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The *rcsB* Gene, a Positive Regulator of Colanic Acid Biosynthesis in *Escherichia coli*, Is Also an Activator of *ftsZ* Expression

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Wild-type genes which, when overexpressed, are capable of restoring the growth deficiency of the division mutant *ftsZ84* of *Escherichia coli* on L medium containing no added NaCl have been isolated. One of these genes is *rcsB*, a positive regulator of colanic acid biosynthesis. A direct relationship between *rcsB* expression and FtsZ activity was observed, suggesting that RcsB specifically increases transcription of *ftsZ*, thus accounting for the restoration of colony formation by *ftsZ84* mutant cells. Analysis of the 5′ upstream sequence of *rcsB* revealed, in addition to the σ^{54} promoter sequence previously reported, a presumptive σ^{70} promoter and LexA-binding site plus an upstream sequence that is found to be essential for the expression of *rcsB* on a plasmid. The absence of the σ^{54} factor does not have a negative effect on the transcription of *rcsB*. The RcsB protein is an activator of its own synthesis, particularly in the presence of NaCl. Evidence which suggests that RcsB can be phosphorylated by a presumably modified EnvZ or PhoM sensor protein leading to a suppression of the growth deficiency of *ftsZ84* mutant cells and to an increase in colanic acid production was obtained. We also demonstrated that the level of colanic acid is reduced when the cells carry a multicopy *rcsC* plasmid, suggesting that the RcsC sensor has phosphatase activity.

Considerable evidence demonstrating a pivotal role for the *ftsZ* gene in the control of cell division in *Escherichia coli* has accumulated (for a review, see reference 21). One would therefore expect *ftsZ* expression or FtsZ activity to be tightly controlled and even to be subject to a multitude of regulatory signals. The genetic structure of *ftsZ* involves several transcriptional units with promoters initiating transcription from upstream genes, while no transcriptional terminators have been found (1, 34). Thus, it is likely that the extent of transcription from each one of these promoters would be influenced by the presence of regulatory signals whose concentrations are likely to be adjusted depending upon growth and environmental conditions.

It has been proposed that the FtsZ activity of *ftsZ84* mutant cells is too low for growth to occur on L agar containing no added NaCl (25). Indeed, the presence of a low-copy-number plasmid carrying the *ftsZ84* allele can restore colony-forming abilities on this medium. Such a conclusion is in agreement with reports demonstrating that FtsZ activity is limiting for cell division (3) and that no growth occurs when its level is decreased below a certain threshold level (6).

In our laboratory, we are engaged in the identification of regulator molecules which increase *ftsZ* expression or FtsZ activity. Our approach consists in cloning wild-type genes which, when overexpressed by their presence on multicopy plasmids, restore colony formation of the *ftsZ84* mutant on salt-free L agar. Using this approach, we observed that overexpression of the *relA* gene suppressed the growth deficiency due to the *ftsZ84* mutation. This appears to be due to the capacity of ppGpp to stimulate the expression of *ftsZ*, thus suggesting a possible coordination between cellular metabolism and cell division regulation (26). Furthermore, Wang et al. (31) recently identified a factor, SdiA, which, at high levels, was capable of restoring division activity in a *ftsZ84* strain at the nonpermissive temperature. This factor was shown to increase the cellular concentration of the FtsZ protein. These findings add further support to the contention that FtsZ is the limiting factor for cell division in the *ftsZ84* mutant (3). They also suggest that any factor that suppresses the mutation is likely to control the expression of *ftsZ* and/or of the FtsZ protein activity in the wild-type strain.

In the present study, we present evidence that RcsB, an effector protein which is known to activate extracellular polysaccharide synthesis in *E. coli* K-12 (12, 28), also activates *ftsZ* expression and restores colony formation in *ftsZ84* mutant cells.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains and their genotypes are listed in Table 1. The *ftsAZ-lacZ* fusion, λTGV3, was obtained from M. Vicente (1). It was transferred to AB1157 (Δ*lacU169*) by lysogenization; 0.1 ml of host cells was preabsorbed with 0.1 ml of phage stock ($<10^6$ phage per ml), and lysogens were selected on L agar containing kanamycin and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal). The integration at the lambda attachment site was confirmed by P1 transduction to a *gal-76*::Tn*10* recipient and by then testing for the loss of tetracycline resistance. L broth or L agar contained (in grams per liter) the following: tryptone (NZ-amine A; ICN Biochemicals), 10; yeast extract, 5; and (unless otherwise specified) NaCl, 5. For the *ftsZ84* phenotype to be fully expressed, NaCl content of the medium must be low and it is therefore important that the ingredients contain low levels of NaCl. For example, different lots of yeast extract, even from a given supplier, were found to have high concentrations of NaCl and could not be used. The composition of the Casamino Acids medium has been described previously (25). In all experiments, the incubation temperature was 30°C.

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TABLE 1. Bacterial strains

Strain[a]	Relevant genotype	Source or reference
AB1157	*rcsB*$^+$ *ftsZ*$^+$	This laboratory
GD113	As AB1157 but *ftsZ84*	2
GD165	As AB1157 but *rcsB-lacZ*-Km^r	This work
GD167	As AB1157 but *rcsB*-Km^r	This work
GD175	As GD113 but *relA1*	26
YMC18	*rpoN208*::Tn*10*	29
GD166	As GD165 but *rpoN*::Tn*10*	P1.YMC18 × GD165
GD185	As GD113 but *rcsB*-Km^r	P1.GD167 × GD113
MC4100	Δ*lacU169*	This laboratory

[a] Strains carrying a *lacZ* fusion had been made Δ*lacU169* by transduction with MC4100-derived P1 lysates with selection for proline independence.

The following antibiotics and/or indicators were added to media at the following concentrations: ampicillin, 100 μg/ml; kanamycin, 100 μg/ml; tetracycline, 15 μg/ml; X-Gal, 20 μg/ml.

DNA manipulations. The in vivo cloning procedure of Mosesson et al. (23) with the mini-Mu replicon pRRA101 was used as described previously (24). Plasmids were extracted by the procedure of Birnboim and Doly (4). Restriction enzyme digestion, ligation, and other molecular genetic manipulations were performed with enzymes purchased from Pharmacia or Boehringer Mannheim Corp. and were used according to instructions supplied by the manufacturer. DNA sequence analysis was performed by the dideoxy nucleotide chain termination method (27) by using the Pharmacia T7 DNA polymerase sequencing kit.

Plasmid construction. The plasmid vectors used were pBR322, pUC18, and pACYC184. The physical maps of relevant plasmids with the direction of transcription indicated for possible vector-encoded sequences into which sequences are cloned are presented in Fig. 2. Plasmid pIP50 containing the *ompR envZ* genes (14) was obtained from T. J. Silhavy. Plasmid pMI42 harboring *phoM* (19) was from A. Torriani. The *lacZ*-Km^r cassette harbored by pKOK6 was obtained from W. Kokotek (17). Plasmids pPG19 and pPG20 were constructed by inserting, in both orientations, the 1.6-kb *Pvu*II fragment from a mini-Mu replicon plasmid carrying both *rcsB* and *rcsC* from GD175 (see Results) at the *Pvu*II site of pBR322. Plasmids pPG8 and pPG9 were made by ligating *Sma*I-cleaved pUC18 with a *Pvu*II fragment from pPG19 in the two orientations. Plasmid pPG11 was constructed by cleaving pPG9 with *Eco*RI and *Hin*dIII and ligating the fragment into pBR322 which had been restricted with the same enzymes. The same approach was used for the construction of pPG12, except that the *Eco*RI-*Hin*dIII fragment was obtained from pPG8. Plasmid pPG28 contains the 1.3-kb *Hin*cII-*Pvu*II fragment isolated from pPG19 which was inserted at the *Sma*I site of pUC18. pPG29 was constructed by inserting the blunt-ended *Kpn*I-*Pvu*II fragment of pPG19 containing *rcsB* in pUC18-restricted with *Sma*I. Plasmids pPG18 and pPG21 contain, respectively, the same fragments as in pPG28 and pPG29. The fragments were obtained by cleavage with *Eco*RI and *Hin*dIII and inserted in pBR322 cleaved with the same enzymes. Plasmid pFG17 contains the *rcsC* gene. This gene, which is adjacent to *rcsB* (28), is flanked by a *Pst*I site (in *rcsB*) and a *Eco*RI site to give a 4.2-kb fragment. This fragment was obtained from the mini-Mu replicon plasmid (used for the construction of pPG19 and pPG20 as described above), blunt ended by the Klenow fragment, and ligated into pACYC184 that had been cleaved with *Eco*RV. For the construction of pPX5, pPG11 was digested partially with *Hin*cII and ligated in the presence of a blunt-ended *lacZ* Km^r cassette from pKOK6. pPX6 was constructed by mixing the same cassette obtained from a *Pst*I-digested pKOK6 followed by its insertion into pPG11 which had been partially digested with *Pst*I. Verification of cassette orientation in these two plasmids was done by restriction analysis. pPG13 was constructed exactly as pPX6 except that it contains the kanamycin resistance cassette of pUC-4K instead of that of pKOK6.

Replacement of chromosomal *rcsB* by a *rcsB-lacZ* transcriptional fusion. Replacement of chromosomal *rcsB* was carried out by the gene replacement procedure described by Winans et al. (33). Plasmid pPX6, which carries the *lacZ* kanamycin resistance cassette at the *Pst*I site of *rcsB*, was linearized and used for the transformation of JC7623 carrying the Δ*lacU169* deletion and plated on L agar containing kanamycin. One transformant was infected with P1, and the lysate was used to transduce AB1157 with selection for resistance to kanamycin. Verification that the *rcsB-lacZ*-Km^r cassette had replaced the wild-type *rcsB* gene on the chromosome was effected by cloning and restriction analysis of the region encompassing *rcsB* which was isolated by the in vivo cloning procedure of Mosesson et al. (23). The construction of strain GD185 which contains the kanamycin resistance cassette was carried out in a similar fashion by using plasmid pPG13.

Determination of colanic acid. The quantity of mucoidy produced was determined by measuring the amount of nondialyzable methylpentose, a constituent of the capsular polysaccharide. Methylpentose was isolated by the method of Kang and Markovitz (15) from cells grown overnight and assayed as described by Dische and Shettles (9). Values from at least two independent experiments were averaged to give the values shown in the figures and tables.

β-Galactosidase assays. Measurement of transcriptional activity of *lacZ* operon fusions through resulting β-galactosidase activity was performed as described by Miller (22). Overnight cultures were diluted 1:100 into L broth containing the appropriate antibiotics and incubated with aeration at 30°C to an optical density at 600 nm of between 0.3 and 0.5. Values reported were averaged from at least three different experiments.

Efficiency of plating. Overnight cultures in L broth were diluted, and 0.1-ml samples were spread on plates of L agar and L agar without added NaCl. The number of colonies that formed on the salt-free medium and L medium was used to calculate the relative efficiency of plating.

RESULTS

Effector genes which, when overexpressed, are capable of restoring colony formation in *ftsZ84* mutant cells on L agar without added NaCl were isolated by using the in vivo cloning procedure. Cells of GD175 (*relA1 ftsZ84*) made lysogenic for Mu*cts62* were transformed with the mini-Mu replicon pRRA101, and lysis was induced. The lysate was used to transduce GD113 (*ftsZ84*) cells lysogenized with Mu$^+$ and then selected on salt-free L agar containing kanamycin at 30°C. One of the recombinant plasmids isolated was chosen for further studies not only on the basis of its capacity to restore colony formation of GD113 cells on the low-salt-containing L medium but also because it conferred a mucoid phenotype. Subcloning of various DNA fragments from this plasmid in pBR322 revealed that both phenotypes were conferred by a plasmid carrying a single *Pvu*II fragment of 1.6 kb (see below). A determination of the nucleotide sequence of this fragment revealed that it contained *rcsB*, a

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1   TGACCCGGAAGTTGAAGAGGAAGAAGAGCGTTTACTGGATGATGTCTGCGTAATGGTGGA   60

61  TGTTACTTCGGCAGAAATTCGGAATATTGTCACTCGCCAGTTAGAAAATTGGGGTGCAAC  120

121 CTGTATCACACCCGATGAAAGATTAATTAGTCAAGATTATGATATCTTTTTAACGGATAA  180

181 TCCGTCTAATCTTACTGCCTCTGGCTTGCTTTTAAGCGATGATGAGTCTGGCGTACGGGA  240

241 AATTGGGCCTGGTCAATTGTGCGTCAACTTCAATATGAGCAACGCTATGCAGGAAGCGGT  300
                      HincII

301 CTTACAATTAATTGAAGTGCAACTGGCGCAGGAAGAGGTGACAGAATCGCCTCTGGGCGG  360

361 AGATGAAAATGCGCAACTCCATGCCAGCGGCTATTATGCGCTCTTTGTAGACACAGTACC  420
                                                    -35 P70
                 LexA
421 GGATGATGTTAAGAGGCTGTATACTGAAGCAGCAACCAGTGACTTTGCTGCGTTAGCCCA  480
                -10 P70
                                    P54
481 AACGGCTCATCGTCTTAAAGGCGTATTTGCCATGCTAAATCTGGTACCCGGCAAAGCAGTT  540
                                    KpnI

541 ATGTGAAACGCTGGAACATCTGATTCGTGAGAAGGATGTTCCAGGAATAGAAAAATACAT  600

601 CAGCGACATTGACAGTTATGTCAAGAGCTTGCTGTAGCAAGGTAGCCTATTACATGAACA  660
    rcsB→
661 ATATGAACGTAATTATTGCC  680
    MetAsnValIleIleAla
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FIG. 1. Nucleotide sequence of 5′ region upstream of the *rcsB* gene. The beginning of the predicted amino acid sequence of RcsB is indicated in three-letter code below the appropriate nucleotide base. The boxed area CTG-N_7-GCA (521 to 533) represents the RpoN-like promoter previously reported (28). The CTG-N_{10}-CAG sequence (437 to 452) represents the LexA box. A presumptive σ^{70} sequence is also underlined. Also shown are the *Hin*cII and *Kpn*I sites.

gene whose sequence has previously been reported (28). In Fig. 1 is presented the nucleotide sequence of the 5′ upstream region of *rcsB*. This 5′ upstream sequence has previously been deposited in GenBank by Stout and Gottesman under accession number M28242. Our sequence is identical to that reported by these authors except for the presence of a single cytosine (position 66 in Fig. 1) instead of two consecutive ones. Examination of this nucleotide sequence revealed, in addition to the presence of the σ^{54} promoter identified previously (28), a sequence, CTG-N_{10}-CAG, that is identical to that of the consensus sequence of a LexA-binding site (30). As for all the LexA-binding sequences that have been identified in *E. coli*, this putative LexA-binding region appears to have a σ^{70} promoter. In fact, there is a presumptive −10 sequence, TATACT, that is identical to that identified for the *lexA* gene. On the other hand, the −35 sequence region has limited similarity to the consensus sequence. No sequence in the region upstream of this LexA-binding site resembling that of a σ^{70} promoter could be identified with any degree of certainty.

We first tested if restoration of colony formation and accumulation of colanic acid were encoded by different regions of the 1.6-kb *Pvu*II fragment of plasmid pPG11. When the kanamycin resistance cassette from pUC-4K was inserted at the *Pst*I site of *rcsB* to form pPG13, mucoidy was not present and growth on salt-free L agar did not occur, suggesting that the two activities are under the control of the *rcsB* gene (Fig. 2). However, the 5′ upstream region was also found to be essential, because plasmids pPG18 and pPG21 which harbored the complete *rcsB* gene but with a partially (pPG18) or a nearly completely deleted (pPG21) 5′ upstream region could neither support growth nor confer mucoidy. That *rcsB* was not expressed in pPG21 was not unexpected, because the alternative σ^{54} promoter sequence preceding *rcsB* (28) had been cleaved by *Kpn*I. However, a similar explanation would not be valid in the case of pPG18, since it carried both the σ^{54} and the presumptive σ^{70} promoter (Fig. 1), suggesting that little or no transcription was initiated from these promoters. This was confirmed by transferring the

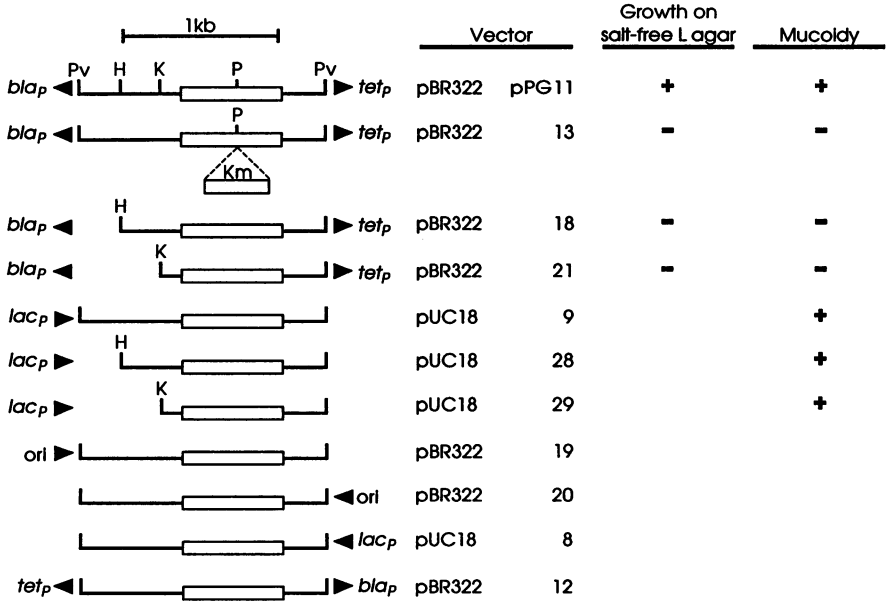

FIG. 2. Physical map of the *rcsB* region and diagram of plasmid chromosomal inserts. The chromosomal inserts in the indicated vectors and the corresponding names of plasmids are shown. Growth on a salt-free L agar column indicates if strain GD113 harboring a given plasmid can grow on this medium. The mucoidy column indicates whether wild-type AB1157 with the given plasmid produces an excess of colanic acid. Only restriction sites relevant to the present study are shown. Pv, *Pvu*II; H, *Hin*cII; K, *Kpn*I; P, *Pst*I. Km is an insertional inactivation cassette from pUC-4K. The rectangle represents the *rcsB* gene. Also shown are the proximal promoters located on each vector and their direction of transcription: bla_p, β-lactamase promoter (Ap^r); tet_p, tetracycline resistance promoter; lac_p, lactose promoter; ori, origin of replication.

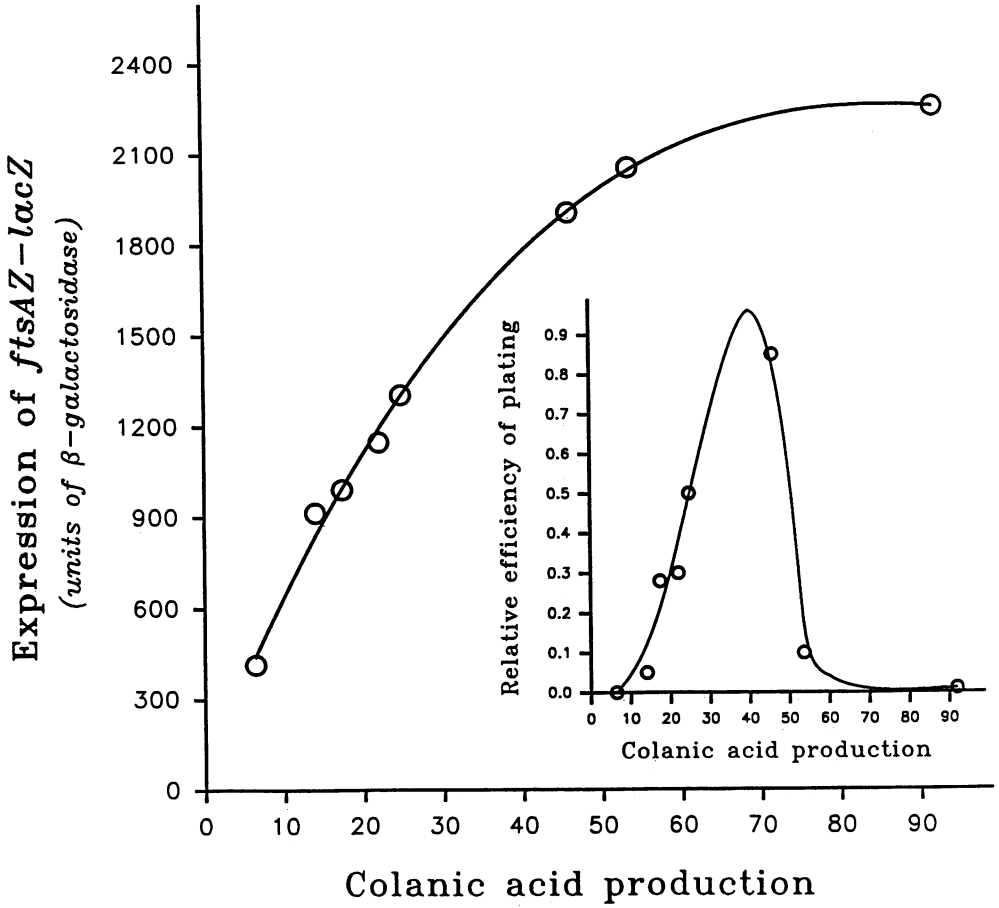

FIG. 3. Expression of *ftsZ* activities in cells harboring various multicopy *rcsB* plasmids exhibiting different levels of *rcsB* expression. Strain AB1157(λTGV3) were assayed for β-galactosidase activities and colanic acid synthesis. Each point represents the units of activities in cells transformed with one of the plasmids described in Fig. 2 and which are in the following order: pPG 13, 19, 11, 8, 12, 20, 9, and 29. (Insert) Relationship between *rcsB* activity (colanic acid production) and the relative efficiency of plating. Overnight cultures of GD113 cells carrying the *rcsB* plasmids were diluted, and samples were spread on plates of L agar and L agar without added NaCl. Colanic acid production is expressed in micrograms of nondialyzable methylpentose per milliliter per unit of optical density at 600 nm.

chromosomal DNA fragments from pPG11, pPG18, and pPG21 into pUC18 and in the same orientation with respect to the plasmid *lac* promoter. The resulting plasmids, pPG9, pPG28, and pPG29, induced large quantities of exopolysaccharides in the harboring cells. Restoration of colony formation (not indicated in Fig. 2) was reduced seemingly because of an overproduction of FtsZ84 in these cells (Fig. 3).

To test whether or not the activity of pPG11 was due to a promoter upstream of the *Hin*cII site, plasmids with a *lacZ* operon fusion inserted at the *Hin*cII site to give pPX5 or at the *Pst*I site to give pPX6 were constructed, and the β-galactosidase activities produced by these two plasmids were compared. The activities determined were 218 U for the fusion inserted at the *Hin*cII site and 4,670 U when the same fusion was at the *Pst*I site. These results indicate that the promoter activity at the level of the *Hin*cII site cannot account for the high activity measured at the *Pst*I site. Thus, the upstream *Pvu*II-*Hin*cII fragment does not seem to contain an active promoter but is, in an unknown capacity, essential for the expression of *rcsB* in pPG11.

Correlation between the RcsB and FtsZ activities. The results obtained above suggest that both colanic acid synthesis and suppression of *ftsZ84* are under the control of the *rcsB* gene. One would therefore expect a correlation to exist between these two activities. Cells of AB1157(λTGV3) were transformed with plasmids harboring the 1.6-kb *Pvu*II DNA fragment, or segments thereof, inserted in the two orientations and/or at different restriction sites (Fig. 2). These plasmids exhibited different levels of *rcsB* expression, presumably because transcription was initiated from different promoters present on the plasmids in addition to the activity associated with the 5′ upstream sequence, when present. It can be seen in Fig. 3 that, concomitant with the accumulation of colanic acid, there was a progressive increase in *ftsZ* expression as expressed by the activities of the *ftsAZ-lacZ* fusion. It is also shown (Fig. 3, insert) that upon an increase in *rcsB* activity, hence in *ftsZ* expression, there was a restoration of colony formation which was optimal when the level of colanic acid was between 30 and 50 μg, corresponding to *ftsAZ-lacZ* activities of between 1,300 to 2,000 β-galactosidase units. At higher levels the efficiency of plating was drastically reduced, an expected finding because a high FtsZ (32) and especially a high FtsZ84 activity have been shown to markedly reduce the colony forming ability on L agar without added NaCl (25).

The RcsB protein is an activator of its own synthesis. A gene

TABLE 2. Expression of chromosomal *rcsB-lacZ* in cells containing plasmids expressing different levels of RcsB

Plasmid	β-Galactosidase (U)
pBR322	215
pPG13	211
pPG19	287
pPG8	360
pPG20	448
pPG29	522

corresponding to *rcsB*, *algR1*, has been identified in *Pseudomonas aeruginosa* and studied (8). The *algR1* gene product has been shown to activate its own promoter when present at low levels (16). To determine whether or not RcsB from *E. coli* shares this property, we transformed GD165, a strain which carries the *lacZ*-Km^r cassette inserted in the chromosomal *rcsB* gene, with plasmids exhibiting different levels of expression of *rcsB*, and the transformants were assayed for β-galactosidase activities. The results are shown in Table 2. It is seen that the expression of the chromosomal *rcsB-lacZ* fusion activities were higher when the cells harbored a *rcsB*-carrying plasmid. It was also observed that as the RcsB levels increased (estimated by the amounts of colanic acid produced [Fig. 2]) there was a proportional increase in the *rcsB-lacZ* fusion activities. These findings demonstrate that, like AlgR1 of *P. aeruginosa*, the RcsB protein of *E. coli* regulates its own synthesis, but unlike AlgR1, no inhibition was observed at the higher RcsB levels.

rcsB promoter activity in the presence of NaCl. The presence of NaCl in L medium permits the growth of *ftsZ84* mutant cells, but since a *rcsB ftsZ84* double mutant can also grow on this medium (results not shown) it is unlikely that this is due to an activation of *rcsB* expression by NaCl. On the other hand, a wild-type strain harboring the multicopy *rcsB* plasmid pPG20 formed mucoid colonies on L agar medium but not when the same medium contained no added NaCl. Therefore, it was of interest to test whether or not the addition of salt to the growth medium stimulated the transcription of *rcsB*. This was determined by using strain GD165 which, as reported above, has a chromosomal *rcsB-lacZ* fusion. It is seen in Table 3 that there was a very small increase in the β-galactosidase activity between the cells grown in the salt-free and the conventional L broth. Furthermore, very little or no activation of *rcsB* expression was observed when the NaCl concentration was increased to 0.3 M, indicating little transcription activation by NaCl. However, as shown in Table 3, a marked increase in the synthesis of RcsB due to NaCl was observed when plasmid pPG20 was present in the cell. Since in this experiment we are measuring the capacity of RcsB to activate its own synthesis (Table 2), it can be concluded that NaCl enhances this capacity for activation. It can also be seen in Table 3 that concomitant with the increase in *rcsB* expression due to NaCl there was a stimulation of the transcription of *ftsZ*, an observation that reinforces the conclusion that RcsB activates *ftsZ* expression. FtsZ expression in the *rcsB* mutant GD167(λTGV3) was found to be slightly but repeatedly lower than in the wild-type strain, suggesting that, at its physiological level, RscB promotes some *ftsZ* expression.

TABLE 3. Expression of *rcsB* and *ftsZ* in cells grown in L medium containing different concentrations of NaCl[a]

Strain and plasmid	β-Galactosidase (U) with NaCl added at following concn (M):		
	0	0.1	0.3
rcsB-lacZ			
GD165/pBR322	231	259	265
GD165/pPG20	481	532	703
ftsAZ-lacZ			
AB1157(λTGV3)/pBR322	351	389	388
AB1157(λTGV3)/pPG20	1,728	1,977	2,208
GD167(λTGV3)/(*rcsB*)	ND	347	ND

[a] ND, not determined.

TABLE 4. Effects of a *rpoN* mutation on *rcsB-lacZ* transcription

Strain and plasmid	Relevant genotype	β-Galactosidase (U)
GD165/pBR322	*rpoN*$^+$	253
GD166/pBR322	*rpoN208*::Tn*10*	291
GD165/pPG12	*rpoN*$^+$	398
GD166/pPG12	*rpoN208*::Tn*10*	501
GD165/pPG20	*rpoN*$^+$	461
GD166/pPG20	*rpoN208*::Tn*10*	624

A possible role for RcsB in cell growth. To obtain evidence that the small difference in FtsZ activity reported in Table 3 between the wild type and the *rcsB* mutant has physiological significance, we tested GD185, an *rcsB ftsZ84* double mutant, for its resistance to nitrofurantoin, a weak SOS inducer which has been shown to induce the synthesis of low levels of the SfiA protein (13). It was postulated that a *rcsB* mutant would be more sensitive to the presence of nitrofurantoin because the inhibition of FtsZ activity by the increased SfiA protein level would not be counteracted by the stimulatory action of RcsB. The efficiency of plating on L agar containing 3 μg of nitrofurantoin was 0.5 for GD113, whereas it was 0.1 for GD185. Moreover, colonies of GD185 appeared only after 36 h of incubation at 30°C instead of 24 h for GD113 (results not shown), suggesting that the RcsB protein probably plays a role in the growth of the bacterial cell under certain growth conditions.

Influence of the RpoN factor on the expression of *rcsB*. Stout and Gottesman have reported that *rcsB* expression was drastically reduced in *rpoN* mutant cells (28). In their studies, the expression of *rcsB* was measured by using a strain lysogenic for a λ*rcsB-lacZ* fusion. Since the extent of the 5′ upstream region in their construction was not specified, it was of interest to compare their results with those obtained with an *rpoN* mutant strain harboring a chromosomal *rcsB-lacZ* fusion. The results are presented in Table 4. It can be seen that the expression of *rcsB* was a little higher in an *rpoN* mutant strain by comparison with its wild-type counterpart. This small increase in the RcsB level appeared to be amplified when the *rpoN* mutant harbored *rcsB* on a multicopy plasmid. Indeed, cells carrying pPG11 and pPG20, two plasmids controlling different levels of RcsB synthesis, had correspondingly higher *rcsB-lacZ* activities, a finding consistent with the fact that RcsB activates its own synthesis. These results indicate that, under the growth conditions that were used, the absence of the σ^{54} factor in the cell does not have an adverse effect on the expression of *rcsB*.

Effects of LexA on the regulation of *rcsB*. The presence of a putative LexA protein-binding site was unexpected considering that *rcsB* is not known to encode a function related to the SOS response. However, a LexA-binding site preceding genes which have no expected SOS functions was

identified in the *rpsU-dnaG-rpoD* macromolecular synthesis operon whose expression was found to be altered in a *lexA* mutant (20). We constructed derivatives of GD165 which had either the noninducible *lexA3* or the defective *lexA71* mutation and measured their β-galactosidase activities. In both cases, the *rcsB-lacZ* activities were slightly lower in the mutants in comparison with that in their wild-type counterpart (results not shown). This suggests that, under laboratory conditions, wild-type LexA had a weak stimulatory effect on the expression of *rcsB*, but because this phenomenon could also be due to an indirect effect of the *lexA* mutations it was not investigated further.

Regulation of RcsB activity by phosphorylation. In a model for RcsB activation, it was proposed that RcsC is converted to an active protein kinase which is capable of phosphorylating RcsB, although the environmental signal that triggers this phosphorylation has not yet been identified, nor has phosphorylation of RcsB been demonstrated (11, 28). *rcsC* null mutations do not block capsule synthesis in *lon* hosts, which have high levels of the positive regulator RcsA, indicating that the nonphosphorylated form of RcsB can activate the *cps* genes. On the other hand, a mutated form of RcsC, encoded by *rcsC137*, has been postulated to constitutively phosphorylate RcsB because high levels of mucoidy are produced in this mutant (5). This implies that phosphorylated RcsB is a better effector than its unphosphorylated form, but since the *rcsC137* allele is recessive in the presence of a wild-type copy of *rcsC*, it suggests that RcsC dephosphorylates RcsB. To obtain information as to whether or not a phosphorylated form of RcsB can be demonstrated and maintained in a wild-type *rcsC* background, we artificially phosphorylated RcsB by cross-talk with mutationally altered EnvZ and PhoM. Of these two sensor proteins, EnvZ has been directly shown to phosphorylate its effector, OmpR (14). Two plasmids, pIP50 and pMI42, which carried the wild-type *envZ* and *phoM* genes, respectively, were transformed into AB1157. Colonies of the transformed cells were nonmucoid, indicating that no cross-talk was occurring between the wild-type sensor proteins and RcsB. However, rare mucoid colonies appeared spontaneously. The plasmids were isolated from these mucoid mutants and retransformed into AB1157 and GD113 cells. All colonies that formed on the selective medium were mucoid, indicating that a mutation had occurred on the plasmid which has presumably altered the substrate specificities of the sensor proteins. It can be seen in Table 5 that cells harboring pFG39 and pFG40, plasmids carrying the presumably altered *envZ* and *phoM* genes, respectively, were mucoid. Furthermore, the presence of these plasmids in GD113 cells restored growth in the low-salt L agar medium. That these activities were conditional to the presence of RcsB is indicated by the fact that the plasmids had no effect in a *rcsB* mutant. It can also be seen that the presence of the compatible multicopy *rcsC* plasmid pFG17 considerably reduced but did not completely prevent the synthesis of colanic acid by the harboring cells. These results provide additional evidence for a role for RcsC in the regulation of RcsB activity and further suggest that RcsC has phosphatase activity.

TABLE 5. Effects of multicopy of mutationally altered *envZ* and *phoM* on colanic acid synthesis on L medium and growth of GD113 and GD185 on L medium without added NaCl

Strain and plasmid	Relevant genotype	Colanic acid[a]	Growth
GD113/pBR322	*ftsZ84*	5.9	−
GD113/pIP50	*ftsZ84*	5.7	−
GD113/pMI42	*ftsZ84*	5.5	−
GD113/pFG39	*ftsZ84*	23.8	+
GD113/pFG40	*ftsZ84*	26.1	+
GD113/pFG39/pACYC184	*ftsZ84*	26.0	+
GD113/pFG40/pACYC184	*ftsZ84*	26.9	+
GD113/pFG39/pFG17	*ftsZ84*	11.1	+
GD113/pFG40/pFG17	*ftsZ84*	11.3	+
GD185/pBR322	*ftsZ84 rcsB*-Km^r	5.3	−
GD185/pFG39	*ftsZ84 rcsB*-Km^r	5.3	−
GD185/pFG40	*ftsZ84 rcsB*-Km^r	5.7	−

[a] Expressed as micrograms of nondialyzable methylpentose as in Fig. 3.

DISCUSSION

We have isolated a gene which, when present on a high-copy-number plasmid, is capable of restoring colony formation in *ftsZ84* mutant cells on a rich low-salt medium. This gene was *rcsB*, a gene which has been characterized in another laboratory and shown to code for an effector protein capable of activating colanic acid synthesis in *E. coli* K-12 (28). We have demonstrated that there is indeed a net correlation of *rcsB* expression as measured by colanic acid accumulation, an increase in *ftsZ* expression, and restoration of colony formation by *ftsZ84* mutant cells at intermediate levels of *ftsZ* expression. We have also demonstrated the capacity of *rcsB* to autoregulate its own synthesis.

It appears that the 5′ upstream sequence of *rcsB* contains several regulatory signals. Deleting this region or part of it results in a gene which is not sufficiently expressed to promote colanic acid synthesis and *ftsZ* expression. This upstream sequence is only poorly translated, since a *lacZ* fusion inserted at its *Hin*cII site expresses little β-galactosidase activities. The regulatory signals present are the σ^{54} promoter which is located immediately upstream of *rcsB* (28), a consensus LexA-binding site sequence containing a presumptive σ^{70} promoter, and an upstream sequence that appears essential for its expression. Its deletion, as in plasmid pPG18, gives an inactive *rcsB*. Thus, the presumptive σ^{70} and the σ^{54} promoters appear not to be recognized by their respective RNA polymerase holoenzyme, at least when the sequence upstream of the *Hin*cII site is absent. Therefore, it is not possible at the present stage of this investigation to postulate a mechanism that would explain how the expression of *rcsB* is promoted. A promoter in the region upstream of the *Hin*cII site appears to be absent, implying that the σ^{70} and σ^{54} are weak promoters which are either used only under certain growth or environmental conditions or are simply vestigial elements that no longer play a role in the expression of *rcsB*. An alternative and more likely possibility would be that transcription from the σ^{70} or the σ^{54} promoter or both is dependent on the presence of an upstream activating sequence to which bind various effector proteins. The available data suggest that σ^{54} promoters are regulated by activation by enhancerlike elements that are located at some distance from the polymerase binding site (18). Our observation that *rcsB* is slightly more expressed in a *rpoN* mutant would not support this hypothesis. Our results are also at variance with those of Stout and Gottesman, who demonstrated the dependence of *rcsB* expression on the σ^{54} promoter (28). This discrepancy is perhaps due to the use of *rcsB-lacZ* fusions with possibly different 5′ upstream DNA sequences and is again indicative of the existence of a complex mechanism for the regulation of *rcsB*.

The results of Table 5 indicate that the presence of NaCl in the growth medium has little effect on the rate of transcription of *rcsB* in cells harboring only a *rcsB-lacZ* fusion. However, in cells carrying a multicopy *rcsB* plasmid, the chromosomal *rcsB-lacZ* expression is higher, suggesting that the presence of salts stimulates the autoactivation process. However, the requirement for NaCl for colony formation by GD113 cells cannot be explained on the basis of a lower RcsB activity in the absence of salt because *rcsB ftsZ84* double mutant cells grow well on L agar (results not shown). Therefore, the reason for the dependence of GD113 cells on NaCl for growth in L medium remains unclear.

rcsB is a dispensable gene since insertional *rcsB* mutants do not appear to differ from their wild-type counterpart except for the mucoid phenotype (5). However, it appears that this gene could, under certain growth or environmental conditions, confer a distinct advantage to the cell as estimated by comparing the sensitivity of a *rcsB* mutant to nitrofurantoin. We have also shown that RcsB has the potential of being phosphorylated by a sensor molecule and is more active in its phosphorylated form. This suggests a mechanism for rapidly increasing RcsB activity should this be required as a result of changes in environmental conditions. In a separate study, we have identified a new gene which maps at the 5-min position of the genetic map and, when overexpressed, suppresses the *ftsZ84* growth deficiency phenotype and induces mucoidy in a wild type but not in an *rcsB* mutant cell (10). This suggests that *rcsB* is a global regulon involved in a variety of cellular functions in addition to extracellular polysaccharide synthesis. This would explain its occurrence even in organisms which do not synthesize colanic acid. For example, *algR1* of *P. aeruginosa* is the gene corresponding to *rcsB* of *E. coli* (7). Both AlgR1 and RcsB are effector proteins with extensive sequence homologies and have nearly identical molecular weights. They are activators of transcription, of their own synthesis, and of genes responsible for extracellular polysaccharide synthesis. The *algR1* is also preceded by a σ^{54} promoter. It will be interesting to investigate how the regulation of *rcsB* compares with that of *algR1*.

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