JOURNAL OF BACTERIOLOGY, May 1989, p. 2424–2434
0021-9193/89/052424-11$02.00/0

Vol. 171, No. 5

Altered Transcriptional Patterns Affecting Several Metabolic Pathways in Strains of *Salmonella typhimurium* Which Overexpress the Fructose Regulon

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Expression of β-galactosidase in transcriptional fusions with the *pps* gene (encoding phosphoenolpyruvate [PEP] synthase), the *aceBAK* operon (encoding malate synthase, isocitrate lyase, and isocitrate dehydrogenase kinase, respectively), and the *phs* operon (encoding either thiosulfate reductase or a regulatory protein controlling its expression) was studied in *Salmonella typhimurium*. β-Galactosidase synthesis in these strains was repressible either by growth in the presence of glucose or by the presence of a *fruR* mutation, which resulted in the constitutive expression of the fructose (*fru*) regulon. Five enzymes of gluconeogenesis (PEP synthase, PEP carboxykinase, isocitrate lyase, malate synthase, and fructose-1,6-diphosphatase) were shown to be repressed either by growth in the presence of glucose or the *fruR* mutation, while the glycolytic enzymes, enzyme I and enzymes II of the phosphotransferase system as well as phosphofructokinase, were induced either by growth in the presence of glucose or the *fruR* mutation. Overexpression of the cloned *fru* regulon genes (not including *fruR*) resulted in parallel repression of representative gluconeogenic, Krebs cycle, and glyoxylate shunt enzymes. Studies with temperature-sensitive mutants of *S. typhimurium* which synthesized heat-labile III^{Fru} proteins provided evidence that this protein plays a role in the regulation of gluconeogenic substrate utilization. Other mutant analyses revealed a complex relationship between *fru* gene expression and the expression of genes encoding gluconeogenic enzymes. Taken together, the results suggest that a number of genes encoding catabolic, biosynthetic, and amphibolic enzymes in enteric bacteria are transcriptionally regulated by a complex catabolite repression/activation mechanism which may involve enzyme III^{Fru} of the phosphotransferase system as one component of the regulatory system.

Previous reports have provided evidence that the phosphotransferase systems in *Salmonella typhimurium* and *Escherichia coli* in some way regulate the process of sugar biosynthesis (gluconeogenesis) by depressing the activities of the gluconeogenic enzymes phosphoenolpyruvate (PEP) synthase (encoded by the *pps* gene) and PEP carboxykinase (encoded by the *pck* gene) (3, 9, 27). This evidence was derived from strains of *Salmonella* which exhibited high-level constitutive expression of the fructose (*fru*) regulon. Mutations in the fructose repressor gene (*fruR*) simultaneously resulted in fructose regulon constitutivity and inability to grow on gluconeogenic substrates such as alanine and Krebs cycle intermediates (3). The same inability to grow on gluconeogenic substances resulted from overproduction of the glucitol enzyme III (III^{Gut}) (31–33). The effects of the *fruR* mutation were shown to be independent of cyclic AMP (cAMP) and its receptor protein (3). Depressed activities of gluconeogenic enzymes appeared to at least partially account for the growth phenotypes of these mutants (10, 12, 21), but the regulatory mechanism and the protein constituent(s) of the fructose regulon which were involved were not identified.

In this report we describe the construction of *lacZ* transcriptional fusions to the *pps* gene and to a hitherto unidentified gene, closely linked to *pps*, which appears to regulate expression of both the *pps* and *pck* genes in *S. typhimurium*. The fusion in the *pps* structural gene caused β-galactosidase to be synthesized in response to the factors and conditions controlling transcription of this gene. β-Galactosidase synthesis in this strain is shown to be repressible by growth in the presence of glucose or by introduction of the *fruR* mutation. However, the degrees of repression both by glucose and by the *fruR* mutation are apparently diminished by construction of the fusion. The same observations were made for β-galactosidase expression in strains of *S. typhimurium* which contain the *lacZ* gene transcriptionally fused to genes in the *aceBAK* operon. This operon normally encodes malate synthase and isocitrate lyase, the enzymes of the glyoxylate shunt, as well as isocitrate dehydrogenase kinase (16). Interestingly, enzyme assays also revealed that fructose-1,6-diphosphatase is repressed by growth in the presence of glucose or by the *fruR* mutation, while enzyme I and enzymes II of the bacterial PEP-dependent phosphotransferase system (PTS) as well as phosphofructokinase activities were enhanced. Similar results were obtained when strains of *Salmonella* which overproduce the plasmid-encoded *fru* regulon gene products were studied. Experiments with temperature-sensitive mutants which synthesize heat-labile III^{Fru} proteins provided evidence that this protein plays a role in the regulation of gene expression promoted by the *fruR* mutation. A novel and complex mechanism of catabolite repression is implied.

MATERIALS AND METHODS

Strain construction and characterization. Bacterial strains used in the present study are listed in Table 1. Transposon Tn*5* mutagenesis was accomplished by growing phage P22 on strain TT3416, which contains a Tn*5* insertion element in a temperature-sensitive F′ plasmid. This strain was obtained from D. Botstein, Massachusetts Institute of Technology.

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TABLE 1. Strains of *Salmonella typhimurium* used in this study[a]

Strain	Genotype	Relevant defect(s)	Construction, source, or reference
ATCC 14028	Wild type	None	E. Groisman
EB222	*phs-101*::Mu dl(*amp lac*)	Thiosulfate reductase (regulatory or structural)	M. A. Clark (4)
JM103[b] (pMYS139)	Δ(*lac pro*)*thi rpsL supE endA sbcB15 hsdR4*(F′ *traD36 proAB lacI*^{q} ZΔ*M15*) (*pMYS139* [*gutB*$^+$])	Overproduction of III^{Gut}	32
MS1309	*aceA112*::Mu dJ	Isocitrate lyase	S. Maloy (30)
MS1311	*aceB113*::Mu dJ	Malate synthase	S. Maloy (30)
PP994	*crr-307*::Tn*10*	III^{Glc}	P. Postma (28)
SA2020	*aroD88*	Aromatic amino acid auxotrophy	27
SB1873	*Wild type*	None	26
SB2026	*ptsH15 crr-6*	HPr, III^{Glc}	25
SGSC647	*galE*::Tn*10*	UDP galactose 4-epimerase	K. Sanderson
LJ712	*fruR51*::Tn*10*	Fructose repressor	3
LJ1229	*phs-101*::Mu dl(*amp lac*) *fruR51*::Tn*10*	Thiosulfate reductase (regulatory or structural), fructose repressor	Transduction of EB222 to Tet^r with LJ712 as donor
LJ1230	*phs-101*::Mu dl(*amp lac*) *crr-307*::Tn*10*	Thiosulfate reductase (regulatory or structural), III^{Glc}	Transduction of EB222 to Tet^r with PP994 as donor
LJ2239	*fruR51*::Tn*10 fru-61*::Tn*5*	Fructose repressor, fructose utilization	Transduction of LJ2301 to Kan^r and fructose negativity with LJ2440 as donor
LJ2240	*ptsH15 crr-6 fru-61*::Tn*5*	HPr; III^{Glc}, fructose utilization	Tn*5* insertion yielding fructose negativity from SB2026
LJ2301	*fruR51*::Tn*10*	Fructose repressor	Transduction of SB1873 to Tet^r with LJ712 as donor
LJ2413	*pps-1*::Mu dJ *ppsC*	PEP synthase, PEP synthase promoter (?)	Spontaneous Lac$^+$ derivative of LJ2490
LJ2415	*pps-1*::Mu dJ	PEP synthase	Transduction of SB1873 to Kan^r with LJ2490 as donor
LJ2416	*pps-1*::Mu dJ *fruR51*::Tn*10*	PEP synthase, fructose repressor	Transduction of LJ2415 to Tet^r with LJ712 as donor
LJ2417	*pps-1*::Mu dJ *ppsC fruR51*::Tn*10*	PEP synthase, PEP synthase promoter, *fru* repressor	Transduction of LJ2413 to Tet^r with LJ712 as donor
LJ2423–LJ2438	*pps-1*::Mu dJ *fruR51*::Tn*10 fru-66–fru-81*	PEP synthase, fructose repressor, Fru$^-$	NNG mutagenesis of LJ2416 to Fru$^-$
LJ2440	*pps-2*::Mu dJ	PEP synthase regulatory gene	Simultaneous transduction of SA2020 to Kan^r and AroD$^+$ with a random pool of Mu dJ insertions
LJ2441	Wild type	None	Transduction of SA2020 to AroD$^+$ with SB1873
LJ2443	*fruR51*::Tn*10*	Fructose repressor	Transduction of LJ2441 to Tet^r with LJ2301 as donor
LJ2463	*fruR51*::Tn*10 fruB62*(Ts)	Fructose repressor, III^{Fru}	NNG mutagenesis of LJ2301 to xylitol resistance
LJ2465	*fruR51*::Tn*10 fruB63*(Ts)	Fructose repressor, III^{Fru}	NNG mutagenesis of LJ2301 to xylitol resistance
LJ2467	*fruR51*::Tn*10 fruB64*(Ts)	Fructose repressor, III^{Fru}	NNG mutagenesis of LJ2301 to xylitol resistance
LJ2469	*fruR51*::Tn*10 fruB65*(Ts)	Fructose repressor, III^{Fru}	NNG mutagenesis of LJ2301 to xylitol resistance
LJ2480	*ptsH15 crr-6 fru-61*::Tn*5* *muC*$^+$ (*amp*)	HPr, III^{Glc}, fructose utilization, *muC*$^+$ (*amp*) lysogen	Transduction of LJ2240 to *amp* with SL4213::*muC*$^+$(*amp*) as donor (11)
LJ2490	*pps-1*::Mu dJ	PEP synthase	Simultaneous transduction of SA2020 to Kan^r and AroD$^+$ with a random pool of Mu dJ insertions
LJ2491	*aceA112*::Mu dJ	Isocitrate lyase	Transduction of LJ2441 to Kan^r with MS1309 as donor
LJ2492	*aceB113*::Mu dJ	Malate synthase	Transduction of LJ2441 to Kan^r with MS1311 as donor
LJ2493	*pps-1*::Mu dJ *fruR51*::Tn*10*	PEP synthase, fructose repressor	Transduction of LJ2490 to Tet^r with LJ712 as donor
LJ2494	*aceA112*::Mu dJ *fruR51*::Tn*10*	Isocitrate lyase, fructose repressor	Transduction of LJ2491 to Tet^r with LJ712 as donor
LJ2495	*aceB113*::Mu dJ *fruR51*::Tn*10*	Malate synthase, fructose repressor	Transduction of LJ2492 to Tet^r with LJ712 as donor
LJ2510	*ptsH15 crr-6 fru-61*::Tn*5* *muC*$^+$(*amp*) *galE*::Tn*10*	HPr, III^{Glc}, fructose utilization, *muC*$^+$ (*amp*) lysogen, UDP galactose 4-epimerase	Transduction of LJ2480 to Tet^r with SGSC647 as donor

Continued on following page

TABLE 1—*Continued*

Strain	Genotype	Relevant defect(s)	Construction, source, or reference
LJ2522	*ptsH15 crr-6 fru-61*::Tn*5* *muC*$^+$ (*amp*) *galE*::Tn*10*(pMC2 *fru*)	HPr, III^{Glc}, fructose utilization, *muC*$^+$ (amp) lysogen; UDP galactose 4-epimerase, pMC2 (random clone of *Salmonella* DNA)	Transduction of LJ2510 to Neo^r with 14028 (pEG5155) as donor (11)
LJ2523	*ptsH15 crr-6 fru-61*::Tn*5* *muC*$^+$ (*amp*) *galE*::Tn*10*(pMC3 *fru*$^+$)	HPr, III^{Glc}, fructose utilization, *muC*$^+$ (*amp*) lysogen, UDP galactose 4-epimerase, pMC3	Transduction of LJ2510 to Neo^r and fructose positivity with 14028 (pEG5155) as donor (11)
LJ2539	*pps-2*::Mu dJ	PEP synthase regulatory gene	Transduction of LJ2441 to Kan^r with LJ2440 as donor
LJ2540	*fruR51*::Tn*10 pps-2*::Mu dJ	Fructose repressor, PEP synthase regulatory gene	Transduction of LJ2443 to Kan^r with LJ2440 as donor
LJ2552	*fruR51*::Tn*10 fruB62*(Ts) *aceB113*::Mu dJ	Fructose repressor, III^{Fru}, malate synthase	Transduction of LJ2463 to Kan^r with MS1311 as donor
LJ2553	*fruR51*::Tn*10 fruB63*(Ts) *aceB113*::Mu dJ	Fructose repressor, III^{Fru}, malate synthase	Transduction of LJ2465 to Kan^r with MS1311 as donor
LJ2554	*fruR51*::Tn*10 fruB64*(Ts) *aceB113*::Mu dJ	Fructose repressor, III^{Fru}, malate synthase	Transduction of LJ2467 to Kan^r with MS1311 as donor
LJ2555	*fruR51*::Tn*10 fruB65*(Ts) *aceB113*::Mu dJ	Fructose repressor, III^{Fru}, malate synthase	Transduction of LJ2469 to Kan^r with MS1311 as donor
LJ2558	*fruR51*::Tn*10 aceB113*::Mu dJ	Fructose repressor, malate synthase	Transduction of LJ2301 to Kan^r with MS1311 as donor

[a] Strains of different numbers which are of identical genotype were constructed in different genetic backgrounds. Construction of these strains allowed rigorous comparison of phenotypes due to specifically altered alleles in appropriate isogenic backgrounds.
[b] *E. coli* strain.

The resulting lysate was used to infect recipients at 42°C, and selection was made for kanamycin resistance (Kan^r). LJ2240 was isolated by this procedure. *lacZ* transcriptional fusions were constructed by mutagenesis with the Mu dJ transposable element (2, 13). P22 was grown on strain TT10288 (13), and the resulting lysate was used to transduce SB1873 to kanamycin resistance. Random Mu dJ mutants were pooled (about 5,000 transductants per pool) for a second round of P22 growth (6). The resulting lysates were used to transduce SA2020 (*aroD88* [27]) simultaneously to amino acid prototrophy and kanamycin resistance on minimal glucose plus kanamycin plates to obtain a collection of *lac* fusion mutations which were clustered near *aroD*. These mutants were screened for the inability to grow on minimal lactate-pyruvate-kanamycin plates on the assumption that the *pps* gene maps near *aroD* in *S. typhimurium* as it does in *E. coli* (1). This assumption was found to be valid, and two lactate- and pyruvate-negative mutants (LJ2440 and LJ2490) were saved for further analysis.

LJ2413 was isolated as a spontaneous mutant from LJ2490 with an enhanced ability to ferment lactose. LJ2415 was selected as a kanamycin-resistant transductant of SB1873 with LJ2490 as the donor. LJ2416 and LJ2417 are the *fruR* derivatives of LJ2415 and LJ2413, respectively, which resulted from transduction to tetracycline resistance with P22 grown on LJ712. The method of P22 transduction was described previously (7). The growth phenotypes of these mutants, summarized in Table 2, are consistent with their genotypes, with the exception of strains LJ2490 and LJ2415, which grew poorly in acetate (see below).

Cloning of the *fru* genes. The cloning of the *fru* regulon was accomplished by an in vivo cloning procedure (11). The donor lysate was prepared by Eduardo Groisman from a derivative of strain ATCC 14028 which contained the mini-Mu cloning vector pEG5155. The recipient was constructed from a *fru-61*::Tn*5* mutant (LJ2240) by successive transduction to Mu lysogeny (ampicillin resistance) and *galE*::Tn*10* (tetracycline resistance). The Mu (Amp^r) P22 lysate was provided by Dr. Groisman, and the *galE*::Tn*10* allele was derived from SGSC647. These genetic alterations conferred plasmid stability and Mu susceptibility, respectively, to the recipient (11). Recipients which received a clone expressing the *fru* genes were selected and screened directly on eosin-methylene blue (EMB) with fructose-neomycin (200 μg/ml) plates. Selection for resistance to high levels of neomycin gave rise to multiple copies of the plasmid-encoded *kan* gene. (The chromosome contained a single copy of the *kan* gene in the Tn*5*.) Fructose-positive clones were obtained at a frequency of 5×10^{-4}. Plasmid DNA was isolated from several fructose-positive clones and used to transform the parent to verify the presence of the *fru* genes on the plasmid.

TABLE 2. Growth phenotypes[a]

Strain (genotype)	Alanine	Pyruvate	Lactate	Acetate	Citrate	Lactose	Fructose	Mannitol
SB1873 (wild type)	+	+	+	+	+	−	+	+
LJ2490 (*pps*::*lacZ*)	−	−	−	±	+	−	+	+
LJ2413 (*pps*::*lacZ ppsC*)	−	−	−	±	+	+	+	+
LJ2415 (*pps*::*lacZ*)	−	−	−	±	+	−	+	+
LJ2416 (*pps*::*lacZ fruR*)	−	−	−	−	−	−	+	+
LJ2417 (*pps*::*lacZ ppsC fruR*)	−	−	−	−	−	±	+	+
LJ2301 (*fruR*)	−	−	−	−	−	−	+	+

[a] Growth was followed for 2 days at 37°C. Symbols: +, strong growth; ±, weak growth; −, no growth.

Fructose-positive (e.g., LJ2523) and fructose-negative (e.g., LJ2522) transductants were retained for characterization.

Selection of mutants expressing temperature-sensitive enzyme III^{Fru}. Temperature-sensitive *fruB* mutations were isolated by taking advantage of the fact that *fruR* mutants are sensitive to the fructose analog xylitol (3). A late-logarithmic-phase culture of LJ2301 was spread (~5 × 10^6 cells per plate) onto minimal arabinose (0.2%)–xylitol (0.1%) plates. After the plates were dry, crystals of 1-methyl-3-nitro-1-nitrosoguanidine (NNG) were sprinkled onto them and incubated at 41°C for 2 days. Xylitol-resistant colonies were clonally isolated and screened for fructose fermentation at 41 and 30°C. Ten of 1,200 mutants which were fructose negative at 41°C were partially or fully positive at 30°C. LJ2463, LJ2465, LJ2467, and LJ2469 were among these mutants.

Media and drugs. LB broth and the minimal salts medium used (medium 63) have been described before (20). All carbon sources in the minimal medium were present at a concentration of 0.2% except for DL-lactate, pyruvate, and citrate, which were present in neutralized form at 0.5%. EMB agar base was obtained from Difco Laboratories (catalog no. 0511-11) and was supplemented with 0.5% sugar except for lactose (1%). Glucose or fructose, when present, was added to LB broth to a final concentration of 0.2% when cultures were grown for extract preparation.

Amino acids (10 μg/ml) and vitamins (2 μg/ml) were added to minimal medium as required (6). Drug concentrations were 25 μg/ml for kanamycin, 20 μg/ml for tetracycline, 40 μg/ml for ampicillin, and 200 μg/ml for neomycin.

Enzyme assays. Crude extracts were prepared from early-stationary-phase bacterial cultures (LB broth supplemented with a carbon source when indicated) by washing the cells three times with 50 mM potassium phosphate, pH 7.4, passing them through a French pressure cell at 10,000 lb/in^2, and removing cellular debris by low-speed centrifugation. β-Galactosidase (20), PEP synthase (method 1 [5]), PEP carboxykinase (15), isocitrate lyase (18), fructose-1,6-diphosphatase (22), phosphofructokinase (8), and the enzymes of the PTS (26) were assayed as described previously. The malate synthase assay was slightly modified from a previously described protocol (18). Preequilibration of the glyoxylate with Tris buffer prevented spurious changes in the OD_{232} during the determinations. Protein was determined by the method of Lowry et al. (17).

RESULTS

Partial suppression of the FruR phenotype by traces of glucose. A wild-type strain of *S. typhimurium* grew on minimal agar plates, as shown in Table 3. It did not grow on plates containing 25 μM glucose as the sole source of carbon. Addition of this concentration of glucose to medium containing a high concentration of acetate, lactate, glycerol, or citrate had no effect on growth of the wild-type cells.

Strain LJ2301 (*fruR51*::Tn*10*) was not able to utilize acetate or lactate as the sole carbon source. It utilized citrate poorly, but glycerol was utilized at the wild-type rate. Unlike the wild-type strain, addition of 25 μM glucose to the solid medium substantially stimulated growth on all gluconeogenic substrates. *E. coli* JM103(pMYS139), a III^{Gut}-overproducing strain (31), exhibited behavior qualitatively similar to that of the *S. typhimurium fruR* mutant (Table 3). The relatively poor utilization of citrate by JM103(pMYS139) is reflective of poor utilization of citrate by *E. coli*.

TABLE 3. Synergistic effect of gluconeogenic substances and 25 μM glucose on the growth of a *fruR* mutant strain and a III^{gut} overproducer[a]

Carbon source	Colony surface area (mm^2)		
	LJ2441 (wild type)	LJ2301 (*fruR*)	JM103 (pMYS139) (III^{Gut} overproducer)
Glucose (25 μM)	<0.2	<0.2	<0.2
Acetate (0.5%)	13	0.2	0.2
Acetate (0.5%) + glucose (25 μM)	13	3	3
DL-Lactate (1%)	19	<0.2	3
DL-Lactate (1%) + glucose (25 μM)	19	3	3
Glycerol (0.2%)	13	13	7
Glycerol (0.2%) + glucose (25 μM)	13	13	16
Citrate (0.5%)	19	3	0.2
Citrate (0.5%) + glucose (25 μM)	19	7	5

[a] Colony surface areas were calculated after plates were incubated for 96 h at 37°C.

Repression of gluconeogenic enzyme synthesis, stimulation of glycolytic enzyme synthesis, and effects of the *fruR* mutation. As shown in Table 4, growth of wild-type cells in the presence of glucose reduced the activities of five distinct gluconeogenic enzymes. The Krebs cycle enzyme isocitrate dehydrogenase and pyruvate kinase showed similar repression. By contrast, the glycolytic enzymes, enzyme I of the phosphotransferase system and phosphofructokinase, exhibited enhanced activities when cells were grown in the presence of glucose. Introduction of the *fruR51* mutation had effects on both the gluconeogenic enzymes and the glycolytic enzymes which paralleled those elicited by growth in the presence of glucose (Table 4).

The effects of the *fruR* mutation on the expression of several PTS permeases were also examined (Table 5). In this

TABLE 4. Effects of glucose and the *fruR* mutation on the activities of selected enzymes of gluconeogenesis, glycolysis, and the Krebs cycle[a]

Enzyme	Relative sp act ± SD (%)		
	LJ2441 (wild type)	LJ2441 (wild type) + 0.5% glucose	LJ2443 (*fruR*)
PEP synthase	100	10 ± 5	5 ± 5
PEP carboxykinase	100	15 ± 2	23 ± 7
Fructose-1.6-diphosphatase	100	46 ± 6	46 ± 6
Isocitrate lyase	100	21 ± 5	45 ± 32
Malate synthase	100	7 ± 5	11 ± 1
Isocitrate dehydrogenase	100	12	28
Pyruvate kinase	100	53	51
Enzyme I	100	140 ± 20	270 ± 70
Phosphofructokinase	100	180	250

[a] All enzymes were measured in the soluble fraction of cell extracts (high-speed supernatants) according to the protocols outlined in Materials and Methods. The values (reported with standard deviation) represent the averages of two independent measurements. The absolute values in the first column are: PEP synthase, 31 nmol of pyruvate phosphorylated/min per mg of protein; PEP carboxykinase, 3.5 nmol of PEP produced per min per mg of protein; fructose-1,6-diphosphatase, 0.2 nmol of fructose-6-phosphate produced per min per mg of protein; isocitrate lyase, 1.0 nmol of isocitrate cleaved per min per mg of protein; malate synthase, 8.4 nmol of malate produced per min per mg of protein; phosphofructokinase, 4.4 nmol of fructose-1.6-diphosphate produced per min per mg of protein; enzyme I, 1.0 nmol of sugar phosphorylated per min per mg of protein; pyruvate kinase, 0.7 nmol of pyruvate formed per min per mg of protein; isocitrate dehydrogenase, 6.7 nmol of isocitrate oxidized per min per mg of protein.

TABLE 5. Effects of *fruR* mutation on uninduced activities of various enzymes II of the PTS[a]

Enzyme	Sp act (nmol of sugar phosphorylated/min per mg of protein) ± SD		
	LJ2441 (wild type)	LJ2443 (*fruR*)	Fold sp act increase by *fruR* mutation
II^{Fru}	0.46 ± 0.08	36 ± 2	78
II^{Mtl}	0.40 ± 0.22	2.4 ± 0.6	6.0
II^{Nag}	1.3 ± 0.1	1.6 ± 0.3	1.2
II^{Gut}/III^{Gut}	0.06 ± 0.02	0.10 ± 0.02	1.7
II^{Glu}	0.22 ± 0.22	0.28 ± 0.18	1.3
II^{Man}/III^{Man}	0.86 ± 0.26	1.3 ± 0.2	1.6

[a] Enzyme II activities were measured in crude extracts of uninduced (LB broth-grown) cultures as described previously (26). The results represent an average of two determinations. Standard deviations are reported. The sugar-specific components of the PTS were ensured of catalyzing the rate-limiting phosphoryl transfer reaction by inclusion of excess quantities of the soluble, energy-coupling components enzyme I and HPr.

study, enzyme II^{Fru} activity was nearly 80-fold higher in the *fruR* mutant than in the parental strain. Enzyme II^{Mtl} activity was also significantly increased. The activities of the other enzymes II were slightly increased by the *fruR* mutation. Therefore, levels of the enzymes II of the PTS, which are glycolytic in nature, are increased by the *fruR* mutation.

Properties of Mu dJ fusion strains and their *fruR* derivatives. The growth phenotypes of isogenic *pps*, *aceA*, and *aceB* mutants, in both the presence and absence of the *fruR* mutation, are summarized in Table 6. The growth properties of all of the *fruR* mutants were consistent with previous observations (3). The *pps* mutants were able to utilize citrate at wild-type rates but were unable to utilize lactate, alanine, or pyruvate as sole sources of carbon (Tables 2 and 6). These mutants also exhibited a diminished ability to grow on minimal acetate plates. To explain this last observation, carbon sources metabolized via the glyoxylate shunt may be converted to PEP by the PEP synthase-catalyzed reaction as well as the PEP carboxykinase-catalyzed reaction (12). The glyoxylate shunt mutants (*aceB* and *aceA*) were unable to use acetate as a sole carbon source but were like or nearly like the wild type with respect to the utilization of lactate and citrate. The growth phenotypes of the double mutants suggested that the mutations in the *pps* and *ace* operons did not interfere with the FruR phenotype.

TABLE 6. Growth phenotypes of *pps*::Mu dJ, *aceA*::Mu dJ, and *aceB*::Mu dJ operon fusions in the presence and absence of a functional *fruR* gene[a]

Strain	Genotype	Growth		
		Acetate	Lactate	Citrate
LJ2441	Wild type	+++	+++	+++
LJ2443	*fruR*	−	−	+
LJ2490	*pps-1*	+	−	+++
LJ2539	*pps-2*	+	−	+++
LJ2491	*aceA*	−	+++	++
LJ2492	*aceB*	−	++	++
LJ2493	*pps-1 fruR*	−	−	+
LJ2494	*aceA fruR*	−	−	+
LJ2495	*aceB fruR*	−	−	+

[a] Minimal medium 63 plates were supplemented with neutralized acetate (0.5%), DL-lactate (1%), or citrate (0.5%). Colony size was scored after 72 h at 37°C. Symbols; +++, wild-type growth rate; ++ and +, progressively smaller colony sizes; −, no growth. Each cell type gave uniformly sized colonies.

TABLE 7. Effects of sugars and various mutations on the activities of PEP synthase and PEP carboxykinase[a]

Strain	Genotype	Sugar added	Relative sp act (%)	
			PEP synthase	PEP carboxykinase
LJ2441	Wild type	None	100	100
		Glucose	14	12
		Fructose	7	16
LJ2443	*fruR*	None	<5	23
		Glucose	<5	7
	pps-1	None	<5	106
		Glucose	<5	13
LJ2539	*pps-2*	None	14	9
		Fructose	9	6

[a] Cells were grown in LB broth and enzymes were assayed as described in Materials and Methods. These determinations were performed on the soluble fraction of cell extracts (high-speed supernatants). The initial concentration of sugar in the growth medium was 0.2%. For PEP synthase, 100% = 24 nmol of PEP produced per min per mg of protein; for PEP carboxykinase, 100% = 112 nmol of PEP produced per min per mg of protein.

The PEP synthase and PEP carboxykinase activities summarized in Table 7 provide information about the extent of hexose repression in various strains, including the two presumptive *pps* fusion strains. In the wild-type strain, PEP synthase and PEP carboxykinase activities were strongly repressed both by glucose and by fructose. The *fruR* mutant exhibited undetectable PEP synthase activity and low but detectable PEP carboxykinase activity which was further repressed by growth in the presence of glucose. Since PEP synthase activity was below the level of detection in the *fruR* and *pps-1* strains, repression by glucose could not be measured. The *pps-1* mutant retained wild-type levels of PEP carboxykinase activity, which was repressed by glucose to an extent similar to that observed in the wild-type strain. In contrast, the *pps-2* mutant exhibited reduced but detectable PEP synthase activity, and it exhibited greatly reduced PEP carboxykinase activity as well. Both activities were further reduced by growth in the presence of fructose. These observations suggest that while the *pps-1* mutation is due to a defect in the structural gene for PEP synthase, the *pps-2* mutation is in a regulatory gene controlling the synthesis of both PEP synthase and PEP carboxykinase.

β-Galactosidase activities in strains which contain Mu dJ transcriptionally fused to a variety of *fruR*-regulated genes are presented in Table 8. The top eight strains share the *pps-1*::Mu dJ allele, and the β-galactosidase activities in these strains reflect the effects of various mutations and growth conditions on the transcription of the fused *pps-1* gene. Measurements of β-galactosidase activities in LJ2490, LJ2493, LJ2415, and LJ2416 represent parallel studies in two distinct isolates (genetic backgrounds) of LT2 (see Table 1 for the origins of these strains). In both cases, the *fruR* mutation caused a reduction in β-galactosidase activity. This change in expression was modest but was the same within experimental error as that elicited by growth in the presence of glucose.

LJ2413 was isolated as a spontaneous lac^+ derivative of LJ2490 (*pps-1*::Mu dJ). Strain SA2020 (*aroD88*) was transduced to kanamycin resistance with P22 grown on LJ2413 (*pps-1*::Mu dJ *ppsC*). The transductants were then scored for lactose fermentation and the ability to grow on minimal medium without the addition of aromatic amino acids. The lac^+ mutation was found to be 99% cotransducible with the *kan* gene within the Mu dJ element. While it is assumed that this mutation enhances the transcription of the *pps-1* gene, it

TABLE 8. β-Galactosidase activities in *lacZ* fusion strains

Strain	Genotype	β-Galactosidase activity[a] ± SD			
		No addition	Glucose	Fructose	cAMP
LJ2490	*pps-1*::Mu dJ	4.8 ± 0.5	3.1 ± 0.4	—[b]	—
LJ2493	*pps-1*::Mu dJ *fruR*	2.9	—	—	—
LJ2413	*pps-1*::Mu dJ *ppsC*	380	260	—	—
LJ2417	*pps-1*::Mu dJ *ppsC fruR*	230	130	—	—
LJ2415	*pps-1*::Mu dJ	3.8	1.9	—	—
LJ2416	*pps-1*::Mu dJ *fruR*	1.7	1.4	—	—
LJ2423	*pps-1*::Mu dJ *fruR fru*	11	—	—	—
LJ2429	*pps-1*::Mu dJ *fruR fru*	0.1	—	—	—
LJ2539	*pps-2*::Mu dJ	18 ± 1.3	22	10	—
LJ2540	*pps-2*::Mu dJ *fruR*	12 ± 2.1	14	8.8	—
LJ2491	*aceA*::Mu dJ	83	19	—	
LJ2494	*aceA*::Mu dJ *fruR*	23	11	—	—
LJ2492	*aceB*::Mu dJ	89	12	—	—
LJ2495	*aceB*::Mu dJ *fruR*	68	11	—	—
EB222	*phs*::Mu dJ	810	—	—	740
LJ1229	*phs*::Mu dJ *fruR*	430	—	—	400
LJ1230	*phs*::Mu dJ *crr*	400	—	—	560

[a] Activities are expressed as nanomoles of *p*-nitrophenyl β-galactoside cleaved per minute per milligram of protein. These determinations were performed on crude extracts of LB broth grown cells. Glucose and fructose were added to a final concentration of 0.2%. cAMP was added to a final concentration of 5 mM.
[b] —, Not determined.

has not been determined whether it is due to a promotorlike or *trans*-acting defect. The mutation has been termed *ppsC*.

The *ppsC* mutation increased β-galactosidase expression by about 80-fold. However, the transcriptional response to the *fruR* mutation or to growth in the presence of glucose remained similar to that of the parent (LJ2490) on a percentage change basis. In other words, the *ppsC* mutation did not alter the regulatory responses to glucose or *fruR*.

LJ2423 and LJ2429 are derivatives of LJ2416 (*pps-1*::Mu dJ *fruR*) which were selected for the simultaneous utilization of citrate as sole carbon source and altered β-galactosidase expression. These two mutants (but not all of the mutants obtained by this procedure) possessed a fructose-weak or fructose-negative phenotype. Mutations selected in this way might be expected to act in *trans* on the expression of *lacZ*. In fact, in neither of these two strains was the mutation which affected *lacZ* transcription cotransducible with the *pps-1* gene. A 7-fold increase and a 16-fold decrease in β-galactosidase activity in LJ2423 and LJ2429, respectively, relative to the parental strain, were observed. These results suggest that the *pps-1* fusion is in the normally transcribed orientation and is subject to regulation. This selection procedure as well as the biochemical and phenotypic properties of these mutants will be more fully described in the next section (see Table 9).

Strains LJ2539 and LJ2540 contain the *pps-2*::Mu dJ fusion in the presence and absence of a functional *fruR* gene, respectively. As for the *pps-1*::Mu dJ mutant, β-galactosidase expression in the *pps-2*::Mu dJ strain was depressed by growth in the presence of glucose or by the presence of the *fruR* mutation. Growth in the presence of fructose also repressed β-galactosidase synthesis. Since the *pps-1* and *pps-2* fusions exhibited quantitatively similar degrees of repression by both sugar and the *fruR* mutation, and since these two presumed cistrons mapped close together, it is reasonable to propose that these two genes constitute part of a regulon.

LJ2491, LJ2494, LJ2492, and LJ2495 contain transcriptional fusions to two genes of the glyoxylate shunt, *aceA* and *aceB* (30) in genetic backgrounds which either lack or contain the *fruR51*::Tn*10* allele. The *fruR* mutation reduced β-galactosidase expression in the *aceA*::Mu dJ fusion by about fourfold, as did growth in the presence of glucose. Growth of the *fruR* derivative in the presence of glucose resulted in further repression (Table 8). Qualitatively, the *aceB* fusion acted like the *aceA* fusion in response to the *fruR* mutation. However, repression of β-galactosidase synthesis by *fruR* was less severe in the *aceB* strain. Repression of β-galactosidase synthesis in the *aceB* mutant by glucose was similar in degree to that observed in the *aceA* fusion. Further, repression by *fruR* and that by glucose were at least partially additive.

The last three strains in Table 8 contain transcriptional fusions to a thiosulfate reductase (*phs*) structural or regulatory gene (4). The *fruR* mutation repressed transcription of this gene about twofold by a cAMP-independent mechanism. A mutation in the *crr* gene, which encodes the enzyme III^{Glc}, also decreased expression of *lacZ*. However, unlike *fruR*-promoted repression, repression caused by the *crr* mutation was partially overcome by growth in the presence of cAMP (Table 8).

Regulatory mutants of LJ2416 with altered citrate utilization, β-galactosidase expression, and fructose regulon expression. LJ2416 was spread on a plate of EMB lactose, and a crystal of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was used to induce mutations. Mutants with altered lactose fermentation were clonally isolated and scored for fructose, mannitol, lactose, and citrate utilization. Mutants which were simultaneously altered in two or three of these characteristics (LJ2423 through LJ2438) occurred with high frequency, suggesting a common regulatory defect. These mutants were kept for further analyses. Some of these strains were characterized biochemically, as shown in Table 9. The resulting data revealed no clearly consistent phenotypic or biochemical pattern. Several observations are, however, worthy of note. (i) All mutants described in Table 9 showed decreased fructose fermentation, although other mutants isolated by the same procedures possessed a fully fructose-positive phenotype. (ii) Some of the mutants showed depressed mannitol utilization, and most mutants with weak mannitol fermentation displayed partially depressed fructose fermentation, but a consistent correlation between fructose and

TABLE 9. Unlinked Lac$^+$ and Cit$^+$ derivatives of LJ2416 (*pps-1*::Mu dJ *fruR*)

Strain	Genotype	Relative sp act[a]						
		β-Galactosidase	PEP carboxykinase	II^{Fru}	III^{Fru}	Fructose-1-phosphate kinase	Enzyme I	HPr
LJ2416	*pps-1*::Mu dJ *fruR*	1.0	1.0	1.0	1.0	1.0	1.0	1.0
LJ2423	*pps-1*::Mu dJ *fruR fru-66*	6.5	1.4	0.35	0.36	0.60	0.96	0.87
LJ2425	*pps-1*::Mu dJ *fruR fru-68*	0.09	1.2	0.02	0.05	0.94	1.04	0.81
LJ2426	*pps-1*::Mu dJ *fruR fru-69*	1.3	1.3	0.62	0.69	0.78	1.36	0.90
LJ2429	*pps-1*::Mu dJ *fruR fru-72*	0.06	1.8	0.04	0.09	1.13	1.27	0.83
LJ2430	*pps-1*::Mu dJ *fruR fru-73*	1.3	0.47	0.02	0.05	0.89	0.76	0.75
LJ2432	*pps-1*::Mu dJ *fruR fru-75*	1.1	0.73	0.35	0.72	0.62	0.77	0.77
LJ2438	*pps-1*::Mu dJ *fruR fru-81*	0.07	1.3	0.06	0.03	0.13	0.91	0.71

[a] The absolute activities of the various enzymes in the crude extract of strain LJ2416 were as follows: β-galactosidase, 1.4 nmol of *p*-nitrophenyl β-galactoside cleaved per min per mg of protein; PEP carboxykinase, 19 nmol of PEP produced per min per mg of protein; II^{Fru}, 3.7 nmol of fructose-1-phospohate produced per min per mg of protein, III^{Fru}, 4.0 nmol of fructose-1-phosphate produced per min per mg of protein; fructose-1-phosphate kinase, 2.6 μmol of fructose-1,6-diphosphate formed per min per mg of protein; enzyme I, 1.4 nmol of sugar phosphate formed per min per mg of protein; HPr, 9.8 nmol of sugar phosphate formed per min per mg of protein.

mannitol utilization was not noted. (iii) Mutants which exhibited the strong mannitol fermentation of the parent had a greatly impaired ability to ferment fructose. (iv) All Lac$^-$ or Lac$^+$ mutants were Fru$^-$ Mtl$^+$, but the positive lactose fermentation characteristic did not consistently correlate with a particular fructose or mannitol phenotype. (v) Mutants with substantially restored citrate utilization always showed a negative fructose fermentation response, but one of these mutants showed depressed mannitol fermentation as well as fructose fermentation. It must therefore be concluded that more than a single class of mutants, pleiotropically affected for growth on several carbon sources, were obtained.

The results of biochemical analyses of several of these regulatory mutants are presented in Table 9. The activities of β-galactosidase and PEP carboxykinase varied independently of each other and of the fructose enzymes. The activities of the enzymes II^{Fru} and III^{Fru} varied in parallel, suggesting that the mutations exerted regulatory effects on expression of the corresponding *fru* structural genes (*fruA* and *fruB*). Furthermore, the activities of the enzymes II^{Fru} and III^{Fru} correlated well with fructose fermentation. Synthesis of the fructose-1-phosphate kinase did not appear to be regulated coordinately with that of II^{Fru} and III^{Fru}. Instead, all of the mutants except LJ2438 possessed essentially normal levels of this enzyme. Finally, in all of the mutants, the activities of enzyme I and HPr appeared to be unaffected by these mutations. Thus, defects in the energy-coupling proteins of the PTS could not account for the poor mannitol fermentation of some of these strains. The results clearly suggest a regulatory interrelationship between *fru* gene expression and gluconeogenesis.

Effects of overexpression of cloned *fru* regulon genes on *fruR*-regulated gene expression. A 7-kilobase-pair fragment of *Salmonella* DNA which complemented a *fru* mutation was cloned into a high-copy-number plasmid (see Materials and Methods). Table 10 shows that when the parental strain harbored this plasmid, the levels of II^{Fru} and III^{Fru} expression were increased by about 50-fold and that of fructose-1-phosphate kinase was increased about 30-fold. These data suggest that the structural genes which code for these three enzymes reside within the cloned insert. FPr activity was increased, but not as dramatically as the other fructose enzymes. pMC2, which contained a random noncomplementing piece of *Salmonella* DNA, exhibited the low fructose PTS activity of the parent. Enzyme I, II^{Mtl}, and phosphofructokinase activities were increased by the presence of the *fru* clone. By contrast, the expression of isocitrate dehydrogenase and PEP carboxykinase was reduced by inclusion of the *fru* plasmid. These alterations were qualitatively, and in some cases quantitatively, similar to those produced by the *fruR* mutation (compare Table 10 with Tables 4 and 5). These data suggest that the effects of the *fruR* mutation may at least partially result from overexpression of one or more of the *fru* gene products. It is also possible, however, that the effect of overexpression of the *fru* operon exerts its regulatory effects by titrating out the *fru* repressor (see Discussion).

Properties of temperature-sensitive *fruB* mutants. A number of temperature-sensitive *fruB* mutants were isolated as described in Materials and Methods. The growth properties of four such mutants are summarized in Table 11. All four mutants could utilize both fructose and citrate for growth at 21°C. At 30°C, LJ2465 partially lost the ability to ferment fructose, and it simultaneously became negative on citrate. The other three mutants showed a transition temperature of about 37°C on both fructose and citrate. These results clearly suggest that if the mutation giving rise to the temperature-sensitive phenotype is due to a defect in the *fruB* gene, then III^{Fru} plays a role not only in fructose utilization, but also in citrate utilization.

TABLE 10. Effects of cloned fructose genes on expression of selected *fruR*-regulated genes[a]

Enzyme	Activity (nmol of reactant consumed/min per mg of protein)	
	LJ2522 [HPr$^-$ III^{Glc-} *fru*(pMC2)]	LJ2523 [HPr$^-$ III^{Glc-} *fru*(pMC3)]
II^{Fru}	0.34	16
III^{Fru}	0.13	6.6
Fructose-1-phosphate kinase	1.1	32
FPr	0.18	0.76
I	1.1	1.9
II^{Mtl}	0.09	0.32
Phosphofructokinase	6.1	7.7
Isocitrate dehydrogenase	5.6	2.0
PEP carboxykinase	0.91	0.21

[a] pMC2 was a random isolate which showed no *fru* regulon activity.

TABLE 11. Growth properties of temperature-sensitive *fruB* mutants at various temperatures

Strain	Genotype	Growth on citrate[a]				Fermentation of fructose[b]			
		21°C	30°C	37°C	41°C	21°C	30°C	37°C	41°C
SB1873	Wild type	++	++	++	++	++	++	++	++
LJ2301	*fruR*	+/−	+/−	+/−	+/−	++	++	++	++
LJ2463	*fruR fruB62*(Ts)	+	+	+/−	−	++	++	+	+
LJ2465	*fruR fruB63*(Ts)	+	−	−	−	++	+/−	+/−	+/−
LJ2467	*fruR fruB64*(Ts)	+	+	+/−	−	++	++	+	+
LJ2469	*fruR fruB65*(Ts)	+	+	+/−	−	++	++	+	+

[a] Citrate growth was assessed after a 5-day incubation period at the temperature indicated on medium 63 minimal plates containing 0.5% citrate. Symbols: ++, wild-type growth; +, +/−, −, progressively poorer growth.

[b] Fructose fermentation was assessed on EMB-fructose (0.5%) plates after a 24-h incubation period at 30, 37, or 41°C. Plates incubated at 21°C were scored after an incubation period of 3 days.

Table 12 provides direct biochemical evidence that these mutants possess thermolabile III^{Fru} proteins. III^{Fru} activity in the parental strain was stable to heat treatment (42°C). By contrast, all four mutants described exhibited thermolabile activities in crude extracts. A 10-min incubation at 42°C resulted in the loss of about 70% of the III^{Fru} activity. Interestingly, growth of one temperature-sensitive mutant at 41°C resulted in synthesis of a heat-stable III^{Fru}. It is not yet clear whether this stable III^{Fru} activity is due to complexation with a stabilizing protein (29) or to expression of a distinct structural gene. Enzyme II^{Fru} showed greater thermolability than other enzymes II which have been studied in this regard. It denatured at about 50°C (data not shown), in contrast to the 80 to 90°C observed for other enzymes II of the PTS (19, 24). It should be noted that LJ2554 grown at 41°C exhibited lower activities of II^{Fru} and fructose-1-phosphate kinase than the parental strain grown at 41°C, suggesting a role of III^{Fru} in expression of the *fru* regulon.

DISCUSSION

In previous communications, gluconeogenic deficiencies have been correlated with, and indeed were indirectly (3) or directly (9) attributed to, the *fruR* mutation in strains of *Salmonella typhimurium*. In this communication, we extended our studies to show that several enzymes in addition to those which function in gluconeogenesis are affected by the *fruR* mutation. Furthermore we probed the mechanism by which the *fruR* mutation regulates expression of distal genes.

The stimulation of growth of *fruR* mutants on gluconeogenic substances by trace amounts of glucose suggests that gluconeogenic deficiencies can at least in part account for the FruR growth phenotype. However, the in vitro studies presented here imply that the effects of this defect may extend beyond the biosynthesis of carbohydrates and indeed may explain why traces of glucose only partially suppressed the FruR phenotype (Table 3).

Recent results indicate that the *fruR* mutation greatly reduces the synthesis of the cytochrome *d* oxidase complex (T. Kaysser, R. B. Gennis, A. M. Chin, and M. H. Saier, Jr., unpublished results). Thus, the intracellular signal generated by the *fruR* mutation affects aerobic metabolism at the level of electron transport. Since growth in the presence of fermentable carbon sources (e.g., glucose) reduces cytochrome *d* expression (14), parallel effects of growth in the presence of glucose and the *fruR* mutation are observed. The recent finding that *fruR* mutations decrease the virulence of our *Salmonella* strains in mice (B. E. Hoertt, R. L. Warren, A. M. Chin, and M. H. Saier, Jr., unpublished results) may be the result of the altered metabolic abilities of these mutants.

Glycolysis and gluconeogenesis appear to be regulated by *fruR* in a reciprocal fashion (Fig. 1 and Table 13). That is, synthesis of some key glycolytic enzymes was stimulated by the *fruR* mutation, while synthesis of the gluconeogenic enzymes was repressed. It is therefore clear that overproduction of the fructose PTS components does not generally inhibit protein synthesis and that the repression of glucone-

TABLE 12. Heat inactivation of III^{Fru} in extracts derived from strains containing temperature-sensitive *fruB* mutations

Strain	Genotype	Growth temp (°C)	Relative sp act[a]					
			III^{Fru}		II^{Fru}		β-Galactosidase	Fructose-1-phosphate kinase
			No heat inactivation	10 min, 42°C	No heat inactivation	10 min, 42°C		
SB1873	Wild type	21	1.0	1.0	1.0	1.0	1.0	1.0
LJ2558	*aceB*::Mu dJ *fruR*	41	3.4	2.9	3.8	3.8	2.9	7.8
LJ2552	*aceB*::Mu dJ *fruR fruB62*(Ts)	21	0.63	0.20	1.7	2.3	1.1	0.94
LJ2553	*aceB*::Mu dJ *fruR fruB63*(Ts)	21	0.76	0.20	0.86	0.82	0.89	0.95
LJ2554	*aceB*::Mu dJ *fruR fruB64*(Ts)	21	0.73	0.27	1.2	1.3	0.95	0.90
		41	0.49	0.57	2.4	2.8	1.6	3.1
LJ2555	*aceB*::Mu dJ *fruR fruB65*(Ts)	21	0.37	0.14	0.81	1.0	1.1	1.3

[a] Absolute specific activities for the enzymes assayed in strain LJ2558 grown at 21°C were: III^{Fru} without heat inactivation, 1.2 nmol of fructose-1-phosphate produced per min per mg of protein; III^{Fru} with heat inactivation, 1.6 nmol of fructose-1-phosphate produced per min per mg of protein; II^{Fru} without heat inactivation, 0.74 nmol of fructose-1-phosphate produced per min per mg of protein; II^{Fru} with heat inactivation, 0.66 nmol of fructose-1-phosphate produced per min per mg of protein; β-galactosidase, 6.2 nmol of *p*-nitrophenyl β-galactoside cleaved per min per mg of protein; fructose-1-phosphate kinase, 31 nmol of fructose-1,6-diphosphate formed per min per mg of protein.

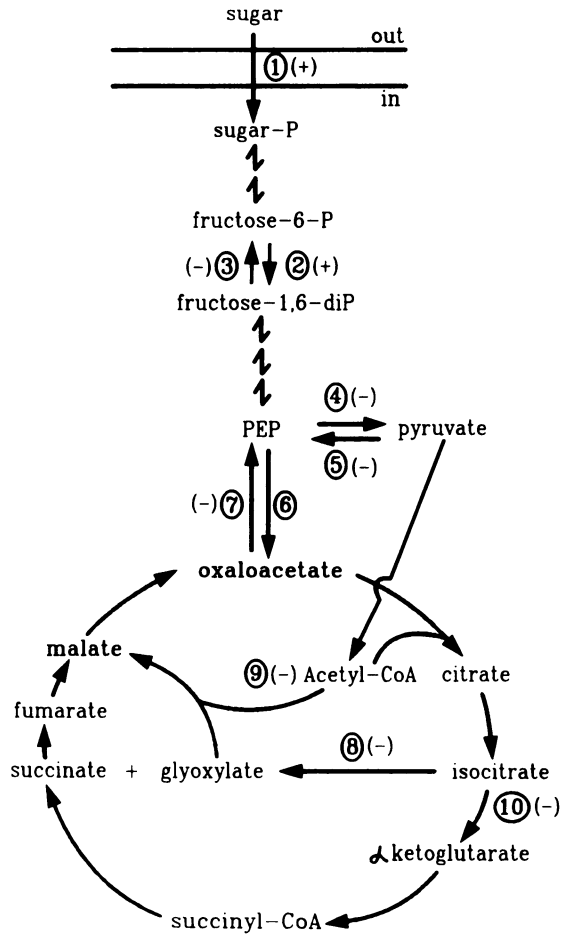

FIG. 1. Metabolic pathways concerned with anaerobic and aerobic sugar degradation (glycolysis [enzymes 1 and 2] and the Krebs cycle [enzymes 4, 6, and 10], respectively) as well as the sugar-biosynthetic pathways (the glyoxalate shunt [enzymes 8 and 9]) and gluconeogenesis (enzymes 7, 5, and 3). The direction of transcriptional regulation of the enzymes indicated by number is induced (+) or repressed (−) by the *fruR* mutation or by the presence of sugar in the medium. The enzymes are indicated by number as follows: 1, PTS; 2, phosphofructokinase; 3, fructose-1,6-diphosphatase; 4, pyruvate kinase; 5, PEP synthase; 6, PEP carboxylase; 7, PEP carboxykinase; 8, isocitrate lyase; 9, malate synthase; and 10, isocitrate dehydrogenase. It can be seen that enzymes of the anaerobic pathway (glycolysis) are induced by the *fruR* mutation or the presence of exogenous sugar, but the enzymes of the aerobic pathway as well as the glyoxalate shunt and gluconeogenic enzymes are repressed. P, Phosphate; CoA, coenzyme A.

ogenic enzyme synthesis exhibits specificity. Activation of one pathway accompanied by repression of the reverse pathway prevents futile cycling and suggests physiological relevance. Furthermore, the effects of growth in the presence of glucose were mimicked by the *fruR* mutation on representative enzymes of glycolysis, gluconeogenesis, the glyoxylate shunt, and the Krebs cycle (Fig. 1 and Table 13).

Since the *fruR* mutation has such wide-ranging effects, it may be a global regulatory gene which incidentally elevates fructose PTS expression. Three lines of evidence argue against this incidental role of the *fruR* gene and argue further for involvement of a *fru* regulon gene product in regulation. (i) Stimulation of PTS protein synthesis was relatively specific for the fructose system (Table 5). (ii) Elevated expression of the *fru* gene products encoded within a multicopy plasmid in the presence of a wild-type *fruR* gene elicited a biochemical pattern of gene expression resembling that of the *fruR* mutants (Table 10) and gave rise to a defective growth phenotype resembling that of *fruR* mutants (data not shown). (iii) Defined structural defects in III^{Fru} due to the presence of temperature-sensitive alleles (Table 12) correlated with a change in the *fruR*-associated growth defects (Table 11). Our working hypothesis is that at least some of the effects of *fruR* are due to overproduction of III^{Fru}.

TABLE 13. Central carbohydrate metabolic enzymes whose synthesis is influenced by *fruR* and exogenous sugar

Class	Enzymes
Glycolytic enzymes (induced by *fruR* or exogenous sugar)	Enzyme I, enzyme I^{Fru}, HPr, FPr, enzymes II or II-III pairs, phosphofructokinase[a]
Gluconeogenic enzymes (repressed by *fruR* or exogenous sugar)	Isocitrate lyase, malate synthase, PEP carboxykinase, PEP synthase, fructose-1,6-diphosphatase
Oxidative metabolic enzymes (repressed by *fruR* or exogenous sugar)	Isocitrate dehydrogenase, pyruvate kinase, cytochrome *d* complex

[a] All of these except phosphofructokinase are part of the PTS.

While our data do not exclude the possible participation of other *fru* gene products, such as the *fru* repressor, in this regulation, a role for III^{Fru} is suggested. Defined structural alterations in III^{Fru} (Table 12) correlated with changes in the *fruR* growth phenotype (Table 11). However, since III^{Fru} is overexpressed in *fruR* mutants (Table 5), which show poor utilization of citrate (Table 2), and since heat inactivation of III^{Fru} also leads to decreased citrate utilization (Table 11), it is possible that different conformations of III^{Fru} function in transcriptional activation and repression. On the other hand, our experiments do not distinguish primary from secondary effects of III^{Fru} thermolability on citrate utilization. The situation is rendered even more complex by the observation that when one of the presumptive temperature-sensitive *fruB* mutants was grown at 42°C, III^{Fru} activity was no longer thermosensitive (Table 12). These observations make simple interpretations of the results impossible. However, it should be noted that there is precedence for enzymes III mediating regulatory phenomena. Overproduction of III^{Gut} gives rise to phenotypic and biochemical properties resembling those of *fruR* mutants (Table 3) (31). A distinct regulatory role for III^{Glc} is also well established (23, 25).

We have obtained or constructed strains of *Salmonella typhimurium* in which Mu dJ is transcriptionally fused to operons which encode *fruR*-regulated enzymes. The fact that the *fruR* mutation reduced β-galactosidase expression in these strains suggests that this mutation acts by altering the transcription of target operons. However, although there was qualitative agreement between the β-galactosidase activity and the corresponding intact target enzymes, quantitative agreement was not always observed. Growth in the presence of glucose exhibited similar inconsistencies. These discrepancies may be due to physical disruption of the operon (e.g., polar effects on downstream regulatory genes) or to disruption of a different regulatory mechanism by inclusion of the insertion element. In vitro mixing experiments failed to demonstrate the presence of allosteric activators or inhibitors of *fruR*-regulated gene products by purified III^{Fru} or by *fruR* crude extracts (unpublished results). Furthermore, *fruR* mutations have no obvious effect on DNA supercoiling (C. F. Higgins, personal com-

munication). We are currently investigating the possibility that III^{Fru} or the *fruR* gene product regulates transcription by directly binding to the DNA of target sequences.

The *fruR* mutation causes repression of at least three genes (*aceA*, *aceB*, and *phs*) which are also repressed by glucose in a cAMP regulatory protein (CRP- and cAMP-) independent fashion (4, 30). Since *fruR*-elicited regulation, like glucose repression, is independent of the CRP-cAMP complex (3), it is possible that both processes function by the same or a related mechanism. Further studies will be required to establish the details of this novel mechanism.

Together, the observations reported in this article suggest that *fruR* mutations may inappropriately signal the presence of carbohydrates in the environment. This "inappropriate" signaling presumably reflects an involvement of the *fruR* gene product and/or the product of a *fru* regulon gene. In this connection it is worth noting that the PTS is the dominant carbohydrate transport system which senses the presence of its substrates in the environment and has been shown to regulate numerous aspects of carbohydrate metabolism in enteric bacteria (23).

The biochemical data described herein indicate that growth in the presence of carbohydrate or inclusion of the *fruR* mutation in the genome causes glycolysis to be stimulated while oxidative metabolism and gluconeogenesis are reduced (Fig. 1, Table 13). Perhaps, in the presence of excess carbohydrate, glycolysis is capable of supplying sufficient ATP by substrate level phosphorylation so that complete oxidation of the carbohydrate by the Krebs cycle and electron transport is not necessary. Advantage may be attained under these conditions by minimizing the number of enzymes which must be synthesized. Sugar biosynthesis (gluconeogenesis) under these conditions is clearly inappropriate.

ACKNOWLEDGMENTS

We thank Brigitte Feucht for valuable assistance with the enzyme assays and Andy Hengstenberg for assistance in the isolation of the temperature-sensitive *fruB* mutants described in Tables 11 and 12. Eduardo Groisman generously provided bacterial strains and assistance in the cloning of the *fru* regulon. Other bacterial strains were generously provided by D. Botstein, M. Clark, S. Maloy, P. Postma, and K. Sanderson. Valuable assistance in the preparation of this manuscript was provided by Mary Beth Hiller.

This work was supported by Public Health Service grants 5RO1AI21702 and 2RO1AI14176 from the National Institute of Allergy and Infectious Diseases.

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