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Physiological Consequences of the Complete Loss of Phosphoryl-Transfer Proteins HPr and FPr of the Phosphoenolpyruvate:Sugar Phosphotransferase System and Analysis of Fructose (*fru*) Operon Expression in *Salmonella typhimurium*

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Mutants of *Salmonella typhimurium* defective in the proteins of the fructose operon [*fruB(MH)KA*], the fructose repressor (*fruR*), the energy-coupling enzymes of the phosphoenolpyruvate:sugar phosphotransferase system (PTS) (*ptsH* and *ptsI*), and the proteins of cyclic AMP action (*cya* and *crp*) were analyzed for their effects on cellular physiological processes and expression of the fructose operon. The *fru* operon consists of three structural genes: *fruB(MH)*, which encodes the enzyme III^{Fru}-modulator-FPr tridomain fusion protein of the PTS; *fruK*, which encodes fructose-1-phosphate kinase; and *fruA*, which encodes enzyme II^{Fru} of the PTS. Among the mutants analyzed were Tn*10* insertion mutants and *lacZ* transcriptional fusion mutants. It was found that whereas a *fruR*::Tn*10* insertion mutant, several *fruB(MH)*::Mu dJ and *fruK*::Mu dJ fusion mutants, and several *ptsHI* deletion mutants expressed the *fru* operon and β-galactosidase at high constitutive levels, *ptsH* point mutants and *fruA*::Mu dJ fusion mutants retained inducibility. Inclusion of the wild-type *fru* operon in *trans* did not restore fructose-inducible β-galactosidase expression in the *fru*::Mu dJ fusion mutants. *cya* and *crp* mutants exhibited reduced basal activities of all *fru* regulon enzymes, but inducibility was not impaired. Surprisingly, *fruB*::Mu dJ *crp* or *cya* double mutants showed over 10-fold inducibility of the depressed β-galactosidase activity upon addition of fructose, even though this activity in the *fruB*::Mu dJ fusion mutants that contained the wild-type *cya* and *crp* alleles was only slightly inducible. By contrast, β-galactosidase activity in a *fruK*::Mu dJ fusion mutant, which was similarly depressed by introduction of a *crp* or *cya* mutation, remained constitutive. Other experiments indicated that sugar uptake via the PTS can utilize either FPr-P or HPr-P as the phosphoryl donor, but that FPr is preferred for fructose uptake whereas HPr is preferred for uptake of the other sugars. Double mutants lacking both proteins were negative for the utilization of all sugar substrates of the PTS, were negative for the utilization of several gluconeogenic carbon sources, exhibited greatly reduced adenylate cyclase activity, and were largely nonmotile. These phenotypic properties are more extreme than those observed for tight *ptsH* and *ptsI* mutants, including mutants deleted for these genes. A biochemical explanation for this fact is proposed.

Recent studies have revealed that bacteria utilize many diverse mechanisms to regulate gene expression (25, 37), including antitermination (29), enhancement (52), competitive repression-activation (55), and protein phosphorylation (1, 11). Most of these mechanisms are believed to have their counterparts in eucaryotes (23, 43). They have been found to operate in a variety of combinations in regulons encoding proteins of the bacterial phosphoenolpyruvate:sugar phosphotransferase system (PTS) (40, 41), which frequently possess multiple promoters or transcriptional terminators within single operons (10, 33, 34, 53, 55).

In previous reports, we demonstrated that the fructose-specific catabolic system in many bacteria exhibits unique features. First, it is the only carbohydrate catabolic system of the PTS that in enteric bacteria possesses its own HPr-like activity (49, 50, 54) and its own enzyme I-like protein (4, 27; R. Geerse, Ph.D. thesis, University of Amsterdam, Amsterdam, The Netherlands, 1989). Second, the protein possessing HPr-like activity (the FruBMH protein) proved to be a fusion protein encoded by the *fruB(MH)* gene and apparently consisting of three domains, an N-terminal enzyme III^{Fru} (III^{Fru}) domain (FruB), a C-terminal HPr-like domain termed FPr (FruH), and a central modulator domain lacking sequence identity with any known PTS protein but possessing sequence similarity to part of the receiver module of bacterial two-component regulatory systems (FruM) (18, 26, 54a). Third, it was shown that the fructose system apparently plays a central role in the regulation of gluconeogenesis (4, 5, 19, 20). Fourth, several bacterial species were found to possess a fructose-specific PTS, and the fructose PTS is more widespread among bacterial species than any other sugar-specific PTS permease (8, 12, 46, 54a). Finally, it was proposed, on the basis of several indirect lines of evidence, that the complex, current-day PTS in numerous eubacteria evolved from a primordial fructose-specific PTS of relatively simple structure (39, 47, 51). All of these observations and postulates led to the possibility that the fructose regulon in enteric bacteria might possess unusual and interesting features.

In this report, we present the results of our genetic, physiological, and biochemical studies concerned with the consequences of the loss of *fru* regulon expression as well as with the transcriptional regulation of the *fru* regulon in

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Salmonella typhimurium. We have isolated and characterized transposon insertion mutants, *lacZ* transcriptional fusion mutants, and point mutants in the *fru* regulon. The induction characteristics of the fructose catabolic enzymes in these mutants and of β-galactosidase in *fru-lacZ* fusion mutants are described. The results of this and a previous study (6) show that expression of the *fru* regulon is influenced by a variety of genetic alterations as follows: (i) *fruR* mutations render expression of the five known activities of the *fru* regulon high-level constitutive; (ii) *fruB*(*MH*)::Mu dJ and *fruK*::Mu dJ mutants synthesize β-galactosidase noninducibly (constitutively) although *fruA*::Mu dJ mutants still exhibit inducibility for *lacZ* expression; (iii) unlike all other carbohydrate catabolic enzyme systems thus far studied, *crp* and *cya* mutations reduce basal activities of *fru* regulon enzymes but do not reduce the extent of induction; and (iv) *fruB*::Mu dJ *crp* or *cya* double mutants exhibit greatly reduced basal β-galactosidase activity with restored fructose inducibility, but *fruK*::Mu dJ *crp* and *cya* mutants which also exhibit reduced β-galactosidase activity do not exhibit restored fructose inducibility. Finally, we show that the simultaneous loss of both HPr and FPr activities results in a more severe phenotype than any genetic defect in the PTS studied thus far.

MATERIALS AND METHODS

Strain constructions and characterization. *lacZ* transcriptional fusions were constructed by mutagenesis with the Mu dJ transposable element (24). P22 was grown on strain TT10288, and the resulting lysate was used to transduce SB1873 to kanamycin resistance (13). The Mu dJ transposon does not contain the Mu A or B gene and therefore cannot by itself transpose. However, when TT10288 (24) is the donor, the Mu A and B functions can be supplied transiently by the Mu A and B genes encoded by the adjacent helper bacteriophage present in strain TT10288. Many independent, kanamycin-resistant, fructose-negative or fructose-sensitive transductants were isolated, and representatives of each phenotypic class were assayed for II^{Fru}, III^{Fru}, and fructose-1-phosphate kinase. Strains LJ1182, LJ2371, LJ2375, LJ2383, LJ2391, and LJ2394 (Table 1) were obtained by this procedure.

All Mu dJ mutants in a wild-type background exhibited normal fermentation properties on mannitol, maltose, xylose, and galactose eosin-methylene blue plates and normal or slightly elevated levels of enzyme I and HPr when assayed in vitro. The Mu dJ markers in strains LJ1182, LJ2371, LJ2375, LJ2383, and LJ2394 were shown to be >90% cotransducible with a Tn*10* insertion mutation in the *fru* operon (in strain LJ1120, which lacked detectable II^{Fru} and III^{Fru} activities) when phage P22 was used as the carrier. Thus, the acquisition of tetracycline sensitivity accompanied the acquisition of kanamycin resistance in greater than 90% of the 30 to 60 transductants examined in each cross.

The *fru*::Mu dJ fusion mutations were transferred from the wild-type background into the genetic backgrounds of two *fruR* strains by transduction with phage P22 as the carrier and selection for kanamycin resistance. These genetic backgrounds were LJ2301 (*fruR51*::Tn*10*) and LJ716 (*manA56 ptsH15 fruR51*::Tn*10* (Table 1). In the latter background, the presence of the FPr domain of the FruBMH protein correlated with mannitol fermentation, since HPr was lacking as a result of the presence of the *ptsH15* mutation. In addition, any fructose fermentation or phosphoenolpyruvate-dependent phosphorylation observed could be attributed exclusively to the fructose-specific PTS, since the mannose PTS (which also phosphorylates fructose with low affinity) was lacking as a result of the *manA56* mutation.

These fusion mutations were assigned to specific cistrons in the *fru* operon as follows (Table 2): *fruB57*::Mu dJ and *fruB58*::Mu dJ, fructose fermentation negative in the presence of the *manA56* mutation, mannitol fermentation negative in the presence of the *ptsH15* and *fruR51* mutations, FPr, fructose-1-phosphate kinase, and II^{Fru} activities absent, and III^{Fru} activity present in *fruB57*::Mu dJ but not

TABLE 1. Bacterial strains used[a]

Strain	Genotype	Reference or source (if other than this study)
S. typhimurium		
SB1873	LT2	
LJ1120	*fru*::Tn*10*	
LJ1182	*fruB57*::Mu dJ (III^{Fru+} FPr^-)	
LJ2371	*fruB58*::Mu dJ (III^{Fru-} FPr^-)	
LJ2375	*fruK59*::Mu dJ	
LJ2383	*fruA62*::Mu dJ	
LJ2391	*fruA63*::Mu dJ	
LJ2394	*fruA64*::Mu dJ	
LJ2301	*fruR51*::Tn*10*	5, 6
LJ2396	*fruR51*::Tn*10 fruB58*::Mu dJ	
LJ1188	*fruR51*::Tn*10 fruB57*::Mu dJ	
LJ2398	*fruR51*::Tn*10 fruK59*::Mu dJ	
LJ2400	*fruR51*::Tn*10 fruA62*::Mu dJ	
LJ2403	*fruR51*::Tn*10 fruA63*::Mu dJ	
LJ2404	*fruR51*::Tn*10 fruA64*::Mu dJ	
LJ716	*manA56 ptsH15 fruR51*::Tn*10*	5
LJ719	*manA56 ptsH15 fruR51*::Tn*10 fruB58*::Mu dJ	
LJ720	*manA56 ptsH15 fruR51*::Tn*10 fruB57*::Mu dJ	
LJ721	*manA56 ptsH15 fruR51*::Tn*10 fruK59*::Mu dJ	
LJ722	*manA56 ptsH15 fruR51*::Tn*10 fruA62*::Mu dJ	
LJ723	*manA56 ptsH15 fruR51*::Tn*10 fruA63*::Mu dJ	
LJ724	*manA56 ptsH15 fruR51*::Tn*10 fruA64*::Mu dJ	
LJ2537	*fruA60*::Tn*5*	
LJ2538	*fruA60*::Tn*5 fruR51*::Tn*10*	
SB1475	*ptsH15*	50
LJ1190	*fruB57*::Mu dJ *ptsH15*	
LJ2046	*ptsH6*	50
LJ1216	*fruB57*::Mu dJ *ptsH6*	
PP994	*crr-307*::Tn*10*	9
LJ1202	*ptsH15 crr-307*::Tn*10*	
SB1477	*ptsI18*	50
LJ703	Δ(*cysK-ptsHI41*)	7
LJ1193	*fruB57*::Mu dJ Δ(*cysK-ptsHI41*)	
LJ1211	*fruB57*::Mu dJ *crr-307*::Tn*10*	
PP1002	*cya*::Tn*10*	P. Postma
PP1037	*crp*::Tn*10*	P. Postma
LJ1223	*fruK59*::Mu dJ *crp*::Tn*10*	
LJ1224	*fruB58*::Mu dJ *crp*::Tn*10*	
LJ1225	*fruK59*::Mu dJ *cya*::Tn*10*	
LJ1226	*fruB58*::Mu dJ *cya*::Tn*10*	
LJ1219	*fruB57*::Mu dJ *cya*::Tn*10*	
LJ1245	*fruB57*::Mu dJ *crp*::Tn*10*	
E. coli		
LU181	F^- *lacI3 glpR galE crp-20b*	22
LU124	F^- *pro cya-8306 lacI3 glpR galR araC$_c$67 rpsL*	22

[a] Additional strains are described in Table 8.

TABLE 2. Properties of Mu dJ insertion mutants specifically defective for *fru* operon expression and function in *S. typhimurium*[a]

Genotype and strain	Property											
	Fermentation of[b]:		FPr activity[c]	Utilization of[d]:				β-Galactosidase activity[e]			Enzyme activity[f]		
	Fructose	Mannitol		Fructose	Citrate	Acetate	Lactate	−Fructose	+Fructose	Fold induction	III^{Fru}	Fructose-1-phosphate kinase	II^{Fru}
fruB58::Mu dJ													
LJ719	−	−	<3										
LJ2371				±	+	+	+	1.4	1.9	1.3			
LJ2396											<1	<0.5	<3
fruB57::Mu dJ													
LJ720	−	−	<1										
LJ1182				±	+	+	+	2.5	3.2	1.3			
LJ1188											7	<1	<5
fruK59::Mu dJ													
LJ721	S	+	32										
LJ2375				S	+	+	+	1.6	1.8	1.1			
LJ2398											7	<0.5	<3
fruA62::Mu dJ													
LJ722	−	+	34										
LJ2383				±	+	+	+	0.07	0.4	6			
LJ2400											7	85	<1
fruA63::Mu dJ													
LJ723	−	+	30										
LJ2391				±	±	−	±	0.14	0.7	5			
LJ2403											11	41	<1
fruA64::Mu dJ													
LJ724	−	+	39										
LJ2394				±	+	+	+	0.09	0.57	6			
LJ2404											13	59	<1

[a] Three genetic backgrounds were used in the analyses reported, as indicated in footnotes *b* to *f* (see Table 1).

[b] Fermentation responses on eosin-methylene blue-sugar plates containing the sugar at 0.5% and growth responses on minimal medium 63 plates containing the carbon source at 0.2% were recorded as follows: +, strong; ±, weak; −, no response. Kanamycin (50 μg/ml) was present in the media for both the fermentation and growth studies. Fermentation responses were performed with strains possessing the genetic background of LJ716 (*manA56 ptsH15 fruR51*::Tn*10*). Consequently, negative fermentation of fructose indicates that at least one of the fructose catabolic enzymes is impaired, while negative fermentation of mannitol indicates that the fructose-inducible HPr-like protein, FPr, is impaired. S, Sensitive.

[c] Cells of the LJ716 genetic background were grown and extracts were prepared as described in footnote *f*. Activities are expressed as percentages of the activity of the isogenic parental strain, LJ716; 100% FPr activity (for strain LJ716) corresponds to 10 nmol of fructose-1-phosphate produced per min per mg of protein.

[d] Growth studies on minimal plates were conducted in the genetic background of strain LT2 (SB1873, wild type), as were the β-galactosidase assays. S, Sensitive.

[e] Cells were grown at 37°C in LB medium with or without fructose (0.5%), harvested in the exponential growth phase, washed three times, resuspended in medium 63 to 10 Klett units (green filter, 16 μg [dry weight] of cells per ml), toluene treated (2 drops of toluene per ml) with agitation, and exposed to 2.5 mM *p*-nitrophenyl-β-galactoside at 37°C for up to 45 min; 3 ml of a 0.2 M Na_2CO_3–10 mM EDTA solution was then added, and the A_{410} was determined. Absolute specific activities are expressed in nanomoles of substrate hydrolyzed per minute per milligram of protein.

[f] Cells (*fruR51*::Tn*10* genetic background) were grown in LB medium, washed, suspended in 50 mM potassium phosphate (pH 7.4) containing 1 mM dithiothreitol, disrupted by passage through a French pressure cell, centrifuged at low speed, and assayed as described in Materials and Methods. Values are expressed in percentages of the activity found in the isogenic parental strain, LJ2301 (*fruR51*::Tn*10*). Absolute specific activities for LJ2301 were as follows: III^{Fru}, 36 nmol of fructose-1-phosphate produced per min per mg of protein; II^{Fru}, 40 nmol of fructose-1-phosphate produced per min per mg of protein; fructose-1-phosphate kinase, 2.6 μmol of fructose 1,6-bisphosphate produced per min per mg of protein.

fruB58::Mu dJ; *fruK59*::Mu dJ, fructose sensitive, mannitol fermentation positive in a genetic background containing the *ptsH15* and *fruR51* mutations, fructose-1-phosphate kinase and II^{Fru} activities absent, and III^{Fru} and FPr present; *fruA62*::Mu dJ, *fruA63*::Mu dJ, and *fruA64*::Mu dJ, fructose fermentation negative in the presence of the *manA56* mutation, mannitol fermentation positive in the presence of the *ptsH15* and *fruR51* mutations, and II^{Fru} activity absent but III^{Fru}, FPr, and fructose-1-phosphate kinase activities present.

To study the effect of the *fru* operon in *trans* on *lacZ* expression in the various *fru*::Mu dJ mutants, the Mu C^+ gene (ampicillin resistant; 3) was transduced into the strains of interest, using strain SL4213 Mu C^+ *amp* #1 (E. A. Groisman, personal communication) as the donor and phage P22 as the carrier. The resultant strains were transformed with plasmid pMC3 carrying the entire *fru* operon (4). Selection was for resistance to neomycin (200 μg/ml) on LB plates. Strains transformed were LJ1182, LJ1237, LJ2375, LJ2391, LJ2394, LJ721, and LJ723 (Table 1), and these transformed strains all proved to be capable of fructose fermentation.

The *fruA60*::Tn*5* mutant was isolated by mutagenizing LJ2301 with Tn*5* and screening for fructose fermentation. Tn*5* mutagenesis was accomplished by using TT3416 as the donor in P22-mediated crosses. This strain possesses a temperature-sensitive F′ plasmid into which the Tn*5* is inserted. Since there is no *Salmonella* DNA in this replicon, incorporation of kanamycin resistance to homologous recombination cannot occur. The transductions were performed at 42°C to prevent replication of the F′ plasmid in the transductants. LJ2538 was tentatively designated *fruA60*::Tn*5* on the basis of its in vitro enzyme activities. The *fru*::Tn*10* mutant (strain LJ1120) used in the mapping experiment described above was isolated as described previously (4–6).

Enzyme assays. ^{14}C-sugar or [^{14}C]citrate uptake rates in vivo were measured as described previously (50), with the radioactive substrate present at a concentration of 10 μM (specific activity, 5 μCi/μmol). Uptake of a metabolizable substrate represents the sum of transport and metabolism and therefore corresponds to a steady-state metabolic rate. For the experiments conducted in Table 6, 1.6 g of nutrient broth per liter and 0.2% DL-lactate were present in the uptake medium as energy sources. Assays for sugar phosphorylation in vitro were performed as described by Saier et al. (50) except that the concentrations of constituents in the in vitro assay mixture were as follows: ^{14}C-sugar, 10 μM; phosphoenolpyruvate, 5 mM; phosphate buffer, pH 7.4, 50 mM; $MgCl_2$, 12.5 mM; KF, 25 mM; and dithiothreitol, 2.5 mM. The incubation time was usually 20 min at 37°C. II^{Fru} was assayed by inclusion of excess quantities of the soluble fraction of LB broth-grown LJ2301 (*fruR51*::Tn*10*) in the assay mixture. III^{Fru} was assayed by inclusion of excess quantities of the soluble fraction of strain LJ144 [*cpd-401 cysA1150*(F'198 *pts*$^+$ *crr*$^+$ *cysA*$^+$)] (44) as well as butanol-urea-extracted membranes (45) from LJ2301 grown in LB medium. Net FPr-plus-HPr activity was assayed by inclusion of a crude extract from LB broth-grown SB1467 (*ptsH6*) cells (50) to the assay mixture. In a similar fashion, net enzyme I plus enzyme I* activity (4) was assayed by inclusion of a crude extract of strain SB1477 (*ptsI18*) (50) in the assay mixture. The radioactive sugar used to assay II^{Fru} and III^{Fru} was [^{14}C]fructose. [^{14}C]fructose or [^{14}C]mannitol was used to assay the HPr/FPr and enzyme I/enzyme I* activities with the knowledge that the relative contributions to the total activity, due to the presence of the two proteins, HPr and FPr, would be different when the two substrates were used. Fructose-1-phosphate kinase assays were performed as described by Fraenkel (16). β-Galactosidase was assayed by the procedure of Miller (30). Protein was determined by the method of Lowry et al. (28).

Cyclic AMP production. Net cyclic AMP production (cells plus medium) was measured after growth of cells from a small inoculum to the stationary phase (12 h) in medium 63 containing 0.5% galactose essentially as described previously (14, 44). Cultures were then brought to 100°C in a boiling water bath; after a 5-min incubation period, cells were removed by centrifugation. The cyclic AMP concentration in the supernatant was measured by the cyclic AMP-binding protein method as described previously (14, 44).

Relative motility in semisolid agar. Freshly grown wild-type and mutant colonies on LB plates were stabbed with toothpicks into semisolid agar medium consisting of 0.13% tryptone, 0.7% NaCl, 0.35% Bacto-Agar (Difco Laboratories), and a sugar (when present) at 1%. Plates were incubated at 30°C for 16 h, and swarm diameters were measured at 2-h intervals thereafter. Alternatively, 1% tryptone, 0.5% yeast extract, 1% NaCl, and 0.35% Bacto-Agar were used, and the freshly grown bacteria were stabbed into the agar with a straight platinum wire and incubated at 37°C. Swarm diameters were measured periodically thereafter. Results obtained with these two media were similar. Cyclic AMP (2 to 10 mM) was included in the agar medium in some experiments.

Materials and media. All radioactive sugars were purchased from Dupont, NEN Research Products. Nonradioactive sugars and reagents were purchased from the Sigma Chemical Co. LB medium was as described by Miller (30). When a sugar was included, its concentration was 0.5%. The minimal medium used was medium 63 without iron (50). Tetracycline, kanamycin, and ampicillin were used at concentrations of 20, 25 or 50, and 50 μg/ml, respectively. Fermentation was recorded on eosin-methylene blue plates without lactose. Sugars were included at a concentration of 0.5 or 1%.

RESULTS

Characterization of *fru*::Mu dJ fusion mutants. Six Mu dJ insertion mutants expressing β-galactosidase and specifically resulting in the loss of fructose utilization were isolated and characterized (Table 2). In a wild-type genetic background, all of these mutants fermented and utilized fructose weakly; in the genetic background of a *manA* mutant (II^{Man^-}), they were negative for fructose utilization (except strain LJ2375, which was sensitive to fructose). They nevertheless fermented other sugars (mannitol, maltose, xylose, and galactose) normally unless the *ptsH* mutation was also present, in which case the two *fruB* mutants did not ferment mannitol. All of the mutants except strain LJ2391 utilized citrate, acetate, and lactate at wild-type rates (Table 2).

β-Galactosidase in strains LJ2371, LJ1182, and LJ2375 was synthesized at high, nearly constitutive levels, but this enzyme in strains LJ2383, LJ2391, and LJ2394 exhibited low basal activity which could be enhanced about sixfold by inclusion of fructose in the growth medium (Table 2). These Mu dJ insertion mutations were transferred by transduction to a *fruR51*::Tn*10* genetic background (see Materials and Methods). Strain LJ2396 exhibited low to negligible activities of the three fructose-specific proteins (III^{Fru}, fructose-1-phosphate kinase, and II^{Fru}); LJ1188 exhibited III^{Fru} activity but nondetectable kinase and II^{Fru} activities; LJ2398 similarly exhibited nondetectable kinase activity; and strains LJ2400, LJ2403, and LJ2404 exhibited low III^{Fru} activity, high fructose-1-phosphate kinase activity, and nondetectable II^{Fru} activity (Table 2). The *fruB58*::Mu dJ mutation evidently abolished the activities of all *fru* operon products, including FPr (measured in a *ptsH* genetic background), but the *fruB57*::Mu dJ mutation apparently left the III^{Fru} domain of the FruBMH protein intact while abolishing the FPr and downstream activities. On the basis of these results, it was concluded that the Mu dJ insertions in strains LJ2371 and LJ1182 were in the B and M or H domains of the *fruB*(*MH*) gene, respectively; the insertion in strain LJ2375 was in the *fruK* gene, and those in strains LJ2383, LJ2391, and LJ2394 were in the *fruA* gene.

Strains bearing the chromosomal *fru*::Mu dJ mutations in the wild-type genetic background (LT2) and also bearing plasmid pMC3 carrying the entire *fru* operon (4) were constructed as described in Materials and Methods. These strains were then examined for β-galactosidase activities after growth in complex medium with or without fructose as described for the experiments presented in Table 2. The presence of the *fru* operon in *trans* had little or no effect on the induction of β-galactosidase in the *fruA*, *fruB*, or *fruK* mutants.

The consequences of mutations in the *fruR*, *crp*, *cya*, and *pts* genes on expression of β-galactosidase in some of the Mu dJ fusion mutants were examined (Table 3). Synthesis of β-galactosidase in the *fruB58*::Mu dJ fusion mutant, which was only slightly inducible, was increased threefold by inclusion of the *fruR51*:Tn*10* mutation. *crp* and *cya* mutations lowered basal β-galactosidase activity in this fusion mutant about 10-fold, but surprisingly, this activity was restored by inclusion of fructose in the growth medium. Similar results were obtained with the *fruB57*::Mu dJ mu-

TABLE 3. Effects of fructose induction and various relevant mutations on β-galactosidase synthesis in *fruB*::Mu dJ and *fruK*::Mu dJ strains of *S. typhimurium*[a]

Strain	Genotype	Relative sp act	
		− Fructose	+ Fructose
LJ2371	*fruB58*::Mu dJ	1.0	2.6
LJ2396	*fruB58*::Mu dJ *fruR51*::Tn*10*	3.0	1.8
LJ1224	*fruB58*::Mu dJ *crp*::Tn*10*	0.14	1.3
LJ1226	*fruB58*::Mu dJ *cya*::Tn*10*	0.10	1.2
LJ1182	*fruB57*::Mu dJ	1.5	2.4
LJ1188	*fruB57*::Mu dJ *fruR51*::Tn*10*	2.4	1.2
LJ1245	*fruB57*::Mu dJ *crp*::Tn*10*	0.09	1.8
LJ1219	*fruB57*::Mu dJ *cya*::Tn*10*	0.17	2.2
LJ1190	*fruB57*::Mu dJ *ptsH15*	0.3	2.7
LJ1193	*fruB57*::Mu dJ Δ(*cysK-ptsHI41*)	0.6	2.9
LJ2375	*fruK59*::Mu dJ	1.8	1.2
LJ1223	*fruK59*::Mu dJ *crp*::Tn*10*	0.15	0.21
LJ1225	*fruK59*::Mu dJ *cya*::Tn*10*	0.15	0.19

[a] Cells were grown in LB medium with or without 0.5% fructose, washed three times, suspended in 50 mM potassium phosphate buffer (pH 7.4) containing 1 mM dithiothreitol, and passed through a French press at 10,000 lb/in^2. β-Galactosidase activity was measured as described in Materials and Methods. In all strains analyzed, fructose-1-phosphate kinase activity was low to negligible and was not inducible by inclusion of fructose in the growth medium. Assays were conducted in duplicate or triplicate with reproducibility of ±30%. The value of 1.0 corresponds to an absolute specific activity of 1.4 nmol of substrate hydrolyzed per min per mg of protein.

tant, although the *fruR* mutation had a lesser effect on the uninduced level of β-galactosidase. Introduction of the *ptsH15* or Δ(*cysK-ptsHI41*) mutation, which like the *cya* mutation reduces cellular cyclic AMP levels (14, 44), similarly reduced basal activity and restored induction by fructose. By contrast, the constitutive expression of β-galactosidase activity in the *fruK59*::Mu dJ mutant, which was likewise depressed by the *cya* or *crp* mutation, was not rendered inducible by fructose when cyclic AMP receptor protein (CRP) or adenylate cyclase function was destroyed.

Effects of mutations in the *pts* operon on *fru* regulon expression. Table 4 summarizes the effects of mutations in the *pts* operon (10, 38, 49, 50) on expression of the four activities associated with the three structural genes of the *fru* operon in *S. typhimurium* as measured by enzyme assays in vitro. In the wild-type strain, FPr, II^{Fru}, and III^{Fru} were apparently about 10-fold inducible, whereas fructose-1-phosphate kinase seemed to be about 25-fold inducible. Two *ptsH* mutants were examined for their effects on *fru* operon expression. The *ptsH15* mutation is a UGA nonsense mutation in the structural gene encoding HPr, and consequently it is somewhat leaky for *ptsH* expression (50). The *ptsH6* mutation is a missense mutation in the structural gene for HPr and exhibits a tighter phenotype (50). Both mutations appeared to depress the basal and induced levels of *fru* operon expression, possibly because of the reduction in adenylate cyclase activity in these strains (14, 41, 44; see also Tables 5 and 8). Similar behavior was observed for the tight *ptsI* mutant, *ptsI18* (data not shown), which resulted from the introduction of an ochre nonsense mutation in the structural gene for enzyme I (50). The Δ(*cysK-ptsHI41*) mutation exhibited a similar effect, but induction was abolished or markedly reduced. Similar results were obtained when the cells were grown in minimal medium rather than complex medium (data not shown). Elimination of III^{Glc} function by introduction of a Tn*10* insertion mutation into the *crr* gene (9, 48) did not appear to appreciably affect *fru* regulon expression either by itself (Table 4) or in the presence of *pts* mutations (data not shown).

Dependency of *fru* operon expression on cyclic AMP and CRP. The dependency of [^{14}C]fructose uptake on cyclic AMP and its receptor protein (CRP) is shown in Table 5. Wild-type cells effectively accumulated radioactive metabolites from [^{14}C]fructose when grown in a nutrient medium lacking fructose, and inclusion of fructose in the growth medium enhanced the uptake rate about threefold. By contrast, *cya* or *crp* mutants, which lack adenylate cyclase or CRP, respectively, accumulated radioactive metabolites from exogenous [^{14}C]fructose poorly when grown in the absence of fructose or cyclic AMP (uptake rate, about 25% of the uninduced wild-type rate). Surprisingly, the uptake rates were enhanced about 10-fold by inclusion of fructose in the growth medium (Table 5). The induced rate of [^{14}C]fructose uptake was essentially the same in the mutants as in the wild-type strain. Inclusion of cyclic AMP in the growth medium lacking fructose restored uptake by the *cya* mutant, but not by the *crp* mutant, to the level observed for the wild-type strain when grown without fructose.

The unusual dependency of fructose uptake on the intact *cya* and *crp* genes suggested that basal synthesis of the proteins of the *fru* operon was dependent on cyclic AMP and CRP but that induction by fructose was not. This conclusion was confirmed by assaying the *fru* enzymes in vitro (Table 5). The basal activities of II^{Fru}, III^{Fru}, and fructose-1-phosphate kinase were all reduced in the *cya* and *crp* mutants relative to the wild-type parental strain. Inclusion of fructose in the growth medium enhanced the activities of all of these enzymes to about the same extent as in the parental strain. The fully induced level of each enzyme was some-

TABLE 4. Effects of mutations in the *pts* operon on expression of the genes of the *fru* operon in *S. typhimurium*[a]

Strain	Genotype	Fermentation of fructose	Enzyme activity[b]							
			FPr (apparent)		II^{Fru}		III^{Fru}		Fructose-1-phosphate kinase	
			−	+	−	+	−	+	−	+
SB1873	Wild type	+	1.0	10 ± 4	1.0	7 ± 1	1.0	10 ± 4	1.0	25 ± 13
SB1475	*ptsH15*	±	0.4 ± 0.2	3.0 ± 2.1	0.6 ± 0.1	4 ± 1	0.7	4.1	1.2 ± 0.6	22 ± 7
LJ2046	*ptsH6*	±	ND	ND	0.5	3.8	0.7	3.3	1.1 ± 0.7	12 ± 7
LJ703	Δ(*cysK-ptsHI41*)	−	ND	ND	0.7	0.4	1.1	1.1	0.9 ± 0.4	2.3 ± 1.3
PP994	*crr-307*::Tn*10*	+	ND	ND	0.9	9.3	1.7	5.7	2.1 ± 1.7	23 ± 12

[a] Cells were grown in LB medium with (+) or without (−) 0.5% fructose and assayed for the various enzyme activities as described in Materials and Methods.
[b] The value of 1.0 corresponds to absolute specific activities (in nanomoles of product formed per minute per milligram of protein) of 2 for FPr plus HPr, 5 for II^{Fru}, 4 for III^{Fru}, and 100 for fructose-1-phosphate kinase. When error values are provided (in standard deviations), the experiments were conducted at least three times. ND, Not determined.

TABLE 5. Effects of Tn*10* insertion mutations in the *cya* and *crp* genes on expression of the structural genes of the *fru* operon in *S. typhimurium*[a]

Strain	Genotype	Fructose uptake[b]			Enzyme activity					
					II^{Fru}		III^{Fru}		Fructose-1-phosphate kinase	
		−	+cAMP	+Fru	−	+Fru	−	+Fru	−	+Fru
SB1873	Wild type	1.0	1.0 ± 0.2	2.6 ± 0.6	1.0	7.1 ± 0.6	1.0	11 ± 8	1.0	25 ± 13
PP1002	*cya*::Tn*10*	0.2 ± 0.1	1.0 ± 0.2	2.8 ± 0.7	0.5 ± 0.1	5.3 ± 2.0	0.5 ± 0.2	6.9 ± 4.1	0.5 ± .03	15 ± 8
PP1037	*crp*::Tn*10*	0.3 ± 0.1	0.3 ± 0.1	2.4 ± 0.5	0.5 ± 0.1	6.0 ± 4.0	0.3 ± 0.2	7.5 ± 3.5	0.5 ± 0.13	12 ± 6

[a] All assays were performed as described in Materials and Methods. Most values were determined three times. Error values are expressed in standard deviations. Absolute specific enzyme activities are reported in Table 4. The value of 1.0 for fructose uptake corresponds to 4 μmol of [^{14}C]fructose taken up per min per g (dry weight) of cells at 37°C. All strains fermented fructose.

[b] —, Cells were grown in LB medium without fructose; +cAMP or +Fru, cells were grown in the same medium in the presence of 5 mM cyclic AMP or 0.5% fructose, respectively, for 3 h before harvesting during exponential growth. The low degree of apparent induction in the fructose uptake experiment (measured in vivo) relative to the phosphorylation experiments (measured in vitro) is believed to reflect the fact that enzyme I and HPr rather than II^{Fru}-III^{Fru} are rate limiting for transport in the fully induced state. Excess amounts of these energy coupling proteins were added for the in vitro assays.

what less than that observed for the parental strain, however. Comparison of the levels of enzyme activities with the uptake rates suggested that for both the wild-type and mutant strains, the II^{Fru}-III^{Fru} pair was rate limiting for uptake of [^{14}C]fructose in the uninduced state but not after induction by fructose.

The effects of the loss of the CRP on the uptake of glucose and fructose after growth under different conditions were determined (Table 6). In the wild-type strain, glucose uptake was mildly inducible by growth in the presence of glucose, in agreement with previous results (36), and the presence of fructose in the growth medium depressed this activity only slightly. Addition of a 100-fold excess of fructose to the cell suspensions during transport inhibited glucose uptake only 5 to 28% (data not shown). In the *crp* mutant, glucose uptake activity was not induced by inclusion of a sugar in the growth medium. In fact, the activity was slightly depressed. The presence of a 100-fold excess of fructose depressed glucose uptake by the *crp* mutant only 5 to 10% (data not shown). In contrast, fructose uptake was markedly induced by growth in the presence of fructose, both in the wild-type and in the *crp* mutant strains. Glucose, when present as the sole sugar in the growth medium, had a slight inductive effect, but it repressed when fructose was also present (Table 6). Inhibition of fructose uptake by a 100-fold excess of glucose was similar in the wild-type and *crp* mutant strains (30 to 62% inhibition). Thus, catabolite repression and catabolite inhibition are both apparently operative in the absence of CRP.

TABLE 6. Effect of carbon source present during growth on relative [^{14}C]glucose and [^{14}C]fructose uptake activities in wild-type and *crp* mutant strains of *S. typhimurium*[a]

Determination	Relative rate in strain:	
	SB1873 (wild type)	PP1037 (*crp*)
[^{14}C]glucose uptake after growth in presence of:		
No sugar	62	100
Fructose	72	80
Glucose	100	67
Glucose + fructose	89	67
[^{14}C]fructose uptake after growth in presence of:		
No sugar	26	7
Fructose	100	100
Glucose	37	13
Glucose + fructose	55	71

[a] Cells were grown in LB medium containing the sugar(s) indicated at a concentration of 0.5%. The uptake experiment was conducted at 37°C, with the radioactive sugar at a concentration of 10 μM (5 μCi/μmol) as described in Materials and Methods with washed cells suspended to a density of 10 Klett units (green filter; 16 μg [dry weight] of cells per ml) in medium 63 containing 1.6 g of nutrient broth per liter and 0.2% DL-lactate as energy sources. Additional experiments revealed that 1 mM nonradioactive fructose only weakly inhibited [^{14}C]glucose uptake (5 to 28% inhibition) but that glucose more strongly inhibited [^{14}C]fructose uptake (30 to 62% inhibition) under the same standard conditions in both the wild-type and the *crp* mutant strains. Absolute rates of uptake, corresponding to 100%, were 12 and 10 μmol of [^{14}C]glucose and [^{14}C]fructose taken up per min per g (dry weight) of cells at 37°C.

Fructose uptake and the activities of the fructose-specific enzymes of the PTS (II^{Fru} and III^{Fru}) were assayed in a wild-type *E. coli* strain, a *cya* deletion mutant, LU124, and a *crp* deletion mutant, LU181 (22), as described in Table 5. The induction properties of these *E. coli* strains were very similar to those reported for the corresponding *Salmonella* strains (data not shown).

Properties of mutants lacking HPr or FPr of the PTS. Growth of the wild-type cells in the presence of fructose induced fructose uptake activity (Table 7). Cells defective for HPr (*ptsH15*; SB1475) or components of the *fru* operon including FPr (*fruB57*::Mu dJ; LJ2529) took up [^{14}C]fructose poorly after growth in complex medium lacking fructose. Of these two mutants, only the *ptsH15* mutant took up fructose appreciably after growth in the presence of fructose, and negligible activity was observed for the *ptsH15 fruB57* double mutant (Table 7).

[^{14}C]mannitol uptake activity showed very different behavior (Table 7). In the wild-type strain, this activity was specifically induced by growth in the presence of mannitol. In uninduced cells, the *fruB57*::Mu dJ mutant took up [^{14}C] mannitol as well as the wild type, whereas the *ptsH15* mutant exhibited exceptionally low activity which was only slightly greater than that of the *ptsH15 fruB57* double mutant. Growth of the *ptsH15* mutant in the presence of fructose (but not mannitol) induced uptake activity, presumably due to FPr induction (49, 50), as suggested by the fact that the double mutant was essentially inactive. When [^{14}C] glucose replaced [^{14}C]mannitol as the radioactive substrate, similar results were obtained (data not shown).

The sums of the activities of HPr and FPr, here designated HPr plus FPr, as well as the enzyme I activities in the same strains, were measured in vitro. When assayed with [^{14}C] mannitol as the sugar substrate (Table 7) or [^{14}C]methyl-α-glucoside as the sugar substrate (data not shown), somewhat higher than wild-type HPr activity was observed in the uninduced *fruB57*::Mu dJ mutant, whereas the *ptsH15* mu-

TABLE 7. Properties of *S. typhimurium* mutants defective for HPr, FPr, or both[a]

Property	Inducer	Value			
		SB1873 (wild type)	SB1475 (*ptsH15*)	LJ1182 (*fruB57*::Mu dJ)	LJ1190 (*fruB57*::Mu dJ *ptsH15*)
Uptake of[b]:					
[^{14}C]fructose	None	2.4 ± 0.7	0.6 ± 0.1	0.1 ± 0.03	0.2 ± 0.04
	Fructose	9 ± 4	6 ± 3	0.1 ± 0.03	0.1 ± 0.03
	Mannitol	1.9 ± 0.6	0.5 ± 0.3	0.1 ± 0.03	ND
[^{14}C]mannitol	None	4.3 ± 1.0	0.4 ± 0.2	5.0 ± 2.0	0.2 ± 0.2
	Fructose	4.7 ± 1.7	4.5 ± 3.2	5.9 ± 1.9	ND
	Mannitol	12 ± 5	0.4 ± 0.2	13 ± 3	0.1 ± 0.03
Relative HPr + FPr activity[c]	None	1.0	0.10 ± 0.01	1.6 ± 0.6	0.06 ± 0.04
	Fructose	2.3 ± 1.0	0.56 ± 0.10	1.2 ± 0.3	0.02 ± 0.01
Relative enzyme I activity[c]	None	1.0	0.05 ± 0.01	1.8 ± 0.3	0.10 ± 0.04
	Fructose	1.5 ± 0.5	0.2 ± 0.02	1.3 ± 0.1	0.11 ± 0.03
Relative cyclic AMP production[d]	None	1.0	0.5	1.5	0.2
Relative citrate uptake[e]	None	1.0	0.4	1.3	0.1
Relative motility in semisolid agar[f]	None	1.0	0.4	1.0	0.2
	Fructose	0.8	ND	1.1	0.2
	Mannitol	0.9	ND	1.0	0.2
Utilization of[g]:					
Citrate	Citrate	+	±	+	−
Alanine	Alanine	+	±	+	−
Lactate	Lactate	+	+	+	±

[a] Strains are listed in Table 1, and experimental procedures were as described in Materials and Methods. All strains fermented galactose, xylose, and arabinose normally. Maltose was fermented by strains SB1873 and LJ1182 but not by strains SB1475 and LJ1190. Error values are expressed in standard deviations (three or more determinations). ND, Not determined.

[b] Determined with cells grown in LB medium with or without the sugar inducer indicated. Cells were harvested in the logarithmic growth phase, washed three times with medium 63, and resuspended in the same salts medium at a cell density of 0.04 to 0.16 mg (dry weight) of cells per ml. Values are expressed in micromoles of ^{14}C-sugar accumulated per minute per gram (dry weight) of cells at 37°C. The uptake experiments were conducted three times, each time with a distinct cell preparation.

[c] The value of 1.0 corresponds to a value of 30 nmol of [^{14}C]mannitol phosphorylated at 37°C per min per mg of protein when HPr or FPr was measured and 140 nmol of [^{14}C]mannitol phosphorylated per min per mg of protein when enzyme I was measured. Assays were conducted as described in Materials and Methods. The phosphorylation assays were conducted three times, each time with a distinct extract. The lower rates of phosphorylation in vitro as compared with the in vivo uptake rates are consistent with previous reports and are believed to be due to disruption of a more active complex of PTS enzymes during cell disruption (17, 42).

[d] The value of 1.0 corresponds to 0.31 μmol of cyclic AMP produced and secreted into the medium per g (dry weight) of cells during growth to the stationary phase as described in Materials and Methods (14).

[e] The value of 1.0 corresponds to a value of 4 μmol of [^{14}C]citrate taken up per min per g (dry weight) of cells. Uptake was quantitated as described in Materials and Methods.

[f] The value of 1.0 corresponds to a swarm diameter of 25 mm in assays conducted as described in Materials and Methods.

[g] Estimated on minimal plates consisting of medium 63 (49) containing Bacto-Agar (1.5%) and the carbon source at a concentration of 0.5%.

tant and the double mutant showed low activities. After fructose induction, the FPr activity increased dramatically in the *ptsH15* mutant strain, but no appreciable increase in activity was observed in the *fruB57*::Mu dJ mutant or the *ptsH15 fruB57* double mutant. In fact, the latter mutant lacked measurable activity. Enzyme I activities were low in the *ptsH15* and *ptsH15 fruB57* mutants relative to the wild-type strain as a result of the polarity of the UGA nonsense mutation in the mutant *ptsH15* gene (50).

Correlation of citrate uptake and motility with cyclic AMP production. Whereas the *ptsH15* mutant produced 50% less cyclic AMP than the parental strain, and the *fruB57*::Mu dJ mutant produced 50% more cyclic AMP than the parent, the double mutant produced very little cyclic AMP (Table 7). These findings correlated with the induction of citrate uptake, motility in semisolid agar medium, and the utilization of various gluconeogenic substrates by the different mutant strains (Table 7).

Earlier observations had suggested that citrate utilization in mutants defective for the proteins of the PTS could serve as an indicator of adenylate cyclase activity (43; M. H. Saier, Jr., unpublished observations). The same appears to be true of alanine and lactate utilization. Since ribose and xylose could still be utilized by the double mutant, it was clear that some basal level of cyclic AMP synthesis occurred. Motility in semisolid agar medium also correlated with cyclic AMP production (Table 7).

The motile behavior of many mutant strains defective for the proteins of the PTS, various catabolic enzymes, and the proteins of cyclic AMP action are summarized in Table 8. It can be seen that leaky *pts* mutants, mutants defective for the enzymes II of the PTS, and mutants lacking specific enzymes of intermediary metabolism exhibited normal motility in the semisolid medium used. Tight *pts* mutants, lacking HPr or enzyme I, and *crr* mutants, defective for III^{Glc}, exhibited depressed motility, whereas *cya* mutants were essentially nonmotile. These results correlate with the known effects of the mutations studied on adenylate cyclase activity in vivo (14, 44). *crp* mutants were also nonmotile. The *fruR51*::Tn*10* mutant, which synthesizes normal levels of cyclic AMP (5), exhibited normal motility. As expected, motility of a *cya* mutant was restored by addition to the semisolid medium of 5 mM cyclic AMP (Table 8). The same was partially true of *pts* mutants and the *ptsH15 fruB57*::Mu dJ double mutant (Table 8 and unpublished experiments).

Table 8 also shows the motile behavior of a number of *Escherichia coli* strains. Once again, in the *pts*, *crr*, and *cya* mutants studied, motility correlated well with adenylate cyclase activities measured previously (14, 41). The partial restoration of motility by the *crr-13* mutation in the *ptsI316*

TABLE 8. Motility of *S. typhimurium* and *E. coli* strains in semisolid agar medium[a]

Strain	Genotype	Defect	Swarm diam (mm)
S. typhimurium			
SB1873	Wild type	None	12
SB1681	*ptsI16*	Enzyme I (leaky)	15
SB1476	*ptsI17*	Enzyme I (leaky)	14
SB1685	*ptsH24*	HPr (leaky)	15
SB1477	*ptsI18*	Enzyme I (tight)	4
SB1682	*ptsI19*	Enzyme I (tight)	6
SB1683	*ptsI20*	Enzyme I (tight)	5
SB1475	*ptsH15*	HPr (tight)	5
SB1686	*pmi-2*	Phosphomannose isomerase	15
SB1687	*man-12*	II^{Man}	14
SB1667	*malQ62*	Amylomaltase	13
SB1669	*malB64*	Maltose transport	13
SB1744	*mtlA61*	II^{Man}	11
SB1796	*ptsI17 crr-1*	Enzyme I (leaky) and III^{Glc}	14
SB1798	*ptsI18 crr-3*	Enzyme I (tight) and III^{Glc}	4
SB1799	*ptsI19 crr-4*	Enzyme I (tight) and III^{Glc}	5
SB2026	*ptsH15 crr-6*	HPr (tight) and III^{Glc}	4
SB1854	*crr-3*	III^{Glc}	2
SB2125	*crr-4*	III^{Glc}	2
SB1786	*cya-202*	Adenylate cyclase (tight)	1
SB1790	*crp-205*	CRP	1
LJ2301	*fruR51*::Tn*10*	Fructose repressor	15
PP1002	*cya*::Tn*10*	Adenylate cyclase	1
PP1002	*cya*::Tn*10* (+ 5 mM cyclic AMP)	Adenylate cyclase	12
LJ1190	*fruB57*::Mu dJ *ptsH15*	HPr and FPr	4
LJ1190	*fruB57*::Mu dJ *ptsH15* (+ 5 mM cyclic AMP)	HPr and FPr	6
E. coli			
1100	*thi* (wild type)	None	12
1101	*thi ptsH315*	HPr	18
1103	*thi ptsI316*	Enzyme I	1
SB2273	*thi ptsI316 crr-13*	Enzyme I and III^{Glc}	7
5336	*thi cya*	Adenylate cyclase	0.5
5333	*thi crp*	CRP	0.5

[a] Motility was measured as described in Materials and Methods. The diameter of the swarm of a given bacterial strain is a measure of the motility of that strain under the conditions used.

genetic background correlates with increased cyclic AMP production as reported previously (44). Increased adenylate cyclase activity is an unusual characteristic of *crr* mutants in this *E. coli* genetic background. The results generally suggest that motility in semisolid agar medium can be used to estimate levels of cyclic AMP production in mutants altered for the regulation of adenylate cyclase activity.

DISCUSSION

We have constructed a double mutant lacking both HPr and FPr activities, and this mutant has proven to possess drastically altered physiological properties. It synthesizes extremely low levels of cyclic AMP, utilizes many carbon sources poorly, and is poorly motile (Table 7). An effect of *fru* mutations on cyclic AMP production has been noted by Gershanovitch et al. (21). It is reasonable to suppose that these extreme physiological properties result from the strong inhibition of adenylate cyclase by the PTS (41). It is interesting to note that tight point and deletion *ptsI* mutants are not as deficient for cyclic AMP synthesis, for motility, or for gluconeogenic carbon utilization as the *ptsH15 fruB57*::Mu dJ double mutant described here. This fact may be attributed to the residual enzyme I activity of tight *ptsI* mutants as a result of the presence of a nearly cryptic gene encoding a second enzyme I that is negatively regulated by the *fruR* gene product (6). The *fruR* gene product, suggested but not yet conclusively shown to be the repressor of the *fru* regulon, exerts a pleiotropic effect on the expression of genes encoding proteins involved in gluconeogenesis, the glyoxylate stunt, oxidative metabolism, and *Salmonella* virulence (4–6, 18, 19; B. Hoertt, R. L. Warren, A. M. Chin, and M. H. Saier, Jr., unpublished results). Mutations in the *fruR* gene also give rise to elevated activities of enzyme I, HPr, and some of the enzymes II (4). Interestingly, loss of FPr due to the *fruB57*::Mu dJ mutation apparently exerts a similar effect (Table 7). A product of the *fru* operon can possibly interact with or phosphorylate a component of the transcriptional apparatus controlling *pts* operon expression.

The simultaneous loss of HPr and FPr functions results in the loss of PTS-mediated transport function (Table 7). In the *fruB57*::Mu dJ mutant lacking FPr but possessing wild-type levels of HPr, fructose uptake was also reduced to near basal activity. Evidence published previously led to the conclusion that HPr could substitute for FPr in vitro (54). The dramatic loss of transport function in the *fruB57*::Mu dJ mutant (Table 7) can be explained either by the imprecise cleavage of FPr from the III^{Fru}-FPr fusion protein or by the loss of II^{Fru} due to a polar effect of the Mu dJ insertion element.

Mutations in the *crp* or *cya* gene that eliminate activity of CRP or adenylate cyclase, respectively, and *pts* mutations that reduce rates of cyclic AMP synthesis (14, 41, 42) resulted in depressed basal *fru* operon expression, as reflected by both in vivo and in vitro assays (Tables 5 and 6) as

well as in vitro assays of β-galactosidase activities of *fru-lacZ* transcriptional fusions (Table 3). This situation is reminiscent of that observed previously for synthesis of functional II^{Glc} and II^{Man} (36). Unlike the latter systems, however, the *crp* or *cya* mutations did not interfere with fructose-promoted induction of *fru* operon expression. To the best of our knowledge, this is a unique finding. The other regulons or operons which encode transport proteins or enzymes that initiate the catabolism of exogenous carbohydrates are not appreciably inducible by the inducer or substrates in tight *crp* or *cya* mutants. The in vitro results reported therefore explain the in vivo transport results (Table 7) as well as the positive fructose fermentation responses which have long been known to be characteristic of these mutants (50).

Lowered cyclic AMP levels cannot explain the lack of *fru* operon induction in a *pts* deletion strain, Δ(*cysK-ptsHI41*) (Table 4). In this case, an insufficient supply of the presumed inducer, fructose-1-phosphate, may provide an explanation. However, if fructose-1-phosphate is the true inducer, then the β-galactosidase induction in *fruA*::Mu dJ mutants (Table 2) is anomalous. Two distinct mechanisms of induction, one by fructose and a second by fructose-1-phosphate, may be operative. It is possible that even free fructose cannot enter the cytoplasm of the *pts* deletion strain to a concentration sufficient to effect induction. Alternatively, a cryptic *fru* gene encoding a second, poorly expressed II^{Fru} may be present on the *Salmonella* chromosome (Y. W. Cao and M. H. Saier, Jr., unpublished results).

Three independently isolated Mu dJ insertion mutants, specifically defective for fructose utilization, lacked II^{Fru} activity but possessed measurable III^{Fru} and fructose-1-phosphate kinase activities (Table 2). On this basis, the insertion elements were presumed to be in the *fruA* gene, the last known gene in the *fru* operon (19, 35). However, the activities of fructose-1-phosphate kinase were high, whereas those of III^{Fru} were low. Considering that the gene order is believed to be *fruB(MH)KA*, the noncoordinate expression of the *fruB* and *fruK* genes is anomalous. Interestingly, this observation correlates with the low *fruB* gene expression and high *fruK* gene expression in a *fruA*::Tn*5* insertion mutant of *Rhodobacter capsulatus* (8). Since the gene order in *R. capsulatus* is *fruB(HI)KA* (54a) (similar to that in *Salmonella* species), this result correlates with those reported here but is not easy to interpret. This observation contrasts with the report of Geerse et al. (19), who found that a Tn*10* insertion mutation in *S. typhimurium* gave rise to loss of II^{Fru} activity but retention of full III^{Fru} and fructose-1-phosphate kinase activities. Since the *fruA*::Mu dJ mutants could ferment mannitol in a *ptsH fruR* genetic background, the *fruB(MH)* gene product must have been synthesized in appreciable amounts, in accordance with the report of Geerse et al. (19). Degradation of III^{Fru} in the absence of II^{Fru} or the presence of a second promoter preceding the *fruH* gene segment may provide an explanation.

Geerse et al. showed with a *galK* fusion that the *Alu*I-*Hin*fI fragment in the region preceding the *fruB* gene (nucleotides 235 to 405) contains a *fru* operon regulatory region that is responsive to the presence of FruR, but they could not find a promoter sequence (18). Computer analyses using five different programs (31, 32) have revealed a single −35 region and a single −10 region within this fragment (TTGCGT at position 304 and CAGACT at position 327, respectively). The −10 region is the same as that for the *E. coli pfkB* gene. The sequence TGAAACGTTTCA (positions 336 to 347) forms a hairpin structure, the only such structure anywhere within this region, and FruR might bind to this hairpin structure, thereby blocking transcription by RNA polymerase.

With regard to transcriptional regulation of the *fru* operon, the results summarized here and previously (6), as well as unpublished results (Cao and Saier) suggest (i) that the *fru* regulon in *S. typhimurium* is complex; (ii) that it may consist of at least three or four operons, one encoding the III^{Fru}-FPr protein, fructose-1-phosphate kinase, and II^{Fru}, a second encoding a fructose-inducible enzyme I-like protein, a third encoding the fructose repressor, FruR, and possibly a fourth encoding a cryptic II^{Fru} (Cao and Saier, unpublished results); (iii) that it is unique in its response to the loss of the cyclic AMP-CRP complex as a result of mutations in the *cya*, *crp*, or *pts* gene; (iv) that it may be regulated at the transcriptional level by two or more distinct mechanisms (possibly involving two distinct inducers, fructose-1-phosphate and fructose) which together account for induction by fructose, or that low-level expression of cryptic *fru* genes accounts for the anomalous *fru* operon induction behavior; and (v) that the proteins of the fructose-specific PTS may play a direct or indirect role in transcriptional regulation. Some of these observations are reminiscent of transcriptional regulation in other operons encoding PTS proteins. For example, the *E. coli pts* operon, the *nag* regulon, and the *gut* operon have multiple promoters (10, 33, 34, 55), the *E. coli bgl* and *Bacillus subtilis sac* operons are controlled by PTS-mediated antiterminator phosphorylation (1, 2, 15, 29, 52), and the *E. coli gut* operon is regulated by antagonistic transcriptional repression-activation (55). A concise discussion of transcriptional regulation involving PTS proteins and the PTS genetic apparatus has recently appeared (40). Thus, while the modes of *fru* regulon transcriptional regulation have not yet been delineated, mechanisms to be considered include (i) regulation involving PTS-mediated protein phosphorylation, (ii) antitermination, (iii) initiation at multiple promoters, (iv) classical repression or activation, and (v) an unusual (if not unique) interaction between the cyclic AMP-CRP complex and fructose-specific transcriptional regulatory proteins. Further studies will be required to establish the mechanistic bases for the complex expressional patterns reported in this communication.

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