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Genetic and Physiological Tests of Three Phosphate-Specific Transport Mutants of *Escherichia coli*

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Phosphate-specific transport system mutations *phoT35*, *pst-2*, and *phoS25*-(Am) were mapped between *bgl* and *glmS*, at about 83 min on the *Escherichia coli* chromosome. All three mutations were recessive to wild-type genes on transducing bacteriophage λ *asn*. The *phoS25*(Am) and *pst-2* mutations were also recessive to transducing phage λ d*glm*; however, the *phoT35* mutation was not. This suggests that *phoT35* lies in a different complementation group from *phoS25*(Am) or *pst-2*. Isogenic series of strains carrying these mutations were constructed in two genetic backgrounds, pit^+ (wild type) and *pit* (relying entirely on the phosphate-specific transport system for phosphate uptake). The *pst-2 pit* double mutant was incapable of P_i utilization, but the *phoT35 pit* double mutant exhibited no such deficiency.

Escherichia coli has two major systems for P_i uptake: the P_i transport (Pit) system, which has no effect on alkaline phosphatase regulation, and the phosphate-specific transport (Pst) system, which exerts negative control over alkaline phosphatase synthesis (19, 23). Both transport systems seem to feed a common internal P_i pool, and their rates of uptake are approximately additive in low concentrations of orthophosphate, but somewhat less than additive in high concentrations of orthophosphate (19, 25). *pit pst* double mutants require organic phosphates for growth (19, 21, 23). Studies by Rosenberg et al. (19, 20) and Willsky and Malamy (25) suggest that the Pit system is typical of a class of low-affinity membrane-bound porters, whereas the Pst system is typical of a class of inducible high-affinity transport systems with osmotic-shock-sensitive binding proteins (3).

In the presence of arsenate, a toxic phosphate analog, the Pst system is distinguishable from the Pit system; bacteria with a functional Pit system show no growth when 10 mM arsenate is present in medium containing 1 mM phosphate, whereas *pit* mutant strains are resistant (19, 26). Strain K10 (wild type for alkaline phosphatase and the Pst system), from which many strains used in alkaline phosphatase research are derived (1, 4), is *pit* (19, 25).

The Pst system is believed to consist of the closely linked genes *pst*, *phoS*, and *phoT* (23). Mutants in *phoS*, the best characterized, lose the phosphate-binding protein (7, 9, 14, 24). The hypothesis that *phoT* and *pst* define two separate genes was based on a 10-fold difference in level of constitutive synthesis of alkaline phosphatase (23). It has been reported (21) that in a *recA* strain a pst^+ episome does not complement a *pst* mutation, which would prevent a *cis-trans* test of the Pst system genes; however, the fertility of the donor strain in this cross was not confirmed. Mutations *phoT* and *pst* could not be resolved into two loci by P1 transduction (23).

Published studies of mutants of K10 classified as *phoT* (19, 23) report that transduction of alkaline phosphatase constitutivity into a *pit* strain results in a requirement for organic phosphate, indistinguishable from the requirement of *pit pst* double mutants. Our studies (data not shown) of the four strains examined in the literature (C10, C90, C101, and C112) (19, 23) as well as other mutants classified as *phoT* (C25, C64, and C84) reveal them to be arsenate sensitive, in contrast to their parent strain K10, a *pit* mutant and arsenate resistant (19). These strains were isolated by selection of constitutivity for alkaline phosphatase on media containing no phosphate source other than glycerol 2-phosphate, which is not taken up by the bacteria, and P_i. Thus, bacteria requiring organic phosphate could not grow, and alkaline phosphatase-constitutive mutants losing the Pst system could only be isolated as pit^+ revertants or pseudorevertants; therefore, the simplest explanation of these findings is that these *phoT* mutations, like the *pst* mutations, abolish the Pst system. Uptake of both orthophosphate and arsenate in these strains must be carried out by a reversion to pit^+ or pseudoreversion in the Pit system. In

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contrast, the isolation of *pst* mutants was in the presence of the transportable organic phosphate source glycerol 3-phosphate (21, 23). Therefore, the *phoT* mutations appear identical to the *pst* mutations in effect on the Pst system, except for their higher level of constitutive synthesis of alkaline phosphatase; this may result from their selection for alkaline phosphatase constitutivity, for which the *pst* mutants were not selected.

However, a single strain of those classified as *phoT*, C4, proved arsenate resistant at the level characteristic of K10 and other *pit* mutants and proved capable of transducing alkaline phosphatase constitutivity into a *pit* mutant without resulting in a requirement for organic phosphate. This mutation, *phoT35*, therefore differs in phenotype from *phoS*, *pst*, and the *phoT* mutations considered above and possibly represents a different gene. Here we study the genetic and physiological properties of the *phoT35* mutation in well-defined *pit* and *pit*$^+$ genetic backgrounds, as compared with two *phoS* and *pst* mutations. Our studies also indicate a different order of the Pst system region, relative to *bgl* and *ilv*, from that previously published (23).

MATERIALS AND METHODS

Strain construction. Table 1 lists the strains used in this series of experiments. Strain W3110 *trpR* is closely related to wild-type *E. coli* K-12, but is F$^-$ and also λ^- (1). A spontaneous streptomycin-resistant mutant was selected from a *bglR ilv* derivative of W3110 *trpR*.

Strain GS5 *pit pst* (21) requires proline for growth; in addition, it cannot utilize P_i and therefore requires glycerol 3-phosphate for growth. These supplements interfere with selection that uses carbon sources such as salicin. A spontaneous revertant utilizing P_i (GS5W1) was selected by growth on P_i as the sole phosphate source. This revertant was repressible for alkaline phosphatase synthesis and still resistant to 10 mM arsenate in the presence of 1 mM phosphate, as was strain GS5. This is the phenotype expected of a *pit pst*$^+$ revertant (19, 23).

GS5W1 was then reverted to Pro$^+$ His$^+$ Ura$^+$ and transduced to *bglR pst*$^+$ *ilv* by selecting Bgl$^+$ (capable of growth on salicin as a carbon source). This strain was designated strain GO.

To facilitate transduction of *glmS*, the kanamycin resistance transposon Tn*5*, inserted in *ilv* (*ilv*::Tn*5*), was transduced by P1 from strain GS1 (gift of L. Guarente) into *glmS* strain DC104, and a lysate of the resulting strain was used to transduce the linked *glmS* and *ilv*::Tn*5* markers.

Table 2 lists the bacteriophages used in this work.

Media. Rich media used were H, LB, and R (7). Synthetic medium was either Tris-buffered medium 121 (11) or MOPS (morpholinepropanesulfonate)-buffered medium (18) with various sterile supplements added per liter: 5 mg of thiamine; 2 g of glucose, maltose, or salicin; and 0.07 or 1 mM KH_2PO_4. Sterile nutritional requirements of auxotrophs were added as necessary. Strains which are *glmS* require supplementation of both minimal and rich media with 0.5 mg of glucosamine per ml. *sn*-Glycerol 3-phosphate was added at 0.4 mg/ml; streptomycin at 100 μg/ml, kanamycin at 80 μg/ml, or chloramphenicol at 12 μg/ml was added for antibiotic selection. MOPS medium was supplemented with 2 μM $ZnSO_4$. MacConkey agar was made according to Miller (17).

MOPS salts, *sn*-glycerol 3-phosphate, salicin, antibiotics, and nutritional supplements were sterilized by filtration. Arsenate resistance was measured by following growth in media containing 1 mM KH_2PO_4 and 10 mM Na_2HAsO_4.

Alkaline phosphatase assays. Alkaline phosphatase was assayed in intact cells, using 10 mM *p*-nitrophenolphosphate (Sigma Chemical Co.) as the substrate in 0.6 M Tris buffer at pH 8.2 (11). The activity is expressed as micromoles of *p*-nitrophenol liberated per minute per milligram (dry weight) of cells at 37°C.

To screen colonies on plates for the presence of alkaline phosphatase, the plates were sprayed with 1 M Tris buffer, pH 8.2, containing 10 mg of *p*-nitrophenolphosphate per ml.

Transduction. P1 *vir* or P1 *kc* transductions were carried out as described by Miller (17). For lysates of strain DC104.1, thermoinducible lysogens of P1 *clr*100 *Cm* were used (17). For transductions requiring expression of the selective marker, such as antibiotic resistance, the mixture of cells and lysate was incubated for 45 min at 37°C before spreading on selective media. Transductants were purified at least twice by single-colony isolation.

Lambda transduction was done by using thermally inducible λ *c*I857 *S*7 bacteriophage according to Miller (17). To reduce the viscosity of the lysate, 1 μg of DNase per ml was added, and the lysate was left at room temperature for 5 min before the lysate was sterilized with chloroform.

RESULTS

Isogenic series of Pst system mutants: *pit*$^+$ and *pit*. Strain W3110 *trpR* is F$^-$ and λ sensitive, allowing construction of diploids by using F′ factors and λ transducing bacteriophage. Mutations *phoT35*, *phoS25*(Am), and *pst-2* (from mutant strains C4, C31, and GS5, respectively), as well as the wild-type Pst region from strain K10 for comparison, were introduced into strain W3110.1.1 by P1 transduction, selecting for Ilv$^+$, in the presence of glycerol 3-phosphate in order not to select against bacteria which were incapable of P_i transport. The transductants were scored for inability to metabolize salicin (Table 3). Three-factor analysis of this cross (Table 3) located all three Pst system genes between *bgl* and *ilv*.

Attempts to transduce *pit* into derivatives of strain W3110 *trpR* failed. Therefore, a series of Pst system mutants was similarly constructed by using a derivative (GO) of the existing *pit* strain, GS5, as the background (Table 4).

Ordering of the Pst genes by reciprocal

TABLE 1. *Bacterial strains*

Strain	Genotype	Source or reference
W3110	F⁻ *trpR*	C. Yanofsky (1)
W3110.1	F⁻ *trpR ilv bglR*[a] *tna*	C. Yanofsky
W3110.1.1	F⁻ *trpR ilv bglR rpsL tna*	Spontaneous mutant of W3110.1
WC4	F⁻ *trpR rpsL phoT35*	This work
WC4.1	F⁻ *trpR rpsL phoT35 ilv*::Tn*5 glmS*	This work
WC4.2	F⁻ *trpR rpsL phoT35 ilv*::Tn*5 glmS* (λ d*glm*)	This work
WC4.3	F⁻ *trpR rpsL phoT35 ilv*::Tn*5 glmS* (λ *asn*)	This work
WC31	F⁻ *trpR rpsL phoS25* (Am)	This work
WC31.1	F⁻ *trp rpsL phoS25* (Am) *ilv*::Tn*5 glmS*	This work
WC31.2	F⁻ *trpR rpsL phoS25* (Am) *ilv*::Tn*5 glmS* (λ d*glm*)	This work
WK10	F⁻ *trpR rpsL*	This work
WK10.1	F⁻ *trpR rpsL ilv*:: Tn*5 glmS*	This work
WK10.2	F⁻ *trp rpsL ilv*::Tn*5 glmS* (λ d*glm*)	This work
WGS5	F⁻ *trpR rpsL pst-2*	This work
WGS5.1	F⁻ *trpR rpsL pst-2 ilv*::Tn*5 glmS*	This work
WGS5.2	F⁻ *trpR rpsL pst-2 ilv*::Tn*5 glmS* (λ d*glm*)	This work
DC104	F⁻ *glmS thi rbs ara bglR gyrA* Δ *lac* λ^s	J. Felton
DC104.1	F⁻ *glmS ilv*::Tn*5 thi rbs ara bglR gyrA* Δ *lac* λ^s	This work
JG85	F'197 *metE*⁺/*metE70 recA56 rha-2* λ⁻	B. Bachmann (12)
JG85.1	F'197 *bglR metE*⁺/*bglR*⁺ *metE70 recA56 rha-2* λ⁻	J. Felton
K10[b]	Hfr *pit-1 rel tonA*	Our collection (4)
C4	Hfr *pit-1 phoT35 rel tonA*	Our collection (7)
C4.1	Hfr *pit-1 phoT35 rel tonA bglR*	This work
C31	Hfr *pit-1 phoS25* (Am) *rel tonA*	Our collection (7)
C31.1	Hfr *pit-1 phoS25* (Am) *rel tonA bglR*	This work
GS5	F⁻ *pit-1 pst-2 his-53 proC24 pyrF30 thyA25 metBl gyrA12 rps L97 tsx-63?*	B. Bachmann (22)
JF217	F⁻ *rbs ara bglR thi glmS gyrA* Δ*lac* (φ80 *h*) (λ d*glm*) (λ Y199)	J. Felton
BH217	F⁻ *rbs ara bglR thi glmS gyrA* Δ*lac* (λ d*glm*) (λ *c*I857 *S7*)	J. Felton
KL728	F' KL728 *pyrE malB met*⁺/*metBl argG6 rpsL104 tonA2 his-1 leu-6 mtl-2 xyl-7 recAl supE44 malAl gal-6 lacYl tsx-1* λ⁻	K. B. Low, KLF11/JC1553 (12)
GS1	F' KLF10/*araD139 ilv*::Tn*5* Δ*lacU169 rpsL97 tsx-63? psi relA* Δ*malB101*	L. Guarente
GO	F⁻ *thyA25 pit-1 metB1 gyrA12 rpsL97 tsx-63? bglR ilv*	This work
GOT4	F⁻ *thyA25 pit-1 metBl gyrA12 rpsL97 tsx-63? bglR phoT35*	This work
GOT4.1	F⁻ *thyA25 pit-1 metBl gyrA12 rpsL97 tsx-63? bglR phoT35 ilv*::Tn*5*	This work
GOS31	F⁻ *thyA25 pit-1 metBl gyrA12 rpsL97 tsx-63? bglR phoS25* (Am)	This work
GOS31.1	F⁻ *thyA25 pit-1 metB1 gyrA12 rpsL97 tsx-63? bglR phoS25* (Am) *ilv*::Tn*5*	This work
GOST5	F⁻ *thyA25 pit-1 metBl gyrA12 rpsL97 tsx-63? bglR pst-2*	This work
GOPST5.1	F⁻ *thyA25 pit-1 metBl gyrA12 rpsL97 tsx-63? bglR pst-2 ilv*::Tn*5*	This work
GOPST5R	F⁻ *thyA25 metBl gyrA12 rpsL97 tsx-63? bglR pst-2*	This work
GS5W1	F⁻ *proC pyrF his thyA25 pit-1 metBl gyrA12 rpsL97 tsx-63?*	This work
GS5W2	F⁻ *proC pyrF his thyA25 pit-1 metBl gyrA12 rpsL97 tsx-63?*	This work
GS5R1	F⁻ *proC pyrF his thyA25 pst-2 metBl gyrA12 rpsL97 tsx-63?*	This work
KY7485	*asn thi rif* (λ *asn*) (λ *c*I857 *S7*)	T. Miki (17)

[a] The wild-type *bglR*⁺ is phenotypically Bgl⁻ (incapable of utilizing salicin as a carbon source), whereas *bglR* mutants are phenotypically Bgl⁺.

[b] Strains K10, C4, and C31 and their derivatives are resistant to phage T2, for which no map position has been assigned.

TABLE 2. *Bacteriophages*

Phage	Source or reference
Lambda	
dglm	J. Felton
c60	M. Fox
asn	T. Miki (17)
vir	B. Wanner (18)
Y199	J. Felton (10)
*c*I857 *S7*	J. Felton
P1	
vir	C. Pratt (18)
kc	B. Wanner (18)
*clr*100 *Cm*	B. Wanner (18)

TABLE 3. *Transduction of Pst system mutations into a pit⁺ strain*

Donor	Ilv⁺ transductant[a]			Name of transductant
	Total	APC[b] (%)	Bgl⁺ (%)	
C31 *phoS25*(Am)	69	64	45	WC31
C4 *phoT35*	70	38	33	WC4
GS5 *pst-2*	44	57	33	WGS5
K10 (wild type)	68	0	32	WK10

[a] The recipient in these crosses was W3110.1.1 *pit⁺ rpsL ilv bglR trpR tna*. Transductants were selected and purified as Ilv⁺ on MOPS-glucose-1 mM KH_2PO_4-glycerol 3-phosphate-streptomycin plates and then replicated onto MacConkey salicin medium to score Bgl and on MOPS-glucose-1 mM KH_2PO_4 with and without glycerol 3-phosphate and MOPS-glucose-0.07 mM KH_2PO_4 for screening of alkaline phosphatase and utilization of P_i.

[b] Constitutivity for alkaline phosphatase synthesis. Percentages given are fraction of total Ilv⁺ transductants.

crosses. To establish the order of *phoS25*(Am), *phoT35*, and *pst-2*, four-factor crosses were carried out, using P1 lysates of the mutant strains C31, C4, and GS5 as the donors and *ilv*::Tn*5* derivatives of the *pit* series of isogenic Pst system mutant strains GOS31, GOT4, and GOPST5 as the recipients. In each cross, *ilv⁺* transductants were selected and purified, and those retaining the recipient *bgl* allele, *bglR*, were scored for alkaline phosphatase constitutivity. In this manner, a crossover between *ilv* and *bgl* was required. Pairs of the various Pst mutants were recombined in reciprocal crosses and screened for wild-type alkaline phosphatase regulation. One cross of each pair would require a double crossover to form wild-type recombinants, whereas the reciprocal cross would require a quadruple crossover. Thus, by comparing the frequencies of wild type in each reciprocal pair of crosses, it is possible to order the Pst system mutations.

Table 5 establishes that *phoS25*(Am) lies between *ilv* and *pst* and that *phoT35* lies between *pst-2* and *bgl*, very close to *pst-2* (Fig. 1).

Complementation of Pst system heterozygotes. Attempts to determine the dominance behavior of the mutations in the Pst region by constructing heterozygous diploid strains, using F′ plasmids KL728 and F′197, showed strong selection against the diploid; therefore, complementation tests were performed with two specialized lambda transducing phages carrying the Pst region of the chromosome. The first of these, λ d*glm*, was derived from a prophage integrated in the *bgl* operon and carries the region between *blgR* and *glmS* (J. Felton, unpublished data). By using a lysate of BH217, lysogens carrying this phage were constructed in *glmS* derivatives of the W3110 *trpR pit⁺* series of Pst system mutants. Complementation of the mutations constitutive for alkaline phosphatase was measured by assaying for alkaline phosphatase specific activity (Table 6). Alkaline phosphatase synthesis was repressed in high-phosphate medium in the *phoS* and *pst* transductants, suggesting that the Pst system genes on the transducing phage complemented these mutant alleles. The *phoT* lysogens (C4.2), however, were not repressible. Seventeen additional lysogens of WC4.1, made by using a lysate of the same bacteriophage from strain JF217, were not complemented either. To determine whether this phage did not carry *phoT35*, we used a second transducing bacteriophage, λ *asn* (Fig. 1), isolated from a lysogen with λ inserted in *bglB* and carrying bacterial chromosomal genes extending to *asn* (16). By using a lysate of strain KY7485, strain WC4.1 was lysogenized with this phage as above. Two isolates were obtained (WC4.3),

TABLE 4. *Transduction of Pst system mutations into pit strain GO*

Donor	Ilv⁺ transductant[a]		Name of transductant
	Total	APC[b] (%)	
C31.1 *phoS25*(Am)	19	68	GOS31
C4.1 *phoT35*	18	39	GOT4
GS5 *pst-2*	37	46	GOPST5

[a] Ilv⁺ transductants selected and purified on MOPS-glucose-1 mM KH_2PO_4-glycerol 3-phosphate-thymine-methionine-thiamine-streptomycin plates and then scored for ability to utilize P_i on MOPS-glucose-1 mM KH_2PO_4-thymine-methonine-thiamine-streptomycin and for alkaline phosphatase constitutivity on MOPS-glucose-1 mM KH_2PO_4-glycerol 3-phosphate-thymine-methionine-thiamine-streptomycin plates by plate spray assay with *p*-nitrophenolphosphate.

[b] See Table 3.

TABLE 5. *Four-factor crosses between ilv and bglR*

Donor	Recipient	Ilv^{+a}	Unselected phenotype[b]	% of Ilv$^+$ transductants with designated phenotype
GS5	GOS31.1	424	Bgl$^-$ AP$^+$	0
ilv$^+$	*ilv*		Bgl$^-$ APC	2.8
pst-2	*phoS25*(Am)		Bgl$^+$ AP$^+$	4.7
bglR$^+$	*bglR*		Bgl$^+$ APC	92.5
C31	GOPST5.1	424	Bgl$^-$ AP$^+$	0
ilv$^+$	*ilv*		Bgl$^-$ APC	35.4
phoS25(Am)	*pst-2*		Bgl$^+$ AP$^+$	0
bglR$^+$	*bglR*		Bgl$^+$ APC	64.6
C4	GOPST5.1	824	Bgl$^-$ AP$^+$	0
ilv$^+$	*ilv*		Bgl$^-$ APC	18.0
phoT35	*pst-2*		Bgl$^+$ AP$^+$	0.2
bglR$^+$	*bglR*		Bgl$^+$ APC	81.8
GS5	GOT4.1	824	Bgl$^-$ AP$^+$	0
ilv$^+$	*ilv*		Bgl$^-$ APC	5.5
pst-2	*phoT35*		Bgl$^+$ AP$^+$	0
bglR$^+$	*bglR*		Bgl$^+$ APC	94.5
C4	GOS31.1	104	Bgl$^-$ AP$^+$	0
ilv$^+$	*ilv*		Bgl$^-$ APC	4.8
phoT35	*phoS25*(Am)		Bgl$^+$ AP$^+$	6.7
bglR$^+$	*bglR*		Bgl$^+$ APC	88.5
C31	GOT4.1	104	Bgl$^-$ AP$^+$	0
ilv$^+$	*ilv*		Bgl$^-$ APC	25
phoS25(Am)	*phoT35*		Bgl$^+$ AP$^+$	0
bglR$^+$	*bglR*		Bgl$^+$ APC	75

[a] P1 transduction of *ilv*::Tn*5 bglR* to *ilv*$^+$ by selecting Ilv$^+$ recombinants (from the donor) and then screening for Bgl$^+$ (the recipient phenotype). Transductants were selected and purified on MOPS-glucose-glycerol 3-phosphate-thymine-methionine medium and then screened for Bgl phenotype on MacConkey salicin agar and for alkaline phosphate phenotype by plate spray assay.

[b] AP$^+$ denotes wild type repressible for alkaline phosphatase; APC denotes constitutive phenotype.

TABLE 6. *Activities of alkaline phosphatase in Pst system transductants*

Strain	Genotype	λ phage	No. of lysogens tested	Sp act[a]		Induction ratio[b]
				High phosphate	Low phosphate	
WK10.2	Wild type	*dglm*	15	0.012 ± .006	2.40 ± .47	197
WC4.2	*phoT35*	*dglm*	15	1.39 ± .22	1.74 ± .43	1.25
WC31.2	*phoS25*(Am)	*dglm*	15	0.023 ± .013	1.17 ± .23	52
WGS5.2	*pst-2*	*dglm*	12	0.023 ± .016	1.29 ± .14	57
WC4.3	*phoT35*	*asn*	2	0.001 ± 0	0.48 ± .25	480

[a] Expressed in micromoles of nitrophenol liberated per minute per milligram of cells; mean ± standard deviation.

[b] Induction ratio is obtained by dividing the specific activity in low-phosphate by that in high-phosphate medium. Cultures in high-phosphate medium (MOPS-glucose-1 mM KH_2PO_4-isoleucine-valine) were grown overnight and limited by glucose starvation to an optical density at 540 nm of 0.5. Cultures in low-phosphate medium (MOPS-glucose-0.07 mM KH_2PO_4-isoleucine-valine) were grown for 40 h to phosphate starvation. Growth was at 30°C to maintain the phage.

both of which were repressible for alkaline phosphatase synthesis (Table 6). Both isolates were immune to λ *c60* and sensitive to λ *vir*, proving that both were lysogens. Spontaneous loss of the prophage resulting in alkaline phosphatase constitutivity occurred in both isolates at a frequency of 3% in overnight cultures, whereas curing the prophage by growth at 37°C resulted

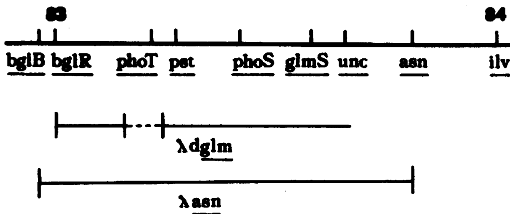

FIG. 1. *Structure of the bgl-ilv region of the chromosome. Order of genes between bglR (83 min) and ilv (84 min) (2) as determined by three- and four-factor crosses. Distances are not precisely to scale. The two phages used in the complementation test and the region of the chromosome they carry (J. Felton, personal communication; 15, 16) are also presented.*

in 99.4% of the bacteria in both isolates becoming constitutive for alkaline phosphatase synthesis. This locates the *phoT*$^+$ allele in these lysogens on the bacteriophage. Thus, bacteriophage λ *asn* complements the *phoT35* mutation, and the *phoT*$^+$ allele is dominant. It follows that λ d*glm* must carry a small deletion of the *phoT* gene.

Growth rate of Pst system mutants. All of the mutations in the W3110 *trpR* background were capable of growth with P_i as the phosphate source, as expected because of the functional Pit system in these strains. Growth rates of the *pit*$^+$ Pst system mutants in low- and high-phosphate media indicated no significant difference between strains and no reduction of growth rate in low-phosphate medium (data not presented).

The isogenic *pit* strains were tested for growth rates in low (0.07 mM)- and high (1 mM)-P_i media (Table 7). None of the mutant strains was particularly sensitive to the phosphate concentrations used; however, a large variation of growth rate was evident among the strains. The growth rate of GOS31 [*phoS25*(Am)] was almost 50% reduced compared with the wild type; this may have been due to the inability of the *phoS* mutant to synthesize the phosphate-binding protein. The *pit pst* double mutant, GOPST5, could not grow in the absence of organic phosphates; in medium supplemented with glycerol 3-phosphate it still exhibited slower growth than did the wild-type strain. These results were expected since GOPST5 duplicates the *pit pst* character of its ancestor, GS5. This confirms the identification of P_i-utilizing revertant GS5W1 as *pit pst*$^+$ and the validity of using strain GO as a background completely dependent on the Pst system for construction of isogenic Pst mutant strains. The results also confirm the requirement for *pst*$^+$ in the Pst system. The *pit phoT35* mutant, however, was able to utilize P_i, as was the *pit phoS25*(Am) mutant at a reduced rate, indicating that these genes play a different role in phosphate transport than does *pst*.

Alkaline phosphatase activities of Pst system mutants. Transductants of each genotype were assayed for induced level of alkaline phosphatase after starvation for phosphate and for repressed level after starvation for glucose in excess phosphate. The Pst system mutants in the *pit*$^+$ background (W3110 *trpR*) were fully constitutive (data not shown), including the *pst* strain (WGS5) (Table 8), whereas the *pst*$^+$ strain exhibited repression of over 200-fold. This is similar to the behavior of the *pit* donor strains K-10, C4, and C31, but not *pst pit* strain GS5, which was partially repressible.

The *pit* strains GO, GOS31, GOT4, and GOPST5 were assayed for alkaline phosphatase-specific activity and compared with parental strain GS5 and P_i-utilizing revertants GS5W1 and GS5W2 (Table 8). The regulation of alkaline phosphatase in the mutants remained the same

TABLE 7. *Growth rates of Pst system mutants in a pit⁻ background*

Strain	Genotype	Doubling time (min)	
		Low phosphate[a]	High phosphate[b]
GO	*pit-1*	75	77
GOS31	*pit-1 phoS25*(Am)	116	104
GOT4	*pit-1 phoT35*	79	80
GOPST5	*pit-1 pst-2*	>1,200	>1,200
GOPST5[c]	*pit-1 pst-2*	97	98

[a] MOPS-glucose–0.07 mM KH_2PO_4–thymine–methionine–thiamine.

[b] MOPS-glucose–1 mM KH_2PO_4–thymine–methionine–thiamine.

[c] Growth rate of strain GOPST5 supplemented with glycerol 3-phosphate (0.4 mg/ml).

TABLE 8. *Alkaline phosphatase activities of Pst system mutations in a pit⁻ background*

Strain	Genotype	Alkaline phosphatase activity[a]	
		Low phosphate	High phosphate
GO	*pit-1*	0.45	0.0028
GOS31	*pit-1 phoS25*(Am)	0.45	0.61
GOT4	*pit-1 phoT35*	0.45	0.62
GOPST5[b]	*pit-1 pst-2*	0.47	0.19
GS5[b]	*pit-1 pst-2*	0.24	0.13
GS5W1	*pit-1*	0.59	0.0026
GS5W2	*pit-1*	0.67	0.0026
GOPST5R	*pst-2*[c]	0.10	0.15
GS5R1	*pst-2*[c]	0.16	0.17
WGS5	*pst-2*	0.36	0.66

[a] Expressed as in Table 6.

[b] Medium supplemented with glycerol 3-phosphate. (0.4 mg/ml).

[c] Revertant of *pst-2 pit-1* to P_i^+ at undetermined site (see text).

upon transduction into the *pit* background (GS5). The *pit pst* double mutants exhibited somewhat reduced activity in high-phosphate medium, indicating partial repressibility similar to that found in the parent strain GS5. To determine whether this reduction in alkaline phosphatase activity was due to the addition of glycerol 3-phosphate to the medium for *pit pst* strains, second-site revertants to P_i utilization which retained constitutivity for alkaline phosphatase synthesis (GS5R1 and GOPST5R) were assayed in media containing no glycerol 3-phosphate. The site of this reversion was not determined. These revertants exhibited constitutive synthesis at the lower level characteristic of the repressed *pit pst* strains. These results demonstrate the independence of regulatory effects on alkaline phosphatase from utilization of P_i.

DISCUSSION

In the three-factor cross (Table 3), all Bgl^- transductants (unable to utilize salicin) were constitutive for alkaline phosphatase synthesis, whereas the constitutive transductants were both Bgl^+ and Bgl^-, indicating that *pst-2*, *phoT35*, and *phoS25*(Am) all lie between *ilv* and *bglR*. The three-factor cross carried out to construct *glmS* derivatives of the Pst system mutants similarly indicated that *glmS* lies between *ilv* and the Pst system genes (data not shown). The results of the four-factor crosses (Table 5) suggest the order *bgl phoT35 pst-2 phoS25*(Am) *glmS ilv*. This order is consistent with the frequencies of cotransduction observed in the strain constructions detailed herein and is in agreement with the work of other researchers (T. Miki, personal communication; J. Felton and A. Wright, personal communication; 15).

Use of the transducing bacteriophage λ d*glm* showed that the *phoS25*(Am) and *pst-2* mutants were recessive to the wild-type alleles in terms of alkaline phosphatase regulation, but the *phoT35* mutation was not complemented by this bacteriophage, which may thus carry a small deletion. Transducing phage λ *asn* did complement the *phoT35* mutant, however. Since the λ d*glm* phage complemented the *pst-2* mutation without complementing the *phoT35* mutation, the two mutations appear to lie in different complementation groups. Although it is possible that this is a spurious result caused by insertion of the phage within the mutant gene, it is consistent with the functional distinction seen between these two alleles in the *pit* background (Table 7), in which only the *pst* mutation caused loss of P_i transport. Thus the *pst-2* and *phoT35* mutations appear to represent two different genes. The one *pst* and several *phoT* mutations not examined herein may actually comprise any number of genes, and it has been suggested that *phoS* mutations lie in the same gene as the *pst*-like *phoT* mutations (12).

All the pit^+ strains can utilize P_i and are arsenate sensitive, as expected. The *pit phoS25*(Am) strain can also utilize P_i, although at a reduced rate, whereas the *pit pst-2* strain cannot, as expected from previous reports (19, 20, 24). However, strain *pit phoT35* utilizes P_i, contrary to published reports that *pit phoT* double mutants cannot (23). This delineates a requirement for the *pst* gene function in uptake of P_i, but the function missing in *phoT35* is apparently not required or can be bypassed in this genetic background. No effect of P_i concentration on growth rate was observed in any of the strains examined in this work. This contrasts with the published report of reduced growth rate in low-P_i medium exhibited by *phoT* mutants (23). These discrepancies may be due in part to the *phoT35* mutation being in a different gene from the mutations previously examined (19, 23).

The *phoT35* and *phoS25*(Am) mutant strains are constitutive for alkaline phosphatase synthesis, but the *pst-2* mutation exhibits three different phenotypes, depending on the strain background: full constitutivity in the pit^+ (W3110 *trpR*) background; partial repressibility in the *pit* strain (GS5); and constitutivity at this lower level in the revertants of GS5 utilizing P_i. The partial repression in the *pit pst* strain unable to utilize P_i must be independent of phosphate uptake, confirming (23) that the cause of induction of alkaline phosphatase synthesis is not depletion of the internal P_i pool of the cell.

The full constitutivity of the *pst* mutants in the pit^+ background (WGS5) eliminates any physiological differences from the *pst*-like *phoT* mutants. From the work described in this paper and the results described in the literature (19, 21, 23), it is possible to assign the Pst system mutants to four classes. The first class comprises *phoS* mutants C31, C48, C61, C78, C86, C72, and C76, which lack the phosphate-binding protein (7). The second class comprises eight *pst*-like *phoT* mutants (C10, C25, C64, C84, C90, C91, C101, C112), all of which exhibit high level of constitutivity as well as loss of Pst system function. The third class comprises two *pst* mutants (GS5 and UR1) exhibiting partial constitutivity in a *pit* background but full constitutivity in a pit^+ background and also loss of Pst system function. These classes may represent alleles of the same gene or two or more genes. Class 4, comprising only one mutant (C4) among those analyzed, represents the *phoT* gene, constitutive at a high level for synthesis of alkaline phosphatase, retaining Pst system function, and geneti-

cally distinguishable from at least two other Pst system mutants, as shown herein.

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