JOURNAL OF BACTERIOLOGY, Mar. 1991, p. 1738–1747
0021-9193/91/051738-10$02.00/0

Vol. 173, No. 5

RcsA, an Unstable Positive Regulator of Capsular Polysaccharide Synthesis

VALERIE STOUT, ANGEL TORRES-CABASSA,† MICHAEL R. MAURIZI,‡ DAVID GUTNICK,§ AND SUSAN GOTTESMAN*

Laboratory of Molecular Biology, Building 37, Room 4B03, National Cancer Institute, Bethesda, Maryland 20892

Received 27 September 1990/Accepted 20 December 1990

RcsA is an unstable positive regulator required for the synthesis of colanic acid capsular polysaccharide in *Escherichia coli*. Degradation of the RcsA protein in vivo depends on the ATP-dependent Lon protease. DNA sequence analysis of the *rcsA* gene reveals a single open reading frame for a 23,500-Da highly basic protein (pI = 9.9), consistent with the observed size of the purified subunit of RcsA. The DNA and protein sequences are highly homologous to the *rcsA* gene and protein from *Klebsiella pneumoniae* and other species. The carboxy-terminal region of RcsA contains a possible helix-turn-helix DNA-binding motif that resembles sequences found at the carboxy terminus of RcsB, another positive regulator of capsule synthesis, and in several other transcriptional regulators including members of the LuxR family. *rcsA62*, a mutation in *rcsA* that leads to increased capsule synthesis, encodes a protein designated RcsA*, which differs from wild-type RcsA only in the replacement of Met-145 by valine. The RcsA* protein is subject to Lon-dependent degradation. The stability of wild-type RcsA in vivo is increased by multicopy RcsB. Conversely, RcsA is degraded more rapidly in *rcsB* mutant hosts than in wild-type hosts. These results suggest that RcsA and RcsB interact in vivo and are consistent with genetic experiments that indicate an interaction between RcsA and RcsB. Based on these experiments, we propose a model for capsule regulation in which RcsA interacts directly with RcsB to promote transcription of the genes for capsule synthesis.

Colanic acid capsular polysaccharide synthesis in *Escherichia coli* is encoded by the *cps* genes (51) and is under the control of two positive regulators, the products of the *rcsA* and *rcsB* genes (21). RcsA has been identified as an unstable protein, which is normally limiting for capsule synthesis (49). Mutations in the ATP-dependent protease Lon result in increased capsule synthesis; we have previously demonstrated that RcsA is stabilized in *lon* mutant cells (49). Therefore, it seems likely that the increase in capsule synthesis in *lon* mutants is due at least in part to the increased accumulation of RcsA in these cells.

Why is RcsA unstable? One rationale for incorporating an unstable protein into a regulatory network is provided by the example of the cell division inhibitor SulA, another target of Lon-dependent degradation (34). SulA synthesis is tightly regulated by the LexA repressor, and SulA is only made as part of the SOS response to DNA damage (24, 33). When SulA is present in cells, no septa are formed and cells do not divide (25). Thus, SulA overproduction or constitutive synthesis of SulA is lethal to growing cells (20, 25, 42). It is essential that a growing cell rid itself of any SulA made during the SOS response. The Lon-mediated degradation of SulA provides a straightforward mechanism for the recovery from this particular emergency response.

Many of the parallel issues for the regulatory circuit for capsule synthesis have not yet been addressed. Overproduction of RcsA from a multicopy plasmid is not itself lethal, but cells grow slowly and frequently lose the plasmid (46a). Although cells tolerate increased capsule synthesis, it is clear that a great deal of energy and synthetic activity are diverted into the capsule. Mutations which lead to increased capsule synthesis map in *lon*, in *rcsA* (RcsA*, dominant mutations in *rcsA*), and in *rcsC*, a complex regulator tightly linked to the *rcsB* positive regulatory gene (5, 21). Such mutants, particularly those with the strong *rcsC* mutation, frequently accumulate second-site mutations which reduce capsule synthesis to background levels (20a). Mutations in other loci which lead to increased capsule synthesis (*capS* and *capT*, described by Markovitz [31]) and *ops* (54) have not been fully characterized. *capS* and *capT* may be allelic to the *rcs* loci.

RcsC and RcsB have homology to a variety of two-component regulatory proteins in bacteria. RcsC, a membrane protein, has homology with histidine kinase environmental sensors, while RcsB has similarities to phosphorylated DNA-binding protein effectors (46, 47). By analogy to the model developed for such sensor-effectors, RcsC may sense environmental stimuli and transmit this information to RcsB via phosphorylation, resulting in the positive stimulation of transcription of *cps* genes. The nature of the environmental signal and the role of RcsA in such a scheme are still unclear. RcsB is absolutely required for capsule synthesis, while the RcsA requirement can be partially bypassed in some *rcsC* mutants or when RcsB is overproduced (5). Genetic evidence is consistent with RcsA acting either directly or indirectly through RcsB to activate transcription of *cps* genes. It might promote DNA binding or act by modulating RcsB activation by RcsC.

Homologs of RcsA have been found in *Erwinia* and *Klebsiella* species (1, 9, 32a, 36a, 50). These genes function in *E. coli* to complement *E. coli rcsA* mutants for expression of *cps* genes (7, 9, 32a, 50). In *Erwinia stewartii*, the *rcsA*

* Corresponding author.
† Present address: Center for Devices and Radiological Health, Food and Drug Administration, Rockville, MD 20850.
‡ Present address: Laboratory of Cell Biology, National Cancer Institute, Bethesda, MD 20892.
§ Present address: Department of Microbiology, Tel Aviv University, Ramat Aviv 69978, Israel.

TABLE 1. Bacterial strains, bacteriophages, and plasmids

Strain, phage, or plasmid	Description	Source[a] or reference
Bacterial strains		
BL21(DE3)(pLysS)	p_{lac}-T7 polymerase lysogen with lysozyme on plasmid	48
JB3019	*rcsA27 cpsB10-lac lon*$^+$	As for JB3012 (5)
JM101	F'i^Q/Δ*lac-pro*	53
JT4000	Δ*lon-510 lac*	SG20250 derivative
SA2691	Δ*lac* Δ*gal su recA*$^+$	HB101 derivative from S. Adhya
SG1132	*lac leu pro* r_k^- m_k^-	SA2691 Gal$^+$
SG1142	*rcsB62*::Δkan	pJB102 linear transformant (5)
SG20043	*cps-3*::Tn*10 lon*-100	51
SG20250	Δ*lac*	21
SG20322	*lon-146*::Tn*10 cpsB10-lac*	32
SG22029	*rcsA62 zed-751*::Δkan	This work
SG23002	*lac rcsB62*::Δkan	SG1132 + P1(VS20183)
SG23004	*lac lon-146*::ΔTn*10*	SG1132 + P1(SG20322)
VS2022	*rcsA62* (RcsA*) *zed-751*::Δkan	SG1132 + P1(SG22029)
VS20183	*rcsB62*::Δkan *cps-3*::Tn*10*	SG20043 + P1(SG1142)
Bacteriophages		
MAT237	*rcsA*$^+$Δ37 in M13mp19	49
MAT100	*rcsA*$^+$ in M13mp18	49
MAT200	*rcsA*$^+$ in M13mp19	49
MVS1	*rcsA62* (RcsA*) in M13	*Bam-Pst*I from pATC462 in M13mp18
SY62	λ *c*I857 *rcsA62* (RcsA*)	21
Plasmids		
pAR2106	T7 promoter vector	38
pATC70	*rcsA-lac* Δ70	pATC591 + *Hin*dIII-*Eco*RI of Δ70 of Fig. 1
pATC118	pUC18 + *rcsA*	This work; from MAT237
pATC119	pUC19 + *rcsA*	49
pATC400	pBR322 + *rcsA*$^+$	49
pATC462	pBR325 + *rcsA62* (RcsA*)	This work
pATC591	pRS591	*Hin*dIII linker in *Sma* site of pRS591
pJB100	pBR322 + *rcsB*$^+$	5
pJB102	pBR322 + *rcsB62*::Δkan	5
pRS591	*lac* fusion vector	44
pVS102	pACYC184 + *rcsB*$^+$	*Hin*dIII-*Bam*HI from pJB100
pVS104	pBR322 + *rcsA62* (RcsA*)	*Eco*RI-*Sph*I from MVS1
pVS110	pAR2106 + *rcsA*	This work

[a] SG1132 + P1(VS20183) indicates P1 transduction from VS20183 into SG1132.

analog is a virulence factor needed for synthesis of a capsular polysaccharide necessary for the infection of corn (10–12, 17). *E. stewartii* RcsA produced in *E. coli* is degraded in a Lon protease-dependent manner (50). Therefore, both the activity of RcsA and its susceptibility to degradation may have been conserved in all gram-negative organisms, suggesting that such a protease-sensitive regulator plays an important role for capsule regulation.

We have determined the sequence of the RcsA gene, compared it with the sequence of its *Klebsiella pneumoniae* analog, and studied its turnover. Our results suggest that RcsA interacts with RcsB and that RcsA and RcsB belong to the same family of transcription activators.

MATERIALS AND METHODS

Bacterial strains, bacteriophages, and plasmids. The bacterial strains, bacteriophages, and plasmids used in this work and their sources are listed in Table 1.

Deletions, construction of RcsA-LacZ fusions, and sequencing. The cyclone subcloning system for M13 from International Biotechnologies, Inc. (IBI) (14), was used to generate *rcsA* deletions in the M13mp18 and mp19 clones MAT100 and MAT200, respectively, following the manufacturer's specifications (49). *Hin*dIII, *Eco*RI, and T4 DNA polymerase (Bethesda Research Laboratories) were sometimes used in place of the enzymes provided by IBI. After deletion and ligation of the DNA, plaques were isolated on *E. coli* JM101 and screened for their ability to complement a chromosomal *rcsA* mutation in an F' host (F' *lon rcsA cpsB10*::*lac*) by screening on lactose-tetrazolium plates. Those plaques which failed to complement the *rcsA* mutation were analyzed further. The deletion endpoints were determined from restriction analyses of the double-stranded replicative form on acrylamide gels. Some of these deletion derivatives have been described previously (49). The endpoints of the deletions are summarized in Fig. 1.

Single-stranded DNA isolated from the M13mp18 and M13mp19 deletions was sequenced by the dideoxy nucleotide chain termination method (41) from the appropriate M13 primer to give overlapping sequences of the *rcsA* gene. The sequence of the gene was later confirmed by using synthetic oligonucleotide primers on pATC400, the pBR322 derivative of MAT200 (49). All oligonucleotide primers and linkers were synthesized on the Applied Biosystems 380B DNA synthesizer.

A substantial portion of cyclone deletions of MAT200 gave Lac$^+$ plaques on JM101. In MAT200, *rcsA*$^+$ is oriented in the same direction as *lacZ*. Since the parental MAT200 phage disrupts *lacZ* and gives Lac$^-$ plaques, Lac$^+$ plaques

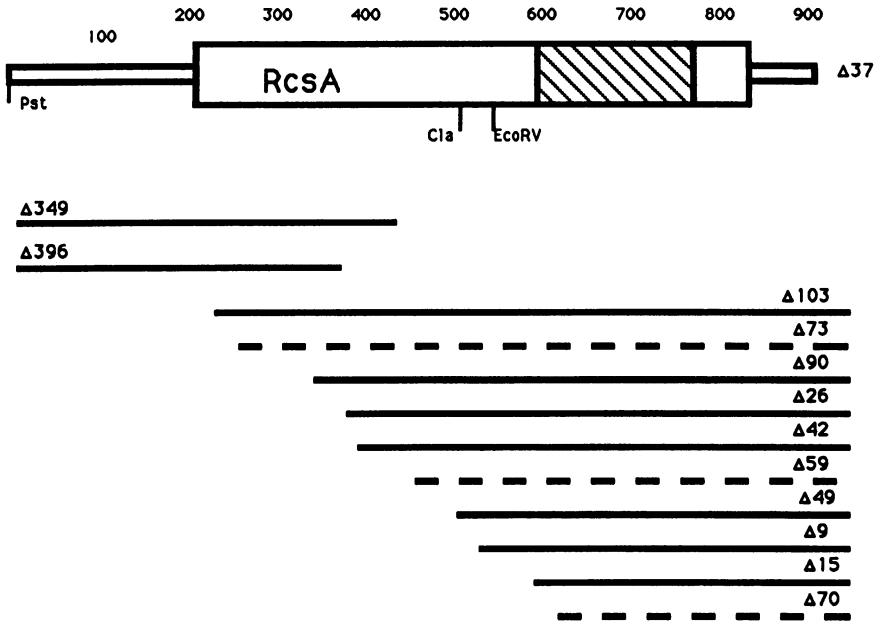

FIG. 1. Deletions of *rcsA*. The Δ37 *rcsA*$^+$ clone is schematically represented at the top of the figure. The open reading frame corresponding to RcsA is shown as a wide box; the striped region is the conserved domain shown in Fig. 4. The extents of deletions into *rcsA* generated in M13 are shown as lines below the gene (lines represent deleted portion). Dotted lines show deletions which yielded *rcsA-lacZ* fusions with a Lac$^+$ phenotype.

could be formed only if the *rcsA* amino terminus was fused in frame to *lacZ* after deletions were made. *rcsA-lacZ*$^+$ fusions were transferred from M13 to a modified version of the pRS591 translational fusion vector pATC591 (44). A 12-bp *Hin*dIII linker was inserted within the *Sma*I site of pRS591 to form pATC591. In this plasmid, proteins fused to *lacZ* in M13 remain in frame when inserted in the *Eco*RI site of the plasmid. *Hin*dIII-*Eco*RI fragments of Lac$^+$ *rcsA* deletion fragments were ligated with *Hin*dIII- and *Eco*RI-digested pATC591. Lac$^+$ plasmids proved to give RcsA-LacZ protein fusions, as expected. The Δ70 Lac$^+$ fusion plasmid pATC70 was subsequently used to isolate an RcsA-LacZ fusion protein for microsequencing (see below).

Cloning and sequencing of *rcsA*62*. The *rcsA** mutation on phage SY62 was isolated after *mutD* mutagenesis of the λ*rcsA*$^+$ phage and screening for plaques which increased *cps-lac* expression in a *lon*$^+$ host. Homologous recombination was used to cross the *rcsA** mutation on phage SY62 onto the chromosome by lysogenization of MC4100. A defective kanamycin-resistant transposon Tn*10* derivative (Δkan) linked to *rcsA* (*zed751*::Δkan) was crossed into this strain by P1 transduction to make SG22029. The *rcsA** mutation on SY62 was also transferred to a plasmid by digesting the phage DNA with *Bam*HI and inserting the 6.2-kb piece into pBR325 that had been digested with *Bam*HI. This plasmid, designated pATC462, was subsequently digested with *Bam*HI and *Pst*I, and the 2.5-kb fragment was inserted into the same restriction sites of M13mp18 to form MVS1. Sequencing of the insert was performed as described above with synthetic oligonucleotides.

Amino-terminal sequence of the RcsA-β-galactosidase fusion protein. A small portion of the fusion protein from pATC70 was prepared by immunoprecipitation with anti-β-galactosidase antibody (Cooper Biomedical) and isolated on a 7% polyacrylamide gel in sodium dodecyl sulfate (SDS). The fusion protein was electroeluted (26) and separated from salts by precipitation and washing with 90% isopropanol. Approximately 100 pmol of the protein was dissolved in trifluoroacetic acid and subjected to sequential Edman degradation on an Applied Biosystems model 470A sequencer (39).

Purification of RcsA and isolation of antibody. A DNA fragment containing *rcsA* with an *Nde*I site at the start codon was obtained by the polymerase chain reaction (PCR) (40). The template used in the PCR was pATC118, a pUC18 derivative similar to pATC119 (49). The primers for the reaction were the −40 17-mer sequencing primer (New England BioLabs) and a 40-base oligonucleotide identical to bases 192 to 237 in the *rcsA* sequence (Fig. 2), except for three nucleotide changes at positions 208 to 210 (from GCC to CAT) to create an *Nde*I site.

To allow transcription of *rcsA* from a T7 promoter, the PCR fragment containing *rcsA* was digested with the restriction enzymes *Nde*I and *Bam*HI (Bethesda Research Laboratories) and ligated to pAR2106 (38) that was digested with the same enzymes. The *rcsA* open reading frame and the promoter region of the resulting plasmid, pVS110, were sequenced to confirm the presence of the promoter and wild-type *rcsA* gene.

Overproduction of RcsA was accomplished after transforming pVS110 into BL21(DE3) carrying pLysS (48). The culture was grown at 37°C in LB medium with ampicillin (125 μg/ml) and chloramphenicol (30 μg/ml). The T7 polymerase gene in BL21 (DE3) (which is controlled by the *lacUV5* promoter) was induced with 1 mM IPTG (isopropylthiogalactopyranoside) at an OD_{600} of 0.3 and expressed for 3 h before the cells were harvested. The pellet was suspended in 1/20 volume of 50 mM Tris (pH 8.0)–2 mM EDTA. This suspension was subjected to two freeze-thaw cycles and brief sonication to lyse the cells and break up DNA polymers. The pLysS plasmid (which carries the gene encoding

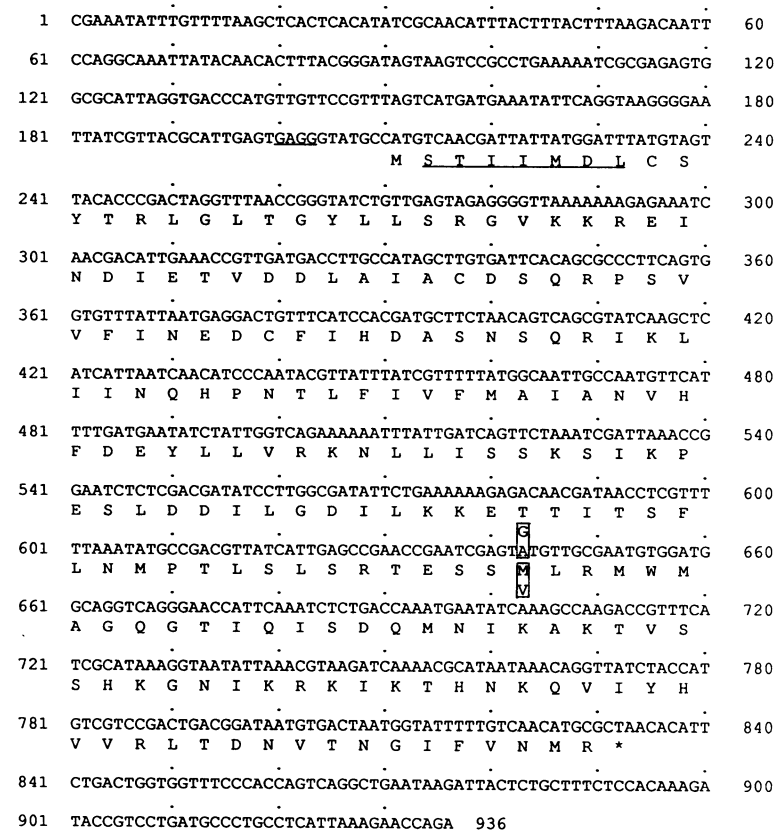

FIG. 2. Sequence of *E. coli rcsA*. The sequence of the Δ37 *rcsA*$^+$ clone is shown. The amino acid sequence obtained by microsequencing of the Δ70 fusion protein is indicated by underlining. The mutational change in *rcsA62* (RcsA*) is shown at bp 653, which resulted in an A to G change, thus changing Met-145 to Val. A possible ribosome-binding site is underlined. The single-letter code for amino acids is used.

T7 lysozyme) allowed the cells to be broken open more easily (38). A low-speed centrifugation step (2,000 × *g*, 20 min) pelleted inclusion bodies that contained most of the RcsA from the cells, while most of the soluble proteins remained in the supernatant (see Fig. 5).

The RcsA-containing pellet was solubilized in 2% SDS and run on an SDS-polyacrylamide gel. The RcsA band (detected by size) was cut out, eluted by electrophoresis (26), and injected into New Zealand female rabbits to raise antibodies. The resulting antiserum was preabsorbed with a cell extract of a strain deleted for *rcsA* (JB3019). The serum and extract were mixed for 2 h at 4°C and centrifuged at 10,000 × *g* for 30 min. The preabsorbed serum was used to detect RcsA by Western immunoblotting as described previously (28).

RcsA protein was further purified by reverse-phase chromatography on a Vydac C-18 column (0.75 by 30 cm). The homogeneous protein was eluted at approximately 50% acetonitrile in a linear gradient of water and acetonitrile, each containing 0.05% trifluoroacetic acid.

Amino acid analysis. RcsA purified by reverse-phase chromatography was hydrolyzed in 6 N HCl at 155°C for 25 and 50 min. The dried sample was derivatized with phenylisothiocyanate, and the phenylisothiocarbamyl amino acids were separated on an Axxiom C-18 reverse-phase column as described by Bidlingmeyer et al. (4).

Turnover of RcsA and RcsA*. Pulse-chase and immunoprecipitation experiments were performed as described by Trun and Silhavy (52) with the following modifications. Cells were grown at 37°C to an OD_{600} of 0.3 and labeled for 1 min with 10 μCi of [^{35}S]methionine (1,000 Ci/mmol; New England Nuclear) per ml in M63 medium (43). The samples were chased with warm M63 medium containing 0.5% methionine, and 1-ml samples were removed at the times indicated in the legends to Fig. 6 and 7. The samples were precipitated with trichloroacetic acid (5% final concentration) and washed with acetone. The precipitates were resuspended in 100 μl of a buffer containing 1% SDS, 1 mM EDTA, and 10 mM Tris-HCl (pH 7.5) and boiled for 5 min. RcsA was immunoprecipitated from a 50-μl portion of the cell extract with 5 μl of RcsA antiserum. After the immunoprecipitates were washed four times, the pellets were resuspended in 40 μl of gel loading buffer and boiled. After centrifugation at 12,000 × *g* for 5 min, 20 μl of the supernatant was loaded onto a 15% SDS–polyacrylamide gel.

Computer search and comparison methods. Sequence comparisons were carried out with the University of Wisconsin Genetics Computer Group package on a VAX computer (16). A profile was generated by using Profile from the *E. coli* RcsA, RcsB, MalT, and UhpA, *Bordetella pertussis* BvgA, and *Rhizobium meliloti* FixJ protein sequences, for a 55-amino-acid region including the 43-amino-acid sequence used by Henikoff et al. (22). This profile sequence was used to search the NBRF data bank. In addition to the proteins already present in the group used to generate the profile, additional members identified included *E. coli* NarL, *Bacillus subtilis* DegU, and two sigma factors, *B. subtilis* sigma H and *E. coli* sigma 70. In all cases except the last two, a criterion for inclusion in the aligned group was conservation

```
E.c.  MSTIIMDLCSYTRLGLTGYLLSRGVKKREINDIETVDDLAIACDSQRPSVVFINEDCFIH   60
      ||| ||||||||||||||||| ||| || ||       ||  | |  | ||| ||||| |
K.p.  MSTMIMDLCSYTRLGLTGYLTSRGIKKQEIVEVNSAADLQKHCTSCCPAVVFLNEDCFVH
                .         .         .         .         .         .
E.c.  DASNSQRIKLIINQHPNTLFIVFMAIANVHFDEYLLVRKNLLISSKSIKPESLDDILGDI   120
      |     | || | | ||| || || ||| || ||||||||||| | || ||
K.p.  DDESNGIIRQIITQNPATLFVIFMSLANIHFDRYLRVRKNLLISSKSITPKDLDVILVNY
                .         *         .         .         .         .
E.c.  LKKETTITSFLNMPTLSLSRTESSMLRMWMAGQGTIQISDQMNIKAKTVSSHKGNIKRKI   180
      ||  |    | |||||| ||| || ||||| || ||| ||||||||||||||||| ||
K.p.  LKYKNTSVGQLTLPTLSLSKTESNMLQMWMAGHGTSQISTQMNIKAKTVSSHKGNIKKKI
                .         .
E.c.  KTHNKQVIYHVVRLTDNVTNGIFVNMR  207
      ||||||||| |||| | | || ||||
K.p.  QTHNKQVIYHIVRLTENITSGIQVNMR
```

FIG. 3. Comparison of *E. coli* and *K. pneumoniae* RcsA proteins. Alignment of predicted amino acid sequences of *E. coli* (E. c.) RcsA and *K. pneumoniae* (K. p.) RcsA (1). Identical amino acids are connected by a vertical line. Alignment done by Bestfit (16). The site of the *rcsA62* (RcsA*) mutation is marked with an asterisk.

at most or all of the sites which had shown absolute conservation in the initial group. Some members of the set with unknown function are not shown.

Nucleotide sequence accession number. The sequence discussed here was assigned GenBank accession number M58003.

RESULTS

Sequence of *rcsA*. We have previously described the isolation of deletions of a cloned *rcsA* gene in M13 phage (49). Because we can assay M13 carrying *rcsA* directly for complementation of a chromosomal *rcsA* mutation, we were able to define the smallest complementing fragment of our original clone as a piece of about 0.9 kb (Δ37, Fig. 1) (49). Other deletions from the M13mp19 derivative MAT200, which carries the *rcsA* gene reading in the same direction as *lac*, were screened for Lac^+ fusions created by deletion between the two open reading frames to create an in-frame RcsA-LacZ fusion protein. A significant proportion of the deletion mutants became Lac^+; a number of these were sized and sequenced. The nucleotide sequence and deduced amino acid sequence from the 0.9-kb fragment are shown in Fig. 2. A single open reading frame encoding a highly basic (pI = 9.9) 23.5-kDa protein was found, in reasonable agreement with the 27-kDa estimate of the size of RcsA from SDS-polyacrylamide gel electrophoresis (PAGE) (49). The clones carry 210 bp upstream of the first ATG. The ability of plasmids to complement *rcsA* mutations, together with the observation that this region is sufficient to promote synthesis of RcsA-LacZ fusion proteins (data not shown), even when present in single copy, suggests that a functional promoter is present within these 210 bp.

One of the *rcsA-lacZ* fusions was subcloned into pBR322, and the fusion protein was purified from overproducing cells by immunoprecipitation with anti-β-galactosidase antibodies. The homogeneous protein extracted from an SDS-polyacrylamide gel was sequenced (Materials and Methods), and the first seven amino-terminal amino acids agreed with those predicted by the translated DNA sequence (underlined in Fig. 2). The fusion protein is encoded beginning at bp 214 and does not retain the amino-terminal methionine.

Allen et al. (1) cloned and sequenced a gene from *K. pneumoniae* which, when present on a multicopy plasmid, increased capsular polysaccharide synthesis in *E. coli*. They called the gene *rcsA*. No function was demonstrated for the gene in *K. pneumoniae* and no attempt was made to complement an *E. coli rcsA* mutation. We noted a strong similarity between the *Klebsiella* gene and the *E. coli rcsA* gene. Recently, two groups have independently demonstrated that the *Klebsiella* gene does indeed complement *E. coli rcsA* mutants (9, 32a). A comparison of the predicted translation products of the *E. coli* and *K. pneumoniae rcsA* genes is shown in Fig. 3. The proteins share 68% exact amino acid identity and are identical in size. When the most conservative changes are considered, about 81% of all amino acids are conserved. Particularly noteworthy in this regard are the 50 amino acids near the carboxy terminus and the 30 amino acids at the amino terminus of the protein.

Comparison of RcsA with other regulatory proteins. Henikoff and coworkers found that *Klebsiella* RcsA was a member of a family of proteins which they called the LuxR group (22). Many but not all of these proteins are the effector components of two-component systems. The LuxR family includes UhpA and FixJ, which we had previously found to be the effectors with the most homology to RcsB (47), the other positive regulator for capsule synthesis. It is likely, therefore, that both RcsA and RcsB belong to the LuxR family. All members of the family are positive regulators of transcription.

An alignment of some members of the family is shown in Fig. 4. In all cases, the aligned region shown ends within 17 amino acids of the C-terminus of the protein. Within the 55-amino-acid stretch shown, 8 positions are identical for all or nearly all family members (marked with an asterisk in Fig. 4). Many other positions are conserved in a majority of the members. The aligned region includes a stretch of 17 amino acids (positions 26 to 42 of Fig. 4) which are absolutely conserved between the *Klebsiella* and *E. coli* RcsA proteins. This region is also conserved in two *Erwinia* RcsA proteins (3, 9, 36a).

By using the pattern for a helix-turn-helix protein defined by Pabo and Sauer (36), the protein segments shown in Fig. 4 were determined to have well-conserved residues which align with these predictions at positions 23 to 36. Such a prediction would put a turn at position 29. The turn and DNA-binding region from such an alignment would include the stretch of absolutely conserved amino acids in the C-termini of the RcsA proteins. If this prediction is correct, the homologies seen upstream of this region (positions 3 to 22 in Fig. 4) may represent a different conserved domain, possibly involved in protein-protein interactions.

Overproduction and purification of RcsA and production of anti-RcsA antibody. Using the sequence shown in Fig. 2, we designed oligonucleotide primers for PCR amplification (40) of *rcsA* and simultaneous introduction of an *NdeI* site at the ATG start codon of *rcsA*. The amplified fragment was inserted into the T7 expression vector pAR2106 (48) to give

```
                                1 *   *  * *     *      *          *           *    50
E. coli          Sig70      agLTaREakV LrMrfgidmN tDytlEevgk qfdVtrerir qieakalrkl rhpsr
B. subtillis     SigH       elLsdlERkV LvLYldGrsy qEIsdELnrh vKsIdnalqr vkrKLekyle ireIs
B. subtillis     DegU       hiLTrREceV LqMLadGksN rgIgesLfIS eKTVknHvsN iLqKMnvndr tqaVv
B. subtillis     GerE       psLTkREReV FeLLvqdktt kEIAsELfIS eKTVrnHisN aMqKLgvkgr sqaVv
E. coli          NarL       nqLTpRERdI LkLiaqGlpN kmIArrLdIt esTVkvHvkh mLkKMklksr veaav
B. pertussis     BvgA       svLsnREltV LqLLaqGmsN kDIAdsMflS nKTVstyktr lLqKLnatsl velId
E. coli          MalT       spLTqREWqV LgLiysGysN eqIAgELeVa atTIktHirN lyqKLgvahr qdaVq
R. meliloti      FixJ       qtLseRERqV LsavvaGlpN ksIAyDLdIS prTVevHraN vMaKMkaksl phlVr
E. coli          UhpA       dpLTkRERqV aekLaqGmav kEIAaELglS pKTVhvHraN lMeKLgvsnd velar
E. coli          RcsB       krLspkEseV LrLFaeGflv tEIAkkLnrS iKTIssqkks aMmKLgvenk ialln
E. coli          RcsA       lsLsrtEssm LrMWmaGqgt iqIskqMnIk aKTVssHkgN ikrKikthnk qviyh

          Consensus         --LT-RER-V L-LL--G--N -EIA-EL-IS -KTV--H--N -M-KL----- ---V-
Helix-Turn-Helix                                  ΦAXXΦXΦX XXXIXXΦ
                                                   G         V
                                                             L
```

FIG. 4. UhpA-LuxR family of regulatory proteins. Selected members of a set of proteins identified by Henikoff et al. (22), with the addition of *E. coli* RcsB, *E. coli* RcsA, and others identified after a search of the NBRF protein data bank with a profile from the set (see Materials and Methods). RcsA sequence starts with amino acid 136. The single-letter code for amino acids is used. Asterisks above the alignment indicate residues which are identical in all or all but one or two members of the set. Majority conserved amino acids are capitalized and shown on the consensus line. The helix-turn-helix line shows residues expected for proteins with a helix-turn-helix motif (36). Φ stands for hydrophobic amino acids (Ile, Leu, Val, and Met); X indicates unspecified. Boldfacing indicates the site of *rcsA62* (RcsA*) that changes M to V at position 10. Sequences are from the NBRF data bank. References for the sequences are NarL (35, 45), GerE (13), BvgA (2), DegU (23), MalT (8), FixJ (15), RcsB (47), UhpA (19), sigma 70 (Sig70) (6), and sigma H (SigH) (18).

the T7 promoter-*rcsA* plasmid pVS110. The sequence of *rcsA* in this vector was confirmed by double-stranded sequencing. After pVS110 was transformed into BL21(DE3) (pLysS), induction with IPTG led to the accumulation of a protein of about 27,000 Da (Fig. 5A, lane 2), consistent with the previously observed and predicted size of RcsA. The majority of the protein was found in the particulate fraction of cell extracts (Fig. 5A, lane 1) after cells were ruptured by lysozyme treatment and sonication. This protein was purified as described in Materials and Methods. The amino acid composition of the purified protein agreed well with that predicted from the DNA sequence (Table 2). Western blots with antibody raised to the purified protein (Fig. 5B) showed the presence of RcsA after induction in cells carrying the multicopy *rcsA* plasmid (lane 2) but not in uninduced cells (lane 3). However, no RcsA was detected in cells carrying only a single copy of *rcsA* in a *lon* mutant (Fig. 5B, lane 4). There was no cross-reactivity with RcsB (data not shown).

Degradation of RcsA by Lon protease. We have shown previously that RcsA is degraded in a Lon-dependent fashion (49). This result was confirmed for RcsA synthesized from a multicopy plasmid by using pulse-labeling and immunoprecipitation to detect RcsA (Fig. 6). We estimate the

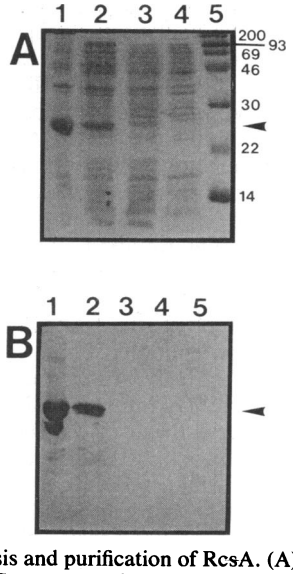

FIG. 5. Synthesis and purification of RcsA. (A) Coomassie blue-stained gel of SDS extracts of cells containing pVS110 (RcsA$^+$) before (lane 3) and after (lane 2) induction with IPTG. Lane 1, Pellet of the induced extract after low-speed centrifugation showing that most of the RcsA is in inclusion bodies; lane 4, extract of *lon* mutant (JT4000); lane 5, molecular mass markers (Amersham rainbow markers) in kilodaltons. (B) Western blot made from second gel run with the same samples as in panel A. Negatively absorbed anti-RcsA antiserum was used. Arrowheads point to position of RcsA protein.

TABLE 2. Amino acid composition of RcsA

Amino acid	Composition (mol%)	
	Determined from amino acid analysis[a]	Predicted from sequence
Asx	11.0	13.0
Glx	7.1	6.8
Ser	8.5	9.2
Gly	4.3	3.9
His	3.3	2.9
Thr	7.1	7.3
Arg	5.2	5.3
Ala	3.8	3.4
Pro	2.4	1.9
Tyr	1.9	1.9
Val	6.6	6.3
Met	4.3	4.3
Ile	11.9	11.6
Leu	10.9	9.7
Phe	3.8	3.4
Lys	6.6	7.3
Cys	ND[b]	1.5
Trp[c]	0.5	0.5

[a] Duplicate samples of RcsA were hydrolyzed in 6 N HCl at 155°C for 25 and 50 min, and amino acids were analyzed as described under Materials and Methods.

[b] ND, Not determined.

[c] Tryptophan was determined from the absorbance spectrum as described previously (29).

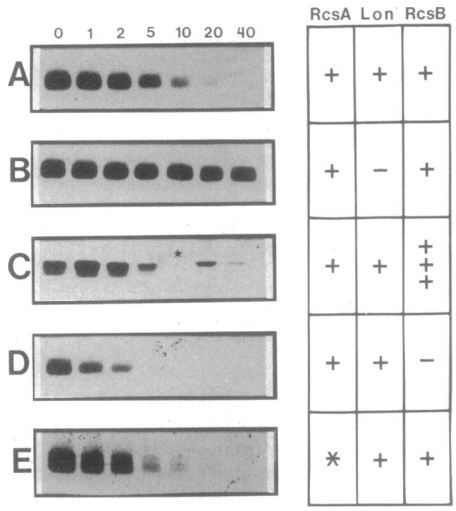

FIG. 6. Turnover of RcsA and RcsA* made from multicopy plasmids. Cells were pulse-labeled with [^{35}S]methionine and chased for the indicated times (in minutes). RcsA was immunoprecipitated from the samples and subjected to SDS-PAGE, followed by autoradiography (described in detail in Materials and Methods). Row A, pATC400 (*rcsA*$^+$) in *lon*$^+$ *rcsB*$^+$ host (SG1132); row B, pATC400 (*rcsA*$^+$) in *lon-146* host (SG23004); row C, pATC400 (*rcsA*$^+$) and pVS102 (*rcsB*$^+$) in *lon*$^+$ *rcsB*$^+$ host (SG1132); row D, pATC400 (*rcsA*$^+$) in *lon*$^+$ *rcsB62* host (SG23002); row E, pVS104 (*rcsA62* [RcsA*]) in *lon*$^+$ *rcsB*$^+$ host (SG1132). The star indicates a lost sample in row C at the 10-min chase time point. Genotypes of strains are indicated to the right. Symbols: +, wild type; −, null mutation in gene; ‡, multicopy plasmid carrying wild-type gene; *, *rcsA62* (RcsA*).

half-life of RcsA synthesized from the plasmid in these wild-type cells to be about 3 min (Fig. 6A); the half-life is about 30 min in a host with a *lon* mutation (Fig. 6B). Despite exposure times 30-fold longer, we have been unable to detect wild-type RcsA made from a single chromosomal copy of the gene in a *lon*$^+$ host (Fig. 7A). Chromosomally encoded

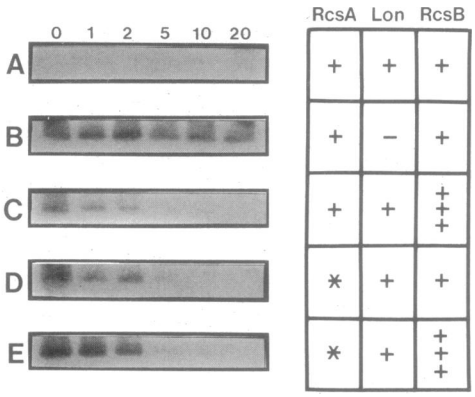

FIG. 7. Turnover of RcsA and RcsA*: single-copy-encoded RcsA. Samples were prepared as described in the legend to Fig. 6 except gels were exposed to film for approximately 30 times longer (approximately 3 weeks) to allow adequate visualization of the RcsA bands. Samples were (A) wild type (SG1132), (B) *lon* mutant (SG23004), (C) wild type with a plasmid encoding RcsB (pJB100 in SG1132), (D) *rcsA62* mutant (SG22029), and (E) *rcsA62* mutant with a plasmid encoding RcsB (pJB100 in SG22029). Symbols to the right are as defined in the legend to Fig. 6.

protein is detected in *lon* mutant hosts, but only after at least 10 days of exposure (Fig. 7B). This suggests that in a *lon*$^+$ cell, either the degradation of single-copy RcsA is so rapid (half-life of less than 1 min) that even for short pulses the accumulation of the protein is minimal, or less RcsA is synthesized. The latter suggestion is not supported by the observation that relative incorporation of label into RcsA during a 1-min pulse in *lon*$^+$ and *lon* mutant cells is not significantly different (Fig. 6A and B, 0 time points). In addition, preliminary results of experiments with *rcs*::*lac* fusions do not indicate any effect of *lon* on RcsA synthesis (data not shown).

In the immunoprecipitations of RcsA and RcsA* made from multicopy plasmids, two or three bands were often detected. All of these bands appear to be RcsA, since they were all degraded in a Lon-dependent manner and all appeared only when RcsA was overproduced. All bands were considered when estimating the half-life of RcsA.

RcsA*. Dominant mutations in *rcsA* which increase capsule expression in *lon*$^+$ hosts (termed RcsA*) have been isolated previously (21). *rcsA62*, a strong RcsA* allele, was originally isolated in an *rcsA*$^+$ lambda transducing phage (Materials and Methods).

rcsA62 was subcloned from lambda to pBR325 and into M13mp18 (Materials and Methods). The DNA sequence of *rcsA62* revealed a single base change resulting in replacement of Met-145 by valine. The methionine at this position is conserved between *E. coli*, *K. pneumoniae* (Fig. 3), *Erwinia stewartii*, and *Erwinia amylovora* (3, 9, 36a). Interestingly, the change from methionine to valine increases the similarity of RcsA to the consensus sequence for the conserved region of the UhpA/LuxR family shown in Fig. 4.

The stability of RcsA* was compared with that of RcsA. When both proteins are overproduced from multicopy plasmids, the half-life of RcsA* (Fig. 6E) was indistinguishable from that of RcsA (Fig. 6A). However, comparison of RcsA* and RcsA made from either single chromosomal gene revealed a dramatic difference (Fig. 7A and D). While RcsA was not detectable in *lon*$^+$ hosts, even after 3 weeks of exposure of the autoradiograms, the RcsA* protein was visible. RcsA in multiple copy and RcsA* in single or multiple copy all have half-lives of about 3 min. If, as we believe, the half-life of single-copy RcsA is less than 1 min, then the stability of the mutant RcsA* is increased compared with RcsA$^+$. Consistent with this model, the synthesis levels of RcsA and RcsA* are not significantly different, as seen in the zero time points of Fig. 6A and E. The increased stability of RcsA* relative to RcsA is only apparent when synthesized from a single copy of the gene, suggesting that RcsA* stability may be affected by other cellular factors which are available in only limited amounts.

RcsA stability is affected by RcsB. One reasonable candidate for a factor which may interact with RcsA to affect its stability is RcsB. Excess RcsA made from the plasmid was slightly more unstable in an *rcsB* mutant host (Fig. 6A and D) and was significantly stabilized in cells that overproduce RcsB from a compatible *rcsB*$^+$ plasmid (Fig. 6C). In addition, in Lon$^+$ cells that carry the *rcsB*$^+$ plasmid, RcsA made from a single chromosomal gene was detectable (Fig. 7C), consistent with either an increase in RcsA synthesis or a significant increase in RcsA stability. Preliminary data from *rcsA*::*lac* fusions indicates that *rcsB* does not affect the synthesis of RcsA. In addition, synthesis levels of RcsA measured from the pulsed samples are not dramatically affected by different levels of RcsB (zero time points in Fig. 6A, C, and D).

The increased stability of RcsA* compared with RcsA$^+$ made from a single-copy chromosomal gene may reflect increased interaction with RcsB. Since RcsA* made from a single chromosomal gene was not significantly stabilized by a multicopy *rcsB*$^+$ plasmid (Fig. 7E), we conclude that RcsA* is saturated with RcsB at the intracellular levels made from a single-copy *rcsB* gene.

Genetic evidence for RcsA-RcsB interaction. We had observed in previous work that *rcsB* mutations which remove the C-terminal portion of the protein (RcsBΔ) prevent capsule synthesis when present on high-copy-number plasmids in *lon* mutant cells (5). We had hypothesized that the truncated form of RcsB made mixed multimers with wild-type RcsB protein; the mixed multimers would be inactive for capsule synthesis. An alternative explanation is suggested by the finding that RcsA and RcsB interact; the formation of mixed multimers of RcsA and RcsBΔ would also be expected to turn down capsule synthesis. To test this possibility, we introduced a compatible RcsA$^+$ plasmid into *lon cps*::*lac* hosts and assayed β-galactosidase at 37°C to see whether increased levels of RcsA would overcome the inhibitory effect of RcsBΔ produced from the *rcsB62*::Δkan plasmid pJB102. While *lon* hosts expressed *cps-lac* fusions at high levels (60 U of β-galactosidase), the *rcsB62*::Δkan plasmid reduced expression to basal level (2 U). Units are as defined by Miller (32b). Introduction of the compatible *rcsA*$^+$ plasmid increased synthesis to 48 U. This result suggests that RcsBΔ interferes with capsule synthesis by interacting with RcsA and preventing it from stimulating capsule expression. If this is the case, then the C-terminal region of RcsB, which is missing in the negatively complementing plasmid, is not necessary for the RcsA-RcsB interaction. The missing region includes the sequence homologous to the UhpA-LuxR family shown in Fig. 4.

DISCUSSION

RcsA is a positive regulator of capsule synthesis in *E. coli*, *Erwinia* spp., and probably many other gram-negative bacteria. Sequence analysis of the protein shows that it is highly basic, consistent with a possible role in interacting with nucleic acids. Previous data had shown that the activity of the protein has been well conserved between these species (50); this conservation is reflected at the amino acid level as well (3, 9, 36a; this work). Stretches of amino acids at the amino terminus and at the C-terminus are exactly conserved between the proteins from *K. pneumoniae* and *E. coli*, as well as in two *Erwinia* homologs. Since the proteins from these different species can substitute for each other, the target(s) for their action must also be conserved.

Both RcsA and the other positive regulator for capsule synthesis, RcsB, are members of a family of regulatory proteins, defined as the LuxR group by Henikoff et al. (22) and slightly redefined here by us with reference to UhpA. The domain conserved within this family (Fig. 4) is always found at the C-terminus of the protein. In one case, GerE, the conserved domain constitutes virtually the entire protein (13). One member of the family, MalT, is known to stimulate transcription at maltose promoters; this stimulation is dependent upon sigma 70 (37). Sigma 70 itself and the alternative *B. subtilis* sigma factor, sigma H, are less conserved members of the family. The conserved region may encode both a helix-turn-helix motif and a second domain that might be involved in protein-protein interactions.

We were unable to detect RcsA made from a single-copy gene unless either Lon was absent or excess RcsB was present. Mutant RcsA* was detected when the gene was present in single copy. When RcsA was made from a multicopy plasmid, *lon* affected the turnover dramatically but did not significantly change the synthesis rate (Fig. 6). We believe the same was true when RcsA was made from a single chromosomal gene. If so, the RcsA synthesized during the 1-min pulse must have been degraded so rapidly in Lon$^+$ cells that it could not be detected. Similarly, our data do not indicate that excess RcsB changed the synthesis levels of RcsA or that the mutant RcsA* was synthesized at a higher rate. Rather, we conclude that excess RcsB and the RcsA* mutation each allowed increased RcsB-RcsA interaction, which in turn partially protected RcsA from degradation. The stabilizing effect of RcsB on RcsA was confirmed by examining the turnover of RcsA encoded by a multicopy plasmid. RcsA had a longer half-life in the presence of excess RcsB and a shorter half-life in the absence of RcsB.

The second type of data consistent with an interaction between RcsB and RcsA is that overproduction of truncated RcsB decreases *cps* expression. This suggests that the truncated protein interferes with RcsA function by binding RcsA in an inactive complex. Consistent with this interpretation, the inhibitory effect of truncated RcsB can be relieved by providing excess RcsA. Also, we had previously found that in an *rcsC137* host, excess truncated RcsB reduces capsule expression to the RcsA-independent value (20 to 40 U of β-galactosidase from a *cps-lac* fusion) rather than the RcsB-independent value (1 to 2 U) (5), again suggesting that truncated RcsB interferes with RcsA function, most likely by binding it in an inactive complex. The ability of RcsA and RcsB to interact and the requirement for both proteins to stimulate capsule expression suggest that RcsA and RcsB form a complex to activate *cps* transcription.

These results are similar to the observations of Jones and Holland (27) for SulA, the unstable division inhibitor of *E. coli*. They showed that SulA stability was modulated by the availability of FtsZ. The inhibitory effects of SulA on cell division can be overcome by increasing the amount of FtsZ in the cell, in parallel with our observations with RcsA and RcsB (30). The results with truncated RcsB proteins suggest that the C-terminal conserved region discussed above cannot be responsible for RcsA-RcsB interaction, since the truncated protein, missing both conserved domains, is unable to stimulate synthesis but is able to interact with RcsA to prevent capsule synthesis.

If RcsA interacts with RcsB, we can imagine at least two steps at which it might work. Since RcsB is probably activated to promote *cps* transcription by RcsC-dependent phosphorylation, RcsA might either stimulate phosphorylation or prevent dephosphorylation of RcsB. Alternatively, RcsA interaction with RcsB might directly promote DNA binding by RcsB or activation of transcription of RcsB bound at the promoter. For either mechanism, one would expect that the RcsA requirement for *cps* expression could be bypassed, which is the case in either an *rcsC137* host or a host carrying *rcsB*$^+$ on a multicopy plasmid. The finding that RcsA as well as RcsB contains a possible helix-turn-helix motif leads us to favor a model in which RcsA and RcsB both bind DNA and RcsA increases the activity of RcsB either by aiding the binding of RcsB or by promoting an active conformation of RcsB.

RcsA* increases *cps* expression in *lon*$^+$ cells. This result can be explained by the increased availability of RcsA* resulting from its increased stability. We assume that since multicopy RcsA* is turned over in a Lon-dependent reaction

at a rate similar to that for RcsA$^+$ protein, the intrinsic stabilities of RcsA$^+$ and RcsA* are very similar. The apparent increased stability of RcsA* over RcsA$^+$ when made from single-copy genes may reflect a more stable association of RcsA* with RcsB, which we have shown affects the degradation of RcsA and RcsA*. As we would predict, single-copy RcsA* is no longer detectable in an *rcsB* mutant (data not shown).

The coupling of an unstable regulatory protein such as RcsA to an effector like RcsB is thus far a unique pattern for gene regulation. It will be interesting to see whether any other members of the UhpA-LuxR family make use of such an auxiliary factor.

ACKNOWLEDGMENTS

We thank J. Pumphrey and S. Rudikoff for microsequencing of the RcsA-LacZ fusion protein and D. Coplin and C. Whitfield for sharing their results on *rcsA* sequencing before publication.

REFERENCES

1. **Allen, P., C. A. Hart, and J. R. Saunders.** 1987. Isolation from *Klebsiella* and characterization of two *rcs* genes that activate colanic acid capsular biosynthesis in *Escherichia coli*. J. Gen. Microbiol. **133**:331–340.
2. **Arico, B., J. F. Miller, C. Roy, S. Stibitz, D. Monack, S. Falkow, R. Gross, and R. Rappuoli.** 1989. Sequences required for expression of *Bordetella pertussis* virulence factors share homology with prokaryotic signal transduction proteins. Proc. Natl. Acad. Sci. USA **86**:6671–6675.
3. **Bernhard, F., K. Poetter, K. Geider, and D. L. Coplin.** 1990. The *rcsA* gene from *Erwinia amylovora*: identification, nucleotide sequence, and regulation of exopolysaccharide biosynthesis. Mol. Plant-Microbe Interact. **3**:429–437.
4. **Bidlingmeyer, B. A., S. A. Cohen, and T. L. Tarvin.** 1984. Rapid analysis of amino acids using pre-column derivatization. J. Chromatogr. **336**:93–104.
5. **Brill, J. A., C. Quinlan-Walshe, and S. Gottesman.** 1988. Fine-structure mapping and identification of two regulators of capsule synthesis in *Escherichia coli* K-12. J. Bacteriol. **170**:2599–2611.
6. **Burton, Z., R. R. Burgess, J. Lin, D. Moore, S. Holder, and C. A. Gross.** 1981. The nucleotide sequence of the cloned *rpoD* gene for the RNA polymerase sigma subunit from *E. coli* K12. Nucleic Acids Res. **9**:2889–2903.
7. **Chatterjee, A., W. Chun, and A. K. Chatterjee.** 1990. Isolation and characterization of an *rcsA*-like gene of *Erwinia amylovora* that activates extracellular polysaccharide production in *Erwinia* species, *Escherichia coli*, and *Salmonella typhimurium*. Mol. Plant-Microbe Interact. **3**:144–148.
8. **Cole, S. T., and O. Raibaud.** 1986. The nucleotide sequence of the *malT* gene encoding the positive regulator of the *Escherichia coli* maltose regulon. Gene **42**:201–208.
9. **Coleman, M., R. Pearce, E. Hitchin, F. Busfield, J. W. Mansfield, and I. S. Roberts.** 1990. Molecular cloning, expression and nucleotide sequence of the *rcsA* gene of *Erwinia amylovora*, encoding a positive regulator of capsule expression: evidence for a family of related capsule activator proteins. J. Gen. Microbiol. **136**:1799–1806.
10. **Coplin, D. L., and D. Cook.** 1990. Molecular genetics of extracellular polysaccharide biosynthesis in vascular phytopathogenic bacteria. Mol. Plant-Microbe Interact. **3**:271–279.
11. **Coplin, D. L., R. D. Frederick, D. R. Majerczak, and E. S. Haas.** 1986. Molecular cloning of virulence genes from *Erwinia stewartii*. J. Bacteriol. **168**:619–623.
12. **Coplin, D. L., and D. R. Majerczak.** 1990. Extracellular polysaccharide genes in *Erwinia stewartii*: directed mutagenesis and complementation analysis. Mol. Plant-Microbe Interact. **3**:286–292.
13. **Cutting, S., and J. Mandelstam.** 1986. The nucleotide sequence and the transcription during sporulation of the *gerE* gene of *Bacillus subtilis*. J. Gen. Microbiol. **132**:3013–3024.
14. **Dale, R. M. K., 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 mitochondrial 18s rDNA. Plasmid **13**:31–40.
15. **David, M., M. L. Daveran, J. Batut, A. Dedieu, O. Domergue, J. Ghai, C. Hertig, P. Biostard, and D. Kahn.** 1988. Cascade regulation of *nif* gene expression in *Rhizobium meliloti*. Cell **54**:671–683.
16. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12**:387–395.
17. **Dolph, P. J., D. Majerczak, and D. L. Coplin.** 1988. Characterization of a gene cluster for exopolysaccharide biosynthesis and virulence in *Erwinia stewartii*. J. Bacteriol. **170**:865–871.
18. **Dubnau, E., J. Weir, G. Nair, L. Carter III, C. Moran, Jr., and I. Smith.** 1988. *Bacillus* sporulation gene *spo0H* codes for σ^{30} (σ^H). J. Bacteriol. **170**:1054–1062.
19. **Friedrich, M. J., and R. J. Kadner.** 1987. Nucleotide sequence of the *uhp* region of *Escherichia coli*. J. Bacteriol. **169**:3556–3563.
20. **George, J., M. Castellazzi, and G. Buttin.** 1975. Prophage induction and cell division in *E. coli*. III. Mutations *sfiA* and *sfiB* restore division in *tif* and *lon* strains and permit the mutator properties of *tif*. Mol. Gen. Genet. **140**:309–332.

20a. **Gottesman, S.** Unpublished data.

21. **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. Bacteriol. **162**:1111–1119.
22. **Henikoff, J., C. Wallace, and J. P. Brown.** 1990. Finding protein similarities with nucleotide sequence databases. Methods Enzymol. **183**:111–132.
23. **Henner, D. J., M. Yang, and E. Ferrari.** 1988. Localization of *Bacillus subtilis sacU*(Hy) mutations to two linked genes with similarities to the conserved procaryotic family of two-component signalling systems. J. Bacteriol. **170**:5102–5109.
24. **Huisman, O., and R. D'Ari.** 1981. An inducible DNA-replication-cell division coupling mechanism in *E. coli*. Nature (London) **290**:797–799.
25. **Huisman, O., R. D'Ari, and S. Gottesman.** 1984. Cell division control in *Escherichia coli*: specific induction of the SOS SfiA protein is sufficient to block septation. Proc. Natl. Acad. Sci. USA **81**:4490–4494.
26. **Hunkapiller, M. W., E. Lujan, F. Ostander, and L. E. Hood.** 1983. Isolation of microgram quantities of proteins from polyacrylamide gels for amino acid sequence analysis. Methods Enzymol. **91**:227–236.
27. **Jones, C. A., and I. B. Holland.** 1985. Role of the SfiB (FtsZ) protein in division inhibition during the SOS response in *E. coli*: FtsZ stabilizes the inhibitor SfiA in maxicells. Proc. Natl. Acad. Sci. USA **82**:6045–6049.
28. **Katayama, Y., S. Gottesman, J. Pumphrey, S. Rudikoff, W. P. Clark, and M. R. Maurizi.** 1988. The two-component, ATP-dependent Clp protease of *Escherichia coli*. Purification, cloning and mutational analysis of the ATP-binding component. J. Biol. Chem. **263**:15226–15236.
29. **Levine, R. L., and M. M. Federici.** 1982. Quantitation of aromatic residues in proteins: model compounds for second-derivative spectroscopy. Biochemistry **21**:2600–2606.
30. **Lutkenhaus, J., B. Sanjanwala, and M. Lowe.** 1986. Overproduction of FtsZ suppresses sensitivity of *lon* mutants to division inhibition. J. Bacteriol. **166**:756–762.
31. **Markovitz, A.** 1977. Genetics and regulation of bacterial capsular polysaccharide biosynthesis and radiation sensitivity, p. 415–462. *In* I. Sutherland (ed.), Surface carbohydrates of the prokaryotic cell. Academic Press, Inc. (London), Ltd., London.
32. **Maurizi, M. R., P. Trisler, and S. Gottesman.** 1985. Insertional mutagenesis of the *lon* gene in *Escherichia coli*: *lon* is dispensable. J. Bacteriol. **164**:1124–1135.

32a. **McCallum, K. L., and C. Whitfield.** 1991. The *rcsA* gene of *Klebsiella pneumoniae* O1:K20 is involved in expression of the serotype-specific K (capsular) antigen. Infect. Immun. **59**:494–502.

32b. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Mizusawa, S., D. Court, and S. Gottesman.** 1983. Transcription of the *sulA* gene and repression by LexA. J. Mol. Biol. **171**:337–343.
34. **Mizusawa, S., and S. Gottesman.** 1983. Protein degradation in *Escherichia coli*: the *lon* gene controls the stability of the SulA protein. Proc. Natl. Acad. Sci. USA **80**:358–362.
35. **Nohno, T., S. Noji, S. Taniguchi, and T. Saito.** 1989. The *narX* and *narL* genes encoding the nitrate-sensing regulators of *Escherichia coli* are homologous to a family of prokaryotic two-component regulatory genes. Nucleic Acids Res. **17**:2947–2957.
36. **Pabo, C. O., and R. T. Sauer.** 1984. Protein-DNA recognition. Annu. Rev. Biochem. **53**:293–321.
36a. **Poetter, K., and D. Coplin.** Personal communication.
37. **Richet, E., and O. Raibaud.** 1989. MalT, the regulatory protein of the *Escherichia coli* maltose system, is an ATP-dependent transcriptional activator. EMBO J. **8**:981–987.
38. **Rosenberg, A. H., B. N. Lade, D. Chui, S. Lin, J. J. Dunn, and F. W. Studier.** 1987. Vectors for selective expression of cloned DNAs by T7 RNA polymerase. Gene **56**:125–135.
39. **Rudikoff, S., and J. G. Pumphrey.** 1986. Functional antibody lacking a variable-region disulfide bridge. Proc. Natl. Acad. Sci. USA **83**:7875–7878.
40. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239**:487–491.
41. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
42. **Schoemaker, J. M., R. C. Gayda, and A. Markovitz.** 1984. Regulation of cell division in *Escherichia coli*: SOS induction and cellular location of the SulA protein, a key to *lon*-associated filamentation and death. J. Bacteriol. **158**:551–561.
43. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
44. **Simons, R. W., F. Houman, and N. Kleckner.** 1987. Improved single and multicopy *lac*-based cloning vectors for protein and operon fusions. Gene **53**:85–96.
45. **Stewart, V., J. Parales, Jr., and S. M. Merkel.** 1989. Structure of genes *narL* and *narX* of the *nar* (nitrate reductase) locus in *Escherichia coli* K-12. J. Bacteriol. **171**:2229–2234.
46. **Stock, J. B., A. J. Ninfa, and A. M. Stock.** 1989. Protein phosphorylation and regulation of adaptive responses in bacteria. Microbiol. Rev. **53**:450–490.
46a. **Stout, V.** Unpublished data.
47. **Stout, V., and S. Gottesman.** 1990. RcsB and RcsC: a two-component regulator of capsule synthesis in *Escherichia coli*. J. Bacteriol. **172**:659–669.
48. **Studier, F. W., A. H. Rosenberg, J. J. Dunn, and J. W. Dubendorff.** 1990. Use of T7 RNA polymerase to direct expression of cloned genes. Methods Enzymol. **185**:60–89.
49. **Torres-Cabassa, A. S., and S. Gottesman.** 1987. Capsule synthesis in *Escherichia coli* K-12 is regulated by proteolysis. J. Bacteriol. **169**:981–989.
50. **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.
51. **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.
52. **Trun, N. J., and T. J. Silhavy.** 1989. PrlC, a supressor of signal sequence mutations in *Escherichia coli*, can direct the insertion of the signal sequence into the membrane. J. Mol. Biol. **205**: 665–676.
53. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.
54. **Zinkewich-Peotti, K., and J. M. Fraser.** 1988. New locus for exopolysaccharide overproduction in *Escherichia coli* K-12. J. Bacteriol. **170**:1405–1407.