JOURNAL OF BACTERIOLOGY, Oct. 1972, p. 183-187
Vol. 112, No. 1

Printed in U.S.A.

Suppressor of Phosphofructokinase Mutations of *Escherichia coli*

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Received for publication 26 June 1972

The known locus of fructose-6-phosphate kinase mutations (*pfkA*) in *Escherichia coli* is at 76 min on the genetic map. We have now found another gene, *pfkB*, at 33 min, mutation of which suppresses *pfkA* mutations. The suppression is not informational, and *pfkB* may be a second gene for fructose-6-phosphate kinase activity.

We have recently described fructose-6-phosphate kinase (phosphofructokinase) mutants of *Escherichia coli* (13) and shown that their genetic locus, *pfk*, is at 76 min on the chromosome (14). During further study of these mutants, we have discovered an extragenic suppressor for them, whose mode of action is not understood. It is probably not an informational suppressor and may be a second gene for this enzyme activity.

MATERIALS AND METHODS

The three *pfk* mutants AM1, -2, and -3, prototrophic derivatives of K-10 (HfrC, str^s) carrying *pfkA1*, *-2*, and *-3* mutations (previously called *pfk-1*, etc.), were described earlier (13). The suppressor, *pfkB1*, was selected in strain AM1. (The wild-type alleles of *pfkA* and *-B* are called *pfkA0* and *pfkB0*; see Results.) In mapping and other experiments, we used another strain described earlier (14), DF1651 (F^-, edd^-, his^-, pps^-, $pyrD^-$, $tyrA^-$, str^r). Figure 1 shows the genetic map positions of relevant markers. *pfkA1* was introduced from AM1 into DF1651 by conjugation, with selection for tyrosine independence ($tyrA^+$) on appropriately supplemented minimal-glycerol plates containing streptomycin; DF85 was a recombinant which had inherited *pfkA1* from the donor and retained other markers (F^-, edd^-, his^-, *pfkA1*, $pyrD^-$, str^r). Strains DF86 and DF87 are like DF85, but carry *pfkA2* and *pfkA3*, respectively. Strains DF85B1 and DF87B1 were derived from DF85 and DF87 by transduction of pps^+ (ability to grow on lactate[3]) from the suppressor strain (*pfkA1*, *pfkB1*) and scoring of transductants (by assay and growth phenotype) to reveal those which had inherited the suppressor (*pfkB1*). The suppressor was similarly introduced into parental strain DF1651 by cotransduction with *pps* (*see below*) to give DF1651B1.

Growth conditions, media, and genetic methods were described earlier (4, 6). Phosphofructokinase was assayed spectrophotometrically (13), with the modification that the crude extracts were usually centrifuged at 30,000 rev/min for 2 hr in a Spinco 50ti rotor to remove NADH oxidase, in which case NaCN was omitted from the assay mixture.

RESULTS

Discovery of the suppressor. Fructose-6-phosphate kinase mutants have a characteristic pleiotropic phenotype: they grow almost normally on fructose, slower than normal on glucose and glucose-6-phosphate, and not at all on mannitol, sorbitol, or mannose (13; A. T. E. Morrissey, Ph.D. thesis, Harvard Univ., Cambridge, Mass., 1971). (This phenotype has since been confirmed by the isolation of *pfk* mutants in other laboratories [11; D. Blangy and H. Buc, *personal communication*].) The genetic locus of the phosphofructokinase mutations (*pfkA*, 76 min [14]) was not known to be the structural gene for the enzyme. To determine whether it was, we selected revertants, with the usual expectation that some second site revertants in the structural gene would have altered enzyme and abnormal growth properties. When 2×10^8 cells of strain AM1 (*pfkA1*) were spread on minimal mannitol plates and incubated at 25 C, 0 to 100 spontaneous revertants were found per plate. One hundred independent revertants (each obtained from a different clone of the mutant) were screened for growth on minimal-mannitol plates at 37 and 42 C; their growth was indistinguishable from that of the wild-type parental strain, K-10. Thus, all appeared to be revertants to wild type, and none were obvious candidates for containing altered enzyme. Another 100 independent 25 C revertants of the same mutant appearing after mutagenesis (a treatment with ultraviolet light which increased the revertant frequency about 10-fold

above spontaneous) also seemed to be wild type, as did 33 independent 25 C revertants of each of the other two mutants (AM2 [*pfkA2*] and AM3 [*pfkA3*]). We then tried to find cold-sensitive revertants, since phosphofructokinase is an allosteric protein (2), and cold-sensitive mutations are likely to affect allosteric interactions (10). Twenty spontaneous revertants of strains AM1 (*pfkA1*) were selected on minimal-mannitol plates at 42 C. None appeared to be cold sensitive but several seemed different from the wild type. For example, one of them (strain AM1R20) had a doubling time in minimal medium with mannitol at 37 C of 72 min; both the wild type and the usual revertant (e.g., AM1R3) had doubling times of 48 min. AM1R20 also grew on sorbitol and mannose and grew better than AM1 on glucose (Table 1). Thus it appeared to be a partial revertant, and, in fact, enzyme assay showed that it contained substantial phosphofructokinase activity (*see below*). Before studying the enzyme, however, we decided to map the reversion mutation, expecting that it would be at the *pfkA* locus. We found, instead, that it was an unlinked suppressor.

pfkA lies between *rha* and *metB* (Fig. 1), and when phage P1 grown on a *pfkA* mutant is used to transduce a *pfkA*$^+$, *rha*$^-$, *metB*$^-$ strain to *rha*$^+$ or to *metB*$^+$, some of the recombinants (e.g., 65 and 43%, respectively [14]) inherit *pfkA*$^-$. When this experiment was done with an apparently wild-type revertant (strain AM1R3, Table 1) as donor, there was no coinheritance of *pfkA1* (mannitol negativity), showing that this particular reversion was probably to *pfkA*$^+$. However, when the partial revertant (strain AM1R20) was used as donor in the transduction with a *rha*$^-$, *metB*$^-$ recipient, there was still normal inheritance of *pfkA1* with these markers. (One hundred *rha*$^+$ transductants were scored for methionine independence [Met$^+$] and ability to grow on mannitol [Mtl$^+$] and gave 6 Met$^+$ Mtl$^+$, 26 Met$^+$ Mtl$^-$, 54 Met$^-$ Mtl$^+$, and 14 Met$^-$ Mtl$^-$.) The phosphofructokinase positivity of AM1R20 therefore must have been caused by a mutation elsewhere, a suppressor.

TABLE 1. *Growth on plates*[a]

Strain	Colony size (mm) on carbon sources		
	Gluconate	Glucose	Mannitol
K-10 (wild type)	3.0	1.8	1.8
AM1 (*pfkA1*)	3.0	0.2	No growth
AM1R3 ("wild-type" 42 C revertant)	3.0	1.8	1.8
AM1R20 (unusual 42 C revertant)	3.0	0.9	0.5

[a] The strains were streaked to minimal plates with the indicated carbon sources and incubated 48 hr at 42 C. Colony sizes are averages for well-isolated colonies.

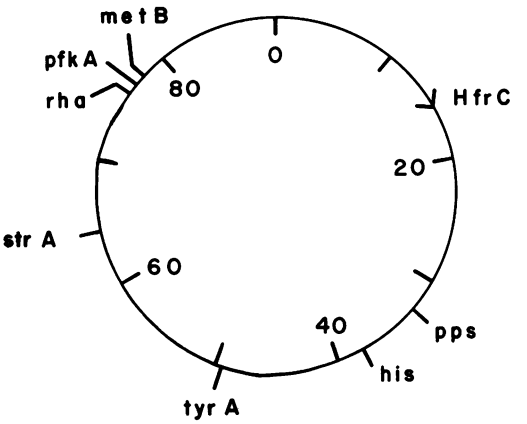

FIG. 1. *Genetic map of E. coli (16). pfkA is the locus previously called pfk; pfkB, the suppressor locus, is near pps (see text).*

Notation. The locus formerly named *pfk* is now called *pfkA*, and the suppressor locus is *pfkB*. Because we do not know the function of either locus we will use the following notation: the wild-type strain K-10 (and, as far as we know, other wild-type strains of *E. coli* K-12) is *pfkA0, pfkB0*. The mutants we described were all *pfkA*$^-$ (strains AM1 [*pfkA1, pfkB0*], AM2 [*pfkA2, pfkB0*], and AM3 [*pfkA3, pfkB0*]). The suppressed mutant AM1R20 is *pfkA1, pfkB1*.

Mapping pfkB. *pfkB* is at about 33 min. Preliminary experiments showed that it was between 15 and 40 min on the chromosome (A. T. E. Morrissey, Ph.D. thesis). We then used strain DF85, which carried *pfkA1* and several markers in the region of interest (e.g., *his*$^-$ and *pps*$^-$; *see* Materials and Methods). When used as recipient in a conjugation with AM1R20 (HfrC, *pfkA1, pfkB1, str*^{s}) with selection for growth on mannitol (*pfkB1*), 93 out of 100 recombinants had inherited *pps*$^+$ from the male, whereas with selection for *pps*$^+$ 96 out of 100 recombinants had inherited *pfkB1*. The genes *pfkB* and *pps* were also linked by transduction: with phage P1 grown on AM1R20 and the same recipient, 64% of *pps*$^+$ recombinants were *pfkB1*, and with selection of *pfkB1* 75% were *pps*$^+$.

Is pfkB1 an informational suppressor? The usual explanation of an unlinked sup-

pressor mutation is alteration of a translation component allowing mistranslation of mutant genotype (7). Thus, if *pfkA1* were a nonsense mutation, then *pfkB1* could be a nonsense suppressor. This does not seem to be the situation. First, there is no known nonsense suppressor mapping at 33 min. Second, strain AM1R20 (*pfkA1*, *pfkB1*) was sensitive to wild-type phage T4 but not to T4 nonsense mutants (16 amber, 1 ochre, and one UGA [A. T. E. Morrissey, Ph.D. thesis]). Third, all three of the *pfkA* mutants were suppressed by *pfkB1*. Thus, with strains DF86 (*pfkA2*, *pfkB0*, *pps*$^-$) and DF87 (*pfkA3*, *pfkB0*, *pps*$^-$), transduction to *pps*$^+$ repaired the phosphofructokinase-negative phenotype in 10 out of 24 and 15 out of 24 recombinants, respectively, when the donor phage was prepared on a *pfkB1* strain, and there was no repair with the donor phage coming from a *pfkB0* strain. The three *pfkA* mutations are unlikely to be identical, since two were selected independently and the three have slightly different phenotypes (A. T. E. Morrissey, Ph.D. thesis). It is still conceivable that *pfkB1* could be a missense suppressor acting on each of them. However, informational suppression is also rendered unlikely by the levels of enzyme activity found in strains carrying *pfkB1* (*see below*).

Construction of a pfkA0, pfkB1 strain. We have mentioned three combinations of the *pfk* alleles: wild type (*pfkA0*, *pfkB0*), mutant (*pfkA1*, *pfkB0*), and the suppressed mutant (*pfkA1*, *pfkB1*). The fourth combination, the suppressor in a strain with wild-type *pfkA* (*pfkA0*, *pfkB1*), was made as follows (A. T. E. Morrissey). DF1651 (*pps*$^-$, *pfkA0*, *pfkB0*) was transduced to *pps*$^+$ by using a lysate prepared on a *pfkA1*, *pfkB1* strain. Because the recipient was already phosphofructokinase positive, recombinants which had inherited *pfkB1* might not be recognizable. However, we found that some of the *pps*$^+$ recombinants had phosphofructokinase activities similar to the recipient DF1651, whereas others had much higher activity (*see below*). To determine whether the two classes were genotypically *pfkA0*, *pfkB0* and *pfkA0*, *pfkB1*, respectively, phage P1 grown on each class was used to transduce DF85 (*pfkA1*, *pfkB0*, *pps*$^-$) to *pps*$^+$. All transductants from the recombinants with normal enzyme level were still phosphofructokinase negative; thus, this class of donor was *pfkB0*. In contrast, about half the transductants from a high-level strain were phosphofructokinase positive; thus, the high-level strain (DF1651-B1) carried *pfkB1* and was *pfkA0*, *pfkB1*.

Enzyme levels. The level of phosphofructokinase as assayed in crude extracts tends to be somewhat variable from day to day and strain to strain, even in the wild type. However, cultures grown and assayed at the same time usually give consistent relative activities. In one experiment, the wild-type K-10 (*pfkA0*, *pfkB0*) had a specific activity of 116, whereas the suppressed mutant AM1R20 (*pfkA1*, *pfkB1*) had an activity of 226. Table 2 shows the levels found in the complete series of strains derived from DF1651. The presence of the suppressor in this strain (DF1651B1) was reflected in a fourfold increase in enzyme activity. The *pfkA* mutations (strains DF85–87) gave low activities, as previously reported (13). (The level of *pfkA1* in this background, however, was definitely higher than the 10% value found for the same mutation in HfrC [13], although the growth phenotype was still phosphofructokinase negative.) The *pfkB1* allele in all cases restored phosphofructokinase activity to the mutant strains. The levels found were exceedingly variable, and in the three suppressed strains the averages ranged from similar to, to considerably above, the wild type level.

Inducibility of phosphofructokinase. One possible cause of variability in enzyme activity is that induction or repression may be occurring in response to uncontrolled cultural factors. We have not yet found any definite indication of such control. For example, in an experiment with strain K-10 (*pfkA0*, *pfkB0*) when cells were harvested from logarithmic growth in minimal media with glucose, glycerol, and

TABLE 2. *Phosphofructokinase activities*[a]

Strain	Genotype	Specific activity (nmoles/min; mg of protein)
DF1651	*pfkA0*, *pfkB0* (wild-type)	171 ± 19 (4)
DF1651B1	*pfkA0*, *pfkB1* ("suppressed wild-type")	693 ± 81 (4)
DF85	*pfkA1*, *pfkB0* (mutant)	46 ± 22 (5)
DF85B1	*pfkA1*, *pfkB1* (suppressed mutant)	158 ± 87 (5)
DF86	*pfkA2*, *pfkB0* (mutant)	7 ± 6 (5)
DF86B1	*pfkA2*, *pfkB1* (suppressed mutant)	224 ± 173 (6)
DF87	*pfkA3*, *pfkB0* (mutant)	28 ± 7 (5)
DF87B1	*pfkA3*, *pfkB1* (suppressed mutant)	640 ± 427 (13)

[a] The strains are all derivatives of DF1651 (*see text*). The cultures were grown in broth to logarithmic phase or to stationary phase. Standard deviations and number of independent cultures assayed are also shown.

fructose as carbon source, the specific activities were 146, 157, and 147, respectively. A stationary-phase culture in glucose minimal medium had an activity of 165. Exponential-phase cultures in broth supplemented with the same three carbon sources had activities of 140, 148, and 175, respectively. (It may be recalled that the properties of the *pfkA* mutants show that phosphofructokinase is important for growth on glucose, but not on glycerol or fructose [13].)

The suppressed mutant AM1R20 (*pfkA1*, *pfkB1*) also contained phosphofructokinase activity which did not vary markedly with the growth conditions. For example, harvested from stationary phase in minimal media with glucose, glucose-6-phosphate, mannitol, fructose, and gluconate, the specific activities were, respectively, 170, 267, 307, 197, and 172. Activities were similar in broth-grown cells.

DISCUSSION

What is the *pfkB1* mutation? It suppresses three *pfkA* mutations previously described and maps elsewhere on the chromosome. Suppressed mutants have at least as high levels of enzyme activity as the wild type, with the level even higher in the "suppressed wild type." The simplest model would be that *pfkB1* species another fructose-6-phosphate kinase.

Several possible explanations may be excluded. These include the following. (i) The suppressor might somehow relate to another "phosphofructokinase", fructose-1-phosphate kinase, an enzyme known to be induced by growth on fructose and involved in fructose metabolism (5). However, the known purified fructose-1-phosphate kinase (of *Aerobacter aerogenes*) does not accept fructose-6-phosphate as substrate (8). Furthermore, extracts of *pfkB1* strains with high fructose-6-phosphate kinase activity did not contain high fructose-1-phosphate kinase activity (A. T. E. Morrissey, Ph.D. thesis). (ii) The activity in suppressed strains might not be a fructose-6-phosphate kinase at all. However, it has the same requirements for substrates and auxiliary enzymes as the activity in the wild type and thus is unlikely to actually be, for example, a fructose-6-phosphate aldolase activity. (iii) The map position at about 33 min on the chromosome might suggest that *pfkB* is related to mannose metabolism because there is a locus for mannose utilization nearby (*man*) (12). For example, if growth on mannose involved induction of another fructose-6-phosphate kinase, linked to *man*, then the *pfkB1* mutation might be to constitutivity of that enzyme. However, the fact that *pfkA* mutants are unable to grow on mannose does not fit that particular model.

Other speculations may be considered. Though phosphofructokinase purified from *E. coli* (2) is a tetramer of equal-sized subunits (1), the subunits conceivably could be nonidentical and map separately. Or, one might consider an explanation involving covalent modification. (Covalent modification was once proposed, but later rejected, for phosphofructokinase of yeast [9].) Or, *pfkB1* might have something to do with normal regulation of phosphofructokinase gene expression. Our preliminary findings of non-inducibility do not exclude such models.

Studies on whether *pfkA* is a structural gene for phosphofructokinase and enzymological work on the enzyme in suppressed strains are in progress.

ACKNOWLEDGMENTS

This work was supported by grant GB-15958 from the National Science Foundation. A.T.E.M. was supported by Public Health Service training grant GM-00177-09 and D.G.F by Public Health Service Career Development Award 5-K3-GM-7344, both from the National Institute of General Medical Sciences.

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