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Defining the *Pseudomonas aeruginosa* SOS Response and Its Role in the Global Response to the Antibiotic Ciprofloxacin

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Pseudomonas aeruginosa **infections can be virtually impossible to eradicate, and the evolution of resistance during antibiotic therapy is a significant concern. In this study, we use DNA microarrays to characterize the global transcriptional response of *P. aeruginosa* to clinical-like doses of the antibiotic ciprofloxacin and also to determine the component that is regulated by LexA cleavage and the SOS response. We find that genes involved in virtually every facet of metabolism are down-regulated in response to ciprofloxacin. The LexA-controlled SOS regulon identified by microarray analysis includes only 15 genes but does include several genes that encode proteins involved in recombination and replication, including two inducible polymerases known to play a role in mutation and the evolution of antibiotic resistance in other organisms. The data suggest that the inhibition of LexA cleavage during therapy might help combat this pathogen by decreasing its ability to adapt and evolve resistance.**

Pseudomonas aeruginosa is a common opportunistic human pathogen which is particularly infamous for the high rates of illness and death it causes in patients with cystic fibrosis (18). Once established, *P. aeruginosa* infections can be controlled with some antibiotics, for example, the fluoroquinolones, but are virtually impossible to eradicate, at least in part due to the pathogen's ability to progress through a series of physiological changes that facilitate infection and persistence (18). Its ability to adapt to environmental stress, such as antibiotic therapy, may be related to the large size of its genome (6.3 Mb) and the large number of genes that encode transcriptional regulators.

One of the most important transcriptional responses to environmental stress in bacteria is the SOS response. In *Escherichia coli* (9) and *Bacillus subtilis* (2), it involves the controlled derepression of 43 and 33 genes, respectively, whose protein products facilitate the repair and/or tolerance of DNA damage. Transcription of these genes is induced by the single-stranded DNA (ssDNA) that results from stalled replication forks or direct damage to DNA (15). RecA forms filaments on the ssDNA that mediate recombinational repair and also bind and induce autocleavage of the SOS gene repressor, LexA, resulting in the transcription of the repressed genes. Interestingly, ciprofloxacin, the prototypical fluoroquinolone and an important antibiotic for treating *P. aeruginosa* infections, induces LexA cleavage and the SOS response in *E. coli* (11, 33).

In this study, we determined the global and SOS-mediated transcriptional response of *P. aeruginosa* PAO1 to clinical-like levels of ciprofloxacin. Experiments and controls were repeated in triplicate, which allowed us to identify changes in transcription with a confidence level of $P \leq 0.001$. The data reveal a complex and coordinated LexA-independent response to ciprofloxacin that involves the down-regulation of metabolism, motility, and permeability. The LexA-mediated response is limited to the induction of 15 genes that appear to provide specialized DNA recombination and replication functions.

In addition to furthering our understanding of how the transcriptional response of *P. aeruginosa* contributes to its pathogenicity, we are interested in understanding the potential utility of LexA autoproteolysis inhibitors. For many bacteria, LexA is known to repress genes that regulate processes such as phage mobilization (17, 21, 34), resistance element transfer (3), toxin production (17, 21, 34, 38), mutation (14, 15, 26, 32), and the evolution of resistance (7, 8). For example, we recently demonstrated both in vivo and in vitro that the acquisition of the chromosomal mutations required for the evolution of ciprofloxacin resistance in *E. coli* requires the autoproteolysis activity of LexA and the subsequent induction of the error-prone SOS polymerases in both wild-type (7) and hypermutator strains (8). Thus, suitably designed inhibitors of LexA could be administered with different antibiotics to prevent the emergence of resistance. Identification of the SOS regulon in *P. aeruginosa* is expected to help define the broader utility of such drugs.

MATERIALS AND METHODS

Bacterial strains and growth. *P. aeruginosa* PAO1 was obtained from G. Sundin. Unless specified, solid medium was Lennox LB (28) plus 1.6% agar (LBA); liquid medium was Miller LB (28) (LB). For selection, antibiotics were used for *E. coli* and *P. aeruginosa* PAO1, respectively, as follows: streptomycin (Sm), 30 μg/ml and 250 μg/ml; gentamicin (Gm), 15 μg/ml and 50 μg/ml. Ciprofloxacin was obtained from MP Biomedicals (Aurora, Ohio) and used at the concentrations indicated below. All bacteria were grown aerobically at 37°C.

Strain construction. Primer sequences were designed based on the *P. aeruginosa* genome database (http://v2.pseudomonas.com) (35, 39). A *lexA* allelic exchange cassette was assembled containing ~800 bp of homology surrounding *lexA*, the *lexA* open reading frame, and the Gm^r marker from vector pBBR1MCS-5 (22) using assembly PCR and the primers listed in Table S1 at the website http://www.scripps.edu/chem/romesberg/. The resulting cassette was cloned into vector pKNG101 (20) to create pRTC0021; the S125A mutation was then introduced using primers PA_lexA_S125A_QCF and PA_lexA_S125A_QCR and the QuikChange site-directed mutagenesis kit (Stratagene) to create vector pRTC0022.

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pRTC0022 was transformed into *E. coli* strain SY-17 and introduced into *P. aeruginosa* by conjugative transfer with selection on M9 plus 0.2% citrate and Gm to select clones that integrated the allelic exchange cassette into the chromosome by either a single- or double-crossover event. Replica plating onto M9 plus 0.2% citrate containing either Gm or Sm identified clones containing the allelic exchange cassette and lacking the vector sequences due to a double-crossover event. Colonies were verified as Gm^r and Sm^s, and the mutation was confirmed by sequencing.

Confirmation that the LexA(S125A) mutant is not cleaved in response to ciprofloxacin. For each strain, five clones were grown in LB for 18 h. Cultures were diluted 1:500 and grown to mid-log phase (optical density at 60 nm [OD_{600}], ~0.4 to 0.5), and then ciprofloxacin was added to a final concentration of 1 μg/ml. At 0, 30, and 120 min following ciprofloxacin addition, cell aliquots were removed and stored at −20°C. During the experiment, the OD_{600} and viable CFU per ml were monitored for each of the cultures (see Fig. 1A and B, below). This protocol is identical to that used to prepare samples for the transcriptional studies (below). Whole-cell lysates were prepared by sonication in phosphate-buffered saline, and the soluble fraction was collected and normalized for total protein concentration (Bio-Rad protein assay). Samples were separated on a 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel and transferred to a 0.2-μm nitrocellulose membrane. Immunostaining was performed with a rabbit polyclonal antiserum to LexA (1:8,000; 2 h; kindly provided by J. Little) and horseradish peroxidase-linked anti-rabbit antibody (1:20,000; 1 h; Upstate Biotechnology), followed by detection with ECL Plus (GE Biosciences).

Growth rate measurement. For each strain, three cultures were inoculated into 2 ml tryptic soy broth (Difco) and grown for 16 h with shaking, followed by continued growth after a 100-fold dilution in tryptic soy broth. At the indicated time points, the OD_{600} was measured and the number of viable CFU per ml was determined by dilution plating.

Sensitivity to UV light and MMS. Three independent cultures of each strain were grown overnight in LB. Appropriate dilutions were plated onto LBA, and UVC irradiations were performed using a G8T5 germicidal tube (Ushio America, Cypress, CA). UV fluences were determined using a UVX radiometer with a UVX-25 sensor (UV Products). After irradiation, plates were protected from light and incubated for 2 days before colonies were counted. To determine the methyl methanesulfonate (MMS; Aldrich) sensitivity, three independent cultures were grown overnight in LB. Appropriate dilutions were plated onto LBA containing MMS at the indicated concentrations and incubated for 2 days before colonies were counted.

MIC determination. For each strain, three independent cultures were grown for 25 h in LB containing no antibiotic. From each culture, ~10^5 CFU were used to inoculate LB containing increasing concentrations of ciprofloxacin in 96-well plates. Inoculations were done in duplicate to yield a total of six data points per strain. After 18 h of incubation, growth was measured by reading the OD_{650} in a V_{max} Kinetic microplate reader (Molecular Devices, California). The MIC was defined as the lowest concentration of ciprofloxacin that prevented any detectable growth.

Transcriptional analysis. *P. aeruginosa* genome arrays containing 25-mer probe sets for over 5,500 open reading frames from PAO1, 199 probe sets corresponding to 100 intergenic regions, and 117 probe sets from other *P. aeruginosa* strains were obtained from Affymetrix (Santa Clara, CA). A complete description and annotation for this *P. aeruginosa* genome array is available at http://www.affymetrix.com.

Sample preparation and data analysis. For each strain, five clones were inoculated in LB and grown for 18 h. Cultures were diluted 1:500 and grown to mid-log phase (OD_{600}, ~0.4 to 0.5), at which point ciprofloxacin was added to a final concentration of 1 μg/ml. At 0, 30, and 120 min following ciprofloxacin addition, appropriate volumes from each of the five cultures per strain were pooled and added to 2 volumes of RNAprotect reagent (QIAGEN); cell pellets were stored at 4°C until RNA extraction. Total RNA was extracted using the RNeasy Mini kit (QIAGEN) at the end of the sample collection period. This procedure was repeated three independent times to generate three samples each just prior to and 120 min post-ciprofloxacin addition. Details of data analysis and reverse transcription-PCR validation have been provided along with our supplementary data sets via the internet (http://www.scripps.edu/chem/romesberg/).

Microarray accession numbers. Microarray data have been deposited at the National Center for Biotechnology Information's Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) under the accession number GSE5443.

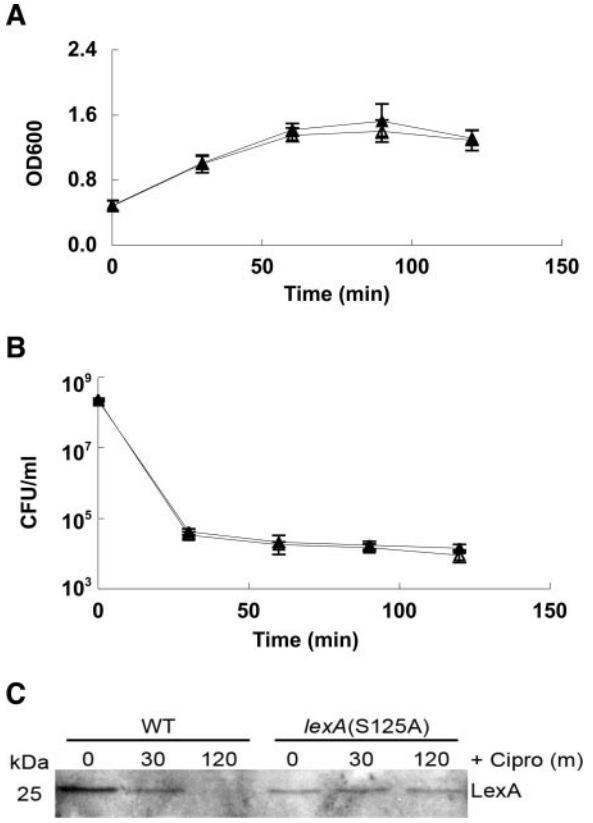

FIG. 1. Population kinetics of *P. aeruginosa* strains during microarray and whole-cell lysate sample preparation monitored by OD (A) and CFU/ml (B). Filled and open triangles represent PAO1 and the LexA(S125A) mutant, respectively. Time zero is defined as the point immediately following ciprofloxacin addition. As described in the text, samples for total RNA extraction and whole-cell lysates were collected at 0, 30, and 120 min post-ciprofloxacin addition. (C) Analysis of total full-length LexA measured by Western blotting with anti-LexA antibody.

RESULTS

General characterization of the *P. aeruginosa* SOS response. We constructed a *lexA*(S125A) mutant of *P. aeruginosa* PAO1, where the catalytic serine of LexA has been replaced with alanine. We monitored the levels of full-length LexA in response to added ciprofloxacin (1 μg/ml ciprofloxacin) and showed that while the wild-type protein underwent cleavage, the mutant protein remained intact (Fig. 1).

No significant growth attenuation was observed with the *lexA*(S125A) strain relative to its isogenic parental strain (log phase doubling times of 45.0 ± 3.5 and 45.5 ± 4.5 min were observed for the LexA mutant and PAO1 strains, respectively [means ± standard deviations]), as was previously observed with analogous mutants of *E. coli* (15). Relative to the wild-type strain, we found that the *lexA*(S125A) mutant was hypersensitive to UV irradiation (Fig. 2A) but not to MMS (Fig. 2B) or ciprofloxacin [the ciprofloxacin MIC for both wild-type PAO1 and the *lexA*(S125A) strain was 0.125 μg/ml]. This suggests that the *P. aeruginosa* SOS response is important for repairing DNA damage associated with UV irradiation but not with MMS or ciprofloxacin.

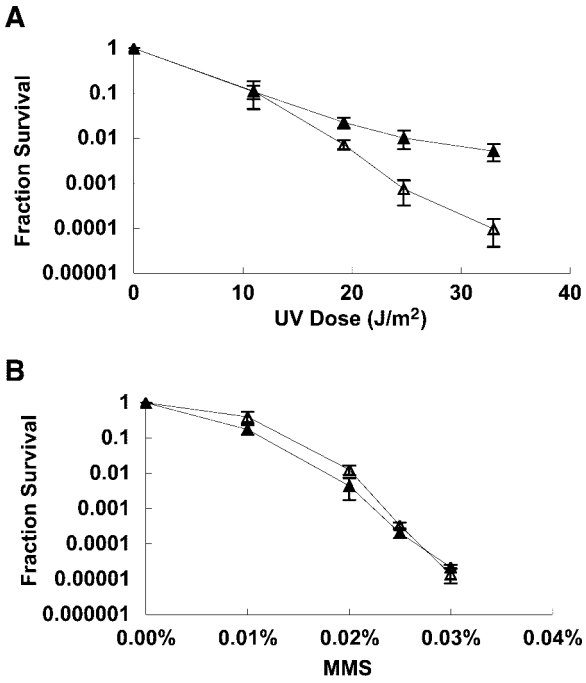

FIG. 2. Killing kinetics of *P. aeruginosa* strains, as shown by survival following UV (A) or MMS (B) treatment. PAO1 and the LexA (S125A) mutant are represented by filled and open triangles, respectively.

Characterization of the *P. aeruginosa* transcriptional response to ciprofloxacin. Using DNA microarrays, we transcriptionally profiled mid-log-phase PAO1 at 30 and 120 min after exposure to suprainhibitory concentrations of ciprofloxacin (1 μg/ml; 8× MIC) (Fig. 1A and B). Only the magnitude of the observed changes was different, and so we focused the analysis on the 120-min data set. We observed that the levels of 196 transcripts increase at least twofold and the levels of 408 transcripts decrease at least twofold relative to levels immediately before the addition of the drug (Table 1; see also our supporting information at http://www.scripps.edu/chem/romesberg/).

Sixty-four of the genes that are up-regulated in response to ciprofloxacin are in regulons that are likely controlled by LexA-like repressors (Table 2). For example, the autoregulated LexA-like Ser-Lys dyad repressor PtrR (25) is up-regulated by 5-fold, and seven genes thought to be directly or indirectly under its control are up-regulated by up to 100-fold. One of these genes, *ptrB*, acts to repress the type III secretion system (40). Thus, via induced cleavage of PtrR, exposure to ciprofloxacin results in a down-regulation of the type III secretion system. In addition, 35 nearby cryptic prophage genes spanning from PA0614 to PA0648 are strongly up-regulated. Another putative phage repressor that shares similarities with LexA and is highly homologous to PtrR is PA0906. PA0906 is divergently transcribed from a putative operon that spans PA0907 to PA0911. PA0906 and the five genes of this operon are up-regulated 20- to 80-fold after exposure to the drug. Overall, the data suggest that at least one-third of the positive transcriptional response to ciprofloxacin is controlled by LexA-like repressors.

The number of genes that are down-regulated in response to ciprofloxacin is more than twice the number that are up-regulated. The down-regulated response appears to involve virtually every facet of cellular metabolism, including general metabolism, cell wall/capsule biosynthesis, DNA replication/repair, cell division, motility, and quorum sensing (Table 1; see also our supporting information at http://www.scripps.edu/chem/romesberg/). Changes observed were similar for all genes in a given operon, supporting the physiological significance of their regulation.

We observed significant and consistent changes in the operons encoding the subunits of ATP synthase (PA5553 to PA5561), which all decrease between 4- and 13-fold, and the subunits of NADH dehydrogenase complex I (PA2637 to PA2649), which all decrease between 2- and 5-fold. Similar decreases were observed in the response to acute H_2O_2 damage (30). In addition, while *nrdA* and *nrdB*, which encode the ribonucleotide reductase complex, are both up-regulated in response to ciprofloxacin four- to eightfold, two genes in a separate operon (PA5496 and PA5497) that are predicted to encode an alternate ribonucleotide reductase are down-regulated three- to fourfold. In addition, genes encoding many other proteins involved in metabolism were down-regulated after exposure to the drug (Table 1; see also our supporting information at the website http://www.scripps.edu/chem/romesberg/).

In addition to the decreased transcription of genes involved in general metabolism, decreases are also observed with genes involved in DNA metabolism. An operon containing genes that encode components of the replication machinery, including *dnaA*, *dnaN*, *recF*, and *gyrB*, is down-regulated ~4-fold in response to ciprofloxacin. In addition, genes encoding DNA polymerase I, the HolB subunit of DNA polymerase III, and the DNA binding protein HU are all down-regulated two- to fivefold. The *recG* and *ruvABC* genes, all encoding proteins thought to be important for repairing ciprofloxacin-induced damage (7, 11), are down-regulated, albeit less than twofold. In contrast, *recA*, *recX*, and *recN* are up-regulated 7- to 17-fold. Interestingly, the three genes encoding damage-inducible DNA polymerases, PA0923, PA0670, and PA0669, are up-regulated in response to ciprofloxacin. PA0923 encodes a *dinB*-like Y-family polymerase and is up-regulated fourfold in response to ciprofloxacin. PA0670 and PA0669 encode two polymerases recently shown to be involved in damage-induced mutagenesis in *Caulobacter crescentus* (16) and are up-regulated two- and sixfold, respectively. The overall pattern of expression in the DNA replication genes suggests a shift from the canonical DNA replication enzymes to the inducible polymerases in response to ciprofloxacin.

Nearly all of the major cell division and lipopolysaccharide genes are significantly down-regulated. Of particular note are the changes observed in the *wbp* region, which encodes the B-band lipopolysaccharide O antigen and spans from PA3141 to PA3160. Transcription of these genes decreased by two- to sixfold after exposure to ciprofloxacin. Another interesting trend is the down-regulation of 41 genes that encode proteins involved in motility. We also observe a two- to fourfold increase in transcription of two major efflux proteins (MexC and MexR) and a four- to fivefold decrease in transcription of three major membrane pore proteins (OprD, OprG, and OprI). These changes in mobility and permeability are consistent with

TABLE 1. Transcriptional response to 1 μg/ml ciprofloxacin

ORF function, regulation, and name	Gene	Annotation	Fold change[a]	
			PAO1	LexA(S125A)
General metabolism				
Downregulated				
PA5553	*atpC*	ATP synthase ε chain	−6.9	−6.4
PA5554	*atpD*	ATP synthase β chain	−12.7	−12.7
PA5555	*atpG*	ATP synthase γ chain	−10.8	−12.5
PA5556	*atpA*	ATP synthase α chain	−10.0	−10.1
PA5557	*atpH*	ATP synthase δ chain	−7.5	−9.4
PA5558	*atpF*	ATP synthase B chain	−8.5	−7.7
PA5559	*atpE*	ATP synthase C chain	−7.3	−8.4
PA5560	*atpB*	ATP synthase A chain	−8.2	−7.5
PA5561	*atpI*	ATP synthase protein I	−4.3	−4.3
PA2637	*nuoA*	NADH dehydrogenase I chain A	−2.5	−2.8
PA2638	*nuoB*	NADH dehydrogenase I chain B	−3.4	−4.1
PA2639	*nuoD*	NADH dehydrogenase I chain C, D	−2.4	−2.3
PA2640	*nuoE*	NADH dehydrogenase I chain E	−2.9	−2.6
PA2641	*nuoF*	NADH dehydrogenase I chain F	−2.9	−3.0
PA2642	*nuoG*	NADH dehydrogenase I chain G	−2.9	−3.6
PA2643	*nuoH*	NADH dehydrogenase I chain H	−3.2	−3.1
PA2644	*nuoI*	NADH dehydrogenase I chain I	−3.0	−3.1
PA2645	*nuoJ*	NADH dehydrogenase I chain J	−5.0	−5.7
PA2646	*nuoK*	NADH dehydrogenase I chain K	−4.6	−4.3
PA2647	*nuoL*	NADH dehydrogenase I chain L	−5.0	−5.4
PA2648	*nuoM*	NADH dehydrogenase I chain M	−4.0	−4.9
PA2649	*nuoN*	NADH dehydrogenase I chain N	−4.7	−4.9
DNA metabolism				
Downregulated				
PA5345	*recG*	Recombination/repair	−1.8	−2.4
PA0966	*ruvA*	Branch migration	−1.7	−1.7
PA0967	*ruvB*	Branch migration	−1.6	−1.5
PA0965	*ruvC*	Holliday junction resolvase	−1.6	−1.3
PA1804	*hupB*	DNA binding protein HU	−4.7	−5.4
PA0004	*gyrB*	Gyrase subunit B	−3.4	−3.6
PA0003	*recF*	Recombination	−4.0	−3.8
PA0001	*dnaA*	Replication initiation factor	−3.3	−2.9
PA0002	*dnaN*	Beta clamp	−3.9	−4.2
PA2961	*holB*	DNA polymerase III δ′ subunit	−2.9	−2.0
PA5493	*polA*	DNA polymerase I	−1.6	−1.4
Upregulated				
PA0923	*dinB*	Y-family polymerase	3.7	2.1
PA0669	*dnaE2*	Damage-inducible polymerase	1.9	1.3
PA0670	*imuB*	Damage-inducible polymerase	6.3	1.1
PA3616	*recX*	RecA regulation	7.1	1.1
PA3617	*recA*	Recombination/repair	7.4	1.0
PA4763	*recN*	Recombination/repair	17.2	1.0
Nucleotide metabolism				
Downregulated				
PA5496		Predicted ribonucleotide reductase	−4.1	−3.4
PA5497		Predicted ribonucleotide reductase	−2.6	−2.7
Upregulated				
PA1155	*nrdB*	Ribonucleotide reductase small chain	3.8	3.7
PA1156	*nrdA*	Ribonucleotide reductase large chain	7.9	7.7
Cell division				
Downregulated				
PA4407	*ftsZ*	Cell division protein FtsZ	−2.7	−2.3
PA4408	*ftsA*	Cell division protein FtsA	−2.9	−2.7
PA4409	*ftsQ*	Cell division protein FtsQ	−3.1	−2.7
PA4413	*ftsW*	Cell division protein FtsW	−2.4	−1.9
Upregulated				
PA0373	*ftsY*	Signal recognition particle receptor	1.5	1.4

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TABLE 1—*Continued*

ORF function, regulation, and name	Gene	Annotation	Fold change[a]	
			PAO1	LexA(S125A)
LPS biosynthesis				
Downregulated				
PA3141	*wbpM*	Nucleotide sugar epimerase/dehydratase	−2.2	−2.1
PA3145	*wbpL*	Glycosyltransferase	−3.8	−2.8
PA3146	*wbpK*	Probable NAD-dependent epimerase/dehydratase	−2.8	−2.8
PA3147	*wbpJ*	Probable glycosyl transferase	−3.8	−4.2
PA3148	*wbpI*	Probable UDP-*N*-acetylglucosamine 2-epimerase	−3.4	−3.9
PA3149	*wbpH*	Probable glycosyltransferase	−5.4	−6.4
PA3150	*wbpG*	LPS biosynthesis protein	−6.1	−5.2
PA3153	*wzx*	O-antigen translocase	−4.3	−3.2
PA3155	*wbpE*	Probable aminotransferase	−4.5	−4.1
PA3156	*wbpD*	Probable acetyltransferase	−3.3	−2.8
PA3157		Probable acetyltransferase	−2.7	−3.0
PA3158	*wbpB*	Probable oxidoreductase	−3.3	−3.9
PA3159	*wbpA*	Probable UDP-glucose/GDP-mannose dehydrogenase	−2.9	−2.8
PA3160	*wzz*	O-antigen chain length regulator	−2.5	−3.1
Motility				
Downregulated				
PA4550	*fimU*	Type 4 fimbrial biogenesis protein FimU	−2.6	−2.5
PA3115	*fimV*	Motility protein FimV	−3.7	−3.3
PA1822	*filM*	Hypothetical protein	−1.8	−1.7
PA0395	*pilT*	Twitching motility protein	−2.2	−2.1
PA0411	*pilJ*	Twitching motility protein	−3.0	−3.0
PA0412	*pilK*	Methyltransferase	−2.1	−1.7
PA2960	*pilZ*	Type 4 fimbrial biogenesis	−1.6	−1.7
PA4525	*pilA*	Type 4 fimbrial biogenesis precursor	−27.8	−19.7
PA4526	*pilB*	Type 4 fimbrial biogenesis	−6.8	−8.0
PA4527	*pilC*	Type 4 fimbrial biogenesis	−6.4	−5.9
PA4528	*pilD*	Type 4 prepilin peptidase	−7.7	−6.4
PA4550	*fimU*	Type 4 fimbrial biogenesis	−2.6	−2.5
PA4551	*pilV*	Type 4 fimbrial biogenesis	−2.9	−2.4
PA4552	*pilW*	Type 4 fimbrial biogenesis	−2.3	−2.0
PA4554	*pilY1*	Type 4 fimbrial biogenesis	−2.2	−2.1
PA4555	*pilY2*	Type 4 fimbrial biogenesis	−1.9	−1.5
PA5040	*pilQ*	Type 4 fimbrial biogenesis outer membrane protein	−5.7	−6.1
PA5041	*pilP*	Type 4 fimbrial biogenesis	−7.5	−6.6
PA5042	*pilO*	Type 4 fimbrial biogenesis	−5.1	−5.1
PA5043	*pilN*	Type 4 fimbrial biogenesis	−4.5	−3.5
PA5044	*pilM*	Type 4 fimbrial biogenesis	−3.7	−4.0
PA1094	*fliD*	Flagellar capping protein	−2.6	−1.9
PA1100	*fliE*	Flagellar hook-basal body complex	−2.8	−2.4
PA1101	*fliF*	Flagellum M-ring outer membrane protein precursor	−2.5	−2.4
PA1102	*fliG*	Flagellar motor switch protein	−1.8	−1.6
PA1103	*fliH*	Flagellar synthesis	−2.0	−1.7
PA1104	*fliI*	Flagellum-specific ATP synthase	−1.6	−1.7
PA1105	*fliJ*	Flagellar synthesis	−2.0	−1.6
PA1443	*fliM*	Flagellar motor switch protein	−2.0	−1.8
PA1445	*fliO*	Flagellar synthesis	−1.6	−1.3
PA1446	*fliP*	Flagellar synthesis	−1.9	−1.5
PA1447	*fliQ*	Flagellar synthesis	−1.8	−1.3
PA1456	*cheY*	Chemotaxis response regulator	−2.8	−2.6
PA1457	*cheZ*	Chemotaxis	−2.4	−2.5
PA1458		Probable chemotaxis signal transduction kinase	−2.0	−1.8
PA1459		Probable chemotaxis signal transduction methyltransferase	−1.7	−1.4
PA2654		Probable chemotaxis transducer	−2.3	−1.7
PA1461	*motD*	Flagellar motor protein	−1.7	−1.3
PA1464		Probable purine binding chemotaxis protein	−2.1	−1.8
PA1097	*fleQ*	Transcriptional regulator	−2.0	−1.7
PA1098	*fleS*	Two-component sensor	−1.8	−1.6
Quorum sensing				
Downregulated				
PA3477	*rhlR*	Transcriptional regulator RhlR	−1.8	−2.0
PA1430	*lasR*	Transcriptional regulator LasR	−1.7	−1.7

Continued on following page

TABLE 1—*Continued*

ORF function, regulation, and name	Gene	Annotation	Fold change[a]	
			PAO1	LexA(S125A)
Efflux				
Upregulated				
PA4599	*mexC*	RND multidrug efflux membrane fusion protein MexC precursor	4.4	3.1
PA0424	*mexR*	Multidrug resistance operon	2.2	1.6
Permeability				
Downregulated				
PA4067	*oprG*	Outer membrane protein OprG precursor	−4.8	−6.6
PA2853	*oprI*	Outer membrane lipoprotein OprI precursor	−1.7	−1.8
PA0958	*oprD*	Membrane porin OprD precursor	−4.7	−6.3
Pyocin synthesis				
Upregulated				
PA0611	*ptrR*	Repressor of ptrN/ptrB (ser-lys dyad)	5.0	4.3
PA0610	*ptrN*	Transcriptional activator of pyocin synthesis	55.6	48.7
PA0985		Pyocin S5	111.1	50.4
PA1150	*pys2*	Pyocin S2	66.7	41.7
PA1151	*imm2*	Pyocin S2 immunity protein	17.5	12.6
PA3866		Pyocin protein	52.6	36.8
Phage region				
Upregulated				
PA0614		Hypothetical/phage related	71.4	52.4
PA0615		Hypothetical/phage related	83.3	70.8
PA0616		Hypothetical/phage related	55.6	43.8
PA0617		Hypothetical/phage related	83.3	66.5
PA0618		Hypothetical/phage related	83.3	57.5
PA0619		Hypothetical/phage related	90.9	59.1
PA0620		Hypothetical/phage related	71.4	56.1
PA0621		Hypothetical/phage related	50.0	38.5
PA0622		Hypothetical/phage related	58.8	52.1
PA0623		Hypothetical/phage related	50.0	38.8
PA0624		Hypothetical/phage related	55.6	40.0
PA0625		Hypothetical/phage related	66.7	55.1
PA0626		Hypothetical/phage related	125.0	75.7
PA0627		Hypothetical/phage related	83.3	57.5
PA0628		Hypothetical/phage related	90.9	64.3
PA0629		Hypothetical/phage related	100.0	84.8
PA0630		Hypothetical/phage related	83.3	54.9
PA0631		Hypothetical/phage related	111.1	76.5
PA0632		Hypothetical/phage related	111.1	84.4
PA0633		Hypothetical/phage related	76.9	62.9
PA0634		Hypothetical/phage related	71.4	54.8
PA0635		Hypothetical/phage related	90.9	71.8
PA0636		Hypothetical/phage related	55.6	45.7
PA0637		Hypothetical/phage related	111.1	70.3
PA0638		Hypothetical/phage related	90.9	70.0
PA0639		Hypothetical/phage related	100.0	74.0
PA0640		Hypothetical/phage related	71.4	46.2
PA0641		Hypothetical/phage related	71.4	51.3
PA0642		Hypothetical/phage related	43.5	25.0
PA0643		Hypothetical/phage related	100.0	52.6
PA0644		Hypothetical/phage related	90.9	53.9
PA0645		Hypothetical/phage related	58.8	37.2
PA0646		Hypothetical/phage related	58.8	32.3
PA0647		Hypothetical/phage related	50.0	32.7
PA0648		Hypothetical/phage related	58.8	46.0
Virulence/toxin				
Downregulated				
PA4315	*mvaT*	Global regulator of virulence/motility	−4.0	−5.4
PA1718	*pscE*	Type III export protein	−7.2	−6.0
PA1719	*pscF*	Type III export protein	−2.7	−2.2
PA1722	*pscI*	Type III export protein	−3.5	−3.7
PA1716	*pscC*	TTSS outer membrane protein precursor	−1.9	−1.3

Continued on following page

TABLE 1—*Continued*

ORF function, regulation, and name	Gene	Annotation	Fold change[a]	
			PAO1	LexA(S125A)
PA1713	*exsA*	Regulator of exoenzyme synthesis	−1.9	−1.5
PA1710	*exsC*	Exoenzyme S synthesis protein C precursor	−3.5	−2.6
PA1711	(*exsE*)		−3.5	−2.5
PA1712	*exsB*	Exoenyzme S synthesis protein B	−4.6	−3.1
Upregulated				
PA0612	*ptrB*	Suppressor of TTSS	166.7	178.1
PA0613		In operon with PA0612	62.5	56.0

[a] Difference in expression of PAO1 or the LexA(S125A) mutant 120 min after ciprofloxacin exposure compared to immediately prior to exposure.

the general trend toward reduced metabolic activity in response to antibiotic exposure.

Contribution of the *P. aeruginosa* SOS genes to the ciprofloxacin-induced transcriptional response. We next characterized the transcriptional response to ciprofloxacin in a *lexA*(S125A) PAO1 mutant under the same conditions as those used to characterize the wild-type strain. As expected, *lexA*, *recA*, *recX*, and *recN* are induced by ciprofloxacin in a LexA cleavage-dependent manner (Table 3). In addition, PA3413 and PA1045 are regulated by LexA. PA3413 is a probable homolog of *E. coli yebG*, which is LexA regulated in *E. coli* (29), but its biological function is not known. PA1045 appears to encode a DinG helicase (37), which is related to the mammalian XPD family of helicases, and it may play a role in transcription-coupled repair.

The induction of several hypothetical genes was found to depend on LexA cleavage: PA2288, PA3414, PA1044, PA0069, and PA0922. PA3414 is predicted to encode a protein of unknown function, but its location next to *yebG* (see above) suggests that these genes may be coordinatively transcribed. PA2288 encodes a hypothetical protein of no known function. However, it has recently been shown that mutation of *wspF*, which disrupts a signal cascade involved in biofilm formation, causes a mild induction of *lexA* and *recA* (each 1.6-fold) and a 4.0- and 2.8-fold increase in PA3414 and PA2288 expression, respectively, supporting the association of these genes with the SOS response (19). PA1044 encodes a hypothetical protein with no known function that is divergently transcribed from PA1045 (see above). PA0922 and PA0069 are predicted to encode a hypothetical protein and a photolyase-like protein, respectively.

TABLE 2. Other damage-inducible Ser-Lys dyads and downstream targets

ORF	Gene	Description	Fold change[a]
PA0906		Predicted transcriptional regulator (Ser-Lys dyad)	5.2
PA0907		Hypothetical, divergent to PA0906	30.3
PA0908		Hypothetical	17.5
PA0909		Hypothetical, related to phage	32.3
PA0910		Hypothetical	83.3
PA0911		Hypothetical	71.4
PA0611	*ptrR*	Transcriptional regulator (Ser-Lys dyad) of pyocin synthesis	5.0
PA0610	*ptrN*	Transcriptional activator of pyocin synthesis (regulated by ptrR)	55.6
PA0612	*ptrB*	Suppressor of TTSS	166.7
PA0613		In operon with PA0612	62.5
PA0985		Pyocin S5	111.1
PA1150	*pys2*	Pyocin S2	66.7
PA1151	*imm2*	Pyocin S2 immunity protein	17.5
PA3866		Probable pyocin protein	52.6

[a] Difference in expression of PAO1 120 min after ciprofloxacin exposure compared to immediately prior to exposure.

In *E. coli* there are three nonessential polymerases, each of which is LexA regulated: Pol II (encoded by *polB*), Pol IV (encoded by *dinB*), and Pol V (encoded by *umuC* and *umuD*). The *P. aeruginosa* genome encodes three nonessential polymerases, PA0923, PA0669, and PA0670. As mentioned above, transcription of PA0923, which encodes a polymerase that is highly homologous to *E. coli dinB*, is induced by ciprofloxacin; however, its induction is not LexA regulated. This agrees with recent findings in other organisms (6, 36) and suggests that a LexA-regulated *dinB* polymerase may be more the exception than the rule. In contrast, PA0669 and PA0670, which appear to be encoded in the same operon, are induced by ciprofloxacin in a LexA cleavage-dependent manner. PA0669 is predicted to encode an alternate alpha-subunit, and PA0670 is predicted to encode a Y-family polymerase. This operon resembles one that was recently found to play a role in damage-induced mutagenesis in the α-*Proteobacteria Caulobacter crescentus* (16), although a PAO1 mutant lacking this operon shows no signs of increased sensitivity to UV, MMS, or ciprofloxacin-mediated damage (R. T. Cirz and F. E. Romesberg, unpublished results). While we were able to detect sufficient levels of PA0670 in the microarray studies, the level of PA0669 was too low to observe a rigorous statistical difference between PAO1 and the LexA mutant directly (supporting information can be found at http://www.scripps.edu/chem/romesberg/). However, using real-time PCR we were able to detect PA0669 mRNA after ciprofloxacin treatment, but not in the LexA mutant, confirming that this gene is LexA regulated (see Table S2 in our supporting information at http://www.scripps.edu/chem/romesberg/).

Analysis of the *P. aeruginosa* LexA box and other potential SOS genes. By identifying the SOS regulon empirically, we were also able to identify a consensus binding sequence for LexA, **CTG**-TATAA-ATATA-**CAG** (bold residues are 100% conserved) (Table 3). The consensus is essentially the same as that in *E. coli* with the exception of position eight, where it is most frequently a dA in *P. aeruginosa* and a dT in *E. coli*.

We searched the *Pseudomonas* genome (35, 39) for other

TABLE 3. LexA-regulated genes identified in the microarray-based whole-genome transcription assay

ORF	Gene	LexA box	Distance (bp) to start codon	Mismatches (bp) from consensus	Fold change PA01 vs LexA(S125A)
Consensus		**CTG** TATAA ATATA **CAG**[a]			
PA3008	*sulA*	Shared with PA3007			33.1
PA3007	*lexA*	CTG GATAA AAACA CAG	9	3	22.7
PA3413	*yebG*	CTG TATGG ATAAC CAG	40	4	13.9
PA4763	*recN*	CTG TATAA ATAAC CAG	24	2	10.4
PA2288		CTG TATGA ATGTA CAG	41	2	8.4
PA3414		Shared with PA3413			6.5
PA3617	*recA*	CTG TCTAC TTATA CAG	43	3	3.4
PA3616	*recX*	Shared with PA3617			3.5
PA1044		CTG GATAA ATTTT CAG	60	3	2.0
PA1045	*dinG*	CTG GATAA ATTTT CAG	54	3	1.7
PA0069	*phl*	CTG TATCC ATATA CAG	20	2	3.4
PA0922		CTG TATAT TCGTA CAG	52	4	5.7
PA0669	*dnaE2*	Shared with PA0671			
PA0670	*imuB*	Shared with PA0671			5.0
PA0671	*sulA2*	CTG TATTT ACATA CAG	137	3	6.3

[a] The bold residues are 100% conserved.

potential LexA binding sites using the sequence $CTGN_2TN_7CAG$ with up to four mismatches in the central 10-bp region. In addition to the sites that regulate the 15 genes identified in our microarray studies, eight potential LexA binding sites were identified (see Table S4 in our supporting information at http://www.scripps.edu/chem/romesberg/). Four are positioned between 128 and 154 bp from a gene, and four are intragenic. The microarray data suggest that either these sites do not bind LexA in vivo or that they do not effectively regulate expression. Thus, the data suggest that the 15 genes identified experimentally represent the entire LexA regulon.

DISCUSSION

The global transcriptional response to ciprofloxacin was previously examined in *P. aeruginosa* PAO1 grown for 2.5 h in the presence of sub-MIC and MIC levels of the drug (5). Ciprofloxacin was found to induce changes in the transcription of >900 and >1,200 genes at sub-MIC and MIC levels, respectively ($P \leq 0.05$). While this study identified the increased transcription of the cryptic prophage genes spanning from PA0614 to PA0648 (5), which we also observed with supra-MIC levels of the drug, it did not identify any consistent and significant changes in genes involved in metabolism or proliferation (see Table S3 in our supporting information at http://www.scripps.edu/chem/romesberg/). In fact, the present study reveals that the largest part of the response involves the down-regulation of genes that encode proteins involved in general metabolism and DNA replication/repair, as well as the down-regulation of genes involved in cell division, motility, quorum sensing, and cell permeability. These changes appear to be specific for higher, clinical-like levels of the drug and may contribute to the pathogen's survival during therapy, as has already been suggested for both planktonic and biofilm cells (1, 24). Indeed, this response is a reasonable survival strategy, considering that ciprofloxacin is more lethal to actively dividing cells than resting cells (11), and it may facilitate survival until a more specific response is orchestrated.

The SOS response is thought to be a major component of the bacterial response to stress and has been characterized thoroughly in *E. coli*, where it includes the derepression of 43 genes that orchestrate virtually the entire positive transcriptional response to UV irradiation (9). The only other globally characterized SOS response is that of *B. subtilis*, where 33 genes have LexA binding sites and are induced by UV irradiation and mitomycin C in a RecA-dependent manner. While the number of LexA-regulated genes in *E. coli* and *B. subtilis* is similar, only seven genes are common to both organisms.

By directly comparing the response to ciprofloxacin of LexA-cleavable and uncleavable strains, we identified 15 *P. aeruginosa* genes that are induced by ciprofloxacin in a LexA cleavage-dependent manner. These genes appear to be controlled from nine LexA binding sites, with five of the sites controlling expression of divergent or polycistronic operons. The consensus binding site is the 16-nucleotide imperfect palindrome **CTG-**TATAA-ATATA-**CAG** (where the bold indicates absolutely conserved nucleotides). As expected, the SOS regulon includes *lexA* and *recA*. It also includes *recN*, *recX*, and probable *yebG*, *dinG*, and *phl* homologs, which are all commonly part of the SOS regulon in other bacteria. Also included is the polycistronic operon containing *imuA*/*sulA*, *imuB*, and *dnaE2*, as observed in several other SOS operons (12). The data suggests that, like other γ-Proteobacteria, both DNA repair and induced mutation are central components of the LexA-regulated SOS response in *P. aeruginosa*. However, *P. aeruginosa* LexA appears to regulate only the recombinational repair proteins RecX and RecN and not the nucleotide excision repair proteins UvrA, UvrB, and UvrD, nor the recombinational repair proteins RuvA and RuvB, all of which are LexA regulated in both *E. coli* (9) and *B. subtilis* (2) and are also predicted to be LexA regulated in other γ-Proteobacteria (13). RecX is thought to associate with RecA and cap filament extension (10), while RecN is thought to cooperate in some forms of recombination (31). Why these recombination proteins are regulated by LexA in *P. aeruginosa*, while ones common to other SOS regulons are not, is unclear but likely reflects the environment in which the pathogen has adapted to survive.

Induced mutation in *E. coli* is controlled by LexA cleavage-mediated derepression of *polB*, which encodes Pol II, *dinB*,

which encodes Pol IV, and *umuDC*, which encodes the Pol V preprotein. *P. aeruginosa* does not have a *umuDC* homolog, and our data show that *polB* is not induced in response to ciprofloxacin and that *dinB*, while induced, is not repressed by LexA. Instead, *P. aeruginosa* appears to control induced mutation from the LexA-repressed *imuA*/*sulA-imuB-dnaE2* operon. *imuB* and *dnaE2* encode inducible polymerases, and their homologs are required for the majority of UV- and mitomycin C-induced mutations in the highly related organism *Caulobacter crescentus* (16). In addition, a *dnaE2* homolog in *Mycobacterium tuberculosis* has been shown to be required for UV-induced mutation (4). The operon also appears in many other species and is predicted computationally to be universally LexA regulated (12). Interestingly, the presence of this operon has been correlated with the absence of a *umuDC* operon (12), suggesting that it may perform a similar function.

The SOS system may play an underappreciated role in the response to several commonly used antibiotics. In *E. coli*, the SOS response is induced by ciprofloxacin (11, 33), rifampin (7), β-lactams (27), and trimethoprim (23). While the LexA regulon of *P. aeruginosa* is significantly smaller than that of *E. coli*, or *B. subtilis*, it appears to have retained control over induced mutation. Thus, it seems likely that the initial reduction in metabolism observed in *P. aeruginosa* provides the SOS response time to induce mutations that allow it to persist and eventually to evolve resistance, as has been observed in *E. coli* (7, 8) and *M. tuberculosis* (4). These results suggest that an inhibitor of LexA cleavage might have a profound and favorable effect on *P. aeruginosa* therapy.

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