the expression of two genes from this region (PP_3873 and PP_3894) that had been shown to be DNA damage inducible in the mitomycin C-treated wild-type strain was analyzed by means of quantitative RT-PCR studies in the PP_3901 mutant derivative.

FIG. 2. (A) Structural arrangement of the *P. putida* PP_3901 gene promoter. LexA1 and LexA2 binding sites and their positions with respect to the ATG translational starting codon are indicated. The bent arrows indicate the positions of the oligonucleotides used to obtain the PP_3901 promoter probe for EMSA experiments. (B) EMSA of the *P. putida* PP_3901 promoter in the absence or in the presence of either LexA1 or LexA2 purified protein. To determine the specificity of binding of both proteins, a 300-fold molar excess of either nonlabeled *lexA1* or *lexA2* promoter was used as a specific or nonspecific competitor fragment depending on the purified LexA protein that was used in each case.

FIG. 3. Relative expression levels of the PP_3901 gene in the *P. putida* wild-type (Wt) strain and in *lexA1* and *lexA2* mutant derivatives of this strain. The expression level was measured by quantitative RT-PCR and is the ratio of the relative mRNA concentration of the PP_3901 gene in mitomycin C-treated (+) and untreated (−) cells to the relative PP_3901 mRNA concentration in the untreated *P. putida* wild-type strain. Relative mRNA concentrations were normalized to *P. putida trpA* gene expression. In each case, the mean value from three independent experiments (each performed in triplicate) is shown.
As Fig. 4 shows, the RT-PCR results indicate that neither prophage gene was induced by mitomycin C treatment in the PP_3901 derivative, suggesting that PP_3901 is involved in the DNA damage-mediated induction of prophage genes. In this respect, the explicit regulation of PP_3901 by both LexA proteins and its involvement in the DNA damage-mediated induction of the prophage genes are reminiscent of previously described mechanisms of SOS-mediated bacteriophage induction. For instance, the Tum protein of coliphage-186 (36) and other prophages (4) has been shown to be explicitly regulated by LexA, and it controls the induction of these prophages by interfering with the lytic cycle repressor. Nonetheless, the protein sequence of PP_3901 has no apparent sequence homology with Tum, suggesting that it may be a key component of a previously unreported pathway of LexA-mediated bacteriophage induction. Moreover, induction of the prophage genes required not only the presence of PP_3901 but also treatment of the P. putida culture with mitomycin C, as shown in Fig. 4 for lexA1 and lexA2 mutant derivatives. Even though both mutant derivatives had an increased amount of the PP_3901 product in nontreated cultures, the prophage gene expression remained unchanged compared with that of the isogenic wild-type strain.

In summary, the results presented above present a picture of pronounced asymmetry between the two cohabiting LexA regulons of P. putida and show that this asymmetry can be extended to other Pseudomonas species harboring two lexA genes. Based on previous results for the phylogeny of the mutagenesis cassette associated with the lexA2 gene (13), where the relationship between the two P. putida LexA proteins has been firmly established, the small size of the lexA2 regulon reinforces the hypothesis that the lexA2 gene resulted from a relatively ancient duplication of the full lexA-imuA-imuB-dnaE2 cassette at the onset of the /H9252-Proteobacteria/H9253-Proteobacteria radiation. As observed in other instances (5, 26), genetic duplication would have freed one of the two copies from selective constraints, while the other copy maintained its former regulatory role. This would explain both the divergence observed in the LexA2 binding motif and the very small number of genes under LexA2 regulation. Ultimately, the most convincing explanation for the survival of the lexA2 gene in several Pseudomonas and Xanthomonas species might well be its direct regulation of the imuA-imuB-dnaE2 mutagenic cassette, which has been shown to be LexA regulated in all the species in which it is present (13). In fact, in the close relative P. aeruginosa, which has lost the lexA2 gene, the imuA-imuB-dnaE2 cassette is regulated by the remaining LexA protein (9), which has been shown to correspond to P. putida LexA1 and which binds to the E. coli LexA binding sequence (9). In this context, the fact that the P. putida LexA1 protein has an E. coli-like binding motif is noteworthy, as it confirms previous suggestions that an E. coli LexA binding motif was already in place when the lexA-imuA-imuB-dnaE2 cassette
duplication took place early in the history of β- and γ-Pro-
extoeba (13). Furthermore, the fact that the regu-
lon of Xanthomonas LexA1, which has a binding sequence diver-
gent from that of E. coli (41), has been shown to regulate only a few genes (22) suggests that preservation of the E. 
coli LexA binding motif may have played a substantial role 
in the conservation of LexA reguolon contents in the γ-Pro-
extoeba. Taking into account the significant overlap be-
tween LexA regulons in different phyla, in spite of man-
ifestly divergent LexA binding sites across the domain 
Bacteria (12, 22), the evidence that motif conservation could 
emulate to some extent with conservation of reguolon contents 
has strong implications for the evolution of the LexA regu-
lon, as it suggests that changes in the LexA binding se-
quence must have been very gradual and continuous follow-
ing an ancestral line. In this context, the puzzling existence of 
markedly divergent LexA binding motifs (6, 7, 11, 12, 41), 
which are often associated with very small regulons, may be 
regarded simply as the end product of relaxed selection of 
the LexA reguolon in some species and phyla.

ACKNOWLEDGMENTS

This work was funded by grants BFM2004-02768/BMC and 
BFU2006-00767/BMC from the Ministerio de Educacio-
ón y Ciencia of Spain, by grant 2005SGR533 from the Generalitat de Catalunya, and by 
the Consejo Superior de Investigaciones Cientificas. S. Campoy is the recipient of a 
educational contract from INIA-IRTA. M. Abella is a recipient of a predoctoral 
award from the Ministerio de Educacio-
n y Ciencia.

REFERENCES

Widespread distribution of a LexA-regulated DNA damage-inducible mul-

Chachau, S. A. Colavito, S. N. Fuller, E. S. Groban, L. A. Hensley, T. C. 
O’Brien, A. Shah, J. T. Tierney, L. L. Tomm, T. M. O’Gara, A. I. Goranov, 

regulatory proteins. Statistical-mechanical theory and application to oper-

regarded simply as the end product of relaxed selection of the LexA regulon in some species and phyla.

5. Erill, I., S. Campoy, and J. Barbe. 2007. Eons of distress: an evolutionar-
y perspective on the bacterial SOS response. FEBS Microbiol. Rev. 28:813– 
826.

of an adaptive mutagenesis cassette in the bacteria domain. Nucleic Acids 
Res. 34:66–77.

reveals substantial variability in the gene contents of the gamma proteobac-

mo- 

H. Ohmori, and R. Woodgate. 2000. Identification of additional genes 
belonging to the LexA regulon in Escherichia coli. Mol. Microbiol. 35: 
1550–1572.

An SOS-regulated operon involved in damage-inducible mutagenesis in 

Lovley, and J. Barbe. 2003. Geobacter sulfurreducens has two autoregulated 
LexA-encoded genes whose products do not bind the recA promoter: differing 

47:399–408.

2007. Study of involvement of ImuB and DnaE2 in stationary-phase mu-


Reconstruction of the evolutionary history of the LexA-binding sequence. 
Microbiology 150:3783–3795.


Harbor Laboratory, Cold Spring Harbor, NY.

18. Nelson, K. E., C. Weinelt, I. T. Paulsen, R. J. Dodson, H. Viltart, V. A. 
Martins dos Santos, D. E. Fouts, S. R. Gill, M. Pog, M. Holmes, L. Brinkac, 
M. Beanan, R. T. DeBoy, S. DavAre, J. Kolonay, R. Madupu, W. Nelson, 
O. White, J. Peterson, H. Khouri, I. Hance, P. Chris Lee, E. Holtzapple, D. 
Scanlan, K. Tran, A. Moazzee, T. Utterback, M. Rizzo, K. Lee, D. Kosack, 
D. Moesi, H. Wieler, J. Lauber, D. Stjepandic, J. Hoheisel, M. Stratez, 


binding sites. Application to Escherichia coli promoters. J. Mol. Biol. 207: 
415–430.


binding sequence motifs in nuclear acids. Proc. Natl. Acad. Sci. USA 95: 
10710–10715.

between transcriptional initiation and elongation in E. coli is highly variable 

numbers to the arrows: parameterizing a gene regulation network by 
10560.

binding sites of cca, the gene encoding coli E. Mol. Gen. Genet. 215: 
483–489.


26. Sassanfar, M., and J. W. Roberts. 1995. Two regulatory DNA motifs to the 
 LexA protein binding site of the 
recA promoter: differing re-

27. Shearwin, K. E., A. M. Brumby, and J. B. Egan. 1998. The Tum protein of 
E. coli regulates recA expression of a mutant muscA operon. J. Bacteriol. 172: 
6223–6231.