JOURNAL OF BACTERIOLOGY, Jan. 1996, p. 280–283 Vol. 178, No. 1
0021-9193/96/$04.00+0

NOTES

FruR Mediates Catabolite Activation of Pyruvate Kinase (*pykF*) Gene Expression in *Escherichia coli*

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Received 28 June 1995/Accepted 18 October 1995

Expression of a *pykF-lacZ* fusion was studied as a function of the carbon source in wild-type strains and strains lacking or overproducing the FruR protein of *Escherichia coli*. FruR controls the response to the carbon source by repressing *pykF* expression more strongly under gluconeogenic than under glycolytic conditions, a phenomenon we term catabolite activation.

Multiple mechanisms underlie the regulation of gene expression in response to carbon source availability in enteric bacteria (13, 15). One mechanism, involving cyclic AMP and the cyclic-AMP receptor protein, is well established but does not account for all types of observed responses. Our recent studies have shown that mutations giving rise to defective functioning of the fructose repressor, FruR, lead to a pleiotropic, cyclic-AMP-independent phenotype. These mutants cannot utilize gluconeogenic substrates as sole sources of carbon for growth (4). This phenotype appears to arise because FruR is a transcriptional activator of essential gluconeogenic genes such as those encoding phosphoenolpyruvate synthase (*ppsA*) and phosphoenolpyruvate carboxykinase (*pckA*) (3, 4, 6, 8). The mechanism of FruR-mediated transcriptional regulation involves the binding of this protein to a well-established FruR binding sequence in the promoter regions of target operons (11, 12).

All FruR-controlled genes encoding enzymes concerned with sugar fermentation are subject to repression by FruR, while those encoding gluconeogenic, Krebs cycle, and glyoxylate shunt enzymes, as well as several electron transport proteins, are subject to activation by FruR (3, 7, 11–12a, 14). Furthermore, FruR has been shown to control the oxidation of at least three dozen carbon sources in *Escherichia coli* (11). In this report we extend the above-mentioned studies by demonstrating that FruR controls the response of one of the two *E. coli* pyruvate kinase genes, *pykF*, to carbon source availability.

Figure 1A shows the physical map of the *pykF* gene control region, Fig. 1B shows the PCR-amplified DNA fragment used for construction of the *pykF-lacZ* fusion, and Fig. 1C shows the PCR-amplified DNA fragments used for in vitro DNA binding studies. A FruR binding sequence is found downstream of the single putative *pykF* promoter region from −35 to −10, but no cyclic-AMP receptor protein binding site was identified. The position of the FruR box is consistent with a repressive function for FruR as discussed previously (11).

Two oligodeoxynucleotides, MB1309 and MB1307 (Table 1), were used in the PCR to amplify a 922-bp DNA fragment harboring the control region of the *E. coli pykF* gene (Fig. 1). This DNA fragment was used for the construction of a single-copy chromosomal protein fusion in the *E. coli* K-12 strain K10 (2), which we had rendered Lac$^-$ by phage P1*vir* transduction by using a lysate grown on strain SH210 (16). The *pykF-lacZ* protein fusion was generated in the vector system described by Simons et al. (17). The presence of the *pykF-lacZ* fusion of the resulting strain, SB431, was verified by PCR with primer MB1309 and a primer which annealed to the *lacZ* gene (MB1090 [Table 1]). In addition, DNA sequencing was performed to verify the preservation of the wild-type sequence in the amplified DNA fragments. Strain SB562 was constructed by raising phage P1*vir* on strain LJ2725 bearing a *fruR*::Kan^r mutation (11) and subsequently transducing this mutation into strain SB431. The phenotype of SB562 was tested as described previously (11).

Figure 2 presents *pykF-lacZ* induction data obtained after growth of the bacteria in the presence of various glycolytic and gluconeogenic substrates. In the *fruR* wild-type genetic background, the β-galactosidase activity was about twofold higher when cells were grown in the presence of a glycolytic substrate (glucose, fructose, galactose, or lactose) than when grown in the presence of a gluconeogenic substrate (succinate, fumarate, acetate, or pyruvate). β-Galactosidase activities were uniformly higher in a *fruR* mutant background than in the wild-type background, and the inductive effect of glycolytic substrates was essentially abolished by the genetic loss of FruR function. The apparent activity enhancement specifically observed when the *fruR* mutant was grown in fructose-containing medium is unexplained.

Figure 3 illustrates how *fruR* overexpression from a high-copy-number plasmid (11) affects *pykF-lacZ* expression in a *fruR* null mutant strain. As expected, overproduction of FruR depressed β-galactosidase activity irrespective of the supplemented carbon source. Most notable, however, is the fact that the repressive effect of FruR was substantially greater in gluconeogenic media than in glycolytic media.

The data in Fig. 2 and 3 demonstrate that FruR negatively regulates *pykF* expression. Further, FruR controls the response to the carbon source by a repression mechanism that is largely abolished under glycolytic conditions but not under gluconeogenic conditions. In this context, it is interesting that expression of the *E. coli pykA* gene, which encodes an isoenzyme of

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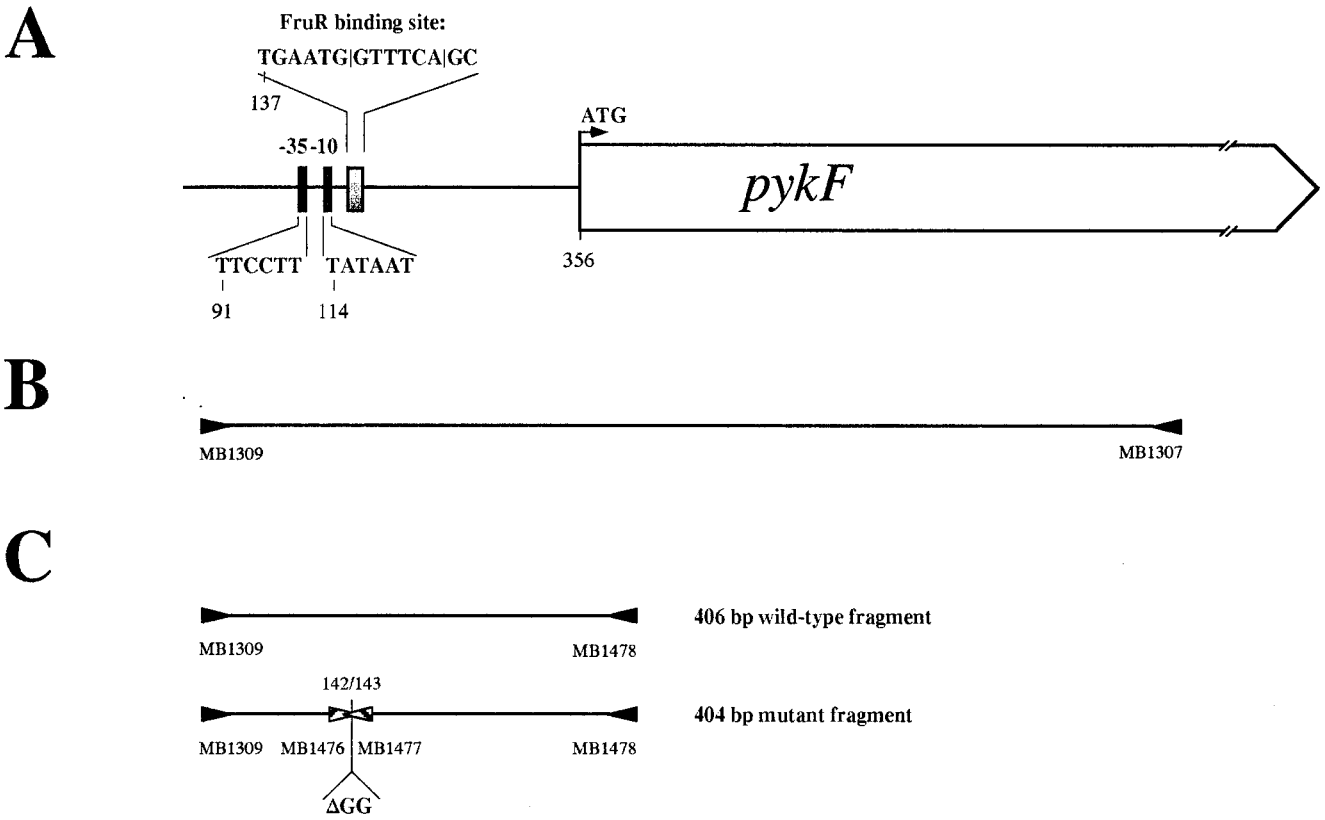

FIG. 1. (A) Physical map of the *pykF* regulatory region. Numbers given correspond to the base pairs provided in the published DNA sequence (accession number M24636). A FruR binding site is present downstream of a single putative promoter element from −35 to −10. The DNA fragments used for the construction of a translational *lacZ* fusion (922-bp fragment [B]) and for in vitro DNA binding studies (fragments used for DNA band migration retardation [C]) are also shown. The solid arrowheads indicate the oligodeoxynucleotides used to amplify DNA fragments by PCR. The sequences for these oligodeoxynucleotides are presented in Table 1. The striped arrowheads indicate primers used for PCR-mediated site-directed mutagenesis (see text for details).

pykF, is independent of FruR action and is not affected by the nature of the carbon source (unpublished results).

In order to establish that FruR regulates *pykF* expression directly by binding to an operator site in the *pykF* control region, DNA band migration retardation experiments were performed as described previously (11, 12). The wild-type DNA fragment used in these studies was amplified by PCR with primers MB1309 and MB1478 (Table 1). This procedure generated a 406-bp DNA fragment encompassing the regulatory region of the *pykF* gene (Fig. 1). In addition, a mutant DNA fragment with a 2-bp deletion in the putative FruR binding site of *pykF* was generated by PCR-mediated site-directed mutagenesis (1). In this mutagenesis procedure, two DNA amplification reactions were carried out, one with the primer pair MB1309 and MB1477 and one with the primer pair MB1478 and MB1476 (Table 1). Both amplified DNA fragments were purified, and they were combined in a third PCR with primers MB1309 and MB1478. The resulting 404-bp DNA fragment was shown by DNA sequencing to contain the expected 2-bp deletion in the center of the putative FruR binding site of *pykF* (Fig. 1). As can be seen in Fig. 4, FruR bound to and shifted the wild-type DNA fragment but not the mutant fragment. Addition of 5 mM fructose 1-phosphate (F1P) to the binding reaction resulted in reversal of FruR binding to the wild-type fragment, indicating that the FruR-F1P complex cannot bind to the *pykF* operator site.

TABLE 1. Designations and sequences of primers used for the synthesis of the control region of the *E. coli pykF* gene

Primer	Oligodeoxynucleotide sequence (5′ to 3′)[a]	Length of primer (nucleotides)
MB1309	4 GGACGAATTCGCGTAACCTTTTCCCTGGAACGTTAA	36
MB1307	906 GGACGGATCCTGTTCGCAACCAAAGATCAGGTCCTG	36
MB1478	391 GGACGGATCCGGTCCGATGGTGCAAACAATTTT	33
MB1477	157 CCAAAGT**GCTGAAA--ATTCA**AGAGTCAATTGGCGCG[b]	35
MB1476	131 GACTCTT**GAAT--TTTCAGC**ACTTTGGACTGTAG[b]	32
MB1090	2349 TAACGCCTCGAATCAGCAACGGCT	24

[a] Numbers are the nucleotide positions provided under the accession numbers for *pykF* (M24636) and *lacZ* (J01636). Underlined DNA sequences designate added nucleotides comprising a restriction enzyme site plus at least three nucleotides at the 5′ end.

[b] Primers MB1477 and MB1476 each contain a deletion of two nucleotides (5′-CC and 5′-GG, respectively) at positions 142 and 143 (indicated by dashes). The FruR binding site is shown in boldface. These two primers were used in combination with primers MB1309 and MB1478 to generate a defective FruR binding site.

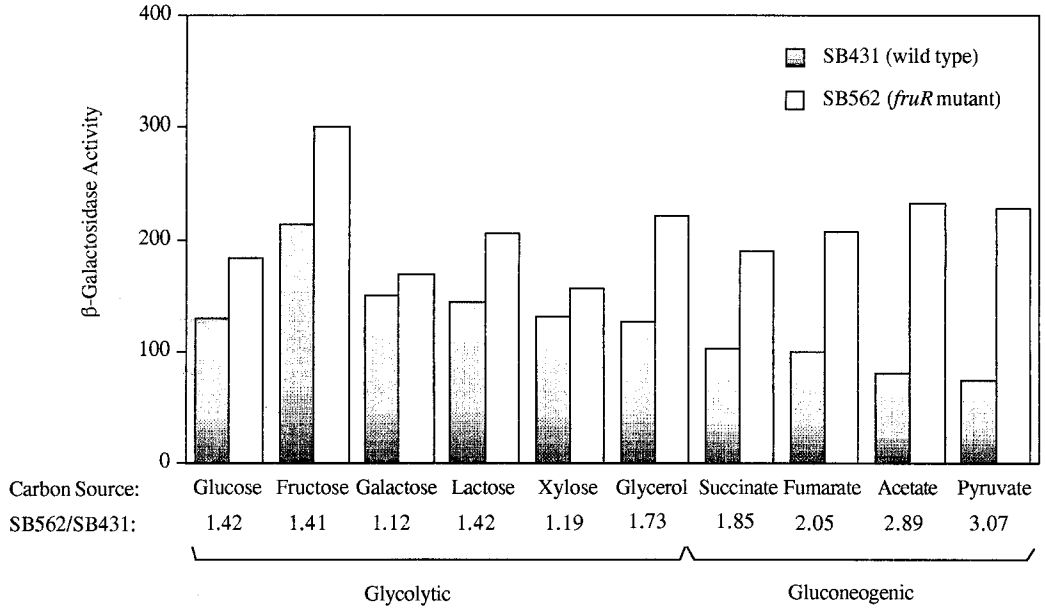

FIG. 2. Effect of carbon source and FruR deficiency on β-galactosidase activity in *pykF-lacZ* fusion strains. Single-copy, chromosomal *pykF-lacZ* fusions were constructed in the genetic backgrounds of a *fruR* mutant (SB562) as well as the isogenic wild-type strain (SB431). The strains were grown in minimal medium M9 (9) supplemented with the carbon source indicated (0.4%) and Casamino Acids (0.5%). The latter was added to allow growth of the *fruR* mutant (11). The β-galactosidase activities are expressed in Miller units (9). The fold derepression was calculated by comparing the specific β-galactosidase activities of the *fruR* mutant with its isogenic wild-type strain, and values are at the bottom. The values are averages of at least two independent expression studies, each using five samples harvested during the logarithmic phase (optical density at 600 nm between 0.4 and 0.8) of bacterial growth. The relative standard deviations did not exceed 15%.

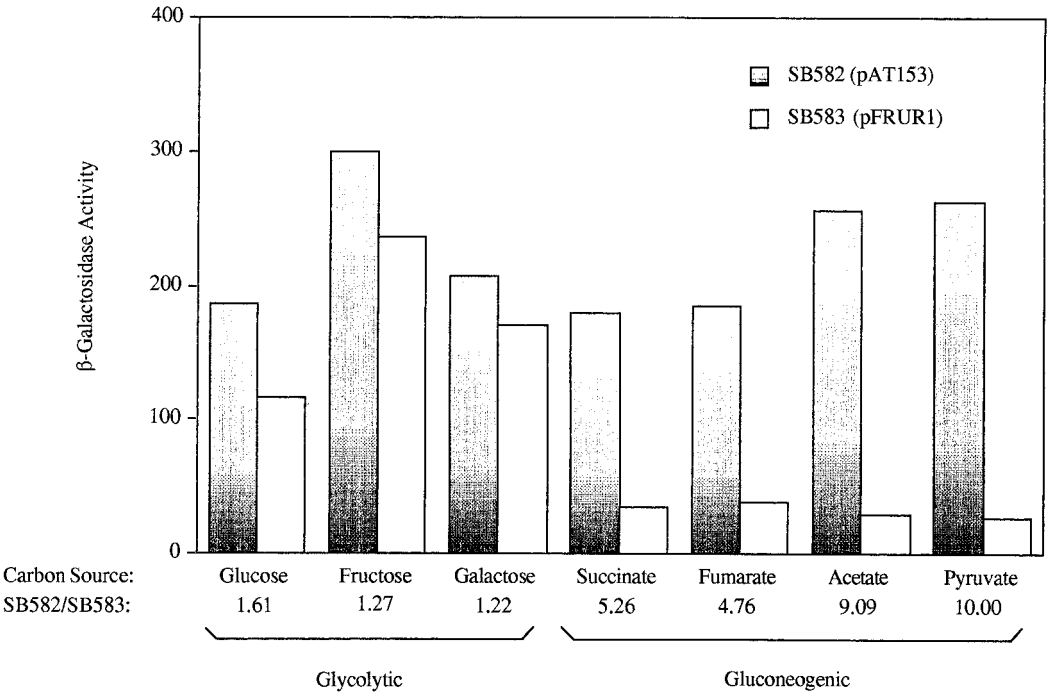

FIG. 3. Effect of carbon source and FruR overexpression on β-galactosidase activity in *pykF-lacZ* fusion strains. Control vector pAT153 and the *fruR* gene, overexpressing plasmid pFRUR1 (11), were transformed into the *fruR* mutant SB562, which resulted in strains SB582 and SB583, respectively. The strains were grown in minimal medium M9 (9) supplemented with the carbon source indicated (0.4%) and Casamino Acids (0.5%). The β-galactosidase activities are expressed in Miller units (9). The fold repression was calculated by comparing the specific β-galactosidase activities of the *fruR* mutant bearing vector pAT153 with the *fruR* mutant harboring plasmid pFRUR1, and values are at the bottom. The values are averages of at least two independent expression studies, each using five samples harvested during the logarithmic phase (optical density at 600 nm between 0.4 and 0.8) of bacterial growth. The relative standard deviations did not exceed 15%.

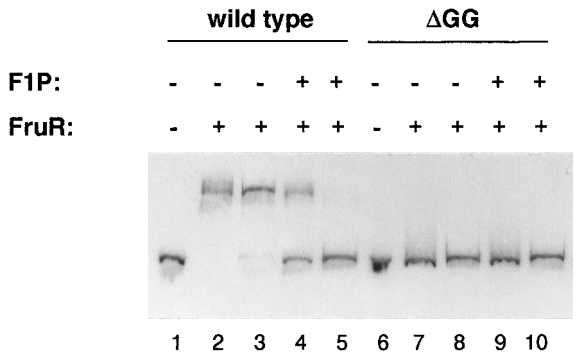

FIG. 4. DNA band migration retardation assay for the analysis of FruR binding to the *E. coli pykF* gene regulatory region. The assay used two distinct DNA fragments of 406 bp (lanes 1 to 5) and 404 bp (lanes 6 to 10 [Fig. 1]) and two different FruR concentrations (200 ng for lanes 2, 4, 7, and 9 and 50 ng for lanes 3, 5, 8, and 10). The second of these fragments (ΔGG) contains a 2-bp deletion in the central position of the FruR binding site. F1P was added to a final concentration of 5 mM where indicated.

The *pykF-lacZ* fusion in the wild-type background exhibited the expected dependency on FruR (Fig. 2). When the 2-bp deletion in the FruR binding site was introduced, the differential response to glycolytic versus gluconeogenic conditions in vivo was abolished as expected. However, the deletion mutation lowered the β-galactosidase activity observed under glycolytic conditions (i.e., with glucose present as the carbon source), so that this activity was the same as for the wild-type strain under gluconeogenic conditions (i.e., with pyruvate as the carbon source). This was not as expected; it was assumed that the 2-bp deletion would affect only FruR binding. We presume that this deletion mutation exhibited an adverse effect on transcriptional initiation of the *pykF* gene by RNA polymerase.

The results reported in this communication establish that FruR binds to the control region of the *pykF* gene, downstream of the single putative promoter from −35 to −10, to cause a repressive effect that mediates the phenomenon of carbon catabolite activation. There is evidence suggesting that this regulation by FruR is mediated by at least one of the cytoplasmic metabolic effectors, F1P and fructose 1,6-bisphosphate (FBP), during glycolytic growth. Previous reports demonstrated that these metabolites inhibit FruR binding in vitro (11, 12). In those studies, F1P was found to be the more potent effector in vitro, since it interfered with FruR binding at 1,000-fold-lower concentrations than were required for inhibition by FBP. Consistent with these findings are the results of the in vivo expression studies reported here (Fig. 2), which show that the strongest derepression of *pykF* expression in the *fruR* wild-type background occurred when the cells were grown in the presence of fructose where F1P levels are elevated. Furthermore, the lower-level derepression of *pykF* expression observed when cells were grown in the presence of other glycolytic substrates is presumably due to mediation by FBP, the levels of which are known to be higher during glycolysis than during gluconeogenesis (8, 10). Under gluconeogenic growth conditions, the concentrations of F1P and FBP are apparently insufficient to cause appreciable dissociation of FruR from its operator site in the control region of the *pykF* gene. The present results, together with earlier mechanistic studies (5, 11, 12), provide clear evidence suggesting that FruR mediates catabolite activation by a cyclic-AMP-independent mechanism as suggested previously (4).

We thank Michael C. O'Neill for performing the computer analysis of the promoter region and Mary Beth Hiller for assistance in the preparation of the manuscript.

This work was supported by USPHS grants 5RO1 AI21702 and 2RO1 AI14176 from the National Institute of Allergy and Infectious Diseases.

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