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Similar and Divergent Effects of ppGpp and DksA Deficiencies on Transcription in *Escherichia coli*▽†

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The concerted action of ppGpp and DksA in transcription has been widely documented. In disparity with this model, phenotypic studies showed that ppGpp and DksA might also have independent and opposing roles in gene expression in *Escherichia coli*. In this study we used a transcriptomic approach to compare the global transcriptional patterns of gene expression in strains deficient in ppGpp (ppGpp^0) and/or DksA (Δ*dksA*). Approximately 6 and 7% of all genes were significantly affected by more than twofold in ppGpp- and DksA-deficient strains, respectively, increasing to 13% of all genes in the ppGpp^0 Δ*dksA* strain. Although the data indicate that most of the affected genes were copositively or conegatively regulated by ppGpp and DksA, some genes that were independently and/or differentially regulated by the two factors were found. The large functional group of chemotaxis and flagellum synthesis genes were notably differentially affected, with all genes being upregulated in the DksA-deficient strain but 60% of them being downregulated in the ppGpp-deficient strain. Revealingly, mutations in the antipausing Gre factors suppress the upregulation observed in the DksA-deficient strain, emphasizing the importance of the secondary channel of the RNA polymerase for regulation and fine-tuning of gene expression in *E. coli*.

Guanosine tetraphosphate and guanosine pentaphosphate, collectively called ppGpp, are modified nucleotides that act as intracellular transducing signals in most gram-negative and gram-positive bacteria and in the chloroplasts of plant cells (47). In 1969, Cashel and Gallant established that the intracellular level of ppGpp was induced in response to nutrient starvation in *Escherichia coli* with important consequences for the pattern of gene expression, a process named the stringent response (7). The hallmark of the stringent response is the downregulation of rRNA and tRNA synthesis that occurs upon amino acid starvation (8, 42, 45).

ppGpp is produced very rapidly from GTP (or GDP) and ATP in response to any stress condition that will result in attenuation of growth (5, 8, 9, 28). In *E. coli*, the synthesis of ppGpp is mediated by the enzymes RelA and SpoT (8, 52). The RelA protein synthesizes ppGpp in response to amino acid starvation by recognizing and binding to stalled ribosomes, which have an uncharged tRNA bound in their A site (8, 50). The SpoT protein has ppGpp synthetase and hydrolase activities, and its synthetase activation mechanism is not well understood (16, 50, 52). In many bacteria, a single RelA-SpoT homologue protein that has both synthetase and hydrolase activities is present (33).

A few years ago it was reported that DksA, a 17-kDa RNA polymerase (RNAP) binding protein, potentiates ppGpp-mediated repression of the rRNA promoter and ppGpp-mediated stimulation of some promoters of amino acid biosynthesis operons (34, 35). These landmark findings encouraged the hypothesis that DksA acts as a cofactor for the ppGpp-dependent effects on transcription (reviewed in reference 19). DksA has an overall structural similarity to the transcription elongation factor GreA, with a prominent coiled-coil domain in the N terminus and a Zn finger motif in the C terminus (36, 44, 49). It has been suggested that the protrusion of the DksA N terminus into the secondary channel of the RNAP would stabilize the interaction between RNAP and ppGpp (36). It has been proposed that the synergistic effect of ppGpp and DksA on transcription occurs by decreasing the energy required for open complex formation and also by reducing open complex stability, which will result in either repression or stimulation of transcription, depending on the kinetic properties of a given promoter (35).

The proposed model of concerted action of ppGpp/DksA to regulate transcription would imply that deficiency of either of the two factors would cause similar phenotypic consequences, as has been shown for several genes (19, 34, 35). However, differential phenotypes of DksA-deficient (Δ*dksA*) and ppGpp-deficient (ppGpp^0) mutant strains in several cellular processes, such as adhesion and motility, indicated that apparent discrepancies with the postulated concerted model exist (2, 20, 29, 38). For example, it was shown that the expression of the type 1 fimbriae was significantly downregulated in ppGpp^0 strains, while it was notably upregulated in DksA-deficient strains (1, 2). In contrast to these in vivo discrepancies, in vitro studies demonstrated that both ppGpp and DksA stimulate transcription from the *fimB* promoter that is responsible for the differences observed in vivo. It was postulated that the discrepancy

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TABLE 1. Bacterial strains used in this study

Strain	Relevant characteristics or description[a]	Reference or source
MG1655	F$^-$ *ilvG rph1*	18
AAG1	MG1655 Δ*lacZ*	2
CF11657	MG1655 *greA*::Cm^r	38
CF11663	MG1655 *greB*::Km^r	38
CF1693	MG1655 *relA251*::Km^r *spoT207*::Cm^r	52
AAG95	MG1655 Δ*dksA*::Km^r	2
TE8114	MG1655 Δ*dksA*::Tc^r	6
AAG98	CF1693 Δ*dksA*::Tc^r	This study
AAG107	MG1655 *greA*::Cm^r *greB*::Km^r	This study
AAG101	MG1655 Δ*dksA*::Tc^r *greA*::Cm^r	This study
JFV2	AAG1 Δ*relA* Δ*spoT*	This study
JFV10	AAG1 *tnaA*::*lacZ* Km^r	This study
JFV11	JFV2 *tnaA*::*lacZ* Km^r	This study
JFV12	JFV10 Δ*dksA*::Tc^r	This study
JFV13	JFV11 Δ*dksA*::Tc^r	This study
JFV14	JFV2 Δ*dksA*::Tc^r	This study
PRG13	AAG1 *fliC*::*lacZ*(+70) Km^r	This study
PRG14	PRG13 Δ*dksA*::Tc^r	This study
PRG15	PRG13 Δ*dksA*::Tc^r *greA*::Cm^r	This study
PRG16	AAG1 *fliC*::*lacZ*(+1210) Km^r	This study
PRG17	PRG16 Δ*dksA*::Tc^r	This study
PRG18	PRG16 Δ*dksA*::Tc^r *greA*::Cm^r	This study

[a] Km, kanamycin; Cm, chloramphenicol; Tc, tetracycline.

between the in vivo and in vitro findings was, at least in part, a consequence of the increased availability of the secondary channel of the RNAP to interact with the antipausing Gre factors in the DksA-deficient strain (2).

In this work, comparative studies of the effects of ppGpp and DksA on the global pattern of gene expression in *E. coli* were performed using a transcriptome approach. Our results let us conclude that deficiency in either ppGpp and/or DksA causes major alterations in the general profile of gene expression. Interestingly, when the transcriptional profiles obtained were compared, the deduced roles of ppGpp and DksA in the expression of specific genes were much more diverse than initially expected. Hence, although many genes are conegatively or copositively affected, there are also genes that are differentially affected by ppGpp and DksA. Studies of the expression of genes involved in flagellum biosynthesis and chemotaxis indicate that the increased RNAP secondary-channel occupancy by the Gre factors in the absence of DksA might be responsible for some of the differential phenotypes detected in the DksA-deficient strains. These results also suggest that competition for the occupancy of the secondary channel of the RNAP may play a key role in transcriptional regulation.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains used are listed in Table 1. Strains were grown in LB medium (3) at 37°C with vigorous shaking (200 rpm), unless otherwise stated. When necessary, antibiotics were used at the following concentrations: chloramphenicol, 15 μg/ml; kanamycin, 25 μg/ml; and tetracycline, 12.5 μg/ml. Bacterial growth was monitored by measuring the optical density at 600 nm (OD_{600}) in a Shimadzu UVmini 1240 spectrophotometer, where 0.5 unit of OD_{600} corresponds to mid-log-phase growth and approximately 5×10^8 bacteria/ml. Given the propensity of ppGpp- and DksA-deficient strains to accumulate suppressor mutations within *rpoBC* and *rpoD* loci, routine controls for the auxotrophy phenotype of the ppGpp- and DksA-deficient strains were performed using minimal M9-salt plates (41) supplemented with 0.4% (wt/vol) glucose and 10 μM thiamine. In addition, the fidelity of the *rpoBCD* genes in the mutant strains was confirmed as wild type (WT) by DNA sequencing as previously described (2).

Genetic techniques. Basic molecular genetic manipulations were performed essentially as described previously (41). DNA sequencing was performed using the DYEnamic ET terminator cycle sequencing kit according to the manufacturer's protocol (GE Healthcare). Gene disruption of the *relA* allele (corresponding to amino acids 6 to 743) and the *spoT* allele (corresponding to amino acids 7 to 699) was carried out by allelic exchange using the suicide plasmid pKO3 as previously described (27, 31). The *tnaA*::*lacZ* and *fliC*::*lacZ* transcriptional gene fusions on the chromosome were constructed as previously described (11, 14). The chloramphenicol resistance of plasmid pKD3 was amplified using the primer pairs tnaA-P1R/tnaA-P2, fliC1/fliC6, and fliC7/fliC2 to obtain the fusions *tnaA*::*lacZ*, *fliC*::*lacZ* (+70) and *fliC*::*lacZ* (+1210), respectively. Primers sequences are specified in Table S1 in the supplemental material. After recombination, a deletion between nucleotides 34 and 1078 of the chromosomal *tnaA* gene and deletions between nucleotides 71 and 790 [*fliC*::*lacZ* (+70) fusion] and between nucleotides 1211 and 1527 [*fliC*::*lacZ* (+1210) fusion] of the chromosomal *fliC* gene were performed. Next, the chloramphenicol resistance gene was replaced by a promoterless *lacZ* gene linked to a kanamycin resistance gene from either plasmid pKG137 (*tnaA*::*lacZ* fusion) or pKG136 (*fliC*::*lacZ* fusions) (pKG plasmids are courtesy of J. M. Slauch). The different alleles were introduced by P1 transductions (51) with the following strains serving as donors: TE8114 for Δ*dksA*::Tc^r, CF11657 for *greA*::Cm^r, CF11663 for *greB*::Km^r, JFV10 for *tnaA*::*lacZ*, PRG13 for *fliC*::*lacZ* (+70), and PRG16 for *fliC*::*lacZ* (+1210).

Microarray analysis. Total RNA was isolated from two independent cultures of MG1655, CF1693 (ppGpp^0), AAG95 (Δ*dksA*), and AAG98 (ppGpp^0 Δ*dksA*) grown to the beginning of stationary phase (OD_{600} of 1.5), and cDNA was synthesized as described previously (2). Four comparisons were made for each combination pair, i.e., WT/ppGpp^0 mutant, WT/Δ*dksA* mutant, and WT/ppGpp^0 Δ*dksA* mutant, using the two different total RNA isolations and both Cy3 and Cy5 labeling. The labeled cDNAs were mixed in hybridization buffer, hybridized to microarray slides (*E. coli* K-12 V2 array, MWG; Ocimum Biosolutions), scanned, and analyzed as previously described (2). The microarray slides contain 4,288 open reading frames (ORFs) of *E. coli* K-12 covered by 4,239 50-mer oligonucleotides. Forty-seven of the oligonucleotides are replicated, and the slide also contains 48 control oligonucleotides (*Arabidopsis* ORFs), giving a total of 4,608 spots on each microarray slide. The microarray chip covers most of the *E. coli* genome, but it does not include any of the noncoding RNAs (rRNA and tRNA).

Statistical analysis of the microarray data. Scanned images were processed using the software Genepix 6 (Axon Inc.), and raw expression values obtained from GPR files were processed as described previously (15). Data quality was tested by MA plots of mean intensities to illustrate existing biases and the need for normalization and by correlation plots to check the quality of technical and biological replicates. Nonspecific filtering of spots which had been flagged as "bad" by the image analysis program was used. Background signal which might be considered due to nonspecific hybridization was removed using the method of Kooperberg et al. (23). Data were normalized using a print-tip loess algorithm (53).

The selection of differentially expressed genes between combination pairs was based on a linear model analysis with empirical Bayes moderation of the variance estimates following the methodology developed by Smyth (43). *P* values were adjusted to obtain strong control over the false discovery rate as previously described (39). Genes were selected as being "statistically significantly differentially expressed" by using volcano plots; these had a significant adjusted *P* value and showed a fold change of at least 2 or −2. The analysis of biological significance was based on an enrichment analysis as described previously (22).

All the statistical analysis, were done using the free statistical language R and the libraries developed for microarray data analysis by the Bioconductor Project (http://bioconductor.org).

Gene distribution in functional groups. Functional classification of the ORFs present in the *E. coli* K-12 V2 array was performed accordingly to the classification made at The Comprehensive Microbial Resource at the J. Craig Venter Institute (http://cmr.jcvi.org/cgi-bin/CMR/CmrHomePage.cgi). The ORF names in each functional group can be found at http://cmr.tigr.org/tigrscripts/CMR/shared/GetNumAndPercentGenesInARole.cgi. The total number of ORFs in each functional group was compared to the number of ORFs affected in each mutant strain (compared to the WT strain), with differentiation between the ORFs that were up- or downregulated. The ORFs in the two hypothetical groups (groups 10 and 11) were combined into one functional group, as were unclassified ORFs and ORFs encoding proteins of unknown function (groups 21 and 22).

RT-PCR assay. Reverse transcription-PCR (RT-PCR) was performed using Ready to-Go RT-PCR beads (GE Healthcare) and the primer pairs shown in Table S1 in the supplemental material, following the manufacturer indications. Saturation curves with increasing amounts of total RNA were made to determine

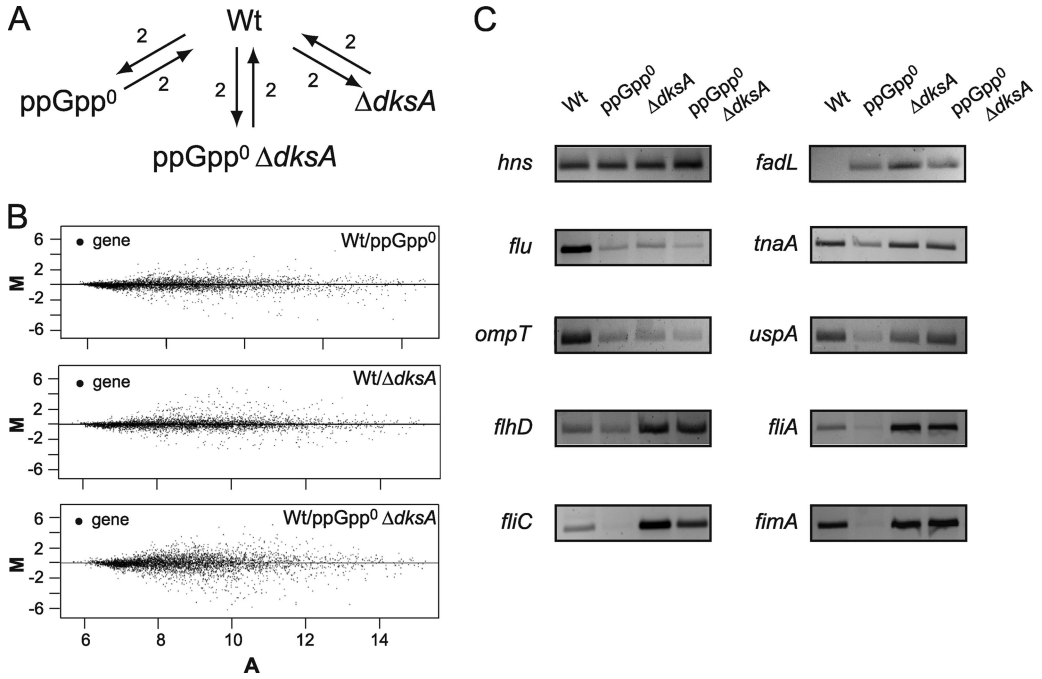


FIG. 1. Experimental setup and validation of the microarray data. (A) Schematic illustration of the experimental layout used in this microarray study. cDNAs from the WT (MG1655), ppGpp^0 (CF1693), Δ*dksA* (AAG95), and ppGpp^0 Δ*dksA* (AAG98) strains, labeled with either Cy3 or Cy5, were hybridized to microarray slides in the different combinations shown. The origins of the arrows show the Cy3-labeled samples, whereas the destinations show the Cy5-labeled samples. The number on the arrow shows the number of replicates for each combination. (B) Statistical MA plots of the normalized raw data obtained from each WT/mutant comparison. M represents the fold change in gene expression between WT and mutant ($\log_2$ values), and A represents the average spot intensity. (C) RT-PCR analysis of transcription of 10 selected genes from the WT (MG1655), ppGpp^0 (CF1693), Δ*dksA* (AAG95), and ppGpp^0 Δ*dksA* (AAG98) strains. The amounts of total RNA used as the template were 10 ng for *tnaA*; 20 ng for *hns*, *ompT*, and *uspA*; 40 ng for *fadL*; 200 ng for *flu*, *flhD*, *fliC*, and *fimA*; and 400 ng for *fliA*. *hns* was included as a control to confirm that equivalent amounts of template were used. Samples from bacterial cultures grown in LB medium to an OD_{600} of 1.5 at 37°C were used for all the studies shown.

empirically the amount of total RNA used for detection of each specific transcript. The total RNA used was isolated as described for the microarray analysis.

Chemotaxis assay. The chemotaxis response was analyzed on TB plates (1% tryptone, 0.5% NaCl) containing 0.25% agar and supplemented with 2 mM final concentrations of L-serine, maltose, glucose, or L-valine. Overnight bacterial cultures in LB at 37°C were spotted (5 μl) on the centers of the plates and incubated 12 h at 30°C. The experiments were repeated twice with four plates of each strain in each experiment. The colony diameter was measured and plotted, and standard errors were calculated.

β-Galactosidase assay. β-Galactosidase activity measurements were performed as described by Miller (32). Data are mean values from duplicate determinations in at least three independent experiments plotted with standard errors.

Western blot analysis. Protein levels were determined from crude extracts of soluble and/or total proteins after separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transfer to polyvinylidene difluoride membrane (PolyScreen) (23a), and Western blot analysis using polyclonal rabbit anti-OmpT, anti-FliA (Neoclone), anti-FliC (50a), and anti-H-NS (21a). Antibody-decorated protein bands were detected with chemiluminescence reagents (GE Healthcare) and quantified using the Fluor-S scanner and Quantity One software (Bio-Rad). To be able to visualize the FliC and FliA proteins in the WT strain (MG1655), highly (~40-fold) concentrated protein extracts of soluble proteins were used.

RESULTS

Experimental layout of the transcriptional analysis. The intriguing debate on the role of ppGpp and DksA in transcription (2, 19, 29, 35, 38) led us to compare the global gene expression pattern of the *E. coli* WT strain MG1655 to that of the extensively studied ppGpp-deficient strain (CF1693) (ppGpp^0), a Δ*dksA* mutant strain (AAG95), and a ppGpp^0 Δ*dksA* mutant strain (AAG98). The bacterial cultures were grown in LB medium at 37°C to the beginning of stationary phase (OD_{600} of 1.5), and total RNA was extracted. To test the response to deficiency of ppGpp and/or DksA on the general transcriptional pattern, *E. coli* K-12 V2 arrays (MWG, Ocimum Biosolutions) were used.

In Fig. 1A, the experimental layout used in the microarray experiment is shown. Every mutant RNA sample (ppGpp^0, Δ*dksA*, and ppGpp^0 Δ*dksA*) was hybridized to WT RNA. For each combination pair, i.e., WT/ppGpp^0, WT/Δ*dksA*, and WT/ppGpp^{0}Δ*dksA*, hybridizations using the two possible dye assignments (dye swaps) were performed using the same RNA preparations (technical replicates). Moreover, each combination was replicated twice using different RNA preparations (biological replicates). Therefore, the data shown are the results of four replicates for each strain combination. Quality control of the raw data obtained shows, according to current standards (15), a very high statistical quality of the experimental data as indicated by MA plots of the data after normalization (Fig. 1B). A tightly centered distribution, symmetrical around zero, was detected. High reproducibility was observed between technical or biological replicates, which ranged from 0.7 to 0.8 for technical replicates and from 0.82 to 0.93 for biological replicates.

To confirm and validate the transcriptomic results obtained, RT-PCR was used to analyze the mRNA levels of 10 repre-

sentative genes in the different strain backgrounds (Fig. 1C). The genes selected were *hns*, *flu*, *ompT*, *flhD*, *fliC*, *fadL*, *tnaA*, *uspA*, *fliA*, and *fimA*. The difference in the levels of mRNAs for the 10 selected genes in the different strains backgrounds was similarly detected by using either the transcriptome approach or RT-PCR methodologies. Further validation experiments with some of the selected genes were performed. The expression of genes involved in flagellum synthesis (*fliA* and *fliC*) and the expression of *ompT*, which encodes an outer membrane protease, were also studied by using Western blot analysis (see Fig. 6 and see Fig. S1B in the supplemental material). The fold difference in the expression of FliC, FliA, and OmpT in the ppGpp^0, Δ*dksA*, and ppGpp^0 Δ*dksA* strains compared to the WT was similar to what was observed at the transcriptional level. The expression of *tnaA*, which encodes the tryptophanase (TnaA) (12), was severely downregulated in the ppGpp-deficient strain compared to the WT (27-fold; see Table S2 in the supplemental material), consistent with earlier results (54). When *tnaA* expression was monitored using a chromosomal transcriptional *tnaA*::*lacZ* fusion under the same experimental conditions as in the microarray study, a 22-fold downregulation was observed in the ppGpp^0 strain. The expression of *tnaA*::*lacZ* in the Δ*dksA* and the ppGpp^0 Δ*dksA* strains was equally consistent with the results obtained by microarray analysis (see Fig. S1A and Table S2 in the supplemental material). Moreover, the results obtained from the microarray data were consistent with previous studies of the transcriptional expression of the *fim* genes performed under the same experimental conditions (1, 2) (see Table S2 in the supplemental material).

Effect of ppGpp and DksA deficiencies on the gene expression pattern of *E. coli* K-12 strain MG1655. An initial comparison of the genes that were up- or downregulated more than twofold ($P < 0.0001$) in the different mutant backgrounds was performed using a Venn diagram (Fig. 2A). The number of genes affected in the ppGpp^0, Δ*dksA*, and ppGpp^0 Δ*dksA* strains compared to the WT were 265, 311, and 556, respectively, representing 6, 7, and 13% of the total number of ORFs represented in the DNA microarray. To obtain an overview of the effects of the single and double mutations on the biology of the bacterial cell, the ORFs with expression altered more than twofold were distributed into different functional groups and presented as percentages of the total number of ORFs of each functional group that were up- or downregulated in the different mutant strains compared to the WT (Fig. 2B). In all three strains, the ORFs involved in transcription; purine, pyrimidine, and protein synthesis; protein fate; and cell envelope biogenesis were mostly upregulated (i.e., repressed by ppGpp and DksA), and the ORFs involved in energy metabolism, transport/binding, and amino acid biosynthesis were mostly downregulated (i.e., stimulated by ppGpp and DksA). By looking at the data as the percentage of the total number of ORFs affected in the different mutant backgrounds (Fig. 2B), the predominantly affected functional categories, in ppGpp- and/or DksA-deficient strains, were found to be energy metabolism and transport/binding proteins, in agreement with the crucial role described for the ppGpp/DksA regulatory system in the adaptation of growing cells to severe nutritional stresses. In almost all the functional categories, higher numbers of genes were found to be altered in the double mutant than in the single mutants, as exemplified by the categories of transcription, protein synthesis, energy metabolism, and central intermediary metabolism (Fig. 2B). Consistent with the concerted model proposed, most of the genes affected in the double mutant were also affected in the single mutants, although to a lesser extent in most cases. Hence, looking at the genes that were strongly (more than sixfold) affected by ppGpp and DksA, 60% of the genes affected in the double mutant were slightly altered in the single mutant strains (Fig. 3C). It is remarkable that of those genes affected more than sixfold in the double mutant (62 genes), almost one-third of them (19) were affected in the ppGpp^0 strain but not in the Δ*dksA* strain, while only 2 genes were affected in the Δ*dksA* strain but not in the ppGpp^0 strain.

Effect of ppGpp and/or DksA deficiencies on the general pattern of transcription stimulation and repression. Next, all ORFs altered more than twofold were classified into ORFs whose transcription was either stimulated or repressed in the different genetic backgrounds (Fig. 3). This classification shows that some ORFs were differentially affected (i.e., were upregulated in one mutant background but downregulated in another mutant background). These differentially affected ORFs were excluded in the comparison shown in Fig. 3 and are discussed in following sections.

The analyses of the ORFs that were up- or downregulated show that in the double mutant, 30% more genes were downregulated than upregulated (293 versus 203) (Fig. 3A). When only ORFs that were up- or downregulated more than sixfold were considered, the relative difference in numbers was even higher (49 versus 14) (Fig. 3B). These results highlight a noteworthy role of ppGpp/DksA in stimulation of gene expression. The distributions of ORFs that were up- or downregulated in the ppGpp-deficient strain are similar (114 versus 106 and 6 versus 12 [genes altered by >2-fold or >6-fold, respectively]). On the other hand, in the single DksA-deficient strain, the number of genes upregulated was higher than the number of genes downregulated (159 versus 81 and 20 versus 9 [genes altered by >2-fold or >6-fold, respectively]). The difference was even greater when looking at the ORFs that were upregulated or downregulated only in the DksA-deficient strains (86 + 54 versus 41 + 6, respectively), suggesting that DksA might independently play a more crucial role in the repression of gene expression than in its stimulation. However, previous results indicate that stimulation of gene expression in a DksA-deficient strain may not necessarily be due to a direct repressive effect of DksA on transcription (2). It might also be consequence of an increased association of other factors (Gre factors) with the RNAP occurring in the absence of DksA.

The array data imply that not all genes are regulated by ppGpp and DksA in a concerted manner. Although our results partially support the proposed concerted model of ppGpp/DksA action on transcription, some data also indicate clear inconsistency with that model. For example, while 60% of the genes affected more than sixfold in a strain deficient in both factors were also altered in the single mutant strains (as would expected from the concerted model), 40% were not (Fig. 3C). Moreover, an elevated number of genes were affected by single ppGpp or DksA deficiencies but not by deficiency of both regulatory factors, strongly suggesting that the concerted model is not valid for all genes (Fig. 2 and 3). Further-

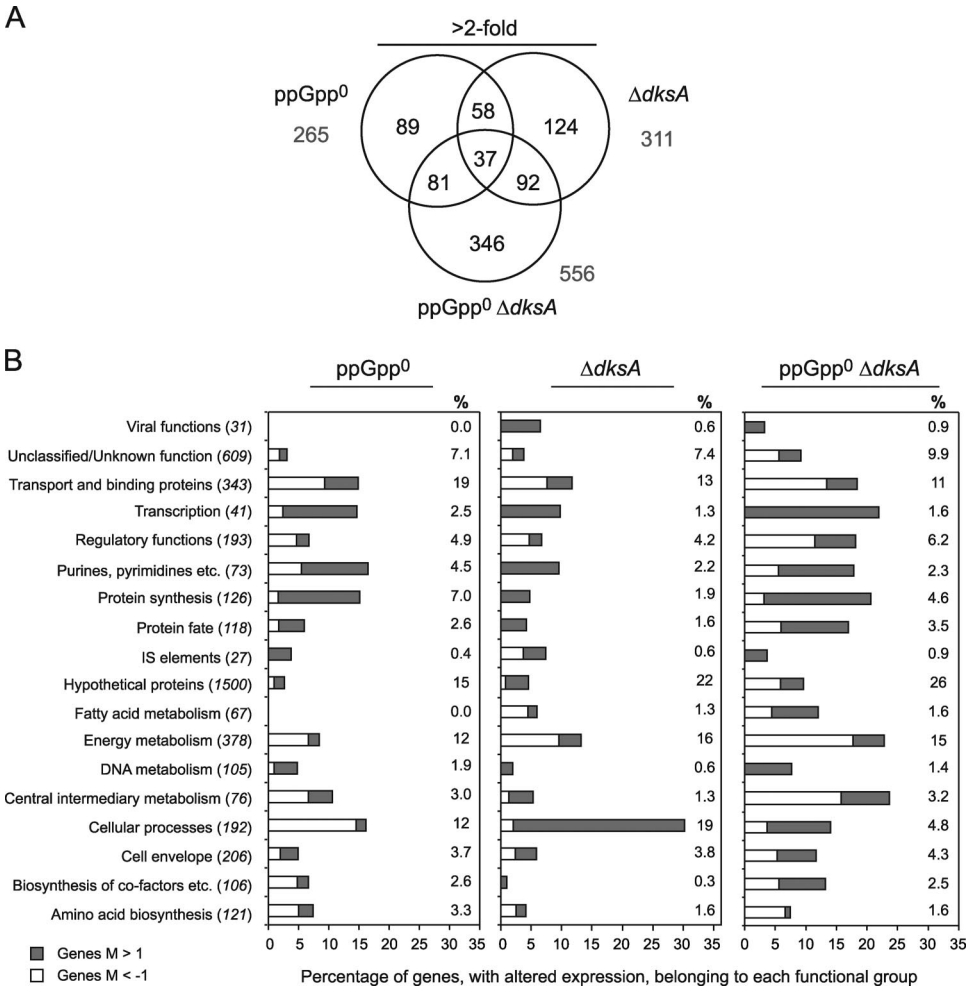
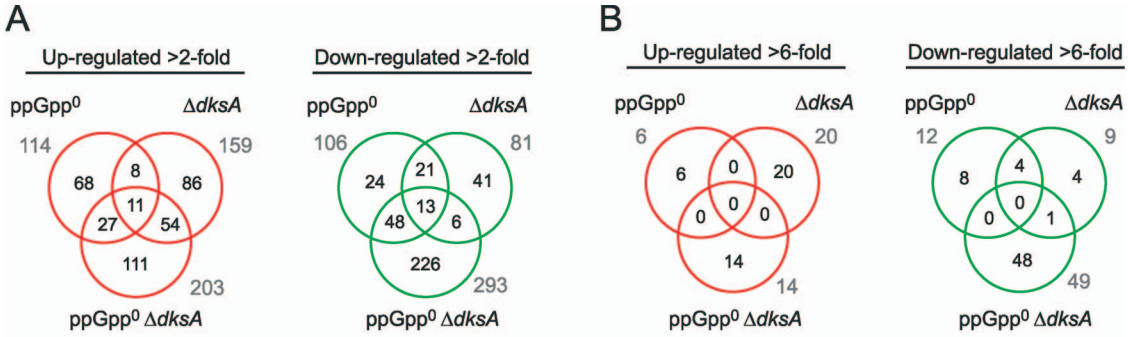


FIG. 2. Effect of ppGpp and/or DksA deficiency on global gene expression pattern in *E. coli*. (A) Venn diagram representing the number of ORFs with altered expression levels (more than twofold) in the different mutant strains (ppGpp^0 [CF1693], Δ*dksA* [AAG95], and ppGpp^0 Δ*dksA* [AAG98]) compared to the WT (MG1655). Numbers in gray indicate the total number of ORFs that were altered in each mutant strain in comparison to the WT ($P < 0.0001$). (B) Distribution of the ORFs with more than twofold-altered expression into functional categories. The bar diagram represents the percentage of ORFs with altered expression relative to the total number of ORFs in each functional group. The open bars represent the percentage of ORFs having decreased expression compared to the WT (M value of <-1). The gray bars represent the percentage of ORFs with elevated expression in the mutant compared to the WT (M value of >1). The total number of ORFs present in each category in *E. coli* is indicated in parentheses after the category designation. The numbers besides the bar diagrams represent the percentage of ORFs affected in each functional category relative to the total number of ORFs affected in each mutant strain (265, 311, and 556 ORFs in the ppGpp^0, Δ*dksA*, and ppGpp^0 Δ*dksA* strains, respectively).

more, ppGpp and DksA deficiencies cause differential effects on the expression of some ORFs. It is noteworthy that several ORFs of the cellular process functional group respond differentially to deficiency in either ppGpp or DksA (Fig. 2B). In the ppGpp-deficient strain, most of the genes in this functional category were downregulated, indicating that ppGpp stimulates their expression. Conversely, in the DksA-deficient strain, most of the genes were upregulated, indicating that DksA represses their expression. Extraordinarily, 77% of the genes (57 genes of 74) in this functional category affected by ppGpp were differentially regulated by DksA. Most of those genes (51 genes) are involved in chemotaxis and motility. The other six genes are *katG*, *cvpA*, *cspF*, *cspD*, *betB*, and *b1503*. A similar pattern of regulation was also found for other gene determinants belonging to other functional categories, such as the *fim* genes (see Table S2 in the supplemental material), represented in the cell envelope group, that were previously reported to have such a pattern of expression (2).

In Fig. 4 the ORFs differentially affected in at least two of the mutant backgrounds (>2-fold compared to the WT) are grouped depending on their pattern of expression in the different mutant strains. Interestingly, most of the genes that were differentially regulated were stimulated by ppGpp (40 genes) (Fig. 4B), whereas only 6 genes (Fig. 4A) were repressed. Among them, in addition to the previously described *fim* genes, many ORFs coding for the flagellum and chemotaxis systems were found (Fig. 4C). Our data suggest that depending on promoter-specific properties, the requirements for ppGpp and DksA might be dissimilar and the mechanisms of repression and stimulation might be diverse.



C

ORF	ppGpp⁰	Δ*dksA*	ppGpp⁰ Δ*dksA*
Up-regulated			
dnaK			8.1
dnaJ			8.4
ybcU		2.7	7.5
pheP	1.4	1.5	6.3
yeeF	2.9	3.1	6.5
ndk	3.2		27
sdaC	3.5	3.8	6.9
sdaB	4.1	2.7	7.6
yhdG	1.4	2.7	6.1
yrfG	1.8	2.3	7.1
lldP			28
lldR			12
lldD			7.4
b2174	1.3	3.1	9.4
Down-regulated			
yaaJ			0.2
ykgE	0.6	0.5	0.1
ykgF	0.4	0.4	0.1
dcuC			0.1
ybeL	0.7	0.7	0.2

ORF	ppGpp⁰	Δ*dksA*	ppGpp⁰ Δ*dksA*
Down-regulated			
wrbA	0.2		0.1
putP			0.1
ychH			0.1
narK			0.1
narG	0.5		0.1
narH	0.4		0.1
narJ	0.4		0.1
narI	0.5		0.1
fdnH	0.4		0.1
fdnI	0.5		0.2
manX	0.4	0.7	0.1
manY	0.3	0.6	0.1
napD	0.7		0.1
napF			0.1
b2343			0.2
yfiD	0.3		0.1
yfiA	0.3		0.1
yjjI			0.1
hypA			0.1
hypB			0.1
hypC			0.1
hypD			0.1

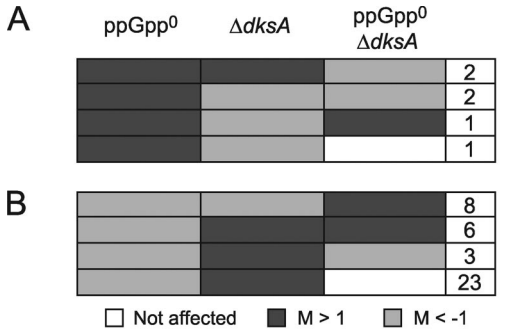
ORF	ppGpp⁰	Δ*dksA*	ppGpp⁰ Δ*dksA*
Down-regulated			
abrB			0.1
cydA	0.5		0.1
cydB	0.5		0.1
ybiW	0.7		0.2
ybjW	0.7		0.1
focA	0.7		0.1
b0919	0.6	0.6	0.1
yccJ	0.3		0.2
yhgG			0.1
nikD			0.1
yibO			0.1
ytfE			0.1
ansB	0.4	0.8	0.1
b2997	0.7	0.6	0.1
tdcC	0.6	0.2	0.1
tdcB		0.3	0.1
tdcD	0.4	0.2	0.1
yhaD			0.2
yhaE			0.1
feoB			0.1
nrfA	0.2		0.1
nfrB	0.2		0.1

The numbers show the fold difference in expression in the mutant strain as compared to the Wt

FIG. 3. General pattern of transcription stimulation and repression in strains deficient in ppGpp and/or DksA. (A and B) The Venn diagrams indicate the number of ORFs upregulated (red) and downregulated (green) more than either twofold (A) or sixfold (B) in the different mutant strains. ORFs that were differentially altered in one of the mutant strains compared to the others have been excluded. (C) Table showing the 63 (49 + 14) ORFs affected more than sixfold in the ppGpp^0 Δ*dksA* strain compared to the WT and the fold difference in expression of each ORF in the three mutant strains compared to the WT ($P < 0.0001$). The absence of number indicates no significant difference in expression level compared to the WT strain.

Effect of ppGpp and/or DksA deficiencies on flagellum biosynthesis and chemotaxis operons. The transcriptional pattern of the 54 ORFs coding for flagellum biosynthesis and chemotaxis is shown in Fig. 5A. These genes are distributed in 18 operons and are grouped into early, middle, and late operon classes depending on their temporal expression (Fig. 5A) (10, 30). All the flagellum- and chemotaxis-encoding ORFs were induced in the DksA-deficient strain compared to the WT strain, whereas 60% of them (32 of the 54 ORFs) were downregulated in the ppGpp-deficient strain (Fig. 5A). Interestingly, the expression of several of those ORFs was not significantly altered in the double mutant strain. The extent of the effects caused by the deficiency in either DksA or ppGpp varies depending on the operon class. In the DksA-deficient strain, expression was slightly induced (2.5-fold) for the early operon (*flhDC*), modestly induced (between 4- and 10-fold) for the middle operons, and greatly induced for the late operons, i.e., *fliC* (36-fold), *motB* (20-fold), and *tar* (23-fold) (Fig. 5A). In the ppGpp-deficient strain, the early and middle operons were slightly repressed (1.4- and 2.9-fold on average, respectively), while most of the late operons were unaffected (Fig. 5A).

The ppGpp/DksA-mediated control of flagellum and chemotaxis genes was assessed by phenotypic characterization of the chemotactic movement in the different strains (WT, ppGpp^0, Δ*dksA*, and ppGpp^0 Δ*dksA* strains) using TB plates with either different chemoattractants (L-serine, maltose, or glucose) or a chemorepellent (L-valine) (Fig. 5B). The WT strain showed modest spreading and a weak response to the different chemoattractants. Nevertheless, the ppGpp-deficient strain showed even less motility, corroborating our transcrip-



C

	ORF		ORF		ORF		ORF
A	*nikA*	B	*b1777*	B	*flgM*	B	*fliG*
	glnQ		*udhA*		*flgA*		*fliH*
	yhaU		*fliA*		*flgB*		*flgH*
	yjfN		*fliS*		*flgC*		*fliJ*
	trxC		*fimI*		*flgD*		*fliK*
	dppA		*carA*		*flgE*		*fliL*
B	*pdhR*		*carB*		*flgF*		*fliM*
	aldA		*fimC*		*flgG*		*fliN*
	fumC		*hdeB*		*flgH*		*pyrI*
	mglA		*artI*		*flgI*		*pyrB*
	mglB		*hdeA*		*fliZ*		*fimA*
			flgN		*fliF*		

FIG. 4. Expression profiles of the ORFs altered more than twofold compared to the WT and differentially regulated in at least one of the mutant strains ($P < 0.0001$). Dark and light gray boxes indicate upregulation (M value of >1) or downregulation (M value of <-1) of gene expression in the mutant strains, respectively. White boxes indicate no significant alteration of gene expression. The number of ORFs found in each "class" is shown on the right. (A) ORFs presumably repressed by ppGpp (upregulated in the ppGpp-deficient strain). (B) ORFs presumably stimulated by ppGpp (downregulated in the ppGpp-deficient strain). (C) Table showing the names of the ORFs represented in the different "classes" represented in panels A and B. Genes are listed following the subclasses in panels A and B from top to bottom.

tional data indicating that the ppGpp-deficient strain expresses, compared to the WT, many fewer flagella although it has a similar level of expression of the chemotaxis receptors (Tsr, Tar, and Trg sensing L-serine, maltose, and glucose, respectively). Conversely, the DksA-deficient strain showed an increased motility and an evident chemotactic response pattern that can be correlated with the transcriptional induction of the flagellum and chemotaxis receptors. The ppGpp^0 Δ*dksA* strain showed an intermediate phenotype, as expected on the basis of the transcriptional data (Fig. 5).

To corroborate that the differences observed in the transcriptional profile and the phenotypic assays correlate with differences in the production of flagella, direct observation by transmission electron microscopy and detection of FliC (a major subunit of the flagella) and FliA (a σ factor and regulator of late operons) were performed with the WT, ppGpp^0, Δ*dksA*, and ppGpp^0 Δ*dksA* strains. Microscopy observation (Fig. 6A) confirmed the massive flagellum production expected in the Δ*dksA* strain compared to the other strains, confirming the transcriptional data. Moreover, an obvious decrease in the levels of FliC and FliA was observed in the ppGpp-deficient strain, whereas a huge increase was observed in the DksA-deficient strains independently of the presence or absence of ppGpp (Fig. 6B) (since the amount of FliC in MG1655 was very low, different amounts of each protein extract were loaded for quantification purposes). As a control, the protein level of H-NS (a major nucleoid-associated protein) was determined using the same protein extracts.

The increased expression of flagellum-related genes is abolished in the absence of the Gre factors. Repression and induction of gene expression caused by deficiency in ppGpp or DksA, respectively, has been previously described for the genes of the *fim* determinant, coding for the type 1 fimbriae (1, 2). In that case, it was shown that the upregulation of gene expression in a DksA-deficient strain in vivo did not imply that DksA has an active repressing effect on transcription. In fact, it was shown that the stimulation of the *fim* determinant in the DksA-deficient strain was suppressed by mutation of the genes coding for the Gre factors. Those results suggest that in the absence of DksA, the availability of the secondary channel of the RNAP to interact with GreA and GreB might increase, to account for the upregulation observed (2). To determine whether the increased expression profile of flagellum genes in the DksA-deficient strain might be explained by a similar mechanism, the effects of *gre* mutations on the expression of FliC were analyzed (Fig. 7). The expression of FliC (a major subunit of the flagella) in the DksA-deficient background was greatly reduced in the presence of a *greA* mutation (Fig. 7A), in agreement with the mechanism proposed to underlie DksA-mediated repression of *fimB* transcription. The results obtained when the *greA* allele was added in the *dksA* background indicate that, under the conditions used, GreA seems to play a relevant role in the control of *fliC*. An interesting question is whether the interplay between the DksA and Gre factors affects *fliC* expression either at transcription initiation or during transcription elongation. To discriminate among those possibilities in vivo, early (+70, first codon of the *fliC* coding sequence) and late (+1210) chromosomal *lacZ* fusions within the *fliC* gene were constructed. Expression from those fusions in the WT, Δ*dksA*, and Δ*dksA* Δ*greA* strains was determined (Fig. 7B). Interestingly, for the early *fliC* fusion, no important response to DksA deficiency was observed. However, a very important increase in expression was detected in the absence of DksA when the late *fliC* fusion was used. Remarkably, this increased expression dropped to WT levels when GreA was eliminated. These results suggest that *fliC* expression is importantly regulated at the elongation level in a GreA-dependent manner. Moreover, the results with the early fusion do not let us rule out that the interplay between DksA and the Gre factors might also contribute to the regulation at the transcription initiation level. Remarkably, the expression of FliC was severely reduced in a Δ*greA* Δ*greB* strain even in the presence of DksA, suggesting that the expression of FliC is strictly dependent on the presence of the antipausing Gre factors (Fig. 7A). To elucidate whether the Gre factors directly or indirectly affect transcription of the *fliC* gene will require further studies.

DISCUSSION

The ppGpp mediator of the stringent response induces major changes in the pattern of gene expression, with consequential effects on different cellular processes (reviewed in refer-

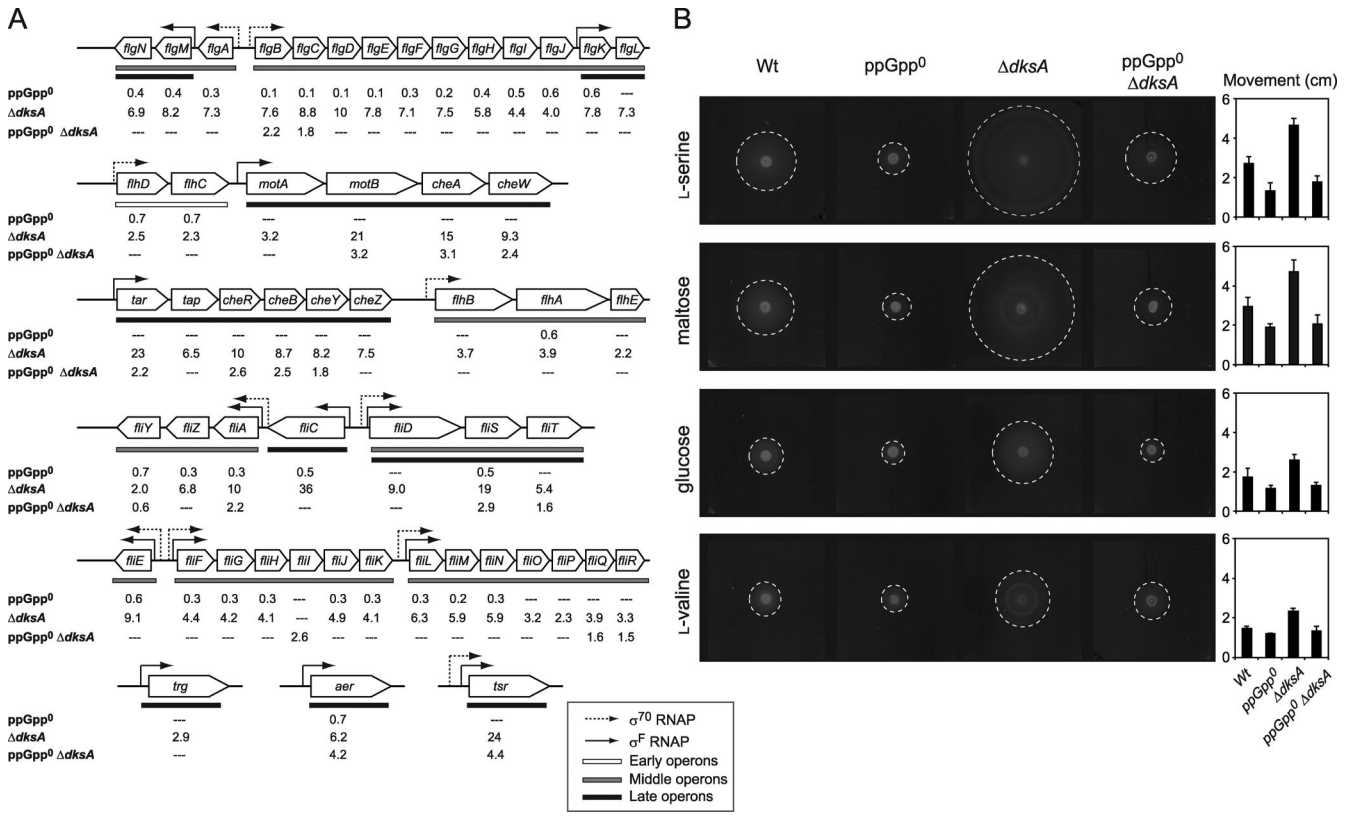


FIG. 5. Effect of ppGpp and/or DksA deficiencies on flagellum biosynthesis and chemotaxis in *E. coli*. (A) Genetic structure of the genes involved in flagellum biosynthesis and chemotaxis. Marked lines indicate whether the operons are expressed in the early (white), middle (gray), or late (black) stage of the temporal induction pathway. Dashed-arrow lines indicate promoters that are σ^{70} dependent and solid arrow lines promoters that are σ^{F} dependent. The numbers below the ORF names show the fold difference in transcriptional expression in each mutant (ppGpp^0 [CF1693], Δ*dksA* [AAG95], and ppGpp^0 Δ*dksA* [AAG98])] compared to the WT (MG1655). Downregulated genes have values of <1, and upregulated genes have values of >1 ($P < 0.0001$) (B) Determination of the effect of ppGpp and/or DksA on motility. Movement on low-agar TB plates containing three chemoattractants (L-serine, maltose, and glucose) and one chemorepellent (L-valine) was measured for the WT (MG1655), ppGpp^0 (CF1693), Δ*dksA* (AAG95), and ppGpp^0 Δ*dksA* (AAG98) strains. The diameter of the perimeter of the bacterial growth was measured, and averages and standard errors from four independent experiments were plotted in the graphs to the left of the pictures.

ence 19). A common experimental approach to establish whether a genetic system is under ppGpp control is to compare expression in a WT strain and its ppGpp-deficient counterpart in the beginning of stationary phase (1, 2, 17, 26). In this report, we describe comparative microarray analysis of the global transcriptional patterns of a widely studied ppGpp-deficient *E. coli* strain (ppGpp^0, CF1693) and its otherwise WT counterpart (MG1655) grown in LB medium at 37°C to the beginning of stationary phase (OD_{600} of 1.5). The discovery that DksA can potentiate the effects of ppGpp on transcription stimulation and repression (2, 29, 35, 38) led us to also compare the global gene expression patterns of a Δ*dksA* strain and a ppGpp^0 Δ*dksA* strain.

Of the total number of protein-encoding ORFs screened (all genes of *E. coli* excluding those for rRNA and tRNA), we found that 6% were up- or downregulated more than twofold in the ppGpp-deficient strain compared to the WT. Our results are reminiscent of microarray studies of the effects of other global regulators in *E. coli*, such as H-NS (21) and Fis (4), where a similar percentage of genes (~5%) were found to be affected. The number of genes affected when comparing the DksA-deficient strain to the WT was similar to the number affected in the ppGpp-deficient strain (approximately 7%). In the strain deficient in both ppGpp and DksA, the number rose to 13% of the ORFs. In accordance with previous reports describing the general effect of ppGpp and DksA on bacterial physiology, the cellular functions mostly affected in the strains deficient in those factors were metabolism, transport, translation, and DNA/RNA metabolism (8, 13, 37). In a recent report by Traxler et al. (48), the gene expression profiles of WT and ppGpp-deficient strains after induction of the stringent response were compared. Although the experimental setup and the growth conditions used were different in the two approaches, we found in the ppGpp-deficient strain (see Table S2 in the supplemental material) altered expression of genes involved in the control of important cellular processes (protein synthesis, transcription, replication, fatty acid biosynthesis, stress response, etc.) that were likewise detected by Traxler et al. (48), corroborating the important role of ppGpp in the control of diverse cellular processes in the bacterial cells. Interestingly, in comparison with our results, a higher number of genes showed altered expression in the ppGpp-deficient strain, which presum-

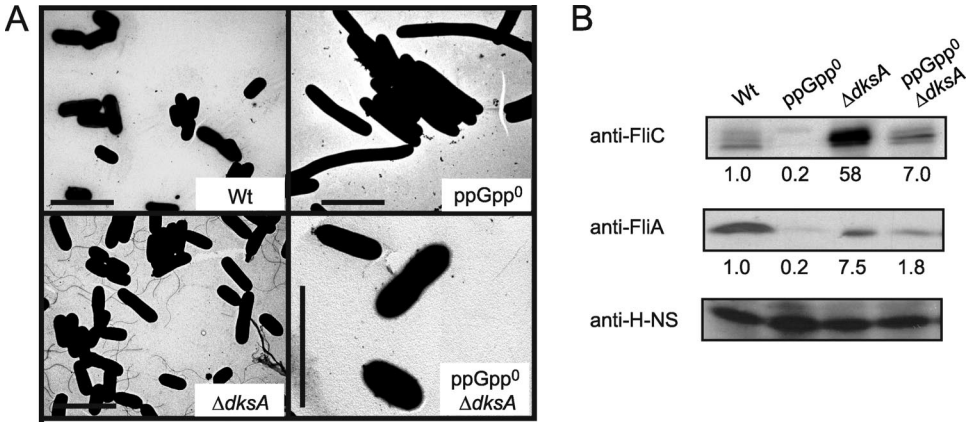


FIG. 6. ppGpp and DksA deficiencies differentially affect flagellum production. (A) Electron micrographs of bacterial cultures of the WT (MG1655), ppGpp^0 (CF1693), Δ*dksA* (AAG95), and ppGpp^0 Δ*dksA* (JVF14) strains, grown under the same conditions as for Fig. 1. Bar, 5 μm. (B) Effect of ppGpp and DksA deficiencies on expression of the major flagellum subunit FliC and the sigma subunit FliA. Determinations of the protein levels of FliC, FliA, and H-NS (control) were performed by Western blot analysis of protein extracts from the WT (MG1655), ppGpp^0 (CF1693), Δ*dksA* (AAG95), and ppGpp^0 Δ*dksA* (AAG98) strains cultured as for Fig. 1. For FliC and FliA immunodetection, 100 μg of protein extracts was used for the WT and ppGpp^0 strains, while 5 μg of the Δ*dksA* extract and 10 μg of the ppGpp^0 Δ*dksA* extract were used. For H-NS detection, 25 μg of each extract was used. The values shown below the gels represent the relative amount of protein found in the mutant strains compared to the WT strain (set as 1), after adjusting for the amount of extract used.

ably might be explained by the fact that the expression profiles were compared after inducing the stringent response by amino acid starvation (48).

Based on expression studies performed with the *rrnB* and amino acid biosynthetic operons (34, 35), a model of concerted coregulation by ppGpp and DksA was proposed, which entails that mutants deficient in either ppGpp or DksA would possess similar features independently of whether the ppGpp/DksA-mediated regulation was direct or indirect. However, phenotypic studies indicated that ppGpp and DksA can have identical but also independent and opposing roles in gene expression in *E. coli* (2, 20, 29, 38). Our transcriptional data support the phenotypic diversity previously described. (i) Most of the ORFs affected in either ppGpp- or DksA-deficient strains were also affected in the strain lacking both factors. Hence, many of the 346 ORFs affected in the ppGpp^0 Δ*dksA* strain are also affected in the ppGpp^0 and the Δ*dksA* strains, but by less than twofold (Fig. 2A). (ii) Interestingly, a notable number of genes whose expression is altered in either the ppGpp-deficient (89 + 58) or DksA-deficient (124 + 58) strain, representing 55 and 58% of all genes affected in the ppGpp- and DksA-deficient strains, respectively, did not show altered expression in the ppGpp/DksA-deficient strain. Moreover, 58 of those genes were affected in both the ppGpp- and DksA-deficient strains (Fig. 2A). (iii) Many genes exhibited opposite effects on transcription by ppGpp and DksA (Fig. 4). Thus, our data indicate that the mechanisms of regulation by ppGpp and DksA are most likely more diverse than initially suggested, and not all effects observed can be explained by the concerted coregulation. Elucidation of the presumably different modes of action of these two regulatory factors will require detailed studies of genetic determinants of regulation of the individual systems.

An extensive group of genes that encode flagellum synthesis and chemotaxis were downregulated in ppGpp-deficient strains while being upregulated in the DksA-deficient counterpart. A similar expression pattern was previously de-

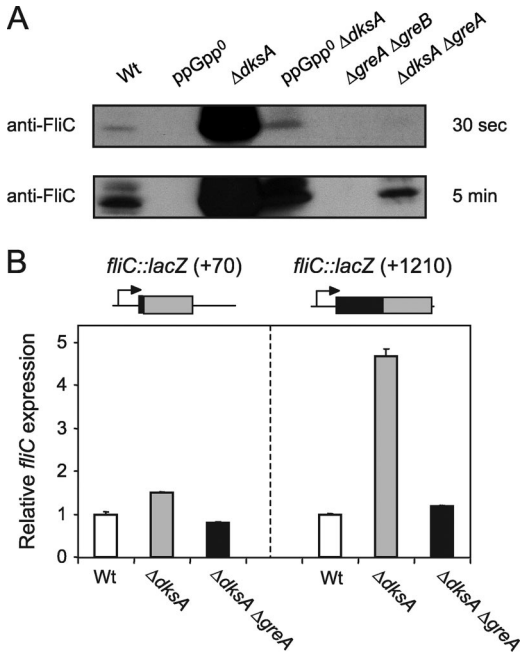


FIG. 7. GreA and GreB regulate expression of the major flagellum subunit FliC. (A) FliC immunodetection in protein extracts (100 μg) from the WT (MG1655), ppGpp^0 (CF1693), Δ*dksA* (AAG95), ppGpp^0 Δ*dksA* (AAG98), Δ*greA* Δ*greB* (AAG107), and Δ*dksA* Δ*greA* (AAG101) strains cultured as for Fig. 1. Detection after 30 s and 5 min of exposure is shown. (B) Expression from two chromosomal *fliC*::*lacZ* transcriptional fusions. *lacZ* fusions within the *fliC* gene at positions +70 (left panel) and +1210 (right panel) were constructed, and their expression in the WT (PRG13 and PRG16), Δ*dksA* (PRG14 and PRG17), and Δ*dksA* Δ*greA* (PRG15 and PRG18) strains was monitored. Cultures were grown in LB medium to an OD_{600} of 1.5 at 37°C. Relative average values and standard deviations from three independent experiments are shown. For each *fliC*::*lacZ* fusion, the value in Miller units in the WT was set as 1, which corresponds to 60 and 106 Miller units for PRG13 and PRG16, respectively.

scribed for the type 1 fimbriation-encoding genes (1, 2, 29); however, it was demonstrated that in vitro, both ppGpp and DksA stimulate the transcription from the responsible promoter, namely, that for *fimB* (2). A model was suggested in which the upregulation of *fimB* detected in the DksA-deficient strain in vivo was a consequence of the increased vacancy of the secondary channel of the RNAP, due to the absence of DksA, that could promote the binding of other regulators such as the GreA/B antipausing factors. Here we showed evidence that the transcriptional stimulation of flagellum- and chemotaxis-related genes observed in DksA-deficient strains was suppressed when the Gre factors were removed (Fig. 7). These results suggest that when analyzing the in vivo DksA-mediated effects on gene expression, the passive consequence of vacancy of the secondary channel of the RNAP should be considered. GreA-regulated genes in *E. coli* have been identified using a microarray approach comparing the expression profiles of a *greA greB* strain, a *greA*$^+$ *greB* strain, and a strain overexpressing GreA from a plasmid in a *greA greB* background (46). Although the growth conditions used were different from those in our study (30°C and mid-log-phase cultures as opposed to 37°C and late mid-log-phase cultures), 39 of the 311 genes with altered expression in the DksA-deficient strain were found to be regulated by GreA, supporting the hypothesis that some of the phenotypes found in a DksA-deficient strain might be related to the interplay between DksA and the Gre factors. The influence of the Gre factors on the expression of ppGpp/DksA-regulated genes presumably depends on the promoter analyzed. As we have shown for *fimB* transcription, the Gre factors had an effect in vivo only in the absence of DksA (2), and for *argI* the Gre factors were dispensable (2), while for FliC, the Gre factors affect expression even in the presence of DksA (Fig. 7).

In contrast to both our transcriptional and chemotactic response results, it has been reported that both ppGpp- and DksA-deficient strains of *E. coli* are defective in motility (29). Differences in the experimental strategies might account for the discrepancies observed, because in those studies no chemoattractants were included in the medium used to monitor motility (29). Accordingly, when our strains were grown under the same conditions, defects in motility were detected in both ppGpp- and DksA-deficient strains (data not shown). Downregulation of motility-related genes was also detected in a previous microarray analysis of ppGpp-deficient strains growing in MOPS (morpholinepropanesulfonic acid) medium containing glucose (48). However, compared with our results, only few of the flagellum synthesis genes were downregulated and to a minor extent, which might be explained by the described repressive effect of glucose at high concentrations on the chemotactic motility of *E. coli* (24).

The interplay between DksA and the Gre factors highlights the importance of the secondary channel of the RNAP in gene regulation. Based on the intracellular concentrations of DksA, GreA, and GreB and their predicted affinities for RNAP, it has been postulated that most of the RNAP would be occupied by DksA rather than GreA and/or GreB (40). Moreover, it has been described that the abundance of DksA, GreA, and GreB does not change throughout the growth curve (40). However, it cannot be ruled out that there may be conditions where the affinities of the different factors change to override differences in their relative abundances. It should also be considered that interactions of RNAP with a specific promoter sequence, with a particular σ subunit, or with any other regulatory factor could potentially alter the conformation of the secondary channel of the RNAP, thereby changing its relative affinity for different secondary-channel regulators. A competition among regulatory factors to interact with the secondary channel of the RNAP has been proposed to provide a new mode for regulating transcription (38). Regulation of the *fimB* promoter (2) and the data presented here lend strong support to this idea. However, further biochemical and in vitro studies would be required to corroborate the interplay among those regulatory factors and its role in the regulation of transcription in bacteria. With respect to competition of the secondary channel for regulatory purposes, Lamour et al. (25) have recently shown that Rnk, which does not affect the RNAP activity per se, has a C-terminal structure similar to that of the Gre family of proteins, and they suggest that it may compete with the Gre factors and DksA for binding to the RNAP secondary channel.

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REFERENCES

1. **Aberg, A., V. Shingler, and C. Balsalobre.** 2006. (p) ppGpp regulates type 1 fimbriation of *Escherichia coli* by modulating the expression of the site-specific recombinase FimB. Mol. Microbiol. **60:**1520–1533.
2. **Aberg, A., V. Shingler, and C. Balsalobre.** 2008. Regulation of the *fimB* promoter: a case of differential regulation by ppGpp and DksA *in vivo*. Mol. Microbiol. **67:**1223–1241.
3. **Bertani, G.** 1951. Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. J. Bacteriol. **63:**293–300.
4. **Bradley, M. D., M. B. Beach, A. P. de Koning, T. S. Pratt, and R. Osuna.** 2007. Effects of Fis on *Escherichia coli* gene expression during different growth stages. Microbiology **153:**2922–2940.
5. **Braeken, K., M. Moris, R. Daniels, J. Vanderleyden, and J. Michiels.** 2006. New horizons for (p) ppGpp in bacterial and plant physiology. Trends Microbiol. **14:**45–54.
6. **Brown, L., D. Gentry, T. Elliott, and M. Cashel.** 2002. DksA affects ppGpp induction of RpoS at a translational level. J. Bacteriol. **184:**4455–4465.
7. **Cashel, M., and J. Gallant.** 1969. Two compounds implicated in the function of the RC gene of *Escherichia coli*. Nature **221:**838–841.
8. **Cashel, M., D. R. Gentry, V. J. Hernandez, and D. Vinella.** 1996. The stringent response, p. 1458–1496. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed., vol. 1. ASM Press, Washington DC.
9. **Chatterji, D., and A. K. Ojha.** 2001. Revisiting the stringent response, ppGpp and starvation signaling. Curr. Opin. Microbiol. **4:**160–165.
10. **Chilcott, G. S., and K. T. Hughes.** 2000. Coupling of flagellar gene expression to flagellar assembly in *Salmonella enterica* serovar Typhimurium and *Escherichia coli*. Microbiol. Mol. Biol. Rev. **64:**694–708.
11. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA **97:**6640–6645.
12. **Di Martino, P., A. Merieau, R. Phillips, N. Orange, and C. Hulen.** 2002.

Isolation of an *Escherichia coil* strain mutant unable to form biofilm on polystyrene and to adhere to human pneumocyte cells: involvement of tryptophanase. Can. J. Microbiol. **48:**132–137.
13. **Durfee, T., A. M. Hansen, H. Zhi, F. R. Blattner, and D. J. Jin.** 2008. Transcription profiling of the stringent response in *Escherichia coli*. J. Bacteriol. **190:**1084–1096.
14. **Ellermeier, C. D., A. Janakiraman, and J. M. Slauch.** 2002. Construction of targeted single copy *lac* fusions using lambda Red and FLP-mediated site-specific recombination in bacteria. Gene **290:**153–161.
15. **Gentleman, R., V. Carey, W. Huber, R. Irizarry, and S. Dudoit.** 2005. Bioinformatics and computational biology solutions using R and Bioconductor. Springer, Berlin, Germany.
16. **Gentry, D. R., and M. Cashel.** 1996. Mutational analysis of the *Escherichia coli spoT* gene identifies distinct but overlapping regions involved in ppGpp synthesis and degradation. Mol. Microbiol. **19:**1373–1384.
17. **Gustavsson, N., A. Diez, and T. Nystrom.** 2002. The universal stress protein paralogues of *Escherichia coli* are co-ordinately regulated and co-operate in the defence against DNA damage. Mol. Microbiol. **43:**107–117.
18. **Guyer, M. S., R. R. Reed, J. A. Steitz, and K. B. Low.** 1981. Identification of a sex-factor-affinity site in *E. coli* as gamma delta. Cold Spring Harbor Symp. Quant. Biol. **45:**135–140.
19. **Haugen, S. P., W. Ross, and R. L. Gourse.** 2008. Advances in bacterial promoter recognition and its control by factors that do not bind DNA. Nat. Rev. Microbiol. **6:**507–519.
20. **Hirsch, M., and T. Elliott.** 2002. Role of ppGpp in *rpoS* stationary-phase regulation in *Escherichia coli*. J. Bacteriol. **184:**5077–5087.
21. **Hommais, F., E. Krin, C. Laurent-Winter, O. Soutourina, A. Malpertuy, J. P. Le Caer, A. Danchin, and P. Bertin.** 2001. Large-scale monitoring of pleiotropic regulation of gene expression by the prokaryotic nucleoid-associated protein, H-NS. Mol. Microbiol. **40:**20–36.
21a. **Johansson, J., and B. E. Uhlin.** 1999. Differential protease-mediated turnover of H-NS and StpA revealed by a mutation altering protein stability and stationary-phase survival of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **96:**10776–10781.
22. **Khatri, P., and S. Draghici.** 2005. Ontological analysis of gene expression data: current tools, limitations, and open problems. Bioinformatics **21:**3587–3595.
23. **Kooperberg, C., A. Aragaki, A. D. Strand, and J. M. Olson.** 2005. Significance testing for small microarray experiments. Stat. Med. **24:**2281–2298.
23a. **Kukkonen, M., K. Lähteenmäki, M. Suomalainen, N. Kaikkinen, L. Emödy, H. Lång, and T. K. Korhonen.** 2001. Protein regions important for plasminogen activation and inactivation of alpha2-antiplasmin in the surface protease Pla of *Yersinia pestis*. Mol. Microbiol. **40:**1097–1111.
24. **Lai, H. C., J. C. Shu, S. Ang, M. J. Lai, B. Fruta, S. Lin, K. T. Lu, and S. W. Ho.** 1997. Effect of glucose concentration on swimming motility in enterobacteria. Biochem. Biophys. Res. Commun. **231:**692–695.
25. **Lamour, V., S. T. Rutherford, K. Kuznedelov, U. A. Ramagopal, R. L. Gourse, K. Severinov, and S. A. Darst.** 2008. Crystal structure of *Escherichia coli* Rnk, a new RNA polymerase-interacting protein. J. Mol. Biol. **283:**367–379.
26. **Lange, R., D. Fischer, and R. Hengge-Aronis.** 1995. Identification of transcriptional start sites and the role of ppGpp in the expression of *rpoS*, the structural gene for the sigma S subunit of RNA polymerase in *Escherichia coli*. J. Bacteriol. **177:**4676–4680.
27. **Link, A. J., D. Phillips, and G. M. Church.** 1997. Methods for generating precise deletions and insertions in the genome of wild-type *Escherichia coli*: application to open reading frame characterization. J. Bacteriol. **179:**6228–6237.
28. **Magnusson, L. U., A. Farewell, and T. Nystrom.** 2005. ppGpp: a global regulator in *Escherichia coli*. Trends Microbiol. **13:**236–242.
29. **Magnusson, L. U., B. Gummesson, P. Joksimovic, A. Farewell, and T. Nystrom.** 2007. Identical, independent, and opposing roles of ppGpp and DksA in *Escherichia coli*. J. Bacteriol. **189:**5193–5202.
30. **McCarter, L. L.** 2006. Regulation of flagella. Curr. Opin. Microbiol. **9:**180–186.
31. **Merlin, C., S. McAteer, and M. Masters.** 2002. Tools for characterization of *Escherichia coli* genes of unknown function. J. Bacteriol. **184:**4573–4581.
32. **Miller, J. H.** 1992. A short course in bacterial genetics: laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
33. **Mittenhuber, G.** 2001. Comparative genomics and evolution of genes encoding bacterial (p) ppGpp synthetases/hydrolases (the Rel, RelA and SpoT proteins). J. Mol. Microbiol. Biotechnol. **3:**585–600.
34. **Paul, B. J., M. M. Barker, W. Ross, D. A. Schneider, C. Webb, J. W. Foster, and R. L. Gourse.** 2004. DksA: a critical component of the transcription initiation machinery that potentiates the regulation of rRNA promoters by ppGpp and the initiating NTP. Cell **118:**311–322.
35. **Paul, B. J., M. B. Berkmen, and R. L. Gourse.** 2005. DksA potentiates direct activation of amino acid promoters by ppGpp. Proc. Natl. Acad. Sci. USA **102:**7823–7828.
36. **Perederina, A., V. Svetlov, M. N. Vassylyeva, T. H. Tahirov, S. Yokoyama, I. Artsimovitch, and D. G. Vassylyev.** 2004. Regulation through the secondary channel—structural framework for ppGpp-DksA synergism during transcription. Cell **118:**297–309.
37. **Perron, K., R. Comte, and C. van Delden.** 2005. DksA represses ribosomal gene transcription in *Pseudomonas aeruginosa* by interacting with RNA polymerase on ribosomal promoters. Mol. Microbiol. **56:**1087–1102.
38. **Potrykus, K., D. Vinella, H. Murphy, A. Szalewska-Palasz, R. D'Ari, and M. Cashel.** 2006. Antagonistic regulation of *Escherichia coli* ribosomal RNA *rrnB* P1 promoter activity by GreA and DksA. J. Biol. Chem. **281:**15238–15248.
39. **Reiner, A., D. Yekutieli, and Y. Benjamini.** 2003. Identifying differentially expressed genes using false discovery rate controlling procedures. Bioinformatics **19:**368–375.
40. **Rutherford, S. T., J. J. Lemke, C. E. Vrentas, T. Gaal, W. Ross, and R. L. Gourse.** 2007. Effects of DksA, GreA, and GreB on transcription initiation: insights into the mechanisms of factors that bind in the secondary channel of RNA polymerase. J. Mol. Biol. **366:**1243–1257.
41. **Sambrook, J., and D. W. Russell.** 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
42. **Sands, M. K., and R. B. Roberts.** 1952. The effects of a tryptophan-histidine deficiency in a mutant of *Escherichia coli*. J. Bacteriol. **63:**505–511.
43. **Smyth, G. K.** 2004. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Stat. Appl. Genet. Mol. Biol. **3:**Article 3.
44. **Stebbins, C. E., S. Borukhov, M. Orlova, A. Polyakov, A. Goldfarb, and S. A. Darst.** 1995. Crystal structure of the GreA transcript cleavage factor from *Escherichia coli*. Nature **373:**636–640.
45. **Stent, G. S., and S. Brenner.** 1961. A genetic locus for the regulation of ribonucleic acid synthesis. Proc. Natl. Acad. Sci. USA **47:**2005–2014.
46. **Stepanova, E., J. Lee, M. Ozerova, E. Semenova, K. Datsenko, B. L. Wanner, K. Severinov, and S. Borukhov.** 2007. Analysis of promoter targets for *Escherichia coli* transcription elongation factor GreA in vivo and in vitro. J. Bacteriol. **189:**8772–8885.
47. **Takahashi, K., K. Kasai, and K. Ochi.** 2004. Identification of the bacterial alarmone guanosine 5′-diphosphate 3′-diphosphate (ppGpp) in plants. Proc. Natl. Acad. Sci. USA **101:**4320–4324.
48. **Traxler, M. F., S. M. Summers, H. T. Nguyen, V. M. Zacharia, G. A. Hightower, J. T. Smith, and T. Conway.** 2008. The global, ppGpp-mediated stringent response to amino acid starvation in *Escherichia coli*. Mol. Microbiol. **68:**1128–1148.
49. **Vassylyeva, M. N., A. A. Perederina, V. Svetlov, S. Yokoyama, I. Artsimovitch, and D. G. Vassylyev.** 2004. Cloning, expression, purification, crystallization and initial crystallographic analysis of transcription factor DksA from *Escherichia coli*. Acta Crystallogr. D **60:**1611–1613.
50. **Wendrich, T. M., G. Blaha, D. N. Wilson, M. A. Marahiel, and K. H. Nierhaus.** 2002. Dissection of the mechanism for the stringent factor RelA. Mol. Cell **10:**779–788.
50a. **Westerlund-Wikström, B., J. Tanskanen, R. Virkola, J. Hacker, M. Lindberg, M. Skurnik, and T. K. Korhonen.** 1997. Functional expression of adhesive peptides as fusions to *Escherichia coli*. flagellin. Protein. Eng. **10:**1319–1326.
51. **Willetts, N. S., A. J. Clark, and B. Low.** 1969. Genetic location of certain mutations conferring recombination deficiency in *Escherichia coli*. J. Bacteriol. **97:**244–249.
52. **Xiao, H., M. Kalman, K. Ikehara, S. Zemel, G. Glaser, and M. Cashel.** 1991. Residual guanosine 3′,5′-bispyrophosphate synthetic activity of *relA* null mutants can be eliminated by *spoT* null mutations. J. Biol. Chem. **266:**5980–5990.
53. **Yang, S., P. Dudoit, Luu, and T. P. Speed.** 2001. Normalization for cDNA microarray data, p. 141–152. *In* M. L. Bittner, Y. Chen, A. N. Dorsel, and E. R. Dougherty (ed.), Microarrays: optical technologies and informatics. Proceedings of SPIE, vol. 4266. Society of Photo-Optical Instrumentation Engineers, Bellingham, WA.
54. **Yoshimoto, A., T. Oki, and T. Inui.** 1978. Effect of guanosine 5′-diphosphate 3′-diphosphate and related nucleoside polyphosphates on induction of tryptophanase and beta-galactosidase in permeabilized cells of *Escherichia coli*. Arch. Microbiol. **119:**81–86.