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A Mutant of *Escherichia coli* Auxotrophic for Organic Phosphates: Evidence for Two Defects in Inorganic Phosphate Transport

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Summary. An inorganic phosphate transport mutant has been isolated as a *sn*-glycerol-3-phosphate auxotroph and characterized genetically. Two lesions are responsible for the transport defect. One lesion, *pst*, is located at minute 74 of the *E. coli* genetic map while the other lesion, *pit*, is located at minute 68. All "K10" strains that were examined carry the *pit* lesion. Evidence is presented that the glycerol phosphate and hexose phosphate transport systems are not important inorganic phosphate transport systems. The mapping data indicate that the genetic distance between *malA* and *xyl* is greater than that now allowed.

Mutants of *Escherichia coli* auxotrophic for *sn*-glycerol-3-phosphate (G3P) have been isolated and shown to be of two types (Bell, 1974). One type of G3P auxotroph is deficient in the biosynthetic *sn*-glycerol-3-phosphate dehydrogenase, (the *gpsA* gene, Cronan and Bell, 1974a), while the other type owes its G3P requirement to a *Km* defect in *sn*-glycerol-3-phosphate acyltransferase (the *plsB* gene, Cronan and Bell, 1974b). In this paper we report the characterization and genetic mapping of a third type of mutant isolated as a G3P auxotroph. This mutant is defective in inorganic phosphate (Pi) transport; and therefore, requires G3P as a source of phosphate.

Mutants defective in inorganic phosphate transport have been isolated previously (Bennett an Malamy, 1970; Medveczky and Rosenberg, 1970, 1971). The biochemical and genetic characterization of these mutants has led to the conclusion that there are at least four inorganic phosphate transport systems in *E. coli*. Two systems, the *pit* system and the *pst* system, function constitutively. Inorganic phosphate is the primary substrate for both these systems. Two additional systems, the G3P transport system and the hexose phosphate transport system, are inducible and are reported to transport inorganic phosphate as a secondary substrate (Willsky and Malamy, 1974). However, the isolation of these inorganic phosphate transport mutants was accomplished by selecting mutants resistant to the phosphate analog, arsenate. Since arsenate is known to affect several metabolic functions other than phosphate transport (for example, see Rae and Strickland, 1975), some of the characteristics of these mutants may be secondary to the inorganic phosphate transport deficiency.

The isolation of a phosphate transport mutant as an organic phosphate auxotroph has been reported (Medveczky and Rosenberg, 1970, 1971). This mutant lacks a phosphate-binding protein that has been shown to be a component of the *pst* system (Gerdes and Rosenberg, 1974). This mutant may be a multiple mutant since some mutants defective in the phosphate-binding protein can still transport Pi, although the *Km* is higher (Willsky and Malamy, 1974). Further genetic characterization has, however, not been performed.

The phosphate transport mutant described here was isolated without the possible complications of treatment with arsenate. Furthermore, the auxotroph was isolated in a strain that is constitutive for G3P transport function. If the conclusions cited above concerning the involvement of the G3P transport system in inorganic phosphate transport are correct, it should not have been possible to isolate the auxotroph in such a strain. For these reasons, we thought it of interest to determine the relationship of this phosphate transport mutant to those described previously.

Materials and Methods

Bacterial and Phage Strains. The properties of the various bacterial strains used are given in Table. 1. Transduction was done using phage P_1 vira.

Strain 10B5 is a G3P auxotroph derived from a K10 strain of *E. coli*, strain 8 (Hayashi, Koch, and Lin, 1964). Strain GS13 is a spontaneous mutant of GS8 constitutive for hexose phosphate transport (*uhp*^{c}) selected by the ability to grow on D-fructose-1-

Table 1. Bacterial strains

Strain	Sex	Genotype[a]	Source
8	HfrC	*glpD*3, *glpR*2, *phoA*8, *tonA*22, *rel*-1, (λ), T_2^R	E.C.C. Lin Strain
6	HfrC	*glpT*13, *phoA*8, *tonA*22, T_2^R, *rel*-1	E.C.C. Lin Strain
10B5	HfrC	*pst*-2, *pit*-1, other markers as in strain 8	this report
KL16	Hfr	*thi*-1, *rel*-1, λ^-	K.B. Low Strain
KL16–99	Hfr	*recA*1, see Fig. 2 for origins of transfer, λ^-	K.B. Low Strain
KL14	Hfr	*thi*-1, *rel*-1, λ^-	K.B. Low Strain
JG85	F′	F197 *metE*$^+$/*metE*71, *recA*56, *rha*-2, *dna*-2?, λ^-	J. Gross Strain
KLF41/JC1553	F′	F141 *argG*$^+$/*argG*6, *metB*1, *his*-1, *leu*-6, *recA*1, *mtl*-2, *xyl*-7, *malA*1, *gal*-6, *lacY*1, *str*-104, *supE*44, *tonA*2, *tsx*-1, λ^-, λ^R	K.B. Low Strain
MAF1/JC1553	F′	F140 *argG*$^+$/(as above)	W. Maas Strain
KLF11/JC1553	F′	F111 *metB*$^+$/(as above)	K.B. Low Strain
X478	F$^-$	*proC*32, *purE*42, *metE*70, *lysA*23, *thi*-1, *leu*-6, *trpE*38, *lacZ*36, *mtl*-1, *xyl*-5, *ara*-14, *azi*-6, *tonA*23, *tsx*-67, *str*-109, λ^-, *supE*44	R. Curtiss Strain
KL218	F$^-$	*proC*24, *purE*41, *thyA*25, *nalA*12, *argG*34, *metB*1, *his*-53 *pyrF*30, *lac*$^-$, *str*-97, *tsx*-63, *mtl*-2, *xyl*-7 or 14, λ^-	K.B. Low Strain
KL185	F$^-$	*thi*-1, *pyrD*34, *trp*-45, *his*-68, *galK*35, *malA*1, λ^R, *xyl*-7, *mtl*-2, *str*-118, λ^-	K.B. Low Strain
AB2277	F$^-$	*thi*-1, *ilvD*145, *metE*46, *his*-4, *trp*-3, *proA*2, *mtl*-1, *malA*1, *ara*-9, *galK*2, *lacY*1 or *Z*4, *ton*-1, *tsx*-3, *str*-8 or 9, λ^R, λ^-, *supE*45	E.A. Adelberg Strain
AB2563	HfrC	*ilvD*188, *rel*-1, *tonA*22, T_2^R	E.A. Adelberg Strain
GS5[b]	F$^-$	*pit*-1, *pst*-2, *proC*24, *purE*41, *thyA*25, *nalA*12, *metB*1, *his*-53, *pyrF*30, *mtl*$^+$, *argG*$^+$	recombinant from conjugation of 10B5 with KL218
GS8	HfrC	*pit*-1, *pst*-2, *metB*1, *pyrE*$^+$	transductant of CS101-4U from 10B5
GS11	F$^-$	*ilvD*145, *pst*$^+$, other markers as in GS5	transductant of GS5 from AB2277
GS12	F$^-$	*thy*$^+$, *recA*1 derivative of GS5	see *Methods*
GS13	HfrC	*uhp*^{c} derivative of GS8	see *Methods*
GS16	HfrC	*nalA* derivative of 10B5	see *Methods*
GS17	HfrC	*uhp*^{c} derivative of 10B5	see *Methods*
GS19	HfrC	*plsB*13, *pit*$^+$, other markers as in 10B5	transductant of 10B5 from BB13
GS24	HfrC	*asd*-1, *pit*$^+$, other markers as in 10B5	transductant of 10B5 from U482
U482	HfrC	*asd*-1, *thi*-1, *rel*-1, λ^-	F. Jacob Strain
CS101-4U	HfrC	*pyrE*41, *metB*1, *tonA*22, *rel*-1, T_2^R	AT 2243, A.L. Taylor Strain
BB13	HfrC	*plsB*13, other markers as in strain 8	R.M. Bell Strain

[a] Genetic symbols are as described by Taylor and Trotter (1972). Abbreviations: *pit, pst*; see text.
[b] Several irrelevent parental markers have not been tested in this and subsequent recombinants. Only those markers which have been tested are given.

phosphate as the carbon source (Ferenchi, Kornberg, and Smith, 1971). Strain GS17 is a spontaneous *uhp*^{c} mutant of 10B5 isolated in the same manner. Strain GS16 is a spontaneous *nalA* mutant of 10B5 selected for the ability to grow in the presence of 100 μg/ml of naladixic acid. Strain GS12 was constructed by mating KL16-99 with GS5, selecting *thyA*$^+$ recombinants, and checking those recombinants for sensitivity to ultraviolet (UV) light (200 ergs/mm^2). Strain GS5 is one such UV^s*thyA*$^+$ recombinant.

Media. Basic growth media were medium E (Vogel and Bonner, 1956) and a modified medium 56LP containing 0.3 or 1 *mM* Pi (Bell, 1974). Carbon sources were added to 0.4%. Sodium DL-glycerol-3-phosphate was added to 0.04%. Media were supplemented with necessary growth factors as required such as L-amino acids and nucleic acid bases at 50 μg/ml. Solid media contained 1.5% agar. Other media used for genetic studies have been described previously (Cronan and Bell, 1974a, b). The growth temperature was 37° C.

Genetic Crosses. The procedures for conjugational and transductional crosses have been described previously (Cronan and Bell, 1974a, b).

Enzyme Assays. sn-Glycerol-3-phosphate dehydrogenase, *sn*-glycerol-3-phosphate acyltransferase, and phosphatidyl glycerol phosphate synthetase were assayed as described previously (Bell, 1974; Bell, Mavis, Osborn, and Vagelos, 1971).

Transport Assays. The initial rate of uptake of inorganic phosphate was determined by adding ^{32}P (final specific activity = 250 cpm/nmole Pi) to cells growing exponentially in medium 56LP containing 0.4% glucose and 0.04% G3P at 37° and withdrawing aliquots at intervals. The cells were collected on membrane filters (Millipore, Type HA) and washed with 10 ml of the growth media lacking the ^{32}Pi. The filters were dried and the radioactivity determined by liquid scintillation counting using Aquasol LSC Cocktail (New England Nuclear, Boston, Massachusetts).

Macromolecular Synthesis. The rates of DNA, RNA, protein, and lipid synthesis were determined by incorporation of radioactively labeled thymidine, uracil, leucine, or acetate as described previously (Bell, 1974).

Chemicals. Oleic acid [9, 10-^{3}H] at 10 curies/mmole and $H_3{}^{32}PO_4$ (carrier-free) were obtained from New England Nuclear. All other chemicals were reagent grade.

Results

1. Isolation and Characterization of G3P Auxotrophs

Strain 8, growing on casamino acids and potassium oleate, was mutagenized with N-methyl-N′-nitro-N-nitrosoguanidine (Adelberg, Mandel, and Chen, 1964) and allowed to undergo phenotypic expression. Half of the culture was grown for 1.2 generations in the presence of tritiated-K oleate (10 curies/mmole) and the other half was treated similarly with nonradioactive K oleate. The culture exposed to ^{3}H-oleate showed a dramatic loss of viability upon storage at 4° C compared to the control culture. After 20 days only 1 out of 10^6 of the cells exposed to ^{3}H-oleate had survived. Among the 2000 survivors, five G3P auxotrophs were identified. One of these, strain 10B5, showed a tight requirement for G3P and was selected for further characterization with the hypothesis that the G3P requirement and resistance to tritium suicide resulted from a defect in phospholipid biosynthesis.

Strain 10B5 grows as well as its parent, strain 8, on minimal glucose plates supplemented with G3P. It, therefore, has no nutritional requirements other than G3P. The growth of strain 10B5 in low phosphate medium was arrested immediately upon removal of G3P. Moreover, the addition of 0.4 unit/ml of purified *E. coli* alkaline phosphatase caused cessation of growth after 0.6 generations, presumably because the G3P had been degraded to glycerol and inorganic phosphate. However, when strain 10B5 grown in medium E which contains 74 *mM* Pi was starved for G3P, growth continued normally for 0.3 generations before slowing to a rate one-fifth of that of the G3P-supplemented culture for at least two generations before growth ceased. The further observation that glycerol could not substitute for the G3P requirement in either the presence or absence of glucose suggests that strain 10B5 is unlike other G3P auxotrophs that have been isolated [glycerol will substitute for the G3P requirement of the *plsB* and *gpsA* auxotrophs if they are grown on sodium succinate or casamino acids as carbon source (unpublished observations)].

Investigation of the effect of G3P deprivation on macromolecular synthesis in strain 10B5 revealed another unexpected feature of this mutant. In G3P auxotrophs previously isolated, phospholipid synthesis was the initial macromolecular process affected by G3P starvation. However, when strain 10B5 was starved for G3P, RNA synthesis was most dramatically affected falling to a rate less than 5% that of the supplemented culture within 10 minutes. A slower decline in the rate of protein and DNA synthesis was observed. After 40 minutes of starvation the rate of protein synthesis was 70% of normal while the rate of DNA synthesis was 40% of normal. The rate of phospholipid synthesis declined only slightly in the same period (data not shown).

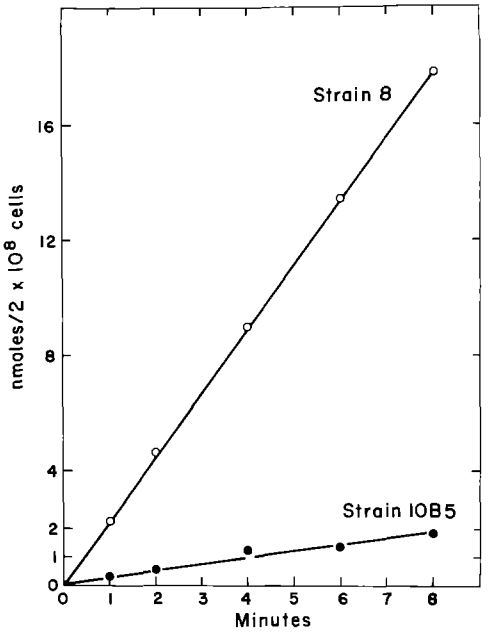

Fig. 1. Uptake of inorganic phosphate in strain 8 and the mutant strain 10B5. ^{32}Pi was added to exponentially growing cells at time zero. At the times indicated, aliquots were removed and processed as described in the Materials and Methods. The Pi concentration is 1 *mM* with a specific activity of 250 cpm/nmole. Cell density was 2×10^8 cells per ml

The sum of these observations suggested that our original hypothesis that the mutant required exogenous G3P to permit normal phospholipid biosynthesis was unlikely. In fact, assays of the biosynthetic G3P dehydrogenase, G3P acyltransferase, and phosphatidyl glycerol phosphate synthetase, the enzymes of phospholipid synthesis known to require G3P, failed to detect any difference between strain 10B5 and its parent, strain 8 (data not shown).

As mentioned above, starvation of strain 10B5 for G3P had a more immediate effect on growth when the cells were growing in medium 56LP than when they were growing in medium E. This observation suggested that inorganic phosphate transport was impaired in the mutant. A direct measure of the initial rate of uptake of Pi demonstrated that strain 10B5 transported Pi at only one-tenth the rate of strain 8 (Fig. 1). The further observation that strains 10B5, GS8, GS13, and GS17 are capable of growth on hexose phosphate as carbon source, even in the absence of G3P, demonstrated that strain 10B5 requires a source of organic phosphate because it is unable to transport Pi at a rate adequate to allow growth.

2. Genetic Mapping of Pi Transport Lesions

a) Conjugational Mapping of Pi⁻ Phenotype. Strain 10B5 is a male strain, and hence, a female derivative of strain 10B5 was obtained by mating with strain X478, a multiply auxotrophic female (a similar mating was done with strain KL218). Pi^- recombinants were observed only when $lysA^+$ recombinants were selected ($argG^+$ in the case of the KL218 mating), which suggested that the lesion(s) responsible for the Pi^- phenotype was located in the 55–80 minute region of the genetic map (Table 2). This was confirmed by mating strain GS5, an $argG^+$, Pi^- recombinant, with several Hfr donor strains (Figure 2). Pi^+ recombinants were numerous when strain KL14 was the Hfr donor but not when strain KL16 was the Hfr donor (data not shown).

Table 2. Mapping of Pi^- by conjugation

Selected recombinants	Number scored	Pi^- recombinants (%)
$proC^+, purE^+$	30	<0.3
leu^+	57	<0.2
$metE^+$	60	<0.2
$lysA^+$	60	10.0
$argG^+$	85	3.5
$trpE^+$	6	<1.7

The $argG^+$ recombinants were progeny of the mating of strain 10B5 with strain KL218. All other recombinants were progeny of the mating of strain 10B5 with strain X478.

Table 3. Mapping of Pi^- by F′ transfer

Episome	Recipient	*recA*	Pi^+ recombinants/ml
F140	GS5	+	1.5×10^4
F140	GS12	–	2.5×10^5
F141	GS5	+	<100
F141	GS12	–	<100
F111	GS5	+	2.4×10^4
F197	GS5	+	1.5×10^4
F197	GS12	–	<100

A more refined mapping of the Pi^- phenotype was performed using various F′ donor strains (Fig. 2). Three derivatives of strain JC1553, each carrying a different F′ factor, were mated with strains GS5 and GS12. In addition, strain JG85 (carrying F197) was used as a F′ donor strain. The donor strains were $recA^-$, thus preventing chromosome mobilization.

The results of these crosses indicate that there are two separate lesions responsible for the Pi^- phenotype observed in strain GS5 (Table 3). One lesion is in the region of the chromosome carried by F140 but not by F141, since matings with the former strain give rise to Pi^+ recombinants, while matings with the latter strain do not. This lesion appears to be recessive as the identical results are obtained when strain GS12, a $recA^-$ derivative of strain GS5, is used as the recipient strain. The second lesion is in the region of the chromosome carried by both F111 and F197. Although the results of the cross of F197 with strain GS12 indicate that this lesion may be *trans*-dominant, the fertility of the donor strain in this cross was not verified. This result was not investigated further.

Thus, one lesion lies between the termini of the chromosomal portions carried by F141 and F140 and is therefore located between minutes 66 and 71 of the genetic map. The second lesion lies in the chromosomal region carried by F197 and is therefore between minutes 72 and 76 of the genetic map.

b) Transductional Mapping of the Pi⁻ Phenotype. In order to localize the two lesions further, we tested for cotransduction by phage P_1 of the lesions with appropriate markers. Because two lesions are responsi-

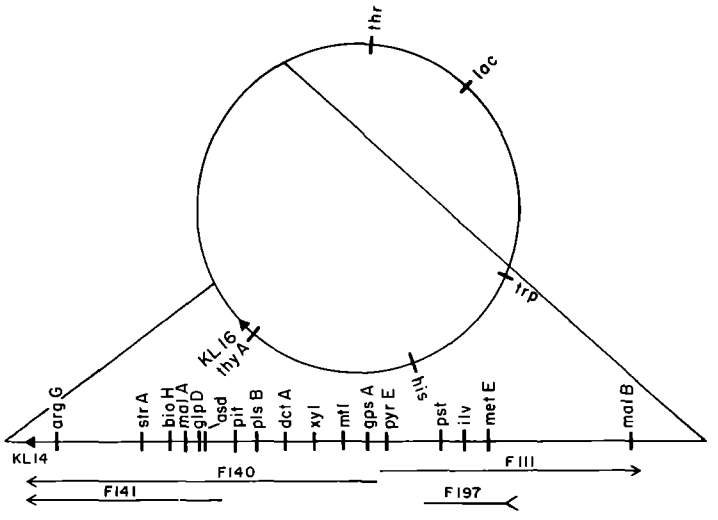

Fig. 2. Genetic map of *E. coli* K-12 adapted from Taylor and Trotter (1972). The Hfr and F′ data are from Low (1972). The map position of *gpsA* and *plsB* are from Cronan and Bell (1974a, b). The enlarged portion of the genetic map was drawn approximately to scale

Table 4. Transductional mapping

Bacterial strains and relevent markers		Marker selected	Colonies with donor marker/total colonies selected	Cotransduction frequency %
Donor	Recipient			
10B5 Pi^-	CS101-4U *pyrE*$^-$	*pyrE*$^+$	4/187	2.2
10B5 Pi^-	AB2563 *ilvD*$^-$	*ilvD*$^+$	58/138	42.1
10B5 Pi^-	GS11 *ilvD*$^-$	*ilvD*$^+$	23/71	32.4
U482 *asd*$^-$	10B5 Pi^-	Pi^+	3/271	1.1
10B5 Pi^-	GS24 *asd*$^-$	*asd*$^+$	4/201	2.0
BB13 *plsB*$^-$	10B5 Pi^-	Pi^+	4/51	7.8
GS16 *nalA*	strain 6 *glpT*$^-$	*glpT*$^+$	52/90	57.8
GS16 *glpT*$^+$	strain 6 *nalA*$^+$	*nalA*	32/55	58.2
X478 *xyl*$^-$	10B5 Pi^-	Pi^+	0/120	<0.8
KL185 *xyl*$^-$	10B5 Pi^-	Pi^+	0/261	<0.4

ble for the Pi^- phenotype, the recipient strain must be defective in one of the two genes if it is to be transduced to Pi^-. Since this prerequisite was often not easily fulfilled, reciprocal transductions were not usually performed.

Phage grown on strain 10B5 were used to transduce strains CS101-4U, AB2563, and GS11, Cotransduction of the Pi^- phenotype with both the *pyrE* locus and the *ilvD* locus was observed (Table 4). [Transduction of these strains to a Pi^- phenotype is possible because they are derivatives of strain "K10" and are therefore defective in the *pit* system (Willsky, Bennett, and Malamy, 1973)]. Because of the great distance between *pyrE* and the *ilv* operon and because of the F′ data already discussed, the lesion must lie between these two markers at about minute 74 of the genetic map. Thus, this lesion is probably identical to the *pst* locus. The cotransduction frequency of this lesion and the *ilv* operon reported here (32–42%) is very similar to that reported previously for the *pst* locus (35%) (Willsky, Bennett, and Malamy, 1973).

The other lesion responsible for the Pi^- phenotype may be identical to the *pit* locus since the F′ data discussed above are consistent with an earlier report that the *pit* locus maps in the *malA-xyl* region of the chromosome (Willsky, Bennett, and Malamy, 1973). Since strain 8, the parent of strain 10B5, is a "K10" strain, this lesion is not new to strain 10B5, but it is necessary for the Pi^- phenotype. This locus was also mapped by transduction. Strain 10B5 was transduced to Pi^+ with phage stocks grown on strains mutant in the appropriate genes and tested for the unselected marker. Cotransduction of *pit* with both the *asd* and *plsB* loci was observed (Table 4). Because the G3P requirement of *plsB* strains can be fulfilled by glycerol when sodium succinate is the carbon source, $G3P^+$ (Pi^+), glycerol-requiring recombinants could be obtained. The reciprocal transduction (with Pi as the unselected marker) is impossible. No cotransduction with *xyl* was observed. Hence the *pit* gene must map between *asd* and *plsB*.

c) Non-dependence of Pi^- Phenotype on glpT or *uhp*. As mentioned above, it has been suggested that the G3P transport system (coded by the *glpT* gene), and the hexose phosphate transport system (coded by the *uhp* gene), secondarily function in inorganic phosphate transport (Willsky and Malamy, 1974). While this may be true (no biochemical evidence was presented by the authors), we offer the following genetic evidence that neither system can facilitate the transport of sufficient Pi to allow strains lacking both the *pit* and *pst* systems to grow.

Strain 10B5 was isolated in a *glpR*^{c} background and is therefore constitutive for all the *glp* regulon functions including transport (Cozzarelli, Freedberg, and Lin, 1968). Strain 10B5 still retains the *glpT*$^+$ character since it was demonstrated that strain GS16 (a spontaneous *nalA* variant of strain 10B5) could donate *glpT*$^+$ to a *glpT*$^-$ strain in a transductional cross. This is true whether *glpT*$^+$ was the selected or unselected marker (Table 4). Thus, strain 10B5 is constitutive for the G3P transport system, yet it still requires G3P as a phosphate source. Similarly, when strain 10B5 (or one of its derivatives, strain GS8) was made constitutive for hexose phosphate transport (*uhp*^{c}) by selecting mutants capable of growth of fructose-1-phosphate (Ferenchi, Kornberg, and Smith, 1971) (strains GS17 and GS13, respectively), these strains were unable to grow on standard minimal media unless a source of organic phosphate was supplied.

Discussion

In agreement with earlier studies, inorganic phosphate transport has been demonstrated to be effected by either of two genetically separable systems. One system, the *pst* system, has previously been mapped at minute 74 of the genetic map (Willsky, Bennett, and Malamy, 1973). We have confirmed that mapping data. In addition, we have mapped a second Pi transport system, the *pit* system, at about minute 68 of the genetic map.

These two transport systems appear to be the only physiologically important inorganic phosphate transport systems. The G3P transport system and the hexose phosphate transport system have been reported also to function as Pi transport systems. However, we find that neither system is capable of transporting sufficient Pi to permit growth. Strains producing these transport systems constitutively (*glpR*^{c}, *glpT*$^+$; *uhp*^{c}) continue to require organic phosphate for growth when both the *pit* and *pst* systems are defective.

It has been suggested that many "K10" strains of *E. coli* lack the *pit* transport system (Willsky, Bennett, and Malamy, 1973). Our results confirm this suggestion since two "K10" strains, strains AB2563 and CS101-4U, can be made Pi$^-$ by transduction to *pst*. Since strain 8 is a "K10" strain, only a single mutational event was necessary to cause the Pi$^-$ phenotype of strain 10B5. Therefore, the unlikely event of two mutations occurring simultaneously need not be invoked to explain the phenotype of strain 10B5.

As was mentioned above, arsenate is known to affect several metabolic functions other than Pi transport. Since strain 10B5 was isolated as a G3P auxotroph rather than as an arsenate-resistant strain, we have examined strain 10B5 and its derivatives with respect to their arsenate sensitivity or resistance. Strain 10B5 is arsenate-resistant. However, its parent, strain 8, is also somewhat arsenate-resistant. Attempts to assign this phenotype to one of the two Pi transport systems have shown that strains that are *pit*$^-$, *pst*$^+$ tend to be arsenate-resistant while strains that are *pit*$^+$, *pst*$^-$ tend to be arsenate sensitive. However, there was sufficient variation among strains to cause some ambiguity. Therefore, arsenate-resistance is a complex phenotype and does not seem suitable for use as a selected phenotype in genetic manipulation.

The mapping of the *pit* system at about minute 68 of the genetic map marks the first time that all the loci in the *malA-xyl* region of the genetic map have been linked by cotransduction. Furthermore, accumulated evidence from this laboratory (this report; Cronan and Bell, 1974b) suggests that the genetic distance between the *malA* and *xyl* loci should be greater than the 4.5 minutes now allowed (Taylor and Trotter, 1972). Earlier studies have mapped three loci between *malA* and *xyl*. The *asd* locus is 60% cotransduced with *malA* (Schwartz, 1966), while the *dctA* locus is 1.5 minutes counterclockwise from the *xyl* locus (as determined by interrupted mating, Kay and Kornberg, 1969). Recently it has been shown that *plsB* is 10–15% cotransduced with *dctA* but is not cotransduced with either *xyl* or *asd* (Cronan and Bell, 1974b). In order for the *pit* locus to be 1–2% cotransduced with *asd* and 8% cotransduced with *plsB* as reported here, the total distance between *asd* and *xyl* should be about one minute greater than allowed at present.

The initial mapping of *malA* and *xyl* was performed using interrupted mating techniques, and the results of that mapping were that the distance between *malA* and *xyl* varied between 4.5 and 5.5 minutes depending on the strain that was used. Even using the same strain, the map distance was found to vary by 0.5 minutes from experiment to experiment (Taylor and Thoman, 1964). Hence, this suggestion is not inconsistent with the data that were used to formulate the present genetic map.

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