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Coordinate Genetic Regulation of Glycogen Catabolism and Biosynthesis in *Escherichia coli* via the CsrA Gene Product

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The carbon storage regulator gene, *csrA*, encodes a factor which negatively modulates the expression of the glycogen biosynthetic gene *glgC* by enhancing the decay of its mRNA (M. Y. Liu, H. Yang, and T. Romeo, J. Bacteriol. 177:2663–2672, 1995). When endogenous glycogen levels in isogenic *csrA*$^+$ and *csrA*::*kanR* strains were quantified during the growth curve, both the rate of glycogen accumulation during late exponential or early stationary phase and its subsequent rate of degradation were found to be greatly accelerated by the *csrA*::*kanR* mutation. The expression of the biosynthetic genes *glgA* (glycogen synthase) and *glgS* was observed to be negatively modulated via *csrA*. Thus, *csrA* is now known to control all of the known glycogen biosynthetic genes (*glg*), which are located in three different operons. Similarly, the expression of the degradative enzyme glycogen phosphorylase, which is encoded by *glgY*, was found to be negatively regulated via *csrA* in vivo. The in vitro transcription-translation of *glgY* was also specifically inhibited by the purified CsrA gene product. These results demonstrate that localization of glycogen biosynthetic and degradative genes within the *Escherichia coli glgCAY* operon facilitates their coordinate genetic regulation, as previously hypothesized (T. Romeo, A. Kumar, and J. Preiss, Gene 70:363–376, 1988). The *csrA* gene did not affect glycogen debranching enzyme, which is now shown to be encoded by the gene *glgX*.

One feature of the complex adaptive response which occurs when *Escherichia coli* or various other bacteria enter stationary phase is the increased expression of genes involved in the biosynthesis of glycogen (reviewed in references 12 and 20–22). A shortage of a nutrient such as nitrogen in the presence of excess carbon is particularly effective in causing the intracellular accumulation of glycogen, which can be metabolized as an endogenous carbon and energy source. The regulatory mechanisms for glycogen synthesis in *E. coli* include (i) allosteric control via the metabolites AMP and fructose-1,6-bisphosphate of the committed step of the pathway, catalyzed by ADPglucose pyrophosphorylase (EC 2.7.7.27); (ii) stimulation of the genetic expression of *glgC* (which encodes ADPglucose pyrophosphorylase [2]) and *glgA* (which encodes glycogen synthase [EC 2.4.1.21] [13]) by guanosine 3′-bisphosphate 5′-bisphosphate and cyclic AMP (cAMP); and (iii) the transcriptional control of *glgS*, a monocistronic gene which stimulates glycogen synthesis in vivo, by cAMP and σ^s (9, 20–22, 24, 28).

We recently identified and characterized the *csrA* gene, which exerts a very strong negative effect on glycogen synthesis in *E. coli* (25, 26). This gene encodes a 61-amino-acid protein, CsrA, which was shown to regulate the expression of *glgB* (encoding glycogen branching enzyme [EC 2.4.1.18]) and *glgC* (26). CsrA has been shown to be a factor which enhances the rate of decay of *glgC* mRNA via a novel though unresolved mechanism (16). The deduced amino acid sequence of CsrA is homologous to a diverse subset of RNA-binding proteins, the KH proteins (16). The *csrA* gene was originally shown to have pleiotropic effects on gluconeogenesis and cell surface properties (26), and we have more recently confirmed that *csrA* negatively regulates gluconeogenesis and also positively regulates glycolysis in *E. coli* (29). The involvement of the *csrA* homolog of *Erwinia carotovora* (*rsmA*, for repressor of stationary-phase metabolites) in the negative regulation of several virulence factors which cause soft-rot disease of plants and its widespread occurrence among members of the family *Enterobacteriaceae* (7) provide further evidence of the important role which *csrA* plays in the regulation of bacterial physiology and metabolism. The finding of an apparent homolog of *csrA* in *Haemophilus influenzae* during the whole-genome sequencing project (8) and our own unpublished hybridization data (35) indicate that *csrA* is present in members of several families of gram-negative bacteria.

The regulation and molecular genetics of endogenous glycogen degradation in *E. coli* have yet to be thoroughly investigated. However, a key enzyme in glycogen metabolism, glycogen phosphorylase (EC 2.4.1.1), is known to be allosterically inhibited by ADPglucose and activated by AMP (5). Glycogen phosphorylase is encoded by a gene that has been alternatively designated *glgY* or *glgP*, which is located distal to *glgA* within the *glgCAY* operon (27, 37). A second enzyme that is believed to be important for the metabolism of intracellular glycogen in *E. coli* is the glycogen debranching enzyme (EC 3.2.1.-), which hydrolyzes the α-1,6 branches of glycogen which has first been partially depolymerized by phosphorylase (11). The *glgBX* operon is located upstream from and in tandem with *glgCAY* and includes the gene for glycogen branching enzyme (*glgB* [3]) and possibly the gene for glycogen debranching enzyme or another catabolic glucanase (27). This was previously hypothesized on the basis of the similarity of the deduced amino acid sequence of GlgX to sequences of glucanotransferases and hydrolases and the fact that *glgX* was not required for glucan biosynthesis (27). Finally, the genes encoding two α-amylases, *malS* (31) and *amyA* (23), have also been characterized for *E. coli*. However, *malS* is part of the maltose regulon, its gene product is not synthesized in glucose-grown cells, and it is a periplasmic enzyme. Therefore, MalS is believed not to be involved in metabolism of endogenous glycogen; the physiological function of AmyA has not been tested.

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description	Source and/or reference
E. coli K-12 strains		
BW3414	Δ*lacU169*	B. Wanner
TR1-5BW3414[a]	BW3414 *csrA*::*kanR*	26
G6	Hfr *his thi* Str^s	M. Schwartz, 10
G6MP11	G6 (*malP*)	A. Pugsley (10)
G6MD3	G6 Δ(*malA-asd*)	32
DH5α	*supE44* Δ*lacU169* (Φ80*lacZ*ΔM15)*hsd R17 recA1 endA1 gyrA96 thi-1 relA1*	1
CAG18642	*zfh-3131*::Tn*10*; 57.5 min	C. Gross and M. Singer
MC4100	F$^-$ Δ(*arg-lacU169*) *araD139 rpsL150 ptsF25 fibB5301 rbsR deoC relA1*	R. Hengge-Aronis (9)
DW18	MC4100 Φ(*glgA*::*lacZ*) (λ*plac*Mu15)	9
RH105	MC4100(λRH704)Φ(*glgS*::*lacZ*)(Hyb)	9
Plasmids		
pOP12	*asd glgBX glgCAY′* in pBR322, Tet^r	19
pMLB1034	Vector for making *glgY*::*lacZ* translational fusion, Amp^r	34
pUC19	Cloning vector, Amp^r	36
pYZ9	Φ(*glgCAY*::*lacZ*) fused at *glgY* codon 161	This study
pBX49	*glgX* in multiple cloning site of pUC19, same orientation as *lacZ*	This study
pBX31	*glgX* in pUC19, opposite of *lacZ* orientation	This study

[a] A strain designation containing the prefix TR1-5 indicates that the *csrA*$^+$ allele has been replaced by the TR1-5 mutant allele (*csrA*::*kanR*) by P1*vir* transduction (26).

The results of the current study establish an expanded role for *csrA* in the genetic regulation of both synthesis of glycogen and its degradation. Furthermore, the previously identified *glgX* open reading frame is now shown to encode the glycogen debranching enzyme. Thus, structural genes needed for glycogen synthesis and catabolism are clustered together in two adjacent operons. The first contains genes required for the synthesis and for the hydrolysis of the α-1,6 linkages of glycogen; the second operon includes the genes required for the formation and for the depolymerization of the α-1,4-glucan linkages of the polymer.

(Some of the experiments described herein were conducted in partial fulfillment of the requirements for a 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. The [α-^{35}S]dATP and [^{14}C]glucose-1-phosphate were purchased from Dupont NEN (Wilmington, Del.). The enzymes α-amylase and amyloglucosidase were from Boehringer Mannheim Biochemicals (Indianapolis, Ind.). Isopropyl β-D-thiogalactopyranoside (IPTG), *o*-nitrophenyl-β-D-galactopyranoside (ONPG), hexokinase, glucose-6-phosphate dehydrogenase, NADH, rabbit muscle phosphorylase *b*, oyster glycogen, maltodextrin, and glucose-1-phosphate were from Sigma Chemical Co. (St. Louis, Mo.). The compound 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), Sequenase version 2.0, and all DNA sequencing reagents were from U.S. Biochemical Corp. (Cleveland, Ohio). Restriction enzymes were purchased from either Bethesda Research Laboratories (Gaithersburg, Md.) or New England BioLabs (Beverly, Mass.). All other biochemical reagents were purchased from commercial sources and were of the highest quality available.

Bacterial strains, plasmids, and growth conditions. Table 1 lists the strains and plasmids that were used in this study, their sources, and relevant genotypes. For experimental determination of glycogen and glycogen-metabolizing enzymes and for genetic expression studies, strains were cultured as previously described (26), with vigorous aeration at 37°C in a medium which allows optimal glycogen biosynthesis, Kornberg medium (1.1% K_2HPO_4, 0.85% KH_2PO_4, 0.6% yeast extract containing either glucose or maltose at 0.5%). Media were supplemented with the following compounds as required: ampicillin, 100 μg/ml; tetracycline, 10 μg/ml; kanamycin, 100 μg/ml; diaminopimelic acid, 50 μg/ml; X-Gal, 40 μg/ml; IPTG, 5 mM.

Molecular biology and genetic techniques. Transduction of the TR1-5 allele (*csrA*::*kanR*) with P1*vir* was conducted (17) by using previously described selection and screening procedures (26). Standard procedures were used for isolation of plasmid DNA and restriction fragments, restriction mapping, transformation, and molecular cloning (1). DNA sequencing was conducted by the chain termination method of Sanger et al. (30) by using the Sequenase version 2.0 kit under the conditions suggested by the manufacturer (U.S. Biochemical Corp.). A plasmid which encodes a *glgCAY*::*lacZ* translational fusion, pYZ9, was obtained by ligating a gel-purified 3.7-kb *Hpa*I restriction fragment of pOP12 into the (alkaline phosphatase-treated) *Sma*I site of pMLB1034 and transforming DH5α to ampicillin resistance. Blue colonies were isolated on LB medium (17) containing ampicillin and X-Gal. The fusion was verified by restriction enzyme mapping and sequencing across the *glgY*::*lacZ* junction by using a primer that anneals within *lacZ* (GATGTGCTGCAAGGCGATTAAGTTGGGTAACG). A 2.3-kb *Kpn*I restriction fragment of pOP12 (19) was subcloned into the (alkaline phosphatase-treated) *Kpn*I site of pUC19 to generate two plasmid clones, pBX49 and pBX31, which contained the *glgX* open reading frame oriented either in the same direction as *lacZ* of the pUC19 vector or in the opposite direction, respectively.

S-30-coupled transcription-translation. Experiments to measure the effects of the CsrA gene product on in vitro expression from the plasmid pYZ9 (encoding *glgCAY*::*lacZ*) were conducted in S-30 extracts prepared from strain TR-1-5BW3414 (*csrA*::*kanR*), as previously described (26). The biologically active CsrA gene product used in these studies was expressed in a recombinant form which contains six histidine residues at the carboxy terminus, which allowed its purification to electrophoretic homogeneity by affinity chromatography on Ni^{2+}-nitrilotriacetic acid resin (as described by the supplier, Qiagen, Inc., Chatsworth, Calif.) (15).

Assays for enzyme activities, glycogen, and total protein. β-Galactosidase, glycogen, and total cell protein were determined as previously described (26). Glycogen phosphorylase and maltodextrin phosphorylase activities were measured by monitoring the rate of incorporation of [^{14}C]glucose from [^{14}C]glucose-1-phosphate by cell extracts into either glycogen or maltodextrin, as previously described (4, 5). One unit of phosphorylase activity incorporates 1 nmol of [^{14}C]glucose into an ethanol-insoluble form in 20 min at 37°C. The *E. coli* debranching enzyme activity was quantified by the method of Jeanningros et al. (11), which uses phosphorylase limit dextrin as a substrate (14), since the *E. coli* debranching enzyme exhibits low-level activity on native glycogen. The assay conditions were previously found to be specific for the debranching enzyme, and other *E. coli* glucanases such as α-amylase did not influence debranching enzyme determinations (11). A unit of debranching enzyme activity releases 1 nmol of reducing end groups (18) per min at 37°C. Control experiments were conducted with heat-inactivated extracts to assure that endogenous glycogen or other factors did not influence the reported activity values of phosphorylase or debranching enzyme, and the assays were determined within the linear range of enzyme activity.

RESULTS AND DISCUSSION

Effects of *csrA* on endogenous glycogen accumulation and metabolism. Figure 1 quantitatively compares glycogen accumulation levels in isogenic *csrA*$^+$ and *csrA*::*kanR* strains throughout the growth curve and into the stationary phase and shows that accumulation reached a level of 1.6 mg/ml of protein in the *csrA*::*kanR* strain, or approximately 50% of the dry weight. The rate of net glycogen accumulation in this strain in

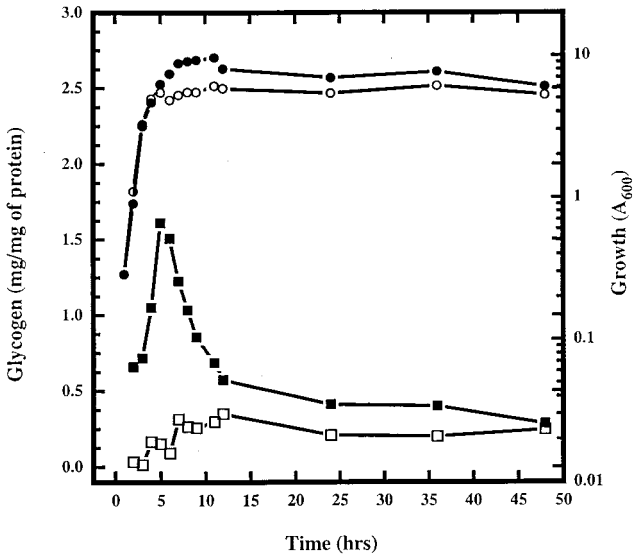

FIG. 1. Effect of *csrA* on endogenous glycogen levels. Strains BW3414 (*csrA*$^+$) and TR1-5BW3414 (*csrA*::*kanR*) were grown in Kornberg medium containing 0.5% glucose, and glycogen and protein levels in cultures were quantified throughout the growth curve and into late stationary phase. Glycogen levels in BW3414 and TR1-5BW3414 are shown as open and closed squares, respectively; growth rates (A_{600}) of these two strains are shown as open and closed circles, respectively.

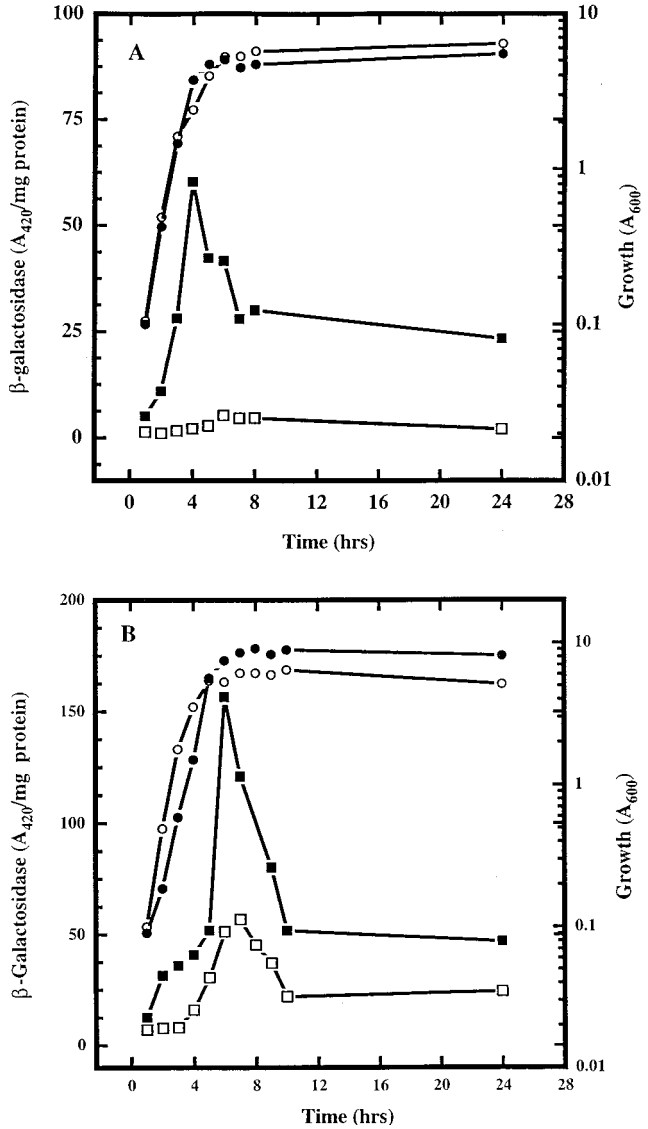

FIG. 2. Effects of *csrA* on expression of *'lacZ* translational fusions of glycogen biosynthesis genes *glgA* and *glgS*. Cultures were grown in Kornberg medium containing 0.5% glucose, and specific β-galactosidase activity was determined. Shown are the effects of *csrA* on the expression of a *glgA*::*lacZ* fusion in DW18 (*glgA*::*lacZ csrA*$^+$) and TR1-5DW18 (*glgA*::*lacZ csrA*::*kanR*) (A) and a *glgS*::*lacZ* fusion in RH105 (*glgS*::*lacZ csrA*$^+$) and TR1-5RH105 (*glgS*::*lacZ csrA*::*kanR*) (B). β-Galactosidase specific activities in the *csrA*$^+$ and *csrA*::*kanR* strains are shown as open and solid squares, respectively; growth rates (A_{600}) of these two strains are shown as open and closed circles, respectively.

the late exponential phase was extremely rapid, approximately 0.3 mg/mg of protein per h. After this strain entered the stationary phase, glycogen levels also decreased rapidly, at a rate of approximately 0.11 mg/mg of protein per h. Both the *csrA*$^+$ and *csrA*::*kanR* cultures remain completely viable under these conditions (26). Thus, both the rate of synthesis of glycogen and its subsequent utilization appeared to be negatively controlled via *csrA*.

Regulation of the glycogen biosynthetic genes *glgA* and *glgS* via *csrA*. We had previously shown that the expression of *glgC* and *glgB* was negatively regulated via *csrA* (26). In order to determine whether the expression of the other glycogen biosynthetic genes is affected by *csrA*, chromosomally encoded *glgA*::*lacZ* and *glgS*::*lacZ* translational fusions were tested for expression in *csrA*$^+$ and *csrA*::*kanR* strains. Figure 2 shows that the expression levels of these two gene fusions in cells grown in Kornberg medium were approximately 12-fold and 3-fold higher in the *csrA*::*kanR* strains, respectively. Thus, the rapid accumulation of glycogen in the *csrA*::*kanR* mutant may be attributed to the fact that *csrA* negatively affects the expression of all of the glycogen biosynthetic genes, *glgB*, *glgC*, *glgA*, and *glgS*, which are located in three different operons, *glgBX*, *glgCAY*, and *glgS*.

Effects of *csrA* on the expression of glycogen phosphorylase. To test whether the rapid catabolism of glycogen in the *csrA*::*kanR* strain subsequent to its accumulation (Fig. 1) was caused by effects of *csrA* on the expression of genes involved in glycogen catabolism, we first determined the specific activity of the key enzyme glycogen phosphorylase in *csrA*$^+$ and *csrA*::*kanR* strains. *E. coli* synthesizes two glucan phosphorylases, one of which, glycogen phosphorylase, shows a substrate preference for native glycogen over short maltodextrins and is the *glgY* gene product (27, 37). The other glucan phosphorylase, maltodextrin phosphorylase (EC 2.4.1.1), exhibits a substrate preference for maltodextrins over native glycogen, and its structural gene *malP* is expressed as one of the genes of the maltose regulon, which are induced by growth on maltose (33). Glycogen phosphorylase is thus involved in the metabolism of endogenous glycogen, while maltodextrin phosphorylase is not (5, 6). Table 2 shows glucan phosphorylase specific activities present in eight strains of *E. coli* during the stationary phase, assayed in the presence of either glycogen (the preferred substrate of glycogen phosphorylase) or maltodextrin (the preferred substrate of maltodextrin phosphorylase). Experiments with strains which lack maltodextrin phosphorylase showed that glycogen phosphorylase activity was elevated three- to four-fold by the disruption of *csrA*. Glycogen phosphorylase specific activity was determined throughout the growth curve in the *malP* mutant strains G6MP11 (*csrA*$^+$) and TR1-5G6MP11

TABLE 2. Effects of the *csrA*::*kanR* mutation on glucan phosphorylase specific activities

Strain (relevant genotype)	Phosphorylase activity (U/mg of protein ± SD) with indicated glucose acceptor and carbon source[a]			
	Glucose		Maltose	
	Glycogen	Dextrin	Glycogen	Dextrin
BW3414	4.5 ± 0.6	1.1 ± 0.2	43 ± 3	49 ± 2
TR1-5BW3414 (*csrA*::*kanR*)	6.9 ± 0.7	1.3 ± 0.2	29 ± 2	37 ± 2
G6	4.4 ± 0.4	2.2 ± 0.1	62 ± 5	100 ± 7
TR1-5G6 (*csrA*::*kanR*)	9.1 ± 0.5	4.4 ± 0.5	42 ± 3	76 ± 6
G6MP11 (*malP*)	1.7 ± 0.4	0.4 ± 0.2	3.4 ± 0.3	1.7 ± 0.3
TR1-5G6MP11 (*csrA*::*kanR malP*)	5.7 ± 0.3	1.3 ± 0.1	5.2 ± 0.5	2.2 ± 0.2
G6MD3 (Δ*glgY* Δ*malP*)	<0.2	<0.2	<0.2	<0.2
TR1-5G6MD3 (*csrA*::*kanR* Δ*glgY* Δ*malP*)	<0.2	<0.2	<0.2	<0.2

[a] Cultures were grown for 18 h in Kornberg medium containing either 0.5% glucose or maltose and were tested for glucan phosphorylase activity by using either glycogen or maltodextrin as the glucose acceptor.

(*csrA*::*kanR*) (Fig. 3) and was found to be induced in the stationary phase and negatively controlled via *csrA*. As expected, growth on maltose resulted in very strong induction of maltodextrin phosphorylase activity. This activity was found to exhibit a weak but reproducible positive effect of *csrA* (Table 2). Finally, deletion of the region of the chromosome which contains both *glgY* and *malP* (in strains G6MD3 and TR1-5G6MD3) resulted in undetectable glucan phosphorylase activity of either kind (Table 2), in support of the existing genetic assignments.

In order to determine whether the effects of *csrA* on glycogen phosphorylase specific activity (Fig. 3) were mediated at the level of genetic expression, a plasmid-encoded *glgCAY*::*lacZ* translational fusion, pYZ9, was constructed and its expression was tested in vivo in the isogenic strains BW3414 (*csrA*$^+$) and TR1-5BW3414 (*csrA*::*kanR*). Up to threefold-higher levels of expression of this fusion in the *csrA*::*kanR* mutant TR1-5BW3414 than in the isogenic strain BW3414 (data not shown) were obtained, a result which was in agreement with results of the glycogen phosphorylase assays and further indicated that *csrA* negatively affects the expression of *glgY*. To determine whether the expression of this *glgY*::*lacZ* fusion was under the direct control of the CsrA gene product, its transcription-translation was tested in S-30 extracts prepared from TR1-5BW3414 (*csrA*::*kanR*) in either the presence or the absence of affinity-purified recombinant CsrA. Figure 4 shows that the expression of the *glgY*::*lacZ* fusion and the *glgC* and *glgA* genes (which were previously shown to be regulated via CsrA in vitro [26]) was potently and specifically inhibited by CsrA.

These results support our previous hypothesis that the location of *glgY* within the *glgCAY* operon facilitates the coordinate genetic regulation of glycogen biosynthesis and catabolism (27).

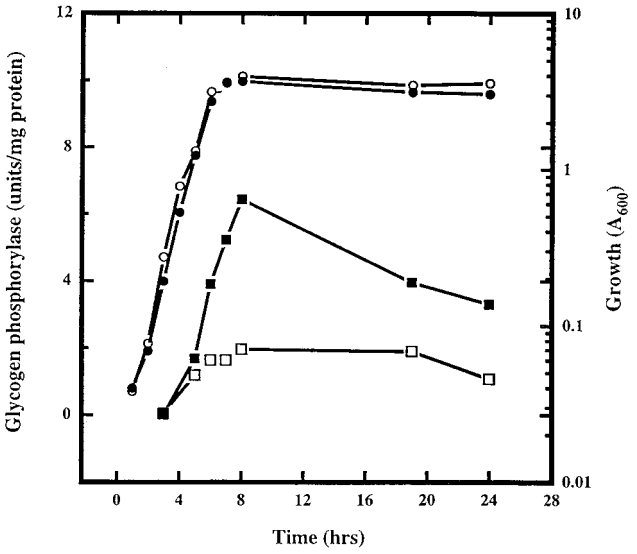

FIG. 3. Effects of *csrA* on glycogen phosphorylase activity. Cultures of G6MP11 (*malP csrA*$^+$) and TR1-5G6MP11 (*malP csrA*::*kanR*) were grown in Kornberg medium containing 0.5% glucose, and specific activity of glucan phosphorylase was determined throughout the growth curve, by using glycogen as the glucose acceptor. Glycogen phosphorylase activities in G6MP11 and TR1-5G6MP11 are shown as open and solid squares, respectively; growth rates (A_{600}) of these two strains are shown as open and closed circles, respectively.

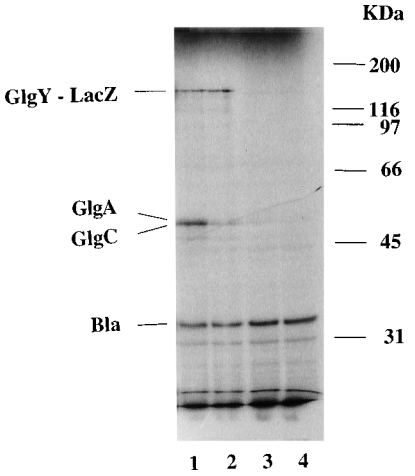

FIG. 4. Effects of CsrA gene product on in vitro expression of a *glgCAY*::*lacZ* translational fusion encoded by pYZ9. Transcription-translation reactions (35-μl volumes) were conducted by using an S-30 extract from TR1-5BW3414 (*csrA*::*kanR*), and results were analyzed as indicated in Materials and Methods. Lanes 1 through 4 depict reaction mixtures (35 μl) which contained 0, 0.1, 0.5, and 1.0 μg of CsrA protein, respectively. The positions of unlabeled standard proteins, glycogen synthase (GlgA), ADPglucose pyrophosphorylase (GlgC), myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase *b* (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), and carbonic anhydrase (31 kDa), were determined by Coomassie blue staining, and the positions of the *bla* gene product (Bla) and the *glgY*::*lacZ* gene product (GlgY-LacZ) were inferred from their respective mobilities.

TABLE 3. Glycogen debranching enzyme specific activity in *E. coli* strains

Strain (relevant description)	Glycogen debranching activity (U/mg of protein ± SD)[a]
BW3414	12 ± 1
TR1-5BW3414 (*csrA*::*kanR*)	10 ± 1
G6MD3 (Δ*malA-asd*)	<1.5
TR1-5G6MD3 (*csrA*::*kanR* Δ*malA-asd*)	<1.5
G6MD3(pUC19)	<1.5
G6MD3(pUC19) (IPTG added)	<1.5
G6MD3(pBX31) (*glgX* in pUC19, reverse)	54 ± 5
G6MD3(pBX31) (IPTG added)	50 ± 9
G6MD3(pBX49) (*glgX* in pUC19, forward)	340 ± 30
G6MD3(pBX49) (IPTG added)	1,090 ± 60

[a] Cultures were grown in Kornberg medium containing 0.5% glucose and harvested at 18 h for determination of glycogen debranching enzyme activity.

Expression of glycogen debranching enzyme, the GlgX gene product. The glycogen debranching enzyme is a second enzyme which has been reported to be involved in glycogen catabolism (11). When glycogen debranching enzyme activity in *csrA*$^+$ and *csrA*::*kanR* strains was tested, no significant difference was observed (Table 3), indicating that *csrA* does not affect the expression of the debranching enzyme. Perhaps the relatively lower number of α-1,6 linkages (approximately 10% relative to the α-1,4 linkages), which are the substrate for the glycogen debranching enzyme, and the lower rate of glycogen catabolism relative to synthesis in *E. coli* do not require *glgX* to be regulated by factors such as CsrA, which modulate the expression of the other *glg* genes.

The structural gene which encodes debranching enzyme had not been previously mapped or cloned. Interestingly, we found that strains which lack the region of the chromosome which contains the *glg* gene cluster (G6MD3 and TR1-5G6MD3) did not express detectable levels of glycogen debranching enzyme (Table 3). This suggested that the debranching enzyme may be encoded by *glgX*, the one remaining unassigned open reading frame within the *glg* gene cluster. When *glgX* was cloned into the multiple cloning site of pUC19 in each orientation with respect to the *lacZ* promoter, the resulting plasmids caused glycogen debranching activity to be expressed at extremely high levels in G6MD3 (Table 3). The level of debranching activity from plasmid pBX49, in which the *lacZ* promoter of the vector is oriented to drive transcription of *glgX*, was significantly higher than that of pBX31, and only the activity from pBX49 was inducible by IPTG. On the basis of its previously determined specific activity (11), debranching enzyme represents approximately 3% of the total protein in the strain containing pBX49. As was expected for the *E. coli* debranching enzyme, less than 6% of the activity seen to occur on phosphorylase limit dextrin was observed when glycogen was used as a substrate (data not shown). Taken together with the known sequence homology of GlgX to glucanases (27), these results indicate that *glgX* encodes the *E. coli* glycogen debranching enzyme.

Conclusions. During growth, glycogen is synthesized at the time when a nutrient such as nitrogen becomes scarce but a carbon source remains available. The present study provides the first evidence that the localization of biosynthetic and catabolic genes within the two tandem operons of the *E. coli glg* gene cluster, *glgBX* and *glgCAY*, facilitates the coordinate genetic expression of enzymes involved in glycogen biosynthesis and catabolism. This coordinate expression may permit the bacterium to prepare for impending starvation by expressing the important catabolic enzyme glycogen phosphorylase (GlgY), along with glycogen synthase and ADPglucose pyrophosphorylase, in the early stationary phase, before biosynthetic precursors for protein synthesis have been expended. The mechanism by which CsrA negatively regulates *glgY* expression is not precisely known. CsrA is an RNA-binding protein which is necessary for the predominant decay pathway of *glgC* mRNA (16), and mobility shift data show that CsrA interacts specifically with the 5′ region of this message (15). If the polycistronic *glgCAY* transcript is not segmentally processed, regulation of *glgY* expression by CsrA may be accomplished quite simply through the net 5′-to-3′ decay of this transcript. We are currently attempting to elucidate the details of this unconventional mechanism of genetic regulation and to further define the role of CsrA in modulating the metabolism and physiological properties of bacteria.

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