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Fine-Structure Mapping and Identification of Two Regulators of Capsule Synthesis in *Escherichia coli* K-12

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Positive and negative regulatory elements involved in the synthesis of colanic acid, the capsular polysaccharide of *Escherichia coli* K-12, have been identified previously. RcsB, a positive regulator for transcription of the structural genes of colanic acid synthesis (*cps*), is a protein of about 26 kilodaltons which probably acts as a multimer. *rcsC*, which maps close to *rcsB* at 48 min on the *E. coli* chromosome, exerts a negative effect on expression of the structural genes and codes for a protein of about 100 kilodaltons. The two genes appear to be transcribed in opposite directions, with the C-terminal ends of the genes being less than 0.3 kilobases apart. Multicopy expression of *rcsB* is lethal in *rcsC* mutants which carry *cps-lac* fusions, probably owing to accumulation of intermediates in the capsule synthesis pathway in these cells. Examination of double mutants and cells carrying multicopy *rcsB*$^+$ plasmids reveal an *rcsA*-independent pathway for capsule synthesis. We hypothesize that RcsC may act as an environmental sensor, transmitting information to the RcsB positive regulator.

The capsular polysaccharide colanic acid is synthesized by a variety of enteric bacteria. Although a function for colanic acid has not been clearly demonstrated, similar capsules in other gram-negative organisms serve to protect cells from dehydration, from bacteriophage infection, and from cellular immunity systems (3, 10, 17). In plant pathogens such as *Erwinia stewartii*, the capsule acts as an important virulence factor (4, 9). Capsular polysaccharides have also been implicated in the plant-bacterium interactions of *Rhizobium* species (21).

We have been investigating the regulation of colanic acid synthesis in *Escherichia coli* K-12 by monitoring the expression of *lac* operon fusions to genes necessary for colanic acid synthesis (*cpsA* to *cpsF*) (13, 39, 41). Using these fusions, we have identified four loci which have major effects on *cps-lac* expression. Two negative regulators, *lon* and *rcsC*, and two positive regulators, *rcsA* and *rcsB*, have been identified (13, 41).

Lon is a major ATP-dependent protease in *E. coli* (6, 8, 11). Among the pleiotropic effects of *lon* are filamentation after DNA-damaging treatments such as UV irradiation and methyl methanesulfonate treatment, failure to degrade abnormal and some normal proteins, and the mucoidy associated with overproduction of colanic acid (12, 15, 16, 25). We have hypothesized that the stabilization of regulatory proteins in *lon* mutants is responsible for the phenotypes of *lon* cells (14). SulA, an inhibitor of cell septation induced after DNA damage, seems to be responsible for the filamentation phenotype; it has a half-life of 1.2 min in *lon*$^+$ cells and 20 min in *lon* mutant cells (31). The overproduction of capsule in *lon* mutants can be explained by the stabilization of the positive regulator, RcsA, in *lon* cells. RcsA has a half-life of 5 min in *lon*$^+$ cells and 20 min in *lon* mutant cells and seems to be limiting for capsule synthesis (39).

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The other two regulators of capsule synthesis, *rcsB* and *rcsC*, map near to each other at 48 min and have opposite effects on the synthesis of capsular polysaccharide. Mutations in *rcsB*, like those in *rcsA*, reduce synthesis in *lon* cells, whereas *rcsC* mutations increase expression in *lon*$^+$ hosts. Preliminary complementation studies with these mutations, carried out with a cosmid vector carrying the nearby *ompC* locus and both *rcsB* and *rcsC*, demonstrated that both *rcsB* and *rcsC* mutations are recessive to the wild type (13). We report here a detailed genetic analysis of the *rcsB* and *rcsC* region and an identification of the *rcsB* and *rcsC* gene products.

MATERIALS AND METHODS

Bacterial strains. *E. coli* K-12 strains and their sources are listed in Table 1. Δ*kan* and ΔTn*10* refer to defective minitransposon insertions (26, 43; see below). Orientation A for Δ*kan* is defined as that with the *Xho*I and *Nru*I sites to the left. For ΔTn*10*, orientation A has IS*10* to the left; the *Hin*dIII site is on the right. P1 transductions were performed as described by Miller (28). *recA* was introduced into strains by two consecutive P1 transductions, the first bringing in *srl*::Tn*10* from SG13182 and the second bringing in *srl*$^+$ *recA* from N100.

λ*rcsB*$^+$ transducing bacteriophage were found in a λD69 bank of *E. coli* DNA (32), screened on *lon rcsB cps-lac* fusion strains, as described in Results. The *cpsB10*::*lacZ* fusion was found to be more sensitive in detecting the transducing phage than was the *cps-11*::*lacZ* fusion (fusions are described in reference 41).

The presence of *ompC*$^+$ on a transducing phage was determined by the ability of that phage to make an *ompC* strain sensitive to the phage *hy2* (37).

While growing cells in various media, we observed that hosts carrying the *lon*::ΔTn*10* insertion mutation grew very poorly on succinate medium. Many of the revertants which eventually arose showed reduced capsule synthesis and carried mutations in *rcsB*. *rcsB* mutations isolated by other means also restored growth on succinate medium to *lon* hosts. *rcsA* mutations, which also abolished *cps* expression,

TABLE 1. *E. coli* strains used

Strain	Relevant genotype	Source or reference
MC4100-derived strains[a]		
SG1041	*lon-100*	41
SG20062	*ilv*::Tn*5*	41
SG20157	*proCYA221 zaj-403*::Tn*10 cpsB10*::*lac*-Mu dl	41
SG20180	*cps-11*::*lac*-Mu dl	13
SG20250	*lon*$^+$ *cps*$^+$	13
SG20308	*cps-11*::*lac*-Mu dl *rcsC145 ompC*::Tn*10*	SG20180 + P1 (SG12017)
SG20322	*cps-11*::*lac*-Mu dl *lon-146*::ΔTn*10*	27
SG20326	*cps-11*::*lac*-Mu dl *rcsC145 ompC*::Tn*5*	SG20308 + P1 (SG4124)
SG20329	*cps-11*::*lac*-Mu dl *rcsC137 ompC*::Tn*5*	13
SG20381	*rcsB10*::ΔTn*10*	SG1041 + SY10
SG20382	*rcsB11*::ΔTn*10*	SG1041 + SY11
SG20383	*rcsB14*::ΔTn*10*	SG1041 + SY14
SG20579[b]	*proC zaj-403*::Tn*10*	41
SG20581[b]	*lon-100*	41
SG20582[b]	*lon*$^+$	41
SG20583[b]	*rcsC145 ompC*::Tn*10*	EA145 + P1 (TK363)
SG20587[b]	*recA*	SG20582 + P1[c]
SG20595[d]	*cps-11*::*lac*-Mu dl *lon-100*	13
SG20598[d]	*cps-11*::*lac*-Mu dl *lon-100 rcsA40 zed-14*::Tn*10*	13
SG20600[d]	*cps-11*::*lac*-Mu dl	13
SG20604[d]	*cps-11*::*lac*-Mu dl *rcsC137 ompC*::Tn*10*	13
SG20611[b]	*lon-146*::ΔTn*10*	SG20582 + P1 (SG20322)
SG20618[d]	*cps-11*::*lac*-Mu dl *rcsB*43 *ompC*::Tn*5 lon-100*	13
SG20643[b]	*rcsC137 ompC*::Tn*5*	SG20581 + P1 (SG20329)
SG20644[b]	*lon-100 rcsA3* (RcsA*) *zed-650*::Tn*10*	SG20581 + P1 (SG12020)
SG20645[b]	*rcsA3* (RcsA*) *zed-650*::Tn*10*	SG20582 + P1 (SG12020)
SG20665[b]	*rcsA40 zed-14*::Tn*10 lon-100*	SG20581 + P1 (SG12014)
SG20682[d]	*cps-11*::*lac*-Mu dl *rcsC145 ompC*::Tn*5*	SG20600 + P1 (SG20326)
SG20685[b]	*lon-100 rcsB10*::ΔTn*10*	SG20581 + P1 (SG20381)
SG20686[b]	*lon-100 rcsB11*::ΔTn*10*	SG20581 + P1 (SG20382)
SG20687[b]	*lon-100 rcsB14*::ΔTn*10*	SG20581 + P1 (SG20383)
SG20688[b]	*lon-100 rcsB15 ompC*::Tn*5*	SG20581 + P1 (JB2015)
SG20696[b]	*rcsB11*::ΔTn*10 rcsC137 ompC*::Tn*5*	SG20581 + P1 (SG12025)
SG20698[b]	*lon-100 rcsB18*::ΔTn*10*	SG20581 + P1 (SG21100)
SG20699[b]	*lon-100 rcsB28*::ΔTn*10*	SG20581 + P1 (SG21101)
SG20702[b]	*rcsC52*::ΔTn*10*	SG20582 + P1 (SG21110)
SG20751[b]	*lon-100 zed-751*::Δ*kan*	SG20665 + P1 (SG20250 *kan* pool)
SG20752[b]	*lon-100 rcsA40 zed-751*::Δ*kan*	As for SG20751 (Lac$^-$)
SG20758[b]	*rcsB15 ompC*::Tn*5*	SG20582 + P1 (JB2015)
SG20759[b]	*rcsA104 zed-751*::Δ*kan*	SG20582 + P1 (SG21044)
SG20761[b]	*rcsC137 ompC*::Tn*10*	SG20582 + P1 (SG12019)
SG20780[b]	Δ*lon-510*	SG20579 + P1 (SG4144)
SG20781[b]	*lon*$^+$	SG20579 + P1 (SG4144)
SG20797[b]	Δ*lon-510 rcsB11*::ΔTn*10*	SG20780 + P1 (SG20382)
SG20798[b]	Δ*lon-510 rcsB11*::ΔTn*10 rcsC137 ompC*::Tn*5*	SG20780 + P1 (SG12025)
SG20799[b]	Δ*lon-510 rcsA104 zed-751*::Δ*kan*	SG20780 + P1 (SG21044)
SG20800[b]	Δ*lon-510 rcsC137 ompC*::Tn*5*	SG20780 + P1 (SG20329)
SG20801[b]	Δ*lon-510 rcsC*$^+$ *ompC*::Tn*5*	SG20780 + P1 (SG20329)
SG20802[b]	*rcsC137 rcsB11*::Tn*10*	SG20781 + P1 (SG12025)
SG20803[b]	*rcsC137 ompC*::Tn*5*	SG20781 + P1 (SG20329)
SG20804[b]	*rcsC137 ompC*::Tn*5 rcsA72*::ΔTn*10*	SG20803 + P1 (ATC5112)
SG20806[b]	Δ*lon-510 rcsC137 ompC*::Tn*5 rcsA72*::ΔTn*10*	SG20800 + P1 (ATC5112)

Continued on following page

did not restore succinate growth. The *rcsB15* mutation was isolated as a succinate$^+$ Lac$^-$ revertant of SG20611 (*lon-146*::ΔTn*10 cps-lac*). The mutation was mapped by P1 transduction to the *rcsB* locus and was complemented by the cosmid containing *rcsB*$^+$ and *rcsC*$^+$, λSV2 *cos* (13).

The *rcsA104* mutation was isolated after nitrosoguanidine mutagenesis (37) of the *lon cpsB*::*lac* host SG21016. Lac$^-$ isolates were screened for complementation by λ *rcsA*$^+$ phage. P1 transduction was used to confirm the location of the mutation.

zed-751::Δ*kan*, a minitransposon linked to the *rcsA* region, was isolated from a pool of Δ*kan* insertions made by infecting SG20250 with λNK1105 (provided by N. Kleckner). Kan^r transductants into a *lon rcsA cps*::*lac* host were screened for Lac$^+$ colonies. The recipient strain, SG20665, also carried a *zed-14*::Tn*10* insertion near *rcsA*; analysis of the segregation pattern for *rcsA* and Tet^r suggested that the Δ*kan* and Tn*10* lay on opposite sides of *rcsA*. Deletions in the *rcsA* region were isolated from hosts carrying *zed-14*::Tn*10*, an *rcsA3* (RcsA*) mutation, and *zed-751*::Δ*kan*, in a *cps*::*lac* background. *rcsA3* (RcsA*) is a dominant allele of *rcsA* which increases capsule synthesis in *lon*$^+$ cells. Tet^s derivatives of these Lac$^+$ hosts were selected on FCT plates (24) and screened for Lac$^-$ and Kan^s. Strains which became

TABLE 1—*Continued*

Strain	Relevant genotype	Source or reference
SG21011	*rcsC137 ompC*::Tn*5 cps*$^+$	SG20250 + P1 (SG20329)
SG21016	*cpsB10*::*lac*-Mu dl Δ*lon-510*	SG20157 + P1 (SG4144)
SG21041	*cpsB10*::*lac*-Mu dl Δ*lon-510 rcsA104*	SG21016 NTG mutagenesis
SG21044	*cpsB10*::*lac*-Mu dl Δ*lon-510 rcsA104 zed-751*::Δ*kan*	SG21041 + P1 (SG20751)
SG21080	*rcsC137 rcsB11*::Tn*10 cps*$^+$ (*ompC*::Tn*5*)	SG21011 + P1 (SG12025)
SG21081	*rcsC137 ompC*::Tn*5 cps*$^+$ *rcsA72*::Tn*10*	SG21011 + P1 (ATC5112)
SG21082	*rcsC52*::ΔTn*10 cps*$^+$	SG21011 + P1 (SG21110)
SG21100	*lon-10 rcsB18*::ΔTn*10*	SG1041 + SY15
SG21101	*lon-100 rcsB28*::ΔTn*10*	SG1041 + SY16
SG21110	*rcsC52*::ΔTn*10 lon-100*	SG1041 + SY19
JB2002[b]	*lon-146*::ΔTn*10 rcsB15*	SG20611, growth on succinate
JB2015[b]	*lon-146*::ΔTn*10 rcsB15 ompC*::Tn*5*	JB2002 + P1 (SG4124)
JB2033[d]	*cps-11*::*lac*-Mu dl *rcsB15 ompC*::Tn*5*	SG20595 + P1 (JB2015)
JB3005[b]	*rcsC137 ompC*::Tn*5 rcsA72*::ΔTn*10*	SG20643 + P1 (ATC5112)
JB3012[b]	*rcsA3* (RcsA*) *zed-14*::Tn*10 zed-751*::Δ*kan lon-100*	SG20644 + P1 (SG20752)
JB3018[b]	*lon-100* Δ*rcsA26*	JB3012, Tet^s Kan^s
JB3030[b]	*lon-100 recA*	SG20581 + P1[c]
JB3031[b]	*rcsC137 ompC*::Tn*5 recA*	SG20643 + P1[c]
JB3032[b]	*lon-100 rcsB15 recA ompC*::Tn*5*	SG20688 + P1[c]
JB3034[b]	*lon-100* Δ*rcsA26 recA*	JB3018 + P1[c]
ATC5112	*cps-11*::*lac*-Mu dl *rcsA72*::ΔTn*10 lon-100*	39
EA145[b]	*rcsC145*	13
Strains from other backgrounds		
C600	*thr leu tonA*	NIH[e] strain collection
DB1255	*rec*BC *sbcB15 supF8 hsdR*	44
N100	*recA*	NIH strain collection
N4956	*thr leu tonA* r$^-$ m$^+$	NIH strain collection
RB132	F$^-$ *lac rpsL gal zxx*::Tn*10* 4HH104(pNK217)	26
SG4124	F$^-$ *galK2 ompC*::Tn*5*	13
SG4144	Δ*lon-510*	27
SG12014	*thr leu tonA zed-14*::Tn*10 rcsA40 lon-100*	13
SG12017	*thr leu tonA rcsC145 ompC*::Tn*10*	C600 + P1 (SG20583)
SG12019	*thr leu tonA rcsC137 ompC*::Tn*10*	13
SG12020	*thr leu tonA rcsA3* (RcsA*) *zed-650*::Tn*10*	13
SG12023	*thr leu tonA rcsC137 ompC*::Tn*5*	C600 + P1 (SG20329)
SG12024	*thr leu tonA rcsC137 ompC*::Tn*5* (*imm*λ *c*I857 *rcsC*$^+$ *rcsB11*::ΔTn*10*)	SG12023 + SY11
SG12025	*thr leu tonA rcsC137 rcsB11*::ΔTn*10 ompC*::Tn*5*	Tet^r temperature resistant λ^s derivative of SG12024
SG12032	*recA thr leu tonA*	C600 + P1 (N100)
SG13182	*srl*::Tn*10 his sulA leu rpsL*	41
TK363	*lac araD ompC*::Tn*10*	13

[a] These strains contain the MC4100 mutations Δ*lacU169 araD flbB rel*.
[b] These strains contain *cpsB10*::*lac*-Mu-*imm*λ.
[c] These *recA* strains were constructed by two consecutive P1 transductions as described in Materials and Methods.
[d] These strains contain the *attB* · *B' bio-936* Δ(*Sal-Xho*) λ *c*I857 ΔH1 prophage.
[e] NIH, National Institutes of Health.

simultaneously Tet^s, Lac$^-$, and Kan^s were assumed to have acquired deletions from the Tn*10* site through *rcsA* and the Δ*kan* insertion. The presence of an *rcsA* mutation was confirmed by complementation with appropriate lambda transducing phage.

Bacteriophage strains and selection of lysogens at the *att* site. The phages and plasmids used in this work are described in Table 2. All transducing phages used here were derived from the D69 vector and therefore carry insertions in the *int* gene (32). The phages are *att*$^+$ and will integrate readily in the presence of Int. A defective, *attB.B' int*$^+$ temperature-inducible prophage was used to provide Int and an appropriate attachment site for lysogenization at the attachment site as previously described (27). Original phage isolates were *imm*^{21}. Lysogens were selected as cells immune to killing by a mixture of *imm*^{21} *c*I *b*538 and *imm*^{21} *c*I *h*80 phage. *imm*λ derivatives were constructed by growing phage stocks on SA431, which carries a defective *c*I857 prophage; *imm*λ recombinants were present in these lysates at 1/10^6 phage.

Phage insertion mutagenesis. Isolation of insertions in the transducing phages was done by the method of Maurizi et al. (27). *imm*λ *c*I857 *rcsB*$^+$ *rcsC*$^+$ (SY8) transducing phages were grown on RB132, which carries a defective Tn*10* derivative capable of transposition but defective in transposase (Tn*10*Δ*16*Δ*17*, called ΔTn*10* here). The strain also carries a mutated Tn*10* element which expresses high levels of transposase (26, 43). The resulting phage lysates carry random ΔTn*10* insertions in about 1/10^6 phage particles. These lysates were used to infect strains carrying a *cps-lac* fusion and a defective heat-inducible prophage. Upon heat induction, the prophage provides Int sufficient to allow efficient lysogenization of the transducing phages. Tetracycline-resistant lysogens were selected and screened on MacConkey lactose indicator agar plates. Phages carrying an *rcsB*::ΔTn*10* insertion will fail to complement an *rcsB lon*

TABLE 2. Phages and plasmids

Phage or plasmid	Genotype	Source or reference
Phage derivatives		
SB16	*imm*^{21} *rcsB*$^+$ *ompC*	D69 pool
SB17	*imm*^{21} *rcsB*$^+$	D69 pool
SB18	*imm*^{21} *rcsB*$^+$ *rcsC*$^+$	D69 pool
SB19	*imm*^{21} *rcsB*$^+$	D69 pool
SY7	*imm*λ *c*I857 *rcsB*$^+$ *ompC*$^+$	From SB16
SY8	*imm*λ *c*I857 *rcsB*$^+$ *rcsC*$^+$	From SB18
SY9	*imm*λ *c*I857 *rcsB*$^+$	From SB19
SY10	*imm*λ *c*I857 *rcsB10*::ΔTn*10 rcsC*$^+$	SY8
SY11	*imm*λ *c*I857 *rcsB11*::ΔTn*10 rcsC*$^+$	SY8
SY14	*imm*λ *c*I 857 *rcsB14*::ΔTn*10*	SY9
SY15	*imm*λ *c*I857 *rcsB18*::ΔTn*10 rcsC*$^+$	SY8
SY16	*imm*λ *c*I857 *rcsB28*::ΔTn*10 rcsC*$^+$	SY8
SY19	*imm*λ *c*I857 *rcsC52*::ΔTn*10*	SY8
Plasmids		
pJB100	*rcsB*$^+$ *bla*$^+$	SY7 + pBR322
pJB101	*rcsB101* Tet^r	pJB100 + pBR322
pJB107	*rcsC*$^+$ *tet*$^+$	SB18 + pBR322
pJB200	*rcsC*$^+$ *bla*$^+$	pUC18 + pJB107
pJB201	*rcsC*$^+$ *bla*$^+$	pUC19 + pJB107
pJB102	*rcsB62*::Δ*kan bla*$^+$	pJB100[a]
pJB110	*rcsB60*::Δ*kan bla*$^+$	pJB100[a]
pJB112	*rcsB10*::ΔTn*10 bla*$^+$	pJB100 + SG20685[b]
pJB113	*rcsB11*::ΔTn*10 bla*$^+$	pJB100 + SG20686[b]
pJB114	*rcsB14*::ΔTn*10 bla*$^+$	pJB100 + SG20687[b]
pJB115	*rcsB18*::ΔTn*10 bla*$^+$	pJB100 + SG20698[b]
pJB116	*rcsB28*::ΔTn*10 bla*$^+$	pJB100 + SG20699[b]
pATC500	*rcsA*$^+$ *bla*$^+$	39
pATC402	*rcsA*::Δ*kan bla*$^+$	39

[a] Δ*kan* mutagenesis of pJB100 with NK1105 as described in Materials and Methods.
[b] ΔTn*10* transferred from chromosome to UV-irradiated pJB100 as described in Materials and Methods.

cps-lac host (SG20618); the cells will remain Lac$^-$. λ *rcsB* carrying ΔTn*10* insertions elsewhere in the phage will complement the *rcsB* mutation, and the lysogens will become Lac$^+$. Similarly, insertions in *rcsC* will allow the isolation of tetracycline-resistant lysogens of *lon*$^+$ *rcsC cps-lac* (SG20682) hosts which remain Lac$^+$. About six independent lysogens of each type (*rcsB*::ΔTn*10* and *rcsC*::ΔTn*10*) were isolated. Purified phages induced from the lysogens were used as sources of insertion mutations in *rcsB* and *rcsC*.

Transfer of mutations from lambda to the bacterial chromosome. ΔTn*10* insertion mutations were transferred from lambda transducing phages to the chromosome by a round of lysogenization and curing. Since the λD69 derivatives carry chromosomal DNA at the *Bam*HI site within the *int* gene, these phages are Int$^-$ and cannot lysogenize by site-specific recombination. Therefore, selection of Tet^r lysogens will result in lysogenization of the phages to the region of bacterial homology (the *rcsB rcsC* region, in this case). Using λ *c*I857 derivatives of the transducing phages, we selected Tet^r lysogens at low temperature. Temperature-resistant derivatives were screened for tetracycline resistance and loss of λ genes. In some cases tetracycline resistance was selected at high temperature.

A *rcsC137 rcsB11*::ΔTn*10* double mutant was constructed in a similar manner, with the *rcsC137* host SG12023 as the recipient for lysogenization. This mucoid strain became nonmucoid and temperature sensitive after selection of tetracycline-resistant lysogens of λ *c*I857 *rcsB11*::ΔTn*10* (SG12024). From these, temperature-resistant tetracycline-resistant derivatives were selected and screened for the presence of the *rcsC137* allele. All temperature-resistant derivatives which remained tetracycline resistant were nonmucoid. Those carrying the *rcsC* mutation could be distinguished from *rcsC*$^+$ hosts by complementing the *rcsB* mutation with a *rcsB*$^+$ (*rcsC*) phage. The *rcsC137 rcsB11*::ΔTn*10* strain (SG12025) will become mucoid under such conditions, whereas a *rcsC*$^+$ *rcsB* host will not. P1 transduction of the *rcsB rcsC* region to an appropriate *cps-lac* host confirmed this behavior for *cps-lac* expression. Transducing phages carrying the *rcsC137* mutation were induced from the original lysogen.

Plasmid constructions. pBR322-, pUC18-, and pUC19-derived plasmids were constructed by restriction enzyme digestion of the transducing phage and plasmid, followed by ligation of the resulting pieces with T4 DNA ligase.

For the *rcsB*$^+$ plasmid pJB100, DNA from the *rcsB*$^+$ *ompC*$^+$ transducing phage SY7 and from pBR322 were cut with the restriction enzymes *Bam*HI and *Hin*dIII, mixed, and ligated. The *Bam*HI site in SY7 and SB16 is not present in the overlapping phages SB17, SB18, and SB19. Therefore, this site is formed from a half *Bam*HI site in the bacterial DNA, which was cut with *Sau*3A in the original cloning into D69, joined to the *Bam*HI site of D69. Amp^r transformants of N4956 were isolated, and the plasmids were extracted and screened on SG20688, a *lon rcsB* strain, for complementation of the *rcsB* mutation. pJB100 complemented *rcsB* mutations and had the expected restriction enzyme cleavage pattern. pJB101 was formed by cloning the *Pst-Pvu*I fragment from pJB100 into the *bla* gene of pBR322. This plasmid fails to complement *rcsB* mutations and negatively complements for capsule synthesis (see Results).

An *rcsC*$^+$ plasmid, pJB107, was constructed by ligating the isolated 4.0-kilobase (kb) *Eco*RI-*Pst*I fragment from SB18 (the *imm*^{21} transducing phage carrying both *rcsC*$^+$ and

rcsB$^+$) to pBR322 DNA cut with the same enzymes. pUC18- and pUC19-derived *rcsC*$^+$ plasmids, pJB200 and pJB201, respectively, were made by inserting this same *Eco*RI-*Pst*I piece from pJB107 into the pUC plasmids.

Mutagenesis of pJB100 and pJB201 with a Δ*kan* transposition element was performed as described by Way et al. (43), using phage λNK1105 provided by Nancy Kleckner.

Insertions in pJB201 (*rcsC*$^+$) were screened by transforming pooled plasmids from kanamycin-resistant cells into C600 and examining these plasmids for complementation of *rcsC137* and location of the Δ*kan* insert.

UV-induced transfer of chromosomal *rcsB* mutations to the plasmid was accomplished by the procedure described by Chattoraj et al. (7). Cells carrying chromosomal mutations and the *rcsB*$^+$ plasmid pJB100 were exposed to UV irradiation at 100 J/m^2. Mutant plasmids were detected by their failure to complement a *lon-100 rcsB*::ΔTn*10 cps-lac* host. Restriction analysis of plasmid DNA from these purified Lac$^-$ colonies indicated that the chromosomal *rcsB* ::ΔTn*10* mutation had indeed been transferred and that the location of the insertion agreed with that determined for the transducing phage mutation from which it was originally isolated.

Transfer of mutations from plasmid to phage or chromosome. Δ*kan* insertion mutations were transferred to the chromosome by cutting the plasmids with *Eco*RI and transforming a *recBC sbc* strain (DB1255) with the resulting linear DNA (18). P1 CM*clr-100* was grown on the resulting Kan^r Amp^s recombinants, and this lysate was used to transfer the mutations into the appropriate backgrounds. Chromosomal Δ*kan* insertions in *rcsB* or *rscC* were subsequently transferred to *c*I857 *rcsB*$^+$ *rcsC*$^+$ (SY8) by selecting Kan^r phages released after heat induction of lysogens of SY8 at the site of bacterial homology. In some cases *rcsC*::Δ*kan* mutations were transferred to the *rcsB*$^+$ *rcsC*$^+$ phages directly by growing the phage on C600 carrying the *rcsC*::Δ*kan* plasmid and selecting, as lysogens in SG20788, the rare phages which had acquired kanamycin resistance.

Enzyme assays. β-Galactosidase assays were performed as described by Miller (28). Cells were grown in glucose or glycerol minimal M56 medium (39) or morpholinepropanesulfonic acid (MOPS)-glucose (34) to an optical density at 600 nm of between 0.05 and 0.6, toluenized, and assayed by the addition of *o*-nitrophenyl-β-D-galactopyranoside (ONPG).

Many of the plasmid-containing strains, particularly those containing the *rcsB*$^+$ plasmid or the *rcsA*$^+$ plasmid, grew very poorly in minimal medium, often losing the plasmid or giving rise to Lac$^-$ revertants that grew much more quickly and overtook the culture. At least some of these Lac$^-$ mutations were localized to the plasmid.

Cells containing ampicillin-resistant plasmids were grown in tryptone broth containing 100 μg of ampicillin per ml since the growth of some of these strains was very poor in minimal media. *recA* strains were used to prevent homologous recombination between the plasmid and the chromosome.

lon cps-lac strains were also somewhat unstable. Strains on lactose MacConkey agar accumulated Lac$^+$ papillae after 1 to 2 days, and cultures of SG20781 gave variable results in β-galactosidase assays, ranging from 1 or 2 to about 30 U of β-galactosidase.

Protein identification. Laemmli (20) polyacrylamide slab gels (12%) were prepared as described by Silhavy et al. (37) with a 3.2% stacking gel. Maxicells were used to determine plasmid gene expression from *rcsB*$^+$, *rcsB*, *rcsC*$^+$, and *rcsC* plasmids. A *lon recA cps-lac* strain, JB3030, was used for the *rcsB* experiments, and a C600 *recA* strain, SG12032, was used for the *rcsC* experiments. The procedure was essentially that described by Silhavy et al. (37). Overnight cultures grown in tryptone broth with 100 μg of ampicillin per ml were suspended in M56 minimal medium containing glucose and 17 amino acids (not including methionine). These cultures were used to inoculate fresh cultures that were then grown to an optical density at 600 nm of 0.45 to 0.5. The cells were UV irradiated at 75 J/m^2 and labeled with 50 μCi of [^{35}S]methionine per ml, with shaking for 1 h at 37°C.

Pulse-chase experiments were performed with pJB100 (*rcsB*$^+$) in JB3030 (*lon*) and in SG20587, the corresponding *lon*$^+$ strain. The strains were grown in 6 ml of glucose minimal M56 medium with 17 amino acids and 100 μg of ampicillin per ml to an optical density at 600 nm of 0.4 and labeled for 1 min with 20 μCi of [^{35}S]methionine per ml. An 800-μl sample was removed to 200 μl of cold 30% trichloroacetic acid on ice, and excess cold methionine was immediately added to the rest of the culture. Samples were taken at 10, 30, and 60 min after the beginning of the chase.

RESULTS

Isolation of λ *rcsB*$^+$ transducing phage. Capsule synthesis and the expression of *lac* fusions to genes necessary for capsule synthesis (*cps*) increase dramatically in *lon* mutant hosts (41). High-level expression of the *cps-lac* fusions is abolished by second-site mutations in either of the two positive regulator genes, *rcsA* and *rcsB* (13). *rcsA* has previously been cloned, and the product has been defined as an unstable protein whose degradation is controlled by *lon* (39). We have used the low expression of *cps-lac* fusions in *lon rcsB* hosts to select λ transducing phages carrying the *rcsB*$^+$ locus from a clone bank made after partial digestion of *E. coli* SG20062 DNA with *Sau*3a, inserted into the *Bam*HI site in the *int* gene of λD69 (32). The phages were screened on Δ*lac lon rcsB cps-lac* strains on lactose tetrazolium agar plates. Since these strains were Lac$^-$, complementation of the *rcsB* mutation by the phage would be expected to produce a Lac$^+$ (red) plaque.

Four phages were found in approximately 1.2 × 10^5 plaques. All four transducing phage candidates were tested for *rcsB* and *rcsC* complementation by making single-copy lysogens in appropriate mutant strains, again with the *cps-lac* fusions. The results of these tests are given in Table 3. Single-copy lysogens of all four isolates complemented *rcsB15* (Table 3, line 1). *rcsC137* increases *cps-lac* expression (and capsule synthesis) in *lon*$^+$ hosts; the cells are Lac$^+$ on MacConkey lactose agar plates. Complementation of *rcsC137* would be expected to reduce *cps-lac* expression to the *lon*$^+$ level (Lac$^-$). Only SB18 was found to complement

TABLE 3. Complementing ability of four *rcsB*$^+$ phage clones

Strain	Genotype	Lac phenotype[a] of strain carrying following phage[b]:				
		None	SB16	SB17	SB18	SB19
JB2033	*lon-100 rcsB15*	−	+	+	+	+
SG20604	*lon*$^+$ *rcsC137*	+	+	+	−	+
SG20600	*lon*$^+$	−	−	−	−	−
SG20595	*lon-100*	+	+	+	+	+
SG20598	*lon-100 rcsA40*	−	−	−	−	−

[a] Phenotypes were observed after overnight incubation at 32°C on MacConkey agar plates containing 1% lactose.

[b] Lysogens were isolated at 32°C by selection for *imm*^{21} after infection of heat-pulsed host strains.

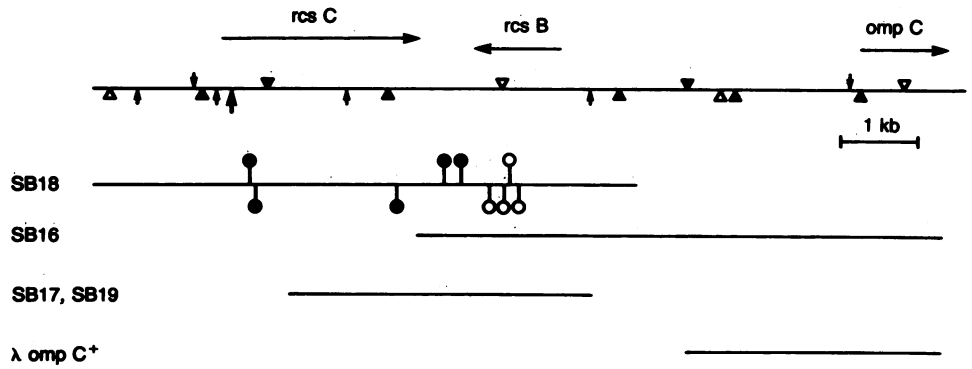

FIG. 1. Composite restriction map of the *rcsB rcsC ompC* region. The map is based on information from clones (see Materials and Methods) and published information on the restriction map for λ *ompC* (29). Symbols: ⚲, ΔTn*10* insertions which inactivate *rcsB*; ⚲, ΔTn*10* insertions which inactivate *rcsC* (symbols above the line are in orientation A, and those below line are in orientation B, as defined in Materials and Methods); the insertions were isolated in the *imm*λ derivative of SB18, SY8. Restriction enzyme sites: ↓, *Eco*RI; ▼, *Hin*dIII; ▽, *Pst*I; ↑, *Pvu*I; △, *Sal*; ▲, *Nru*; ⚲, *Sph*I.

rcsC137 (Table 3, line 2), and only SB16 complemented *ompC* (data not shown). None of the lysogens had any effect on the phenotypes of *lon*$^+$, *lon*, or *lon rcsA40* strains (Table 3). The presence of phages carrying either *rcsB*$^+$ alone, *rcsB*$^+$ *rcsC*$^+$, or *rcs*B$^+$ *omp*C$^+$ suggested that the order of the genes is *ompC-rcsB-rcsC*. These results are consistent with and extend those observed with a cosmid clone carrying *rcsB*$^+$ and *rcsC*$^+$ (13).

Restriction analysis of the transducing phages confirmed the genetic conclusions. The composite restriction map for the regions carried by the four phages is shown in Fig. 1. All four phages share a common 2-kb segment including a *Pst-Pvu*I fragment. SB18 carries an additional 3 kb in one direction, and SB16 carries an additional 3 kb from the other side. The bacterial DNA in SB16 has the restriction pattern expected for the N-terminal side of the *ompC* gene (28, 29). The combined map agrees with that for the region from kb 2260 to 2280 of *E. coli*, as described by Kohara et al. (19).

Isolation of insertions in *rcsB* and *rcsC*. To define the physical limits of the *rcsB* and *rcsC* genes on the transducing phages, we isolated a series of independent ΔTn*10* insertions in the phages, and screened for those disrupting *rcsB* or *rcsC* complementation activity (see Materials and Methods). Restriction enzyme analysis of the sites of the insertion mutations was carried out; the results of this analysis are summarized in Fig. 1, line 2. All insertions which inactivated *rcsB* complementing activity fell within a small region (about 0.5 kb) near the *Pst*I site that is carried by all four *rcsB* transducing phages. Insertions which inactivated *rcsC* complementing activity spanned a region of almost 3 kb, extending from just downstream of the *rcsB* region into the unique region beyond the *Hin*dIII site, carried only on the *rcsC* transducing phage.

rcsB::ΔTn*10* derivatives of *imm*λ *rcsB*$^+$ *rcsC*$^+$ (SY8) complemented *rcsC* mutations and failed to complement three different *rcsB* mutations. Similarly, *rcsC*::ΔTn*10* insertion phages still complemented *rcsB* mutations, but failed to complement three independent *rcsC* mutations. Therefore, *rcsB* and *rcsC* act like independent genes independently expressed.

Transfer of insertion mutations from phage to chromosome. The ΔTn*10* insertions on the phage define *rcsB* and *rcsC* complementing activity. To determine whether either gene is essential for *E. coli*, we transferred the ΔTn*10* insertions from the transducing phage to the host chromosome by homologous recombination (see Materials and Methods). A substantial portion (5 to 50%) of the temperature-resistant derivatives of *rcsB*::ΔTn*10* or *rcsC*::ΔTn*10* lysogens were still tetracycline resistant. These temperature-resistant strains had lost lambda immunity; we assume the tetracycline-resistant, temperature-resistant derivatives have substituted the ΔTn*10* insertion mutation for the wild-type chromosomal allele. P1 transduction of tetracycline resistance from these recombinants into appropriate recipients demonstrated that tetracycline resistance was closely linked to the *ompC*::Tn*5* region, as expected for insertions in the *rcsBC* region. Because the frequency of tetracycline resistance is relatively high, there is no apparent need for a secondary event to allow the growth of hosts carrying these insertions. Therefore, we tentatively conclude that *rcsB* and *rcsC* are dispensable functions for *E. coli*.

The *rcsB*::ΔTn*10* insertion mutations act like the previously described *rcsB* mutations (13). They reduce capsule and *cps-lac* expression to basal levels and can be complemented by the λ *rcsB*$^+$ transducing phage.

The properties of the *rcsC*::ΔTn*10* hosts, on the other hand, were distinctly different from the properties of the previously isolated *rcsC* mutations. Whereas *rcsC137* and *rcsC145* hosts are mucoid and express *cps-lac* at high levels in *lon*$^+$ hosts (13), *rcsC*::ΔTn*10* *lon*$^+$ mutants are nonmucoid and express *cps-lac* at the same level as *lon*$^+$ hosts. *lon* derivatives of these insertion mutations were able to express capsule at high levels. These results suggest that the *rcsC137* mutation does not represent a complete inactivation of the gene and that *rcsC* may not act as a simple negative regulator of capsule synthesis.

Epistasis of regulatory mutations. *rcsA* and *rcsB* mutations were originally isolated by their phenotype of reducing capsule synthesis and *cps-lac* expression in *lon* hosts (13), whereas *rcsC137* mutations increase synthesis in *lon*$^+$ hosts. We should be able to define the interactions of these regulators by the properties of appropriate double mutants. The availability of tetracycline-resistant insertions in *rcsB* on a lambda transducing phage simplified the construction of *rcsB rcsC137* double mutants; *rcsA rcsC137* double mutants were also constructed in *lon*$^+$ and *lon* hosts carrying *cps-lac* fusions. The phenotypes of the resulting strains (Table 4) indicate that both *rcsA*$^+$ and *rcsB*$^+$ are essential for high-level *cps-lac* expression in *rcsC137* mutant hosts in both *lon*$^+$ and *lon* hosts. These results suggest that *rcsC137* is not

TABLE 4. Epistasis of regulatory mutations

Genotype[a]	Expression of *cps-lac* fusions (β-galactosidase assay [units][b])	
	rcsC$^+$	*rcsC137*
lon$^+$	37	699
rcsA	1.2	33
rcsB	1.1	1.1
Δ*lon-510* host	409	680
rcsA	1.3	26
rcsB	1.2	1.2

[a] All strains carry a *cpsB10*::*lac-imm*λ fusion in the MC4100 background. Isogenic set (genotypes described in Table 1): SG20781, SG20759, SG20758, SG20801, SG20799, SG20797, SG20803, SG20804, SG20802, SG20800, SG20806, and SG20798.

[b] β-Galactosidase units are as defined by Miller (28). Cells were grown in MOPS-Glu (34) at 30°C. Results are averages of at least three determinations.

a bypass for the *rcsA rcsB* pathway, but may affect the activity or availability of one or both of these proteins. The significantly higher expression of *cps-lac* fusions in *rcsC137 rcsA* mutants (33 or 26 units) than in *rcsC137 rcsB* mutant hosts (1 unit) suggests that *rcsA* activity is partially dispensable (see below). We explored this possibility further by providing *rcsB* on a multicopy plasmid.

Cloning of *rcsB* into a plasmid. An *rcsB*$^+$ plasmid clone was obtained by digesting SY7 DNA (the *imm*λ derivative of SB16) with *Hin*dIII and *Bam*HI and inserting the phage fragments into the Tet^r gene of pBR322 (Fig. 2). Some of the Amp^r Tet^s transformants of the *lon*$^+$ recipient, N4956, appeared somewhat mucoid. Plasmid DNA isolated from mucoid transformants was able to complement SG20688, a *lon-100 rcsB15 cps-lac* strain. Restriction analysis of the plasmid confirmed that it contained the expected 3.8-kb insert. The plasmid, pJB100, complemented all *rcsB* alleles, including point mutations and ΔTn*10* insertion mutations (data not shown).

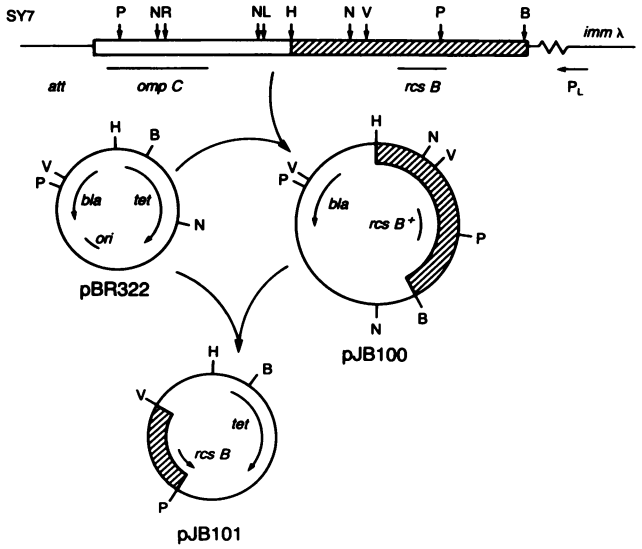

FIG. 2. Construction of pJB100 and pJB101 from SY7. Symbols: ▭, bacterial DNA; ▨, bacterial DNA transferred during restriction and ligation. Restriction enzyme sites: R, *Eco*RI; N, *Nru*; H, *Hin*dIII; B, *Bam*HI; P, *Pst*I; V, *Pvu*I. Only the bacterial insert region of SY7 is drawn to scale; the *Pvu*I-*Pst*I fragment of *rcsB* is 1.1 kb.

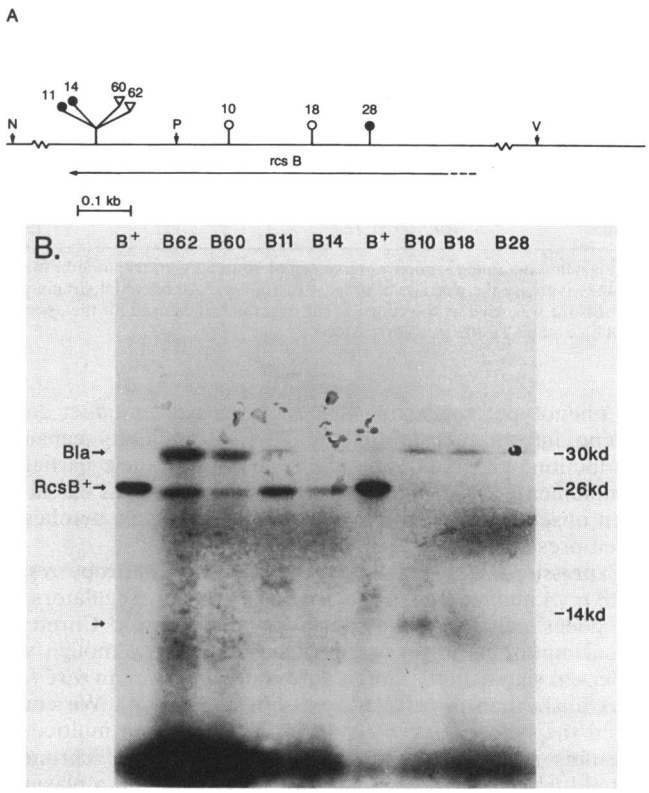

FIG. 3. Identification of RcsB in maxicells. (A) Fine-structure map of *rcsB* insertion mutations. Symbols: ♀, ♦, ΔTn*10* insertions in orientations A and B, respectively; ⅄, Δ*kan* insertions in orientation B. Numbers above insertions are allele numbers for *rcsB* mutations. The line below the map indicates extrapolated start point for *rcsB* protein-coding region and proposed direction of transcription. Restriction enzyme sites are as for Fig. 2. (B) Autoradiography of proteins synthesized in maxicells of JB3030. Lanes: B$^+$, pJB100 (*rcsB*$^+$); B62, pJB102 (*rcsB62*::Δ*kan*); B60, pJB110 (*rcsB60*::Δ*kan*); B11, pJB113 (*rcsB11*::ΔTn*10*); B14, pJB114 (*rcsB14*::ΔTn*10*); B10, pJB112 (*rcsB10*::ΔTn*10*); B18, pJB115 (*rcsB18*::ΔTn*10*); B28, pJB116 (*rcsB28*::ΔTn*10*). Bla, β-Lactamase.

Insertion mutagenesis of pJB100. Mutagenesis of the *rcsB*$^+$ plasmid pJB100 with a Δ*kan* transposon from λNK1105 (43) resulted in the isolation of two insertion mutations. Six chromosomal *rcsB*::ΔTn*10* mutations, originally isolated on the λ transducing phage, as well as two point mutations, *rcsB15* and *rcsB42*, were transferred from the chromosome to the plasmid by UV treatment of pJB100 and selection of Lac$^-$ homogenotes (see Materials and Methods). Insertions were mapped by restriction enzyme analysis to a 0.5-kb region spanning the *Pst*I site in *rcsB*, the same region identified from *rcsB* insertions in the transducing phage (Fig. 3A).

All of these plasmid mutations with the exception of *rcsB42* failed to complement any *rcsB* mutations in the chromosome. When plasmids containing *rcsB*::ΔTn*10* or *rcsB*::Δ*kan* mutations were introduced into a *lon rcsB*$^+$ (Lac$^+$) host, those mapping on one side of the *Pst*I site (*rcsB10*, *rcsB18*, and *rcsB28* in Fig. 3A) had no effect, whereas the others caused the cells to become Lac$^-$ (see Table 5). This apparent negative complementation was not observed in single-copy lysogens of phages carrying the same ΔTn*10* insertions. pJB101, an Δ*rcsB* plasmid made by cloning the *Pst*I-*Pvu*I piece from pJB100 into the *bla* gene of pBR322 (Fig. 2), also exhibited this negatively complement-

TABLE 5. Expression of *cps-lac* in *rcsB* plasmid-containing strains

Strain	Host genotype	β-Galactosidase sp act[a] for following plasmid[b]:					
		pBR322	pJB100	pJB115	pJB113	pATC500	pATC402
JB3030	*lon-100*	168	928	266	1.0	822	274
SG20587	*lon*$^+$	2	479	1.7	0.6	949	372
JB3034	*lon-100 rcsA26*	0.8	232	1.2	0.8	771	4
JB3032	*lon-100 rcsB15*	1.1	804	1.3	1.3	1.6	4
JB3031	*lon*$^+$ *rcsC137*	1,123	Lethal	1,045	42	1,038	386

[a] Plasmid-containing strains were grown at 30 to 32°C in tryptone broth containing 100 μg of ampicillin per ml to an optical density at 600 nm of 0.05 to 0.6. Results given are the average of at least two points. All bacterial strains were Δ*lac recA* and contained the *cpsB10*::*lacZ* fusion.

[b] pBR322 was used as the control. The other plasmids used for the assays were pJB100 (*rcsB*$^+$), pJB115 (*rcsB18*::ΔTn*10*), pJB113 (*rcsB11*::ΔTn*10*), pATC500 (*rcsA*$^+$), and pATC402 (*rcsA161*::Δ*kan*).

ing phenotype, suggesting that a partial gene product containing information up to the *Pst*I site inhibits capsule production. Plasmids containing the *rcsB42* allele partially complemented *rcsB* mutations in the host. *rcsB42* has also been observed to be somewhat leaky for capsule synthesis when present in single copy.

Expression of *cps-lac* in strains carrying multicopy *rcsB*. Both *rcsA* and *rcsB* act as positive, *trans*-acting regulators of the genes necessary for capsule synthesis, *cps*. Chromosomal mutations in these genes act similarly, although we observed significantly more expression of *cps-lac* in *rcsC137 rcsA* hosts than in *rcsC137 rcsB* hosts (Table 4). We compared the behavior of *rcsA* and *rcsB* carried on multicopy plasmids in mediating the expression of *cps-lac* chromosomal fusions. An *rcsA*$^+$ plasmid (pATC500) and a plasmid carrying an *rcsA161*::Δ*kan* mutation (pATC402) were used in parallel with pJB100 (*rcsB*$^+$), pJB115 (*rcsB18*::ΔTn*10*, an early insertion in *rcsB*), and pJB113 (*rcsB11*::ΔTn*10*, a late insertion in *rcsB*) to transform *recA* hosts carrying various *rcs* and *lon* mutations (Table 5). The *rcsB*$^+$ plasmid could not be introduced into these *rcsC cps-lac* hosts; this observation is explored further below. Although the *rcsA*$^+$ plasmid could be transformed into *rcsC137* strains, the transformants were sick and grew poorly.

The *rcsB*$^+$ plasmid dramatically increased capsule synthesis in both *lon*$^+$ (2 to 479 units) and *lon* hosts (168 to 928 units). As expected, pJB100 also complemented the *rcsB15* mutation (from 1 unit to 804 units). Unexpectedly, it also increased expression in the *lon rcsA* host from 0.8 unit to 232 units. This increase in *cps* gene expression in *lon rcsA* hosts has been observed in strains carrying point mutations and insertions in *rcsA*, as well as the deletion used in Table 5, in both *rec*$^+$ and *recA* hosts. In parallel experiments, the *rcsA*$^+$ plasmid, pATC500, increased synthesis in *lon*$^+$ (2 to 949 units), *lon* (168 to 822 units), and *lon rcsA* (0.8 unit to 771 units) hosts, but not in *rcsB* hosts (1.1 to 1.6 units). A number of other *rcsA* plasmids were tested, including pUC derivatives. None showed any increase in capsule synthesis in *rcsB* hosts. Thus, in cells carrying *rcsB*$^+$ in multicopy, the requirement for RcsA for capsule synthesis can be at least partially bypassed, but the requirement for RcsB is not similarly dispensable.

A plasmid carrying the *rcsB18* allele (pJB115; Table 5) had no apparent effect on *cps* expression. The *rcsB* negatively complementing allele, *rcsB11*, when present on a multicopy plasmid (pJB113), decreased *cps-lac* expression in both the *lon-100* (168 units to 1 unit) and *lon*$^+$ *rcsC137* (1,123 to 42 units) strains (Table 5). The *rcsA* null allele *rcsA161* differs from the *rcsB18* allele only in its behavior in the *lon*$^+$ host (372 units compared with 1.7 units). The increase in capsule synthesis in *lon*$^+$ hosts has been observed previously (39) and has been interpreted as the titration by the plasmid of a negative regulator of *rcsA* expression. Apparently the *rcsB* plasmid does not titrate a negative regulator in the same fashion.

Plasmid lethality in *rcsC* mutant hosts. The *rcsB*$^+$ plasmid, which increases capsule expression significantly, could not be introduced into the *rcsC137 cps-lac* host, although an isogenic *rcsB* plasmid could be. We have further investigated the basis for this lethality in the experiments summarized in Table 6. Transformation of the same strains with a *rcsC*$^+$ plasmid (see below) or the *rcsB* mutant plasmid were used as controls. Neither *rcsA* nor *rcsB* chromosomal mutations allowed the introduction of the *rcsB*$^+$ plasmid into an *rcsC137 cps*::*lac* host (Table 6), although the transformation efficiency with control plasmids improved. The few transformants observed with the *rcsC rcsB* host may be due to homogenization of the *rcsB* mutation onto the plasmid.

rcsC::ΔTn*10* mutations do not express *cps-lac* at high levels; these strains are still unable to accept the *rcsB*$^+$ plasmid (Table 6). These strains can be transformed by either the *rcsB* plasmid (Table 6) or an *rcsA*$^+$ plasmid (data not shown). Therefore, the *rcsC137* phenotype alone is not required for lethality. Apparently, an *rcsC*$^+$ host is necessary for the *rcsB*$^+$ plasmid.

Since all of these strains contained a *lac* fusion in one of the *cps* genes and were therefore unable to synthesize

TABLE 6. Transformation efficiency of plasmids carrying *rcs* loci

Host genotype[a]	% Efficiency of transformation[b] of plasmid with following genotype[c]		
	rcsB$^+$	*rcsB11*	*rcsC*$^+$
rcsC137 cps::*lac*	<0.2	2	19
rcsC137 cps$^+$	19	20	3
rcsC::ΔTn*10 cps*::*lac*	<0.2	26	91
rcsC::ΔTn*10 cps*$^+$	254	487	356
rcsC137 rcsB11::ΔTn*10 cps*::*lac*	0.5	300	412
rcsC137 rcsB11::ΔTn*10 cps*$^+$	135	243	105
rcsC137 rcsA cps::*lac*	<0.2	58	152
rcsC137 rcsA cps$^+$	4	30	240

[a] *cps*::*lac* strains: SG20582 (*rcsC*$^+$ strain used to normalize transformation efficiencies), SG20761, SG20702, SG20696, and JB3005; *cps*$^+$ hosts; SG20250 (*rcsC*$^+$ strain used to normalize transformation efficiencies), SG21011, SG21082, SG21080, and SG21081.

[b] Transformation efficiencies are expressed as the percentage of colonies observed in transformation of the same plasmid into the wild-type (*lon*$^+$ *rcsC*$^+$) host.

[c] Plasmids used were pJB201 (*rcsC*$^+$), pJB100 (*rcsB*$^+$), and pJB113 (*rcsB11*::ΔTn*10*).

capsule, it seemed possible that accumulation of an intermediate in the capsule synthesis pathway or failure to make capsule was contributing to the lethality of the *rcsB*$^+$ plasmid in *rcsC* hosts. This seems to be the case. Although the *rcsB*$^+$ plasmid could not be introduced into an *rcsC137* or *rcsC*::ΔTn*10* *cps-lac* host, a similar set of transformations into a *cps*$^+$ host gave transformation efficiencies similar to those for the *rcsB* plasmid (Table 6).

RcsB protein identification. The *rcsB* gene product was identified in maxicells of a *lon recA* strain, JB3030. In addition to the β-lactamase protein band, a band was seen at approximately 25,000 to 26,000 daltons (Da) (Fig. 3B). Maxicells containing plasmids with negatively complementing *rcsB*::ΔTn*10* mutations all produced a band slightly smaller than that from the *rcsB*$^+$ plasmid (Fig. 3B; *rcsB62*, *rcsB60*, *rcsB11*, and *rcsB14*). The null mutation that mapped closest to the *Pst*I site, *rcsB10*::ΔTn*10*, produced a truncated protein of approximately 14,000 Da. The two insertions (*rcsB18* and *rcsB28*) mapping farthest from *Pst*I on the *Pvu*I side produced neither a wild-type RcsB band nor observable truncated gene products (Fig. 3B). These data suggest that the 26,000-dalton band is RcsB; transcription of the gene would proceed from the *Pvu*I end of the gene through the *Pst*I site (Fig. 1 and 3). Maxicells of JB3030 carrying pJB118 (*rcsB15*) show no protein made from the plasmid other than β-lactamase; pJB119 (*rcsB42*) makes a protein identical with RcsB$^+$ in gel migration (data not shown).

Experiments with proteins expressed from the λ *rcsB* transducing phage were consistent with this interpretation of both the identification and direction of transcription of *rcsB* (data not shown; M. Maurizi, personal communication).

Stability of *rcsB* gene product. Previous data suggested that the half-life of the *rcsA* gene product was less than 5 min in wild-type cells and was increased substantially (16 to 20 min) in *lon* cells (39). To verify that the *rcsB* gene product was not similarly regulated, we carried out pulse-chase experiments with *lon*$^+$ and *lon* cells. The *rcsB* gene product showed a half-life of almost 3 h, and more than 10 h in *lon*$^+$ and *lon* cells, respectively (data not shown). These data indicate that RcsB is significantly more stable than RcsA. Although *lon* affects RcsB stability, it seems unlikely that *lon* turnover of RcsB has a significant effect in regulating capsule synthesis.

Cloning of *rcsC* and identification of the *rcsC* gene product. *rcsC* was cloned into the *bla* gene of pBR322 by isolation of a 3.8- to 4.0-kb *Pst*I-*Eco*RI fragment from SB18, the *rcsB*$^+$ *rcsC*$^+$ transducing phage (Fig. 4). The resulting plasmid, pJB107, decreased *cps-lac* expression in the *lon*$^+$ *rcsC137* strain, SG20643. A *Pvu*I deletion in this plasmid removed *rcsC* complementing activity.

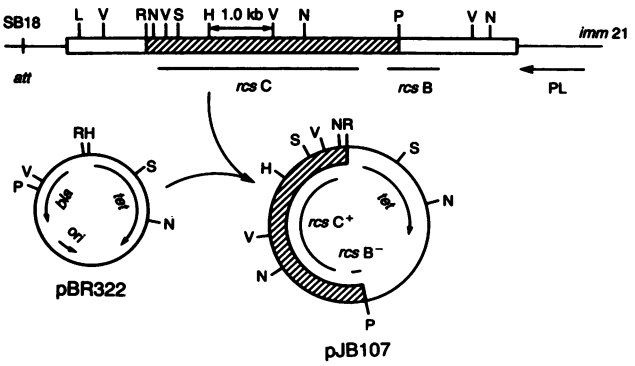

FIG. 4. Construction of pJB107 (*rcsC*$^+$). Symbols: ▭, bacterial DNA; ▨, bacterial DNA transferred during restriction and ligation. Restriction enzyme sites are abbreviated as in Fig. 2; S, *Sph*I. Only the bacterial insert region of SB18 is drawn to scale.

TABLE 7. Complementation of *rcsC137* by plasmids containing *rcsC*[a]

rcsC allele of plasmid[b]	β-Galactosidase units[c]
rcsC$^+$	5
Δ*rcsC202*	743
Δ*rcsC203*	654
rcsC5::Δ*kan*	575
rcsC10::Δ*kan*	843
rcsC16::Δ*kan*	30
rcsC15::Δ*kan*	80
rcsC$^+$ *zei-30*::Δ*kan*	2

[a] The host strain was SG20803.
[b] Plasmids are all derivatives of pJB201.
[c] Cells were grown in glucose M56 plus ampicillin at 32°C and assayed as described by Miller (28).

pUC18 and pUC19 derivatives containing the *Pst*I-*Eco*RI *rcsC*$^+$ fragment both complemented SG20643 or the similar strain, SG20803 (Table 7). Deletion derivatives of the pUC19-*rcsC*$^+$ plasmid were constructed. pJB202, carrying Δ*rcsC202*, has lost the fragment from the *Hin*dIII site located in *rcsC* through the *Pst*I site to the *Hin*dIII located in the multicloning site of pUC19 to the right of the region in Fig. 5A. pJB203, carrying Δ*rcsC203*, was deleted for bacterial sequences from the *Sph*I site through the *Hin*dIII and *Pst*I sites to the multicloning site. Both deletion plasmids failed to complement *rcsC137* (Table 7).

Δ*kan* insertions in pJB201 were mapped (Fig. 5A) and assayed for complementation of *rcsC137* (Table 7). Although insertions close to the *Hin*dIII site (*rcsC5* and *rcsC10*) abolished all *rcsC137* complementation, *rcsC16* and *rcsC15*, which map to the right of the *Nru*I site, in the middle of the fragment, partially complemented *rcsC137* (Table 7). When any of these mutations was crossed from the plasmid into the chromosome by linear transformation, none gave elevated levels of capsule synthesis in *lon*$^+$ hosts. *cps-lac* expression was high in *lon* hosts. This behavior is similar to that seen with *rcsC*::ΔTn*10* mutations originally isolated on λ. A comparison between the Δ*kan* insertions in the plasmid and the ΔTn*10* insertions in *rcsC* in the phage suggest that both carry insertions to the right of the *Nru*I site (Fig. 1 and 5A). Those in the plasmid complement *rcsC137*, whereas those in the phage do not. To determine whether this reflects the difference in copy number or a real difference in the insertion phenotypes, we crossed *rcsC15* and *rcsC16* from the chromosome onto SY8. The resulting Kan^r phages failed to complement *rcsC137*. Therefore, insertions late in *rcsC* apparently have *rcsC* activity sufficient for complementation only if present in multicopy. Δ*kan* insertion 3, which maps far to the right in Fig. 5A, allows full complementation of *rcsC137* when present on the plasmid (Table 7) or the phage (data not shown). The phages carrying this insertion are also *rcsB*$^+$. Therefore, this insertion, which we have designated *zei-30*::Δ*kan*, must lie between *rcsB* and *rcsC*.

Maxicells of SG12032 containing the *rcsC*$^+$ plasmid pJB201 synthesized a unique protein of about 100,000 Da (labeled *rcsC* in Fig. 5B, lanes 1 and 4). pJB202 (*Hin*dIII) and pJB203 (*Sph*I) both lacked this high-molecular-mass band. In the *Hin*dIII deletion (lane 2) two new bands appeared, one of approximately 27,000 Da and one of approximately 24,000 Da (Fig. 5B), whereas in the *Sph*I deletion (lane 3) no new bands were present. Maxicells containing the pJB201 deriv-

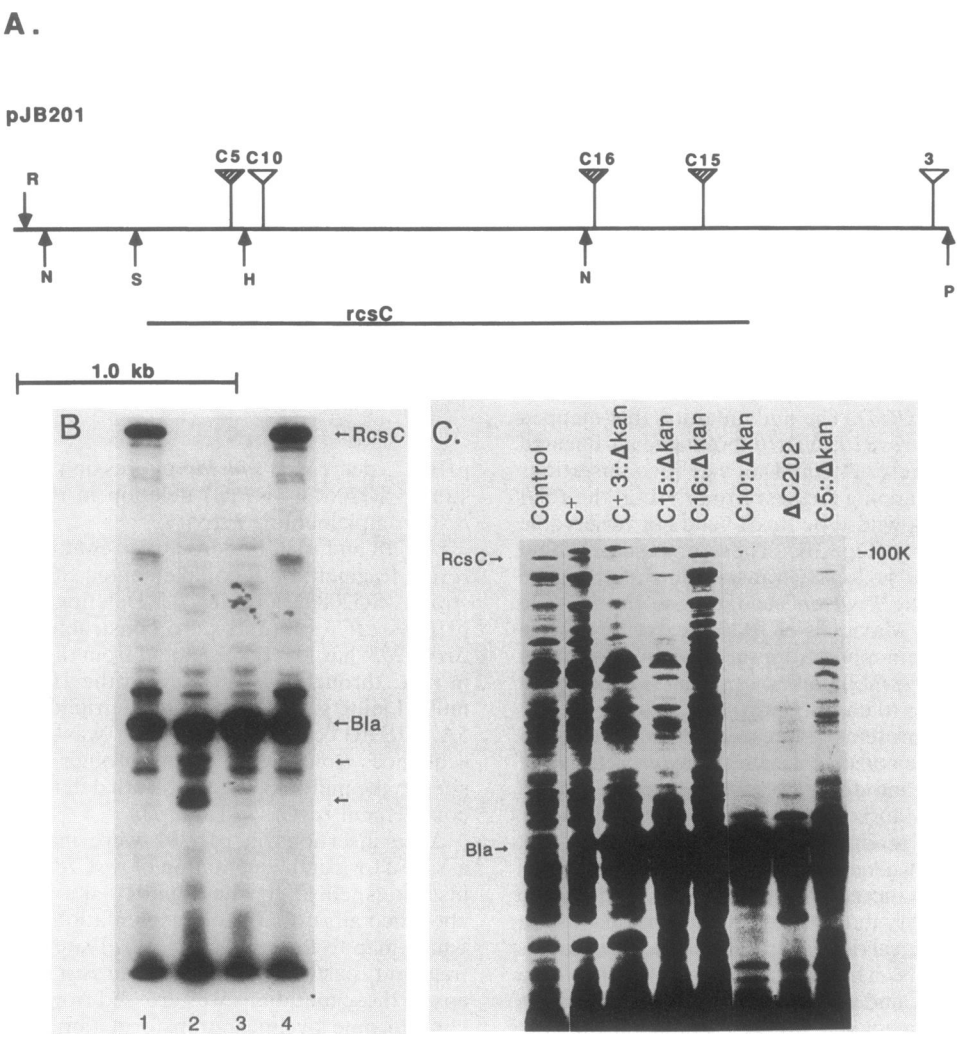

FIG. 5. Identification of RcsC in maxicells. (A) Fine-structure map of *rcsC* region. Symbols: ▼, Δ*kan* insertions in orientation A; ▽, Δ*kan* insertions in orientation B. Restriction site abbreviations are defined in the legend to Fig. 2. Allele numbers are shown above insertions. (B) Proteins synthesized in maxicells containing derivatives of pJB201 (*rcsC*$^+$). All plasmids are in SG12032. Lanes: 1, pJB201; 2, pJB202; 3, pJB203; 4, pJB201. (C) Proteins synthesized in maxicells containing pJB201 derivatives with *kan* insertions. *rcsC* alleles are indicated in the figure. 100k, 100,000-dalton protein.

atives with Δ*kan* insertions were examined for their synthesis of the 100,000-Da protein (Fig. 5C). As expected, the Δ*kan* insertion farthest to the right, no. 3, makes a protein of wild-type molecular mass. *rcsC15* and *rcsC16* make slightly shorter proteins, and *rcsC10* and *rcsC5* make no visible protein of greater than 30,000 Da (Fig. 5C).

The data are consistent with a single *rcsC* gene, beginning between the *Eco*RI and *Hin*dIII sites and transcribed toward the *Pst*I site, opposite to the direction of *rcsB* (Fig. 1).

***cps-lac* expression in strains carrying multicopy *rcsC*.** Both *lon* and *rcsC* act genetically like negative regulators of the *cps* genes. We compared the behavior of *lon*$^+$ plasmids to that of *rcsC*$^+$ plasmids. Multiple copies of *lon*$^+$ on plasmids reduce capsule expression in all strains tested thus far, including Δ*lon-510*, *rcsC137*, and *rcsA3* (RcsA*) (39; Table 8). *rcsA3* (RcsA*) is a dominant allele of *rcsA* which increases capsule production. If RcsA concentrations are normally rate limiting for capsule synthesis, we interpret the reduction in capsule synthesis in strains carrying the *lon*$^+$ plasmid as further evidence that increasing Lon serves to increase degradation of RcsA, thereby leading to decreased capsule synthesis (39). In contrast, pJB201, carrying *rcsC*$^+$, reduces expression to *lon*$^+$ levels only in *rcsC137* hosts (Table 8). *rcsA3* (RcsA*) hosts (line 2) are affected very little by pJB201; expression in *cps-lac lon* hosts (line 3) was reduced about fourfold.

TABLE 8. Effect of multicopy *rcsC* on *cps-lac* transcription

Strain	Host genotype	β-Galactosidase sp act of[a]: pUC19 (control)	pJB201 (pUC19 *rcsC*$^+$)	pJB202 (pJB201 Δ*Hin*dIII)	p*lon*$^+$[b]
SG20643	*lon*$^+$ *rcsC137*	1,152	4.4	692	4
SG20645	*lon*$^+$ *rcsA3* (RcsA*)	96	61	44	0.2
SG20581	*lon-100*	438	107	404	0.3
SG20582	*lon*$^+$	0.3	0.7	1.8	0.01

[a] Plasmid-containing strains were grown at 30 to 32°C in tryptone broth containing 100 μg of ampicillin per ml.
[b] Data from reference 39.

DISCUSSION

The transcription of genes necessary for the synthesis of the colanic acid capsular polysaccharide of *E. coli* is regulated by at least three genes: *rcsA*, *rcsB*, and *rcsC* (13). RcsA has been identified as an unstable positive regulator of capsule synthesis (39). *rcsB*, a positive regulator, and *rcsC*, a negative regulator, map close to each other at 48 min (13).

We have identified the RcsB and RcsC products as proteins of molecular mass 26,000 and 100,000 Da respectively. Insertion mutations which inactivate either of these functions do not affect the other. The mapping of insertions, correlated with the appearance of truncated proteins from plasmids carrying inserts, suggests the genetic map summarized in Fig. 1, in which *rcsB* and *rcsC* are transcribed toward each other.

RcsB, like RcsA, is a positive regulator of capsule synthesis. However, although single-copy lysogens of the *rcsA*$^+$ transducing phage or cells infected with the λ *rcsA*$^+$ phage show an increase in *cps-lac* expression, small increases in the copies of *rcsB*$^+$ have no apparent effect on *cps-lac* transcription (39). Since RcsA is unstable, we have postulated that it is normally limiting for capsule synthesis. RcsB is stable, and is apparently not normally limiting for capsule. The half-life of RcsB, more than 2 h in *lon*$^+$ cells, is significantly longer than the doubling time of the cells and is therefore unlikely to play a significant part in determining the amount of RcsB in cells.

Multicopy plasmid clones of insertion or deletion mutants late in the *rcsB* gene show negative complementation of *rcsB*$^+$ present in single copy on the chromosome. The insertion mutations code for a protein of close to wild-type size (Fig. 3). The same insertion mutations do not show negative complementation when present in single copy (data not shown). This suggests that RcsB may normally act as a multimer in promoting capsule synthesis. By this model, the truncated protein, present in excess, forms mixed, nonfunctional multimers with the wild-type RcsB.

Expressing either RcsA or RcsB from multicopy plasmids increases the expression of capsule of *cps-lac* in both *lon*$^+$ and *lon* strains (Table 5). The *rcsA*$^+$ plasmid, however, requires an intact *rcsB*$^+$ chromosomal gene for expression of capsule, whereas the *rcsB*$^+$ plasmid increases capsule synthesis in either an *rcsA* or an *rcsA*$^+$ host. These results suggest that although the high copy number of RcsA simply amplifies the normal increase in capsule synthesis associated with high RcsA levels (as, for instance, in *lon* hosts), the high copy number of RcsB bypasses the normal RcsA requirement and causes capsule synthesis by an abnormal mechanism. At the least, this suggests that RcsB cannot act solely to stimulate the synthesis of RcsA. It is consistent with models in which RcsA acts to stimulate RcsB synthesis or activity or in which both RcsA and RcsB act as positive regulators directly on *cps* transcription. In the latter case, we might imagine that RcsA acts as an auxiliary, partially dispensable factor, or that some other regulator in the cell is capable of substituting for RcsA at low efficiency. We have not yet been successful in isolating an allele of *rcsB* which in single copy will allow synthesis of high levels of capsule in the absence of RcsA.

The *rcsC* locus has been defined by the isolation of recessive chromosomal mutations which increase capsule synthesis, such as *rcsC137* (13), and by the mapping of insertion mutations in an *rcsC rcsB* transducing phage or an *rcsC* plasmid which abolish complementation of these *rcsC* mutations. These insertions span 3 kb of the bacterial DNA. Multicopy plasmids derived from pUC18 and pUC19 which carry this 3-kb region complement *rcsC* chromosomal mutations, consistent with the idea that this region is sufficient to provide *rcsC* activity.

The properties of these insertion mutations, when crossed from the phage or plasmid into the chromosome, suggest that the *rcsC* locus is more complex than we had suspected. *rcsC* insertion mutations, when present in cells in single copy, have essentially the phenotype of *rcsC*$^+$ cells: low capsule synthesis in *lon*$^+$ hosts and high capsule synthesis in *lon* hosts. This phenotype is inconsistent with RcsC acting as a simple negative regulator of capsule synthesis. However, cells carrying such insertion mutations differ from *rcsC*$^+$ cells in at least two ways: (i) they are unable to support *rcsB*$^+$ plasmids (Table 6); and (ii) when a second copy of the *rcsB rcsC* region is introduced into *rcsC*::Tn*10* *lon*$^+$ hosts, they express capsule at high levels (S. Gottesman, unpublished observation).

One tempting explanation for the very different behavior of *rcsC* alleles such as *rcsC137* and *rcsC* insertion mutations is provided by parallels with other complex regulatory circuits. In the nitrogen-regulatory circuit, *glnL* acts to modify a positive regulator, *glnG*, and allow it to function to stimulate the expression of *glnA*, in response to low availability of fixed nitrogen (23, 35). Although mutations in *glnL* exist which constitutively synthesize GlnA, null mutations in *glnL* have more subtle phenotypes (2, 5, 22). Nitrogen regulation is still observed, but the kinetics of adjustment to nitrogen depletion are significantly slower (22). Nixon et al. have identified a class of "environmental sensors," based on DNA homology, of which *glnL* is one example (36). Others include the phosphate sensor, *phoR*, and the regulator of outer membrane proteins, *envZ*. One hallmark of these types of regulators is that of rather complex genetics (42) and somewhat unpredictable dominance relationships (33). Mutations which abolish the environmental sensor may be bypassed by mutations in the regulatory protein which is the target for the sensor (38). It may be that *rcsC* represents another such environmental sensor, in this case for capsule synthesis regulation.

If *rcsC* regulates capsule synthesis, a number of observations suggest that it may not act through *rcsA*. In *rcsC137* mutant hosts, there is some residual capsule synthesis, even in an *rcsA* deletion strain (Table 4). Since this *rcsA*-independent synthesis is seen both in *rcsC137* mutants and in hosts carrying multicopy *rcsB*$^+$ plasmids, it is tempting to speculate that *rcsC* acts on *rcsB* synthesis or activity, rather than on *rcsA*. Our inability to introduce the *rcsB* plasmid into strains carrying either the *rcsC137* allele or *rcsC*::ΔTn*10* mutations also suggests an interaction between RcsB and RcsC. If lethality is due to accumulation of an intermediate in capsule synthesis, the finding that only the *rcsB* plasmid is lethal may suggest that there are steps in capsule synthesis which are under *rcsB* control and do not require RcsA. Finally, the close physical proximity of *rcsB* and *rcsC* on the *E. coli* map might suggest that they travel together genetically. Whatever the mechanism of *rcsC* action, the observation that increasing levels of RcsC by providing a multicopy source of RcsC does not decrease *cps-lac* expression in either *lon* hosts or in the *rcsA3* (RcsA*) host (Table 8) contrasts sharply with the behavior of *lon*$^+$ plasmids. Therefore, if *rcsC* is a negative regulator of transcription of *rcsB*, it is no better at decreasing *rcsB* synthesis when present in multicopy than when present in single copies. Possibly it acts to modify the activity of RcsB rather than its synthesis, as GlnL does for GlnG.

Although we do not thoroughly understand what environmental conditions colanic acid synthesis responds to, temperature appears to be one important signal. Colanic acid synthesis has always been observed to be higher at low temperatures (25), and *cps-lac* expression in *lon* cells decreases more than 50-fold when cells are grown at 42°C rather than at 30°C (S. Gottesman, unpublished observations). *rcsC137* was isolated as a mutation which increases capsule synthesis in *lon*$^+$ hosts at high temperature (13); not surprisingly, the temperature effect on *cps-lac* expression is much less striking in an *rcsC137* or *lon rcsC137* host than in a *lon* host (T. Klopotowski and S. Gottesman, unpublished observations). *rcsC* insertion mutations, however, still show the temperature response (S. Gottesman, unpublished observations). By analogy once more with *glnL*, we noted above that cells carrying null mutations in *glnL* still retain a response to nitrogen levels, suggesting the existence of an alternative nitrogen-sensing system. If *rcsC* is a temperature sensor for capsule synthesis, an alternative must also exist in this case.

The abundance of colanic acid capsule at low temperatures suggests that it is not likely to be particularly important in helping the bacterial cell in warm-blooded hosts. Recent experiments by Allen et al. (1) confirmed that the presence of colanic acid capsule does not significantly increase *E. coli* resistance to serum killing or phagocytosis. Preliminary experiments (S. Gottesman, unpublished results) suggest that the presence of high capsule levels can help the cell survive dehydration. A possible role in the environment is also suggested by the finding that *Erwinia stewartii* uses an RcsA-like function to regulate synthesis of a capsular polysaccharide which is an important component of virulence for this plant pathogen (40).

A variety of results, on the other hand, confirm that constitutive high-level synthesis of capsular polysaccharide is not healthy for *E. coli* growing or stored in the laboratory. *rcsC137* mutants and *rcsA** mutants grow more slowly than wild-type cells, whether they are *cps*$^+$ or *cps* (S. Gottesman, unpublished observations). We frequently isolate second-site revertants while working with strains that express high levels of capsule. The *rcsA* and *rcsB* plasmids, both of which increase capsule synthesis dramatically, are not well tolerated by cells and are lost very rapidly when selection for the plasmid is removed. Therefore, high-level expression of the capsule pathway is detrimental to cell growth. At least a portion of the problem is due to accumulation of some intermediate, since *cps*$^+$ strains tolerate *rcsB*$^+$ plasmids better than *cps-lac* hosts do.

On the basis of the genetic evidence presented here and elsewhere (13, 39, 41), we believe that both RcsB and RcsA act as positive regulators for capsular polysaccharide synthesis by transcriptionally activating the *cps* genes either directly or indirectly. RcsA is normally limiting for capsule expression; its availability is limited by Lon protease. It is unclear whether the synthesis or degradation of RcsA varies under different cell growth conditions. RcsB availability or activity may be directly controlled by RcsC, a possible environmental sensor. In vivo and in vitro biochemical analysis of this system should provide evidence for the precise mechanism of *rcsA*, *rcsB*, and *rcsC* activity in modulating *cps* expression.

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LITERATURE CITED

1. **Allen, P. M., D. Fisher, J. R. Saunders, and C. A. Hart.** 1987. The role of capsular polysaccharide K21b of *Klebsiella* and of the structurally related colanic-acid polysaccharide of *Escherichia coli* in resistance to phagocytosis and serum killing. J. Med. Microbiol. **24**:362–370.
2. **Backman, K. C., Y.-M. Chen, S. Ueno-Nishio, and B. Magasanik.** 1983. The product of *glnL* is not essential for regulation of bacterial nitrogen assimilation. J. Bacteriol. **154**:516–519.
3. **Bayer, M. E.** 1979. The fusion sites between outer membrane and cytoplasmic membrane in bacteria: their role in membrane assembly and virus infection, p. 167–202. *In* M. Inouye (ed.), Bacterial outer membranes: biogenesis and functions. John Wiley & Sons, Inc., New York.
4. **Bradshaw-Rouse, J. J., M. A. Whatley, D. L. Coplin, A. Woods, L. Sequeira, and A. Kelman.** 1981. Agglutination of strains of *Erwinia stewartii* with a corn agglutinin: correlation with extracellular polysaccharide production and pathogenicity. Appl. Environ. Microbiol. **42**:344–350.
5. **Bueno, R., G. Pahel, and B. Magasanik.** 1985. Role of *glnB* and *glnD* gene products in regulation of the *glnALG* operon of *Escherichia coli*. J. Bacteriol. **164**:816–822.
6. **Charette, M. K., G. W. Henderson, and A. Markovitz.** 1981. ATP hydrolysis-dependent protease activity of the *lon* (*capR*) protein of *Escherichia coli* K12. Proc. Natl. Acad. Sci. USA **78**:4728–4732.
7. **Chattoraj, D. K., K. Cordes, M. L. Berman, and A. Das.** 1984. Mutagenesis and mutation transfer induced by ultraviolet light in plasmid-cloned DNA. Gene **27**:213–222.
8. **Chung, C. H., and A. L. Goldberg.** 1981. The product of the *lon* (*capR*) gene in *Escherichia coli* is the ATP-dependent protease, protease La. Proc. Natl. Acad. Sci. USA **78**:4931–4935.
9. **Coplin, D. L., R. D. Frederick, D. R. Majerczak, and E. S. Haas.** 1986. Molecular cloning of virulence genes for *Erwinia stewartii*. J. Bacteriol. **168**:619–623.
10. **Costerton, J. W., R. T. Irvin, and K.-J. Cheng.** 1981. The role of bacterial surface structures in pathogenesis. Crit. Rev. Microbiol. **8**:303–338.
11. **Goldberg, A. L., K. H. Sreedhara Swamy, C. H. Chung, and F. S. Larimore.** 1983. Proteases of *Escherichia coli*. Methods Enzymol. **80**:680–702.
12. **Gottesman, S., M. E. Gottesman, J. E. Shaw, and M. L. Pearson.** 1981. Protein degradation in *E. coli*: the *lon* mutation and bacteriophage lambda *N* and *cII* protein stability. Cell **24**:225–233.
13. **Gottesman, S., P. Trisler, and A. Torres-Cabassa.** 1985. Regulation of capsular polysaccharide synthesis in *Escherichia coli* K-12: characterization of three regulatory genes. J. Bacteriol. **162**:1111–1119.
14. **Gottesman, S., P. Trisler, A. Torres-Cabassa, and M. R. Maurizi.** 1985. Regulation via proteolysis: *Escherichia coli lon* system, p. 350–354. *In* L. Leive (ed.), Microbiology—1985. American Society for Microbiology, Washington, D.C.
15. **Gottesman, S., and D. Zipser.** 1978. Deg phenotype of *Escherichia coli lon* mutants. J. Bacteriol. **133**:844–851.
16. **Howard-Flanders, P., E. Simson, and L. Theriot.** 1964. A locus that controls filament formation and sensitivity to radiation in *Escherichia coli* K12. Genetics **49**:237–246.
17. **Jann, K., and B. Jann.** 1985. Cell surface components and virulence: *Escherichia coli* O and K antigens in relation to virulence and pathogenicity, p. 157–176. *In* M. Sussman (ed.), The virulence of *Escherichia coli*. Academic Press, Inc. (London), Ltd., London.
18. **Kanaya, S., and R. J. Crouch.** 1984. The *rnh* gene is essential for growth of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **81**:3447–3451.
19. **Kohara, Y. K., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library.

Cell **50**:495–508.

20. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
21. **Leigh, J. A., E. R. Signer, and G. C. Walker.** 1985. Exopolysaccharide-deficient mutants of *Rhizobium meliloti* that form ineffective nodules. Proc. Natl. Acad. Sci. USA **82**:6231–6235.
22. **Magasanik, B.** 1982. Genetic control of nitrogen assimilation in bacteria. Annu. Rev. Genet. **16**:135–168.
23. **Magasanik, B., and F. C. Neidhardt.** 1987. Regulation of carbon and nitrogen utilization, p. 1318–1325. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger, (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
24. **Maloy, S. R., and W. D. Nunn.** 1981. Selection for loss of tetracycline resistance by *Escherichia coli*. J. Bacteriol. **145**: 1110–1112.
25. **Markovitz, A.** 1977. Genetics and regulation of bacterial capsular polysaccharide synthesis and radiation sensitivity, p. 415–462. *In* I. Sutherland (ed.), Surface carbohydrates of the prokaryotic cell. Academic Press, Inc. (London), Ltd., London.
26. **Maurer, R., B. C. Osmond, E. Shekhtman, A. Wong, and D. Botstein.** 1984. Functional interchangeability of DNA replication genes in *Salmonella typhimurium* and *Escherichia coli* demonstrated by a general complementation procedure. Genetics **108**:1–23.
27. **Maurizi, M., P. Trisler, and S. Gottesman.** 1985. Insertional mutagenesis of the *lon* gene in *Escherichia coli*: *lon* is dispensable. J. Bacteriol. **164**:1124–1135.
28. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Mizuno, T., M.-Y. Chou, and M. Inouye.** 1983. A comparative study on the genes for three porins of the *Escherichia coli* outer membrane. J. Biol. Chem. **258**:6932–6940.
30. **Mizuno, T., M.-Y. Chou, and M. Inouye.** 1983. DNA sequence of the promoter region of the *ompC* gene and the amino acid sequence of the signal peptide of pro-OmpC protein of *Escherichia coli*. FEBS Lett. **151**:159–164.
31. **Mizusawa, S., and S. Gottesman.** 1983. Protein degradation in *Escherichia coli*: the *lon* gene controls the stability of *sulA* protein. Proc. Natl. Acad. Sci. USA **80**:358–362.
32. **Mizusawa, S., and D. Ward.** 1982. A bacteriophage lambda vector for cloning with *Bam*HI and *Sau*3A. Gene **20**:317–322.
33. **Nara, F., T. Mizuno, and S. Mizushima.** 1986. Complementation analysis of the wild type and mutant *ompR* genes exhibiting different phenotypes of osmoregulation of the *ompF* and *ompC* genes of *Escherichia coli*. Mol. Gen. Genet. **205**:51–55.
34. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119**:736–747.
35. **Ninfa, A. J., and B. Magasanik.** 1986. Covalent modification of the *glnG* product, NRI, by the *glnL* product, NRII, regulates the transcription of the *glnALG* operon in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **83**:5905–5913.
36. **Nixon, B. T., C. W. Ronson, and F. M. Ausubel.** 1986. Two-component regulatory systems responsive to environmental stimuli share strongly conserved domains with the nitrogen assimilation regulatory genes *ntrB* and *ntrC*. Proc. Natl. Acad. Sci. USA **83**:7850–7854.
37. **Silhavy, T. O., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
38. **Slauch, J. M., S. Garrett, D. E. Jackson, and T. J. Silhavy.** 1988. EnvZ functions through *ompR* to control protein gene expression in *Escherichia coli* K-12. J. Bacteriol. **170**:439–441.
39. **Torres-Cabassa, A., and S. Gottesman.** 1987. Capsule synthesis in *Escherichia coli* K-12 is regulated by proteolysis. J. Bacteriol. **169**:981–989.
40. **Torres-Cabassa, A., S. Gottesman, R. D. Frederick, P. J. Dolph, and D. L. Coplin.** 1987. Control of extracellular polysaccharide synthesis in *Erwinia stewartii* and *Escherichia coli* K-12: a common regulatory function. J. Bacteriol. **169**:4525–4531.
41. **Trisler, P., and S. Gottesman.** 1984. *lon* transcriptional regulation of genes necessary for capsular polysaccharide synthesis in *Escherichia coli* K-12. J. Bacteriol. **160**:184–191.
42. **Wanner, B. L.** 1987. Phosphate regulation of gene expression in *Escherichia coli*, p. 1326–1333. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger, (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
43. **Way, J. C., M. S. Davis, D. Morisato, D. E. Roberts, and N. Kleckner.** 1984. New Tn*10* derivatives for transposon mutagenesis and for construction of *lacZ* operon fusions by transposition. Gene **32**:369–379.
44. **Wyman, A. R., L. B. Wolfe, and D. Botstein.** 1985. Propagation of some human DNA sequences in bacteriophage λ vectors requires mutant *Escherichia coli* hosts. Proc. Natl. Acad. Sci. USA **82**:2880–2884.