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The Product of the Pleiotropic *Escherichia coli* Gene *csrA* Modulates Glycogen Biosynthesis via Effects on mRNA Stability

MU YA LIU,[1] HONGHUI YANG,[2] AND TONY ROMEO[1, 2]*

Department of Microbiology and Immunology[1] and Department of Biochemistry and Molecular Biology,[2] University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76107-2699

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The carbon storage regulator gene, *csrA*, modulates the expression of genes in the glycogen biosynthesis and gluconeogenesis pathways in *Escherichia coli* and has been cloned, mapped and sequenced (T. Romeo, M. Gong, M. Y. Liu, and A. M. Brun-Zinkernagel, J. Bacteriol. 175:4744–4755, 1993; T. Romeo and M. Gong, J. Bacteriol. 175:5740–5741, 1993). We have now conducted experiments that begin to elucidate a unique mechanism for *csrA*-mediated regulation. Steady-state levels of *glgC* transcripts, encoding ADP-glucose pyrophosphorylase, were elevated by up to sixfold in a *csrA*::*kanR* mutant and were less than 6.5% of wild-type levels in a strain containing pCSR10 (*csrA*$^+$), as shown by S1 nuclease protection analysis. The rate of chemical decay of these transcripts after adding rifampin to cultures was dramatically reduced by the *csrA*::*kanR* mutation. Deletion studies of a *glgC′-′lacZ* translational fusion demonstrated that the region surrounding the initiation codon was important for *csrA*-mediated regulation and indicated that neither *csrA*-mediated regulation nor stationary phase induction of *glgC* expression originates at the level of transcript initiation. Cell-free (S-200) extracts containing the CsrA gene product potently and specifically inhibited the in vitro transcription-translation of *glg* genes. The deduced amino acid sequence of CsrA was found to contain the KH motif, which characterizes a subset of diverse RNA-binding proteins. The results indicate that CsrA accelerates net 5′-to-3′ degradation of *glg* transcripts, potentially through selective RNA binding.

During the transition from exponential growth into the stationary phase, *Escherichia coli* and many other bacteria convert available carbon into α-1,6-branched α-1,4-D-glucan or glycogen, which subsequently is degraded as an endogenous source of carbon and energy. Glycogen biosynthesis and utilization depend on several structural and regulatory genes (for reviews, see references 27 to 29). In *E. coli*, two adjacent operons, *glgBX* and *glgCAY* contain genes that are essential for glycogen synthesis. The *glgB* gene encodes glycogen branching enzyme (EC 2.4.1.18) (5), *glgC* encodes ADP-glucose pyrophosphorylase (EC 2.7.7.27) (4), and *glgA* encodes glycogen synthase (2.4.1.21) (19). The coding regions of *glgC* and *glgA* overlap by 1 bp, and each gene is preceded by a Shine-Dalgarno sequence indicative of a ribosome-binding site (40). Interestingly, two genes which apparently encode enzymes involved in glycogen degradation are also encoded in this gene cluster, glycogen phosphorylase (EC 2.4.21) is encoded by *glgY* or *glgP* (33, 46), and *glgX* encodes a putative glucanotransferase or hydrolase (33). A third unlinked monocistronic operon consists of the gene *glgS*, which stimulates glycogen synthesis by an unknown mechanism (15).

The expression of the *glg* structural genes in part determines the amount of glycogen that is accumulated by cultures. The expression of the *glgCAY* operon is induced in stationary phase and is positively regulated by cyclic AMP (cAMP)-cAMP receptor protein (CRP) and by ppGpp, which mediate the catabolite repression and stringent response global regulatory systems, respectively (30, 35). The transcription of *glgCAY* depends on σ^{70} RNA polymerase (29) and is not regulated by the alternative sigma factors σ^s (which is the gene product of *rpoS* or *katF* [15]), σ^{54} (29, 35), or σ^{32} (29). The 5′ termini of four stationary-phase-induced transcripts have been mapped within a 0.5-kb noncoding region upstream from *glgC*, further suggesting complex transcriptional control (35). The expression of the *glgBX* operon is also induced in stationary phase but is not influenced by cAMP or ppGpp. Transcription of the *glgS* gene has been shown to involve both cAMP-CRP and σ^s (15).

We recently described the molecular cloning, mapping, and characterization of a pleiotropic gene, *csrA*, which dramatically alters the level of glycogen that is accumulated under a variety of growth conditions and which also affects gluconeogenesis and cell surface properties (31, 32). The *csrA* gene was shown to encode a 61-amino-acid polypeptide which somehow negatively regulates the expression of *glgB*, *glgC*, and *pckA* (encoding the gluconeogenic enzyme phosphoenolpyruvate carboxy kinase [EC 4.1.1.49]). Each of these genes was still induced in the stationary phase in a *csrA*::*kanR* insertion mutant, indicating that *csrA*-mediated regulation is superimposed on the growth-phase regulation. The expression of *glgC* was strongly regulated via *csrA*; 10-fold higher levels of ADP-glucose pyrophosphorylase were present in a *csrA*::*kanR* insertion mutant. The effects of *csrA* on glycogen synthesis were mediated independently of the catabolite repression and stringent response systems, and it was suggested that *csrA* may encode a component of a novel global regulatory system. The present study explores the possible mechanism of *csrA*-mediated regulation and suggests that the CsrA gene product is a factor which controls messenger RNA stability.

(Some of the experiments described herein were conducted in partial fulfillment of the Master of Science Degree by H. Yang at the University of North Texas Health Science Center at Fort Worth.)

MATERIALS AND METHODS

Chemicals and reagents. Radiolabeled [γ-^{32}P]ATP, α-^{35}S-dATP, and translation grade [^{35}S]methionine were purchased from Dupont NEN (Wilmington, Del.). Rifampin was purchased from Sigma Chemical Co. (St. Louis, Mo.). The CRP, ppGpp, cAMP, S1 nuclease, and enzymes for DNA manipulation were

* Corresponding author. Phone: (817) 735-2121. Fax: (817) 735-2118.

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description	Source and/or reference
E. coli K-12		
BW3414	Δ*lacU169*	Barry Wanner
TR1-5BW3414	BW3414 *csrA*::*kanR*	32
G6MD3	Hfr *his thi* Str^s Δ(*malA-asd*)	39
HB101	*supE44 hsdS20*(r_B^- m_B^-) *recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1*	3
DH5α	*supE44* Δ*lacU169* (ϕ80 *lacZ*ΔM15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	3
GM161	F^- *thr-1 leuB6 dam-4 thi-1 hsdS1 lacY1 tonA21* λ^- *supE44*	Arnold Revzin (1)
MBM7060	F^- *araC* Am *araD* Δ*lacU169 trp* Am *malB* Am *rpsL relA thi supF* (λp 1048)	41
Plasmids		
pUC19	Cloning vector, high copy number, Amp^r	44
pOP12	Contains *asd* and *glgBXCA* in pBR322, Tet^r	23
pPR1	0.5 kb of *glgC* flanking DNA in pUC19, Amp^r	35
pPR2	*glgC* and *glgA* in pUC19, Amp^r	35
pPR2b	Same as pPR2 except for insert orientation, Amp^r	This study
pMLB1034	Vector for making *′lacZ* translational fusions, Amp^r	41
pCSR10	*csrA* subcloned into pUC19, Amp^r	32
pCZ3-3	Φ*glgC′-′lacZ* in pMLB1034, Amp^r	30
pCV1	Vector for making Φ*glgC′-′lacZ* deletions, Amp^r	This study
pΔCZ	Prefix designation for clones with nested upstream deletions from Φ*glgC′-′lacZ*, Amp^r	This study
pTΔCZ	Prefix designation for five Φ*glgC′-′lacZ* deletion clones with an upstream *trpA* terminator, Amp^r	This study
pCΔZ	Prefix designation for clones with *glgC* coding region deletions from Φ*glgC′-′lacZ*, Amp^r	This study
pΔCΔZ509	Φ*glgC′-′lacZ* containing 50 bp of *glgC* upstream flanking DNA and 9 bp of coding DNA, Amp^r	This study
pMLC1	Contains the *lacZ* promoter and part of coding region from pUC19 cloned into pMLB1034, Amp^r	This study

from the sources previously indicated (35). Polynucleotide kinase, Sequenase 2.0, DNA sequencing reagents, and *Bal* 31 exonuclease were from U.S. Biochemical Corp. (Cleveland, Ohio). Moloney murine leukemia virus reverse transcriptase was from Promega (Madison, Wis.). Protein and DNA molecular weight standards were purchased from Bethesda Research Laboratories (Gaithersburg, Md.).

Bacterial strains and plasmids. Table 1 lists the strains and plasmids that were used in this study, their sources, and relevant genotypes.

Growth conditions. Kornberg medium (1.1% K_2HPO_4, 0.85% KH_2PO_4, 0.6% yeast extract, 0.5% glucose [35]) was used to grow strains for all *glg* gene expression studies, and LB medium (1% tryptone, 1% NaCl, 0.5% yeast extract, 0.2% glucose; pH 7.4 [22]) was used for routine laboratory cultures. Liquid cultures were grown at 37°C with gyratory shaking (250 rpm). For growth curve experiments, cultures were inoculated with 1 volume of an overnight culture per 400 volumes of fresh medium. Solid Kornberg medium containing 1% glucose was routinely used to grow colonies for semiquantitative staining of glycogen with iodine vapor (33). Antibiotics were used at the following concentrations (in micrograms per milliliter): ampicillin, 100; tetracycline, 10; kanamycin, 100; and rifampin, 200.

Molecular biology and nucleotide sequencing. Standard procedures were used for isolation of plasmid DNA and restriction fragments, restriction mapping, transformation, and molecular cloning, as previously described (33, 35). Dideoxynucleotide sequencing (37) was performed using the Sequenase version 2.0 kit under the conditions described by the manufacturer (U.S. Biochemical Corp.). For sequencing plasmid DNA containing the upstream *glgC′-′lacZ* deletions, the pBR322 *Eco*RI Clockwise Primer (Bethesda Research Laboratories) was used; for sequencing the *glgC′-′lacZ* fusions that had deletions in the *glgC* coding region, a primer that anneals within *lacZ* (GATGTGCTGCAAGGC GATTAAGTTGGGTAACG) was used.

Transcript mapping and stability studies. The appropriate conditions for quantitative S1 nuclease protection analysis of chromosomally encoded *glgC* transcripts were previously determined and described in detail, including RNA isolation, hybridization and S1 nuclease reactions, resolution of protected fragments on 4% polyacrylamide gels containing 6 M urea, and autoradiography (35). In experiments measuring the effect of *csrA* on steady-state levels of *glgC* transcripts, the RNA was purified beyond the standard procedure by an additional extraction with phenol and was dissolved in GT solution (4.0 M guanidinium isothiocyanate, 0.1 M Tris [pH 7.5], 1% β-mercaptoethanol) and centrifuged through a 5.7 M CsCl cushion (3). For the RNA stability studies, exponentially growing cultures were treated with rifampin to inhibit the initiation of transcription (38) and were sampled at 2-min intervals. The cells were harvested at 14,000 rpm in a microcentrifuge and frozen in solid CO_2-ethanol, with no more than 2 min allowed to elapse between sampling and freezing.

The rRNA species present in each RNA preparation were examined by formaldehyde agarose gel electrophoresis (20) to assess the general quality of the RNA. In order to ensure that probe DNA was free from nicks, the probes were examined by denaturing polyacrylamide gel electrophoresis. To ensure that the protected (S1 nuclease-resistant) products were dependent on *glgC* expression, RNA from the *glg* deletion strain G6MD3 was hybridized to probes. The probe that was used for S1 mapping of *glgC* transcripts in steady-state RNA analyses was a previously described uniquely labeled 5′-^{32}P-labeled *Bam*HI-*Bgl*I restriction fragment of pPR2 (35). The RNA stability studies used a uniquely labeled 5′-^{32}P-labeled *Bam*HI-*Hin*cII fragment of pPR2b, which is 70 bp longer than the *Bam*HI-*Bgl*I fragment. For all S1 mapping experiments, labeled probe was hybridized to 50 μg of total RNA at a ratio of greater than 100-fold excess relative to the *glgC* mRNA. The protected fragments which were generated within each reaction were applied to a single well for electrophoretic analysis. Steady-state analysis and mRNA stability experiments were conducted twice.

The labeled fragments that were protected from S1 nuclease digestion were quantified by densitometric analysis of the autoradiograms on a Discovery Series scanning densitometer utilizing RFLPrint version 2.0 software (PDI, Inc., New York). Several exposures of each gel were prepared and scanned to ensure that the data were collected within the linear ranges of the film and the densitometer.

Transcripts encoding the *glgC′-′lacZ* fusion of pTΔCZ40 were mapped by primer extension analysis (3) with the oligodeoxynucleotide primer CCCAGT CACGACGTTGTAAAACG.

Enzyme and protein assays. Total cell protein and β-galactosidase activity were quantified as previously described (32).

S-30 coupled transcription-translation. Experiments to measure the effects of CsrA-containing extracts on the in vitro transcription-translation of plasmid-encoded genes were conducted with S-30 extracts, as previously described (35). S-30 extracts were centrifuged at 4°C for 1 h at 200,000 × *g*, and the supernatant solutions were stored at −80°C to provide S-200 extracts. Proteins were labeled during in vitro synthesis by incorporation of [^{35}S]methionine and denatured, and equal volumes of each reaction were subjected to electrophoresis on 9.5% sodium dodecyl sulfate-polyacrylamide slab gels. Radiolabeled proteins were detected by fluorography using sodium salicylate (8).

Preparation of deletion derivatives of the *glgC′-′lacZ* translational fusion. Plasmid clones containing nested 5′ deletions from an in-frame *glgC′-′lacZ* translational fusion were prepared by first constructing a cloning vector, pCV1. This was accomplished by ligating three restriction fragments together in a single reaction: the 0.6-kb *Eco*RI-*Kpn*I fragment of pCZ3-3 (30), the 35-bp *Eco*RI-*Hin*cII polylinker fragment of pUC19, and the 140-bp *Kpn*I-*Bgl*I fragment from pPR1. The last fragment was made blunt at the *Bgl*I end by using the Klenow fragment prior to ligation (35). The DNA to be inserted into pCV1 was prepared by linearizing pCZ3-3 (grown in GM161) with *Eco*RI and by digestion for various times with *Bal* 31 exonuclease to generate nested deletions. The DNA was then treated with Klenow fragment to generate blunt ends and was digested with *Bcl*I. Fragments of less than 2 kb in length were isolated by electrophoresis through low-melting-point agarose and were ligated into the 5-kb *Bcl*I-*Sma*I fragment of pCV1 (grown in GM161). Approximately 200 of the resulting clones were analyzed by restriction analysis with *Hin*cII, and selected clones were sequenced to determine the precise endpoint of each deletion. Each of these clones was given the prefix designation pΔCZ, which was followed by numbers indicating the extents of *glgC* upstream noncoding DNA (in base pairs) that were present.

In experiments that were designed to block potential read-through transcription from the plasmid vector into the *glgC'-'lacZ* translational fusion, the *trpA* Rho-independent terminator (9) was cloned upstream from the *glgC'-'lacZ* fusion in five of the clones to generate the clones which were designated by the prefix pTΔCZ. For these experiments, a synthetic oligodeoxynucleotide (AAT TCAGCCCGCCTAATGAGCGGGCTTTTTTTTGGATCCG) was annealed with a complementary oligonucleotide to generate a double-stranded fragment containing 5′ overhangs (prepared by Biosynthesis, Inc., Lewisville, Tex.) and was phosphorylated and cloned into the dephosphorylated *Eco*RI sites of the plasmids. Nucleotide sequencing was used to identify clones that contained only a single terminator inserted in the desired orientation.

Plasmid clones containing in-frame deletions within the coding region of the *glgC-lacZ* fusion were obtained by transforming strain MBM7060 with a plasmid containing an out-of-frame *glgC'-'lacZ* translational fusion and selecting for spontaneous in vivo deletions that confer the Lac^+ phenotype, as described for pCZ3-3 isolation (30). The resulting 120 clones were restriction mapped with *Hin*cII and *Bam*HI, and 12 of them were sequenced to determine the extent of the deletions. Each of these clones was given the prefix designation pCΔZ which was followed by the amount of *glgC* coding DNA (in base pairs) that each contained. A clone that contained only 50 bp of *glgC* upstream flanking DNA and 8 bp of coding DNA, pΔCΔZ508, was constructed by subcloning the *Bam*HI-*Ava*II fragment, which was made blunt at the *Ava*II end with the Klenow fragment, from pCΔZ8 into pMLB1034. The control plasmid pMLC1 was constructed by subcloning the 0.2-kb *Bam*HI-*Pvu*II fragment of pUC19 into pMBL1034. pMLC1 contains the *lacZ* promoter region upstream from an in-frame *lacZ* coding region.

Computer-assisted secondary-structure analysis. The secondary structures of KH proteins were analyzed by the method described by Garnier et al. (12) on a Macintosh computer using GeneWorks (Intelligenetics, Inc.). Sliding windows of 17 residues were chosen for the predictions.

RESULTS

Effects of the carbon storage regulator gene of *E. coli*, *csrA*, on steady-state levels of *glgC* transcripts. Previous measurements of the levels of ADP-glucose pyrophosphorylase and *glgC'-'lacZ*-encoded β-galactosidase in $csrA^+$ and *csrA*::*kanR* strains indicated that the gene product of *csrA* (CsrA) strongly regulates *glgC* expression. Therefore, *glgC* was chosen as the model gene for studying the regulatory mechanism of CsrA. The 5′ ends of chromosomally encoded *glgC* transcripts were mapped by S1 nuclease protection analysis, and their relative levels of abundance were determined for four isogenic strains that differed in their *csrA* genotypes (Fig. 1 and 2) (Table 2). The overall pattern of transcription was observed to be identical to that of *E. coli* B and *E. coli* K-12 3000 (35); four transcripts occur within the upstream flanking region of *glgC*, each of which is present in higher levels in early-stationary-phase versus exponential-phase growth. The levels of these transcripts were found to be negatively affected by *csrA*. Strain TR1-5BW3414 (*csrA*::*kanR*) accumulated approximately 4- to 6-fold higher levels of transcripts A, B, and C than did BW3414 ($csrA^+$). The probe that was used to map transcripts in Fig. 1 was not resolved from the fragment protected by transcript D. However, densitometric analysis of data from the experiment shown in Fig. 2 indicated that transcript D was elevated 1.7-fold by the *csrA*::*kanR* mutation.

Transformation of *E. coli* with the *csrA*-encoding plasmid pCSR10 was previously shown to strongly inhibit accumulation of glycogen (32). This plasmid caused a severe decrease in the steady-state levels of the *glgC* transcripts. The relative concentration of the major *glgC* transcript (transcript B) was less than 2% in a pCSR10-containing strain compared with that of an isogenic *csrA*::*kanR* strain and was less than 6.5% of that of a $csrA^+$ strain. Consistently with the previous observation that *csrA* affects the expression of *glgB*, *glgC*, and *pckA* in both the exponential and the stationary phases of growth (32), the *csrA*::*kanR* mutation affected *glgC* transcript levels in both growth phases.

Effects of *csrA* on *glgC* mRNA stability. The increase in levels of *glgC* transcripts in the *csrA*::*kanR* strain could be explained by an increase in the rate of synthesis of these transcripts or by a decrease in the rates of their degradation, i.e., by increased

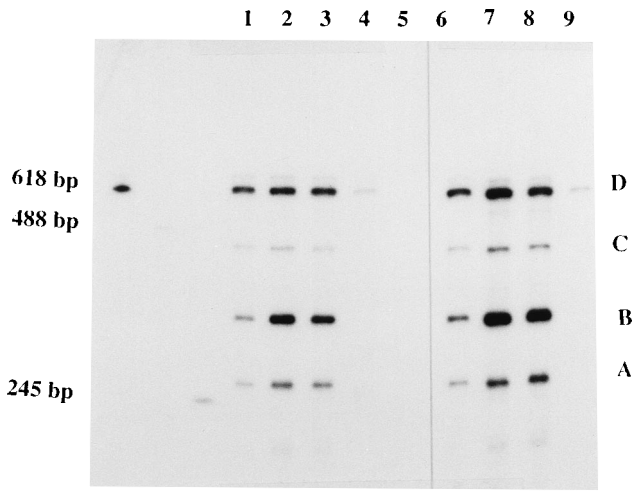

FIG. 1. S1 nuclease protection analysis of *glgC* transcripts from isogenic strains BW3414 ($csrA^+$), TR1-5BW3414 (*csrA*::*kanR*), TR1-5BW3414(pUC19), and TR1-5BW3414(pCSR10) (*csrA* overexpressing). Total RNA was extracted from BW3414 (lanes 1 and 6), TR1-5BW3414 (lanes 2 and 7), TR1-5BW 3414(pUC19) (lanes 3 and 8), TR1-5BW3414(pCSR10) (lanes 4 and 9), and G6MD3 (Δ*glg*) (lane 5), and transcripts were analyzed by S1 nuclease protection as described in Materials and Methods. Samples in lanes 1 through 5 show results from cells harvested in log phase; lanes 6 through 9 were from early-stationary-phase cells. The letters A through D represent protected fragments. In this analysis, the fragment protected by transcript D was not resolved from the full-length *Bam*HI-*Bgl*I probe. The indicated size markers consisted of the intact probe (618 bp) and fragments prepared from the probe by digestion with *Hin*fI (488 bp) or *Ava*II (245 bp).

mRNA stability. Therefore, we examined the effect of *csrA* on the chemical decay of the *glgC* message after the addition of rifampin to exponentially growing cultures of BW3414 ($csrA^+$) or TR1-5 (*csrA*::*kanR*). Steady-state levels of *glgC* transcripts in the pCSR10-containing strain were not sufficient for half-life analysis. RNA isolated from the cultures was subjected to S1 nuclease protection mapping and was quantified by densitometry. As shown in Fig. 2 and 3, the *csrA*::*kanR* mutation had a striking effect on the stability of *glgC* transcripts in these strains. The decay curves of the transcripts exhibited biphasic decay, with a lag period or period of slow decay that was followed by exponential decay. The major transcripts A and B did not exhibit exponential decay within 12 min after the addition of rifampin (approximately one-half of a generation). In two independent experiments, it was observed that >90% of transcripts A and B were degraded in the $csrA^+$ strain before any significant changes were seen in the *csrA*::*kanR* mutant. Figure 2 also shows that some of the full-length probe was protected against S1 digestion. This may have been due to read-through transcription from the upstream *glgBX* operon, since it was not observed when RNA was prepared from a strain from which the *glg* genes had been deleted (Fig. 1). The stability of the full-length probe was less dramatically affected than the *glgC* proximal transcripts by the *csrA*::*kanR* mutation (Fig. 2; densitometry data not shown), which is consistent with the observation that *glgB* expression is less strongly regulated than that of *glgC* (32).

A series of transcripts which appear to be degradation products resulting from endonucleolytic cleavage of the primary transcripts were observed on extended exposure of the autoradiogram (Fig. 2B). The overall patterns of these products were identical in the two strains, although their levels were higher in the *csrA*::*kanR* strain and their rate of decay was greater in the $csrA^+$ strain.

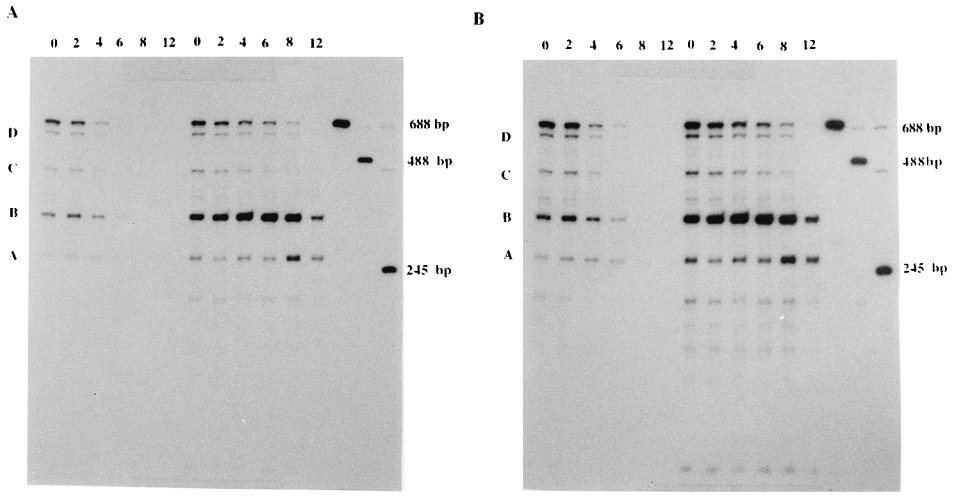

FIG. 2. Stability of *glgC* transcripts in *csrA* and *csrA*::*kanR* strains. Total RNA was isolated and analyzed by S1 nuclease protection from exponential-phase cells (A_{600} = 1.0) of BW3414 (*csrA*$^+$) (lanes 1 through 6) and TR1-5BW3414 (*csrA*::*kanR*) (lanes 7 through 12). The times elapsed (in minutes) after the addition of rifampin are indicated above each lane. Shown are 6 (A) and 24 (B) h of exposure of the same polyacrylamide gel, such that apparent degradation products are allowed to be visualized. Note that in this analysis the fragment protected by transcript D was resolved from the full-length *Bam*HI-*Hin*cII probe. The indicated size markers consisted of the intact probe (688 bp) and fragments prepared from the probe by digestion with *Hin*fI (488 bp) or *Ava*II (245 bp).

CsrA-containing S-200 extracts inhibit the in vitro transcription-translation of *glg* genes. We previously observed that the expression of *glgC* and *glgA* genes in S-30 extracts was activated by the *trans*-acting factors cAMP, CRP, and ppGpp (35). In order to reconstruct *csrA*-mediated regulation in vitro, S-30 extracts were prepared from the *csrA*::*kanR* strain TR1-5BW3414, and high-speed (S-200) extracts were prepared from this strain and from a strain that overexpressed the *csrA* gene, TR1-5BW3414(pCSR10). Extracts from the latter strain contained elevated levels of CsrA protein, as determined by Western blot (immunoblot) analysis using polyclonal antiserum prepared against a synthetic peptide composed of residues 38 to 48 of the deduced amino acid sequence of CsrA, KEVSVHREEIY (data not shown). The S-30 extract contained the nondialyzable cellular factors needed for transcription-translation of plasmid-encoded *glg* genes; S-200 extracts were further centrifuged to remove ribosomes and other large macromolecular complexes. Both kinds of extracts were treated with micrococcal nuclease to degrade endogenous nucleic acids (35), and expression in the S-30 extracts was observed to be completely plasmid dependent (data not shown).

TABLE 2. S1 nuclease protection analysis of the effects of *csrA* on *glgC* transcripts[a]

Strain	Growth phase	Relative levels of transcripts (± range)[b]		
		A	B	C
BW3414	Exponential	6.1 (0.7)	12 (1)	2.0 (0.4)
	Stationary	17 (4)	27 (4)	7.0 (1.7)
TR1-5BW3414	Exponential	38 (6)	69 (6)	6.5 (1.9)
	Stationary	49 (1)	93 (7)	20 (3)
TR1-5BW3414 (pUC19)	Exponential	24 (4)	57 (1)	4.2 (0.4)
	Stationary	56 (7)	93 (8)	19 (1)
TR1-5BW3414 (pCSR10)	Exponential	<1.5	<1.5	<1.5
	Stationary	<1.5	<1.5	<1.5

[a] BW3414 and TR1-5BW3414 are *csrA*$^+$ and *csrA*::*kanR* strains, respectively; pCSR10 contains the wild-type *csrA* gene cloned into pUC19 and results in overexpression of the CsrA protein.

[b] Densitometry data were collected from the experiment shown in Fig. 1 and from an identical, independently conducted experiment. Arbitrary integration units were normalized for each datum set with respect to the highest value of that experiment (designated as 100), and the mean values and ranges were determined.

As shown in Fig. 4, when the S-30 extracts were programmed with pOP12 DNA, expression of *glgB* and *glgC* was clearly observed, as was that of the *asd* gene, which encodes aspartate semialdehyde dehydrogenase, an enzyme that is not involved in glycogen synthesis (35). As previously observed, the expression of *glgC* under these conditions was stimulated upon the addition of the activators cAMP-CRP, and ppGpp, while the expression of *glgB* was not affected. However, the expression of *glgA* was weaker than that previously observed by using S-30 extracts prepared from *csrA*$^+$ strains (35). Addition of the CsrA-containing S-200 extract severely inhibited *glgC* expression in both the basal reactions and in reactions which were activated via cAMP, CRP, and ppGpp. The CsrA-containing extract also inhibited *glgB* expression but appeared to cause little or no inhibition of *glgA* expression, indicating that relative expression of *glgA* versus *glgC* was greater in the presence of CsrA. In contrast to these results, the in vivo expression of a chromosomally encoded *glgA′-′lacZ* fusion exhibited strong negative regulation via *csrA* (43).

The expression of *asd* showed little or no effect of the CsrA-containing extract in the absence of the *glgCA* activators cAMP-CRP and ppGpp. The expression of *asd* was enhanced by the addition of the CsrA-containing extract to reaction mixtures which contained these activators. We suspect that in the latter case, inhibition of *glg* gene expression by CsrA simply relieves competition between *asd* and the *glg* genes for one or more components of the transcription-translation reaction, since no stimulatory effect on *asd* occurred in the absence of the activators, in which case *glg* expression represented a smaller fraction of the total expression. The specific inhibitory effects of CsrA-containing extracts on *glg* gene expression have been reproducibly observed in several experiments and in the two different S-30 extracts which have been tested.

The deduced amino acid sequence of CsrA contains the KH motif, a putative RNA-binding domain. Previously, we were unable to identify genes or proteins that are homologous to

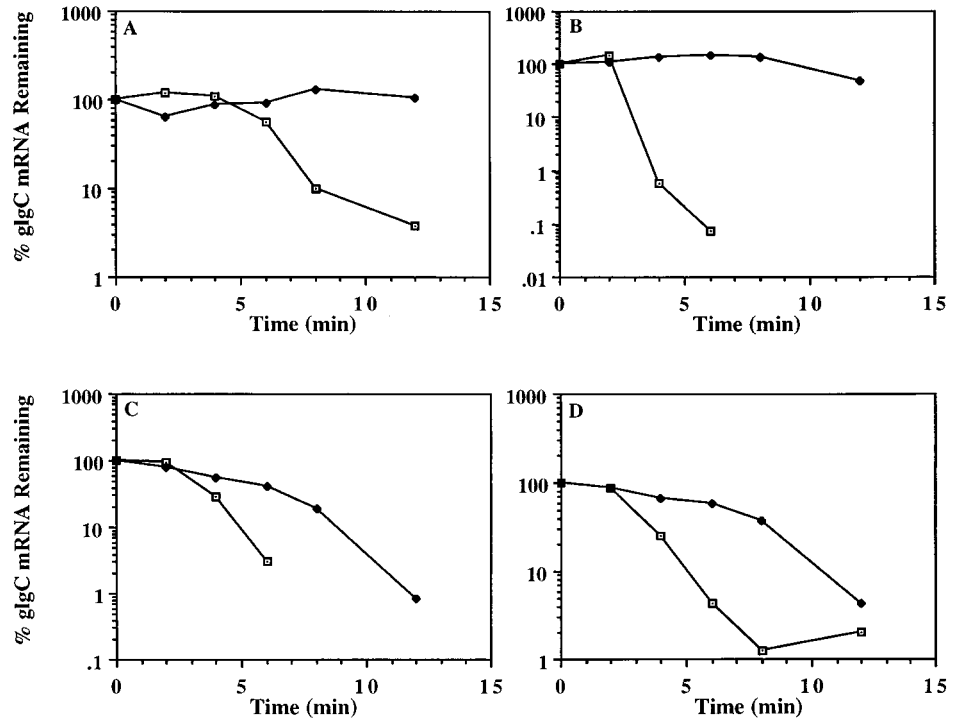

FIG. 3. Densitometric analysis of *glgC* transcript stability. Autoradiograms from the experiment shown in Fig. 2 were analyzed by densitometric scanning, as described in Materials and Methods. The values for a given transcript in each strain were normalized relative to the amount of that transcript at the time of addition of rifampin ($T = 0$ min). (A through D) Results for transcripts A through D, respectively, from strains BW3414 (*csrA*$^+$) (open squares) and TR1-5BW3414 (*csrA*::*kanR*) (closed symbols).

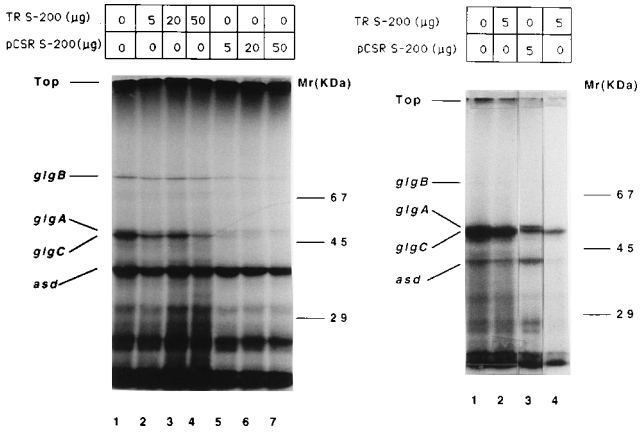

FIG. 4. Effects of S-200 extracts from *csrA*-deficient or *csrA*-overexpressing strains on the expression of pOP12-encoded genes. Transcription-translation reactions (35-μl mixtures) were conducted using an S-30 extract prepared from TR1-5BW3414 (*csrA*::*kanR*) and were analyzed as described in Materials and Methods. The positions of unlabeled standards of glycogen branching enzyme (*glgB*), ADP-glucose pyrophosphorylase (*glgC*), and glycogen synthase (*glgA*) and of bovine serum albumen (67 kDa) ovalbumen (45 kDa), and carbonic anhydrase (29 kDa) were determined by Coomassie blue staining. The quantities of S-200 protein (in micrograms) from strain TR1-5BW3414 (TR) or from TR1-5BW3414(pCSR10) that were added to each reaction mixture prior to starting the reactions with pOP12 plasmid DNA (2 μg) are indicated. The reaction mixtures shown in the right panel contained 2 μg of CRP, 100 μM cAMP, and 250 μM ppGpp; the reaction mixtures in the left panel lacked these factors. Different exposure times were used in the two panels to allow the effects of the CsrA to be readily observed under each condition. Lane 4 of the right panel shows a lighter exposure of lane 2 to allow a better assessment of the effect of the CsrA-containing extract on the relative levels of expression of *glgC* and *glgA*.

csrA or the CsrA gene product (32). However, subsequent manual comparison of the deduced amino acid sequence of CsrA with those of proteins containing the putative RNA-binding KH motif indicated that CsrA is a member of this group of proteins. As shown in Fig. 5, the KH motif is characteristic of a diverse subset of RNA-binding proteins (2, 13, 14, 42). The consensus amino acid sequence of KH is not extremely strict, explaining why previous data base searches were unsuccessful in matching CsrA with other members of the group and why the KH domain was only recently recognized (42). Nevertheless, centrally located in the motif there are two almost invariant residues (GxxG) and there is amino acid conservation over a region of approximately 50 residues (14). Thirty-nine of the amino acid residues of CsrA are compared with 16 other KH domains in Fig. 5. CsrA is also related to the other KH-containing proteins in a region downstream from the 39 residues that are shown. However, this region of similarity is located at variable distances from the rest of the motif among the different KH proteins and is not as well conserved as the region that is shown. Secondary-structure predictions performed by Garnier analysis suggested that the KH domain constitutes a region of conserved secondary structure within this family of proteins (data not shown). The possible secondary structure consists of the central region of KH, which forms a loop (a coil or turn [c/t]), bracketed by two regions of β-sheet structure, followed by another loop, i.e., β-c/t-β-c/t. Inconsistently predicted structures are at the upstream end of this β-c/t-β-c/t region and downstream from it. A previously predicted secondary structure for KH included a helix-loop-helix centered at the GxxG and a complete domain structure of βααββ with loops punctuating the helices and sheets (14).

Expression of *glgC'-'lacZ* translational fusions in *csrA*$^+$ and *csrA*::*kanR* strains. Previously, we had established that the

Consensus	I I f VIG<u>KK</u>GffIR L f VSIf Ef P
E. coli CsrA	VGETLMIGDEVTVTVLGVKGNQVRIGVNAPK EVSVHREE
Chicken vigilin (6)	RTKDLIIEQKFHRTIIGQKGERIREIREKPP EVIINFPD
H. sapiens RP S3	TRTEIIILATRTQNVLGEKGRRIRELTAVVQ KRFGFPEG
E. coli S3	KSIRVTIHTARPGIVIGKKGEDVEKLRKVVA DIAGVPAQ
H. halobium S3	MGMQIVLKAEKPGMVIGKGGKMIRKITTQL EERFDLED
E. coli PNP	RIHTIKINPDKIKDVIGKGGSVIRALTEETG TTIEIED
X. laevis hnRNP (1)	VELRILLQSKNAGAVIGKGGKMIKALRTDY NASVSVPD
(2)	ITTQVTIPKDLAGSIIGKGGQRIKQIRHRSG ASIKIKE
S. cerevisiae MER-1	VTLEIKLNKTQITFLIGAKGTRIESLREKSG ASIKIIP
S. cerevisiae HX (1)	SKMTVNIPANSVARLIGNKGSNLQQIREKFA CQIDIPN
(2)	ITKELIVPVKFHGSLIGPHGTYRNRLQEKY NVFINFPR
(3)	HKMVINVPAEHVPRIIGKNGDNINDIRAEYG VEMDFLQ
(4)	VTKTIDIPAERKGALIGPGGIVRRQLESEF NINLFVPN
H. sapiens FMR1 (1)	FHEQFIVREDLMGLAIGTHGANIQQARKVPG VTAIDDL
(2)	AEDVIQVPRNLGGKVIGKNGKLIQEIVDKSGV VRVRIEA
E. coli NusA (1)	AVKTNDKRIDPVGACVGMRGARVQAVSTELGGERIDIVLWD
(2)	HTMDIAVEAGNLAQAIGRNGQNVRLASQLSGWELNVMTVDDL

FIG. 5. Alignment of the deduced amino acid sequence of CsrA with KH domains of RNA-binding proteins. The deduced amino acid sequence of the CsrA gene product (starting with residue 8) was compared with sequences of 16 putative RNA-binding KH domains from 10 previously aligned proteins (2, 13, 14, 42). The single-letter amino acid code is used; a lowercase f indicates a conserved polar residue. *H. sapiens*, *Homo sapiens*; *H. halobium*, *Halobacterium halobium*; *X. laevis*, *Xenopus laevis*; *S. cerevisiae*, *Saccharomyces cerevisiae*. Numbers in parentheses identify the particular domains shown for proteins that contain multiple KH domains. Boldface lettering in the CsrA sequence indicates that a particular amino acid is found at this position in at least one other KH domain from this set. Boldface lettering in the other protein sequences indicates amino acid identity with the aligned CsrA sequence. The asterisks identify the highly conserved glycine residues, and the underlined KK in the consensus sequence indicates a preponderance of lysine at either of these two positions.

glgC′-′lacZ fusion encoded on pCZ3-3 is regulated by all of the factors that are known to control the chromosomal *glgC* gene, including *csrA* (30, 32), and that expression of the chromosomal *lacZ*$^+$ gene is not affected by *csrA* in either the presence or absence of the gratuitous inducer isopropyl-β-D-thiogalactopyranoside (43). In order to determine the *cis*-acting region(s) necessary for *csrA*-mediated regulation of *glgC* expression, three classes of deletions from a *glgC′-′lacZ* translational fusion were constructed, sequenced, and analyzed for expression in BW3414 (*csrA*$^+$) and TR1-5 (*csrA*::*kanR*) strains (Fig. 6, 7, and 8).

The collection of 5′ nested deletions was prepared from pCZ3-3 (Fig. 6A). The specific β-galactosidase activity expressed from each plasmid clone was determined after 16 h of growth. Figure 7 shows that β-galactosidase activity was higher in TR1-5 throughout the series of plasmids. Deletion of *glgC* upstream flanking DNA to position −87 appeared not to dramatically alter the ratio of expression in BW3414 and TR1-5BW3414, indicating that these regions were not critical for regulation via *csrA* (Fig. 7B). In BW3414, the β-galactosidase activity that was expressed from the deletions extending to −40 and −32 was higher than those from the other the plasmids, as was the ratio of activity of these two clones in *csrA*$^+$ versus *csrA*::*kanR* strains. In several repetitions of this experiment, we consistently observed elevated expression levels from these clones in the *csrA*$^+$ strain and elevated ratios of expression in *csrA*$^+$ versus *csrA*::*kanR* strains, which ranged from 0.3 to 0.6 for the −40 clone (data not shown). Further deletion to −18 resulted in a decrease in the level of expression without further alteration of the ratio of expression in the two strains. This demonstrates that *csrA* can regulate the expression of this fusion even when only 18 bp of *glgC* upstream DNA is present and suggests that the region between −87 and −40 plays a role in the regulation but is not absolutely essential.

The observation that clones lacking most of the upstream noncoding region of *glgC* were nevertheless able to express β-galactosidase under the regulatory control of *csrA* suggested that transcription originating in the plasmid vector contributes to the expression of the *glgC-lacZ* fusion. Therefore, a *trpA* Rho-independent terminator was introduced immediately upstream from the *glgC* DNA in five of the clones (Fig. 6). As shown in Fig. 7, *glgC′-′lacZ* expression from the three terminator-containing clones that possessed the greatest amounts of *glgC* DNA, which included the upstream flanking region of the major *glgC* transcript (transcript B in Fig. 6), was not significantly altered by introduction of the terminator. However, the clones with deletions up to positions −110 and −40 did show weaker expression when the *trpA* terminator was present, indicating that read-through transcription from the vector was contributing to the expression observed. Primer extension analysis of in vivo transcription from pTΔCZ40 (the −40 deletion) showed that transcription was originating from the vector in this clone and that the *trpA* terminator was only partially effective in terminating this transcription. This vector-derived transcription allowed the *glgC′-′lacZ* fusion to be expressed in clones that lacked a *glgC* promoter and permitted the use of deletion analysis to assess the regulatory role of *glgC*-flanking DNA up to the region that is essential for translation.

After having observed that almost all of the upstream flanking DNA of *glgC* was dispensable with respect to *csrA*-mediated regulation, deletions within the *glgC* coding region were isolated and characterized. The three deletions that were studied in detail contained the entire 487 bp of upstream noncoding DNA and either 31, 17, or 8 bp of the coding region (Fig. 6B). As shown in Fig. 7C, the clone that contained 31 bp of the coding region was strongly regulated via *csrA*. The clone containing only 8 bp of coding DNA was very poorly expressed in both strains, which is consistent with the fact that sequences which have been deleted in this clone match the consensus for ribosome binding to the initiation codon-distal region (26). The clone containing 17 bp of coding DNA was not deficient in expression in the *csrA*$^+$ strain. However, its capacity for expression in the *csrA*::*kanR* strain was greatly diminished. Finally, a clone that contained only 50 bp of upstream noncoding DNA and 8 bp of *glgC* coding DNA (Fig. 6C) was also expressed very poorly but still exhibited some negative regulation by *csrA* (Fig. 7C).

The levels of expression of β-galactosidase from eight of the *glgC′-′lacZ*-containing plasmids throughout the growth curve in *csrA*$^+$ and *csrA*::*kanR* strains are shown in Fig. 8. Interestingly, the stationary-phase induction of the *glgC′-′lacZ* fusion was not abolished by upstream deletions extending up to −18, showing that neither the effect of *csrA* nor the stationary-phase induction of *glgC* expression requires transcription from the *glgC* promoters; this suggests that neither type of regulation is mediated at the level of transcript initiation. A control plasmid, pMLC1, which expressed β-galactosidase under the control of the *lacZ* promoter did not exhibit the kind of regulation observed for the *glgC* fusions, and in fact β-galactosidase expression from this plasmid was somewhat higher in the *csrA*$^+$ strain than in the *csrA*::*kanR* strain (Fig. 7C).

DISCUSSION

Numerous observations indicate that mRNA stability in bacteria plays an important role in determining genetic expression and may be selectively altered in response to environmental conditions. Nevertheless, our understanding of the control of

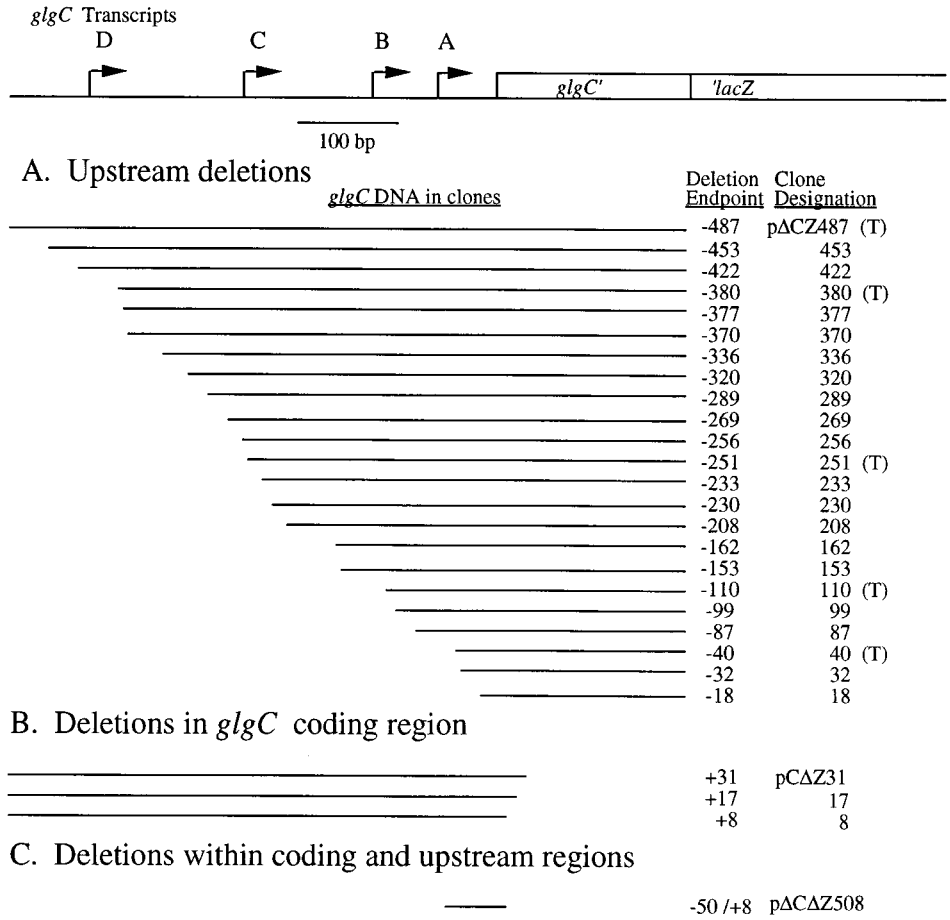

FIG. 6. Deletion derivatives of a *glgC'-'lacZ* translational fusion. The transcription pattern of *glgC* is shown at the top in relation to the plasmid-encoded *glgC'-'lacZ* fusions. Horizontal lines indicate the *glgC* DNA that remains in each of the clones. (A) Clones that have upstream deletions are identical to each other except for the extent of *glgC* DNA that was removed by *Bal* 31 exonuclease digestion. Deletion endpoints are indicated relative to the start of translation. All of the upstream deletion clones were derived from pCZ3-3 (30). Some of these clones were further modified by insertion of a single *trpA* terminator at the distal end of the upstream flanking region, as indicated by (T). (B) Clones with deletions within the *glgC* coding region prepared by in vivo mutagenesis of an out-of-frame *glgC'-'lacZ* translational fusion as described in Materials and Methods. The fusion junctions of clones pCΔZ31, pCΔZ17, and pCΔZ8 were sequenced, and it was determined that the β-galactosidase portions of the respective fusion products should start at amino acids 9, 12, and 9, relative to $LacZ^+$. (C) A single clone that contained 50 bp of upstream noncoding region and 8 bp of the *glgC* coding region, pΔCΔZ508, prepared by subcloning the *Bam*HI-*Ava*II fragment of pCΔZ8 into pMLB1034.

mRNA stability has lagged behind that of the other genetic regulatory processes (for a discussion, see references 7, 11, and 19). Stem-loop-binding proteins that increase the resistance of certain messages against degradation in the 3′-to-5′ direction have been proposed to exist both in *E. coli* and in chloroplasts (for a review, see reference 16). However, many bacterial mRNAs are primarily degraded in the 5′-to-3′ direction. This probably involves the combined action of endo- and exonucleolytic enzymes, since exonucleases that remove nucleotides sequentially from the 5′ ends of transcripts have not been found in bacteria (6). Factors that are known to affect 5′-to-3′ degradation include secondary structure in the 5′ region, specific endolytic ribonuclease activities, and the translational status of the message, as influenced by autogenously regulating proteins, mutations, or antibiotics that affect translation (for a discussion, see references 6, 10, 17, 19, 21, 24, 25, and 45). On the basis of this background information, it seems reasonable to predict that bacterial gene expression may be modulated at the level of 5′-to-3′ mRNA degradation by *trans*-acting factors which alter the rate of degradation of specific mRNAs or classes of messages. The present study suggests that the CsrA gene product may be such a *trans*-acting factor.

Our working hypothesis is that the CsrA gene product, alone or in the presence of other factors, interacts with mRNAs of *csrA*-regulated genes and affects the rates of 5′-to-3′ net degradation of these messages. Several observations from this study support this hypothesis. (i) Each of the four discrete, chromosomally encoded *glgC* transcripts is stabilized by a *csrA*::*kanR* insertion. (ii) Deletion studies indicate that a *cis*-acting region that is required for *csrA*-mediated regulation of *glgC* expression is close to the start of the coding region, e.g., the ribosome-binding region, which extends beyond the Shine-Dalgarno sequence (26) and/or sequences near the ribosome-binding region. This allows the steady-state levels of all four of the *glgC* transcripts to be modulated via *csrA*. (iii) The deduced amino acid sequence of the CsrA gene product contains the KH motif, which is characteristic of a subset of RNA-binding proteins and probably functions as an RNA-binding domain of these proteins (14).

The region of *glgC* that was deduced to be involved in *csrA*-mediated regulation of *glgC'-'lacZ* fusions suggests an association with translation, and the effect of CsrA on message stability could be secondary to translational inhibition. This region is highly conserved in *E. coli* and *S. typhimurium* (34).

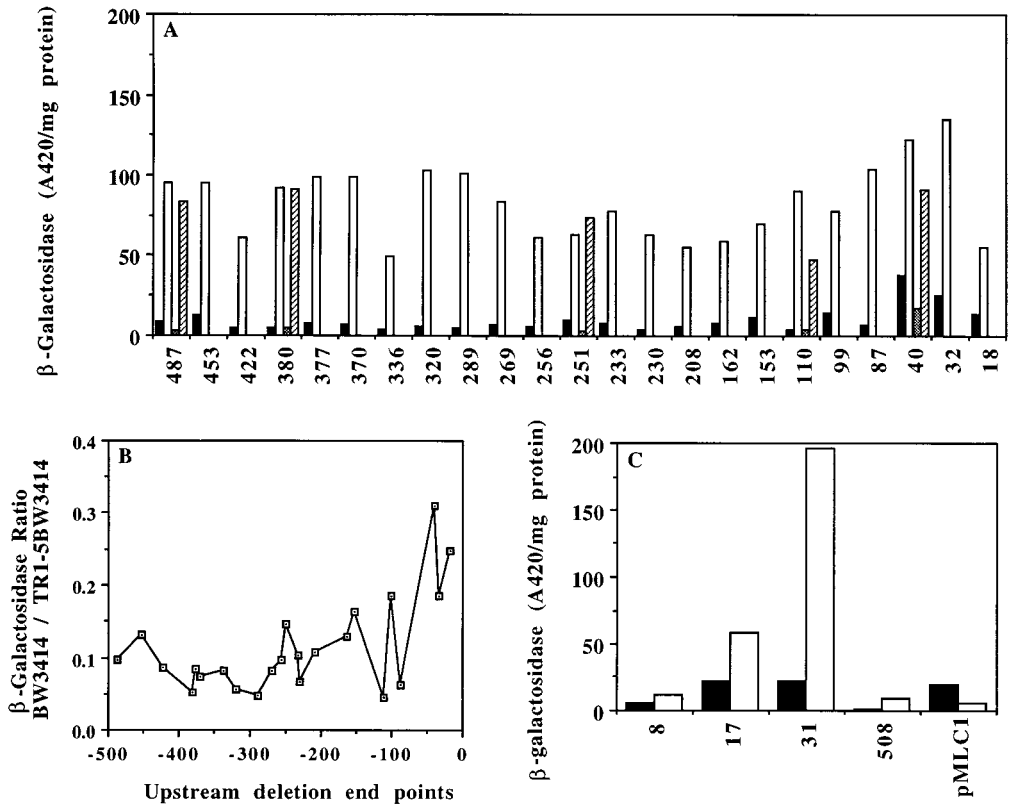

FIG. 7. Expression of plasmid-encoded *glgC′-′lacZ* translational fusions in *csrA*$^+$ and *csrA*::*kanR* strains. Plasmids containing the deletion derivatives shown in Fig. 6 were transformed into the isogenic *csrA* strains. (A) After 16 h of growth in Kornberg medium, β-galactosidase specific activities encoded by the clones containing the nested upstream deletions (Fig. 6A) determined for strains BW3414 (*csrA*$^+$) (black bars) and TR1-5BW3414 (*csrA*::*kanR*) (open bars). Activities expressed from the corresponding clones that contain a *trpA* terminator inserted upstream from the *glgC* DNA are indicated by stippled bars for BW3414 and by hatched bars for TR1-5BW3414. (B) The ratio of β-galactosidase specific activity encoded by each plasmid in BW3414 versus TR1-5BW3414 calculated and plotted against the endpoint of each of the deletions (i.e., the ordinate shows the amount of upstream noncoding *glgC* DNA present in each clone). (C) β-Galactosidase specific activities from clones that contained the entire upstream flanking region of *glgC* but had deletions within the *glgC* coding region (Fig. 6B), a clone that contained only 50 bp of upstream noncoding DNA and 8 bp of the coding region (Fig. 6C), and the control plasmid, pMLC1, which contained no *glgC* DNA. Plasmid pMC1 contained the 0.2-kb *Bam*HI-*Pvu*II fragment of pUC19 subcloned into pMBL1034 and used the promoter region of *lacZ* to express *lacZ*. The open bars and black bars indicate activities from TR1-5BW3414 and BW3414, respectively.

CsrA could interact with the ribosome-binding site, alter secondary structure in the region of the ribosome-binding site, or interact with the ribosome, or a combination of these possibilities could occur. However, the *glgC* and *glgA* genes overlap by 1 bp and are probably translationally coupled (33). Therefore, if *CsrA* directly inhibits *glgC* translation, polarity effects should decrease the expression of *glgA*. This did not occur in the in vitro experiments, although more studies are required to directly determine whether CsrA is a primary regulator of translation.

An observation that should be considered in any model of *csrA*-mediated regulation is that sequence between +17 and +31 of the *glgC* coding region appears to be important. Deletion of this region did not significantly affect expression in the *csrA*$^+$ strain but greatly diminished the expression in the *csrA*::*kanR* strain. This result is not consistent with a simple *cis*-acting negative (operator-like) region, since deletion of this kind of *cis*-acting site should enhance expression in the *csrA*$^+$ strain and should not alter expression in the *csrA*::*kanR* strain. Either of two simple models could explain this observation. (i) This region has a positive function which is the target for CsrA. For example, this region may help to protect the transcript against degradation. (ii) Two processes are simultaneously mediated within this region, i.e., CsrA-mediated transcript decay and a second process that serves a positive role in expression. For example, this region could be needed for efficient translation. In the *csrA*::*kanR* strain, deletion of the dual-functioning region would affect only the positive process, thereby decreasing expression, while in the *csrA*$^+$ strain both the positive and the negative processes are disrupted by the deletion, resulting in no net change in expression.

Although CsrA-containing extracts exhibited little effect on the in vitro expression of *glgA*, the in vivo expression of a chromosomal *glgA′-′lacZ* fusion is strongly regulated via *csrA* (43). This discrepancy may be due to the facts that the pOP12 plasmid, which was used as the in vitro template for *glg* expression, does not contain the entire *glgY* gene and that the *glgCAY′* transcript that it encodes lacks half of the *glgY* coding region and a putative stem-loop structure following *glgY* (33), which may protect *glgA* against 3′-to-5′ degradation (16). The major route of *glgC* transcript decay in vivo is clearly *csrA*-mediated degradation and requires the 5′ end of the transcript. Furthermore, processing between *glgC* and *glgA* cannot occur, since these genes overlap. However, a truncated *glgCAY′* transcript might be degraded in the 3′→5′ direction in the absence of CsrA, favoring the expression of *glgC* relative to that of *glgA*. The addition of the CsrA-containing S-200 extract to an S-30 extract that lacks CsrA or the use of S-30 extracts prepared from a *csrA*$^+$ strain for transcription-translation (35) resulted in levels of *glgC* and *glgA* expression from the pOP12 plasmid that were more similar.

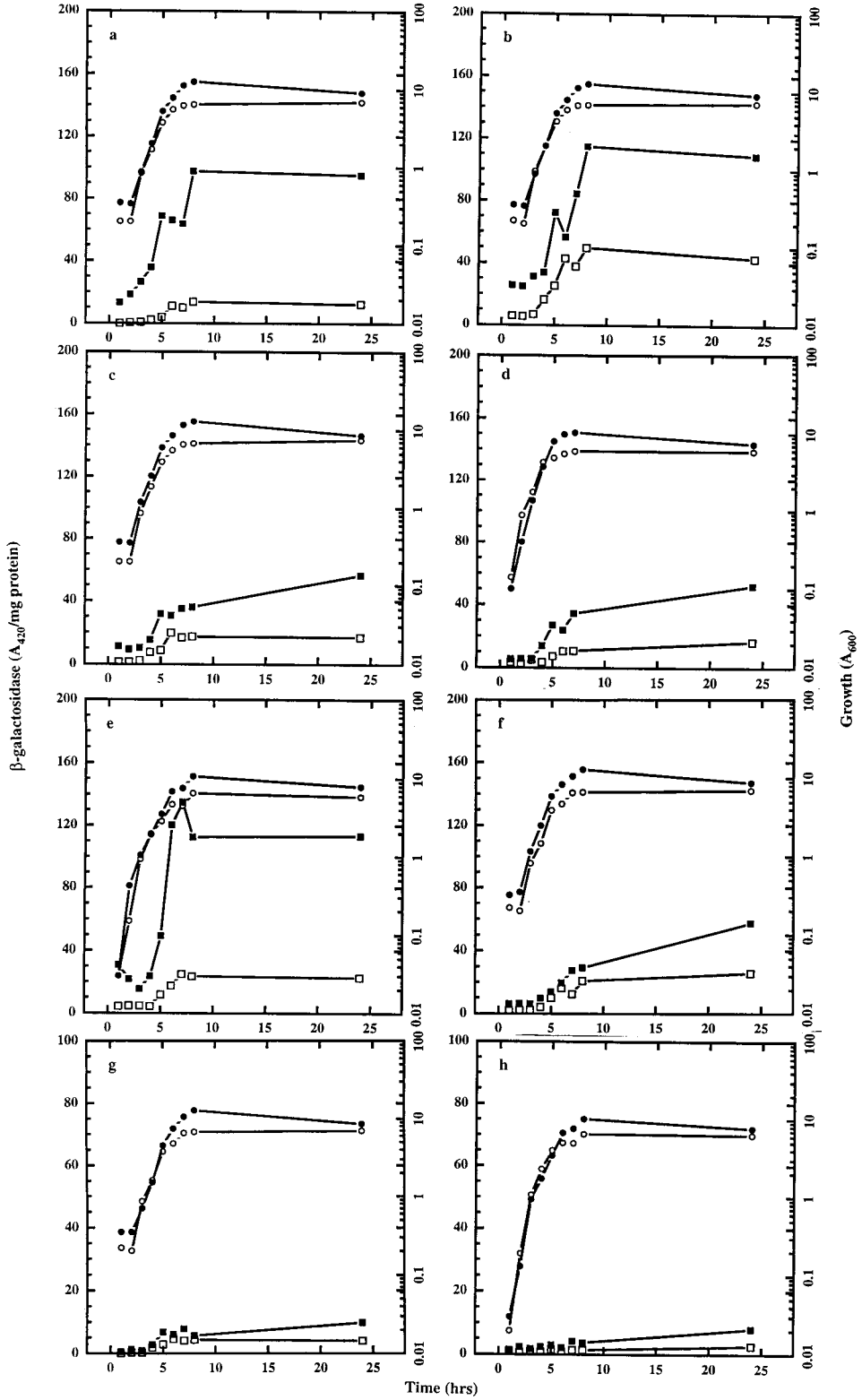

FIG. 8. Effects of *csrA* on the expression of selected *glgC'-'lacZ* fusions throughout the growth curve. β-Galactosidase specific activities expressed from *glgC'-'lacZ* fusions from eight plasmid deletion derivatives in strains BW3414 (open symbols) and TR1-5BW3414 (closed symbols) are indicated by squares; turbidity readings of the cultures (A_{600}) are indicated by circles. (A through H) Activities from clones pΔCZ487, pΔCZ40, pΔCZ18, pTΔCZ40, pCΔZ31, pCΔZ17, pCΔZ8, and pΔCΔZ508, respectively.

The demonstration that *csrA* decreases *glgC* mRNA stability establishes yet another level at which glycogen synthesis is regulated. The negative effects of *csrA* on glycogen synthesis (32) and on *glgC* expression are mediated independently of positive regulation via cAMP-CRP and ppGpp. The expression of *glgB* is not affected by either of the positive regulators but, similarly to *glgC*, is induced in stationary phase and is regulated via *csrA* (32, 35). Furthermore, *csrA* probably controls glycogen synthesis via additional effects on carbon metabolism. We have observed that *csrA* negatively regulates the gluconeogenic genes and positively regulates several of the glycolytic genes (32, 36). The 20- to 30-fold higher levels of glycogen accumulated in a *csrA*::*kanR* mutant (up to 1.6 mg of glycogen per mg of protein [32]) suggest that *csrA* plays a major role in directing carbon flux in *E. coli.*

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REFERENCES

1. **Arraj, J. A., and M. G. Marinus.** 1983. Phenotypic reversal in *dam* mutants of *Escherichia coli* K-12 by a recombinant plasmid containing the *dam*$^+$ gene. J. Bacteriol. **153:**562–565.
2. **Ashley, C. T., K. D. Wilkinson, D. Reines, and S. Warren.** 1993. *FMR1* protein: conserved RNP family domains and selective RNA binding. Science **262:**563–566.
3. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. Seidman, J. A. Smith, and K. Struhl.** 1989. Current protocols in molecular biology. John Wiley & Sons, Inc., New York.
4. **Baecker, P. A., C. E. Furlong, and J. Preiss.** 1983. Biosynthesis of bacterial glycogen. Primary structure of *Escherichia coli* ADPglucose synthetase as deduced from the nucleotide sequence of the *glgC* gene. J. Biol. Chem. **258:**5084–5088.
5. **Baecker, P. A., E. Greenberg, and J. Preiss.** 1986. Biosynthesis of bacterial glycogen. Primary structure of *Escherichia coli* 1,4-α-D-glucan:1,4-α-D-glucan 6-α-D-(1,4-α-D-glucano)-transferase as deduced from the nucleotide sequence of the *glgB* gene. J. Biol. Chem. **261:**8738–8743.
6. **Bechhofer, D.** 1993. 5′ mRNA stabilizers, p. 31–50. *In* J. G. Belasco and G. Brawerman (ed.), Control of messenger RNA stability. Academic Press, San Diego, Calif.
7. **Belasco, J. G.** 1993. mRNA degradation in prokaryotic cells: an overview, p. 3–11. *In* J. G. Belasco and G. Brawerman (ed.), Control of messenger RNA stability. Academic Press, San Diego, Calif.
8. **Chamberlain, J. P.** 1979. Fluorographic detection of radioactivity in polyacrylamide gels with the water-soluble flour, sodium salicylate. Anal. Biochem. **98:**132–135.
9. **Christie, G. E., P. J. Farnham, and T. Platt.** 1981. Synthetic sites for transcription termination and a functional comparison with tryptophan operon termination sites in vitro. Proc. Natl. Acad. Sci. USA **78:**4180–4184.
10. **Court, D.** 1993. RNA processing and degradation by RNase III, p. 71–108, *In* J. G. Belasco and G. Brawerman (ed.), Control of messenger RNA stability. Academic Press, San Diego, Calif.
11. **Ehretsmann, C. P., A. J. Carpouis, and H. M. Krisch.** 1992. mRNA degradation in procaryotes. FASEB J. **6:**3186–3192.
12. **Garnier, J., D. J. Osguthorpe, and B. Robson.** 1978. Analysis of the accuracy and implications of simple methods for predicting the structure of globular proteins. J. Mol. Biol. **120:**97–120.
13. **Gibson, T. J., P. M. Rice, J. D. Thompson, and J. Heringa.** 1993. KH domains within the *FMR1* sequence suggest that fragile X syndrome stems from a defect in RNA metabolism. Trends Biochem. Sci. **18:**331–333.
14. **Gibson, T. J., J. D. Thompson, and J. Heringa.** 1993. The KH domain occurs in a diverse set of RNA-binding proteins and is probably involved in binding to nucleic acid. FEBS Lett. **324:**361–366.
15. **Hengge-Aronis, R., and D. Fischer.** 1992. Identification and molecular analysis of *glgS*, a novel growth-phase-regulated and *rpoS*-dependent gene involved in glycogen synthesis in *Escherichia coli*. Mol. Microbiol. **6:**1877–1886.
16. **Higgins, C. F., H. C. Causton, G. S. C. Dance, and E. A. Mudd.** 1993. The role of the 3′ end in mRNA stability and decay, p. 13–27. *In* J. G. Belasco and G. Brawerman (ed.), Control of messenger RNA stability. Academic Press, San Diego, Calif.
17. **Kennell, D. E.** 1987. The instability of messenger RNA in bacteria, p. 101–143. *In* W. Reznikoff and L. Gold (ed.), Maximizing gene expression. Butterworths Publishers, Stoneham, Mass.
18. **King, T. C., and D. Schlessinger.** 1987. Processing of RNA transcripts, p. 703–718. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Shaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
19. **Kumar, A., C. E. Larsen, and J. Preiss.** 1986. Biosynthesis of bacterial glycogen. Primary structure of *Escherichia coli* ADPglucose:α-1,4-glucan, 4-glucosyl transferase as deduced from the nucleotide sequence of the *glgA* gene. J. Biol. Chem. **261:**16256–16259.
20. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Melefors, O., U. Lundberg, and A. von Gabain.** 1993. RNA processing and degradation by RNase K and RNase E, p. 53–57. *In* J. G. Belasco and G. Brawerman (ed.), Control of messenger RNA stability. Academic Press, San Diego, Calif.
22. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Okita, T. W., R. L. Rodriguez, and J. Preiss.** 1981. Biosynthesis of bacterial glycogen. Cloning of the glycogen biosynthetic enzyme structural genes of *Escherichia coli*. J. Biol. Chem. **256:**6944–6952.
24. **Petersen, C.** 1992. Control of functional mRNA stability in bacteria: multiple mechanisms of nucleolytic and non-nucleolytic inactivation. Mol. Microbiol. **6:**277–282.
25. **Petersen, C.** 1993. Translation and mRNA stability in bacteria: a complex relationship, p. 117–141. *In* J. G. Belasco and G. Brawerman (ed.), Control of messenger RNA stability. Academic Press, San Diego, Calif.
26. **Petersen, G. B., P. A. Stockwell, and D. F. Hill.** 1988. Messenger RNA recognition in *Escherichia coli*: a possible second site of interaction with 16S ribosomal RNA. EMBO J. **7:**3957–3962.
27. **Preiss, J.** 1984. Bacterial glycogen synthesis and its regulation. Annu. Rev. Microbiol. **38:**419–458.
28. **Preiss, J., and T. Romeo.** 1989. Physiology, biochemistry and genetics of bacterial glycogen synthesis. Adv. Microb. Physiol. **30:**183–233.
29. **Preiss, J., and T. Romeo.** 1994. Molecular biology and regulatory aspects of glycogen biosynthesis in bacteria. Prog. Nucleic Acid Res. Mol. Biol. **47:**301–327.
30. **Romeo, T., J. Black, and J. Preiss.** 1990. Genetic regulation of glycogen synthesis in *Escherichia coli*: *in vivo* effects of the catabolite repression and stringent response systems in *glg* gene expression. Curr. Microbiol. **21:**131–137.
31. **Romeo, T., and M. Gong.** 1993. Genetic and physical mapping of the regulatory gene *csrA* on the *Escherichia coli* K-12 chromosome. J. Bacteriol. **175:**5740–5741.
32. **Romeo, T., M. Gong, M. Y. Liu, and A.-M. Brun-Zinkernagel.** 1993. Identification and molecular characterization of *csrA*, a pleiotropic gene from *Escherichia coli* that affects glycogen biosynthesis, gluconeogenesis, cell size, and surface properties. J. Bacteriol. **175:**4744–4755.
33. **Romeo, T., A. Kumar, and J. Preiss.** 1988. Analysis of the *Escherichia coli* gene cluster suggests that catabolic enzymes are encoded among the biosynthetic genes. Gene **70:**363–376.
34. **Romeo, T., and J. Moore.** 1991. Comparison of the 5′ flanking regions of the *Salmonella typhimurium* and *Escherichia coli glgC* genes, encoding ADP glucose pyrophosphorylases. Nucleic Acids Res. **19:**3452.
35. **Romeo, T., and J. Preiss.** 1989. Genetic regulation of glycogen biosynthesis in *Escherichia coli*: in vitro effects of cyclic AMP and guanosine 5′-diphosphate 3′-diphosphate and analysis of in vivo transcripts. J. Bacteriol. **171:**2773–2782.
36. **Sabnis, N., H. Yang, and T. Romeo.** Unpublished data.
37. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
38. **Schlessinger, D., K. A. Jacobs, and R. S. Gupta.** 1977. Decay of individual *Escherichia coli trp* messenger RNA molecules is sequentially ordered. J. Mol. Biol. **110:**421–439.
39. **Schwartz, M.** 1966. Location of the maltose A and B loci on the genetic map of *Escherichia coli*. J. Bacteriol. **92:**1083–1089.
40. **Shine, J., and L. Dalgarno.** 1974. The 3′-terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosomal binding sites. Proc. Natl. Acad. Sci. USA **71:**1342–1346.
41. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
42. **Siomi, H., M. J. Matunis, W. M. Michael, and G. Dreyfuss.** 1993. The pre-mRNA binding K protein contains a novel evolutionarily conserved motif. Nucleic Acids Res. **21:**1193–1198.
43. **Yang, H., and T. Romeo.** Unpublished data.
44. **Yanish-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.
45. **Yarchuk, O., N. Jacques, J. Guillerez, and M. Dreyfus.** 1992. Interdependence of translation, transcription and mRNA degradation in the *lacZ* gene. J. Mol. Biol. **226:**581–596.
46. **Yu, F., Y. Jen, E. Takeuchi, M. Inouye, H. Nakayama, M. Tagaya, and T. Fukui.** 1988. α-Glucan phosphorylase from *Escherichia coli*. Cloning of the gene, and purification and characterization of the protein. J. Biol. Chem. **263:**13706–13711.