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Research in Microbiology 154 (2003) 559–572
Research in Microbiology
www.elsevier.com/locate/resmic

DNA array analysis of gene expression in response to UV irradiation in *Escherichia coli*

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Received 4 April 2003; accepted 30 June 2003

First published online 4 July 2003

This article is dedicated to the memory of Maurice Hofnung

Abstract

The capacity of DNA macroarrays that contain all 4290 predicted open reading frames of the *E. coli* K12 genome was evaluated by measuring changes in gene expression in response to irradiation by ultraviolet light (UV). UV and other DNA damaging agents are known to trigger the induction of the SOS response. This is a coordinated increase in the level of expression of a set of approximately 30 unlinked genes, the SOS genes, negatively regulated by the LexA repressor. The analysis was performed on a set of isogenic strains with mutations that affect expression of genes of the SOS system: (i) the *lexA*$^{+}$ strain, in which the SOS system can be induced after DNA damage, (ii) *lexAind*$^{-}$ mutants in which the SOS system cannot be induced, and (iii) *lexAdef* mutants in which the SOS system is induced constitutively. We found that a large set of genes appeared to be either upregulated or downregulated following UV irradiation. Among the genes which appeared to be upregulated in a LexA-dependent manner, we correctly identified 9 out of 27 SOS genes printed on the arrays and one gene containing a LexA binding site. One gene, *dnaN*, encoding the beta subunit of DNA polymerase III holoenzyme, was identified as an upregulated gene in a LexA-independent manner. Our results were compared to those of similar studies previously published. Although the SOS response as a whole could not be illustrated by using DNA arrays, the data suggest that regulation of some SOS genes might be more complex than previously thought.

Keywords: Escherichia coli; DNA arrays; UV irradiation; SOS response

1. Introduction

Bacteria have evolved mechanisms that permit responses to changes in environmental conditions by rapidly adapting their structure and physiology. These mechanisms are based on the existence of multiple regulatory systems in which gene expression is controlled in a coordinate manner in response to environmental stress. Experiments aimed at defining such stress-related global regulatory responses have relied upon either the isolation of operon fusions induced by a particular stress [8] or proteomic experiments in which protein fractions from stressed and unstressed cultures are separated by two-dimensional gel electrophoresis prior to comparison [22]. The recent availability of complete genomic sequences coupled with technical advances in gene array technology offers new approaches to investigating the global effects of environmental stresses by studying genome-wide patterns of gene expression. High-throughput cDNA arrays allow simultaneous measurements of the expression levels of tens of thousands of different genes (for reviews see [7,11]). In the case of the relatively small genomes of bacteria, it is possible to analyze the expression of all predicted genes.

In *Escherichia coli*, one of the best-known stress-related global regulatory responses is the SOS response (for reviews see [6,21,23]). The SOS response is a coordinated increase in the level of expression of a set of unlinked genes, the SOS genes, in response to DNA damage induced by ultraviolet light (UV) or other DNA damaging agents. The SOS response to DNA damage requires the *recA* and *lexA* gene products. LexA is a repressor protein that binds to a site (the "SOS box") located near the promoters of the SOS genes and interferes with the binding of RNA polymerase. The

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0923-2508/$ – see front matter
doi:10.1016/S0923-2508(03)00149-9

induction of SOS genes depends on the cleavage of the LexA protein. Upon DNA damage, the RecA protein is activated for its role in SOS induction when it forms a nucleoprotein filament by binding to single-strand DNA generated by the cell's failed attempts to replicate damaged DNA. The RecA/ssDNA nucleoprotein filament then functions as a coprotease that mediates LexA cleavage by stimulating the latent ability of the LexA repressor to cleave itself into two parts via a proteolytic autodigestion mechanism. The resulting decrease in the cellular pool of intact LexA results in the induction of the SOS regulon. The precise number of proteins induced as part of the global SOS response is presently unknown, but approximately 30 unlinked genes are known to be induced after the cell is exposed to DNA damaging agents. In addition, other genes can be upregulated following DNA damage but are believed to be independent of the LexA regulon.

In the present work, which used *E. coli* as a model, we took advantage of DNA arrays containing all annotated open reading frames (ORFs) [2] to analyze changes in the level of gene expression in response to DNA damage induced by UV. Our objectives were: (1) to evaluate the capacity of DNA arrays to detect the induction of the SOS genes, and possibly to detect the induction of new SOS genes and/or genes whose expression was independent of the LexA regulon; and (2) to compare our results with previously published studies [4,9].

2. Materials and methods

2.1. Bacterial strains

The *E. coli* K12 control *lexA*$^{+}$ strain was PQ65 [17]. This strain harbors a *sulA*::*lacZ* fusion, which allowed the determination of the UV dose and the expression time, giving a maximum induction of the *sulA* SOS gene [16]. The genotype of this strain is: F^{-}, *thr, leu, his, pyrD, thi, rpoB, trp*::Muc^{+}, lacΔU169, *galE*, *sulA*::Mud(Ap *lac*)^{cts}. Isogenic strains PQ200 *lexAind*$^{-}$(*lexA3*) (uncleavable LexA repressor) and PQ210 *lexAdef* (*lexA*::Tn5) (deficient LexA repressor) were constructed by P1-mediated transduction of *lexA* alleles from strains DM1623 [15] and GW1040 [8], respectively, into strain PQ65. Transfer of *lexA* alleles was verified by uninducibility or constitutive inducibility of the *sulA*::*lacZ* fusion.

2.2. Growth and irradiation conditions

Strains were grown in rich Luria–Bertani (LB) medium [12]. Overnight cultures were diluted 1:200 with fresh LB medium and grown at 37 °C with agitation until OD_{600} reached 0.5 (middle-log phase). 8-ml portions of cultures were placed into 9-cm in diameter glass petri dishes and irradiated (30 J m^{-2}). UV irradiation was carried out with a mineral lamp having a maximum output at 254 nm at a dose rate of 0.2 J m^{-2} s^{-1}. The dose rate was measured with a Latarjet dosimeter. Before RNA extraction, 6 ml portions of irradiated culture were incubated for 30 min at 37 °C with agitation for gene expression. In control experiments, cells were treated in exactly the same manner but were not exposed to UV.

2.3. RNA extraction and purification

Total RNA was extracted from bacterial cultures using RNeasy spin columns (QIAGEN, Chastworth, CA). Briefly, 5 ml cell culture samples were pelleted by centrifugation. Cell pellets were rapidly resuspended in 100 µl of a boiling lysis solution (2% SDS, 3 mM EDTA, 30 mM sodium acetate, pH 8). After incubation for 3 min, samples were purified on spin columns according to the recommendation of the manufacturer. RNA samples were eluted in 50 µl of diethylpyrocarbonate (DEPC)-treated water. After digestion with 1 U/µl of DNase I, samples were purified using RNeasy spin columns and finally eluted in 40 µl of (DEPC)-treated water. They were stored at −80 °C until use. Quality and concentration of RNA samples were estimated by agarose gel electrophoresis and spectrophotometry.

2.4. cDNA labelling and hybridization to DNA arrays

A total of 2–5 µg of total RNA was mixed with 0.33 mM of each dATP, dGTP, and dTTP and cDNA labelling primers (Sigma–Genosys Biotechnologies Inc.) in a volume of 25 µl of first-strand buffer heated for 90 °C for 2 min and cooled to 42 °C in 25 min. Then 200 U of Super-Script II (GIBCO BRL, Gaithersburg, MD), 10 U of RNase inhibitor and 20 µCi of (α-^{33}P]dCTP (2000–3000 Ci/mmol) were added, bringing the total volume to 30 µl. This cDNA synthesis reaction mixture was incubated at 42 °C for 2 h. The unincorporated nucleotides were removed on a Sephadex G-25 spin column (Amersham Pharmacia Biotech, Piscataway, NJ). cDNA samples were used in the hybridization experiments only if more than 50% of labelled nucleotides were incorporated.

The DNA arrays (Panorama *E. coli* gene arrays) used in the hybridization experiments were purchased from Sigma–Genosys Biotechnologies Inc. They consist of 12 by 24 cm nylon membranes, on which all of the 4290 PCR-amplified open reading frame (ORFs) of the *E. coli* K12 genome are printed in duplicate, side by side. The membranes were soaked in 50 ml 2× SSPE (10× SSPE: 1.8 M NaCl, 100 mM sodium phosphate (pH 7.7), 10 mM EDTA) for 5 min and prehybridized in 6 ml hybridization solution (5× SSPE, 2% SDS, 1× Denhardt's reagent and 100 µg/ml sonicated salmon sperm DNA) for 1 h at 65 °C using roller bottles. The entire labelled cDNA probe was heated for 10 min at 95 °C and added to 6 ml of hybridization solution at 65 °C. After removing the prehybridization solution, the hybridization solution was added to the membranes. Hybridization was carried out for 16–18 h at 65 °C. Following hybridization,

each membrane was washed with 100 ml of washing solution (0.5× SSPE, 0.2% SDS) three times for 5 min at room temperature and three times for 20 min at 65 °C. The membranes were then wrapped in clear plastic food wrap and exposed to a Phosphoimager screen (Molecular Dynamics) for 48 h. Arrays were stripped with 1 l of boiling dehybridation solution (10 mM Tris (pH 7.5), 1 mM EDTA, 1% SDS) for 30 min. The same array was used up to 5 times.

2.5. Image acquisition and data analysis

Exposed Phosphoimager screens were scanned with a pixel size of 100 µm on a STORM 840 Phosphoimager (Molecular Dynamics). The Array vision 6.0 commercial software (Imaging Research inc.) was used to quantify the intensity for each spot after correcting for background using the "corners between spots" method.

2.6. Experimental design

At least three independent experiments (independent cultures, cDNA synthesis and DNA array hybridization) were performed for each condition (unirradiated or UV-irradiated) or for each strain (*lexA*$^+$, *lexAind*$^-$ or *lexAdef*). Membrane arrays have duplicate spots for each gene, and each spot provides a separate measurement, so that each experiment provided two replicates available for statistical analysis.

2.7. Statistical methods

ArrayStat (Imaging Research Inc.) commercial software was used for statistical evaluation of the data. First, the data are log-transformed (base 10) and centered across replicates, according to conditions. Then the data from the entire array are used to compute an estimate of the relevant error for each gene using a pooled curve fit method. In this method, a curve is computed for which the x-axis represents the average of the log signal intensities for each particular gene. The corresponding standard deviation is plotted along the y-axis. A curve is fitted to these data. The error for any particular gene is based on the plotted curve which fits all the available data, rather than simply using the observed standard for that gene. Then, outliers (data points that are not very reproducible) are removed. They are detected by examining standardized residuals. These residuals are based on the deviations of each point within a row from the mean of the row (there is one row for each gene). Finally, a statistical test (z-test) is performed with a normalization (centering across conditions by the median) and a false-positive error correction by the Bonferroni procedure.

3. Results and discussion

Sixty-nine potential lexA-regulated genes have been identified by computational searches of LexA binding sites in the chromosome, and 29 of these genes have been experimentally identified as LexA-regulated genes, inducible following DNA damage in response to UV irradiation [5]. Two of these genes (*ydsAB* and *dinQ*) were not included in the DNA arrays so that 67 LexA-binding site and 27 SOS genes demonstrated to be lexA regulated were considered in our analysis. The changes in gene expression were examined following a UV dose of 30 J m^{-2} after a post-treatment incubation time of 30 min. These conditions were selected because they provide a maximum induction of the *sulA* SOS gene as measured via the β-galactosidase activity of the *sulA*::*lacZ* fusion in strain PQ65 (data not shown). Although the time of expression following DNA damage may vary from one SOS gene to another, most of the SOS genes are fully induced within 30 min following UV irradiation and remain fully induced up to 60 min [4]. In order to differentiate UV-induced genes that are directly regulated by the LexA repressor from genes that are not directly LexA-regulated, the analysis was performed on a set of isogenic strains carrying mutations that affect expression of the genes of the SOS system: (i) the *lexA*$^+$ strain, in which the SOS system can be induced after DNA damage, (ii) *lexAind*$^-$ mutants in which the SOS system cannot be induced; and (iii) *lexAdef* mutants in which the SOS system is constitutively overexpressed. For each strain (*lexA*$^+$, *lexAind*$^-$, *lexAdef*) and/or for each condition (unirradiated versus UV-irradiated or mutants versus non-mutants), at least three independent experiments were performed. Each independent experiment compared hybridization of two arrays: one hybridized with labelled cDNA from the untreated culture and the other hybridized with labelled cDNA from the same culture but UV-irradiated or with labelled cDNA from a mutant strain grown in the same batch of medium. Since membrane arrays have duplicate spots for each gene, two measurements for each condition were available for analysis. The measurements obtained in each set of experiments were validated by a rigorous statistical analysis (see Section 2). In addition, genes whose ratio of relative expression varied by less than 30% were not considered. The total number of genes for which a modification of expression was observed in each of the conditions tested is summarized in Table 1.

Table 1
Number of genes with modified expression

Comparison	*lexA*$^+$ (UV) / *lexA*$^+$	*lexAind*$^-$ (UV) / *lexAind*$^-$	*lexAdef* / *lexA*$^+$
Induced genes			
Total	268	15	503
(SOS genes)	(15)	(0)	(20)
Repressed genes			
Total	9	1	685
(SOS genes)	(0)	(0)	(2)

3.1. Effects of UV irradiation in a lexA$^+$ wild type context

In the *lexA*$^+$ strain (PQ65), in which the SOS system is fully inducible by UV, changes in gene expression after UV irradiation were measured in 6 independent experiments (i.e., 12 replicates). UV irradiation promoted a significant increase in the level of expression of 268 genes and a decrease in the level of expression of 9 genes (Table 2).

Among the UV-induced genes, we found 13 (*lexA*, *recA*, *recN*, *sulA*::*lacZ*, *uvrB*, *dinD*, *dinI*, *dinJ*, *dinP*, *yebG*, *ruvA*, *ftsK*, *ybfE*) that are known to be members of the SOS regulon and 2 (*rob* and *yafL*) that contain a potential LexA binding site but that have not been previously shown to be LexA-regulated. Among the 10 genes which displayed the highest expression level following UV irradiation (ratio from 2.8 to 12.6), 5 (50%) (*sulA*::*lacZ*; *recA*, *dinI*, *recN* and *uvrB*) are genes known to belong to the SOS regulon. No LexA-regulated genes or genes containing a potential LexA binding site were part of the 9 genes showing a decrease in their expression after UV irradiation. Thus, in our experimental conditions, using Panorama *E. coli* gene arrays, 13 of the 27 known DNA damage-inducible genes could be evidenced.

A similar study was previously performed by Courcelle et al. [4] using glass slide DNA microarrays. In good agreement with our experiments, *recA*, *recN* and *sulA* were the most heavily induced LexA-regulated genes in that study. However, 20 of the 27 known LexA-regulated genes had been found to be induced in a previous study. Except for *ftsK* and *dinJ*, the genes that were not UV-induced in a previous study (i.e., *dinG*, *hokE*, *molR*, *uvrA*, *uvrD*, *ssb* and *ybiA*) were not UV-induced in our experiments. It is possible that some of these genes are UV-inducible only under particular experimental conditions. For example, although a plasmid-encoded *ssb* has been shown to be slightly upregulated under SOS induction, no induction of the chromosomally encoded *ssb* has been reported [14].

In another, similar study by Khil and Camerini-Otero [9], changes in gene expression after treatment of *E. coli* with mitomycin C (MMC), a DNA damaging agent, were assessed using Panorama *E. coli* gene arrays. In that study only a small number of known LexA-regulated genes were induced by MMC (i.e., *dinB*, *dinD*, *sulA*, *recA*, *recN* and *sbmC*). Except for *sbmC*, all the MMC-induced genes were UV-induced in our experiments.

The level of expression of 262 other genes, which are not members of the SOS regulon, was changed (253 upregulated and 9 downregulated) following UV irradiation. Changes in the level of expression of a large number of genes were also observed in [4] (200 genes) and [9] (more than 1000 genes). Three different consequences, at least, could account for the UV-induced modification in the level of expression of a large number of non-LexA-regulated genes: (1) a direct effect of UV irradiation on some cellular components; (2) a cascade of regulatory events triggered by the induction of some SOS genes; and (3) catalytic cleavage of repressors other than LexA by the activated RecA protein. Strikingly, gene expression response patterns observed in the three studies were very different. Only 25 genes, 11 of which are SOS genes, were modified in their level of expression in this work and in [4] (Table 3). Similarly, only 22 genes, 5 of which are SOS genes, were modified in their level of expression in this work and in [9]. Finally, only 6 genes were induced in all three studies: 5 were SOS genes (*sulA*, *recA*, *recN*, *dinD*, *dinP*) and one (*dnaN*) was a non-LexA-regulated gene (Table 3).

Another striking difference between our work and those cited above [4,9] is that we observed a lack of symmetry in the number of genes repressed and induced (Table 2): we found only 9 repressed genes. For numerous genes, a rapid decrease in transcript levels following UV irradiation was observed in [4]. However, this decrease occurred soon after UV irradiation, and then, in the *lexA*$^+$ strain, a number of transcripts recovered pre-irradiation levels prior to 40 min post-irradiation. In our conditions, it is possible that most transcripts with decreased levels following irradiation are not seen because they have recovered their original levels after 30 min of post-irradiation incubation. Interestingly, transcripts of several genes failed to recover in *lexAind*$^-$ mutants within 40 min post-irradiation [4]. This could explain why we did not observe a lack of symmetry in the number of genes repressed and induced in *lexAind*$^-$ mutants (see below). In addition, the fold change of repressed genes did not exceed a factor of 2 and for a number of genes it varied from one experiment to another, so that a large number of repressed genes was not considered significant in the conservative statistical analysis we used. All of the repressed genes were taken into account in [4] and [9] because those authors did not perform statistical analyses (see below).

The large discrepancy between the gene expression response patterns observed in the three studies could possibly be explained by experimental differences. In the three studies, *E. coli* K12 strains of different origins were used. In our study and in [9], experiments were performed in rich medium, whereas in [4] they were performed in minimal medium. As in [9], we used gene-specific primer mixtures for labelling whereas in [4] random primer mixtures were used. It has been shown that the use of gene-specific primer mixtures for labelling results in a decrease in hybridization signals [1]. Moreover, the quality of the arrays used (glass slide versus nylon membranes) might be questioned. In [9], and in our work, the nylon membrane imposed use of radioactive cDNA labelling and separate processing of control and treated samples. In [4] the use of a glass slide enabled labelling of cDNA from control and treated samples with different fluorescent nucleotides in a single reaction, and the simultaneous hybridization and detection of both populations on one array. Finally, data analysis might also be involved. In [4] the fold change in transcript levels was measured versus 6 different times of post-irradiation incubation. No criteria for accuracy of differential gene expression levels

Table 2
Genes with modified expression following UV irradiation in a *lexA*$^+$ genetic context

Gene	b-number	Possible function	Ratio
trpB	b1261	Enzyme; amino acid biosynthesis: tryptophan	12.64
sulA::lacZ	b0958	Enzyme; degradation of small molecules: carbon compounds	7.48
recA	b2699	Enzyme; DNA replication, repair, restriction/modification	7.32
lacA	b0342	Enzyme; degradation of small molecules: carbon compounds	5.88
ibpB	b3686	Factor; adaptations, atypical conditions	5.64
dinI	b1061	Phenotype; not classified	5.26
recN	b2616	Putative enzyme; DNA replication, repair, restriction/modification	4.37
hflX	b4173	Enzyme; degradation of proteins, peptides, glyco	2.82
uvrB	b0779	Enzyme; degradation of DNA	2.80
asnA	b3744	Enzyme; amino acid biosynthesis: asparagine	2.79
valS	b4258	Enzyme; aminoacyl tRNA synthetases, tRNA modification	2.75
tra5_1	b0372	IS, phage, Tn; transposon-related functions	2.62
rpoA	b3295	Enzyme; RNA synthesis, modification, DNA transcription	2.61
yi22_2	b1402	IS, phage, Tn; transposon-related functions	2.60
dinD	b3645	Phenotype; DNA replication, repair, restriction/modification	2.57
ilvG_1	b3767	Enzyme; amino acid biosynthesis: isoleucine, valine	2.52
lacY	b0343	Transport; transport of small molecules: carbohydrates, organic acids, alcohols	2.50
trs5_2	b0552	IS, phage, Tn; transposon-related functions	2.41
clpP	b0437	Enzyme; degradation of proteins, peptides, glyco	2.38
uxuR	b4324	Regulator; degradation of small molecules: carbon compounds	2.38
dinP	b0231	Putative enzyme; not classified	2.36
rpoC	b3988	Enzyme; RNA synthesis, modification, DNA transcription	2.35
b1470	b1470	orf; unknown	2.30
ptr	b2821	Enzyme; degradation of proteins, peptides, glyco	2.30
yfbL	b2271	Putative enzyme; not classified	2.29
malI	b1620	Regulator; degradation of small molecules: carbon compounds	2.29
sdhA	b0723	Enzyme; energy metabolism, carbon: TCA cycle	2.23
rpoB	b3987	Enzyme; RNA synthesis, modification, DNA transcription	2.23
b0362	b0362	orf; unknown	2.21
yeaT	b1799	Putative regulator; not classified	2.18
ompR	b3405	Regulator; global regulatory functions	2.17
uidR	b1618	Regulator; degradation of small molecules: carbon compounds	2.17
bglB	b3721	Enzyme; degradation of small molecules: carbon compounds	2.17
spoT	b3650	Enzyme; global regulatory functions	2.17
malZ	b0403	Enzyme; degradation of small molecules: carbon compounds	2.15
recD	b2819	Enzyme; degradation of DNA	2.13
b1030	b1030	orf; unknown	2.13
dinJ	b0226	Phenotype; not classified	2.13
atpC	b3731	Enzyme; ATP-proton motive force interconversion	2.13
evgS	b2370	Putative regulator; not classified	2.12
b1284	b1284	Putative regulator; not classified	2.11
dsbC	b2893	Enzyme; surface structures	2.11
b1372	b1372	Putative membrane; not classified	2.10
ydeB	b1529	orf; unknown	2.10
pth	b1204	Enzyme; aminoacyl tRNA synthetases, tRNA modification	2.08
yjjQ	b4365	Putative regulator; not classified	2.08
hrsA	b0731	Enzyme; proteins translation and modification	2.07
yhaJ	b3105	Putative regulator; not classified	2.07
yjjG	b4374	Putative enzyme; not classified	2.06
rob	b4396	Factor; DNA replication, repair, restriction/modification	2.05
pflC	b3952	Putative enzyme; energy metabolism, carbon: anaerobic respiration	2.04
b1980	b1980	orf; unknown	2.04
ptrB	b1845	Enzyme; degradation of proteins, peptides, glyco	2.03
yebG	b1848	orf; unknown	2.02
b1327	b1327	orf; unknown	2.02
phoB	b0399	Regulator; global regulatory functions	2.01
yi22_4	b2860	IS, phage, Tn; transposon-related functions	2.01
nfo	b2159	Enzyme; degradation of DNA	2.00
fecA	b4291	Membrane; transport of small molecules: cations	2.00
lexA	b4043	Regulator; global regulatory functions	2.00
ydiF	b1694	Putative enzyme; not classified	1.99
hypF	b2712	Regulator; energy metabolism, carbon: anaerobic respiration	1.98

(*continued on next page*)

Table 2
(Continued)

Gene	b-number	Possible function	Ratio
cyoD	b0429	Enzyme; energy metabolism, carbon: aerobic respiration	1.98
ftsY	b3464	Membrane; cell division	1.98
yafA	b0239	orf; unknown	1.95
rpmC	b3312	Structural component; ribosomal proteins synthesis, modification	1.95
b2496	b2496	Putative factor; not classified	1.95
ruvA	b1861	Enzyme; DNA replication, repair, restriction/modification	1.94
ubiB	b3844	Enzyme; energy metabolism, carbon: electron transport	1.94
gmk	b3648	Enzyme; purine ribonucleotide biosynthesis	1.94
yhiV	b3514	Putative transport; not classified	1.94
aceF	b0115	Enzyme; energy metabolism, carbon: pyruvate dehydrogenase	1.94
yccA	b0970	Putative transport; not classified	1.93
phoH	b1020	Regulator; central intermediary metabolism: phosphorus compounds	1.93
ygfI	b2921	Putative regulator; not classified	1.93
lldP	b3603	Transport; transport of small molecules: carbohydrates, organic acids, alcohols	1.93
deaD	b3162	Enzyme; RNA synthesis, modification, DNA transcription	1.92
ymdC	b1046	Putative enzyme; not classified	1.92
nrdB	b2235	Enzyme; 2′-deoxyribonucleotide metabolism	1.92
mgtA	b4242	Enzyme; transport of small molecules: cations	1.92
b2789	b2789	Putative transport; not classified	1.91
plsC	b3018	Enzyme; macromolecule synthesis, modification: phospholipids	1.91
menF	b2265	Enzyme; biosynthesis of cofactors, carriers: menaquinone, ubiquinone	1.91
cyoC	b0430	Enzyme; energy metabolism, carbon: aerobic respiration	1.91
b1685	b1685	orf; unknown	1.90
yeeY	b2015	Putative regulator; not classified	1.90
modF	b0760	Transport; transport of small molecules: anions	1.89
yhhX	b3440	Putative regulator; not classified	1.89
yafL	b0227	Putative membrane; not classified	1.88
cyaA	b3806	Enzyme; global regulatory functions	1.88
b1567	b1567	orf; unknown	1.88
ydfC	b1573	orf; unknown	1.87
ybhP	b0790	orf; unknown	1.87
napB	b2203	Carrier; energy metabolism, carbon: electron transport	1.87
sfcA	b1479	Enzyme; central intermediary metabolism: gluconeogenesis	1.86
rnt	b1652	Enzyme; degradation of RNA	1.86
sieB	b1353	Phenotype; phage-related functions and prophages	1.86
tolR	b0738	Putative transport; not classified	1.86
yejO	b2190	Putative transport; not classified	1.85
uspA	b3495	Putative regulator; adaptations, atypical conditions	1.85
rnc	b2567	Enzyme; degradation of RNA	1.84
rplO	b3301	Structural component; ribosomal proteins synthesis, modification	1.84
insA_3	b0275	IS, phage, Tn; transposon-related functions	1.83
mLc	b1594	Putative regulator; not classified	1.83
rhlB	b3780	Putative enzyme; not classified	1.83
pncB	b0931	Enzyme; biosynthesis of cofactors, carriers: pyridine nucleotide	1.83
rpoH	b3461	Factor; global regulatory functions	1.83
ybiB	b0800	Putative enzyme; not classified	1.82
yjgD	b4255	orf; unknown	1.82
b1374	b1374	IS, phage, Tn; not classified	1.81
intC	b2349	IS, phage, Tn; not classified	1.81
yjbH	b4029	orf; unknown	1.80
ynfC	b1585	orf; unknown	1.80
purA	b4177	Enzyme; purine ribonucleotide biosynthesis	1.80
b2325	b2325	orf; unknown	1.80
aldB	b3588	Enzyme; degradation of small molecules: carbon compounds	1.79
soxR	b4063	Regulator; global regulatory functions	1.79
ylbF	b0520	Putative enzyme; not classified	1.79
hisD	b2020	Enzyme; amino acid biosynthesis: histidine	1.79
glcC	b2980	Regulator; degradation of small molecules: carbon compounds	1.78
prfC	b4375	Factor; proteins translation and modification	1.78
crl	b0240	Regulator; surface structures	1.77
gutM	b2706	Regulator; degradation of small molecules: carbon compounds	1.77
ycjC	b1299	orf; unknown	1.77

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Table 2
(Continued)

Gene	b-number	Possible function	Ratio
nrdA	b2234	Enzyme; 2′-deoxyribonucleotide metabolism	1.77
yjhF	b4296	Putative transport; not classified	1.76
fucU	b2804	Phenotype; degradation of small molecules: carbon compounds	1.76
thdF	b3706	Putative enzyme; detoxification	1.76
pstA	b3726	Transport; transport of small molecules: anions	1.76
yeiL	b2163	Putative regulator; not classified	1.75
acrB	b0462	Transport; drug/analog sensitivity	1.75
corA	b3816	Transport; transport of small molecules: cations	1.75
recJ	b2892	Enzyme; degradation of DNA	1.75
yeaL	b1789	orf; unknown	1.75
fliG	b1939	Structural component; surface structures	1.74
ebgA	b3076	Enzyme; degradation of small molecules: carbon compounds	1.74
yagD	b0261	Putative enzyme; not classified	1.74
ydfB	b1572	orf; unknown	1.74
ndh	b1109	Enzyme; energy metabolism, carbon: aerobic respiration	1.73
agaW	b3134	Enzyme; central intermediary metabolism: amino sugars	1.73
ascB	b2716	Enzyme; degradation of small molecules: carbon compounds	1.72
leuB	b0073	Enzyme; amino acid biosynthesis: leucine	1.72
yi81_1	b0016	IS, phage, Tn; transposon-related functions	1.72
fabD	b1092	Enzyme; fatty acid and phosphatidic acid biosynthesis	1.72
rpsO	b3165	Structural component; ribosomal proteins synthesis, modification	1.72
b1392	b1392	Putative enzyme; not classified	1.72
dnaN	b3701	Enzyme; DNA replication, repair, restriction/modification	1.71
nupG	b2964	Transport; transport of small molecules: nucleosides, purines, pyrimidines	1.70
ygfS	b2886	Putative enzyme; not classified	1.70
yddB	b1495	orf; unknown	1.70
yaeT	b0177	orf; unknown	1.69
mepA	b2328	Enzyme; murein sacculus, peptidoglycan	1.69
ymcB	b0985	orf; unknown	1.69
b3914	b3914	orf; unknown	1.69
ygeT	b2867	Putative enzyme; not classified	1.69
yjdG	b4124	Putative regulator; not classified	1.69
fucR	b2805	Regulator; degradation of small molecules: carbon compounds	1.69
inaA	b2237	Phenotype; adaptations, atypical conditions	1.68
proA	b0243	Enzyme; amino acid biosynthesis: proline	1.68
yedU	b1967	orf; unknown	1.68
pphA	b1838	Regulator; not classified	1.68
yjiR	b4340	Putative regulator; not classified	1.68
sfmD	b0532	Putative membrane; not classified	1.68
atpE	b3737	Enzyme; ATP-proton motive force interconversion	1.68
mtr	b3161	Transport; transport of small molecules: amino acids, amines	1.68
gltS	b3653	Transport; transport of small molecules: amino acids, amines	1.67
fhuA	b0150	Membrane; outer membrane constituents	1.67
pflD	b3951	Enzyme; energy metabolism, carbon: anaerobic respiration	1.66
nrfG	b4076	Enzyme; energy metabolism, carbon: anaerobic respiration	1.66
yihT	b3881	Putative enzyme; not classified	1.66
yiiF	b3890	orf; unknown	1.66
radC	b3638	Phenotype; not classified	1.65
ybeT	b0647	orf; unknown	1.65
mutY	b2961	Enzyme; DNA replication, repair, restriction/modification	1.65
ccmF	b2196	Putative enzyme; energy metabolism, carbon: electron transport	1.64
tag	b3549	Enzyme; DNA replication, repair, restriction/modification	1.64
nusB	b0416	Factor; RNA synthesis, modification, DNA transcription	1.64
div	b2321	Factor; cell division	1.64
ftsK	b0890	Phenotype; cell division	1.64
trs5_10	b3218	IS, phage, Tn; transposon-related functions	1.64
fecB	b4290	Transport; transport of small molecules: cations	1.63
b1173	b1173	orf; unknown	1.63
thrC	b0004	Enzyme; amino acid biosynthesis: threonine	1.63
yifB	b3765	Putative regulator; not classified	1.63
ybbY	b0513	Putative transport; not classified	1.63
b2253	b2253	Putative enzyme; not classified	1.63

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Table 2
(Continued)

Gene	b-number	Possible function	Ratio
treB	b4240	Transport; transport of small molecules: carbohydrates, organic acids, alcohols	1.63
avtA	b3572	Enzyme; amino acid biosynthesis: alanine	1.63
b0836	b0836	Putative factor; not classified	1.62
nuoL	b2278	Enzyme; energy metabolism, carbon: aerobic respiration	1.62
ykfF	b0249	orf; unknown	1.62
purD	b4005	Enzyme; purine ribonucleotide biosynthesis	1.62
b2372	b2372	Putative factor; not classified	1.61
b0671	b0671	Putative RNA; not classified	1.59
b0753	b0753	Putative regulator; not classified	1.58
yaiH	b0376	Putative enzyme; not classified	1.58
yphG	b2549	orf; unknown	1.58
b2291	b2291	Phenotype; not classified	1.56
b2878	b2878	Putative enzyme; not classified	1.56
ybeQ	b0644	orf; unknown	1.56
pdxY	b1636	Enzyme; biosynthesis of cofactors, carriers: pyridoxine	1.56
uxuB	b4323	Enzyme; degradation of small molecules: carbon compounds	1.56
acrD	b2470	Putative transport; drug/analog sensitivity	1.55
gatR_2	b2090	Regulator; degradation of small molecules: carbon compounds	1.55
focB	b2492	Putative transport; transport of small molecules: carbohydrates, organic acids, alcohols	1.55
ycdB	b1019	orf; unknown	1.55
ydaK	b1339	Putative regulator; not classified	1.55
ydbA_2	b1405	orf; unknown function	1.55
ytfT	b4230	Putative transport; not classified	1.54
yihX	b3885	Putative enzyme; not classified	1.54
yeeA	b2008	orf; unknown	1.54
yeeD	b2012	orf; unknown	1.53
dsrB	b1952	orf; unknown	1.53
yjiH	b4330	orf; unknown	1.53
tsf	b0170	Factor; proteins translation and modification	1.53
dnaQ	b0215	Enzyme; DNA replication, repair, restriction/modification	1.53
b1192	b1192	orf; unknown	1.52
lpxA	b0181	Enzyme; surface polysaccharides and antigens	1.52
yagI	b0272	Putative regulator; not classified	1.52
cspB	b1557	Phenotype; adaptations, atypical conditions	1.52
b1010	b1010	orf; unknown	1.51
yifE	b3764	orf; unknown	1.51
uhpB	b3668	Enzyme; transport of small molecules: carbohydrates, organic acids, alcohols	1.51
uhpC	b3667	Regulator; transport of small molecules: carbohydrates, organic acids, alcohols	1.50
fecE	b4287	Transport; transport of small molecules: cations	1.49
araJ	b0396	Transport; degradation of small molecules: carbon compounds	1.49
nagE	b0679	Enzyme; transport of small molecules: amino acids, amines	1.49
ycjW	b1320	Putative regulator; not classified	1.49
yceH	b1067	orf; unknown	1.48
yadK	b0136	orf; not classified	1.48
ybeF	b0629	Putative regulator; not classified	1.47
b1201	b1201	Putative regulator; not classified	1.47
b1755	b1755	Putative transport; not classified	1.45
yahM	b0327	orf; unknown	1.44
b0395	b0395	orf; unknown	1.44
yedL	b1932	orf; unknown	1.43
yeiE	b2157	Putative regulator; not classified	1.43
yjgU	b4266	Putative enzyme; not classified	1.43
galE	b0759	Enzyme; degradation of small molecules: carbon compounds	1.43
insA_5	b1894	IS, phage, Tn; transposon-related functions	1.43
yacL	b0119	orf; unknown	1.43
cysD	b2752	Enzyme; central intermediary metabolism: sulfur metabolism	1.42
mtlR	b3601	Regulator; degradation of small molecules: carbon compounds	1.42
ynaE	b1375	orf; unknown	1.42
ychH	b1205	orf; unknown	1.41
yjdC	b4135	orf; unknown	1.40
ymfO	b1151	orf; unknown	1.40
aceE	b0114	Enzyme; energy metabolism, carbon: pyruvate dehydrogenase	1.40

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Table 2
(Continued)

Gene	b-number	Possible function	Ratio
galS	b2151	Regulator; degradation of small molecules: carbon compounds	1.39
b1771	b1771	orf; unknown	1.39
prsA	b1207	Enzyme; purine ribonucleotide biosynthesis	1.39
yjcU	b4085	Putative enzyme; not classified	1.39
yjiL	b4334	Putative enzyme; not classified	1.39
cchA	b2457	Phenotype; not classified	1.37
sucD	b0729	Enzyme; energy metabolism, carbon: TCA cycle	1.37
amyA	b1927	Enzyme; degradation of polysaccharides	1.36
mrdA	b0635	Enzyme; cell division	1.35
ftsI	b0084	Enzyme; cell division	1.35
ybfE	b0685	orf; unknown	1.35
ebgR	b3075	Regulator; degradation of small molecules: carbon compounds	1.35
b2362	b2362	orf; unknown	1.34
yfcU	b2338	Putative membrane; not classified	1.34
fnr	b1334	Regulator; global regulatory functions	1.34
yahL	b0326	orf; unknown	1.33
lhr	b1653	Enzyme; DNA replication, repair, restriction/modification	1.33
yiaC	b3550	orf; unknown	1.33
ytfP	b4222	orf; unknown	1.31
aspS	b1866	Enzyme; aminoacyl tRNA synthetases, tRNA modification	1.30
gatD	b2091	Enzyme; degradation of small molecules: carbon compounds	−1.43
fliK	b1943	Structural component; surface structures	−1.44
hycE	b2721	Enzyme; energy metabolism, carbon: fermentation	−1.47
rpsV	b1480	Putative structure; not classified	−1.51
ykgK	b0294	Putative regulator; not classified	−1.63
b1648	b1648	orf; unknown	−1.68
barA	b2786	Enzyme; global regulatory functions	−1.82
fimI	b4315	Structural component; surface structures	−1.83
cspC	b1823	Phenotype; not classified	−1.97

LexA binding site is indicated in bold.
The b-number is the number for each ORF in the *E. coli* genome (Blattner et al. 1997).
The ratio is the fold increase in transcript level in treated culture compared to untreated culture.

was given. It was only the kinetic patterns which indicated whether the expression of a given gene was changed. The fold change in transcript level was also measured at various times of incubation post-treatment in [9] and genes showing more dramatic changes (threefold or higher) were selected and submitted to cluster analysis. Thus no statistical significance tests were applied to the results of studies [4] and [9]. It has been pointed out that there was little correlation between the fold change in transcript levels and the accuracy of differential gene expression levels [1]. The statistical analysis of our data can lower the number of detected genes but provides more conservative results. However, when the inducibility of the SOS genes is taken as a guarantee of quality, the experiments of Courcelle [4] give the most impressive results: both the number of SOS genes induced and the induction factors observed were higher than in our own experiments.

3.2. *Effects of an SOS non-inducible* (lexAind$^-$) *context on gene expression*

In the *lexAind*$^-$ strain (PQ200), in which UV cannot induce the SOS system because of a mutation introducing an amino acid change at a position essential for cleavage and inactivation of the LexA protein [10], changes in gene expression after UV irradiation were measured in 3 independent experiments (i.e., 6 replicates). UV irradiation promoted a significant increase in the expression of only 15 genes and a decrease in the expression of one gene. As expected, no significant increase in the expression of LexA-regulated genes or genes containing a potential LexA binding site was observed (Table 4).

Compared to the 277 genes whose expression was affected by UV effects upon the *lexA*$^+$ strain, expression of only 16 genes was affected by UV in the *lexAind*$^-$ mutant. This could indicate that the modification in gene expression observed in a *lexA*$^+$ strain might be essentially due to induction of LexA-regulated genes, triggering a cascade of regulatory events. In addition, the *lexAind*$^-$ mutation largely affects the ability of cells to survive UV irradiation [15]. It is possible that UV-induced DNA damage which is not repaired in the *lexAind*$^-$ cells leads to a decrease in the transcription rate and lowers the ability to detect genes with changed expression.

Two genes (*dnaN* and *b1392*, an ORF with unknown function) showed an increase in their expression in both the UV-irradiated *lexAind*$^-$ strain (PQ200) and the *lexA*$^+$ (PQ65) strain. This result indicates that these 2 genes could be induced in a LexA-independent manner as a direct consequence of UV irradiation rather than as a consequence

Table 3
Comparison of genes with modified expression following DNA damage obtained in 3 similar studies

Gene	b-number	Possible function	Ratio		
			a (UV)	b (UV)	c (MMC)
sulA*::*lacZ	b0958	Enzyme; degradation of small molecules: carbon compounds	7.48	9.56	+
recA	b2699	Enzyme; DNA replication, repair, restriction/modification	7.32	10.08	+
recN	b2616	Putative enzyme; DNA replication, repair, restriction/modification	4.37	20.18	+
dinD	b3645	Phenotype; DNA replication, repair, restriction/modification	2.57	10.5	+
dinP	b0231	Putative enzyme; not classified	2.36	7.75	+
dnaN	b3701	Enzyme; DNA replication, repair, restriction/modification	1.71	1.68	+
dinI	b1061	Phenotype; not classified	5.26	4.46	
uvrB	b0779	Enzyme; degradation of DNA	2.80	4.14	
yebG	b1848	orf; unknown	2.02	8.85	
lexA	b4043	Regulator; global regulatory functions	2.00	4.8	
ruvA	b1861	Enzyme; DNA replication, repair, restriction/modification	1.94	3.55	
ybfE	b0685	orf; unknown	1.35	1.68	
ibpB	b3686	Factor; adaptations, atypical conditions	5.64	2.22	
deaD	b3162	Enzyme; RNA synthesis, modification, DNA transcription	1.92	1.48	
nrdB	b2235	Enzyme; 2′-deoxyribonucleotide metabolism	1.92	2.11	
modF	b0760	Transport; transport of small molecules: anions	1.89	0.67	
prfC	b4375	Factor; proteins translation and modification	1.78	1.66	
nrdA	b2234	Enzyme; 2′-deoxyribonucleotide metabolism	1.77	2.05	
corA	b3816	Transport; transport of small molecules: cations	1.75	1.8	
yddB	b1495	orf; unknown	1.70	0.38	
yeeA	b2008	orf; unknown	1.54	1.75	
yacL	b0119	orf; unknown	1.43	1.5	
aspS	b1866	Enzyme; aminoacyl tRNA synthetases, tRNA modification	1.30	1.66	
gatR_2	b2090	Regulator; degradation of small molecules: carbon compounds	1.55	0.76	
gatD	b2091	Enzyme; degradation of small molecules: carbon compounds	−1.43	−2.78	
asnA	b3744	Enzyme; amino acid biosynthesis: asparagine	2.79		+
sdhA	b0723	Enzyme; energy metabolism, carbon: TCA cycle	2.23		+
b1284	b1284	Putative regulator; not classified	2.11		+
ygfI	b2921	Putative regulator; not classified	1.93		+
lldP	b3603	Transport; transport of small molecules: carbohydrates, organic acids, alcohols	1.93		+
uspA	b3495	Putative regulator; adaptations, atypical conditions	1.85		+
purA	b4177	Enzyme; purine ribonucleotide biosynthesis	1.80		+
aldB	b3588	Enzyme; degradation of small molecules: carbon compounds	1.79		+
pstA	b3726	Transport; transport of small molecules: anions	1.76		+
yagD	b0261	Putative enzyme; not classified	1.74		+
nuoL	b2278	Enzyme; energy metabolism, carbon: aerobic respiration	1.62		+
purD	b4005	Enzyme; purine ribonucleotide biosynthesis	1.62		+
ycdB	b1019	orf; unknown	1.55		+
yihX	b3885	Putative enzyme; not classified	1.54		+
nagE	b0679	Enzyme; transport of small molecules: amino acids, amines	1.49		+
sucD	b0729	Enzyme; energy metabolism, carbon: TCA cycle	1.37		+

Genes are sorted according to the ratio obtained in our own experiments (a).
LexA-regulated genes are indicated in bold.
The b-number is the number for each ORF in the *E. coli* genome (Blattner et al. 1997).
The ratio is the fold increase in transcript level in treated culture compared to untreated culture.
(a) Results from this study.
(b) Results from Courcelle et al. 2001.
(c) Results from Khil and Camerini-Otero, 2002.

of the induction of the SOS genes. The observed increase in the transcript level of *dnaN* in the UV-irradiated *lexA*$^+$ strain can be explained. The *dnaN* gene encodes the beta subunit of a DNA polymerase III holoenzyme that functions as a sliding DNA clamp responsible for tethering the polymerase to DNA and endowing it with the high processivity required for DNA replication [18]. It has been found that UV irradiation of *E. coli* induces the synthesis of a smaller form of the beta subunit, transcribed from a UV-inducible internal gene located in-frame inside the coding region of *dnaN* [13,19,20]. However, UV induction was abolished in a strain carrying a *lexAind*$^-$ mutation, although a binding site for the LexA repressor was not present in the *dnaN* gene and no specific binding of LexA to the *dnaN* promoter could be detected [13]. Since, in our experiments, an increase in the transcript level of *dnaN* was also observed in the UV-irradiated *lexAind*$^-$ mutant, our results argue for a LexA-independent induction process. An increase in the transcript level of *dnaN* was also observed in both UV-irradiated *lexA*$^+$ and *lexAind*$^-$ strains in a similar study [4] and in an MMC-

Table 4
Genes with modified expression following UV irradiation in a *lexAind*$^-$ context

Gene	b-number	Possible function	Ratio
minE	b1174	Factor; cell division	1.66
b1590	b1590	Putative enzyme; degradation of small molecules: carbon compounds	1.66
yjgG	b4247	orf; unknown	1.65
b1392	b1392	Putative enzyme; not classified	1.59
ykgA	b0300	Putative regulator; not classified	1.59
ybcR	b0554	orf; unknown	1.57
hlyE	b1182	Phenotype; not classified	1.55
pheL	b2598	Leader; amino acid biosynthesis: phenylalanine	1.51
ycgB	b1188	Putative factor; not classified	1.47
ymgC	b1167	orf; unknown	1.43
mopB	b4142	Factor; chaperones	1.40
b1582	b1582	orf; unknown	1.40
dnaN	b3701	Enzyme; DNA replication, repair, restriction/modification	1.34
cysI	b2763	Enzyme; central intermediary metabolism: sulfur metabolism	1.31
ackA	b2296	Enzyme; energy metabolism, carbon: electron transport	1.31
tnaA	b3708	Enzyme; degradation of small molecules: amino acids	−2.24

Genes are sorted according to the ratio.
The b-number is the number for each ORF in the *E. coli* genome (Blattner et al. 1997).
The ratio is the fold increase in transcript level in UV-irradiated culture compared to untreated culture.

treated *lexA*$^+$ strain [9]. It was the only non-LexA-regulated gene induced by DNA damage in all 3 studies (Table 3). These observations makes *dnaN* the only gene possibly UV-induced in a LexA-independent manner.

The effects of a *lexAind*$^-$ mutation in the absence of any treatment were also examined. The expression profile of *lexA*$^+$ PQ65 cultures was compared to that of *lexAind*$^-$ PQ20 cultures. A significant modification in the expression of 440 genes (214 genes with increased expression and 226 genes with decreased expression) could be observed between the *lexAind*$^-$ mutant and the *lexA*$^+$ strains (data not shown). A decrease in the expression of 2 SOS genes (*uvrD* and *dinD*) and two genes with potential LexA binding sites (*yaFL* and *ybiT*) was observed. No increase in the expression of SOS or potential SOS genes could be observed. This result could indicate that the *lexAind*$^-$ mutant repressor binds more tightly than the wild type repressor to some LexA binding sites. In addition, the large number of non-SOS genes whose expression was modified by the *lexAind*$^-$ mutation, in the absence of UV irradiation, could indicate that the LexA protein can bind to a site other than the well-characterized SOS boxes and thereby modify the expression of genes which are not members of the SOS regulon.

3.3. *Effects of an SOS constitutive* (lexAdef) *context on gene expression*

The expression profile of the *lexA*$^+$ culture PQ65 was compared to that of a *lexAdef* mutant, PQ210, deficient in the LexA repressor and thereby constitutive in the expression of the SOS genes [8]. The changes in gene expression were compared in 3 independent experiments (i.e., 6 replicates per strain). A significant modification in the expression of more than 1000 genes could be observed between the *lexAdef* mutant and the *lexA*$^+$ strains. The expression of 503 genes was increased whereas the expression of 685 genes was decreased in the *lexAdef* mutant (data not shown).

The modification in the expression of such a considerable number of genes, in the absence of the LexA repressor, could result from a cascade of regulatory events as a consequence of the overexpression of some lexA-regulated SOS genes. Such an interpretation is consistent with the observation that: (i) the expression of only a small number of genes was modified following UV irradiation in a *lexAind*$^-$ genetic context, in which the SOS genes cannot be overexpressed; and (ii) the number of genes whose expression was modified following UV irradiation in a *lexA*$^+$ genetic context, in which the SOS genes could not be fully induced, is lower than in the *lexAdef* mutant, constitutive in the expression of the SOS genes. It should be noted that unlike the *lexAind*$^-$ mutant, the ability of cells to survive UV irradiation is similar in *lexAdef* and *lexA*$^+$ cells. However, the expression of only 32 genes, 8 of which were SOS genes or potential SOS genes, was modified both in *lexA*$^+$ genetic context following UV irradiation and in a *lexAdef* genetic context (Table 5). Among the 503 genes whose expression was increased in the *lexAdef* mutant, 14 are genes known to belong to the SOS regulon and 6 are genes containing a potential LexA binding site (i.e., 20 SOS genes or potential SOS genes) (Table 6). Except for *recN*, the most heavily UV-induced LexA-regulated genes (*sulA*::*lacZ*, *recA*, and *dinI*) were also the most heavily overexpressed in the *LexAdef* mutant. This result is far from what was expected. We expected that overexpression of most of the known LexA-regulated genes (27 genes) would be observed, especially since, in this experiment, the two strains (*lexA*$^+$ and *lexAdef*) are isogenic, and the cultures are grown under similar conditions and are independent of any treatments.

One possible explanation is that, under our conditions and/or because of the strain used, partial induction of the

Table 5
Genes with modified expression in at least 2 of the 3 genetic contexts examined, *lexA*$^+$, *lexAdef* or *lexAind*$^-$

Gene	b-number	Functional categories	Ratio		
			lexA$^+$ (UV) / *lexA*$^+$	*lexAdef* / *lexA*$^+$	*lexAind*$^-$ (UV) / *lexAind*$^-$
hlyE	b1182	Phenotype; not classified	(n.s.)	3.93	1.55
trpB	b1261	Enzyme; amino acid biosynthesis: tryptophan	12.64	29.22	(n.s.)
sulA::lacZ	b0344	Enzyme; degradation of small molecules: carbon compounds	7.48	9.74	(n.s.)
recA	b2699	Enzyme; DNA replication, repair, restriction/modification	7.32	4.45	(n.s.)
dinI	b1061	Phenotype; not classified	5.26	19.40	(n.s.)
uvrB	b0779	Enzyme; degradation of DNA	2.80	2.70	(n.s.)
dinD	b3645	Phenotype; DNA replication, repair, restriction/modification	2.57	6.36	(n.s.)
dinP	b0231	Putative enzyme; not classified	2.36	2.54	(n.s.)
b1030	b1030	orf; unknown	2.13	4.25	(n.s.)
yjjG	b4374	Putative enzyme; not classified	2.06	3.04	(n.s.)
yafL	b0227	Putative membrane; not classified	1.88	2.60	(n.s.)
b1374	b1374	IS, phage, Tn; not classified	1.81	2.01	(n.s.)
ynfC	b1585	orf; unknown	1.80	2.91	(n.s.)
dnaN	b3701	Enzyme; DNA replication, repair, restriction/modification	1.71	(n.s.)	1.34
b3914	b3914	orf; unknown	1.69	2.68	(n.s.)
sfmD	b0532	Putative membrane; not classified	1.68	3.22	(n.s.)
gltS	b3653	Transport; transport of small molecules: amino acids, amines	1.67	2.62	(n.s.)
yihT	b3881	Putative enzyme; not classified	1.66	2.46	(n.s.)
b0753	b0753	Putative regulator; not classified	1.58	1.75	(n.s.)
yaiH	b0376	Putative enzyme; not classified	1.58	2.84	(n.s.)
b2878	b2878	Putative enzyme; not classified	1.56	1.59	(n.s.)
ybeQ	b0644	orf; unknown	1.56	4.55	(n.s.)
ytfT	b4230	Putative transport; not classified	1.54	3.04	(n.s.)
b1192	b1192	orf; unknown	1.52	2.82	1.59
yagI	b0272	Putative regulator; not classified	1.52	3.27	(n.s.)
b1010	b1010	orf; unknown	1.51	4.01	(n.s.)
uhpB	b3668	Enzyme; transport of small molecules: carbohydrates, organic acids, alcohols	1.51	4.93	(n.s.)
ybeF	b0629	Putative regulator; not classified	1.47	2.90	(n.s.)
b0395	b0395	orf; unknown	1.44	2.64	(n.s.)
yeiE	b2157	Putative regulator; not classified	1.43	1.82	(n.s.)
cysD	b2752	Enzyme; central intermediary metabolism: sulfur metabolism	1.42	5.19	(n.s.)
galS	b2151	Regulator; degradation of small molecules: carbon compounds	1.39	2.96	(n.s.)
ybfE	b0685	orf; unknown	1.35	4.57	(n.s.)
hlyE	b1182	Phenotype; not classified	(n.s.)	3.93	1.55
cspC	b1823	Phenotype; not classified	−1.97	−1.56	(n.s.)

Genes are sorted according to the ratio measured in the UV-irradiated *lexA*$^+$ culture.
Known LexA-regulated genes or genes containing a potential LexA-binding site are indicated in bold.
The b-number is the number for each ORF in the *E. coli* genome (Blattner et al. 1997).
The ratio is the fold increase in transcript level in the UV-irradiated culture compared to the unirradiated culture (in the *lexA*$^+$ and *lexAind*$^-$ genetic context) or the mutant compared to the non-mutant culture (in the *lexAdef* genetic context).
(n.s.) = non-significant.

SOS system occurs in the *lexA*$^+$ genetic context and in the absence of UV irradiation. If such an effect did occur, overexpression of SOS genes in a *lexAdef* genetic context would be lower than expected and even hidden for some of these genes. To test this possibility we compared the expression profile of the *lexAind* culture, PQ200, to that of a *lexAdef* mutant, PQ210. This comparison showed that two SOS genes (*yebG* and *recN*) and two genes containing a potential LexA binding site (*ydbH* and *tyrS*) were overexpressed, whereas their overexpression was not evidenced when the expression profile of the *lexA*$^+$ culture was compared to that of the *lexAdef* mutant (Table 6). However some genes overexpressed when the expression profile of the *lexA*$^+$ genetic context was compared to that of the *lexAdef* mutant could not be evidenced when the expression profiles of the *lexAind*$^-$ culture and of the *lexAdef* mutant were compared. It is possible that the *sulA* mutation present in our strain prevents the expression of some SOS genes.

Surprisingly, 2 genes known to be SOS-regulated (*yjiW* and *ydjM1*) and 2 genes containing a potential LexA binding site showed a decrease in their expression in the *lexAdef* mutant. This result, which is not easily interpretable without involving complex regulation pathways, could also reveal the presence of false-positive results that parasitize DNA array experiments.

Altogether, these results suggest that the induction of individual SOS genes is possibly more complex than previ-

Table 6
LexA-regulated genes and genes containing a potential LexA-binding site with modified expression in a *lexAdef* genetic context

Gene	b-number	Possible function	Ratio	
			a	b
dinI	b1061	Phenotype; not classified	19.40	14.81
sulA::lacZ	b0958	Enzyme; degradation of small molecules: carbon compounds	9.74	7.56
uvrA	b4058	Enzyme; DNA replication, repair, restriction/modification	7.03	
brnQ	b0401	Transport; transport of small molecules: amino acids, amines	6.82	
dinD	b3645	Phenotype; DNA - replication, repair, restriction/modification	6.36	3.12
yfiE	b2577	Putative regulator; not classified	5.70	
molR_2	b2116	Regulator; biosynthesis of cofactors, carriers: molybdopterin	5.32	
t150	b3558	IS, phage, Tn; transposon-related functions	5.28	
sbmC	b2009	orf; unknown function	5.16	6.32
ycgL	b1179	orf; unknown	4.62	3
ybfE	b0685	orf; unknown	4.57	
recA	b2699	Enzyme; DNA replication, repair, restriction/modification	4.45	11.89
molR_3	b2117	Regulator; biosynthesis of cofactors, carriers: molybdopterin	3.90	
umuD	b1183	Putative enzyme; DNA replication, repair, restriction/modification	3.09	2.25
molR_1	b2115	Regulator; biosynthesis of cofactors, carriers: molybdopterin	2.90	
uvrB	b0779	Enzyme; degradation of DNA	2.70	
yafL	b0227	Putative membrane; not classified	2.60	
dinP	b0231	Putative enzyme; not classified	2.54	
ybiT	b0820	Putative transport; not classified	2.39	
metR	b3828	Regulator; amino acid biosynthesis: methionine	1.77	
yebG	b1848	orf; unknown		3.03
recN	b2616	Putative enzyme; DNA replication, repair, restriction/modification		3
umuC	b1184	SOS mutagenesis and repair		3.11
ydbH	b1381	orf, hypothetical protein		2.1
tyrS	b1637	Tyrosine tRNA synthetase		2.27
yjiW	b4347	orf; unknown		- 2.5
ydjM1	b1728	orf; unknown	−1.86	
b3020	b3020	Putative transport; not classified	−1.97	
yi81_2	b0582	IS, phage, Tn; transposon-related functions	−2.17	

Genes are sorted according to the ratio.
Known LexA-regulated genes are indicated in bold. The others are genes containing a potential LexA binding site.
The b-number is the number for each ORF in the *E. coli* genome (Blattner et al. 1997).
The ratio is the fold increase in transcript level, (a) in the *lexAdef* culture compared to the *lexA*$^+$ culture or (b) in the *lexAdef* culture compared to the *lexAind*$^-$ culture.

ously thought. It is possible that for some LexA-regulated genes the absence of a LexA repressor is not sufficient to observe an increase in their expression. However, we cannot exclude that the array experiments generate a large number of false–negative as well as false–positive results.

3.4. Identification of SOS genes by membrane arrays

Finally, if one defines an SOS gene as a gene that exhibits the following responses—(i) basal expression in a *lexA*$^+$ cell enhanced by UV irradiation; (ii) basal expression in a *lexAind*$^-$ mutant but not enhanced by UV irradiation; (iii) expression at a derepressed level in a *lexAdef* mutant irrespective of whether the cell has been UV-irradiated—then our array experiments led us to correctly identify 7 SOS genes (*sulA, recA, dinI, uvrB, dinD, dinP* and *ybfE*) out of the 27 known SOS genes. To this list might be added 2 genes, *recN* and *yebG*, for which the overexpression in the *lexAdef* mutant could be seen by comparison to the *lexAind*$^-$ mutant. One gene (*yafL*), containing a LexA binding site but not previously shown to be LexA regulated, was identified as an SOS gene.

On the other hand, we did not observe upregulation of several other known SOS genes in our experiments. In some cases it is possible that lack of sensitivity in the cDNA array hybridization did not allow the detection of SOS genes that are weakly UV-induced. Surprisingly, the *umuC* gene could not be identified as an SOS gene in our experiments, nor in the experiments of Khil and Camerini-Otero [9], although it is highly UV-inducible [4,23]. The UV doses required for *umuC* induction and the kinetics of induction are similar to those observed for the induction of the *sulA* gene, an SOS gene strongly UV-induced in our experiments. This result could reveal some manufacturing defects in the arrays.

In addition, 22 genes, not previously found to contain a LexA binding site or to be LexA-regulated corresponded to the above definition of the SOS genes. Most of them are unknown ORFs or putative proteins. One of them, *trpB*, was one of the most UV-inducible and one of the most overexpressed in the *lexAdef* mutant. Since the *trpB* gene is included in the Mud(Ap *lac*) bacteriophage vector [3], it will be transcribed upon induction of the SOS response as a part of the *sulA*::*lacZ* fusion.

3.5. Conclusions

In conclusion, the use of DNA arrays allowed us to clearly display 9 of 27 known SOS genes. In addition, our data suggest that regulation of some SOS genes might be more complex than previously thought. The comparison of our results with similar studies previously published showed a large discrepancy between the gene expression response patterns observed in the three studies. This discrepancy could be explained by the occurrence of a large number of false-positive results that arise from DNA array experiments and obscure the final data. A result can be considered as highly significant when similar changes in the expression of one gene are found in several similar studies. Thus, *dnaN*, encoding the beta subunit of DNA polymerase III holoenzyme, can be clearly identified as a UV-inducible gene regulated in the LexA-independent manner.

Acknowledgements

This work was supported by the Programme de Recherche Fondamentale en Microbiologie et Maladies Infectieuses et Parasitaires (PRFMMIP microbio 2000) which is subsidized by the Ministère de l'Education nationale, de la Recherche et de la Technologie. We would like to thank Michael Herdman (Unité des Cyanobactéries, Institut Pasteur) for his help in the language revision and correction of this article.

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