Pleiotropic Regulation of Central Carbohydrate Metabolism
in Escherichia coli via the Gene csrA*

Nirupama A. Sabnis‡, Honghui Yang§, and Tony Romeo‡§

From the Departments of ‡Microbiology and Immunology and §Biochemistry and Molecular Biology,
University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76107-2699

The carbon storage regulator gene csrA has been shown previously to dramatically affect the biosynthesis of intracellular glycogen in Escherichia coli through its negative control of the expression of two glycogen biosynthetic operons and the gluconeogenic gene pckA (Romeo, T., Gong, M., Liu, M. Y., and Brun-Zinkernagel, A. M. (1993) J. Bacteriol. 175, 4744–4755). Examination of the effects of csrA on several enzymes, genes, and metabolites of central carbohydrate metabolism now establishes a more extensive role for csrA in directing intracellular carbon flux. Phosphoglucomutase and the gluconeogenic enzymes fructose-1,6-bisphosphatase and phospho(en)pyruvate synthetase were found to be under the negative control of csrA, and these enzyme activities were maximal during the early stationary phase of growth. The enzymes glucose-6-phosphate isomerase, triose-phosphate isomerase, and enolase were positively regulated by csrA. Thus, csrA exerts reciprocal effects on glycolysis versus gluconeogenesis and glycogen biosynthesis. The glycolytic isozymes pyruvate kinase F and A (encoded by pykF and pykA, respectively) and phosphofructokinases I and II (pfkA and pfkB, respectively) exhibited differential regulation via csrA. Since the individual members of these isozyme pairs are allosterically regulated by different cellular metabolites, csrA is also capable of fine-tuning the allosteric regulation of glycolysis. In contrast, the expression of genes of the pentose phosphate pathway was weakly or negligibly affected by csrA.

* This work was supported by Research Grant MCB 9218796 from the National Science Foundation (to T. R.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Dept. of Microbiology and Immunology, University of North Texas Health Science Center at Fort Worth, 3500 Camp Bowie Blvd., Fort Worth, TX 76107-2699. Tel.: 817-735-2121; Fax: 817-735-2118.

‡ The abbreviations used are: PfK, 6-phosphofructokinase; FBP, fructose-1,6-bisphosphatase; FBP, fructose-1,6-bisphosphatase; PEP, phosphoenolpyruvate; Psp, phosphoenolpyruvate synthetase; Pgm, phosphoglucomutase; Pgi, glucose-6-phosphate isomerase; Tpi, triose-phosphate isomerase; Eno, enolase; Pyk, pyruvate kinase; Pdk, phosphoenolpyruvate carboxykinase; bp, base pair(s).

Although the allosteric regulation of central carbohydrate metabolism has been well studied in E. coli and in other bacteria, its genetic regulation has not. The structural genes of these pathways are generally regarded to be constitutively expressed (1). While it is true that the levels of these enzymes do not change dramatically in response to various physiological requirements, it is also clear that the levels of many, if not all, of these enzymes respond to conditions such as oxygen availability and growth rate (11–16), suggesting that the genetic regulation of these pathways is also physiologically significant.

During the transition from exponential growth into stationary phase the demand for biosynthetic metabolism decreases and E. coli as well as many other bacteria rapidly convert available carbohydrate into glycogen, which appears to function as a source of stored carbon and energy. The regulation of glycogen synthesis is complex and includes both allosteric and genetic components (reviewed in Refs. 17–20). Our laboratory recently discovered a regulatory gene, csrA, which dramatically affects the biosynthesis of glycogen. A csrA::kanR insertion mutation results in the accumulation of approximately 20-fold higher levels of glycogen, which can reach a level of 1.6 mg of glycogen/mg of protein in the early stationary phase in this mutant (21). The csrA gene was found to negatively control the expression of the two structural genes of the glycogen biosynthetic pathway, glgC encoding ADP-glucose pyrophosphorylase (EC 2.7.7.27) and glgB encoding glycogen branching enzyme (EC 2.4.1.18), as well as the gluconeogenic gene phosphoenolpyruvate carboxykinase (EC 4.1.1.49) (PckA). The gene csrA was mapped at 58 min on the E. coli genome, between altS and serV (22), and was shown to encode a 61-amino acid protein, CsrA (21). Recent studies on the mechanism of csrA-mediated regulation of glgC have shown that the CsrA protein greatly enhances the decay of glgC mRNA, an effect that involves the region overlapping or close to the ribosome binding site of glgC (23). The deduced amino acid sequence of the CsrA gene product was found to contain a KH domain, which has been proposed to function as an RNA-binding region of a diverse subset of RNA-binding proteins (24).

Because the csrA::kanR mutation also affects cell surface properties, as exhibited by the adherence of mutant cells to glassware, and because the regulation of glycogen biosynthesis by csrA is mediated independently of the known global regulators of the glycogen operon glgCAP, cAMP, and ppGpp (25, 26,
39), it was previously suggested that csrA may encode a component of a novel global regulatory system (21). The present study is consistent with this possibility and firmly establishes a role for csrA in the regulation of central carbohydrate metabolism.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains, Bacteriophage, and Plasmids**—The relevant properties of the strains, phage, and plasmids used in the present study are listed in Table I.

**TABLE I**

| Strain/phage/plasmid | Genotype/relevant characteristics | Source and/or Ref. |
|----------------------|----------------------------------|-------------------|
| E. coli K-12 strain  |                                  |                   |
| BW3414               | lA derivative, lacA169            |                   |
| TR1-5<sup>a</sup>    | csrA::kanR                      |                   |
| G6MD3                | Hfr his thi str-<sup>a</sup> (malA-asd) |                   |
| H9534                | W3110(1 argF-lacU169)            |                   |
| HB585                | W3110(1 argF-lacU169)           |                   |
| HB301(aDR52)         | Δ(lacZF1 lacZ126)                |                   |
| HB301(aDR104)        | Δ(lacZF1 lacZ126)                |                   |
| SB588                | ΔpykF- lacA2, Δlysoyzac        |                   |
| SB589                | Δlysoyzac                      |                   |
| Phage                | P1vir                           |                   |
| Plasmid              | pUC19                            |                   |
|                      | ccsA<sup>-</sup> cloned into pUC19 |                   |
|                      | pCSR10                           |                   |

<sup>a</sup> A strain designation containing the prefix TR1–5 indicates that the wild type (csrA<sup>-</sup>) allele has been replaced by the TR1-5 mutant allele (csrA::kanR) by P1vir transduction.

39), it was previously suggested that csrA may encode a component of a novel global regulatory system (21). The present study is consistent with this possibility and firmly establishes a role for csrA in the regulation of central carbohydrate metabolism.

**Experimental Procedures**

**Bacterial Strains, Bacteriophage, and Plasmids**—The relevant properties of the strains, phage, and plasmids used in the present study are listed in Table I.

**Media, Growth Conditions, and General Procedures**—Cultures were inoculated for growth curves by adding 1 volume of an overnight culture to 400 volumes of freshly prepared Kornberg medium (containing 0.5% glucose) and were grown at 37 °C with rapid gyratory shaking. For detection of endogenous glycogen, cultures were streaked onto Kornberg agar medium containing 1% glucose, grown overnight at 37 °C, and stained with iodine vapor (21). MacConkey agar (Difco) was used to determine the Lac phenotype. Ampicillin (100 μg/ml), kanamycin (100 μg/ml), and diaminopimelic acid (50 μg/ml) were sterilized by filtration and were included as needed in the media. To study enzyme activities throughout the growth cycle, cells were harvested at the indicated time points by centrifugation at 4 °C, washed twice in the appropriate buffer, as described in the corresponding references below, weighed, and stored at −80 °C until use. The cells (0.5 g) were thawed and suspended in 10 ml of lysing buffer and disrupted using an ice-cold French pressure cell at 10,000 p.s.i. The cell lysate was collected on ice and centrifuged for 10 min at 14,000 × g. The supernatant solution was used to determine enzyme activity on the day of preparation and was assayed for total protein. Each of these experiments was performed in entirety at least twice.

**Enzyme and Protein Assays**—Previously published methods were used to assay the activities of fructose-1,6-bisphosphatase (31), phosphofructokinase (32), phosphoglucomutase (Pgm) (EC 5.4.2.2) (33), glucose-6-phosphate isomerase (Pgi) (EC 5.3.1.9) (34), phosphofructokinases I and II (2), triose-phosphate isomerase (Tpi) (EC 5.3.1.1) (35), enolase (Eno) (EC 4.2.1.11) (36) and pyruvate kinases F and A (2). Each of the respective reactions was started by the addition of enzyme extract and was coupled to the reduction of NADH or the oxidation of NADPH, following the change in absorbance at 340 nm in a Gilford recording spectrophotometer, with the exception of the phosphoenolpyruvate carboxykinase, pckA, was elevated approximately 2-fold in a csrA::kanR mutant. Second, a multicopy plasmid which overexpresses the csrA gene, pCSR10, resulted in the inability of strains to grow on gluconogenic compounds as sole sources of carbon (21). To further explore the potential role of csrA in regulating gluconeogenesis, the effects of csrA on the specific activities of Fbp and Pps were determined. Fig. 1 (A and B) shows that Fbp specific activity increased from 7- to 10-fold as cultures progressed from the exponential phase into early stationary phase of growth and thereafter declined to mid-log levels. During the early stationary phase, the levels of Fbp were approximately 1.5–2-fold higher in the csrA::kanR mutant than in the isogenic csrA<sup>-</sup> strain. As shown in Fig. 1B, the effects of the csrA::kanR mutation on Fbp were complemented by pCSR10. In fact, pCSR10-containing cells possessed significantly lower levels of Fbp activity than the csrA<sup>-</sup> strain. The specific activity of Pps was 2–4-fold higher in the csrA::kanR mutant, and similar to that of Fbp, it was highest in both strains during the early stationary phase (Fig. 1C).

Both gluconeogenesis and the uptake of glucose via the phosphoenolpyruvate-dependent phosphotransferase system generate glucose 6-phosphate in the cell, which must be converted by the enzyme Pgm into glucose 1-phosphate to be used as a biosynthetic precursor of polysaccharides such as glycogen. The specific activity of Pgm was up to 4-fold higher in the csrA::kanR strain relative to the isogenic csrA<sup>-</sup> strain (Fig. 1D). Pgm activity in both strains increased approximately 2-fold as cultures entered the stationary phase. The kinetics of expression of Fbp, Pps, and Pgm in the csrA::kanR mutant were approximately parallel to those of the isogenic csrA<sup>-</sup> strain, as shown previously for the expression of the glycogen biosynthetic genes and pckA, indicating that the regulation of these systems by csrA is superimposed on the station-
ary phase control (21).

Positive Regulation of Glycolysis by csrA—Carbon flux through the Embden-Meyerhof pathway must be regulated if a glycolytic/gluconeogenic futile cycle is to be avoided. In view of the impact of csrA on glycogen biosynthesis and on the gluconeogenic enzymes, it was of interest to determine whether csrA affects the activities of the glycolytic enzymes. The specific activities of three of the bidirectional enzymes of this pathway, Pgi, Tpi, and Eno, were determined throughout the growth curve, as were the unidirectional, allosterically regulated glycolytic enzymes, PfkI, PfkII, PykA, and PykF.

Fig. 2 shows that Pgi (A), Tpi (B), and Eno (C) activities were from 1.5- to 3-fold higher in the csrA1 strain relative to the csrA::kanR strain throughout the growth curve, indicating that these enzymes are under positive control of csrA. Comparison of Figs. 1 and 2 also shows that the specific activities of the glycolytic enzymes were much higher than the gluconeogenic enzymes. This is consistent with the idea that levels of Embden-Meyerhof enzymes generally are present in significantly higher levels to meet the glycolytic requirements relative to the gluconeogenic requirements and indicates that csrA is a positive regulator of glycolysis.

In E. coli, phosphofructokinase exists as two isozymes, PfkI and PfkII, which differ in their allosteric properties (1, 2). The effect of csrA on each isozyme was examined, and the specific activity of the major isozyme, PfkI, was found to be significantly higher (up to 7.4-fold) in the csrA+ parent strain relative to the csrA::kanR mutant (Fig. 3A). In contrast, the activity of PfkII (Fig. 3B) was higher (1.3–4-fold) in the mutant than in the wild type strain. Therefore, these two glycolytic isozymes exhibited reciprocal regulation by csrA. In the wild type strain, PfkI represented approximately 80% of the total Pfk activity throughout the growth curve. In the csrA::kanR mutant, PfkI constituted approximately 30% of the total Pfk activity as the culture entered stationary phase. Therefore, under conditions that were optimal for glycogen synthesis, i.e. in the csrA::kanR mutant during the early stationary phase of growth, PfkI was the predominant isozyme.

Fig. 3 (C and D) also shows the specific activities of the pyruvate kinase isozymes PykF and PykA in the csrA+ and the csrA::kanR strains. PykF activity was 4.3–9.4-fold higher in the wild type strain during the growth curve. However, PykA levels were similar in the two strains. Therefore, under the conditions of these studies, csrA appears to positively regulate the levels of PykF without altering the level of PykA.

In order to test whether the changes observed in the levels of these enzymes are due to the effect of csrA on the expression of their structural genes, β-galactosidase activity expressed from chromosomally encoded pykF-1acZ or pykA-1acZ translational fusions was measured in isogenic csrA+ and csrA::kanR strains (Fig. 4). β-Galactosidase expressed from the pykF-1acZ fusion was up to 3.8-fold higher in the csrA+ strain versus the csrA::kanR mutant (Fig. 4A). On the other hand, no appreciable difference was observed in the expression of the pykA-1acZ fusion in the relevant strains (Fig. 4B). Therefore, csrA determines PykF specific activity by enhancing pykF gene expression, rather than by altering PykF enzymatic activity.

Complementation of the csrA::kanR Mutation by pCSR10—It was possible that the effects of the csrA::kanR mutation on the
plasmid pCSR10 also complemented the effect of the csrA::kanR mutation on the levels of the enzymes that were observed to be positively regulated via csrA, Pgi, Tpi, PKl, Eno, and PykF. However, overexpression of csrA from pCSR10 did not result in greater than wild type expression of these enzymes. It may be concluded from the results of this experiment that the inactivation of the csrA gene itself was responsible for the effects of the csrA::kanR mutation on gluconeogenic and glycolytic enzyme levels.

Effect of csrA on Enzymes Expressed in a Δ-glg Mutant—To determine whether the csrA::kanR mutation alters levels of glycolytic and gluconeogenic enzymes as an indirect consequence of the dramatic effect that it has on carbon flux into glycogen (21), the csrA::kanR mutation was transduced into G6MD3, a strain in which the glgBX and glgC operons have been deleted and which is incapable of synthesizing either glycogen or ADP-glucose. Levels of glycolytic and gluconeogenic enzymes were assayed in the resulting isogenic strains. Table III summarizes these experiments and shows that at 8 h the specific activities of Fbp, Pps, Pgm, and PKlII were 2.3-, 2.5-, 1.6-, and 2-fold higher in the csrA::kanR mutant than in the csrA+ strain. Specific activities of Pgi, PKl, Tpi, Eno, PykF, and PykA were 2.1-, 6.0-, 1.4-, 2.0-, 2.8-, and 1.1-fold higher in the wild type strain as compared with the csrA::kanR mutant. These values are reasonably consistent with values from the strains that synthesize glycogen (Figs. 1–3) and show that the effects of csrA on glycolytic and gluconeogenic enzyme levels is not a secondary regulatory response to the enhanced carbon flux toward glycogen in the csrA::kanR mutant. Of course, these results do not exclude the possibility that intracellular metabolites other than those affected by glycogen synthesis may serve to indirectly mediate the effects of csrA on the levels on one or more of these enzymes.

Effect of csrA on the Expression of Genes of the Pentose Phosphate Pathway—The pentose phosphate pathway provides NADPH and biosynthetic intermediates for the cell, and two genes of the pathway, zwf, encoding glucose-6-phosphate dehydrogenase, and gnd, encoding 6-phosphogluconate dehydrogenase, have been shown to exhibit growth rate-regulated expression (28, 40). The possibility that csrA regulates the expression of these two genes was tested by determining β-galactosidase specific activities expressed from the chromosomally encoded gnd-’lacZ transcriptional and translational fusions of strains HB354 and HB582, respectively, and zwf-’lacZ transcriptional and translational fusions of strains HB301[ΔDR52] and HB301[ΔDR104], respectively, in isogenic csrA+ and csrA::kanR strains throughout their growth curves (data not shown). The gnd-’lacZ translational fusion exhibited very weak but reproducible positive regulation by csrA, i.e. slightly higher expression was observed in the csrA+ strain. No appreciable effect was observed for the zwf-’lacZ transcriptional or translational fusions or the gnd-’lacZ transcriptional fusion, indicating that at least under our experimental conditions, csrA is probably not a significant regulator of the pentose phosphate pathway.

Effects of csrA on Metabolite Levels and Energy Charge—The above observations established that csrA is an important modulator of intermediary carbohydrate metabolism in E. coli. Further evidence for such a role was obtained by measuring the levels of five key intracellular metabolites in csrA+ and csrA::kanR strains. Tables IV and V show the effects of the csrA mutation on adenylates and energy charge, FBP and PEP. The metabolite levels and energy charge in the csrA+ strain were similar to those previously determined for E. coli (38, 42). On the other hand, the csrA::kanR mutant had lower levels of ATP and higher levels of AMP and ADP throughout the growth
The intracellular concentrations of FBP and PEP were both elevated in the csrA::kanR mutant. The decrease in energy charge in the mutant is consistent with a decrease in glycolysis and an increase in gluconeogenesis and glycogen synthesis and was predictable based upon the effect of the mutation on enzyme specific activities. The elevated level of PEP in the mutant reflects enhanced synthesis of this metabolite via Pps and Pck, as well as a decrease in its utilization by Pyk. The elevated FBP levels appear to result from a decrease in glycolytic flux following the Pfk reaction, and in particular, the effect of the csrA::kanR mutation on the expression of pykF is probably important.

**DISCUSSION**

The pleiotropic phenotype of a csrA::kanR insertion mutant originally indicated that the role of csrA in E. coli is broader...
Effects of csrA on Carbohydrate Metabolism

TABLE II

Complementation of the TR1-5 mutation (csrA::kanR) by pCSR10 (csrA−)
Specific enzyme activities were determined in stationary phase (24 h) cultures of E. coli BW341[pUC19], TR1–5BW341[pUC19], and TR1–5BW341[pCSR10] grown in Kornberg medium as described under “Experimental Procedures.”

| Enzyme                          | Specific activity |
|---------------------------------|------------------|
|                                 | BW[pUC19] | TRBW[pUC19] | TRBW[pCSR10] |
| Fructose-1,6-bisphosphatase     | 0.001 ± 0.0000 | 0.0019 ± 0.0000 | 0.0001 ± 0.0000 |
| PEP synthetase                  | 0.0164 ± 0.0001 | 0.040 ± 0.000 | 0.0129 ± 0.000 |
| Phosphoglucomutase              | 0.297 ± 0.001   | 1.26 ± 0.005  | 0.204 ± 0.000  |
| Glucose-6-P-isomerase           | 1.48 ± 0.03     | 0.89 ± 0.01   | 1.15 ± 0.00    |
| Triose-phosphate isomerase      | 12.3 ± 0.0      | 7.05 ± 0.00   | 12.7 ± 0.00    |
| Enolase                         | 1.34 ± 0.0001   | 0.574 ± 0.001 | 1.37 ± 0.02    |
| Phosphofructokinase I           | 0.115 ± 0.000   | 0.014 ± 0.000 | 0.080 ± 0.000  |
| Phosphofructokinase II          | 0.013 ± 0.000   | 0.035 ± 0.000 | 0.012 ± 0.000  |
| Pyruvate kinase F               | 0.494 ± 0.002   | 0.055 ± 0.000 | 0.445 ± 0.001  |
| Pyruvate kinase A               | 0.216 ± 0.000   | 0.161 ± 0.000 | 0.185 ± 0.000  |

TABLE III

Effect of csrA on specific enzyme activities in G6MD3 (Δg6p)
Specific enzyme activities were determined in early stationary phase (8 h) cultures of E. coli strains G6MD3 (csrA−) and TR1–5G6MD3 (csrA::kanR) grown in Kornberg medium as described under “Experimental Procedures.”

| Enzyme                          | Specific Activity |
|---------------------------------|------------------|
|                                 | G6MD3      | TR1–5G6MD3 |
| Fructose-1,6-bisphosphatase     | 0.017 ± 0.000 | 0.043 ± 0.000 |
| PEP synthetase                  | 0.021 ± 0.000 | 0.051 ± 0.000 |
| Phosphoglucomutase              | 1.21 ± 0.0   | 2.03 ± 0.00  |
| Glucose-6-P-isomerase           | 0.23 ± 0.000  | 0.11 ± 0.00  |
| Triose-phosphate isomerase      | 10.5 ± 0.000  | 4.2 ± 0.00  |
| Enolase                         | 1.82 ± 0.000  | 0.93 ± 0.00  |
| Phosphofructokinase I           | 0.12 ± 0.0000 | 0.020 ± 0.00  |
| Phosphofructokinase II          | 0.011 ± 0.0000 | 0.048 ± 0.00  |
| Pyruvate kinase F               | 0.23 ± 0.0000 | 0.080 ± 0.000 |
| Pyruvate kinase A               | 0.090 ± 0.0000 | 0.080 ± 0.000 |

which allow optimum glycogen synthesis in the early stationary phase. An important implication of this study is that under these conditions gluconeogenesis occurs during a fairly restricted period of time, coincident with glycogen biosynthesis, indicating that the primary role of gluconeogenesis in glucose-grown cells is to enhance glycogen synthesis. The growth-phase response demonstrated here for the gluconeogenic enzymes should help the cell to conserve energy. A futile cycle of gluconeogenesis and glycogen synthesis would be prevented or at least minimized in the exponential phase; the conversion of carbohydrate into endogenous glycogen should be favored as cells enter the stationary phase; and later in the stationary phase, during glycolysis, a futile cycle of glycogen synthesis would again be avoided.

The decrease in the activities of the gluconeogenic enzymes which occurs later in stationary phase indicates that enzyme inactivation is also an important determinant of the gluconeogenic capacity of a cell. This may involve the specific inactivation of these enzymes later in the stationary phase or these enzymes may be labile throughout the growth cycle and their patterns of activity in the growth curve reflect genetic expression. The induction profile of a pckA−lacZ transcriptional fusion (21, 43) favors the latter alternative. In fact, the cell regulates the levels and activities of the gluconeogenic enzymes in a variety of ways, including (i) regulation at the level of transcript initiation via cAMP and the repressor of the PEP:fructose phosphotransferase system, FruR (43, 44 and references therein), (ii) allosteric control of Fbp by AMP and potential allosteric control of Pck by calcium ions and by ATP and PEP (10, 45, 46), and (iii) based upon extrapolation from studies on the mechanism of glgC regulation via CsrA (23), post-transcriptional control of mRNA stability may also be an important determinant of the gluconeogenic capacity of the cell.

A surprising finding of these studies was that the isozymes which catalyze the phosphofructokinase and pyruvate kinase reactions are differentially affected by csrA. In both cases the individual isozymes have been shown to be allosteric (2–9). They exhibit distinct and fairly complex regulation via several intracellular metabolites and are also expressed differently in intracellular carbon flux by observing the effects of disrupting or overexpressing csrA on the enzymes of central carbohydrate metabolism. The csrA gene has now been shown to affect several of these essential enzymes and thereby modulate intracellular carbon flux on a wide scale in E. coli. As summarized in Fig. 5, csrA exerts reciprocal effects on enzymes of glycolysis versus those of gluconeogenesis and glycogen biosynthesis. The studies on adenylyl energy charge and metabolite levels presented here provide additional evidence of the role of csrA in determining central carbohydrate flux.

Previous experiments had suggested that csrA negatively regulates gluconeogenesis (21). This hypothesis is supported by the current study, which indicates that Fbp, Pps, and Pgm are negatively regulated by csrA. This study also shows that these activities are induced in the stationary phase, as was shown previously to be true for Pck by Goldie (43). An earlier study had also indicated that Fbp activity is higher in stationary phase than in exponential phase in glucose-grown cells, although the difference was less than observed here, approximately 2-fold (31). In the current study, gluconeogenic enzyme activities were found to increase sharply in early stationary phase and thereafter decrease to pre-stationary phase levels. Since this response was not known previously for Fbp, the greater stationary phase induction of Fbp observed in our studies (7–10-fold) may be explained by the possibility that in the earlier experiment (31) the culture was harvested later in the stationary phase, after Fbp levels had dropped. The experiments of the current study were conducted under conditions than the control of glycogen synthesis (21). The primary goal of the current study was to explore the potential involvement of csrA in the regulation of intracellular carbon flux by observing the effects of disrupting or overexpressing csrA in E. coli.
Effects of csrA on Carbohydrate Metabolism

Table IV
Metabolite levels in BW3414 (csrA⁻) and TR1-BW3414 (csrA::kanR) strains of E. coli
Cultures were grown in Kornberg medium as described under “Experimental Procedures.”

| Time | Strain | Metabolite | ATP (μmol/g protein) | ADP (μmol/g protein) | AMP (μmol/g protein) | PEP (μmol/g protein) | FBP (μmol/g protein) |
|------|--------|------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| h    | BW     | 4.26 ± 0.95| 2.03 ± 0.05          | 0.05 ± 0.01          | 0.03 ± 0.02          | 0.04 ± 0.01          | 0.14 ± 0.02          |
|      | TR     | 5.36 ± 1.06| 3.27 ± 0.08          | 0.10 ± 0.02          | 0.06 ± 0.02          | 0.11 ± 0.03          | 0.23 ± 0.04          |

*The transition from exponential growth into stationary phase occurred between 5 and 6 h.

Table V
Calculated intracellular metabolite concentrations and adenylate energy charge in BW3414 (csrA⁻) and TR1-BW3414 (csrA::kanR) strains of E. coli

| Time | Strain | Metabolite concentrations | ATP (μmol/g protein) | ADP (μmol/g protein) | AMP (μmol/g protein) | PEP (μmol/g protein) | Energy charge |
|------|--------|---------------------------|----------------------|----------------------|----------------------|----------------------|--------------|
| h    | BW     |                           | 4.45 ± 0.95          | 2.03 ± 0.05          | 0.05 ± 0.01          | 0.03 ± 0.02          | 0.14 ± 0.02 |
|      | TR     |                           | 5.36 ± 1.06          | 3.27 ± 0.08          | 0.10 ± 0.02          | 0.06 ± 0.02          | 0.23 ± 0.04 |

a Metabolite concentrations were calculated from the data shown in Table IV, assuming that protein constitutes 55% of the dry weight, 70% of the protoplasm is water, the density of protoplasm is 1.1, and there is no compartmentalization of metabolite pools (41).
b Adenylate energy charge is defined as the concentration ratio of ATP + 0.5ADP/ATP + ADP + AMP (42).

On the other hand, PfkI1 has generally been considered to be dispensable to the cell, since a pknB mutant has no observable growth defect. However, it should be emphasized that the PfkI1 isozone is capable of performing this step of glycolysis, and suppressors of the pknA mutation have been isolated and shown to result from the overexpression of pknB (47). The current studies show that the “minor“ form of Pfk (PfkI1) predominates when csrA has been disrupted. We may conclude that a pknB⁺ cell has the capacity to produce more PfkI1 than normally is present and that the elevation of PfkI1 in the csrA::kanR strain is not a compensatory response to the decrease in PfkI.

In viewing our findings in relation to those of others (2), it was noted that in addition to being positively regulated via csrA, the isozones PfkI and PykF are related in other ways. (i) They are allosterically strongly regulated by glycolytic intermediates, PEP in the former case and FBP in the latter. The isozones which are either under negative control or unregulated by csrA, PfkI1 and PykA, respectively, are allosterically regulated by other kinds of metabolites or show very weak effects of glycolytic intermediates. (ii) The expression of PfkI and PykF was also regulated according to the carbohydrate source used for growth, glucose being superior to pyruvate in both cases (2). The expression of the other two isozones shows at best minimal effects of different carbon sources. Clearly, positive regulation of gene expression via csrA favors that glycolysis is responsive to intermediates of carbohydrate metabolism. Neither the pentose phosphate genes nor the glycolytic isozone PykA, which can be allosterically activated by ribose phosphate, are substantially regulated via csrA.

We have previously hypothesized that csrA is part of an adaptive response pathway (21), which carries the implicit assumption that the csrA::kanR mutation reflects a physiological condition under which this pathway is inactive. When csrA is inactivated glycolysis decreases, but it is still needed to provide ATP for the cell, while flux through the gluconeogenesis and glycogen biosynthesis pathways is increased. Thus, it is significant that when csrA is inactivated, PfkI1 becomes the major Pfk isozone. PfkI1 is activated by ADP or other NDPs and is strongly inhibited by low concentrations of PEP (2). It has been shown that PEP levels can become quite high under
involved differences in anaerobic versus aerobic conditions, and were excluded from being involved. Regulation via ccrA::kanR is dated. However, two potential physiological conditions can be favored glycogen synthesis.

does not regulate gene expression in response to the growth phase, since the disruption of ccrA did not alter the temporal regulation of the glg genes (21) or the glycogenolytic enzymes. An additional relevant observation is that ccrA regulates some genes that are induced in the stationary phase and others that are expressed only in the exponential phase.

The product of the ccrA gene is capable of exerting either positive or negative effects on gene expression and is thus able to regulate gluconeogenesis and glycolysis in an opposite fashion. Studies are in progress to determine whether ccrA regulates additional metabolic pathways. While the CcrA gene product is directly involved in the negative regulation of gene expression, a direct role in the positive regulation of genes (e.g. pykF) remains to be established. The complementation studies described herein present one kind of evidence suggesting that genetic activation via ccrA may be intrinsically different from inhibition. When the ccrA gene was overexpressed from the plasmid pCS10, the negatively regulated genes were consistently found to be expressed at levels lower than in the ccrA" strain. On the other hand, none of the genes which are apparently subject to positive regulation by ccrA were expressed at higher levels in the pCS10-containing strain than in the wild type ccrA" strain. These and many other questions concerning the biological function and mechanism of ccrA remain to be answered.

Acknowledgments—We are grateful for the generous gifts of strains by the individuals listed in Table I, especially Stefan Bledig (NSC Technologies), who provided strains prior to publication. We thank Bob Gracey and Paul Cook and Bill Karsten who provided instrumentation and/or advice for conducting some of the enzyme and metabolite assays. We also thank Wayne Nicolson, Umit Yuksel, and Mark Hart for providing critiques of the manuscript.

REFERENCES

1. Fraenkel, D. G. (1987) Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology (Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M., and Umbarger, H. E., eds) Vol. 1, pp. 142-150, American Society for Microbiology, Washington, D. C.

2. Kotlarz, D., Garreau, H., and Buc, H. (1975) Biochim. Biophys. Acta 381, 257-268

3. Waygood, E. B., Mort, J. S., and Sanwal, B. D. (1976) Biochemistry 15, 277-282

4. Kotlarz, D., and Buc, H. (1976) Eur. J. Biochem. 117, 569-574

5. Blangy, D., Buc, H., and Monod, J. (1969) J. Mol. Biol. 31, 13-35

6. Balub, J. (1978) J. Biol. Chem. 253, 4330-4335

7. Raicu, M., and Valentin, M. (1977) J. Biol. Chem. 252, 170-179

8. Garrido-Pertierra, A., and Cooper, R. A. (1977) J. Bacteriol. 129, 1208-1214

9. Garrido-Pertierra, A., and Cooper, R. A. (1983) FEBS Lett. 162, 420-422

10. Fraenkel, D. G., Pontremoli, S., and Horecker, B. L. (1966) Arch. Biochem. Biophys. 114, 4-12

11. Farrish, E. E., Baker, H. V., II, and Wolf, R. E., Jr. (1982) J. Bacteriol. 152, 594-594

12. Hidy, N., and Doolle, H. W. (1976) Microbiol. 17, 23-33

13. Rechelt, J. L., and Doolle, H. W. (1971) Antonie van Leeuwenhoek J. Microbiol. Ser. 37, 497-506

14. Thomas, A. D., Doelle, H. W., Westwood, A. W., and Gordon, G. L. (1972) J. Bacteriol. 112, 1099-1105

15. Smith, M. W., and Neidhardt, F. C. (1983) J. Bacteriol. 154, 336-343

16. Doolle, H. W., and Hidy, N. W. (1978) Eur. J. Biochem. 83, 479-484

17. Krebs, E. G., and Preiss, J. (1975) MTP Int. Rev. Sci. Carbohydr. Biochem. Ser. 1, 337-389

18. Press, J. (1984) Annu. Rev. Microbiol. 38, 419-458

19. Press, J., and Romeo, T. (1989) Adv. Microb. Physiol. 30, 183-233

20. Press, J., and Romeo, T. (1994) Prog. Nucleic Acids Res. Mol. Biol. 47, 299-329

21. Romeo, T., Gong, M., Li, M. Y., and Brun-Zinkernagel, A.-M. (1993) J. Bacteriol. 175, 4744-4755

22. Romeo, T., and Gong, M. (1993) J. Bacteriol. 175, 5740-5741

23. Liu, M. Y., Yang, H., and Romeo, T. (1993) J. Bacteriol. 175, 2663-2672

24. Gibson, T. J., Thompson, J. D., and Heringa, J. (1993) FEBS Lett. 324, 361-366

25. Romeo, T., and Preiss, J. (1990) J. Bacteriol. 171, 131-137

26. Romeo, T., and Preiss, J. (1989) J. Bacteriol. 171, 2773-2782

27. Schwartz, M. (1966) J. Bacteriol. 92, 1083-1089
29104  Effects of csrA on Carbohydrate Metabolism

28. Rowley, D. L., Pease, A. J., and Wolf, R. E., Jr. (1991) J. Bacteriol. 173, 4660–4667
29. Singer, M., Baker, T. A., Schnitzler, G., Delschel, S. M., Goel, M., Dove, W., Jaacks, K. J., Grossman, A. D., Erickson, J. W., and Gross, C. A. (1989) Microbiol. Rev. 53, 1–24
30. Yanish-Perron, C., Vieira, J., and Messing, J. (1985) Gene (Amst.) 33, 103–119
31. Fraenkel, D. G., and Horecker, B. L. (1965) J. Bacteriol. 90, 837–842
32. Cooper, R. A., and Kornberg, H. L. (1969) Methods Enzymol. 13, 309–314
33. Adhya, S., and Schwartz, M. (1971) J. Bacteriol. 108, 621–626
34. Fraenkel, D. G., and Levsohn, S. R. (1967) J. Bacteriol. 93, 1571–1578
35. Rozacky, E. E., Sawyer, T. H., Barton, R. A., and Gray, R. W. (1971) Arch. Biochem. Biophys. 146, 312–320
36. Maitra, P. K., and Lobo, Z. (1971) J. Biol. Chem. 246, 475–488
37. Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K.,gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Klenk, D. C. (1985) Anal. Biochem. 150, 76–85
38. Lowry, O. H., Carter, J., Ward, J. B., and Glaser, L. (1971) J. Biol. Chem. 246, 6511–6521
39. Romeo, T., Kumar, A., and Preiss, J. (1988) Gene (Amst.) 70, 363–376
40. Pease, A. J., and Wolf, R. A., Jr. (1994) J. Bacteriol. 176, 115–122
41. Neidhardt, F. C. (1987) in Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology (Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M., and Umbarger, H. E., eds) Vol. 1, pp. 3–6, American Society for Microbiology, Washington, D. C.
42. Dietzler, D. N., Lais, C. J., and Leckie, M. P. (1974) Arch. Biochem. Biophys. 160, 14–24
43. Goldie, H. (1984) J. Bacteriol. 159, 832–836
44. Postma, P. W., Lengeler, J. W., and Jacobson, G. R. (1993) Microbiol. Rev. 57, 543–594
45. Goldie, A. H., and Sanwal, B. D. (1980) J. Biol. Chem. 255, 1399–1405
46. Krebs, A., and Bridger, W. A. (1980) Can. J. Biochem. 58, 309–318
47. Daldal, F. (1983) J. Mol. Biol. 168, 285–305
Pleiotropic Regulation of Central Carbohydrate Metabolism in *Escherichia coli* via the Gene *csrA*

Nirupama A. Sabnis, Honghui Yang and Tony Romeo

*J. Biol. Chem.* 1995, 270:29096-29104.
doi: 10.1074/jbc.270.49.29096

Access the most updated version of this article at [http://www.jbc.org/content/270/49/29096](http://www.jbc.org/content/270/49/29096)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 47 references, 21 of which can be accessed free at [http://www.jbc.org/content/270/49/29096.full.html#ref-list-1](http://www.jbc.org/content/270/49/29096.full.html#ref-list-1)