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pfkB and *pfkC* Loci of *Escherichia coli*

R. T. VINOPAL[1] AND D. G. FRAENKEL*

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115

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Mutants lacking *Escherichia coli* phosphofructokinase (*pfkA*, 78 min) are suppressed by the unlinked *pfkB1* mutation, which restores some enzyme activity (Morrissey and Fraenkel, 1972). We here describe a secondary mutation at *pfkB*, "*pfkB*$^-$," which abolishes the suppression as well as the low residual activity of unsuppressed *pfkA* mutants. *pfkB* is at about 33 min, with the gene order *aroD-pps-pheS-pfkB*. A positive selection was found that yielded both the *pfkB*$^-$ mutations and a new similar mutation, *pfkC*$^-$. *pfkC* is an early marker in Hfr KL16 (ca. 50 to 55 min). Some *pfkC*$^-$, but no *pfkB*$^-$, mutations were amber. A temperature-sensitive *pfkB*$^-$ was also obtained. Strains carrying *pfkB*$^-$ or *pfkC*$^-$, but wild type at *pfkA*, were not markedly affected in growth on sugars. A new search for suppressors such as *pfkB1* gave five independent candidates, all of which suppressed both *pfkA1* and *pfkA2* and occurred in the *pfkB* region; none occurred at *pfkC*. Neither the *pfkB* nor the *pfkC* loci have assigned functions. It is likely that they are somehow involved in expression of phosphofructokinase activity 2 (Fraenkel, Kotlarz, and Buc, 1973).

This paper and the succeeding one continue our mutant analysis of phosphofructokinase in *Escherichia coli*. We first described phosphofructokinase-deficient mutants (9) and the locus (*pfkA*) of their lesions on the chromosome (10). The growth phenotype of such mutants is generally as expected: slow or no growth on substances primarily metabolized via fructose 6-phosphate (such as glucose and mannitol), and relatively little growth impairment on other compounds (such as fructose or glycerol). Details of this phenotype were complex and could be influenced by various mutations allowing partial phenotypic suppression (18). In the present papers we return to the biochemical genetics of the phosphofructokinase activity itself.

In an early search for *pfkA* revertants with altered enzyme, done to show whether the *pfkA* locus was the structural gene, we had found an unlinked suppressor mutation, *pfkB1*, which restored growth and phosphofructokinase activity to several *pfkA* mutants (11). In a strain carrying the suppressor together with the wild-type allele at *pfkA* (i.e., *pfkA*$^+$, *pfkB1*), there were two easily separable phosphofructokinase activities: activity 1, like the enzyme in the wild-type strain, and activity 2, without allosteric kinetics (3). F. Kotlarz and H. Buc (personal communication) have found that antiserum to activity 1 hardly, if at all, binds activity 2. A small amount of activity 2 could also be found in wild-type strains. Thus, the simplest type of explanation for *pfkB1* might be that it derepresses activity 2.

In this paper we describe a new type of allele, "*pfkB*$^-$," which reduces to zero the residual phosphofructokinase activity found in all *pfkA* mutants. We also describe mutations at a third locus, *pfkC*, that affect the amount of phosphofructokinase activity 2. We have ordered the *pfkB* locus with respect to several nearby genes, and we describe the selection of several new mutations similar to *pfkB1*.

In an accompanying paper (17) we describe new mutant alleles in *pfkA* and various experiments showing that this locus is probably the structural gene for activity 1.

MATERIALS AND METHODS

Media. Minimal medium 63 (2) supplemented with 1 μg of thiamine hydrochloride per ml, 25 μg of amino acids or uracil per ml as required (aromatic mutants were supplemented with phenylalanine, shikimic acid, tryptophan, and tyrosine), and the carbon source at 0.4% was used. Minimal plates were of this medium with 2% agar (Difco). Broth was medium 63 with 1% tryptone (Difco) and 0.4% yeast extract (Difco). Media used for phage P1 propagation and assay were those of Lennox (6).

Bacterial strains. The principal strains of *E. coli* K-12 used in this work are listed in Table 1, with genotype and derivation.

[1] Present address: Microbiology Section, Biological Sciences Group, University of Connecticut, Storrs, Conn. 06268.

TABLE 1. *E. coli K-12 strains used*[a]

Strain	Sex	Genotype	Derivation, source, or reference
AM1	HfrC	*pfkA1 tonA22*	(9)
CA8000	HfrH	*thiA*	J. R. Beckwith
DF85	F⁻	*edd-1 galK his pfkA1 pps-1 pyrD str tonA22*	(11)
DF86	F⁻	*edd-1 galK his pfkA2 pps-1 pyrD str tonA22*	(11)
DF86B1	F⁻	*edd-1 galK his pfkA2 pfkB1 pyrD str tonA22*	(11)
DF88	F⁻	*edd-1 galK his pfkA2 pfkB1 pfkB2 pyrD str tonA22*	DF86B1, EMS, pcs for Man⁻
DF89	F⁻	*edd-1 galK his pfkB1 pfkB2 pyrD str tonA22*	DF88, Man⁺ trans.
DF1651	F⁻	*edd-1 galK his pps-1 pyrD str tyrA*	(11)
DF1651B1	F⁻	*edd-1 galK his pfkB1 pyrD str tyrA*	(11)
K8-5m	F⁻	*aceA iclR met(BF) str*	C.G.S.C. (18)
KL16	Hfr		(8)
KL1699	Hfr	*recA*	(7)
KL98	Hfr		(8)
KLF48/KL159	F⁻	F'148/*aroD5 his4 proA2 recA1 thi1 xyl5*	C.G.S.G. 4302[b]
NP37	F⁻	*pheS5 (TS) rel thi*	B. Weiss (11)
PA3	Hfr P4X	*kdgA kdgP metB*	J. Pouyssegur (see ref. 8)
RT207-	F⁻	like RT501, but: -1, *pfkB1, pfkB5;* -3, *pfkC1;* -195 *pfkB1, pfkB6;* -42, *pfkC2;* -99, *pfkC3;* -108, *pfkC4;* -118, *pfkC5;* -143, *pfkC6;* 162, *pfkC7*	RT501, 502, 504, Gly⁺ revts.[c]
RT211	F⁻	*aroD6 edd-1 galK his pfkA1 pyrD str*	RT500, Lact,⁺ (*pfkB*⁺) trans. (DF1651B1)
RT212	F⁻	*aroD6 edd-1 galK his pfkA1 pfkB1 pyrD str*	RT500, Lact.⁺ (*pfkB1*) trans. (DF1651B1)
RT213	F⁻	*aroD6 edd-1 galK his pfkA1 pfkB1 pfkB2 pyrD str*	RT500, Lact.⁺ (*pfkB*⁻) trans. (DF89)
RT214	F⁻	*aroD6 edd-1 galK his pfkA1 pfkB1 pfkB3 pyrD str*	RT500, Lact.⁺ (*pfkB*⁻) trans. (RT518)
RT220, 221, 222 223	F⁻	*recA, his*⁺ derivatives of RT211, 212, 213, 214	RT211-214, His⁺ (*recA*, ultraviolet sensitive) conj. KL1699[d]
F148/RT220	F'	F'148/RT220	RT220, Aro⁺, conj. (KLF48/KL159[e]
F148/RT221	F'	F'148/RT221	RT221, Aro⁺, conj. (KLF48/KL159)[e]
F148/RT222	F'	F'148/RT222	RT222, Aro⁺, conj. (KLF48/KL159)[e]
F148/RT223	F'	F'148/RT223	RT223, Aro⁺, conj. (KLF48/KL159)[e]
RT225	F⁻	*edd-1 galK his metB pfkB1 pyrD str*	—[f]
RT500	F⁻	*aroD6 edd-1 galK his pfkA1 pps-2 pyrD str*	(18)
RT501, 502, 504	F⁻	*edd-1 galK his pfkA2 pfkB1 pyrD str*	—[g]
RT518	F⁻	*edd-1 galK his pfkA2 pfkB1 pfkB3 pyrD str tonA22*	DF86B1, Gly⁺ revt.
RT519	F⁻	*edd-1 galK his pfkA2 pfkB1 pfkB7 pyrD str tonA22*	DF86B1, Gly⁺ revt.
RT676	F⁻	*edd-1 galK his pfkA2 pfkB1 pfkC6 pyrD str supF*	—[h]
RT678	F⁻	*edd-1 galK his pfkA2 pfkB1 pfkC6 pyrD str supF*⁺	—[h]
RT800	F⁻	*edd-1 galK his pfkA2 pfkB1 pfkB8(TS) pyrD str*	RT501, Gly⁺ revt.
RT855	F⁻	*edd-1 galK his pfkB1 pfkB2 pyrD str tyrA*	DF1651, Lact.⁺ (*pfkB*⁻) trans. (DF89)[i]
RT857	F⁻	*edd-1 galK his pyrD str tyrA*	DF1651, Lact.⁺ (*pfkB*⁺) trans. (DF89)[i]
RT871-	F⁻	like DF85, but: -10, *pfkB10;* -12, *pfkB11;* -13, *pfkB12*	DF85, NTG, Man⁺ revt.
RT873	F⁻	*edd-1 galK his met(BF) pfkB1 pfkC6 pyrD str supF*	—[j]
RT874	F⁻	*edd-1 galK his met(BF) pfkB1 pfkC6 pyrD str supF*⁺	—[j]

[a] Designations of genes are according to Taylor and Trotter (16), except *pfkB* and *pfkC* (this study). We have included all known markers and allele numbers. Phenotypic abbreviations are capitalized gene designations (e.g., Met⁺); others are Man (mannose utilization), Lact. (lactate utilization), and Gly (glycerol utilization). EMS, ethyl methane sulfonate mutagenesis; NTG, nitrosoguanidine mutagenesis; TS, heat sensitive; revt., revertant, spontaneous unless otherwise specified; pcs, penicillin counterselection; trans., transduction with phage P1 (followed with donor strain in parentheses); conj., conjugational recombinant or F-prime partial diploid (donor strain in parentheses). Thus, the derivation of strain RT212 given as RT500, Lact.⁺ (*pfkB1*) trans. (DF1651B1) is read "a transductant of RT500, using P1 grown on DF1651B1, with selection on lactate and scoring for a *pfk* suppressor." CGSC indicates that the strain was obtained through the courtesy of B. Bachmann, Coli Genetic Stock Center, Yale University.

[b] With the episome present this strain will grow on minimal medium if proline is added. The F-prime 148 episome carried the *his* and *aroD* loci, but not *pyrD* (8). Plate matings between this strain and RT500 (*aroD his pfkA1 pps pyrD*), selecting Aro⁺ on glycerol plates with histidine and uracil, gave uracil-requiring strains able to grow on lactate, so the episome carried the *pps* locus. Some Aro⁺, uracil⁻ isolates were histidine independent and thus presumably diploid. All recombinants resembled RT500 in slow growth on gluconate; hence the episome probably carried the *pfkB*⁺ allele (rather than *pfkB1*).

[c] RT207-1, -3, -99, -118 from RT501; -19, -108, -143 from RT502; -42, -162 from RT504.

TABLE 1—*Continued*

[d] Strains RT220–RT223 are also uracil independent as a result of conjugational repair, but the *aroD* and *pfkB* loci were retained as in RT211–RT214.

[e] Selected on lactate plates. Partial diplody of these strains was confirmed by restoration of endogenote phenotype after acridine orange curing of the episome.

[f] RT225 derives from RT142 (*edd galK his metB pps-1 pyrD str;* ref. 18) in three steps: Lact.$^+$ (*aroD*) trans; EMS, pcs for Lact.$^-$; Lact.$^+$ (Aro$^+$, *pfkB1*) trans. (DF1651B1).

[g] RT501, -502, and -504 are ϕ80-sensitive (*tonA*$^+$) derivatives of DF86B1, derived in two steps: EMS, pcs for Leu$^-$; then Leu$^+$ (ϕ80 sensitive) conj. (CA8000), three different recombinants.

[h] RT676 and -678 were derived from RT207-143 in two steps: EMS, pcs for Trp; then Trp$^+$ (*sup*$^+$ or *sup*$^-$) trans. (RT686, ref. 17).

[i] The *pfkB* allele was determined by transducing RT500 to Aro$^+$ and scoring.

[j] RT873 and -874 were derived from RT207-143 in three steps: EMS, pcs for Trp$^-$; then Man$^+$ (Met$^-$) trans. (K8-5m); finally Trp$^+$ (*sup*$^+$ or *sup*$^-$) trans. (RT686).

Genetic procedures. Phage P1 lysates were prepared and used in transductions as previously described (18). Conjugations were done as described by Sesnowitz-Horn and Adelberg (15). Interrupted matings used broth with 0.4% glucuronic acid. Counterselection of Hfr cells was by streptomycin sulfate (100 μg/ml). F-prime factors were transferred to F$^-$ recipients by spreading a late exponential-phase culture of the recipient as a lawn on moist selective plates and streaking a loopful of the F-prime donor onto the plates. The donor strain was counter-selected by omitting a required amino acid from the plates. Partial diploid recombinants were confirmed by testing for restoration of the original F$^-$ phenotype after curing of the episome by growth from a small inoculum in pH 7.8 broth with acridine orange (50 μg/ml). Mutagenesis with ethyl methane sulfonate was as described previously (18). Scoring of strains for possession of a nonsense suppressor, using a T4 amber mutant, and detection of amber mutations using ϕ80*psupF* were as described in the accompanying paper (17).

Chemicals. Sources were described previously (18).

RESULTS

The pfkB$^-$ mutations. If *pfkB1* were a mutation somehow conferring a higher level of a phosphofructokinase normally present as a minor constituent (activity 2) in the wild-type strain (*pfkA*$^+$ *pfkB*$^+$), then if the phosphofructokinase mutant phenotype were selected in a suppressed *pfkA* mutant (*pfkA*$^-$ *pfkB1*), one might expect to find, among other possibilities, mutation again at *pfkB*, either back to the wild-type allele *pfkB*$^+$ or to some other type of inactivation. This selection was first done with strain DF86B1 (*pfkA2 pfkB1*), using ethyl methane sulfonate mutagenesis and a penicillin enrichment step in minimal medium with mannose as carbon source. Among survivors, two strains appeared which again had the phosphofructokinase mutant phenotype and lacked phosphofructokinase activity. These new strains (e.g., DF88) were actually more negative in growth phenotype than the original mutant DF86 (*pfkA2 pfkB*$^+$), being absolutely unable to grow on glucose or gluconate, and showed zero phosphofructokinase activity in extracts, rather than the usual 10% level of *pfkA* mutants (Table 2).

The new mutation in these strains was in the *pfkB* region. We showed earlier that *pfkB* is co-transduced with *pps* at approximately 32 min on the map (11). The new mutants no longer served as transductional donors of mannitol or mannose positivity (*pfkB1*) to the *pfkA* mutant strain DF86 (*pfkA2 pfkB*$^+$ *pps*$^-$), and selection for growth on lactate (*pps*$^+$) in the same cross showed co-inheritance of the new extreme negative phenotype (e.g., in a cross using strain DF88 as donor, five out of seven lactate transductants showed the new phenotype and the remaining two showed the same phenotype on sugars as strain DF86 itself). Since we were able to recover *pfkB1* from the new strain as a very rare recombinanat (see below), the new mutation is presumably very closely linked to it, and we name the combination *pfkB1,pfkB2* (indicated casually as *pfkB*$^-$).

A positive selection for pfkB$^-$. We found by chance a positive selection for *pfkB*$^-$. Some of the *pfkB1*-suppressed *pfkA* mutants grew somewhat more slowly on glycerol than did the unsuppressed mutants. This effect was not extreme (compare colony sizes [Table 2] on glycerol of strains DF86 [*pfkA2 pfkB*$^+$] and DF86B1 [*pfkA2 pfkB1*]) and is not even seen in all the suppressed mutants (however, glycerol had often been used as a permissive medium for these strains). Nonetheless, the effect in strains such as DF85B1 (ref. 11, *pfkA1 pfkB1*) and DF86B1 (*pfkA2 pfkB1*) was marked enough on plates spread to confluence that spontaneous revertants to strong growth on glycerol were readily obtained. Some of these glycerol revertants had the phenotype of the unsuppressed *pfkA* mutant again or, indeed, of *pfkA*$^-$ *pfkB*$^-$ strains. This was first observed with glycerol revertants of strain DF86B1, in which case 5 out of 20 isolates showed zero phosphofructokinase

TABLE 2. *pfk mutant series*[a]

Strain	Casual designation	Alleles			Colony sizes (mm)						Phospho-fructokinase activity
		pfkA	*pfkB*	*pfkC*	Glu-cose	Man-nose	Man-nitol	L-Arab-inose	Gluco-nate	Glyc-erol	
DF1651	Wild type	+	+	+	2.6	1.8	1.2	1.0	2.0	3.0	0.512
DF1651B1	A^+B1	+	1	+	2.8	1.7	0.7	1.4	2.2	2.4	1.765
DF86	A^-	2	+	+	0.4	0.5	0.2	1.4	1.2	1.4	0.026
DF86B1	A^-B1	2	1	+	2.8	2.0	1.5	3.5	2.2	0.4	0.804
DF88	A^-B^-	2	1, 2	+	0.0	0.0	0.0	0.2	0.0	1.1	0.002
DF89	A^+B^-	+	1, 2	+	2.6	1.7	1.4	1.5	1.8	1.8	0.482
RT855	A^+B^-	+	1, 2	+	3.0	2.0	1.5	1.3	ND[b]	2.0	0.391
RT857	Wild type	+	+	+	2.9	2.0	1.3	1.2	1.7	2.7	0.442
RT207-19	A^-B^-	2	1, 6	+	0.0	0.0	0.0	0.5	0.0	1.5	0.005
RT207-99	A^-B1C^-	2	1	3	0.3	0.5	0.3	1.1	0.9	2.1	0.040
RT678	A^-B1C^-	2	1	6	0.5	0.5	0.2	0.7	0.7	2.0	0.029
RT676	$A^-B1C^-su^-$	2	1	6	0.9	1.1	0.8	2.2	1.2	1.2	0.050
RT874	A^+B1C^-	+	1	6	1.9	1.8	1.1	3.0	2.0	1.7	0.520
RT873	$A^+B1C^-su^-$	+	1	6	2.6	1.4	0.7	3.2	2.8	2.0	0.777

[a] Cultures were grown aerobically in broth supplemented with 0.4% fructose, and total phosphofructokinase activity in crude extracts was assayed as usual (3). The same cultures were also spread on minimal plates with the indicated carbon sources (0.4%), all supplemented with histidine, methionine, tyrosine, and uracil (each 25 μg/ml) so as to give approximately 100 colonies/plate, and average colony sizes were estimated after 48 h at 37 C. For any one strain the number of colonies was about the same on each medium, with exceptions in certain cases of slow growth. For example, with strain RT207-99 the small colonies appeared on glucose and mannitol at less than 10% of their frequency on the other plates; in a few cases tested this minority fraction had the same phosphofructokinase activity as the original culture.

[b] ND, Not determined.

activity and contained $pfkB^-$ mutations (by co-transduction with *pps*; e.g., strains RT518 and RT519, Table 3).

Because a positive selection for $pfkB^-$ seemed a useful source for new *pfkB* mutants (including, hopefully, temperature-sensitive or nonsense mutations), the selection was repeated using strains RT501, -502, and -504, which, like DF86B1, carry *pfkA2* and *pfkB1* but are also sensitive to φ80. Of 126 independent glycerol revertants, 45 had the general phosphofructkinase mutant phenotype, and some members of this set were $pfkB^-$ (e.g., strains RT207-1 and RT207-19, Table 3; Table 2 gives the phenotype of strain RT207-19 [line 9]).

(Although the glycerol selection is useful, its physiological basis is not understood. It may be that since *pfkB1*-suppressed *pfkA* mutants seem to contain a phosphofructokinase activity related to activity 2 [to be reported] the non-allosteric nature of such an enzyme leads to difficulties in growth on some gluconeogenic carbon sources and hence selective pressure for its loss [e.g., $pfkB^-$].)

We also identified a heat-sensitive $pfkB^-$ mutation. It was found among a set of glycerol revertants of strain RT501 selected at 42 C; one isolate (RT800) was mannose negative at 42 C but grew normally on mannose at 32 C. The temperature conditional marker was mapped at *pfkB* by transduction (Table 3, strain RT800) and thus was given the designation *pfkB1,pfkB8* ("$pfkB^{ts}$"-like $pfkB^-$ at high temperature and *pfkB1* at low).

The pfkC locus. Not all the glycerol revertants of strains RT501, -502, and -504 that had regained the *pfk* mutant phenotype were $pfkB^-$. Of the nine isolates examined, seven still carried *pfkB1* in normal linkage (Table 3). The latter isolates (e.g., RT207-99, Table 2) were not quite as negative in growth phenotype and enzyme activity as $pfkA^-$ $pfkB^-$ strains (compare lines 10 and 11 with line 5, Table 2). The *pfkA* locus in these strains seemed unaffected, since it could be transduced as usual with *metB* and was still suppressible by *pfkB1* (we used RT225 as recipient for the latter test). Therefore, these strains contained a new locus, *pfkC*, affecting phosphofructokinase.

Mapping the pfkC locus. $pfkC^-$ was obtained as a mutation in strains carrying *pfkA2* and *pfkB1*. Presumably, repair of this phenotype could occur either at *pfkA* (i.e., $pfkA^+$ *pfkB1* $pfkC^-$) or at *pfkC* (i.e., *pfkA2 pfkB1* $pfkC^+$). In fact, when strain RT207-143 (*pfkA2 pfkB1* $pfkC^-$) was transduced to growth on mannose, with a $metB^-$ $pfkA^+$ donor, 44 out of 176 transductants were Met^- (i.e., $pfkA^+$ *pfkB1*

TABLE 3. *Transductional testing of pfkB allele in mannose-negative glycerol revertants of pfkA2 pfkB1 strains*

Transductional donor	Allele[a]	Recipient	Transductants	*pfkB* allele[b] in transductants		
				pfkB$^+$	*pfkB*$^-$	*pfkB1*
RT518[c]	*pfkB3*	DF86[d]	19 Lactate$^+$	10	9	0
RT519	*pfkB7*	DF86[d]	20 Lactate$^+$	9	11	0
DF1651B1[e]	(*pfkB1*)	RT500[f]	19 Aro$^+$	8	0	11
RT207-1[g]	*pfkB5*	RT500[f]	22 Aro$^+$	13	9	0
RT207-3	*pfkC1*	RT500[f]	13 Aro$^+$	3	0	10
RT207-19	*pfkB6*	RT500[f]	103 Aro$^+$	64	49	0
RT207-42	*pfkC2*	RT500[f]	12 Aro$^+$	6	0	6
RT207-99	*pfkC3*	RT500[f]	3 Aro$^+$	0	0	3
RT207-108	*pfkC4*	RT500[f]	5 Aro$^+$	3	0	2
RT207-118	*pfkC5*	RT500[f]	15 Aro$^+$	6	0	9
RT207-143	*pfkC6*	RT500[f]	8 Aro$^+$	2	0	6
RT207-162	*pfkC7*	RT500[f]	11 Aro$^+$	4	0	7
RT800	*pfkB8*	RT500[f]	44 Aro$^+$	24	20[h]	

[a] New mutation in the revertants, assigned according to these results; all carry *pfkB1* also.

[b] *pfkB*$^+$: very weak growth on mannose, some growth on gluconate (like DF86, Table 2). *pfkB*$^-$: no growth on mannose, weak or no growth on gluconate (like DF88, Table 2). *pfkB1*: strong growth on mannose (like DF86B1, Table 2). Selection and scoring was at 37 C.

[c] RT518 and -519 are glycerol revertants of DF86B1.

[d] DF86 carried *pfkA2*, *pfkB*$^+$, and *pps*$^-$; selection of *pps*$^+$ was on lactate (11).

[e] *pfkB1* control.

[f] RT500 carried *pfkA1*, *pfkB*$^+$, *aroD*$^-$, and *pps*$^-$ (Table 1); *aroD* is also linked with *pfkB* (see text). Selection was of *aroD*$^+$ on glycerol plates. (RT500 derivatives carrying *pfkB1* are not weak on glycerol.)

[g] The RT207 series are glycerol revertants of RT501, -502, and -504 (ϕ80-sensitive derivatives of DF86B1). RT800 is a temperature-sensitive glycerol revertant of RT501.

[h] See text. *pfkB1*, *pfkB8* did not give the identical phenotype in the transductants as in RT800, but the scoring was by comparison with the *pfkB1* control cross (relatively weaker growth on glucose and mannose at 42 C).

pfkC$^-$); the frequency of this class (25%) was lower than the usual simple linkage (40%) between *pfkA* and *metB*, suggesting that some of the other transductants might have been repaired at *pfkC*. In any case, this result showed that conjugational mapping of *pfkC* might be difficult if donors carried *pfkA*$^+$.

Fortunately, it was possible to use Hfr strains in which *pfkA* was transferred as a late marker (Fig. 1). With the six strains of series RT207(*his*$^-$)-*pfkC*$^-$ tested by conjugation with KL16 (Table 4), some His$^+$ recombinants were Man$^+$. No such restoration of growth on mannose was found when the recipient was *pfkB*$^-$ (line 1, Table 4), showing that the repairing gene in strain KL16 was not a generalized suppressor of phosphofructokinase. His$^+$ selection using a different Hfr, strain KL98, did not introduce Man$^+$. Therefore, *pfkC*$^+$ must lie between the origin of KL16 and KL98 (Fig. 1). A similar position could be inferred from a cross using Hfr strain PA3 (data not shown). An interrupted mating using Hfr strain KL16 gave Man$^+$ recombinants approximately 15 min before His$^+$ recombinants, which would place *pfkC* near the origin of strain KL16. However, there was no co-transduction of likely markers such as *lysA* when auxotrophs (with wild-type *pfk* genes) were used as donors of Man$^+$ to *pfkC*$^-$ strains.

Amber mutants. The 45 glycerol-revertant strains of interest (series RT207) were screened for suppressibility of the phosphofructokinase-negative phenotype by ϕ80*psupF* by cross-streaking with this phage on minimal media with mannose and with glucose; controls used ϕ80 and ϕ80*psupF*$^+$. Seven of the strains, all *pfkC*$^-$ (RT207-3, -42, -99, -108, -143, and -162, Tables 3 and 4), showed suppression. (Nonsuppressible *pfkC*$^-$ mutants might also exist, but have not yet been mapped.)

Although the suppression by ϕ80*psupF* was clear, the ϕ80*psupF* lysogens easily segregated the phage unless selective pressure was maintained (e.g., growth on glucose). To avoid this problem and to study stable suppressed and unsuppressed *pfkC*$^-$ strains, we constructed derivatives from one of them, strain RT207-143 (*pfkA2 pfkB1 pfkC*$^-$), by selecting a *trp*$^-$ mutation and then introducing *supF* and *supF*$^+$ by phage P1 transduction (strains RT676 and RT678, Table 1). The inheritance of *supF* was

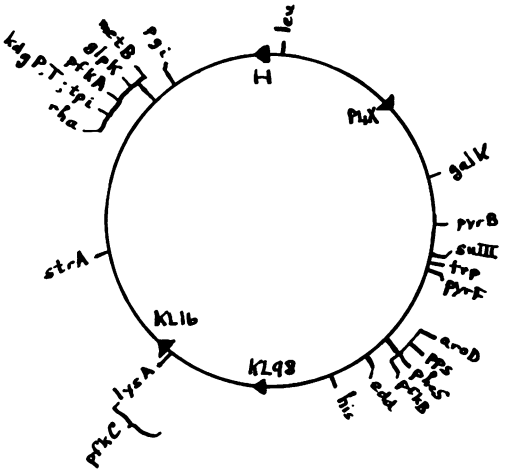

FIG. 1. *Genetic map of E. coli with markers used in this and in an accompanying paper (17). Origins of Hfr strains are indicated inside circle. Most gene abbreviations are in Taylor and Trotter (16); pfkC is described in this paper.*

TABLE 4. *Conjugational mapping of pfkC*[a]

Donor Hfr	Recipients (all *pfkA2 his*$^-$)	Fraction of His$^+$ recombinants that grew on mannose
KL16	RT207-9 (*pfkB*$^-$ *pfkC*$^+$)	0/70
KL16	RT207-42 (*pfkB1 pfkC*$^-$)	65/97
KL16	RT207-99 (*pfkB1 pfkC*$^-$)	32/48
KL16	RT207-108 (*pfkB1 pfkC*$^-$)	20/93
KL16	RT207-118 (*pfkB1 pfkC*$^-$)	33/106
KL16	RT207-143 (*pfkB1 pfkC*$^-$)	17/82
KL16	RT207-162 (*pfkB1 pfkC*$^-$)	45/98
KL98[b]	RT207-99 (*pfkB1 pfkC*$^-$)	0/98

[a] Donors are presumably wild type in *pfk*. Matings were 3 h in broth without added sugar and plated for His$^+$ recombinants.

[b] KL98 was used in crosses with the whole series of *pfkC*$^-$ mutants; no linkage was found in any cases, and only one cross is shown.

tested by using a known phage T4 amber mutation. All transductants carrying *supF* were indeed also suppressed for growth on glucose and mannose, whereas *supF*$^+$ transductants retained the phosphofructokinase-negative phenotype. Table 2 (lines 11 and 12) shows one such isogenic pair.

Although the evidence for *pfkC*$^-$ mutations being ambers seems strong, we would be cautious about considering the matter proven for two reasons. First, the suppressed *pfkC*$^-$ strains, like the unsuppressed strains, had little phosphofructokinase activity by assay (Table 2, lines 11 and 12). Second, among a group of 10 independent revertants of each of six amber mutant strains (RT207-42, -99, -108, -119, -143, -162) selected on glucose or on mannose plates, none was reverted by virtue of a new amber suppressor, as tested by sensitivity to a phage T4 amber mutant which is known to be suppressible by most amber suppressors.

Phenotype of strains carrying pfkB$^-$ or pfkC$^-$ together with pfkA$^+$. To this point we have described the *pfkB*$^-$ and *pfkC*$^-$ mutations as loss of *pfkB1* suppression of *pfkA2* or *pfkA1*. We have also constructed such strains carrying the wild-type locus *pfkA* in several ways (see Table 1): e.g., strains DF89 and RT855 (*pfkA*$^+$ *pfkB1 pfkB2*), RT874 (*pfkA*$^+$ *pfkB1 pfkC6*), and RT873 (*pfkA*$^+$ *pfkB1 pfkC6 supF*). The growth characteristics and enzyme levels of these strains resembled wild type (lines 7, 8, 13, and 14, Table 2).

Diploids at pfkB. Table 5 shows characteristics of haploids carrying *pfkA1* and, respectively, *pfkB*$^+$, *pfkB1*, *pfkB1 pfkB2*, and *pfkB1 pfkB3* and the corresponding diploids carrying *pfkB*$^+$ on the exogenote. The haploids had the usual growth phenotypes and enzyme activities. The diploids were interesting in several respects. The "wild-type control" strain (*pfkB*$^+$/*pfkB*$^+$) grew more slowly than the haploid on all media; indeed, it hardly grew at all on the rich medium tryptone-yeast extract. By assay, phosphofructokinase activity was greater in diploid than haploid. The second diploid (*pfkB*$^+$/*pfkB1*) resembled the *pfkB1* haploid, both in phenotype and assay. The two *pfkA*$^-$ *pfkB*$^-$ strains resembled each other as haploids, but

TABLE 5. *pfkB diploids*[a]

Strain	Casual genotype	Growth		Phospho-fructo-kinase activity
		Glu-cose	TYE[b]	
(RT220)	A1B$^+$	±	+	0.007
F'148/RT220	B$^+$/A1B$^+$	−	−	0.048
(RT221)	A1B1	+	+	0.200
F'148/RT221	B$^+$/A1B1	+	+	0.261
(RT222)	A1B1B2	−	+	0.000
F'148/RT222	B$^+$/A1B1B2	±	±	0.024
(RT223)	A1B1B3	−	+	0.000
F'148/RT223	B$^+$/A1B1B3	−	±	0.062

[a] Construction of the diploids is described in Table 1. The haploids used in this particular experiment were spontaneous or acridine-orange-induced segregants of the diploids. Cultures were grown in minimal medium with glycerol as carbon source before plating and assay. Growth on plates is indicated by + (normal), ± (intermediate), and − (no growth).

[b] TYE, Tryptone-yeast extract (4).

the diploids differed in growth, *pfkB*$^+$/*pfkB1*,-*pfkB2* being like the *pfkB*$^+$ haploid (as if *pfkB1,pfkB2* was recessive), whereas *pfkB1*,-*pfkB3* was somewhat dominant. These results are preliminary, and we draw only these conclusions from them: that *pfkB1* is probably dominant to *pfkB*$^+$ and that *pfkB*$^-$ mutations might include more than one class.

Gene order at pfkB. We earlier showed that the *pfkB1* mutation was at about 32 min on the *E. coli* map, being co-transduced with *pps* (11). The *pps* locus was reported to be closely linked to *aroD* by Brice and Kornberg (1). A *pfkA1 pps aroD* strain, RT500, was prepared for three-point mapping of *pfkB*. Phage lysates prepared on a number of strains differing at the *pfkB* locus were used to transduce strain RT500 to *aro*$^+$ or *pps*$^+$, and transductants were scored for the unselected recipient marker and for *pfk* suppression (Table 6). The gene order *aroD-pps-pfkB* is indicated (Fig. 2). When *aro*$^+$ was selected, linkage between *aroD* and *pps* was 85% (406 out of 477), and between *aroD* and *pfkB* it was 53% (192 out of 361). When *pps*$^+$ (growth on lactate) was selected, 52% (85 out of 165) of the transductants were *aro*$^+$, and 56% (61 out of 109) were recombinant at *pfkB*. The frequent occurrence of mutations suppressing both the *pps* and *pfk* phenotypes (18) complicated the *pps* transductions.

Russell and Pittard placed the gene for the phenylalanine-activating enzyme, *pheS*, close to *pps* on the side opposite *aroD* (14). We determined the order of *aroD*, *pfkB* and *pheS* by transductions using phage grown on a *pheS* heat-sensitive mutant (13) and recipient strains which were *pfkA1 pfkB1 aroD* or *pfkA1 pfkB*$^-$ *aroD* (RT212 and RT213). Results are shown in Table 6 and the order *aroD-pheS-pfkB* fits best (Fig. 2). Co-transduction frequencies were 66% (180 out of 273) between *aroD* and *pheS* and 55% (149 out of 273) between *aroD* and *pfkB*. Thus, the overall order is *aroD-pps-pheS-pfkB*.

Since "*pfkB*$^-$" strains, according to their derivation, carry two mutations at *pfkB*, one should be able to recover *pfkB1* from them. This was rare, with only one instance in Table 6 (cross 3). If this low frequency mainly reflected a gene order *aroD-pfkB2*(or *3*)-*pfkB1*, then reciprocal crosses might yield more *pfkB1* segregants. These were done using a *pfkB*$^+$ donor and *pfkB1,pfkB2* or *pfkB1,pfkB3* (RT213 and 214) as recipients and gave only one apparent *pfkB1* segregant out of 437 Aro$^+$ transductants. Thus, *pfkB2* and *pfkB3* must indeed be close to *pfkB1*, and the order is not known.

Selection of new suppressors at pfkB. The experiments on *pfkB* and *pfkC* began with the chance finding of *pfkB1*, an unlinked suppressor of *pfkA1*. We have done an experiment to determine the general frequency of *pfkB1*-like mutations. We spread 30 independent cultures of strain DF85 (*pfkA1 pfkB*$^+$ *pps*) onto appropriately supplemented mannose minimal plates, adding 1 drop of broth and, on one side of the plates, a few crystals of nitrosoguanidine. After 2 days at 37 C revertants were evident, and one from each plate was purified and scored by patching on plates containing, respectively, mannose, mannitol, glucose, lactate, and glycerol. All the revertants had regained, at least partially, the ability to grow on the first three carbon sources. Two of the 30 also grew on lactate and were presumably mutations to constitutivity of isocitrate lyase (18). The other 28 were tested for linkage to *pfkA* and *pfkB*, by (i) transduction of strain K8-5m (*pfkA*$^+$, *metB*) to methionine independence (on plates with glycerol as carbon source) and scoring of transductants for *pfkA* (according to growth on mannitol), and (ii) transduction of strain RT500 (*pfkA2 aroD*) to aromatic independence with scoring of the transductants for co-inheritance of a mutation allowing growth on mannose (i.e., a suppressor like *pfkB1*). Twenty-three out of 28 of the revertants no longer transduced the mutant phenotype linked to *metB* and thus probably were *pfkA* revertants. The other five retained *pfkA* mutation and showed linkage as suppressors with *aroD*. Since the latter test used a *pfkA2* recipient and the new revertants were selected in *pfkA1*, these results show that the five strains are likely to have general *pfkA* suppressors in the *pfkB* region. The new suppressors have not yet been characterized. Like *pfkB1* they restored substantial phosphofructokinase activity; e.g., parent (*pfkA1 pfkB*$^+$) specific activity was 0.042, and for revertants RT871-10, -12, and -13 it was 0.315, 0.855, and 0.146, respectively. It is interesting that this experiment did not yield any primary *pfkA1* suppressors at *pfkC*.

DISCUSSION

Our initial aim was to explain *pfkB*, and the present results, which are mainly genetic, do not do that. They introduce two new types of mutation, *pfkB*$^-$ and *pfkC*$^-$, each of which results in loss of phosphofructokinase in *pfkB1*-suppressed *pfkA* mutants. According to the simplest model for *pfkB1*—that it somehow increases the amount of a minor phosphofructokinase—the new mutations would somehow decrease it. Then one of the genes, *pfkB* or *pfkC*, might be a structural gene for activity 2 and the other a regulatory gene.

TABLE 6. *Transductional ordering of pfkB with respect to aroD, pheS, and pps*

Donor	Recipient	No. of transductants and selected marker	Scoring[a]			
			Lact⁻ Man⁻	Lact⁺ Man⁻	Lact⁺ Man⁺	Lact⁻ Man⁺
AM1 (*pfkA1 pfkB*⁺)	RT500 (*pfkA1 pfkB*⁺ *aroD pps*)	116 *aro*⁺ [b]	20(*pps*)	96(*pps*⁺)	0	0
DF1651B1	RT500 (*pfkA1 pfkB*⁺ *aroD pps*)	110 *aro*⁺	15(*pfkB*⁺ *pps*)	39(*pfkB*⁺ *pps*⁺)	55(*pfkB1 pps*⁺)	1(*pfkB1 pps*)
			Lact⁻ Gna⁺	Lact⁺ Gna⁺	Lact⁺ Gna⁻	Lact⁻ Gna⁻
DF89 (*pfkA*⁺ *pfkB1 pfkB2*)	RT500 (*pfkA1 pfkB*⁺ *aroD pps*)	181[c] *aro*⁺	25(*pfkB*⁺ *pps*)	55(*pfkB*⁺ *pps*⁺)	101(*pfkB*⁻ *pps*⁺)	0(*pfkB*⁻ *pps*)
RT518 (*pfkA*⁺ *pfkB1 pfkB3*)	RT500 (*pfkA1 pfkB*⁺ *aroD pps*)	70 *aro*⁺	10(*pfkB*⁺ *pps*)	25(*pfkB*⁺ *pps*⁺)	35(*pfkB*⁻ *pps*⁺)	0(*pfkB*⁻ *pps*)
			Aro⁺ Man⁻	Aro⁻ Man⁻	Aro⁺ Man⁺	Aro⁻ Man⁺
AM1	RT500 (*pfkA1 pfkB*⁺ *aroD pps*)	56 *pps*⁺ [d]	35(*aro*⁺)	21(*aro*)	0	0
DF1651B1	RT500 *pfkA1 pfkB*⁺ *aroD pps*)	60 *pps*⁺	8(*aro*⁺ *pfkB*⁺)	12(*aro pfkB*⁺)	20(*aro*⁺ *pfkB1*)	20(*aro pfkB1*)
			Aro⁺ Gna⁺	Aro⁻ Gna⁺	Aro⁺ Gna⁻	Aro⁻ Gna⁻
DF89 and RT518 (pooled)	RT500 (*pfkA1 pfkB*⁺ *aroD pps*)	49 *pps*⁺	7(*aro*⁺ *pfkB*⁺)	21(*aro pfkB*⁺)	15(*aro*⁺ *pfkB*⁻)	6(*aro pfkB*⁻)
			PheS⁺ Man⁺	PheS^{ts} Man⁺	PheS^{ts} Man⁻	PheS⁺ Man⁻
NP37 (*pheS*^{ts})	RT212 (*pfkA1 pfkB1 aroD*)	145 *aro*⁺	37(*pfkB1 pheS*⁺)	24(*pfkB1 pheS*^{ts})	80(*pfkB*⁺ *pheS*^{ts})	4(*pfkB*⁺ *pheS*⁺)
			PheS⁺ Gna⁻	PheS^{ts} Gna⁻	PheS^{ts} Gna⁺	PheS⁺ Gna⁺
NP37	RT213 (*pfkA1 pfkB1 pfkB2 aroD*)	128 *aro*⁺	49(*pfkB*⁻ *pheS*⁺)	14(*pfkB*⁻ *pheS*^{ts})	62(*pfkB*⁺ *pheS*^{ts})	3(*pfkB*⁺ *pheS*⁺)

[a] Scoring of *pfkB*⁺ (in a *pfkA* mutant strain) was no growth on mannose, moderate growth on gluconate as carbon source; *pfkB1*, strong growth on mannose; *pfkB*⁻, no growth on mannose or gluconate; *pps*⁺, growth on lactate as carbon source; *aro*⁺, growth without an aromatic amino acid and shikimate supplement; *pheS*^{ts}, no growth at 42 C, normal growth at 30 C; *pheS*⁺, normal growth at 42 C. Abbreviations are Aro⁺, aromatic independence; Lact⁺, Gna⁺, and Man⁺, growth on lactate, gluconate, and mannose, respectively.

[b] Transduction mixtures were plated on glycerol, with histidine and uracil, at 37 C.

[c] One additional transductant grew well on mannose and gluconate; hence it presumably had segregated *pfkB2*, leaving *pfkB1*.

[d] Transduction mixtures were plated on lactate, with histidine, uracil, and an aromatic supplement, at 37 C.

The present results might accord best, although not easily, with *pfkB* being regulatory and *pfkC* the structural gene (e.g., *pfkB*⁺ a repressor of *pfkC* expression, *pfkB1* a constitutive mutation, and *pfkB*⁻ a mutation to uninducibility), but such a model would be speculative and quite different ones could be suggested. (For example, one could fit a model along the lines suggested by Kemper for the *supQ* suppressor of *leuD* mutations in *Salmonella typhimurium* [5]; thus, the *pfkB*⁺ gene product would normally bind and inactivate the *pfkC* product, etc.) The usefulness of the new mutations will be that they provide strains for biochemical testing of

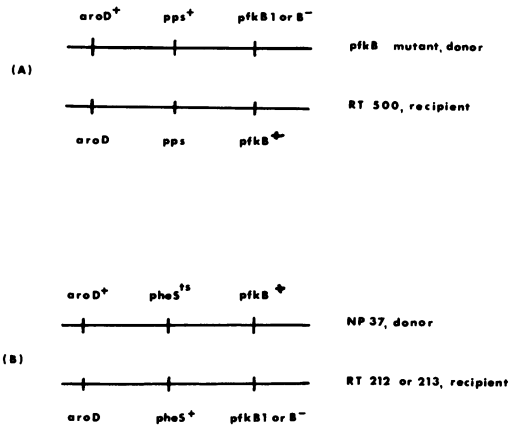

FIG. 2. *Transductional ordering of aroD, pps, pheS, pfkB. (A) shows genes used in crosses with strain RT500; (B) shows the crosses using RT213 and RT213 (Table 6).*

such models, and an obvious approach is to characterize the phosphofructokinase activities in certain of them.

The other approach will be to search for other functions of *pfkB* and *pfkC*. It is true that in certain strains carrying the wild-type allele *pfkA* (e.g., *pfkA*$^+$ *pfkB*$^-$ *pfkC*$^+$, and *pfkA*$^+$ *pfkB1 pfkC*$^-$, Table 2) aerobic growth on a few sugars is not markedly affected by comparison with wild-type strains, but this should be studied more thoroughly. We note also that, so far, *pfkB*$^-$ has only been recognized as a secondary mutation linked to *pfkB1* (e.g., *pfkB1 pfkB2*), and we do not know what the phenotype of *pfkB2* would be alone. Likewise, *pfkC*$^-$ has only been studied in strains also carrying *pfkB1*, and so *pfkB*$^+$ *pfkC*$^-$ strains will have to be prepared.

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