JOURNAL OF BACTERIOLOGY, Jan. 2007, p. 531–539 Vol. 189, No. 2
0021-9193/07/$08.00+0 doi:10.1128/JB.01464-06

Complete and SOS-Mediated Response of *Staphylococcus aureus* to the Antibiotic Ciprofloxacin▽

Ryan T. Cirz,[1] Marcus B. Jones,[2] Neill A. Gingles,[1] Timothy D. Minogue,[2] Behnam Jarrahi,[2] Scott N. Peterson,[2] and Floyd E. Romesberg[1]*

Department of Chemistry, The Scripps Research Institute, La Jolla, California 92037,[1] and Pathogen Functional Genomics Resource Center (PFGRC), The Institute for Genomic Research, Rockville, Maryland 20850[2]

Received 15 September 2006/Accepted 26 October 2006

Staphylococcus aureus **infections can be difficult to treat due to both multidrug resistance and the organism's remarkable ability to persist in the host. Persistence and the evolution of resistance may be related to several complex regulatory networks, such as the SOS response, which modifies transcription in response to environmental stress. To understand how *S. aureus* persists during antibiotic therapy and eventually emerges resistant, we characterized its global transcriptional response to ciprofloxacin. We found that ciprofloxacin induces prophage mobilization as well as significant alterations in metabolism, most notably the up-regulation of the tricarboxylic acid cycle. In addition, we found that ciprofloxacin induces the SOS response, which we show, by comparison of a wild-type strain and a non-SOS-inducible *lexA* mutant strain, includes the derepression of 16 genes. While the SOS response of *S. aureus* is much more limited than those of *Escherichia coli* and *Bacillus subtilis*, it is similar to that of *Pseudomonas aeruginosa* and includes RecA, LexA, several hypothetical proteins, and a likely error-prone Y family polymerase whose homologs in other bacteria are required for induced mutation. We also examined induced mutation and found that either the inability to derepress the SOS response or the lack of the LexA-regulated polymerase renders *S. aureus* unable to evolve antibiotic resistance in vitro in response to UV damage. The data suggest that up-regulation of the tricarboxylic acid cycle and induced mutation facilitate *S. aureus* persistence and evolution of resistance during antibiotic therapy.**

Staphylococcus aureus is a gram-positive pathogen and a leading cause of both nosocomial and community-acquired infections, ranging from food poisoning and skin abscesses to more serious diseases, such as pneumonia, meningitis, endocarditis, septicemia, and toxic shock syndrome. Before antibiotics were available, mortality associated with *S. aureus* infections approached 80% (38). Even with antibiotics, *S. aureus* infections are often difficult to treat due to the unique ability of this pathogen to persist and adapt. Adaptability is highly correlated with virulence (8) and is thought to result from the transcriptional regulation of stress response genes (8).

For a growing number of bacteria, the SOS response has been recognized as a critical component of the response to environmental stress, in particular to antibiotics such as ciprofloxacin (11, 15, 34). Ciprofloxacin induces double-stranded DNA breaks and stalled replication forks, both of which are processed to single-stranded DNA. RecA forms filaments on the single-stranded DNA, and these nucleoprotein filaments facilitate recombinational repair (15) as well as bind the SOS gene repressor LexA, stimulating its autoproteolysis. This cleavage inactivates the LexA repressor and results in the induction of the SOS genes. Interestingly, in addition to the fluoroquinolones, e.g., ciprofloxacin, other antibiotics, such as the rifamycins (5), trimethoprim (26), and β-lactams (29, 31), have all been shown to induce LexA cleavage and the SOS response. Epidemiological studies have also documented a link between the severity of some infections and the use of several of these antibiotics (45).

The genome-wide experimental characterization of the genes regulated by LexA, and therefore the genes that constitute the SOS response, has been reported for *Escherichia coli* (9), *Bacillus subtilis* (2, 19), and *Pseudomonas aeruginosa* (6). In *E. coli* and *B. subtilis*, the LexA regulon includes relatively large numbers of genes, i.e., at least 43 and 63, respectively, although only 7 genes are common between them. In contrast, the regulon of the more pathogenic bacterium, *P. aeruginosa*, contains only 15 genes. In all cases, LexA controls the expression of *recA* and *lexA*, the positive and negative regulators of the response, respectively, as well as genes that encode proteins involved in DNA repair or recombination and genes that encode nonessential DNA polymerases. Interestingly, several of these polymerases have been shown to be required for induced mutation, suggesting that it is an important part of the SOS response.

In this study, we report the global transcriptional responses of *S. aureus* 8325 30 and 120 min after exposure to ciprofloxacin. Ciprofloxacin-induced changes in transcription were monitored in both the wild-type strain and a mutant strain expressing a noncleavable mutant of LexA. We also characterized the contribution of the SOS response to induced mutation in *S. aureus*. We found that the general and SOS-specific responses appear to facilitate survival and the evolution of resistance by altering metabolism and inducing mutation.

MATERIALS AND METHODS

Bacterial strains and growth. The strains and plasmids used in this study are listed in Table 1. Unless specified otherwise, the solid medium was tryptic soy agar (TSA; Difco), and the liquid medium was tryptic soy broth (TSB; Difco).

* Corresponding author. Mailing address: Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037. Phone: (858) 784-7290. Fax: (858) 784-7472. E-mail: floyd@scripps.edu.

▽ Published ahead of print on 3 November 2006.

TABLE 1. Strains used in this work

Strain or plasmid	Description	Source
Strains		
8325	Wild-type reference strain	NARSA[a]
RN4220	Restriction-negative strain	NARSA
RTC3001	8325 Spec^r *lexA* control strain	This work
RTC3002	8325 *lexA*(*S130A*)::Spec^r	This work
RTC3003	8325 ΔSACOL1955::Spec^r	This work
RTC3004	8325 ΔSACOL1400::Spec^r	This work
Plasmids		
pMAD	Allelic exchange vector	J. Penadés
pRTC0071	pMAD-Spec^r-*lexA*	This work
pRTC0072	pMAD-*lexA*(*S130A*)::Spec^r	This work
pRTC0074	pMAD-ΔSACOL1955::Spec^r	This work
pRTC0075	pMAD-ΔSACOL1400::Spec^r	This work

[a] NARSA, Network on Antimicrobial Resistance in *Staphylococcus aureus*.

For selection of *S. aureus*, antibiotics were used as follows: spectinomycin (Spec), 100 μg/ml; and erythromycin (Erm), 1.5 μg/ml. For *E. coli*, ampicillin was used at 100 μg/ml. Ciprofloxacin was obtained from MP Biomedicals (Aurora, OH) and used at the concentrations indicated below. For blue-white screening, 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) was added to the medium at 150 μg/ml. All bacteria were grown aerobically at 37°C.

Strain construction. All primer sequences were designed based on sequence information obtained from the *S. aureus* genome database (http://www.genome.ou.edu/staph.html) (23). Allelic exchange constructs (Table 1) were created for each of the mutants (oligonucleotide primer sequences are available at http://www.scripps.edu/chem/romesberg/PublicationsMain.htm). Briefly, a linear cassette consisting of the Spec^r cassette from vector pIC333 (41) surrounded by ~700 bp of DNA homologous to the target insertion site was constructed for each mutant by assembly PCR and cloned into vector pMAD (1) at the BamHI site, generating constructs pRTC0071, pRTC0074, and pRTC0075. The S130A mutation was introduced into pRTC0071, using primers SA_lexA_S130A_QCF and SA_lexA_S130A_QCR and a QuikChange site-directed mutagenesis kit (Stratagene) to create vector pRTC0072.

Each vector was transformed into strain RN4220 by electroporation. Vector DNA was repurified using a plasmid miniprep kit (QIAGEN) following the manufacturer's protocols, with the exception that cell pellets were suspended in 250 μl buffer P1 containing 100 μg/ml lysostaphin (Sigma) and incubated for 1 h at 37°C. Following purification, vector DNA was transformed into strain 8325 by electroporation. Allelic exchange was performed as described previously (1). Erm^s Spec^r white colonies were confirmed by PCR followed by DNA sequencing. The Spec^r *lexA* control strain generated using pRTC0071 served to discount any possible polar effects on the surrounding genes.

Sample preparation for transcriptional analysis. For each strain, three clones were inoculated into TSB and incubated for 18 h. Cultures were diluted 1:100 and incubated until they reached early log phase (optical density at 600 nm [OD_{600}] = 0.5 to 0.6), at which point ciprofloxacin was added to a final concentration of 0.8 μg/ml. Immediately prior to ciprofloxacin addition and again 30 and 120 min following its addition, appropriate volumes from each of the three cultures per strain were pooled and added to 2 volumes of RNAprotect reagent (QIAGEN). Cultures were centrifuged, and cell pellets were stored at 4°C until RNA extraction. During the experiment, OD_{600} and viable CFU per ml were monitored for each of the cultures (see Fig. 2A and B). Total RNA was extracted using an RNeasy Mini kit (QIAGEN) at the end of the sample collection period. This procedure was repeated three independent times to generate three samples at each time point for each strain.

Microarray design. Epoxy-coated *S. aureus* microarrays were acquired from the Pathogen Functional Genomics Resource Center at The Institute for Genomic Research. Microarray slides contained 21,504 elements, including control oligonucleotides (10 *Arabidopsis thaliana* amplicons and 500 *A. thaliana* 70-mers). Oligonucleotides were designed based on 4,546 unique open reading frames (ORFs) and used in quadruplicate with sequences from *S. aureus* strains COL, Mu50, MW2, N315, MRSA252, and MSSA476 and from plasmid pLW043.

Generation of probes for microarray experiments. cDNA probes for microarray experiments were generated as follows. Briefly, 2 μg of total RNA was incubated at 42°C overnight in a mixture containing 6 μg of random hexamers (Invitrogen, Carlsbad, CA); 0.01 M dithiothreitol; an amino-allyl-deoxynucleoside triphosphate mixture containing 25 mM (each) dATP, dCTP, and dGTP, 15 mM dTTP, and 10 mM amino-allyl-dUTP (Sigma); reaction buffer (Clontech Laboratories, Mountain View, CA); and 400 units of Powerscript reverse transcriptase (Clontech Laboratories). The resulting RNA template was hydrolyzed by adding NaOH and EDTA to final concentrations of 0.2 and 0.1 M, respectively, and incubating the mixture at 65°C for 15 min. Unincorporated amino-allyl-dUTP was removed with a Minelute column (QIAGEN). The cDNA probes were eluted with phosphate buffer (4 mM KPO_4, pH 8.5, in ultrapure water), dried, and resuspended in 0.1 M sodium carbonate buffer (pH 9.0). To couple the amino-allyl cDNA with fluorescent labels, normal human serum-Cy3 or normal human serum-Cy5 (Amersham Biosciences, Piscataway, NJ) was added at room temperature and incubated for 2 h. Uncoupled label was removed using a Minelute column (QIAGEN).

Microarray hybridization and scanning. Epoxy-coated slides were prehybridized in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (Invitrogen), 0.1% sodium dodecyl sulfate (SDS), and 1% bovine serum albumin at 42°C for 60 min. The slides were then washed at room temperature with distilled water, dipped in isopropanol, and spun dry. Equal volumes of the appropriate Cy3- and Cy5-labeled probes were combined, dried, and then resuspended in a solution of 40% formamide, 5× SSC, and 0.1% SDS. Resuspended probes were denatured by being heated to 95°C prior to hybridization. The probe mixture then was added to the microarray slide and allowed to hybridize overnight at 42°C. Hybridized slides were washed sequentially in solutions of 1× SSC-0.2% SDS, 0.1× SSC-0.2% SDS, and 0.1× SSC at room temperature, dried, and then scanned with an Axon GenePix 4000 scanner.

Microarray normalization and analysis. Microarray data were normalized as previously described (37). Briefly, scanned TIFF images were processed through TIGR Spotfinder software (available at http://www.tigr.org/software/tm4). Processed data were normalized by LocFit (LOWESS) normalization in block mode, using TIGR MIDAS software (available at http://www.tigr.org/software/tm4). Signal intensities of <10,000 were removed from the analysis to avoid spurious signal intensity measurements. All hybridizations were performed with a minimum of one flip-dye experiment to compensate for dye bias. Each microarray slide contained four in-slide replicates of each unique oligonucleotide. Median signal intensity values, calculated from each set of in-slide replicates and flip-dye experiments, were used to calculate $\log_2$ and *x*-fold changes in gene expression.

Reverse transcription-PCR (RT-PCR) validation of microarray data. RNA samples used in the microarray analysis were treated with amplification-grade DNase (Invitrogen) according to the manufacturer's suggested protocol. DNase-treated RNA was repurified using an RNeasy kit (QIAGEN). No contaminating DNA was detected by PCR. DNase-treated RNA was reverse transcribed using an iScript kit (Bio-Rad), and real-time PCR was performed with an iCycler (Bio-Rad), using an iQ SYBR green kit (Bio-Rad) according to the manufacturer's recommended protocol and an initial denaturing step of 95°C for 3 min followed by 40 cycles of 95°C for 10 s and 55°C for 30 s. Expression of 16S rRNA (14) was monitored to allow for sample normalization. Results and all primers used are shown at http://www.scripps.edu/chem/romesberg/PublicationsMain.htm.

UV light-induced mutagenesis. For each strain, five cultures were grown in TSB at 37°C for 18 h. Each culture was diluted 1:100 in TSB and grown for approximately 2 h to an OD of 0.5 (~1×10^8 CFU/ml). For each culture, cells were harvested by centrifugation, washed once in 0.9% NaCl, and resuspended in 0.9% NaCl at one-fifth the original volume (to ~5×10^8 CFU/ml). Cell suspensions were irradiated with 8.6 J m^{-2} UV light. For each sample, serial dilutions were plated on TSA to determine viable cell counts, and two 200-μl samples of the cell suspension were plated onto each of two TSA plates for recovery prior to drug addition. After a 2-h incubation at 37°C (a condition previously shown to be sufficient for recovery [43]), TSA top agar (TSB plus 0.6% agar) containing antibiotic was added to yield a final concentration of 100 μg/ml rifampin or 500 μg/ml streptomycin. Plates were incubated at 37°C, and viable CFU and resistant colonies were counted at 24 and 48 h. Each sample was normalized by viable CFU, and spontaneous mutants were subtracted by counting the number of resistant mutants from unirradiated samples of identical cultures and correcting for UV-mediated killing.

Microarray accession numbers. Microarray data have been deposited at the National Center for Biotechnology Information's Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE6258.

RESULTS

Characterization of *S. aureus* transcriptional response to ciprofloxacin. To understand how *S. aureus* responds to cipro-

floxacin, we transcriptionally profiled *S. aureus* strain 8325 30 and 120 min after exposure to suprainhibitory concentrations of ciprofloxacin (0.8 μg/ml [4× the MIC]), using DNA microarrays. At 30 min posttreatment, 118 and 117 transcripts were found at increased and decreased levels, respectively, and at 120 min, 252 and 258 transcripts were found at increased and decreased levels, respectively (Table 2).

Ciprofloxacin affects the expression of many genes involved in toxin production and virulence (Table 2). An interesting trend observed is that while ciprofloxacin induced the up-regulation of prophage genes, it also induced the down-regulation of many chromosomal virulence factors. For example, *hlY* and MW1942, which encode an alpha-hemolysin and a leukocidin family toxin, respectively, were both down-regulated more than fourfold at 120 min. In addition, *sarA*, *sarR*, *rot*, and *agrB*, which encode positive regulators of complex transcriptional responses associated with stress and virulence, were down-regulated.

Genes involved in almost every facet of metabolism were down-regulated by ciprofloxacin, including amino acid, cofactor, nucleotide, fatty acid, and cell wall biosynthesis, energy metabolism, and electron transport (Table 2). While generally less uniform, significant changes in expression were also observed with genes involved in nutrient uptake and metabolism. Components of the phosphotransferase system responsible for carbohydrate uptake were altered, apparently in favor of glucose- and sucrose-specific import. Transcripts encoding components of the glycolysis/glucogenesis and fermentation pathways were both up- and down-regulated by exposure to ciprofloxacin.

Remarkably, transcripts encoding enzymes that catalyze essentially every step of the tricarboxylic acid (TCA) cycle were found at increased levels after exposure to ciprofloxacin (Table 2). Only the step catalyzed by malate dehydrogenase did not appear to be up-regulated at both time points, but transcription of one of the malate dehydrogenase isoforms (encoded by *ldh-2*) was elevated at 30 min. In addition, transcripts of genes that encode components of the pyruvate dehydrogenase complex (SACOL1448 and SACOL1449), which converts pyruvate to succinyl-coenzyme A (succinyl-CoA) and funnels it into the TCA cycle, were found at ~3-fold elevated levels.

Ciprofloxacin also affected the expression of a variety of genes involved in DNA metabolism and repair (Table 2). Interestingly, while genes involved in the synthesis of purines and pyrimidines were down-regulated, ribonucleotide reductase was up-regulated, as were the genes encoding the β-subunit of the replicative polymerase (Pol III) and the inducible Y-family polymerases SACOL1955 and SACOL1400. SACOL1955 was up-regulated 2-fold, but only at 120 min, while SACOL1400 was up-regulated 8- and 11-fold at 30 and 120 min, respectively. Several other DNA metabolism genes were significantly up-regulated in response to ciprofloxacin, including *lexA* and *recA* as well as *uvrA*, *uvrB*, *parE*, *parC*, *recF*, *gyrA*, and *gyrB*.

Interestingly, a variety of DNA repair genes that are strongly damage inducible in other organisms did not exhibit a change in expression in *S. aureus* in response to ciprofloxacin, and in some cases, they were actually down-regulated (Table 2). For example, transcript levels of *recX* (which encodes a modulator of RecA function) and SACOL0751 (which encodes a putative photolyase) were both essentially unchanged, while *recN*, *xerC*, *xerD*, and *ruvB*, which encode proteins involved in DNA repair and recombination, were all down-regulated by ciprofloxacin. In addition, the mismatch repair genes *hexA* (encoding a MutS homolog) and *hexB* (encoding a MutL homolog) were both slightly down-regulated at 120 min. Whether the modest decrease in the transcription of *hexA* and *hexB* has biological significance is unknown.

***S. aureus* SOS response.** To characterize the contribution of the SOS response to DNA damage repair, we constructed a *lexA*(*S130A*) mutant of strain 8325. The S130A mutant cannot undergo autoproteolysis due to mutation of the mechanistically essential (6, 27, 29) catalytic serine to alanine and is thus unable to derepress the SOS genes. The *lexA*(*S130A*) strain was more sensitive to both UV light and the alkylating agent methyl methanesulfonate (MMS) (Fig. 1A and B) but not to ciprofloxacin (Fig. 2).

We next compared the ciprofloxacin-induced transcriptional response of the *lexA*(*S130A*) mutant strain to that of the wild-type strain. Eighteen genes were identified that were induced by ciprofloxacin in the wild-type strain but not in the mutant strain (Table 3). To address whether derepression of these genes is controlled directly or indirectly by LexA, we searched for potential LexA binding sites. Ten potential sites were identified that appear to control 16 of the 18 identified genes (Table 3). The consensus sequence of these 10 sites, CGAAC-AAAT-GTTCG, is essentially identical to that of *B. subtilis*, CGAAC-RNRY-GTTYC (20). The presence of an upstream consensus binding site suggests that the genes are directly regulated by LexA. The two genes lacking a LexA binding site are likely regulated indirectly.

To identify additional LexA-regulated genes, we performed a genome-wide sequence search of *S. aureus* strain N315 (http://genolist.pasteur.fr). We searched for potential SOS boxes within 200 nucleotides upstream of each ORF, using the motif CGAAC-N_4-GTTCG and allowing for one mismatch. This analysis identified two potential LexA binding sites in addition to the 16 sites identified experimentally. One site is 121 nucleotides upstream of the nearest gene (SACOL0790), which is significantly further than any gene identified empirically in our study, and the corresponding transcript was found at equally decreased levels in both strains after exposure to ciprofloxacin, implying that it is not LexA regulated. The second potential LexA binding site is 76 nucleotides from the start codon of SACOL0901, which is a pathogenicity island gene. However, transcripts of this gene were consistently below the detection limit of the microarray analysis. It is possible that LexA regulates SACOL0901 under conditions where the promoter is more highly transcribed but not in response to ciprofloxacin.

As expected, the positive and negative regulators of the SOS response, i.e., *recA* and *lexA*, respectively, are included among the identified LexA-regulated genes. In addition, several genes that encode proteins involved in DNA metabolism are also part of the LexA regulon, including *uvrA*, *uvrB*, *parC*, *parE*, SACOL1381, and SACOL1382. *uvrA* and *uvrB* encode two subunits of the nucleotide excision repair endonuclease UvrABC, and they appear to be carried on a single transcript that is regulated by the *uvrB* promoter and its LexA binding sequence. Likewise, *parC* and *parE*, which encode the two subunits of topoisomerase IV, a primary target of ciprofloxacin, are also carried on a single transcript, which is regulated

TABLE 2. Transcriptional response of *S. aureus* 8325 to 0.8 μg/ml ciprofloxacin

ORF and functional category	Gene	Annotation	Fold change	
			30 min	120 min
General metabolism				
Down-regulated ORFs				
SACOL2094	*atpC*	ATP synthase F1, epsilon subunit	1.3	−1.6
SACOL2095	*atpD*	ATP synthase F1, beta subunit	1.1	−1.4
SACOL2096	*atpG*	ATP synthase F1, gamma subunit	1.3	−1.9
SACOL2097	*atpA*	ATP synthase F1, alpha subunit	1.1	−1.6
SACOL2098	*atpH*	ATP synthase F1, delta subunit	1.1	−2.0
SACOL2099	*atpF*	ATP synthase F0, B subunit	1.2	−1.6
SACOL2100	*atpE*	ATP synthase F0, C subunit	1.2	−1.9
SACOL2101	*atpB*	ATP synthase F0, A subunit	1.4	−1.6
SACOL0494	*nuoF*	NADH dehydrogenase subunit L	1.1	−2.2
SACOL0975		Coenzyme A disulfide reductase	−1.2	−2.5
SACOL2392	*narI*	Nitrate reductase gamma chain	−2.8	−5.7
SACOL2393	*narJ*	Similar to nitrate reductase delta chain	−2.6	−9.7
SACOL2394	*narH*	Nitrate reductase beta chain	−2.1	−7.7
SACOL2395	*narG*	Respiratory nitrate reductase, alpha subunit	1.0	−4.9
Up-regulated ORFs				
SA1245	*sucA*	2-Oxoglutarate dehydrogenase, E1 component	1.7	2.5
SACOL1158	*sdhC*	Succinate dehydrogenase cytochrome b_{558}	2.0	2.1
SACOL1159	*sdhA*	Succinate dehydrogenase	1.8	2.1
SACOL1160	*sdhB*		1.6	2.5
SACOL1262	*sucC*	Succinyl-CoA synthetase beta subunit	2.1	1.7
SACOL1263	*sucD*	Succinyl-CoA synthetase alpha subunit	1.8	3.0
SACOL1385	*acnA*	Aconitate hydratase	1.8	2.7
SACOL1448	*sucB*	Dihydrolipoamide acetyltransferase	1.6	3.3
SACOL1449	*sucA*		1.6	2.5
SACOL1741	*icd*	Isocitrate dehydrogenase	1.3	2.1
SACOL1908	*fumC*		1.7	2.1
SACOL2424	*bioW*	6-Carboxyhexanoate-CoA ligase	8.4	7.8
SACOL2426	*bioB*	Biotin synthase	8.8	6.9
SACOL2427	*bioA*	Adenosylmethionine-8-amino-7-oxononanoate aminotransferase	7.8	10.2
SACOL2428	*bioD*	Dethiobiotin synthase	8.5	7.4
DNA metabolism				
Down-regulated ORFs				
SACOL1267	*topA*	Topoisomerase I	−1.6	−2.3
SACOL1269	*xerC*	XerC site-specific recombinase	−1.7	−2.0
SACOL1315	*hexA*		−1.1	−1.9
SACOL1316	*hexB*		1.1	−1.7
SACOL1523	*recQ2*		−1.1	−1.5
SACOL1540	*xerD*		—[a]	−2.1
SACOL1564	*recN*		1.1	−2.1
SACOL1619	*dnaG*	Primase	−1.3	−1.5
SACOL1696	*ruvB*		−1.1	−1.5
SACOL1867			1.0	−4.1
ORFs with no change				
SACOL1931	*recX*	RecX regulatory protein	−1.2	−1.4
SACOL0751		Putative photolyase	1.1	−1.1
Up-regulated ORFs				
SACOL0001	*dnaA*	Chromosomal replication initiation protein	−1.1	2.7
SACOL0002	*dnaN*	DNA polymerase III subunit beta	−1.0	2.6
SACOL0004	*recF*	Recombination protein RecF	1.2	2.8
SACOL0005	*gyrB*	DNA gyrase subunit B	1.2	2.9
SACOL0006	*gyrA*	DNA gyrase subunit A	−1.0	2.4
SACOL0823	*uvrB*	Exinuclease ABC subunit B	2.8	2.5
SACOL0824	*uvrA*	Exinuclease ABC subunit A	2.7	2.9
SACOL1304	*recA*	Recombinase A	4.9	4.7
SACOL1374	*lexA*	LexA repressor	4.1	3.8
SACOL1381	*sbcD*	SbcD nuclease	4.2	3.4
SACOL1400		UmuC family polymerase	7.9	10.7
SACOL1955	*dinB*	DNA polymerase IV	−1.1	2.2
SACOL2089		Similar to single-stranded DNA binding protein	1.6	2.5
Nucleotide metabolism				
Down-regulated ORFs				
SACOL0018	*purA*	Adenylosuccinate synthase	−3.8	−3.8

Continued on facing page

TABLE 2—*Continued*

ORF and functional category	Gene	Annotation	Fold change	
			30 min	120 min
SACOL0458	*xpt*	Xanthine phosphoribosyltransferase	1.0	−5.3
SACOL0460	*guaB*	Inositol-monophosphate dehydrogenase	1.1	−2.1
SACOL0554	*hpt*	Hypoxanthine-guanine phosphoribosyltransferase homolog	−1.4	−2.9
SACOL1073	*purE*	Putative phosphoribosylaminoimidazole carboxylase	−7.1	−8.7
SACOL1074	*purK*	Phosphoribosylaminoimidazole carboxylase carbon dioxide fixation chain PurK homolog	−6.8	−12.7
SACOL1075	*purC*	Phosphoribosylaminoimidazole-succinocarboxamide synthase	−6.4	−11.5
SACOL1076	*purS*	Hypothetical protein	−6.4	−11.0
SACOL1077	*purQ*	Phosphoribosylformylglycinamidine synthase I	−5.2	−11.4
SACOL1078	*purL*	Phosphoribosylformylglycinamidine synthetase	−4.4	−7.1
SACOL1079	*purF*	Phosphoribosylpyrophosphate amidotransferase	−3.3	−7.3
SACOL1080	*purM*	Phosphoribosylaminoimidazole synthetase	−1.9	−5.5
SACOL1081	*purN*	Phosphoribosylglycinamide formyltransferase	−1.1	−7.2
SACOL1082	*purH*	Bifunctional purine biosynthesis protein	1.2	−3.8
SACOL1083	*purD*	Phosphoribosylamine-glycine ligase PurD	1.2	−3.1
SACOL1210	*pyrR*	Pyrimidine regulatory protein PyrR	−5.8	−4.3
SACOL1212	*pyrB*	Aspartate carbamoyltransferase catalytic subunit	−9.3	−3.8
SACOL1214	*carA*	Carbamoyl-phosphate synthase small subunit	−9.6	−2.4
SACOL1215	*carB*	Carbamoyl-phosphate synthase large subunit	−5.3	1.1
SACOL1216	*pyrF*	Orotidine-5-phosphate decarboxylase	−3.5	1.3
SACOL1371	*guaC*	GMP oxidoreductase	−2.5	−4.1
SACOL1969	*purB*	Adenylosuccinate lyase	−1.6	−2.0
SACOL2130	*deoD2*	Purine nucleoside phosphorylase	−1.4	−2.6
SACOL2606	*pyrD*	Dihydroorotate dehydrogenase	−1.7	−3.0
SACOL2635	*nrdD*	Anaerobic ribonucleoside triphosphate reductase	−1.2	−2.0
Up-regulated ORFs				
SACOL0790.1		Putative ribonucleoside reductase 2	1.5	3.1
SACOL0791	*nrdI*		1.8	3.4
SACOL0792		Ribonucleotide reductase alpha subunit	2.0	2.7
SACOL0793	*nrdF*	Ribonucleotide reductase beta subunit	1.5	2.3
Lipid biosynthesis				
Down-regulated ORFs				
SACOL0987	*fabH*	3-Oxoacyl-(acyl carrier protein) synthase	−1.3	−4.3
SACOL0988	*fabF*	3-Oxoacyl synthase	−1.2	−3.3
SACOL1243	*plsX*	Fatty acid/phospholipid synthesis protein	−1.1	−3.8
SACOL1244	*fabD*	Malonyl CoA-acyl carrier protein transacylase	−1.1	−3.7
SACOL1245	*fabG1*	3-oxoacyl-(acyl carrier protein) reductase	−1.1	−2.3
SACOL1571	*accC*	Acetyl-CoA carboxylase	−1.1	−2.1
SACOL1572	*accB*	Acetyl-CoA carboxylase biotin carboxyl carrier subunit	−1.3	−2.3
SACOL2079	*cls-2*	Hypothetical protein	−1.4	−2.2
Toxin production/resistance and pathogenesis				
Down-regulated ORFs				
SACOL1173	*hlY*	Alpha-hemolysin precursor	1.4	−4.9
SACOL1812	*rot*	Repressor of toxins Rot	−1.8	−4.1
SACOL0766	*saeR*	Response regulator	1.4	−3.4
SACOL2287	*sarR*	Staphylococcal accessory regulator A homolog	−1.3	−3.1
SACOL0765	*saeS*	Histidine protein kinase	—[a]	−3.0
SACOL1060		ATL autolysin transcription regulator	−1.6	−2.4
SACOL2023	*agrB*	Accessory gene regulator protein B	−1.6	−2.3
SACOL0672	*sarA*	Staphylococcal accessory regulator A	−2.1	−1.5
SACOL0754	*norA*	Quinolone resistance protein	−1.3	−2.0
SACOL0472		Exotoxin, putative	−3.2	1.1
MW0387	*set21*	Exotoxin 2; genomic island Nu Sa Alpha2	−2.3	1.1
MW0385	*set19*	Exotoxin 2; genomic island Nu Sa Alpha2	−3.3	1.2
SACOL0470		Exotoxin, putative	−2.6	1.2
Up-regulated ORFs				
SACOL0096	*sarS*	Staphylococcal accessory regulator A homolog	−1.3	2.6
SACOL1184		Exfoliative toxin A	3.0	—[a]
Phage related				
Up-regulated ORFs				
SAV0910		Tail fiber	−1.2	16.2
SACOL0336		Phi PVL ORF 39-like protein	—[a]	15.4

Continued on following page

TABLE 2—*Continued*

ORF and functional category	Gene	Annotation	Fold change	
			30 min	120 min
SACOL0335		Hypothetical protein	3.2	14.4
MW1926		Phi PVL ORF 39-like protein	2.9	14.1
SAV1991		Hypothetical protein	2.9	13.4
MW1912		Phi PVL ORF 61-like protein	2.1	12.6
SACOL0349		Conserved hypothetical protein	5.3	11.9
MW1896		Hypothetical protein	—[a]	11.8
SAV1996		Phi PVL ORF 32-like protein	3.1	11.6
MW1932		Antirepressor	3.4	11.5
SAV0911		Phi ETA ORF 63-like protein	−1.2	11.2
SAV0886		Large terminase	2.5	11.1
SACOL0357	*dut*		2.5	10.4
SACOL0358		Hypothetical protein	2.7	10.4
SACOL0348		Conserved hypothetical protein	3.5	10.0
MW1899		Conserved hypothetical protein	—[a]	10.0

[a] —, microarray signal was below the detection limit.

by the *parE* promoter and its LexA binding sequence. SACOL1381 and SACOL1382 are LexA regulated and encode the SbcCD endonuclease, which is involved in processing stalled replication forks. These two genes appear to be carried by a single transcript regulated by the SACOL1381 promoter.

The polycistronic operon SACOL2162 to SACOL2160 is also LexA regulated and encodes a protein of unknown function, a putative hemolysin, and a protein involved in cell envelope biosynthesis. Four hypothetical genes, namely, SACOL0436, SACOL1375, SACOL1986, and SACOL1999, also appear to be LexA regulated.

Lastly, SACOL1400, which encodes one of the two *S. aureus* Y-family polymerases, is also LexA regulated. As detected in both the microarray and RT-PCR experiments, this gene was strongly up-regulated by ciprofloxacin in a LexA cleavage-dependent manner. SACOL1400 appears to be very tightly regulated by LexA, as transcription of this gene was barely detectable in wild-type cells prior to ciprofloxacin addition and in *lexA*(*S130A*) mutant cells prior to and following ciprofloxacin addition in our microarray (Table 2) and RT-PCR (data not shown) experiments.

Contribution of *S. aureus* Y-family polymerases to growth, DNA damage repair, and induced mutation. Y-family polymerases have been shown to be important for the induction of mutation and the evolution of ciprofloxacin resistance in other bacteria, such as *E. coli* (5, 7). To examine the functions of the

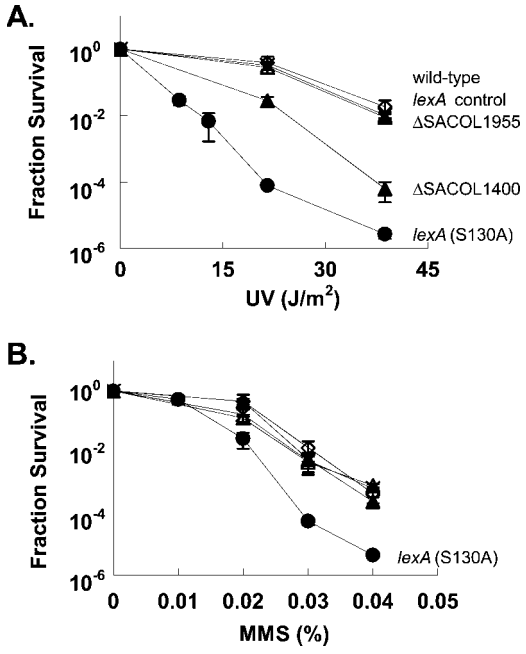

FIG. 1. UV- and MMS-mediated killing of *S. aureus* strains. Fractions of survival after irradiation with UV light (A) or treatment with MMS (B) are shown for wild-type (△), Spec^r *lexA* control (×), *lexA*(*S130A*) mutant (●), ΔSACOL1955 (◇), and ΔSACOL1400 (▲) strains.

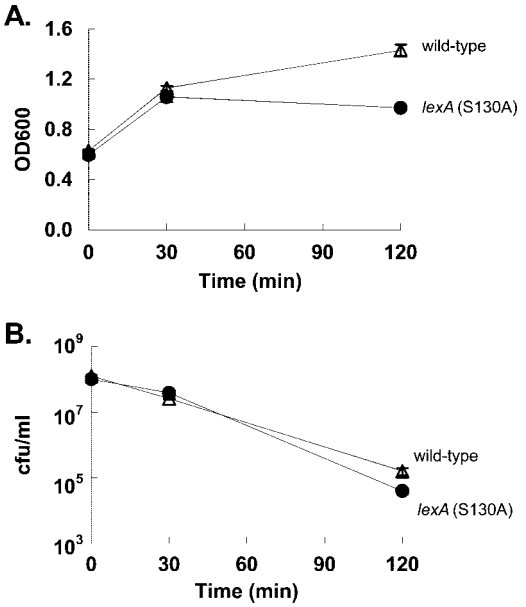

FIG. 2. Ciprofloxacin-mediated killing of *S. aureus* strains. Killing kinetics are shown for wild-type (△) and *lexA*(*S130A*) (●) strains, monitored by optical density (A) and CFU/ml (B) during collection of microarray samples.

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

ORF	Gene	LexA box sequence or description	Distance (bp) to start codon	No. of bp mismatches from consensus	Fold difference[a]	
					30 min	120 min
Consensus		**CGAAC** AAAT GTTCG[b]				
SACOL0436		CGAAC GCAT GTTCT	−25	3	4.9	2.5
SACOL0823	*uvrB*	CGAAC AAAC GTTTG	−92	2	4.0	2.7
SACOL0824	*uvrA*	In operon with *uvrB*			4.2	3.0
SACOL1304	*recA*	CGAAC AAAT ATTCG	−86	1	4.7	5.8
SACOL1374	*lexA*	CGAAC AAAT GTTTG	−57	1	3.4	5.4
SACOL1375		Divergent from *lexA*	−71	2	17.7	7.9
SACOL1381	*sbcD*	CGAAC AAAT GTTCT	−15	1	3.6	2.8
SACOL1382	*sbcC*	In operon with *sbcD*			1.4	2.1
SACOL1389	*parE*	CGAAC GTAC GTTTG	−16	4	2.1	2.0
SACOL1390	*parC*	In operon with *parE*			2.5	2.3
SACOL1400		CGAAC ACGT GTTCT[c]	−35	3	24.8	9.6
SACOL1986		CGAAC ATGT GTTCT[c]	−35	3	2.1	16.7
SACOL1999		CGAAC ATAT GTTCT	−69	2	4.9	5.9
SACOL2160		In operon with SACOL2162			2.8	3.0
SACOL2161		In operon with SACOL2162			2.0	2.3
SACOL2162		CGAAC ATAT TTTCG[c]	−50	2	2.2	2.7

[a] Fold difference in expression between wild-type and *lexA*(*S130A*) mutant strains.
[b] Residues in bold are 100% conserved.
[c] The LexA box shown is the reverse complement to orient CGAAC at the 5′ end.

two *S. aureus* Y-family polymerase genes, SACOL1400 and SACOL1955, deletion strains lacking these genes were constructed. While deletion of either polymerase gene did not significantly affect growth (not shown), deletion of SACOL1400 conferred sensitivity to UV light (Fig. 1A). Neither polymerase deletion strain showed increased sensitivity to MMS or ciprofloxacin (Fig. 1B).

We next examined whether these polymerases contribute to damage-induced mutability. Exposing *S. aureus* to 8.6 J m^{-2} of UV light caused minimal killing but resulted in the induction of mutations that conferred both streptomycin and rifampin resistance in the wild-type strain and a strain lacking SACOL1955 (Table 4). However, in the strain lacking SACOL1400, the mutation frequency, as measured by streptomycin resistance, was reduced >30-fold, and no detectable induction of mutation to rifampin resistance was observed. In the *lexA*(*S130A*) strain, which cannot derepress SACOL1400, no detectable induction of mutation was observed (Table 4), consistent with the requirement of LexA cleavage for the induction of SACOL1400.

TABLE 4. Survival and mutation after treatment with 8.6 J/m^2 UV light

Strain	Mean survival fraction ± SD	No. of colonies (10^8)/viable cell (mean ± SD)	
		Strep^r	Rif^r
Wild type	0.79 ± 0.17	11.7 ± 3.1	29.6 ± 9.3
lexA(*S130A*) mutant	0.67 ± 0.28	—[a]	—[a]
ΔSACOL1955	0.72 ± 0.16	11.4 ± 4.3	33.4 ± 12.6
ΔSACOL1400	0.83 ± 0.20	0.38 ± 1.3	—[a]

[a] —, no measurable induced mutation.

DISCUSSION

Fluoroquinolones have emerged as important antibiotics for the treatment of *S. aureus* infections; however, *S. aureus* remains difficult to treat due to its adaptability and the facility with which it acquires resistance-conferring mutations. To further understand the global response of *S. aureus* to ciprofloxacin, the prototypical fluoroquinolone, as well as the contribution of the SOS response, we analyzed the transcriptional changes induced by ciprofloxacin in both the wild-type *S. aureus* strain 8325 and a *lexA*(*S130A*) mutant. In wild-type cells, ciprofloxacin induced twofold or greater changes in the transcription of 235 genes after 30 min of exposure and of 510 genes after 120 min of exposure. Approximately equal numbers of genes were found at higher and lower levels. The most general response of *S. aureus* 8325 to ciprofloxacin is a significant decrease in the transcript levels of genes corresponding to various facets of metabolism, including amino acid, protein, cofactor, nucleotide, fatty acid, and cell wall biosynthesis as well as energy metabolism and electron transport. However, genes involved in glycolysis/glucogenesis, fermentation, and carbohydrate uptake were both induced and repressed by ciprofloxacin.

Interestingly, ciprofloxacin induced the expression of a number of genes that encode proteins in or related to the TCA cycle. While this has not been observed previously with an antibiotic, TCA cycle gene induction has been reported in response to neutrophil phagocytosis (44) and during entry into stationary-phase growth (25). The up-regulation of carbon flux into and through the TCA cycle is particularly interesting because TCA cycle activity has been shown to be associated with virulence, survival, and persistence of *S. aureus* and several other pathogens (3, 10, 17, 28, 30, 39, 40). In total, the data suggest that ciprofloxacin induces significant changes in carbohydrate uptake and metabolism, with an increased reliance on the TCA cycle for metabolism and/or energy production.

As expected, the resident prophages of *S. aureus* strain 8325 are strongly induced by ciprofloxacin. Overall, 73 prophage genes are up-regulated >2-fold at 120 min, and 50 of these are up-regulated >5-fold (Table 2). An important consequence of inducing prophage genes is the up-regulation of many viru-

lence factors encoded within these elements (29). In contrast, many of the native virulence factors of strain 8325 are down-regulated in response to ciprofloxacin. This includes several key transcriptional regulators, such as *sarA*, *sarR*, *rot*, and *agrB*, which participate in the regulation of numerous virulence factors. The opposing responses of chromosomally and phage-encoded virulence factors may reflect the differing survival strategies of the bacterial host and pathogen and also emphasize the influential role that phages play in controlling the host response to environmental stress (45).

While treatment with ciprofloxacin causes decreases in the transcription of the operons that control purine and pyrimidine biosynthesis, it also induces the transcription of ribonucleotide reductase as well as Pol III and the Y-family polymerases SACOL1955 and SACOL1400. These results suggest that the cell responds to ciprofloxacin by reducing metabolism and funneling resources into DNA synthesis. The strong induction of SACOL1400, in particular, suggests that DNA synthesis might be error-prone, which is further supported by the observed down-regulation of mismatch repair, which has been associated with the accelerated accumulation of mutations (21, 33). In addition, several other DNA repair systems, including *recN* and *ruvB*, which encode proteins involved in recombinational repair of damaged DNA, are down-regulated.

Comparison of the transcriptional responses of the wild-type and *lexA*(*S130A*) mutant strains identified 16 SOS genes. As expected, the SOS regulon includes *recA* and *lexA*, the positive and negative regulators of the response, respectively. Nucleotide excision repair (NER) genes *uvrA* and *uvrB* are LexA regulated in *S. aureus*, but *uvrD* and *uvrC* are not. This regulation of NER genes is different from that in both *E. coli* and *B. subtilis*, where LexA regulates three or all four genes, respectively, and also different from the case for *P. aeruginosa*, which does not regulate any of the NER genes. The only LexA-regulated genes in *S. aureus* that encode proteins with obvious recombinational repair function are SACOL1381 and SACOL1382, which encode homologs of *E. coli* SbcD and SbcC, respectively. The organization of this operon is identical to that of *E. coli*, *B. subtilis*, and *P. aeruginosa*, but it is not LexA regulated in any of these organisms (6, 9, 19). Finally, *parE* and *parC*, which encode the subunits of topoisomerase IV, are weakly repressed by LexA in *S. aureus*, similar to what is observed in *B. subtilis* (19); however, these genes are not LexA regulated in *E. coli* or *P. aeruginosa* (6, 9). Notably, several genes that encode important DNA repair proteins that are commonly LexA regulated in other organisms are not part of the *S. aureus* SOS response, including *ruvA*, *ruvB*, *recN*, and *recX*.

Induced mutation is a central component of all SOS systems characterized to date. In *E. coli*, induced mutation 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 (15). Pol IV and Pol V are Y-family polymerases, which have been shown to synthesize DNA with reduced fidelity (18, 32). In addition, LexA-repressed Y-family polymerases or DnaE2 homologs appear to be required for induced mutation in *B. subtilis* (12, 42), *Caulobacter crescentus* (16), and *Mycobacterium tuberculosis* (4). Our results show that the *S. aureus* genome encodes a single LexA-regulated polymerase, the SACOL1400 protein, and that its deletion or prevention of its derepression by mutation of LexA renders *S. aureus* virtually immutable in response to UV irradiation. In addition, this protein (and not that encoded by SACOL1955) is predicted to have the QLXIF motif required for β-clamp binding, suggesting that its regulation during induced mutation may be similar to that for Pol IV and Pol V in *E. coli* (24).

In total, ciprofloxacin appears to induce a down-regulation of metabolism in *S. aureus*, but with a concomitant increase in TCA cycle activity and in error-prone DNA replication. A general down-regulation of metabolism was also observed for *P. aeruginosa* in response to ciprofloxacin (6) and may represent a common response to this antibiotic. However, induction of the TCA cycle appears to be unique to *S. aureus*. Interestingly, increased utilization of the TCA cycle in this pathogen has been associated with virulence (3, 10, 17, 28, 30, 39, 40). Thus, induction of the TCA cycle, like phage mobilization and the associated lateral transfer of virulence and antibiotic resistance determinants, may be an unintended consequence of ciprofloxacin use that complicates therapy. Ciprofloxacin also induces the SOS response. However, the DNA repair functions orchestrated by the SOS response are very different in *S. aureus* from those in previously characterized bacteria. Interestingly, what is conserved in the different SOS regulons is *recA*, *lexA*, and at least one error-prone polymerase. This suggests that induced mutation is an ancient and central function of the SOS response. Although the idea remains controversial (36), induced mutation may have been selected to facilitate evolution at times of environmental stress, as suggested by others (13, 22, 35). Further understanding of how the TCA cycle and induced mutation contribute to the pathogenicity of *S. aureus* and how these processes are affected by antibiotic therapy should help in the development of improved therapeutic strategies.

ACKNOWLEDGMENTS

This research was supported by the Office of Naval Research (award no. N00014-03-1-0126 to F.E.R.) and the National Institute of Allergy and Infectious Diseases (award N01-AI15447 to S.N.P.).

We thank J. Penadés for sending vector pMAD and C. Lee for technical advice. We thank the Pathogen Functional Genomics Resource Center at The Institute for Genomic Research for DNA microarrays.

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