JOURNAL OF BACTERIOLOGY, Mar. 1987, p. 981–989
0021-9193/87/030981-09$02.00/0

Vol. 169, No. 3

Capsule Synthesis in *Escherichia coli* K-12 Is Regulated by Proteolysis

ANGEL S. TORRES-CABASSA AND SUSAN GOTTESMAN*

Laboratory of Molecular Biology, National Cancer Institute, Bethesda, Maryland 20892

Received 29 September 1986/Accepted 24 November 1986

***lon* mutants of *Escherichia coli* K-12 are defective in an ATP-dependent protease, are UV sensitive, and overproduce the capsular polysaccharide colanic acid. Six structural genes needed for capsular polysaccharide synthesis (*cps*) are transcriptionally regulated by *lon* as well as by three other regulatory genes, *rcsA*, *-B*, and *-C* (S. Gottesman, P. Trisler, and A. S. Torres-Cabassa, J. Bacteriol. 162:1111–1119, 1985). We have cloned *rcsA*, the gene for a positive regulator of capsule synthesis, onto multicopy plasmids and defined the gene by both insertions and deletions. The product of *rcsA* has been identified as an unstable protein of 27 kilodaltons. RcsA has a half-life of 5 min in *lon*$^+$ cells and one of 20 min in *lon* cells. The availability of RcsA is the limiting factor for capsule synthesis; doubling the gene dosage of *rcsA*$^+$ significantly increases expression of *cps* genes. Our results are consistent with a model in which the presence of a *lon* mutation increases the synthesis of capsular polysaccharide via stabilization of RcsA.**

Proteolytic degradation in bacteria and their viruses has been implicated in such cellular events as the destruction of abnormal polypeptides and the regulation of the availability of a number of unstable regulatory proteins (8, 10, 26). We have postulated that the expression of some short-lived "timing" polypeptides needed only for brief periods of time may be regulated at the post-transcriptional level via a proteolytic degradative process (12, 26).

The *lon* gene of *Escherichia coli* K-12 codes for an ATP-dependent protease (3, 4, 9). The Lon protease has been shown to affect the degradation of abnormal proteins in vivo (2, 13, 24, 32) and of the bacteriophage λ N protein both in vivo (10) and in vitro (M. Maurizi, J. Biol. Chem., in press). Mutations in *lon* are pleiotropic and result in increased sensitivity to DNA-damaging treatments (13, 14) and overproduction of the capsular polysaccharide colanic acid (13, 21, 33). We have shown previously that the sensitivity of *lon* mutants to DNA damage is due to a defect in proteolysis which permits the accumulation of an unstable cell division inhibitor (SulA) by prolonging its half-life in the cell (26).

If other effects observed in *lon* cells are a direct result of a defect in proteolysis, as is the case for sensitivity to DNA damage, we would predict the existence of an unstable positive regulator of polysaccharide synthesis which is normally limiting in the cell and which would be a target of the Lon protease.

Several of the enzymes involved in capsular polysaccharide synthesis are present in higher levels in *lon* mutants (18, 21, 22), and at least six structural genes required for capsule production (*cpsA–F*) are negatively regulated by *lon* at the transcriptional level (33).

Secondary *trans*-acting regulatory mutations which affect capsule synthesis have been isolated and mapped (11). Two positive regulatory genes, *rcsA* (regulator of capsule synthesis) and *rcsB*, as well as a negative regulator, *rcsC*, affect the levels of colanic acid capsular polysaccharide in *cps*$^+$ strains and also change the expression of β-galactosidase synthesis produced in strains which carry *lacZ* fusions to the structural genes for capsule synthesis (*cps*::*lac*) (11). The existence of these regulatory genes suggests that the defect in regulation of capsule expression found in *lon* strains may be mediated indirectly by the Lon protease through its action on one of the two positive regulators; thus, either *rcsA* or *rcsB* might code for a gene product which is normally limiting in the cell and the stability of which is regulated by proteolysis.

In this work we have identified the product of the *rcsA* gene and determined its half-life in *lon*$^+$ and *lon* cells. The evidence presented show that RcsA protein is indeed unstable and that its stability is affected by the *lon* protease.

MATERIALS AND METHODS

Bacterial strains and bacteriophage. All bacterial strains relevant to this work and their sources or construction are listed in Table 1.

Plasmids and bacteriophage are listed in Table 2. Isolation of λ *rcsA*$^+$ phage and construction of λ *c*I857 derivatives have been described elsewhere (11, 24).

Transductions with P1 Cmclr100 (28) were done as described by Miller (25).

Deletions of the *rcsA* gene were generated by selecting for tetracycline-sensitive derivatives of strains carrying a Tn*10* insert linked to *rcsA* by the method of Maloy and Nunn (19).

Media and enzyme assays. Unless otherwise indicated, cells were routinely grown in Luria broth (30). When required, the following antibiotics were added at the final concentrations indicated: tetracycline (20 μg/ml), ampicillin (75 μg/ml), or kanamycin (50 μg/ml).

M56 salts [Na_2HPO_4, 8.2 g/liter; KH_2PO_4, 2.7 g/liter; $(NH_4)_2SO_4$, 1.0 g/liter; $FeSO_4 \cdot 7H_2O$, 0.25 mg/liter; $MgSO_4 \cdot 7H_2O$, 0.1 mg/liter; $CA(NO_3)_2 \cdot 4H_2O$, 5 mg/liter, pH 7.2) was supplemented with amino acids, thiamine, glucose (0.2%), and $MgSO_4$ (0.01 M). M56 minimal medium contained 3.2% agar.

MutD mutagenesis of λ *rcsA* was carried out as described by Silhavy et al. (30).

β-Galactosidase expression in the fusion strains was monitored on lactose-MacConkey indicator plates as described previously (33). β-Galactosidase production was assayed as described by Miller (25).

Insertional mutagenesis. Insertions of the transposase-deficient Δ16Δ17Tn*10* (ΔTn*10*) transposon (35) in λ *rcsA*$^+$ transducing phage were obtained by growing the phage on

* Corresponding author.

TABLE 1. Bacterial strains

Strain	Relevant genotype	Source or reference
MC4100 derivatives[a]		
SG20180	*cps-11*::*lac*-Mu *dI lon*$^+$	33
SG20303	*cps*-11::*lac*-Mu *dI lon-100 ton*	11
SG20329	*ompC*::Tn*5 rcsC137 cps-11*::Mu *dI*	11
SG20581	*cpsB10*::*lac* (*imm*$^\lambda$) *lon-100*	33
SG20582	*cpsB10*::*lac* (*imm*$^\lambda$) *lon*$^+$	33
SG20587	*cpsB10*::*lac* (*imm*$^\lambda$) *recA*	SG20582 + P1(SG13182) + P1(N100)
SG20595	*cps-11*::*lac*-Mu *dI lon-100*[b]	11
SG20597	*cps*-11::*lac*-Mu *dI lon-100 rcsB43 zeh-50*::Tn*10*[b]	11
SG20598	*cps-11*::*lac*-Mu *dI lon-100 rcsA40 zed-14*::Tn*10*[b]	11
SG20600	*cps-11*::*lac*-Mu *dI*[b]	11
SG20643	*cpsB10*::*lac* (*imm*$^\lambda$) *ompC*::Tn*5 rcsC137*	SG20581 + P1(SG20329)
SG20645	*cpsB10*::*lac* (*imm*$^\lambda$) *lon*$^+$ *rcsA3* (RcsA*3)	SG20582 + P1(SG12020)
SG20681	*cps-11*::*lac*-Mu *dI lon*$^+$[b]	24
ATC5112	*cps-11*::Mu *dI lon-100 rcsA72*::ΔTn*10*	SG20303 + λ SY72
JB3030	*lon-100 rcsA*$^+$ *recA cpsB10*::*lac*(*imm*$^\lambda$)	Brill and Gottesman, in prepn
JB3034	*lon-100* Δ *rcsA26 recA cpsB10*::*lac* (*imm*$^\lambda$)	Brill and Gottesman, in prepn
JB3035	*lon*$^+$ Δ *rcsA27 cpsB10*::*lac* (*imm*$^\lambda$)	Brill and Gottesman, in prepn
Other strain backgrounds		
C600	F$^-$ *thr leu fhuA rpsL supE*	NIH[c] strain collection
DB1255	*hsdR recBC sbcB15 supF8*	36
N99	F$^-$ *galK2*	NIH strain collection
N100	F$^-$ *galK2 recA*	NIH strain collection
RB132	F$^-$Δ*lac rpsL gal zxx*::Tn*10*Δ4HH104(pNK217)	23
SG12020	*thr leu fhuA zed-650*::Tn*10 rcsA3* (RcsA*3)	11
SG13182	*his sulA* Δ*leu srl*::Tn*10 rpsL*	33

[a] All strains contain Δ(*lac*)U169 *araD flbB relA*.
[b] Contain *attB bio-936* Δ(*Sal-Xho*) cI857 ΔH1 prophage.
[c] NIH, National Institutes of Health.

donor strain RB132, which harbors pNK217 (*his*::ΔTn*10*), as described previously (16, 23, 24). In this strain, transposition functions are provided in *trans* from a chromosomal "high hopper" Tn*10* insertion (23). Phage carrying inserts were detected as tetracycline-resistant lysogens. *rcs*A-72::ΔTn*10* was isolated by the same method from λ *rcsA62* (RcsA*) (SY62).

Strain SG20681 (*lon*$^+$ *cps*::*lac*) carries a defective heat-inducible prophage (*int*$^+$ *xis*$^+$ *c*I857 ΔH1) and has a Lac$^-$ phenotype on indicator plates. This strain was grown at 32°C in TBMM (10) with biotin (0.003%), and the cells were shifted to 41°C for 20 min to provide *int*$^+$ function. They were then infected with the *rcsA* mutagenized phage at a multiplicity of infection of 0.1 to 0.5 and adsorbed at 32°C for 1 h. Lysogens were selected on either lactose-MacConkey or Luria broth agar with tetracycline. Of the Tet^r colonies 2 to 4% were Lac$^-$. Five independent Lac$^-$ Tet^r lysogens from as many independent mutagenized lysates were purified, and secondary lysates were induced. Tet^r phage that failed to complement an *rcsA* chromosomal mutation and which retained their Int$^-$ phenotype, as determined from the red plaque assay (7), were considered to carry an inactivated *rcsA* gene. Phage stocks from purified plaques were used in further analyses and in DNA isolation for restriction digestions.

The ΔTn*10* insertions isolated on the phage were crossed onto the bacterial chromosome by adsorbing the λ *c*I857 *rcsA*$^+$ mutagenized phage on strain SG20303 (*cps*::*lac lon*) at 32°C. Lysogens were cured of the phage at 37°C on Luria broth plates with tetracycline and screened for the Lac phenotype and loss of immunity to the phage. P1 bacteriophage was grown on purified Tet^r Lac$^-$ recombinants, and the lysates were used in transductional mapping of the insertions.

Selection of ΔTn*10* insertions linked to *rcsA*$^+$. A lysate induced from a pool of Tet^r lysogens of strain SG20681 was used to isolate random ΔTn*10* insertions linked to *rcsA*$^+$. The lysate was adsorbed to strain SG20180 at 32°C to select for Tet^r lysogens, which were then cured of the phage by selecting for tetracycline resistance at 37°C. These colonies were then pooled, and a P1 lysate prepared on the cells was used to transduce SG20581 (*lon cps*::*lac*) to Tet^r. The Lac$^+$ Tet^r transductants obtained carried ΔTn*10* insertions near *rcsA*$^+$.

Isolation of ΔKan insertions on the plasmid. λNK1105 carries a defective kanamycin-resistant Tn*10* derivative (ΔKan) and a p*tac*-transposase fusion outside the transposon (35). λNK1105 was adsorbed to an N99 transformant of pATC395 at 39°C, and the resulting Kan^r colonies were then pooled. A small-scale DNA preparation was made and used to transform strain C600 selecting for Kan^r nonmucoid colonies. Three independent insertions were verified by their failure to complement two different *rcsA* chromosomal mutations (*rcsA*40 and *rcsA*72::ΔTn*10*).

Recombination of the ΔKan insertions from pATC395 to the phage was done by growing SY25 or SY26 on N99 derivatives harboring the plasmid to produce a lysate which was then used to transduce C600 to kanamycin resistance. Tet^r Kan^r nonmucoid colonies were purified twice and a secondary lysate was induced. Presence of the insertions on the transducing phage was verified by their failure to complement an *rcsA* mutation and by restriction analysis of phage DNA.

ΔKan insertions in pATC400 were generated as for

pATC395. Recombination into the chromosome was done as follows. Strain DB1255 (*recBC sbcB15*) (36) is defective for a major nuclease activity but is able to carry out recombination when transformed with linearized plasmid DNA (15). Strain DB1255 was transformed with DNA from the ΔKan plasmid digested with *Eco*RI. Kan^r recombinants were selected and screened for their sensitivity to ampicillin. P1 lysates prepared on purified colonies of strains carrying the insertion mutations *rcsA161*::ΔKan, *rcsA163*::ΔKan, and *rcsA162*::ΔKan were used to transduce SG20581 (*lon-100 cps*::*lac*, Lac$^+$) to Kan^r. All Kan^r transductants became Lac$^-$ and were complemented by λ *rcsA*$^+$ as expected if the *rcsA*::ΔKan insertions had been transferred from the plasmid to the chromosome.

Preparation of DNA. Small-scale isolation of phage DNA was done as described by Silhavy et al. (30). For large-scale phage preparations, high-titer phage lysates were obtained by the method of Yamamoto et al. (34) and the DNA was isolated as described previously (30). Small- and large-scale plasmid DNAs were prepared by the alkali method described by Maniatis et al. (20).

TABLE 2. Bacteriophage and plasmids

Name	Relevant genotype	Source or reference
λ derivatives		
SB6	*imm*^{21} *rcsA*$^+$	11
SB18	*imm*^{21} *rcsB*$^+$	Brill and Gottesman, in prepn
SB25	*imm*^{21} *rcsA*$^+$	This work
SB26	*imm*^{21} *rcsA*$^+$	This work
SB57	*imm*^{22} *rcsA57*::Tn*10*	This work
SY25	*imm*$^\lambda$ cI857 *rcsA*$^+$	This work; *imm*$^\lambda$ derivative of SB25
SY26	*imm*$^\lambda$ cI857 *rcsA*$^+$	This work; *imm*$^\lambda$ derivative of SB26
SY50	*imm*$^\lambda$ cI857 *rcsA50*::ΔKan	This work
SY51	*imm*$^\lambda$ cI857 *rcsA51*::ΔKan	This work
SY60	*imm*$^\lambda$ cI859 *rcsA*$^+$	This work; *imm*$^\lambda$ derivative of SB6
SY62	*imm*$^\lambda$ cI857 *rcsA62* (RcsA*62)	This work
SY72	*imm*$^\lambda$ cI857 *rcsA72*::ΔTn*10*	This work
λ NK1105	*b522* cI857 *P*am80 *nin5*	35
Other phage		
P1CMc1r100	cI *ts*100 Cm^r	28
M13mp19 derivatives		
MAT200	*rcsA*$^+$	This work
MAT237	Δ*rcsA37*	This work
Plasmids		
pATC352	*rcsA*$^+$ (Cm^r Ap^r)	pBR325 derivative
pATC395	*rcsA*$^+$ (Tet^r)	pBR322 derivative
pATC400	*rcsA*$^+$ (Amp^r)	pBR322 derivative
pATC401	*rcsA160*::ΔKan (Amp^r)	pATC400 derivative
pATC402	*rcsA161*::ΔKan (Amp^r)	pATC400 derivative
pATC408	*rcsA166*::ΔKan (Amp^r)	pATC400 derivative
pATC410	*rcsA168*::ΔKan (Amp^r)	pATC400 derivative
pATC450	*rcsA50*::ΔKan (Tet^r)	pATC395 derivative
p*lon*$^+$500	(Amp^r)	24
p*lon*Δ510	(Amp^r)	24
pATC119	*rcsA*$^+$ (Amp^r)	pUC19 derivative
pNK217	Δ16Δ17Tn*10*	23, 35

Restrictions, ligations, and transformations. All enzymes used for digestions were obtained from New England BioLabs, and restrictions were carried out according to the supplier's specifications. pBR322, pBR325, pUC19, and M13mp19 DNAs were obtained from Bethesda Research Laboratories.

Ligation reactions and transformations were done by standard methods (20, 30).

Generation of *rcsA* deletions in m13mp19. Construction of *rcsA* deletions in M13mp19 were done in its derivative MAT200 by the rapid single-stranded cloning strategy for producing overlapping clones described by Dale et al. (6). The cyclone subcloning system for M13 and pBI derivatives (system RDS) from International Biotechnologies, Inc., was used for this purpose. The endpoints of the deletions were determined by sizing the single-stranded DNAs on agarose gels and restriction enzyme analysis of the double-stranded replicative forms.

Protein labeling. In vivo determination of protein produced by the *rcsA* recombinant plasmids was done in maxicells as described by Sancar et al. (29). The maxicell strain JB3034 and its plasmid-containing derivatives were grown overnight in M56 salts supplemented with 17 amino acids, 0.2% glucose, 0.1% vitamin B_1, $MgSO_4$ (1 mM), and the required antibiotic, but lacking methionine. At an optical density at 600 mm of 0.6, the cultures were exposed to a UV dose of 70 J/m^2 for 30 s and incubated at 37°C for 30 min, protected from light. D-Cycloserine was then added, and the cultures were grown overnight, starved for 1 h, and labeled with [^{35}S]methionine (50 μCi/ml) for 20 to 30 min (30). Labeled cells were washed, suspended in 2% sodium dodecyl sulfate loading buffer, and boiled for 5 min; 10 μl was run in a 12% acrylamide gel, using the buffers described by Laemmli (17). Electrophoresed gels were fixed for 15 min in 45% methanol–10% acetic acid and immediately soaked in Enlightning (Du Pont Co.) for 20 min, dried, and subjected to autoradiography at −70°C.

The in vivo stability of the RcsA protein produced from the recombinant plasmids was determined in growing cells cultured as indicated above. After a 1-min pulse with [^{35}S]methionine (20 μCi/ml), excess nonradioactive methionine (2 mM) was added to stop label incorporation and 0.5-ml samples were taken during a 30-min period. The samples were mixed with 0.125 ml of 30% trichloroacetic acid to precipitate the proteins. The pellet was washed in cold acetone, suspended, and boiled in 2% sodium dodecyl sulfate buffer, and 10 μl was run in a 13% acrylamide gel. After electrophoresis and autoradiography, the relative amounts of the labeled proteins were determined by densitometry.

RESULTS

Isolation and characterization of λ *rcsA*$^+$ transducing phage. We have previously identified and described mutations at two regulatory loci, *rcsA* and *rcsB*, which abolish transcription from the promoters of the structural genes involved in capsular polysaccharide synthesis in *lon* cells (11). It was postulated that the *lon* protease may inhibit capsule formation by degrading an unstable activator of transcription, possibly a product of one of the two positive regulatory genes required for *cps* gene expression (11, 12).

If a *lon* mutation results in capsule overproduction by increasing the steady-state level of a positive regulator which is subject to proteolytic degradation in *lon*$^+$ cells, that positive regulator should be limiting for capsule synthesis.

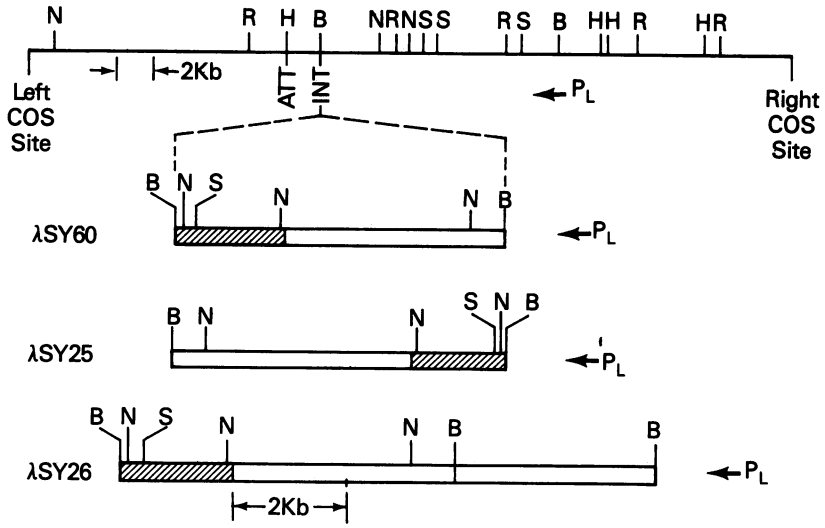

FIG. 1. Schematic diagram showing the restriction map of *imm*$^{\lambda}$ D69 and its λ *rcsA*$^{+}$ derivatives SY60 (from SB6), SY25 (from SB25), and SY26 (from SB26). The cloned fragment was inserted in the *Bam*HI site within the *int* gene of λ D69 (27). The hatched area represents the region within the clone in which all insertions that disrupt the *rcsA* gene mapped. The restriction sites shown are as follows: *Nru*I (N), *Eco*RI (R), *Hin*dIII (H), *Bam*HI (B), and *Sal*I (S).

Furthermore, if capsule overproduction in *lon* mutants results from a decrease in proteolysis, introduction of a second copy of the gene which codes for the activator protein may be sufficient to mimic the *lon* mucoid phenotype in *lon*$^{+}$ strains. Conversely, increasing the amount of the *lon* protease in the cell should abolish capsule production even if the activator protein is overproduced.

To determine if one of the two positive regulatory genes, *rcsA* or *rcsB*, is normally limiting in the cell, we studied the effect of changing gene dosage on transcription of the *cps*::*lacZ* operon fusions by using a λ transducing phage carrying either *rcsA*$^{+}$ or *rcsB*$^{+}$ (J. Brill and S. Gottesman, manuscript in preparation).

The *rcsA* gene was cloned from a *Sau*3A partial *E. coli* digest into the *Bam*HI site of λ D69 (27). Several *rcsA*$^{+}$ transducing phage were isolated by their ability to form Lac^{+} plaques on a lawn of a *lon cps*::*lac rcsA* strain on lactose-tetrazolium plates (11). Three independent λ *rcsA*$^{+}$ clones, SB6 (previously described [11]), SB25, and SB26, were isolated; the *imm*$^{\lambda}$ derivatives of these phage, SY60, SY25, and SY26, were used to determine the restriction enzyme pattern (Fig. 1).

Both phage SY25 and SY60 contained a 6.3-kilobase (kb) *Bam*HI insert. Results from *Bam*HI-*Sal*I, *Bam*HI-*Nru*I, and *Sal*I-*Nru*I double digests indicated that the 6.3-kb insert was present in opposite orientations in these two clones. Phage SY26 carried the 6.3-kb fragment in the same orientation as SB6 plus an additional 1.9-kb *Bam*HI piece. All three phage complemented *rcsA* mutations either in plaques or as lysogens. *rcsA* missense mutations of two sorts were isolated after MutD mutagenesis of the phage: recessive *rcsA* (RcsA^{-}) and dominant *rcsA* (RcsA*) (11).

Insertional mutagenesis of the *rcsA*$^{+}$ phage. We observed that increasing the number of copies of *rcsA*$^{+}$ in strain SG20681 (*lon*$^{+}$ *cps*::*lac*) resulted in a Lac^{+} phenotype, indicating that RcsA is normally limiting (see below). We supposed that lysogens of the transducing phage carrying an inactivated *rcsA* gene would remain Lac^{-}.

SB6 was mutagenized with a defective ΔTn*10* transposon (see Materials and Methods), and Tet^{r} lysogens were screened for their Lac phenotype. About 2% of the colonies remained Lac^{-} and therefore were considered to have been lysogenized by phage carrying an inactivated *rcsA* gene. The phage lysates induced from these lysogens are Lac^{-} on a lawn of a *lon rcsA* strain (11), which confirmed that the inserts inactivated the *rcsA* gene.

Restriction analysis of four independent *rcsA* phage isolated in this manner demonstrated that the insertions mapped within a 2.0-kb *Nru*I fragment in the 6.3-kb *Bam*HI clone (cross-hatched area in Fig. 1). The ΔTn*10* insertions were crossed into the chromosome by homologous recombination. They abolished capsule overproduction in *lon* hosts and were fully complemented by the λ *rcsA*$^{+}$ transducing phage.

***rcsA* is limiting for capsule synthesis.** Lysates of comparable titers of λ *rcsA*$^{+}$, λ *rcsA*::ΔTn*10*, or λ *rcsB*$^{+}$ were used in spot tests on lawns of both *lon*$^{+}$ and *lon cps*::*lac* strains. Expression of β-galactosidase from the fusion was monitored on MacConkey-lactose indicator plates (Table 3). Introduction of additional copies of the *rcsA*$^{+}$ gene was sufficient to increase transcription from the fusion in both backgrounds. In contrast, no apparent effect on *cps*::*lac* expression was seen with λ *rcsB*$^{+}$ (Table 3).

TABLE 3. Gene dosage effect of *rcsA*$^{+}$ and *rcsB*$^{+}$[a]

Strain genotype	Lac phenotype[b]			
	No phage	λ *rcsA*$^{+}$ (SB6)	λ *rcsA*::Tn*10* (SB57)	λ *rcsB*$^{+}$ (SB18)
lon^{+}	−	++	−	−
lon-100	+	+++[c]	+	+
lon-100 rcsA40	−	+	−	−
lon-100 rcsB43	−	−	−	+

[a] The strains used were SG20600 (*lon*$^{+}$), SG20595 (*lon-100*), SG20597 (*lon-100 rcsB43*), and SG20598 (*lon-100 rcsA40*).

[b] Equal volumes (10 μl) from phage lysates of comparable titers were placed over dried spots of the strains on lactose-MacConkey agar and incubated at 32°C for 10 to 20 h. All phage were *imm*^{21}.

[c] +++ indicates more rapid appearance of the Lac^{+} phenotype.

All three *rcsA*$^+$ transducing phage (SY25, SY26, and SY60) also complemented *flaP* and *flaR* mutants. As previously shown, episome F'1334 which contains the *fla* operons confers mucoidy on *lon*$^+$ hosts (11, 31). Clegg and Koshland (5) also observed mucoidy in cells harboring pCK202, which contains the *flaARQP* operon. Comparison of the restriction map of this plasmid and that of the *rcsA*$^+$ phage confirms that the 6.3-kb *Bam*HI fragment overlaps a *Sal*I-*Hin*dIII fragment in pCK202 (see below and Fig. 6). Furthermore, a deletion which eliminated essentially all of the 6.3-kb *Bam*HI fragment abolished the plasmid's ability to confer the mucoid phenotype (5). These observations are consistent with the mapping of *rcsA*. We have shown elsewhere that *rcsA* is not involved in the synthesis of flagella (11). Therefore, *rcsA* exhibits the genetic properties expected of a limiting positive regulator of the *cps* genes, while moderate increases in *rcsB* gene dosage apparently have little effect on *cps*::*lac* expression.

Overproduction of *lon* protease suppresses capsule synthesis. We have shown previously that a *lon* mutation increases expression of the *cps* operons (33). Two types of regulatory mutations which increase capsule synthesis in *lon*$^+$ hosts have been described: the dominant mutations in *rcsA* such as *rcsA3* or *rcsA62* (RcsA*), or a recessive mutation in *rcsC* (11). When a multicopy *lon*$^+$ plasmid (24) was used to transform either a *lon*$^+$ *rcsA3* (RcsA*) host (Table 4) or a *lon*$^+$ *rcsC* host (Table 4), capsule synthesis was suppressed. In transformants of the same strains with plasmid that carries the *lon-510* deletion, *cps*::*lac* expression remained high (Table 4). Therefore, while *rcsA* (RcsA*) and *rcsC* increase capsule synthesis, this increased synthesis remains sensitive to high levels of the Lon protease.

Construction and characterization of *rcsA* recombinant plasmids. The 6.3-kb fragment containing the *rcsA* gene was initially subcloned into the *Bam*HI site of pBR325 to generate plasmid pATC352 (Fig. 2). C600 (*lon*$^+$) transformants of this plasmid were extremely mucoid. When pATC352 was introduced into a *lon cps*::*lac* strain in the presence or absence of an *rcsA* chromosomal mutation, the levels of β-galactosidase increased dramatically (data not shown). The plasmid was found to be very unstable and was lost at a high frequency in media without ampicillin.

A 4.5-kb *Pst*I fragment containing 2.4 kb of the original insert was subcloned into pBR322 to generate pATC395 (Fig. 2). This 2.4-kb *Bam*HI-*Pst*I piece included the region of *rcsA*::ΔTn*10* insertions. The plasmid was found to be significantly more stable than pATC352 in the absence of antibiotic selection and fully complemented *rcsA* chromosomal mutations. While pATC352 had complemented *flaP* and *flaR* mutants, pATC395 failed to do so. This confirmed that *rcsA* was entirely within the *Bam*HI-*Pst*I fragment and was separate from the *fla* operons. Apparently, DNA segments which cause rapid plasmid loss are also separate from *rcsA*.

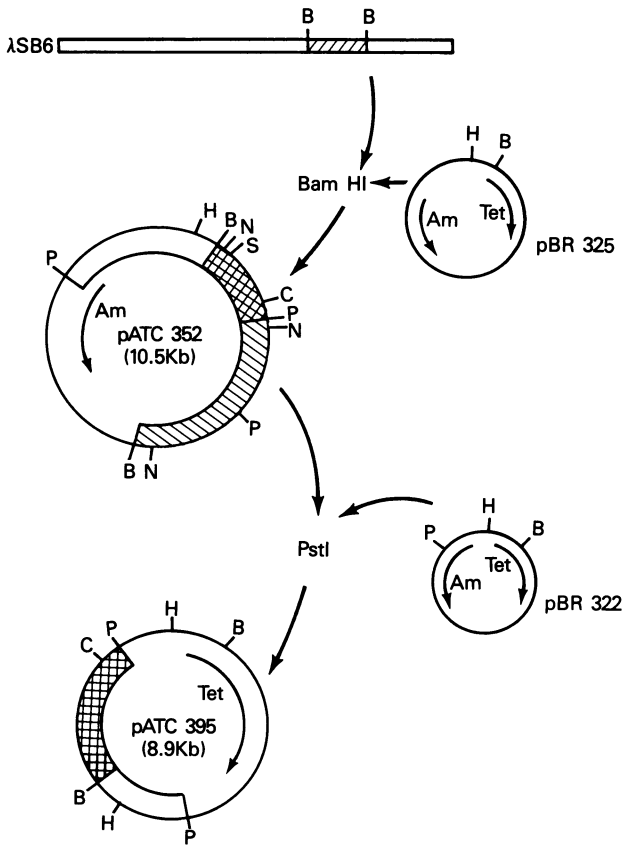

FIG. 2. Plasmid constructions. The 6.2-kb *Bam*HI bacterial DNA insert from λ SB6 was inserted into pBR325 to make pATC352 (*rcsA*$^+$). A 2.4-kb *Bam*HI-*Pst*I fragment (cross-hatched area) was subcloned by restricting pBR322 and pATC352 with *Pst*I followed by ligation with T4 DNA ligase to produce pATC395, which was *rcsA*$^+$ as well. Restriction sites are as follows: *Bam*HI (B), *Hin*dIII (H), *Nru*I (N), *Sal*I (S), *Pst*I (P), and *Cla*I (C).

TABLE 4. Effect of a *lon*$^+$ plasmid on capsule synthesis[a]

Strain genotype	β-Galactosidase units[b]	
	p*lon*$^+$	p*lon*Δ510
lon-100	0.3	201
lon$^+$	0.01	2.1
lon$^+$ *rcsA3* (RcsA*3)	0.2	456
lon$^+$ *rcsC137*	4	475

[a] The *cps-11*::*lac* fusion strains were SG20581 (*lon-100*), SG20582 (*lon*$^+$), SG20645 [*rcsA3*(RcsA*3)], and SG20643 (*rcsC137*).

[b] Cells were grown in glucose-M56 medium supplemented with Casamino Acids and ampicillin (50 μg/ml) at 32°C.

Smaller plasmids containing the *rcsA*$^+$ gene were generated as shown in Fig. 3. A 2.4-kb *Bam*HI-*Pst*I fragment in the M13mp19 derivative MAT200 complemented *rcsA* mutations to increase expression of *cps*::*lac* and of capsular polysaccharide. Deletions of the *rcsA*$^+$ clone were generated in this phage by using T4 DNA polymerase (6). Deletions from the *Eco*RI site that went beyond the *Eco*RV site inactivated the gene (Fig. 4). The largest deletion that still complemented an *rcsA* chromosomal mutation, Δ37, left a 0.86-kb fragment. This piece was subcloned in two orientations into pUC18 and pUC19 to generate pATC118 and pATC119, respectively. The 2.4-kb piece was also subcloned from MAT200 into pBR322 to produce pATC400. All of these plasmids confer mucoidy to *lon*$^+$ hosts.

Insertional mutagenesis of plasmids. Insertions linked to and in the *rcsA* gene cloned in pATC395 were isolated by using the ΔKan transposon as indicated in Materials and Methods. *rcsA*::ΔKan insertions were identified by the plasmid's loss of its ability to complement chromosomal *rcsA* mutations. In plasmids with linked ΔKan inserts, *rcsA* remained fully active. Similar ΔKan insertion mutations were isolated in pATC400 (Fig. 4).

pATC395 and its derivative pATC450, which carries the *rcsA*-50::ΔKan insertion, were used to transform an isogenic

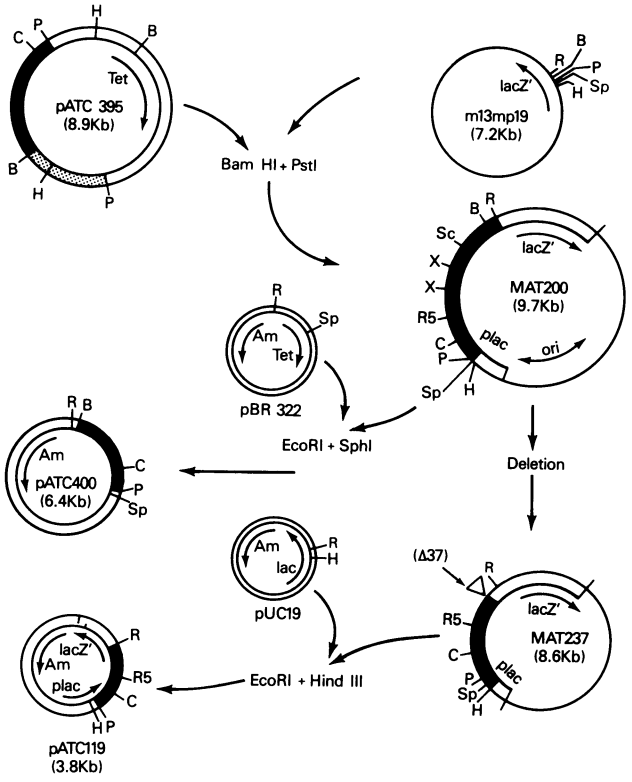

FIG. 3. Construction of pATC400 and pATC119. Plasmid derivatives are shown on the left; M13 derivatives are shown on the right. The 2.4-kb *Bam*HI-*Pst*I fragment was subcloned into the polylinker site of M13mp19 to produce phage MAT200. The RcsA deletion derivative Δ37 was generated by the cyclone method in MAT200 (6). Δ37 was subcloned from phage MAT237, using *Eco*RI and *Hin*dIII, into pUC19 to generate pATC119. Restriction sites are as follows: *Bam*HI (B), *Eco*R5 (R5), *Hin*dIII (H), *Sal*I (S), *Nru*I (N), *Cla*I (C), *Sph*I (Sp), *Pst*I (P), *Eco*RI (R), *Sca*I (Sc), and *Xmn*I (X).

set of *lon*$^+$ and *lon cps*::*lac* fusion strains, and their effect on β-galactosidase expression from the *cps*::*lac* fusion was determined (Table 5). The level of the enzyme increased about 300-fold in pATC395 transformants of *lon*$^+$ strains. In *lon* hosts, which already express high levels of β-galactosidase, pATC395 increases expression fourfold. The same plasmid conferred high levels of *cps*::*lac* expression on cells carrying a chromosomal *rcsA* deletion. The plasmid complementation data confirm that *rcsA* is normally limiting in the cell.

While the level of enzyme obtained from *lon*$^+$ *rcsA* or *lon rcsA* transformants of pATC450 was similar to that of the untransformed control (Table 5), a 100-fold increase in synthesis was observed in a *lon*$^+$ *rcsA*$^+$ transformant with the same *rcsA*::ΔKan plasmid (cf. columns 1 and 3 of line 1, Table 5). Similar results were observed with several other pATC395 derivatives carrying different ΔKan inserts as well as with pATC400 ΔKan insert plasmids (data not shown). These results suggest that the high-copy plasmid titrates a negative effector, allowing expression of the chromosomal copy of *rcsA*$^+$ and, consequently, escape synthesis of *cps*::*lac*. It is unclear whether such escape synthesis is seen in *lon-100* hosts, which already show relatively high levels of β-galactosidase synthesis.

Identification of the *rcsA* gene product. Determination of the proteins synthesized by plasmids pATC119 and pATC400 and several of its ΔKan insertion derivatives was done in the *lon-100* maxicell strain JB3034 (Fig. 5). One major protein of about 27 kilodaltons (kDa) was synthesized by pATC119 (lane 1) and pATC400 (lane 2). All four *rcsA*::ΔKan insertions in pATC400 failed to produce this polypeptide (lanes 3 through 6). No unique protein was synthesized by pATC401 (*rcsA160*::ΔKan) (lane 3). In cells harboring pATC410 (*rcsA168*::ΔKan) a band of about 16 kDa was seen (lane 4). A 23-kDa protein was synthesized by pATC408 (*rcsA166*::ΔKan) (lane 5), and pATC402 (*rcsA161*::ΔKan) produced a 19-kDa protein (lane 6). If these new bands are truncated RcsA proteins, then the direction of transcription of the gene would be from the *Pst*I site to the *Cla*I site (Fig. 6). The results are consistent with the identification of the 27-kDa protein as the *rcsA* gene product.

Stability of RcsA protein. To determine if the RcsA gene

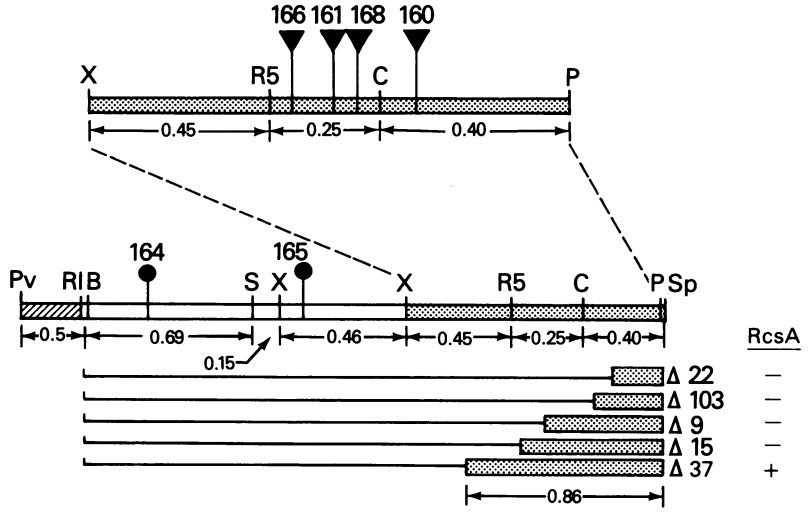

FIG. 4. Detailed restriction map of the *rcsA* clone in pATC400 and deletions generated in phage MAT200. The extent of the deletions are shown by thin lines. Closed circles indicate ΔKan inserts linked to *rcsA*$^+$. Plasmids carrying these inserts confer mucoidy upon *cps*$^+$ hosts and a Lac$^+$ phenotype to *cps*::*lac rcsA* strains. Closed triangles indicate ΔKan inserts which inactivate the *rcsA* gene.

product is affected by *lon* proteolysis, we assayed its stability in growing cells, using isogenic *lon*$^+$ and *lon* cells carrying pATC119 transformants. This plasmid, derived from pUC19, is stable even in the absence of an F′i^Q episome and expresses RcsA at high levels. Exponentially growing cells were pulse-labeled with [^{35}S]methionine for 1 min and chased with an excess of unlabeled methionine for up to 30 min. Samples were removed at various times during the chase, and the proteins were analyzed by electrophoresis and autoradiography (Fig. 7). The amount of *rcsA* determined by densitometry of this gel and others, using both pATC119 and other *rcsA*$^+$ plasmids, indicate that whereas the half-life of the RcsA protein is about 5 (±2) min in *lon*$^+$ cells, it is increased to 20 (±5) min in *lon* strains. Other proteins synthesized from the same plasmids, the Kan^r or the Bla protein, are stable in both *lon*$^+$ and *lon* cells under these conditions (half-life much greater than 30 min). Therefore, as predicted by our model, RcsA is an unstable protein in wild-type cells.

DISCUSSION

Mutations at the *lon* locus of *E. coli*, which codes for an ATP-dependent protease, are pleiotropic and result in overproduction of the capsular polysaccharide colanic acid and sensitivity to UV irradiation (3, 4, 12, 13). The UV sensitivity phenotype of *lon* has been shown to result directly from the defect in proteolysis that allows for the accumulation of SulA, an unstable inhibitor of septation in the cell (26). We show here that, as for UV sensitivity, capsule overproduction in *lon* cells can be explained by the accumulation of an unstable protein, RcsA, which is sensitive to the *lon* proteolytic system.

Two *trans*-acting positive regulatory loci, *rcsA*$^+$ and *rcsB*$^+$, have been previously identified (11). Introduction of an additional copy (in λ *rcsA*$^+$ lysogens) or multiple copies (in strains carrying *rcsA* plasmids) of *rcsA*$^+$ in a *lon*$^+$ background increases both β-galactosidase synthesis from *cps*::*lacZ* fusions and capsule synthesis in *cps*$^+$ strains. These results confirm the observations of Silverman and Simon (31) and Clegg and Koshland (5) that introduction of the episome F′1334 and of plasmids carrying the *flaA* and *flaP* operons resulted in a mucoid phenotype. *rcsA* is tightly linked to *fla*, at min 43 on the *E. coli* map (1, 11). We conclude from our results that *rcsA* is normally limiting in the cell. Small increases in *rcsB*$^+$ gene dosage, on the other hand, did not increase *cps* gene expression.

Our hypothesis was that the limiting positive regulator should also be unstable. We have now demonstrated that the *rcsA* protein is a 27-kDa polypeptide which turns over with a half-life of about 5 min in *lon*$^+$ cells. The RcsA half-life increases to 20 min in *lon* strains. In contrast, the *rcsB* gene product is completely stable in *lon*$^+$ cells (Brill and Gottesman, in preparation). Thus the RcsA protein is unstable and its degradation is affected by the *lon* protease.

TABLE 5. Expression of β-galactosidase in transformants[a]

Strain genotype	β-Galactosidase units[b]		
	No plasmid	pATC395 (*rcsA*$^+$)	pATC450 (*rcsA50*::ΔKan)
lon$^+$ *rcsA*$^+$	1.5	598	118
lon$^+$ Δ*rcsA27*	1.1	419	1
lon-100 rcsA$^+$	73	296	34
lon-100 Δ*rcsA26*	0.1	200	0.8

[a] The strains used were the *recA* derivatives SG20587 (*lon*$^+$ *rcsA*$^+$), JB3035 (*lon*$^+$ *rcsA*), JB3030 (*lon-100*), and JB3034 (*lon-100 rcsA*).

[b] Cells were grown in minimal-M56 Casamino Acids medium with tetracycline at 37°C. Data are an average of two samples.

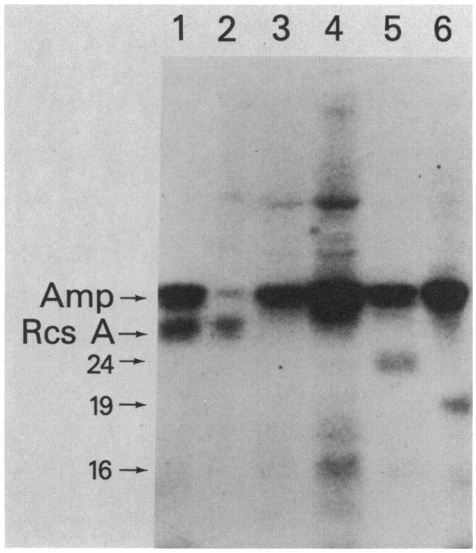

FIG. 5. In vivo labeling of proteins expressed by recombinant plasmids in maxicells. Maxicell strain JB3034 containing different plasmids was labeled as described in Materials and Methods. The proteins were separated on a 12% sodium dodecyl sulfate-polyacrylamide gel. Lanes: 1, pATC119 (*rcsA*$^+$); 2, pATC400 (*rcsA*$^+$); 3, pATC401 (*rcsA160*::ΔKan); 4, pATC410 (*rcsA168*::ΔKan); 5, pATC408 (*rcsA166*::ΔKan); 6, pATC402 (*rcsA161*::ΔKan). Amp, Ampicillin.

The *lon* effect on RcsA stability is similar to that seen for λN protein, an in vivo substrate for Lon proteolysis (10). In both cases, the half-lives of the proteins in *lon* hosts are about 20 min. We believe this residual turnover is due to degradation of these proteins by other cellular proteases. It seems unlikely that Lon is acting indirectly to affect turnover of these proteins. In the case of λN protein, the in vitro degradation of purified N protein by purified *lon* protease (Maurizi, in press) confirms that the in vivo effects of *lon* mutations are reflected by the direct degradation of N by Lon in vitro.

The increase in *cps*::*lac* expression caused by *rcsA62* (RcsA*62), a dominant allele of *rcsA*, can be reversed by introduction of a *lon*$^+$ multicopy plasmid. This suggests that the protein made by this mutant is still sensitive to high levels of the Lon protease. The exact nature of *rcsA* (RcsA*)

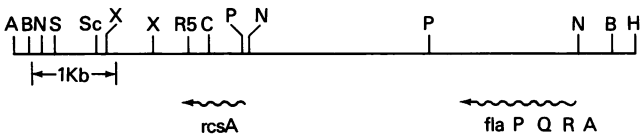

FIG. 6. Fine-structure map of the region of the bacterial chromosome carrying the *rcsA* gene. The map includes restriction information from the λ *rcsA*$^+$ phage and the *rcsA* plasmids as well as from plasmid pCK202, which contains the *flaARQP* operon (5). The *Sal*I-*Hin*dIII fragment is contained in both pCK202 and λ *rcsA*$^+$. The deletion in plasmid pCK202 (Δ7) which abolishes its ability to confer the mucoid phenotype extends from *Ava*I to *Hin*dIII. The wavy arrows show the direction of transcription of the *fla* operon (5) and that postulated for the *rcsA* gene. Restriction sites are as follows: *Ava*I (A), *Bam*HI (B), *Nru*I (N), *Sal*I (S), *Sca*I (Sc), *Xmn*I (X), *Eco*RV (R5), *Cla*I (C), *Pst*I (P), and *Hin*dIII (H).

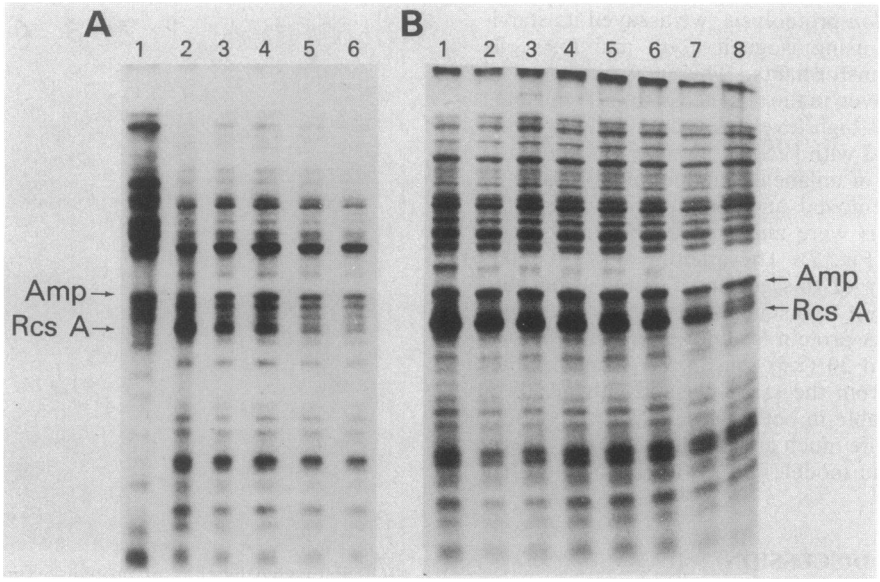

FIG. 7. Pulse-chase labeling of RcsA in *lon*$^+$ and *lon* cells. Cells were pulse-labeled with [^{35}S]methionine for 1 min and chased with excess unlabeled methionine (2 mM). (A) *lon*$^+$ (JB3035): lane 1, pUC19; lane 2, pATC119, 1-min pulse; lane 3, 2-min chase; lane 4, 4-min chase; lane 5, 6-min chase; lane 6, 8-min chase. (B) *lon* (JB3034): lane 1, pATC119, 1-min pulse; lanes 2 to 8, chases of 2, 4, 6, 8, 10, 15, and 30 min. Amp, Ampicillin.

is not known. Its gene product could be a protein with increased but not complete resistance to proteolysis. Even relatively small differences in RcsA protein half-life would be expected to increase capsule synthesis significantly. Alternatively, the RcsA* phenotype may reflect higher levels of synthesis of the wild-type protein or a more active RcsA protein.

The regulation of capsule synthesis and possibly that of *rcsA* may be part of a complex regulatory pathway. Our results indicate that *rcsA* itself may be under the negative control of some effector. *rcsC*, a negative regulator of capsule synthesis (11), may act by controlling the level of RcsA synthesis. We observed that high-level expression of *cps*::*lac* in *rcsC* backgrounds is still sensitive to proteolysis when a multicopy *lon*$^+$ plasmid is introduced in the cell (Table 4). In addition, *rcsC137 rcsA* hosts are defective for *cps*::*lac* expression (data not shown). These results are consistent with models in which *rcsC* acts before *rcsA* to regulate capsule synthesis. We are currently examining the role of *rcsA*, *-B*, and *-C* in capsule synthesis regulation.

Many bacterial species produce capsules in nature and under laboratory growth conditions. For example, nonmucoid mutants of the normally mucoid corn pathogen *Erwinia stewartii* have been isolated and characterized (D. Coplin, personal communication). Some of these mutants are defective in a positive regulator of capsule synthesis with similarities to *rcsA* (D. Coplin, A. S. Torres-Cabassa, and S. Gottesman, manuscript in preparation). Capsule synthesis could be a cellular response to some environmental stress condition; environmental factors such as temperature, medium, and growing conditions influence capsule expression. It is possible that a mechanism for capsule regulation could have been evolutionarily conserved and that similar pathways are involved in the synthesis of other complex polysaccharides.

ACKNOWLEDGMENTS

We thank Julie Brill and Patsy Trisler for constructing some of the strains used in this work and David Coplin for communicating his unpublished results to us. Sankar Adhya, Michael Maurizi, and Olivier Huisman provided helpful comments on the manuscript. We also acknowledge the help of Althea Gaddis in preparing the manuscript for publication.

LITERATURE CITED

1. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
2. **Bukhari, A. I., and D. Zipser.** 1973. Mutant of *Escherichia coli* with a defect in the degradation of nonsense fragments. Nature (London) New Biol. **243**:238–241.
3. **Charette, M. F., G. W. Henderson, and A. Markovitz.** 1981. ATP hydrolysis-dependent protease activity of the *lon* (*capR*) protein of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. USA **78**:4728–4732.
4. **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.
5. **Clegg, D. D., and D. Koshland, Jr.** 1985. Identification of a bacterial sensing protein and effects of its elevated expression. J. Bacteriol. **162**:398–405.
6. **Dale, R. M., B. A. McClure, and J. P. Houchins.** 1985. A rapid single-stranded cloning strategy for producing a sequential series of overlapping clones for use in DNA-sequencing: application to sequencing the corn mitochondrial 18S rDNA. Plasmid **13**:31–41.
7. **Enquist, L. W., and R. A. Weisberg.** 1977. A genetic analysis of the *att-int-xis* region of coliphage lambda. J. Mol. Biol. **111**: 97–120.
8. **Goldberg, A. L.** 1972. Degradation of abnormal proteins in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **69**:422–426.
9. **Goldberg, A. L.** 1985. Role of ATP and protease La, the *lon* gene product, in protein breakdown in *Escherichia coli*, p. 340–345. *In* L. Leive (ed.), Microbiology—1985. American Society for Microbiology, Washington, D.C.
10. **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.
11. **Gottesman, S., P. Trisler, and A. S. Torres-Cabassa.** 1985. Regulation of capsular polysaccharide synthesis in *Escherichia coli* K-12: characterization of three regulatory genes. J. Bacte-

riol. **162**:1111–1119.
12. **Gottesman, S., P. Trisler, A. S. Torres-Cabassa, and M. Maurizi.** 1985. Regulation via proteolysis: the *Escherichia coli lon* system, p. 350–354. *In* L. Leive (ed.), Microbiology—1985. American Society for Microbiology, Washington, D.C.
13. **Gottesman, S., and D. Zipser.** 1978. Deg phenotype of *Escherichia coli lon* mutants. J. Bacteriol. **133**:844–851.
14. **Howard-Flanders, P., E. Simson, and L. Theriot.** 1964. A locus that controls filament formation and sensitivity to radiation in *Escherichia coli* K-12. Genetics **49**:237–246.
15. **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.
16. **Kleckner, N., J. Roth, and D. Botstein.** 1977. Genetic engineering *in vivo* using translocatable drug-resistance elements. New methods in bacterial genetics. J. Mol. Biol. **116**:125–159.
17. **Laemmli, U. K.** 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
18. **Mackie, G., and D. B. Wilson.** 1972. Regulation of the *gal* operon of *Escherichia coli* by the *capR* gene. J. Biol. Chem. **247**:2973–2978.
19. **Maloy, S. R., and W. D. Nunn.** 1981. Selection for loss of tetracycline resistance by *Escherichia coli*. J. Bacteriol. **145**: 1110–1112.
20. **Maniatis, T. E., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **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.
22. **Markovitz, A., M. M. Liebermann, and N. Rosenbaum.** 1967. Derepression of phosphomannose isomerase by regulator gene mutations involved in capsular polysaccharide synthesis in *Escherichia coli* K-12. J. Bacteriol. **94**:1497–1501.
23. **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.
24. **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.
25. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **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.
27. **Mizusawa, S., and D. Ward.** 1982. A bacteriophage lambda vector for cloning with BamHI and Sau3A. Gene **20**:317–322.
28. **Rosner, J. L.** 1972. Formation, induction and curing of bacteriophage P1 lysogens. Virology **48**:677–689.
29. **Sancar, A., A. M. Hack, and W. D. Rupp.** 1979. Simple method for identification of plasmid-encoded proteins. J. Bacteriol. **137**: 692–693.
30. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Silverman, M., and M. Simon.** 1973. Genetic analysis of flagellar mutants in *Escherichia coli*. J. Bacteriol. **113**:105–113.
32. **Simon, L. D., M. E. Gottesman, K. Tomczak, and S. Gottesman.** 1979. Hyperdegradation of proteins in *Escherichia coli rho* mutants. Proc. Natl. Acad. Sci. USA **76**:1623–1627.
33. **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.
34. **Yamamoto, K. R., B. M. Alberts, R. Benzinger, L. Lawhorne, and G. Treiber.** 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus preparation. Virology **40**:734–744.
35. **Way, J. C., M. A. Davis, D. Morisato, D. E. Roberts, and N. Kleckner.** 1984. New Tn10 derivatives for transposon mutagenesis and for construction of *lacZ* operon fusions. Gene **32**: 369–379.
36. **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.