Characterization of the Global Transcriptional Responses to Different Types of DNA Damage and Disruption of Replication in Bacillus subtilis‡

Alexi I. Goranov, Elke Kuester-Schoeck,† Jue D. Wang, and Alan D. Grossman*

Department of Biology, Building 68-530, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received 9 March 2006/Accepted 31 March 2006

DNA damage and perturbations in DNA replication can induce global transcriptional responses that can help organisms repair the damage and survive. RecA is known to mediate transcriptional responses to DNA damage in several bacterial species by inactivating the repressor LexA and phage repressors. To gain insight into how Bacillus subtilis responds to various types of DNA damage, we measured the effects of DNA damage and perturbations in replication on mRNA levels by using DNA microarrays. We perturbed replication either directly with \( p \)-hydroxyphenylazo-uracil (HPUra), an inhibitor of DNA polymerase, or indirectly with the DNA-damaging reagents mitomycin C (MMC) and UV irradiation. Our results indicate that the transcriptional responses to HPUra, MMC, and UV are only partially overlapping. recA is the major transcriptional regulator under all of the tested conditions, and LexA appears to directly repress the expression of 63 genes in 26 operons, including the 18 operons previously identified as LexA targets. MMC and HPUra treatments caused induction of an integrative and conjugative element (ICEBs1) and resident prophages (PBSX and SPb), which affected the expression of many host genes. Consistent with previous results, the induction of these mobile elements required recA. Induction of the phage appeared to require inactivation of LexA. Unrepaired UV damage and treatment with MMC also affected the expression of some of the genes that are controlled by DnaA. Furthermore, MMC treatment caused an increase in origin-proximal gene dosage. Our results indicate that different types of DNA damage have different effects on replication and on the global transcriptional profile.

Proper maintenance of genomic content is a major task for all organisms. A variety of cellular processes are devoted to faithfully replicating and segregating completed genomes prior to cell division. Cells have also evolved several mechanisms for increasing the chances of survival by monitoring and responding to the status of genomic integrity. When replication does not proceed normally, these mechanisms are triggered (22, 23, 71). The role of these mechanisms is to repair the DNA replication defect and to delay subsequent cell cycle events, such as cell division, thus increasing the chances of cell survival (7, 22, 23, 27, 43, 71). The effects of these surveillance mechanisms and subsequent responses are mediated in large part by altering the transcription of many genes (7, 20, 23, 27).

One of the most well-characterized mechanisms that detects DNA damage and replication arrest is the bacterial SOS response (22, 69). There are two regulatory components to this response: RecA and LexA. RecA is a highly conserved protein, with homologs present in eukaryotes (e.g., scRad51 and scDmc1) (42, 43). In the cell, there are usually limited amounts of single-stranded DNA (ssDNA) at the replication forks, but when DNA damage occurs, the amount of ssDNA is thought to increase in both bacteria and eukaryotes (8, 22). RecA binds to ssDNA exposed during DNA damage and catalyzes recombination processes inside the cell (22, 32, 43). RecA has also been shown to mediate replication fork reversal (a mechanism used to repair a stalled replication fork on the leading strand) and to alter the accessibility of the 3′ ends of DNA to DNA polymerase (43).

When RecA is bound to ssDNA, it also stimulates the autocleavage of LexA (38, 41, 45, 59), also called DinR in Bacillus subtilis (56, 67, 68). LexA is a transcriptional repressor that binds within the promoter region of target genes as a dimer and prevents transcription from these promoters (1, 24, 45). Autocleavage of LexA relieves repression, thereby allowing transcription of LexA-repressed genes (1, 45). Subsequent degradation of the cleaved LexA is important, at least in Escherichia coli, for the proper induction of LexA repressed genes (48).

The composition of the LexA regulon has been the subject of many studies in different bacteria. Analysis of the global transcriptional response to DNA damage in E. coli established that treatment with mitomycin C (MMC) causes a large transcriptional effect (approximately 1,000 genes) but that only approximately 50 genes are likely to be regulated directly by LexA (28). The regulation of the remaining genes was speculated to involve other stress-response transcriptional regulators. Treatment of E. coli with UV light (UV) showed a similar number of LexA-regulated genes, but the overall response was smaller than that caused by MMC (14, 52). These studies concluded that RecA and LexA regulate 50 genes (14). RecA
has also been demonstrated to regulate the activation of mobile genetic elements in *E. coli*, most notably the lysogenic phage lambda (57). Similar experiments with MMC treatment in *Mycobacterium tuberculosis* also revealed that approximately 50 genes are regulated to some extent in a RecA-dependent manner following DNA damage (54).

The LexA regulon of *Bacillus subtilis* has also been investigated (1, 9, 39). Genes known to be repressed by LexA in *B. subtilis* are involved in transcriptional regulation (lecA and recA), DNA repair (uvrAB and uvrC), recombination (recA and uvrAB), and cell division (yneA) (1, 9, 27, 39).

A total of 18 operons (approximately 30 genes) were identified as likely direct targets of LexA by using a computational approach combined with in vitro DNA binding experiments and in vivo analysis of recA-dependent genes induced by UV irradiation (1). In addition to the genes repressed by LexA, genes in several lysogenic phages (PBSX, SPβ, and phi-105) and the integrative and conjugal element ICEBs1 are also known to be induced by DNA damage in *B. subtilis* (2, 50, 61, 66).

We examined the global transcriptional response to different types of DNA damage and replication arrest in *B. subtilis* by using whole-genome DNA microarrays. We also tested the roles of recA and lexA in regulating the transcriptional response to various perturbations in DNA replication. Replication was inhibited directly by inhibiting the PolC (catalytic) subunit of DNA polymerase with p-hydroxyphenylazo-uracil (HPUra) (6, 23). We also perturbed replication indirectly by treating cells with two different agents, MMC and UV irradiation, that cause DNA damage. MMC causes the formation of intra- and interstrand DNA cross-links and MMC monoadducts (17). UV irradiation causes the formation of pyrimidine dimers (22). As expected, our results show that RecA plays a major part in regulating the transcriptional response under all of the conditions tested. In addition to the approximately 30 genes in 18 operons previously identified as direct targets of LexA (1), we identified another 31 genes in 8 operons that are also likely to be direct targets of LexA. This brings the total number of operons likely to be regulated directly by LexA to 26 (approximately 63 genes).

In addition to bacterial genes, we found that many genes from lysogenic bacteriophage and the mobile genetic element ICEBs1 required recA but not lexA for induction, as expected because RecA facilitates cleavage of many phage repressor proteins (57). However, the induction of many of these genes was blocked in a mutant defective in the cleavage of LexA, indicating that LexA plays a role in the regulation of these mobile elements. The induced phage genes and their indirect effects comprised more than half of the transcriptional response to replication arrest and MMC treatment.

In addition to the recA-dependent transcriptional response, replication arrest also induces a recA-independent response, which is mediated in part by the replication protein DnaA (23). Our results indicate that replication fork arrest and DNA damage caused by MMC, UV irradiation, or HPUra all induce a recA-dependent SOS response but that the recA-independent response to these perturbations was qualitatively and quantitatively different. MMC also caused a relative increase of the dosage of the genes near the chromosomal origin of replication. This increase in gene dosage was most likely caused by a reduced rate of elongation of replication. Our results demonstrate that cells respond differently to various types of perturbations to DNA replication. Those differences probably allow cells to respond to each challenge more specifically and efficiently.

### Materials and Methods

#### General methods and strain construction. *B. subtilis* strains are listed in Table 1. Genetic manipulations were performed by using standard protocols (25).

#### Media and growth conditions. For all experiments, cells were grown with vigorous shaking at 30°C or 37°C in S7 defined minimal medium with morpholinepropanesulfonic acid buffer at a concentration of 50 mM rather than 100 mM (26); the medium was supplemented with 0.1% glutamate, the required amino acids (at 40 μg/ml), and 1% glucose. HPUra (a generous gift from Neal Brown) and MMC (Sigma) were used at final concentrations of 38 and 1 μg/ml, respectively. For treatment with UV, cultures were transferred to a shallow dish and irradiated with 25 mJ/m² (~50% killing) as previously described (1).

#### Use of DNA microarrays for gene expression profiling. DNA microarrays were prepared either using PCR products from >99% of the annotated *B. subtilis* open reading frames (ORFs) spotted onto Corning GAPS slides or a 65-mer oligonucleotide library representing all of the annotated ORFs of the *B. subtilis* genome (Sigma-Genosys) essentially as described previously (1, 2, 5, 23). Oligonucleotide microarrays were used only for the lexA::ind set of experiments [lexA::ind], and respective isogenic lexA− control strain JJS39. The observations from the oligonucleotide microarrays with respect to JJS39 were verified on PCR arrays to demonstrate compatibility between oligonucleotide and PCR microarray results.

Exponentially growing cultures were treated with HPUra, MMC, or UV, and samples were collected immediately before and 15, 30, and 60 min after treatment and processed as previously described to generate labeled cDNA (1, 23). A similarly processed reference sample was hybridized with each experimental sample for normalization. The reference sample contained pooled total RNA from cell cultures grown in defined minimal medium and cultures treated with DNA damaging agents, thus ensuring that all genes expressed under these conditions are represented in the sample. Our microarray analysis includes every spot that has ≥80% of the pixels at least one standard deviation over background in one or both Cy3 or Cy5 channels. Since virtually all phage genes are represented in the reference sample (Cy3 channel), we obtained data for most of these genes even when we compared test samples of strains missing these genes (phage defective strains for example).

All microarray experiments were done with at least three independent replicates and evaluated by using significance analysis of microarrays (65). For all experiments, we picked the most stringent criteria that resulted in a predicted number of false-positive results (false positives) of one or less. Because the number of statistically significant genes varies from experiment to experiment but the predicted number of false positives was always 1 or less, the calculated rate of identification of false positives varied. For most experiments there was less than 1% probability of identifying random effects (false-positive discovery rate of <1.0%). However, due to the limited number of affected genes in phage-cured strain backgrounds, the false-positive discovery rate increased to 2.9% in the MMC or UV treatment experiments. For all experiments we also

### Table 1. *B. subtilis* strains used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>JH642</td>
<td>trpC2 pheA1</td>
<td>51</td>
</tr>
<tr>
<td>IRN444</td>
<td>trpC2 pheA1 recA260::mls::cat</td>
<td>37</td>
</tr>
<tr>
<td>AIG4</td>
<td>trpC2 pheA1 recA::neo Δspa</td>
<td>23</td>
</tr>
<tr>
<td>AIG105</td>
<td>trpC2 pheA1 ΔneoAB::Tc</td>
<td>23</td>
</tr>
<tr>
<td>JJS39</td>
<td>trpC2 Δupp</td>
<td>21</td>
</tr>
<tr>
<td>AIG246</td>
<td>trpC2 Δupp lexAind</td>
<td>21</td>
</tr>
<tr>
<td>AIG250</td>
<td>trpC2 Δupp lexAind K7(phenol)</td>
<td>21</td>
</tr>
<tr>
<td>AIG266</td>
<td>trpC2 pheA1 lexA::K7(phenol) ΔneoAB::Tc</td>
<td>This study</td>
</tr>
<tr>
<td>YB886</td>
<td>metB10 trpC2 xin-1 SPβ1 ICEBs10 (phage defective, pd)</td>
<td>2, 70</td>
</tr>
<tr>
<td>YB3000</td>
<td>metB10 trpC2 xin-1 SPβ1 recA260::mls::cat ICEBs10 (phage defective, pd)</td>
<td>10</td>
</tr>
</tbody>
</table>
introduced cutoff values of 1.5-fold, i.e., only effects that were ≥1.5-fold were considered significant and are reported.

Microarray data are deposited at the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo) database with accession number GSE4673.

Use of genomic microarrays to study DNA replication. The same type of PCR product-based microarray slides used for expression profiling was used for determining the relative chromosomal content. Asynchronous cultures were treated with MMC or mock treated, and samples were collected at various times after the treatment by mixing them with an equal volume of ice-cold methanol. Chromosomal DNA was extracted and purified by using G-100 QIAGEN genomic DNA purification columns, fragmented by digestion with HaeIII, and purified with QIAGEN QiaQuick PCR purification columns. The DNA fragments were mixed with random hexamers and heated at 95°C for 5 min and then rapidly cooled on ice. Primer extension was conducted at 30°C overnight with Klenow fragment (3’ to 5’ exo-) and a deoxyxycylidine triphosphate mixture including aminoallyl-dUTP. DNA labeled with aminoallyl-dUTP was purified with QIAGEN MinElute columns. Test and reference samples (DNA obtained from cells with a single unrepliliated chromosome) were coupled to Cy5 and Cy3 dyes, respectively, mixed, and hybridized to a microarray using the same procedures as for expression profiling to obtain ratios of test sample to reference for each chromosomal locus. Different test samples were then compared by obtaining the ratio of these ratios. The results presented here are from a single representative experiment.

Thymidine incorporation. Experiments were performed with cells grown to mid-exponential phase at 37°C. For pulse-labeling, 10 μl of [3H]methyl-thymidine (80 and 1 μCi/ml; Perkin-Elmer) was added to 200 μl of culture to a final concentration of 0.05 μCi/ml. The cells were incubated with the radioactive label for 1 min at 37°C, then mixed with an equal volume of 20% ice-cold trichloroacetic acid, and allowed to precipitate for at least 30 min on ice. Samples were then loaded onto glass fiber filters (GF6; Schleicher & Schuell), filtered with a vacuum, washed three times with 10 ml of ice-cold 5% trichloroacetic acid each time, and dried with ethanol. The dried filters were added to scintillation vials, mixed with 10 ml of scintillation fluid, shaken vigorously, and processed with a scintillation counter.

RESULTS AND DISCUSSION

DNA damage and replication fork arrest cause a broad transcriptional response, largely mediated by RecA. We compared the mRNA levels of virtually all B. subtilis genes in cells treated with different agents that block replication directly or that cause DNA damage. The goal was to identify the genes affected under each condition and to understand, at a global level, the types of cellular processes affected by the separate agents. We also sought to identify the genes induced in a recA-dependent manner under each condition. Our experimental approach was to treat exponentially growing cultures with the selected agent and to compare mRNA levels of each gene in treated cultures to the levels in mock-treated cultures. The majority of changes in mRNA abundance were expected to be due to changes in the rate of gene transcription, as opposed to effects on mRNA stability. For each experiment, recA+ and recA-null strains were analyzed in order to determine which changes were recA dependent.

We arrested DNA replication in exponentially growing cells at a concentration of the drug HPUra at which replication appears to be completely inhibited. Comparison of the mRNA levels from cells treated with HPUra for 60 min to levels from mock-treated cells at the same time point revealed that the abundance of 668 mRNA species (~17% of all B. subtilis genes) was significantly affected. Of these 668 genes, the expression of 357 increased, and the expression of 311 decreased (Fig. 1A, column 3, and Table 2). When we analyzed the effect of DNA damage 60 min after treatment with MMC, we found that a total of 464 genes were affected significantly. The expression of 347 genes increased and 117 genes decreased significantly (Fig. 1A, column 1, and Table 2). Of the 464 genes affected, 334 were also affected by HPUra. Of these 334 genes in common, 266 had increased expression and 68 had decreased expression.

Since RecA is a known regulator of the transcriptional response to DNA damage, we tested the effect of MMC treatment on cells lacking recA. Of the 464 genes affected by MMC in recA+ cells, only 35 changed significantly in a recA-null mutant after treatment with MMC (Fig. 1A, column 2, and Table 2). These results demonstrate, as expected, that recA largely mediates the transcriptional response to MMC.

We previously analyzed the recA-independent, sda-independent response to replication arrest with HPUra and found that only 108 of the 668 genes affected in wild-type (WT) cells were also affected in a recA sda double mutant (23). Replication arrest negatively affects the expression of many developmental genes through the induction of sda (7, 23; A. I. Goranov and A. D. Grossman, unpublished results). Sda is an inhibitor of sporulation. It inhibits activation of the transcription factor Spo0A, thereby affecting the expression of at least 100 genes (7, 46, 58). These results indicate that of the 668 total genes affected by HPUra, recA affects the expression of approximately 450 genes, sda affects ~100, and 108 are independent of both sda and recA. These results provide further evidence that RecA is a main, but not the only, contributor to the transcriptional response to DNA damage and perturbations in replication. We also note that some of the differences in gene expression between WT and recA strains may be due to more extensive DNA degradation which happens in the recombination-deficient recA cells (13).

LexA represses 26 operons containing 63 genes. Since RecA is a major regulator of the transcriptional response to DNA damage and replication arrest, we were interested in determining how RecA affects the expression of so many genes. RecA is known to affect gene expression, in part, by catalyzing the autocleavage of the repressor LexA (45) and the cleavage of some phage repressors (57). To address how many genes are directly repressed by LexA, we identified genes that have increased expression in a lexA-null mutant. This analysis was performed in a yneAB-null mutant. yneA encodes a division inhibitor that is repressed by LexA (27). In the absence of lexA, cells filament and grow poorly in minimal medium due to the expression of yneA. In our strain background, the deletion of yneA suppresses the lexA growth defect in minimal medium (data not shown).

Expression of 139 genes increased significantly in the lexA yneAB mutant compared to the lexA+ yneAB strain (Fig. 1, columns 12, 13, and 28; Table S1 in the supplemental material). We searched the 300-bp region upstream of the start of these genes for the relaxed LexA binding site consensus sequence GAA(C/NN)TTC (where n = A, G, T, or C) (1, 9), allowing for one mismatch. Twenty-six of the genes (all were the first gene in their putative operon) had at least one recognizable LexA binding site with no more than one mismatch to the consensus sequence. These 26 operons accounted for approximately 70 of the 139 genes whose expression was increased in a lexA-null mutant. Two other operons have also been reported to be regulated by LexA: aprX and ybaK-cwlD.
FIG. 1. DNA damage and replication arrest affect the expression of large number of genes in a recA-dependent manner. The relative mRNA levels in cells treated with MMC, HPUrA, or UV or untreated or in various mutants were determined by using microarrays. Samples of parallel treated or untreated cell cultures, or mutant and WT, were taken at various times for a direct comparison. Samples were taken during mid-exponential growth and, when treated with HPUrA, MMC, or UV, samples were taken 60 min after treatment. The average from three biological replicates is presented. The data are presented as colored boxes (more like lines in panel A), with each box representing a gene. In panels A and C, the brightest green represents a ≥8-fold decrease, and the brightest red represents a ≥8.0-fold increase in relative levels of mRNA. In panel B, the brightest green represents a ≥3.5-fold decrease, and the brightest red indicates a ≥3.5-fold increase in relative mRNA levels. Gray cells indicate no data for that particular gene in the particular experiment. Black indicates no change. The letters M, H, and UV above a column indicate treatment with MMC, HPUrA, and UV, respectively. Relevant strain properties are also indicated, with pd—indicating the phage-defective strains. (A) The results are shown for the 798 genes that were significantly affected in WT by either HPUrA or MMC treatment. Genes are ordered according to their position in the chromosome. (B) Data from the subset of genes that are likely to be directly regulated by LexA are presented here. These data are extracted from panel A and include the 28 operons whose expression was significantly affected in the lexA-null mutant and that also have putative LexA binding sites in the regulatory regions. All of the previously proposed direct targets of LexA (1) are also included. The data on expression of the previously proposed direct targets of LexA, in phage-defective strains, both recA + and recA null, were presented previously (1) and are included here for completeness. The numeric data for all of the proposed direct targets of LexA are presented in Table S1 in the supplemental material. (C) Data from the subset of genes that are in the mobile genetic elements ICEBs1, PBSX, and SPβ are presented. These data, except for column 24, are from panel A. Columns: 1, 14, and 22, WT strain (JH642) treated with MMC compared to the same strain untreated; 2, 19, and 31, recA strain (IRN444) treated with MMC compared to the same strain untreated; 3, 15, and 23, WT strain (JH642) treated with HPUrA compared to the same strain untreated; 4, 17, and 30, recA sda double mutant (AIG4) treated with HPUrA compared to the same strain untreated. The data from these experiments were previously published (23) and are presented here for comparison. Columns 5 and 31,
TABLE 2. DNA damage and perturbations in replication change causes in the expression of many genes

<table>
<thead>
<tr>
<th>Category</th>
<th>WT HPUra</th>
<th>recA sda HPUra</th>
<th>HPUra</th>
<th>WT MMC</th>
<th>recA MMC</th>
<th>pd− MMC</th>
<th>pd− recA MMC</th>
<th>pd− UV</th>
<th>pd− recA UV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of genes</td>
<td>668</td>
<td>108</td>
<td>464</td>
<td>80</td>
<td>138</td>
<td>30</td>
<td>35</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Up</td>
<td>357</td>
<td>21</td>
<td>347</td>
<td>69</td>
<td>119</td>
<td>30</td>
<td>35</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Down</td>
<td>311</td>
<td>87</td>
<td>117</td>
<td>11</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>No. of genes regulated&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57</td>
<td>20</td>
<td>12</td>
<td>12</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>No. of LexA genes</td>
<td>60</td>
<td>0</td>
<td>55</td>
<td>0</td>
<td>31</td>
<td>0</td>
<td>24</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>recA dependent</td>
<td>-450</td>
<td></td>
<td>429</td>
<td>125</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overlap&lt;sup&gt;b&lt;/sup&gt;</td>
<td>108</td>
<td></td>
<td>35</td>
<td>13</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;sup&gt;c&lt;/sup&gt;/ proximal genes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mobile elements&lt;sup&gt;c&lt;/sup&gt;</td>
<td>193</td>
<td>0</td>
<td>193</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PBSX (32)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>30</td>
<td>0</td>
<td>30</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>SPB (187)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>149</td>
<td>0</td>
<td>143</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ICEBs1 (24)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>14</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The numbers of genes whose mRNA levels are significantly affected by a given treatment in the indicated strain are given. The strains and data are the same as those in Fig 1. pd− refers to phage-defective strains.

<sup>b</sup> Number of genes previously proposed to be directly regulated by DnaA that are significantly affected under the indicated condition (the number of operons is given in parentheses).

<sup>c</sup> Number of genes that are affected under the indicated conditions in recA<sup>+</sup> cells that are also affected under the same conditions in the recA null mutant.

<sup>d</sup> Number of genes within ~1.5% of the chromosome (~50 kb) on each side of oriC whose expression was increased after the indicated treatment.

<sup>e</sup> Total number of genes that belong to mobile genetic elements (ICEBs1, PBSX, and SPB) that were affected significantly under the indicated conditions.

<sup>f</sup> Total number of genes that belong to the element.

(1). Au et al. demonstrated that LexA bound these promoters in vitro. Although the expression of these two operons was not affected significantly in our lexA-null versus lexA<sup>+</sup> comparisons, they did exhibit recA-dependent regulation (1).

Since LexA is inactivated in a RecA-dependent fashion, then the operons we suspect to be LexA repressed should be induced by DNA damage and replication arrest in a recA-dependent manner. We examined whether the 28 operons we suspect to be LexA repressed are induced by DNA damage and replication arrest in a recA-dependent manner in the experiments above (Fig. 1). Twenty-six of the proposed LexA-repressed operons were significantly induced under at least one of these conditions in WT cells and were not induced in a recA-null mutant (Fig. 1 and Table S1 in the supplemental material). The other two operons, yokEF and dltABCDE, were not affected by HPUra, MMC, or UV.

We also tested the effects of the lexA-null mutation on global changes in gene expression in response to replication arrest. More than 600 genes had altered expression 60 min after replication arrest (Fig. 1A, column 11). These results indicate that, as expected, there are several regulatory factors other than LexA that control gene expression in response to replication arrest.

In the lexA-null mutant, the LexA-repressed genes are constitutively expressed and replication arrest should have little or no effect on their expression, unless there are other mechanisms controlling expression of these genes. We found that of the 26 operons proposed to be regulated directly by LexA, only the genes that belong to SPB were induced 60 min after replication arrest with HPUra in the lexA-null mutant (Fig. 1, columns 11, 21, and 27; see also below).

There are mutations in lexA, lexA(ind), that prevent (or greatly reduce) autocleavage of LexA but still allow LexA to function as a repressor (21). Genes that are repressed by LexA should not be induced in the lexA(ind) mutant in response to DNA damage or arrest of DNA replication. We arrested replication elongation with HPUra in lexA(ind) strains and performed microarray analysis to assess the effects on global gene expression. Of the 26 operons regulated by LexA, only tagC (dinC) was increased significantly in the lexA(ind) mutant 60 min after replication fork arrest (Fig. 1B, column 18). The change in tagC expression was less than 20% that in lexA<sup>+</sup> cells. The tagC promoter is one of the most highly induced promoters after DNA damage (Table S1 in the supplemental material) and is probably extremely sensitive to LexA levels. We speculate that there might be some limited proteolysis of the LexA(ind) protein and that the most sensitive promoters become slightly induced. Furthermore, additional results indicate that the partial induction of tagC is not due to lexA-independent regulation of tagC by DNA damage (see above).

Based on our results, we propose that a total of 26 operons containing at least 63 genes are directly repressed by LexA,
since these 26 operons displayed *lexA*- and *recA*-dependent induction after replication arrest or DNA damage. These 26 operons include the 18 operons previously proposed by Au et al. to be regulated directly by LexA. Although two operons, *yokEF* and *dllABCDEF*, had increased expression in *lexA*-null mutants and contained putative LexA binding sites, they were not induced by DNA damage. We suspect that they are probably not directly regulated by LexA and instead are indirectly affected by the constitutive expression of LexA-regulated genes.

Of the eight additional operons we propose to be LexA regulated, one had previously been proposed to be LexA repressed: *dnaE*, encoding an essential, error-prone DNA polymerase (15, 36). Another operon, *yqhH* (*polYI*) encodes a Y family DNA polymerase that has been demonstrated to have a role in stationary-phase mutagenesis and can interfere with replication (18, 19, 63). In addition, the genes in the *yozK* operon are also similar to those encoding the Y-family polymerases, so their regulation by DNA damage is not surprising, although their role in repair of DNA damage is yet to be determined. At least five of the proposed LexA-repressed operons (23 genes) are a part of the prophage SP in the *B. subtilis* genome: *yofD-yox*, *yokHIJKL*, *yopTUVXYZ-yogABC*, *yorHI*, and *yorBCDEFG* (35). The presence of potential LexA binding sites in the promoters of these five *sp* operons indicates that LexA may be directly involved in their regulation. Indeed, LexA also directly regulates at least one gene, *-sdkA*, in the genome of another *B. subtilis* phage, PBSX (Fig. 1) (1). Also, one of the proposed LexA regulated *sp* genes, *yuvk*, is homologous to the Y-family DNA polymerases (e.g., *E. coli* umuC and *B. subtilis* *yqkl* (*polYI*2), *yqhH* (*polYI*1)), which are regulated by LexA in both *E. coli* and *B. subtilis* (Fig. 1) (47, 60, 63).

LexA is also known to directly regulate gene expression in other bacteriophages. In coliphage 186, lytic gene expression is repressed by the phage repressor, c1 (34). The activity of c1 is antagonized by an antirepressor, Tum, whose transcription is repressed by LexA (33). In the *Vibrio cholerae* CTX prophage, LexA binds directly to a promoter required for phage development (53). The removal of LexA repression results in increased expression from this promoter and subsequent prophage induction (53).

In *B. subtilis*, additional genes also appear to be regulated by LexA. The 139 genes that increased in expression in *lexA*-null mutants, 75 had no discernible LexA binding sites and are probably controlled indirectly by LexA. Approximately half of these genes are in SPβ. Most of the 75 genes were also induced in a *recA*-dependent manner after MMC or HPUra treatment (Fig. 1 and data not shown). None of the genes repressed by LexA appears to be a transcriptional regulator, but many are involved in DNA metabolism and can interfere with replication (64). It is plausible that the interference with replication by LexA regulated genes causes many of the detected changes in gene expression. Interference with replication also might be how the *t3i-23* mutation (gene not known) causes temperature-induced SOS-related phenomena, most of which are *recA* dependent (39). *t3i-23* [22% linked to the *abl* (*dal*) locus (39)] does not map near any of the LexA regulated genes described here.

Some of the genes encoding DNA recombination and repair proteins are induced during the development of genetic competence (4, 16, 39, 40, 49, 55). However, most of the LexA-regulated genes described here are not induced during competence development, the exceptions being *recA*, *lexA*, *yneAB*, *tugC*, and *dinB*.

**Induction of phage gene expression by DNA damage and replication arrest requires RecA and the inactivation of LexA.** Our results indicate that a large set of genes induced by DNA damage in *B. subtilis* belong to the genomes of the prophage PBSX (32 total genes) and SPβ (187 total genes) and to the integrative and conjugative element ICEBsl (24 total genes). At 60 min after treatment with HPUra or MMC, 193 of the 243 genes associated with PBSX, SPβ, and ICEBsl were induced (Fig. 1C, columns 22 and 23, and Table 2).

The induction of genes in these elements was dependent on *recA* as phage and ICEBsl genes were not induced in *recA*-null strains (Fig. 1C, columns 29 and 30 [compared to columns 22 and 23], and Table 2). However, unlike most LexA-repressed genes, some phage and ICEBsl genes were still induced by HPUra in a *lexA*-null strain (Fig. 1C, column 27), indicating that genes in these elements are regulated, at least in part, independently of LexA. The induction of SPβ genes by HPUra in a *lexA*-null strain was less than that in WT, at least in part, due to the increased background expression level of SPβ genes in untreated *lexA*-null cultures (Fig. 1C, columns 23 and 27). It is likely that the SPβ induction in untreated *lexA*-null cells is due to the constitutive expression of LexA-regulated genes.

The induction of at least one LexA-repressed gene must play a role in the induction of PBSX and SPβ gene expression, since their induction was prevented in a noncleavable *lexA*(*ind*) mutant (Fig. 1C, column 26). Since the *lexA*(*ind*) mutation was in a different genetic background, we confirmed that HPUra induces the expression of prophage genes in the isogenic *lexA* strain (Fig. 1C, column 25). Curiously, in that strain background we saw no induction of ICEBsl by HPUra in either the *lexA* strain or the *lexA*(*ind*) mutant, though ICEBsl was present in the genome (data not shown). The explanation of this observation will require further study.

Our results are consistent with previously published reports that showed the induction of SPβ and ICEBsl is *recA* dependent (2, 44). These mobile genetic elements are regulated similarly to several other phages and the integrative and conjugative element SXT, which are activated by the *recA*-dependent DNA damage response (3, 57). It is thought that this regulation allows the mobile genetic element a better chance of survival through leaving if the host cell is being damaged.

We also found that expression of genes in PBSX and SPβ is partially controlled by the repressor, LexA, indicating that the full expression of at least one LexA repressed gene is necessary for their induction of the phage genes. It is likely that the lack of increased expression of *recA* or the insufficient activation of RecA due to low abundance of ssDNA in the *lexA*(*ind*) mutant strain prevents full induction of phases (22). In addition, LexA also inhibits expression of at least one operon in PBSX and SPβ, and phage induction might be partly inhibited if these operons are not induced. We were unable to determine whether LexA regulates the expression of genes in ICEBsl, since this element was not induced in a *lexA* strain isogenic to the *lexA*(*ind*) mutant strain. Our data also indicate that LexA...
TABLE 3. MMC and UV treatments affect a few of the genes proposed to be regulated by DnaA

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>No. of operons</th>
<th>Putative DnaA-regulated genes also affected by the indicated treatment (fold effect)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>MMC</td>
<td>8</td>
<td>citZ (2.1), flgB (−1.7), kgdR (−1.9), nap (−2.0), nrdE (1.7) [nrdF (2.4), ymaB (1.9)], yclN (−2.1) [yclP (−1.8), yclQ (−2.0)], ywFO (1.0), yybB (−1.5)</td>
</tr>
<tr>
<td>recA</td>
<td>MMC</td>
<td>3</td>
<td>ymaA (3.4) [nrdE (3.4), nrdF (3.5), ymaB (3.1)], pyrA4 (−18) [pyrB (−21), pyrC (−15), pyrD (−5.8), pyrE (−7.6), pyrK (8.5)], ywFO (2.2) [ywA (2.5)]</td>
</tr>
<tr>
<td>pd−</td>
<td>MMC</td>
<td>2</td>
<td>ypeH (−2.4) [aroD (−2.7), ywFO (3.2), ywA (3.0)]</td>
</tr>
<tr>
<td>pd− recA</td>
<td>MMC</td>
<td>1</td>
<td>ymaA (3.7) [nrdE (3.5), nrdF (3.9), ymaB (3.5)]</td>
</tr>
<tr>
<td>pd−</td>
<td>UV</td>
<td>1</td>
<td>ywFO (2.3)</td>
</tr>
<tr>
<td>pd− recA</td>
<td>UV</td>
<td>6</td>
<td>aroD (−1.7), dnaA (−3.5) [dauN (−3.6)], sda (2.2), yllB (−2.0) [ylcA (1.6), ywFO (2.3) [ywA (2.5)]]</td>
</tr>
</tbody>
</table>

* The relevant genotype is indicated. The strains used were as indicated in Fig. 1.

** Of the 57 genes proposed to be regulated directly by DnaA (23), only those that are also affected by the indicated treatment are indicated. Numbers in parentheses indicate the fold effects, with negative numbers indicating decreased expression. Genes that are internal in an operon are in brackets.

inactivation alone is not sufficient to trigger the full expression of most of the genes of the mobile elements. This is a key difference between the majority of the genes of the mobile elements and the direct targets of LexA.

We also observed that expression of 15 to 20 genes from the skin element had appeared in increased expression after HPUra and MMC treatment (Fig. 1A, columns 1 and 3). The skin element is a defective prophage that disrupts a gene important for sporulation, sigK, and precise excision of skin during sporulation restores the sigK open reading frame (31, 62). The increase in the expression of skin element genes was detected only for genes with high similarity to genes in PBSX and was not detected when the same RNA was hybridized to spotted oligonucleotide arrays (data not shown). Our interpretation of these results is that mRNA from some of the PBSX genes, which are induced in response to DNA damage, cross-hybridizes with the probes to skin element genes, thus giving the appearance of upregulation of the skin element. Consistent with this, previous reports showed that the skin element is not induced by MMC treatment (30).

Phage gene expression and its indirect effects constitute a large portion of the genes induced by DNA damage and replication arrest. As outlined above, many of the genes induced in WT cells after DNA damage belong to prophage. The induction of phage genes is the first step toward assembling phage particles and lysing the host cell to release these particles. PBSX and SPβ are both capable of assembling such particles and releasing them (50, 61, 66). The induction of phage genes and subsequent particle assembly almost certainly have many effects on cellular metabolism that could cause indirect effects on the host gene expression. We were interested in assessing the transcriptional profiles of cells unable to induce the prophage genes after DNA damage, thus eliminating any secondary effects we might observe due to phage induction. We used a strain (YB886) that lacks SPβ and cannot induce PBSX due to a mutation, xin-1 (70), and also is missing ICEBs1 (J. M. Auchtung and A. D. Grossman, unpublished data).

In the phage-defective strain (YB886), 60 min after addition of MMC, under conditions identical to the experiments outlined above, 138 genes were significantly affected compared to the 464 genes affected by MMC in a phage" background. Of the 138 genes, expression of 119 increased and expression of 19 decreased after treatment with MMC. This response was primarily mediated by RecA, since the effect of MMC on most genes (125 of the total 138 affected genes) was absent in the recA-null mutant (Table 2).

The reduction of the overall number of genes affected by MMC in the phage-defective strain indicates that there is a large effect of phage induction on host gene expression. Our analysis indicates that in addition to the 198 phage and ICEBs1 genes, there were 184 nonphage genes that were affected in the WT but not in the phage-defective background after MMC treatment (e.g., Fig. 1A, compare columns 1 and 5, and Table S2 in the supplemental material). Changes in expression of most of the host genes were dependent on both recA and the presence of the phage (Table S2 in the supplemental material). These nonphage genes fall in various categories, indicating that the induction of phages impinges on many cellular processes: 49 genes in cell envelope functions; 35 genes in intermediate metabolism; 24 genes in information pathways; 10 genes in antibiotic production, stress, and detoxification; and 66 genes of unknown function. The observation that a large number of genes involved in cell-envelope functions are affected by phage induction is not entirely surprising since phages need to modify the cell envelope so that the phage particles will be released into the environment. Understanding what events in the cell envelope bring about the observed changes in gene expression and whether there is a different mechanism for the phages to affect gene expression will require further studies.

Replication arrest and unrepaired UV damage affect the expression of genes regulated by DnaA. DnaA, the replication initiation protein in bacteria, mediates a transcriptional response to perturbations in replication that is independent of RecA (23). We had previously established that replication arrest caused by HPUra affects the expression of DnaA-regulated genes, including dnaA, dnaN, sda, ywcC, and fisL (23). We were interested in investigating whether this DnaA-mediated response was induced under any of the other conditions studied. MMC and UV treatments affected a small subset of the genes regulated by DnaA (Table 2 and Table 3). In the WT strain, MMC affected 8 operons (12 genes) of the 20 operons proposed to be regulated directly by DnaA (23). HPUra affects the expression of all 20 operons in a recA-independent manner (23), and yet MMC affected only three operons (total of 12 genes) in a recA-null background. MMC affected two putative DnaA operons (four genes) in the phage-defective strain and only one operon (four genes) in the phage-defective recA strain.
These results indicate that DnaA-regulated genes are affected differently by MMC compared to replication arrest. Because MMC treatment did not change the expression of dnaAN and sda, the best-characterized DnaA targets, it is possible that the genes affected by MMC are controlled by factors other than DnaA under these conditions. In addition, it is as likely that after MMC treatment, DnaA may affect the expression of only a subset of genes, due to different sensitivity of the promoters to DnaA activity.

UV treatment affected only one operon in the phage-defective strain (Table 3). UV treatment in the phage-defective recA strain affected the well-characterized DnaA targets dnaAN and sda, as well as four more proposed DnaA targets (total of eight genes in six operons). Only one of the operons regulated by DnaA, ynzC-ywfO-ywgA, was affected by treatment with UV, MMC, and HPura. The direction of the effects (either increased or decreased) on all operons except yclNOP was the same under all conditions in which the operons were significantly affected.

The effects on more DnaA-regulated genes after UV damage in the recA-null versus recA+ strain are likely due to the inability of recA mutants to repair the DNA damage, thus causing prolonged replication arrest (data not shown) (11, 12, 29). The magnitude of the effect on DnaA-regulated genes after UV damage was not as large as in the HPura experiments. One possibility is that UV partially blocks replication in all cells. For example, the replisome may stall for some period of time and then replication may restart until the replisome stalls again. Another possibility is that replication is completely blocked in a subpopulation of cells and only these cells exhibit effects on DnaA-regulated genes; thus, in the context of the whole population the effects on DnaA-regulated genes appears less than when cells are treated with HPura. These possibilities could be distinguished by analysis of gene expression in single cells.

In HPura-treated cells, the changes in expression of sda and yllB are less than that of other affected genes. However, UV irradiation affected these genes and not others that are more strongly affected by HPura. One possible explanation for seeing only a subset of the proposed DnaA-regulated genes after UV treatment is that expression of the proposed DnaA-regulated genes may require factors in addition to DnaA and that
these additional factors are differentially regulated by UV and HPUra treatments. Our results indicate that the recA-independent, DnaA-dependent responses to the various DNA-damaging treatments and replication arrest are qualitatively and quantitatively different. These differences are likely due to mechanistic differences of how MMC, UV, and HPUra affect replication. We speculate that since different events occur at the replication fork when replication is arrested with various treatments, replication is affected differently, which leads to different effects on DnaA and the DnaA-dependent transcriptional response. Furthermore, in addition to causing DNA damage, MMC has the potential to damage proteins and, thus, could disrupt protein function and disrupt other pathways that occur inside the cell (17). These effects on protein function may influence the transcriptional response after DNA damage.

**MMC causes a relative increase in origin-proximal chromosomal loci.** The expression of a large number of genes within the origin-proximal region appeared to be significantly increased after treatment with MMC in recA^- and recA-null strains when grown in rich as well as in minimal medium (Fig. 2A, Table 2, and data not shown). An analysis of the mRNA abundance, irrespective of statistical significance, revealed that in cells treated with MMC >80% of the genes in the origin-proximal region have higher levels of gene transcripts in most experiments (Fig. 2A). In only one experiment did we observe an effect on <80% of the origin-proximal genes, with only 56% of the origin-proximal genes having higher mRNA abundance. These effects on gene expression were usually less than twofold.

This apparent increase in origin-proximal gene expression might be caused by an increase in the rate of transcription of these genes. Alternatively, the effect on mRNA abundance of the oriC-proximal genes might be caused by increased copies of the origin region. Such an effect on gene dosage could be achieved if MMC were causing overinitiation of DNA replication, with the newly assembled replication forks proceeding for a relatively short distance (~50 kb). Alternatively, MMC could be slowing DNA replication elongation without affecting the rate of initiation, thus effectively increasing replication in the origin region.

To test the hypothesis that there is a higher copy number of the genes near oriC in MMC-treated cells, we performed genomic microarray experiments. This type of experiment allows a comparison to be made between the genomic compositions of different bacterial cultures. The genomic microarray experiments revealed that cultures treated with MMC have an increased (~2-fold) relative amount of origin-proximal DNA compared to untreated cells (Fig. 2B). The fold increase of a particular chromosomal locus was inversely proportional to the distance from the origin, with virtually no increase in DNA content after 60 kb away from the origin. Thymidine incorporation experiments demonstrated that the rate of replication decreases after MMC treatment but not to the level of that in HPUra-treated cultures (Fig. 2B), indicating that MMC inhibits, although it does not abolish, DNA replication. This intermediate effect of MMC is consistent with the notion that MMC slows down elongation, causing a relative increase in origin-proximal DNA and a relative increase in transcripts from origin-proximal genes. Under these conditions MMC has a more toxic effect than HPUra 60 min after the addition of the drug (data not shown), indicating that the more severe effects on DNA replication by HPUra are not due to higher toxicity.

Treatment of recA^- cells with UV irradiation also caused an increase in mRNA levels of the origin-proximal genes (Fig. 2F), indicating increased gene dosage in this region. However, in a recA-null mutant, this increase was not observed (Fig. 2E). In contrast to these effects and those of MMC, treatment of either recA^- or recA-null mutant cells with HPUra (to block replication) did not cause an increase in mRNA levels of origin proximal genes (Fig. 2C and D). These results are consistent with the notion that different mechanisms for blocking DNA replication have different regulatory effects. We suggest that HPUra in recA^- and recA mutant cells and UV damage in recA mutant cells causes a strong arrest of replication forks throughout the chromosome such that there is little or no relative change in chromosomal content compared to untreated cells. We suggest that UV light has different effects in recA^- and recA cells because the recA mutant cells are unable to repair the damage, whereas WT cells repair the damage and resume replication within 40 min. We propose that MMC causes a relative increase in the chromosomal content of origin-proximal regions by slowing down replication fork progression without causing a decrease in the rate of initiation of replication.

Our results demonstrate that the inhibition of replication elongation by different DNA-damaging agents or inactivation of replication components can have both unique and overlapping effects on gene expression. The results also indicate that *B. subtilis*, and likely other bacteria, can induce several different response pathways after DNA damage, but the extent of induction of each pathway may vary such that the organisms have the best chance of survival.

**ACKNOWLEDGMENTS**

This study was supported in part by NIH grant GM41934 to A.D.G.
We thank Neal Brown for the generous gift of HIPUra and Philippe Noirot and S. D. Ehrlich for providing JJS39, lexA, and lexA(ind) strains. We thank C. Lee and J. Auchtung for technical help and T. Baker, C. Lee, M. Berkmen, J. Auchtung, and L. Uittenbogaard for suggestions and comments on the manuscript.

REFERENCES

5. Kropg, S., M. O’Reilly, N. Nolan, and K. M. Devine. 1996. The phage-like element PBSX and part of the skin element, which are resident at different locations on the Bacillus subtilis chromosome, are highly homologous. Microbiology 142(2):2031–2040.
21. Quillardet, P., M. A. Roufaud, and P. Bouige. 2003. DNA repair analysis of...


