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Global Impact of *sdiA* Amplification Revealed by Comprehensive Gene Expression Profiling of *Escherichia coli*

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In *Escherichia coli* the amplification of *sdiA*, a positive activator of *ftsQAZ*, genes that are essential for septation, results in mitomycin C resistance. To help us understand this resistance phenotype, genes whose expression was altered by increased *sdiA* dosage were identified using a DNA microarray-based, comprehensive transcript profiling method. The expression of 62 genes was reduced by more than threefold; of these, 41 are involved in motility and chemotaxis. Moreover, the expression of 75 genes, 36 of which had been previously characterized, was elevated at least threefold. As expected, increased *sdiA* dosage led to significantly elevated *sdiA* and *′ddlB-ftsQAZ-lpxC* operon expression. Transcription of two genes, *uvrY* and *uvrC*, located downstream of *sdiA* and oriented in the same direction, was elevated about 10-fold, although the intervening gene, *yecF*, of opposite polarity was unaffected by increased *sdiA* dosage. Three genes (*mioC* and *gidAB*) flanking the replication origin, *oriC*, were transcribed more often when *sdiA* dosage was high, as were 12 genes within 1 min of a terminus of replication, *terB*. Transcription of the *acrABDEF* genes, mapping in three widely spaced loci, was elevated significantly, while several genes involved in DNA repair and replication (e.g., *nei, recN, mioC*, and *mcrC*) were moderately elevated in expression. Such global analysis provides a link between septation and the response to DNA-damaging agents.

A key early element in cell division is the formation of a septation ring composed of FtsZ polypeptides (19). FtsZ levels must be buffered; overproduction in *Escherichia coli* leads to a hyperdivision phenotype, while an FtsZ deficiency leads to filamentation (19). Transcription of *ftsZ* is regulated in a complex manner (see the schematic in Fig. 1 [34]). The pQ1 promoter is *rpoS* regulated, while the pQ2 promoter is activated by the *sdiA*-encoded protein. The pA promoter is responsive to the RcsB-RcsC two-component system (13). SdiA is homologous to LuxR (34, 38), the quorum-sensing positive activator of *Vibrio fischeri* luminescence (21).

Besides the *ftsQAZ* genes, the *sdiA* gene product of *Salmonella enterica* serovar Typhimurium positively regulates expression of several genes of unknown function resident in an operon on its virulence plasmid (1), suggesting that its action is pleiotropic. Such pleiotropy has also been suggested by the findings that multicopy plasmids harboring *sdiA* overcome the inhibitory action of mitomycin C (40), a DNA-damaging agent that intercalates and forms adducts with the genetic material (36). Together, these results suggest definition of the *sdiA* controlled modulon (23) as a worthwhile exercise since interconnections between cell division, virulence, and DNA metabolism may be unearthed. To this end, we have utilized a recently described *E. coli* whole-genome, high-density microarray method (39) for obtaining comprehensive expression profiles of strains with either a normal or an elevated dose of *sdiA*.

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MATERIALS AND METHODS

Strains and growth conditions. The strains used in this work were *E. coli* K-12 derivatives (Table 1). They were grown at 37°C in Luria-Bertani (LB) broth (8). When necessary, 150 μg of ampicillin per ml was included in the medium. Soft-agar plates contained 0.3% agar. Liquid cultures were aerated by rotary shaking at 250 rpm. Strains DPD2668 and DPD2669 were grown overnight in LB broth with ampicillin before subculturing by a 250-fold dilution in the same medium. The cells were rapidly collected for total RNA extraction (39) when the culture reached an optical density at 600 nm of 0.45. Electrotransformation was used to introduce plasmids into host strains (32).

RNA purification, cDNA labeling, and hybridization to DNA microarrays. These methods have been described previously (39). Total RNA was purified from cell pellets using Qiagen RNeasy Mini Columns (Qiagen, Inc., Valencia, Calif.), with slightly revised protocols. Next, 25 μg of purified total RNA was used as a template for cyanine (Cy3)-labeled cDNA synthesis using random hexamers as primers. cDNA synthesis incorporating Cy5-labeled nucleotide and hybridization with an *E. coli* whole-genome, high-density microarray were performed as described elsewhere (39).

Determination of specific transcripts levels by quantitative, real-time PCR following reverse transcription. Bulk cDNA samples were synthesized from total RNA derived from strains DPD2668 and DPD2669 using TaqMan Reverse Transcription Reagents (PE Applied Biosystems, Foster City, Calif.) and random hexamers as primers. Specific primer pairs were designed with the ABI PRISM Primer Express software (PE Applied Biosystems) for several genes as listed in Table 2. The genetic nomenclature of Blattner et al. (6) was followed. A real-time PCR reaction was performed with each specific primer pair using SYBR Green PCR Master Mix (PE Applied Biosystems). Equal amounts of cDNA (0.55 μg), derived from each bulk RNA sample, were used as the initial template in amplification reactions. The reactions were run on an ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems), during which the fluorescence signal from SYBR Green intercalation was monitored to quantify the double-stranded DNA product formed after each PCR cycle. The threshold cycle (Ct) is the first cycle for which a statistically significant increase in the amount of the PCR product is detected. Ct values are thus inversely proportional to the amount of the RNA species in the original bulk RNA sample. The Ct was determined for each amplification reaction. ΔCt between samples derived from DPD2668 and DPD2669 was calculated for each tested gene. Since PCR prod-

TABLE 1. Strains and plasmids

Strain or plasmid	Genotype	Reference
E. coli		
RFM443	*rpsL200 galK2 lac*Δ*74*	22
DPD2668	RFM443/pUC19	40
DPD2669	RFM443/pDEW140	40
Plasmid		
pDEW140	pUC19 plus *sdiA* (*Eco*RI)	40

ucts double with each amplification cycle, the fold difference in the initial concentration of each transcript equals $2^{\Delta Ct}$.

Motility tests. Fresh single colonies of strain DPD2668 or strain DPD2669 were picked and stabbed to the center of LB soft-agar plates supplemented with ampicillin. The plates were incubated at 37°C. The sizes of the swarm zones were compared both after 8 h and after overnight incubation.

Determination of MICs in liquid medium. Cultures were grown in LB medium supplemented with 100 μg ampicillin per ml to ca. 3×10^8 cells/ml. After a 1:1,000 dilution in the same medium, 50-μl aliquots (ca. 15,000 CFU) were inoculated into the wells of a microtiter plate seeded with an equal volume of the same medium containing a second drug whose MIC was to be determined. Tetracycline, nalidixic acid, rifampin, kanamycin sulfate, chloramphenicol, and spectinomycin were tested in duplicate, twofold dilution series yielding the final concentration ranges of 10 to 0.078, 20 to 0.16, 50 to 0.39, 25 to 0.2, 20 to 0.16, and 100 to 0.78 μg/ml, respectively. The negative controls contained medium that was not supplemented with a second drug. After a static, overnight incubation in a humidified chamber, the absorbance of each well at 590 nm was read using a PE HTS 7000 Plus BioAssay Reader (Perkin-Elmer, Boston, Mass.) running the program HTSoft1.0. In this assay, MICs were defined as the lowest concentration of a drug reducing the final culture absorbance by a factor of 2.

RESULTS AND DISCUSSION

Amplification of *sdiA* has global impact on gene expression. DPD2668 has a single, chromosomal copy of *sdiA* and harbors pUC19, while DPD2669 bears *sdiA* on both the chromosome and pDEW140 (40), a pUC19-based, high-copy plasmid. The growth rates of the two strains were indistinguishable (data not shown). The comprehensive transcript profiles of the two strains from exponential-phase, broth-grown cultures were compared. The presence of *sdiA* in high copy elevated the expression of ca. 9% of the *E. coli* protein encoding genes (open reading frames [ORFs]) by a factor of at least 2 while more than 2% of the ORFs appeared to be repressed by a factor of at least 2. Table 3 lists genes whose expression was elevated at least threefold due to the *sdiA* plasmid, while Table 4 indicates genes that were repressed threefold or more by the presence of a high *sdiA* copy. These tables follow the functional groupings proposed by Riley and Labedan (28) and used previously in analyses of microarray experiments (39).

Elevated level of *sdiA* transcripts due to gene amplification. The presence of pDEW140, the pUC19 derivative harboring the *sdiA* promoter and ORF, resulted in a 30-fold elevation in the detection of *sdiA* transcripts. Thus, an increased gene dosage elevated the amount of the cognate mRNA.

Enhanced expression of genes near *sdiA*. Amplification of *sdiA* elevated the expression of two genes downstream of *sdiA*; the *uvrY* transcript was increased 12-fold, while expression of *uvrC* increased by a factor of 9. These two genes are transcribed in the same direction as *sdiA* (6). *uvrC* specifies a subunit of an excision nuclease that removes bulky lesions (30), while *uvrY* encodes a cognate response regulator to the BarA sensor kinase (D. Georgillis, A. K. Pernestig, S. J. Normark, and O. Melefors, Abstr. 100th Gen. Meet. Am. Soc. Microbiol. 2000, abstr. H89, p. 368). Both BarA and UvrY are needed for the production of extracellular siderophores (Georgillis et al., Abstr. 100th Gen. Meet. Am. Soc. Microbiol. 2000). Within the large region (751 bp) between *sdiA* and *uvrYC* is predicted to be an intervening gene, *yecF*, of opposite transcriptional polarity (6). The expression of *yecF* was unaffected by *sdiA* amplification. The lack of a heightened *yecF* hybridization signal in response to *sdiA* amplification suggests that *sdiA* and *uvrYC* are not cotranscribed. In analogy to the adjacent genomic locations of regulatory gene, target operon pairs such as *lacI* with *lacZYA* (4), and *araBAD* with *araC* (33), SdiA may directly activate *uvrYC* transcription.

Elevated expression of the *ftsZ*-containing *operon*. *sdiA* encodes a positive activator that drives transcription of the *ftsQAZ* genes from promoter Q2 located within the upstream

TABLE 2. Primer pairs used to compare transcript levels

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
acrA	CAGGCGCAGGAAGTCGTT	CAACCGTGCAGCAACTTGAT
acrB	CAGGGTGCTTCGCTGCTT	GCCTTGGTCACGAAAAACTGA
acrR	GCGTCGCGCAGCAATTAT	GGCAAAGAGCCAGTTTTCCA
b0517	GCCTGCTGCACAAAATAAGAGA	TTGCGGTGGTCGGTATCAG
b1606	CGCGGTAGCGACAAACATATT	ACGAGCGGGTCATATTATCCA
b1707	AGCATGACGCGGGTGAA	AGGTAATTTGGGAGCAGGCAAT
dniR	GGCTGTTAAGCGGCGTATTG	GGCTTCAGGCGAAATTGCT
feoA	TCGGCGACCCCATTCAT	GGCCAGATCTTTTTTGCGTAAT
gusR	TGACGATCACGGAATTTGTTG	CTGGGCTTATCTCGCCTTTTT
icdA	CCGGCACAAGGCAAGAAG	AGGGATAATCGGATTTTCAGGAA
mdh	GCCCATAGACAGGGTTGCA	AACGCGGGTACTGAAGTGGTT
mioC	AGGAGAAAGGTTGTCCGGAATAT	CAGGGATCTGGCTGGTTATCA
nei	GATGTGCTGGATCCGAATCTG	AAAGCGCGGCGACAATAA
sdiA	TGCAACGGGAAAAGGACAA	GCGGTGTCACTCAGTATTTAATGC
uvrC	GAACGCCACACCAATAACGTC	ACGCGTCACCGAAAAACAAT
uvrY	CTGTGGAACGCGCGATTT	ACATGAGTATGCCGGGCATT
ybgI	GAACAGCGCGGCGATTAG	CACCGTCTCTTTGCCTTCCA
ybgJ	CAGGCGGACCGGATTTG	CAACAACCTGTTTTTCGCTCAA
ybgK	GGTTTTCGCCAGTCGGGTAT	ATGCGTAACGCGGGCATA
ybgL	GATTCGTGCAGGCAAGCAA	CCGCGATCGGCAAACA

TABLE 3. Transcript levels elevated at least threefold by *sdiA* amplification as determined by probing of microarrays

Functional group and gene	Fold increase
Cell division	
ftsA	10.0
ftsQ	8.8
ftsZ	11.0
sdiA	30.0
Transport of amino acids, carbohydrates, and cations	
glnH	4.0
xylF	3.8
trkG	4.1
DNA degradation	
mcrC	3.5
uvrC	9.3
DNA replication, repair, restriction, and modification	
gidA	4.1
mioC	7.0
nei	8.6
recN	3.6
Metabolism of small molecules	
argA	3.4
lysR	3.6
gst	3.1
Central intermediary metabolism	
agaI	3.3
agaD	4.5
speC	9.5
gcvA	4.6
pntA	10.4
pntB	8.2
Degradation of carbon compounds	
galE	3.8
galK	4.1
galT	4.9
gusR	8.0
Energy metabolism (carbon)	
dniR	5.8
pdhR	4.4
Drug or analog sensitivity	
acrA	6.8
acrD	3.0
acrE	14.0
acrF	6.3
acrR	4.5
Structural elements	
ddlB	4.6
lpxC	3.4
Not classified, *uvrY*	11.9
ORFs of unknown function	
sprT	3.8
b0135	6.4
b0141	4.6
b0517	11.2
b0530	6.5
ybgI	5.2
ybgJ	4.7
ybgK	6.4
ybgL	3.8
b0767	3.3
b1069	3.8
b1113	3.8
b1498	3.7
b1542	3.2
b1545	4.4
b1601	4.0
b1606	8.0
b1657	4.7
b1707	30.0
b1721	6.5
b1756	3.2
b1875	3.4
b1955	5.1
b1956	14.0
b1967	6.6
b2015	3.6
b2016	3.5
b2017	3.8
b2301	3.8
b2302	7.5
b2420	3.2
b2632	4.2
b2642	15.0
b2649	3.0
b2874	3.3
b3395	4.6
b3397	3.5
b3513	5.3
b3888	3.4
b4191	5.1
b4221	5.0

gene *ddlB* (34). It is not known where the transcript from the SdiA-dependent promoter ends. Increased quantities of RNA corresponding to this operon were thus expected. In strain MG1655 grown to exponential phase in LB broth, these transcripts range in quantity (the fraction of a particular transcript/summed transcripts hybridizing to all ORFs on the microarrays) from 240 to 540 ppm relative to the total ORF-specifying mRNA population (39). Amplification of *sdiA*, due to its presence on a multicopy plasmid, elevated the expression of transcripts hybridizing to the *ddlB, ftsQ, ftsA*, and *ftsZ* genes 5-, 9-, 10-, and 11-fold, respectively, relative to the strain that harbored pUC19 (Table 3). *lpxC*, the gene 101 bp downstream of *ftsZ* (6), also appeared to be induced fourfold (Table 3). Since the *lpxC* transcript is elevated in the pDEW140-containing strain and since a transcriptional terminator is not annotated between *lpxC* and *ftsZ* (6), it is reasonable to presume that *lpxC* is cotranscribed with *ftsQAZ* from the SdiA-dependent promoter. As expected, expression of the operon whose transcription is known to be activated by SdiA was highly elevated in a strain carrying an *sdiA*-containing, high-copy plasmid.

The complex structure of this operon (Fig. 1) suggests that the signal hybridizing to *ddlB* arises from a transcript that lacks the 5′ end of this coding sequence and is thus nonfunctional. This measurement indicates one limitation of microarray analyses using entire ORFs as the immobilized, capture reagents.

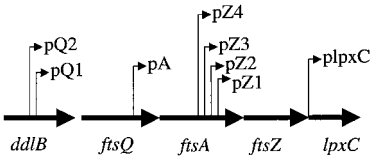

FIG. 1. Transcriptional organization of the *ftsZ* region.

TABLE 4. Transcript levels decreased at least threefold by *sdiA* amplification as determined by probing of microarrays

Functional group and gene	Fold decrease
Taxis and mobility	
air	4.6
cheA	3.7
cheB	3.3
cheW	5.3
cheY	4.6
cheZ	4.0
tar	5.3
tsr	5.9
Transport of amino acids, carbohydrates, or cations	
sdaC	3.9
glpF	3.6
malE	3.7
treB	5.3
fecA	3.5
fecE	3.2
kdpA	3.5
Metabolism of small molecules, *glpK*	3.5
Degradation of amino acids	
sdaB	3.2
tnaA	4.0
Energy metabolism (carbon)	
ackA	3.1
aldA	3.3
Cell exterior constituents, *nanA*	3.7
Surface structures	
flgB	14.3
flgC	16.7
flgD	16.7
flgE	16.7
flgF	7.1
flgG	12.5
flgH	5.9
flgI	5.6
flgJ	6.3
flgK	6.3
flgL	11.1
flgM	7.1
flgN	5.6
flhA	3.1
fliA	10.0
fliC	14.3
fliD	4.6
fliE	5.9
fliF	11.1
fliG	9.1
fliH	5.0
fliI	3.9
fliJ	7.1
fliK	4.8
fliL	7.7
fliM	12.5
fliN	5.0
fliO	4.4
fliP	6.3
fliR	3.1
fliS	5.9
fliT	5.6
fliZ	8.3
ORFs of unknown function	
b0290	3.5
b1194	5.0
b1880	3.6
yjiZ	3.3
yjbP	4.0
yhjF	4.8

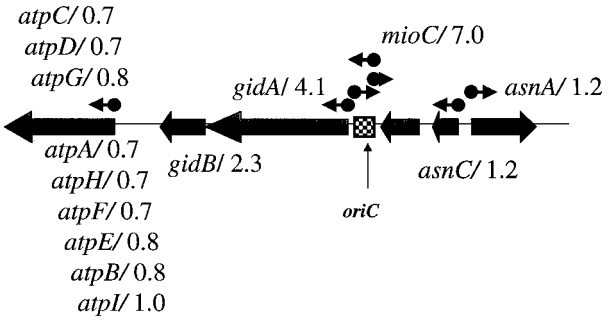

FIG. 2. *oriC* region. Genes are indicated by broad arrows placed on the central line representing a small portion of the circular chromosome. The box indicates the origin of replication. Arrows above the line indicate annotated promoters (6). The *atp* operon is indicated by the leftmost, broad arrow. The numbers following each gene indicate the fold elevation of gene expression caused by *sdiA* amplification.

This signal is misleading since it most likely does not lead to productive expression of DdlB at the protein level. Such inaccuracies may be avoided by subdivision of the ORFs serving as capture agents, a practice that is routinely used in an alternative methodology of microarray analysis (18). Moreover, this result indicates that transcript profiles will be most informative when interpreted within the context of previous studies.

Actions near the origin and a terminus of replication. Figure 2 depicts the genetic organization of the *oriC* region, the origin of DNA replication. The three genes immediately flanking *oriC* were overtranscribed relative to the vector-containing control strain. The cellular content of the *mioC* transcript was elevated sevenfold, whereas the expression of *gidA* and *gidB* were elevated four- and twofold, respectively. This effect was localized since the expression of flanking genes was not increased (Fig. 2).

Figure 3 depicts the region (min 35.3 to 37.3) surrounding *terB*, a terminus of DNA replication. The transcription of 38 genes within this 88-gene region was elevated more than twofold by the presence of pDEW140; The expression of 12 genes was enhanced more than threefold. Unlike the action observed around the origin, *oriC*, the stimulation seen in the vicinity of *terB* resulted in a mosaic pattern relative to the gene order. Interestingly, the expression of *tus*, encoding the terminus-binding factor, was not affected.

Since SdiA enhances the expression of cell division genes (38), it may also directly or indirectly modulate DNA structure around the origin and terminus so that these regions are more exposed for the mechanical operations resulting in proper chromosomal segregation. The *oriC* site is attached to the cell membrane (19). *mioC, gidA*, and *gidB*, stimulated by *sdiA* amplification, are involved in DNA replication (26) and clustered with the chromosomal replication origin, *oriC* (5). If the role of SdiA is to stimulate the expression of genes required for septation during the cell division cycle, coordinate expression of the *ftsZ*-containing operon with those of *mioC* and *gidAB* might be built into the cellular regulatory logic. Alternatively, local structural alterations of the chromosome could be the cause of the observed changes in gene expression. It is possible that the elevated expression level of these three genes was the result of an SdiA-enhanced replication of the origin relative to the remainder of the chromosome, a copy number effect. An-

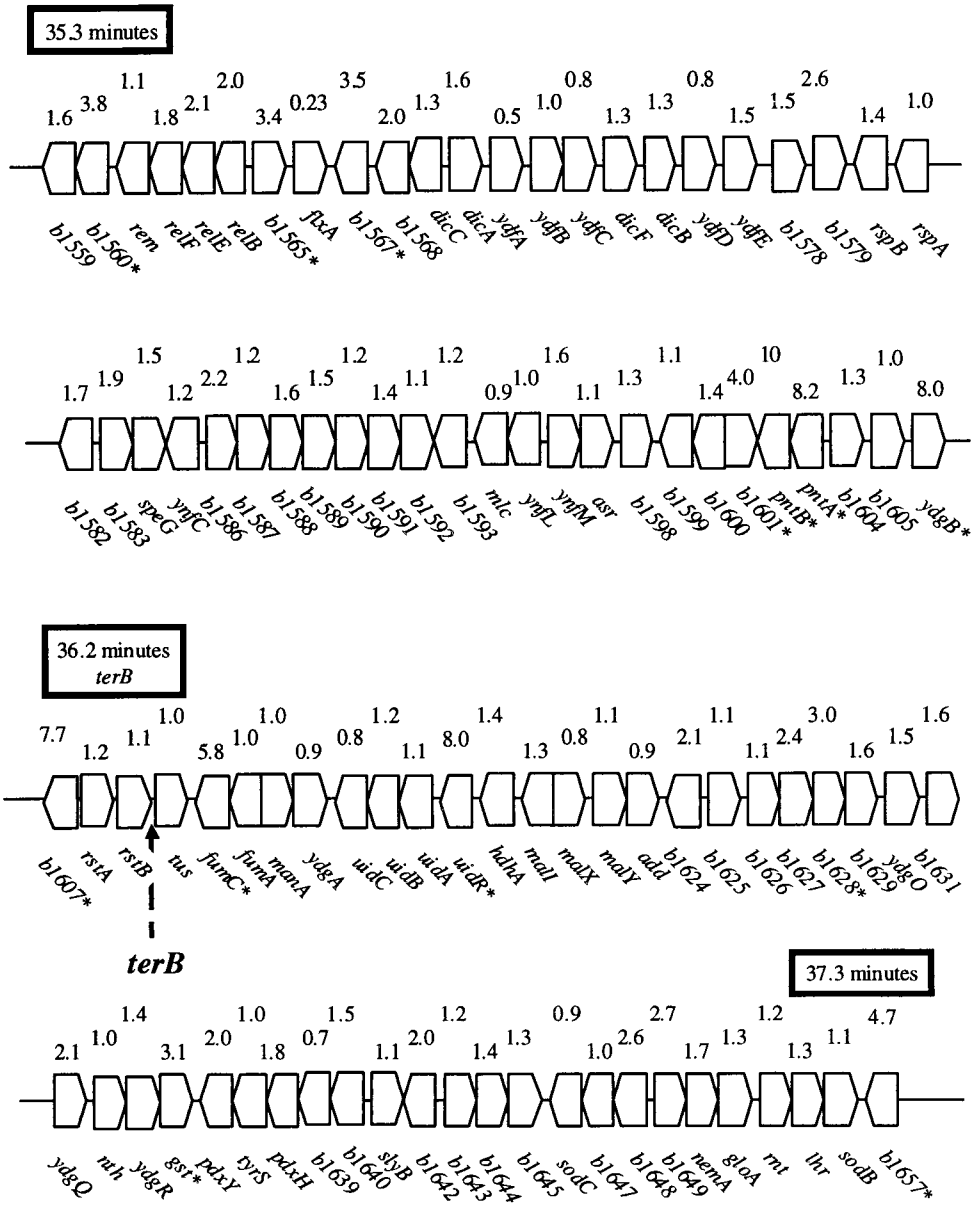

FIG. 3. *terB* region. The 2-min region of the chromosome surrounding *terB* (36.2 min) is depicted. The fold elevation, due to *sdiA* amplification as determined by comprehensive transcript profiling, is indicated above each gene. Genes whose transcripts were elevated >3-fold are marked with an asterisk.

other possibility is that the elevated SdiA titer "relaxed" the structure of the *oriC* region, providing RNA polymerase with greater access to the *gidAB* and *mioC* promoters, an unmasking scenario. Thus, the precise mechanism of enhanced *mioC* and *gidAB* expression remains to be determined.

The transcript content of most genes surrounding the *terB* replication terminus also appeared to be induced moderately when *sdiA* was amplified. This region is subjected to aberrant recombination and replication arrest (11). Such DNA "gymnastics" could also make the region accessible to RNA polymerase when replication is enhanced by *sdiA* amplification. Garrido et al. observed that transcription of *ftsZ* peaked at the time of both the initiation and the termination of replication (9). This observation, which is consistent with the transcript profiling described here, is suggestive of coordination between SdiA action and the timing of cell division.

Heightened expression of *acr* genes. Loss-of-function *acr* mutants (5) display enhanced sensitivity to a broad range of inhibitory agents. The *acr* genes of *E. coli* are organized into five operons, *acrC* (4.6 min), *acrAB* (10.4 min), *acrR* (10.4 min), *acrD* (55.6 min), and *acrEF* (73.5 min [5]). *acrC* encodes a transmembrane protein (5), while *acrD* specifies an aminoglycoside efflux pump (29). Interestingly, *acrE* mutants form filamentous chains of cells indicative of a septation defect, while *acrF* encodes a lipoprotein (5). The *acrAB* operon (25)-encoded proteins are components of a major drug efflux pump (2) that, together with TolC, a protein that forms an outer membrane porin, constitute an efflux channel from the cyto-

plasm out to the culture medium (2, 24, 25). Perhaps, an increased SdiA concentration within the cell, like increased levels of the transcriptional activator Rob (35), leads to elevated AcrA, AcrB, and TolC titers and hence efflux pores for mitomycin C expulsion. This hypothesis is consistent with the heightened sensitivity of a *tolC* mutant to mitomycin C (7).

In addition to the dramatic, 14-fold change in expression of *acrE* in response to *sdiA* amplification, other *acr* structural genes also displayed an increase in transcript quantity. The *acrA, acrD*, and *acrF* transcripts were elevated seven-, three-, and sixfold by multiple copies of *sdiA*. *acrB*, however, is 1 of 61 genes not present in the *E. coli* microarray (39). The inability to detect increased expression of this gene is thus expected. Another method, however, indicated that *acrB* expression was also elevated (see below). Evidence for elevated expression of *acr* genes in each locus was found as indicated by the fold expression reported in Table 3. *tolC* expression was also heightened by a factor of 2.6 due to the presence of pDEW140 (data not shown).

Enhanced expression of other operons that may contribute to the mitomycin C resistance phenotype. Elevated transcription of the *gal* operon genes (*galEKTM*) at min 17 (with basal expression levels of 221, 285, 140, and 198 ppm, respectively) was observed in the strain bearing the *sdiA* amplification. These genes, which were moderately expressed when strain MG1655 was grown in LB broth (average ppm = 210; ranked 841st, 599th, 1,512th, and 963rd, respectively [39]) were elevated 3.8-, 4.9-, 4.1-, and 2.3-fold, respectively. The expression of other sugar catabolic genes was not induced. Since galactose is incorporated into the K-12 lipopolysaccharide core (27), *gal* operon elevation could reflect an altered envelope structure.

At min 16 is the *ybgIJKL-nei* region. *sdiA* amplification elevated expression of these genes 5.2-, 4.7-, 6.4-, 3.8-, and 8.6-fold. These genes, transcribed in the same orientation, are organized into an operon (10) since the ORFs are densely packed, at times overlapping (6) without internal promoters (10). *nei* encodes endonuclease VIII, an enzyme that removes oxidized pyrimidine bases from DNA (16, 31), but the functions of the other genes in this operon are unknown. Since reactive oxygen species are implicated in mitomycin C-mediated cell killing (14) and mutagenesis (17), elevated expression of this operon could contribute to the mitomycin C resistance of cells harboring the *sdiA* amplification (40).

Elevated expression of other genes. ORF *b1707* expression was elevated most drastically, displaying a 30-fold induction. In addition, *speC, b0517, b1606* and *b2642* were all induced at least eightfold (see Table 3), comparable to the fold induction of the *ftsQAZ* mRNA. The expression of *b2015* and *b2016*, two adjacent genes at min 45, was elevated 3.6- and 3.5-fold, respectively. *b2015* is predicted to be a LysR-type transcriptional regulator (6), and the putative *b2016* gene product has low overall homology (E value = 10^{-5} by the BLASTn program [3]) to hexose 4,6-dehydratase from several gram-positive bacteria (6). Similarly, the expression of two other linked genes, *b4221* and *b4222*, of unknown function was elevated 5.0- and 4.8-fold while transcripts of *b2301* and *b2302* were elevated 3.8- and 7.5-fold, respectively. The latter pair of ORFs encodes putative glutathione *S*-transferases (6). Six putative fimbria-like protein genes (6) (*b0135, b0136, b0137, b0138, b0141*, and *b0530*) were induced ca. fivefold, among which the first four appeared to be in an operon. Thus, the first experimental evidence for the cotranscription of at least five potential operons was provided by the microarray analysis; elevated transcripts of *uvrYC, b2015-b2016, b2301-b2302, b4221-b4222*, and *b0135-b0136-b0137-b0138* were each observed upon *sdiA* amplification.

Downregulation of motility-related genes. Amplification of *sdiA* caused the expression of 62 genes to decrease by a factor of 3 or more; of these, 41 were involved in motility and chemotaxis. If a more-stringent cutoff of fivefold was imposed, then expression of 34 the genes were downregulated, 30 of which function in chemotaxis or motility (*cheW, flgBCDEF GHIJKLMN, fliACEFGHJLMNPSTZ, tar*, and *tsr*). Interestingly, the expression of master regulator genes *flhC* and *flhD* (basal levels of 230 and 70 ppm, respectively [39]), controlling flagellum operon expression, was lowered by only about one-third.

Motility defect associated with multiple copies of *sdiA*. Since many genes involved in flagellum biosynthesis, chemotaxis, and motility were dramatically repressed in the *sdiA* overproducing strain, a motility defect was predicted. The swarming of strains having single or multiple copies of *sdiA* was examined by spotting four single-colony isolates of each strain onto semisolid medium. After 8 h of incubation at 37°C, DPD2668 harboring pUC19 had swarmed (diameter, 32 ± 2.5 mm), while DPD2669 containing pDEW140 had not (diameter, 3.2 ± 0.4 mm). After 23 h of incubation, DPD2668 had filled the petri plate (diameter, 100 mm), while DPD2669 had expanded to a lesser extent, covering about one-half of each plate. There was no obvious difference in the growth rate in LB broth supplemented with ampicillin between the two strains when monitored by absorbance. Thus, the leaky motility phenotype described above could be explained by either (i) plasmid loss allowing swarming of a revertant (*sdiA*$^+$ haploid) population as ampicillin was exhausted from the medium or (ii) *sdiA* amplification only partially compromising motility. To distinguish between these possibilities, the site of inoculation and the edge of the swarm after 23 h were streaked for single colonies to an ampicillin-containing LB agar plate. Massive *sdiA*$^+$ plasmid loss from cells at the edge of the swarm was not observed, suggesting that the motility defective phenotype was not an absolute one.

Comparison of the transcript profiles of the *sdiA* overexpression strain and its control indicated that *sdiA* overexpression may result in a deficiency in movement, as confirmed by motility tests. It is not clear how genes involved in chemotaxis and movement are regulated by SdiA. It is possible that control cascades through the regulatory genes *flhCD*. Since the quantity of these transcripts in cells grown in LB broth is relatively low (39), the small reduction in *flhCD* expression might be enough to limit the cells' capacity to swarm.

Independent method confirms elevation of gene expression by *sdiA* amplification. Comprehensive transcript profiling is well suited to surveying the global changes associated with an altered genotype. An alternative method, quantitative real-time RT-PCR, was used to verify the expression level changes of representative genes caused by increased *sdiA* dosage. For these confirmatory experiments, total RNA samples, extracted from fresh cultures, were used.

The expression of *acrB*, 17 genes scored as inducible in the

TABLE 5. Fold induction of specific transcripts attributed to *sdiA* amplification as determined by probing of microarrays and amplification of cDNA samples

Gene	Fold change[a]	
	Amplification	Probing
sdiA	12.7	30.0
b1707	8.9	30.0
b1606	4.8	8.0
uvrY	4.9	11.9
uvrC	4.1	9.3
gusR	3.2	8.0
nei	3.2	8.6
ybgL	2.7	5.2
ybgK	2.4	4.7
ybgJ	2.4	6.4
ybgI	2.1	3.8
acrA	2.3	6.8
acrB	2.0	ND
dniR	2.0	5.8
feoA	2.0	5.8
mioC	1.9	7.0
b0517	1.4	11.2
acrR	0.8	4.5
icdA	1.1	<1.5
mdh	1.1	<1.5

[a] Amplification results were obtained by reverse transcription of bulk RNA followed by PCR amplification. Probing results were obtained with fluorescently labeled cDNA hybridized to DNA microarrays. ND, not determined.

comprehensive transcript profiling analysis (Table 3) and 2 control genes (*icdA* and *mdh*), was quantified. The results are summarized in Table 5. A twofold increase in *acrB* expression was observed upon *sdiA* amplification, a result similar to the 2.3-fold elevation of *acrA* expression determined by the same method. Discrepancies in the measurements of the *b0157* (<1.5-fold increase in reverse transcriptase PCR (RT-PCR) versus an ~11-fold increase in the comprehensive microarray experiment) and *acrR* (decreased 1.2-fold as measured by RT-PCR versus a 4.5-fold increase as monitored by the microarray-based approach) transcripts were observed between the two methods. Since AcrR is a negative regulator of *acrAB* operon expression (20), we postulate that the microarray measurement of the *acrR* transcript was erroneous. Reassuringly, the expression changes of the other 15 genes, scored as elevated in the comprehensive transcript profile, were confirmed by the RT-PCR method; they showed the same trend and a similar magnitude of change with both methodologies. Overall, smaller changes were routinely observed with RT-PCR, perhaps attributable to high background fluorescence associated with SYBR Green.

***sdiA* in high copy conferred resistance to antibiotics with different modes of action.** The responses of a control strain, DPD2668, and DPD2669 harboring pDEW140, the *sdiA* plasmid, to the antibiotics rifampin, chloramphenicol, nalidixic acid, tetracycline, spectinomycin, and kanamycin were examined in liquid medium. The MICs were identical between the two strains when challenged with rifampin, chloramphenicol, spectinomycin, and kanamycin, having values of 6.3, 2.5, 12.5, and 3.1 μg/ml, respectively. The presence of the *sdiA* plasmid in DPD2669, however, raised the MIC for tetracycline from 0.6 to 5 μg/ml and that for nalidixic acid from 1.3 to 10 μg/ml. These results are consistent with the role of AcrA and AcrB in efflux (2, 24). They differ, however, in some details compared to studies of *acr* loss-of-function mutants (25). This may reflect differences in information garnered from genetic alterations causing either loss or gain of function (15).

Summation. *E. coli* high-density microarrays have been successfully used to quantify the entire complement of individual mRNA transcripts (39). It has also been used to profile the gene expression level changes upon chemical treatment (T. K. Van Dyk et al., unpublished results; Y. Wei and R. A. LaRossa, unpublished results; M. Zheng et al., unpublished results; Y. Wei, D. G. Söll, and R. A. LaRossa, unpublished). This work showed its utility in determining the global effects of gene dosage amplification. Expression of *sdiA* is very low (15 ppm) in broth-grown, exponential-phase *E. coli* (39). The 30-fold elevation of the *sdiA* transcript in DPD2669 containing *sdiA* in high copy, compared with that in DPD2668, reflected the amplification of the *sdiA* gene due to its location on both the chromosome and a multicopy plasmid. As a consequence of *sdiA* hyperexpression, transcription of the *'ddlB-ftsQAZ-lpxC* operon, the only *E. coli* genes previously known to be activated by SdiA in *E. coli* (38), was greatly increased. In addition, the expression of genes falling into a few other functional categories (e.g., cell division, DNA replication and repair, drug sensitivity, and macromolecular metabolism) was raised significantly by *sdiA* amplification. Perhaps SdiA serves as a positive activator of these genes, as well as a few other genes whose functions are obscure.

We previously found that *sdiA* in high copy conferred resistance to mitomycin C upon *E. coli* (40). This phenotype (40) is not dependent upon the SOS response (37). When examining the expression profiles from microarray experiments, we found that many genes involved in DNA replication, degradation, repair, transposition, and the stability of chromosomal structure in addition to *nei* and *uvrC* were induced moderately. This suggests increased capacity for chromosomal replication and repair upon *sdiA* overexpression. This may explain why the amplification of *sdiA* confers mitomycin C resistance.

Alternatively, *acr* mutations cause sensitivity to acriflavines, molecules that intercalate into double-stranded DNA containing monotonic runs of base pairs (12). Most *acr* mutants display a defect in acridine efflux; moreover, they are often pleiotropic, being hypersensitive to a wide variety of chemicals (2, 24). Thus, hyperexpression of these genes in a strain harboring an *sdiA*-bearing multicopy plasmid could lead to mitomycin C expulsion and therefore result in the observed resistance to this DNA-damaging agent. It may also explain why *sdiA* in multicopy could confer resistance to a broad spectrum of antibiotics. Thus, microarray methods, like other technologies, become part of the scientific method, generating hypotheses requiring further study.

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