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Genetic Analysis of Mutants Affected in the Pst Inorganic Phosphate Transport System

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A number of mutant alleles affecting the Pst phosphate transport system have been divided into three complementation groups on the basis of constitutive alkaline phosphatase activity in appropriate partial diploid strains. The three complementation groups were represented by the alleles *pstA2* and *phoT32* and the newly described allele *pstB401*. The two alleles *phoS28* and *phoS21* appeared to be polar. The *phoS28* allele affected both the *phoT* and *pstB* genes but not the *pstA* gene, whereas the *phoS21* allele appeared to be a mutation in the *pstA* gene exerting polar effects on both the *pstB* and *phoT* genes. It was concluded that the three genes *pstA*, *pstB*, and *phoT* were part of an operon and that the phosphate-binding protein was not coded for by any of these genes. The *phoS* gene, defined as the structural gene for the phosphate-binding protein, is also part of the operon, but the *phoS28* and *phoS21* alleles are not mutations in the *phoS* gene and were reclassified as *pho-28* and *pho-21* alleles. The gene order was concluded to be *pstA*-(*pstB*-*phoT*)-*phoS*, with the *pstA* gene promotor proximal and the direction of transcription opposite to that of the nearby *unc* operon.

The discovery of the repression by P_i of the synthesis of alkaline phosphatase in *Escherichia coli* (18, 35) opened up an extensive inquiry into the mechanism of this regulatory process. Echols et al. (11) reported that two regions on the *E. coli* genome, R1 and R2, are involved. Mutations in the latter region resulted in the constitutive synthesis of alkaline phosphatase during growth on media containing high concentrations of phosphate. The R2 region was further subdivided into R2a and R2b by Garen and Otsuji (12), who also isolated the protein coded for by the R2a gene. The region of the genome including the R2a and R2b genes was later named *phoS* (1). Medveczky and Rosenberg (23) isolated, from the periplasmic space of *E. coli*, a phosphate-binding protein which was later shown to be identical with the product of the R2a gene (13). Its involvement in phosphate transport was demonstrated by its restoration of P_i transport in spheroplasts (14). Unexpectedly, the protein was only slightly effective in restoring transport to the spheroplasts of a number of R2a mutants.

Willsky et al. (36) retained the mnemonic designation *phoS* for the gene coding for the binding protein (R2a) and designated the R2b gene as *phoT*. Mutations in the *phoT* gene caused alkaline phosphatase to be produced constitutively, but did not affect the binding protein. A third class of mutants (*pst*) was also described (36). This mutation was closely linked to *phoS* and *phoT* and was similar to *phoT* in that it did not affect the binding protein but differed from it in the level of constitutive alkaline phosphatase produced. All three alleles affected the phosphate transport system termed Pst (phosphate-specific transport) as distinct from another system, termed Pit (P_i transport). The mutation affecting the Pit system mapped in a different part of the chromosome and was present in derivatives of strain K10 (36). It should be noted that all of the strains used in the original work on alkaline phosphatase regulation (11, 12, 18, 35) were derivatives of *E. coli* K10.

The status of the three genes *phoT*, *phoS*, and *pst* has not been fully resolved. Levitz et al. (21) reported that complementation tests between *phoS* and *phoT* mutant alleles placed them in the same cistron. Zuckier and Torriani (38), on the other hand, obtained evidence that the *phoT35* allele represents a different complementation group than that of *phoS25* or *pst-2*.

Phosphate deprivation of *E. coli* results in the production of three periplasmic proteins in addition to alkaline phosphatase (24). Of these, one has been identified as the phosphate-binding protein (37); another was shown (2) to be a high-affinity binding protein for *sn*-glycerol-3-phosphate. The function of the third protein is still

unknown. In addition, phosphate-starved *E. coli* cells produce the outer membrane protein e (E, Ic), the synthesis of which appears to be coregulated with that of the four periplasmic proteins (3, 34). Thus, mutations affecting the Pst system result in the constitutive synthesis of protein e and the four periplasmic proteins even during growth in excess P_i.

In the present work, a genetic analysis of a number of mutant alleles affecting the Pst phosphate transport system is carried out, and a tentative gene order is proposed.

MATERIALS AND METHODS

Enzymes. Restriction endonucleases and T4 DNA ligase were obtained from New England Biolabs, Beverly, Mass.

Bacterial strains and plasmids. All of the bacterial strains used were derived from *E. coli* K-12 and are described in Table 1 or in the text. Plasmids used are described in Table 1, Fig. 3, and the text. Plasmid DNA was prepared as described by Selker et al. (31).

Genetic techniques. The techniques used for genetic experiments were as outlined previously (15, 17). Transformations were carried out as described by Lederberg and Cohen (20).

TABLE 1. *Strains of E. coli and plasmids used*

Bacterial strain or plasmid	Relevant genotype[a] and phenotype	Source/reference
NK5304	*srl-1300*::Tn*10 recA56*	Obtained from D. Botstein and N. Kleckner (Massachusetts Institute of Technology)
AN346	*ilvC argH pyrE entA*	5
AN732	*argH pyrE entA metE*	9
G102	*argH pyrE entA metE unc-449 pstB401*	Isolated from strain AN732 after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine
AN1117	*unc-449 pstB401 argH pyrE entA*	Isolated after transduction of strain AN346 with strain G102 as donor
AN1088	*pit argH entA*	30
AN710	*phoT32 argH entA*	29
AN1237	*pAN26/argH pyrE purE recA gyrA*	
AN1703	*pAN112/ilvC pyrE argH purE recA gyrA*	
AN1704	pAN113/*ilvC pyrE argH purE recA gyrA*	
AN1702	pAN111/*ilvC pyrE argH purE recA gyrA*	
AN1708	pAN119/*ilvC pyrE argH purE recA gyrA*	
AN1403	*unc-449 pstB401 argH pyrE entA srl::Tn10 recA*	Isolated after transduction of strain AN1117 with strain NK5304 as donor
AN1697	*phoS28 argH pyrE entA srl::Tn10 recA*	Isolated after successive transductions of strain AN346 with strains C78[b] and NK5304 as donors
AN1698	*phoS21 argH pyrE entA srl::Tn10 recA*	Isolated after successive transductions of strain AN346 with strains C86[b] and NK5304 as donors
AN1696	*phoT32 argH pyrE entA srl::Tn10 recA*	Isolated after successive transductions of strain AN346 with strains AN710 and NK5304 as donors
AN1685	*pstA2 argH pyrE entA srl::Tn10 recA*	Isolated after successive transductions of strain AN346 with strains 5506[b] and NK5304 as donors
AN1664	*ilvC argH pyrE entA srl::Tn10 recA*	Isolated after transduction of strain AN346 with strain NK5304 as donor
CGSC 5506	*pit-1 pstA2 glpR2 glpD3 phoA8 relA1 tonA22* $T2^r$	
AN1861	pAN127/*phoS28 argH pyrE entA srl::Tn10 recA*	Isolated after transformation of strain AN1697 with pAN127
pAN26	*pyrE*$^+$ *unc-449 pstB401 ilvC*$^+$ *argH*$^+$	F-plasmid isolated as described previously (26)
pAN112	*pyrE*$^+$ *phoS28 ilvC*$^+$ *argH*$^+$	F-plasmid isolated as described previously (16)
pAN113	*pyrE*$^+$ *phoS21 ilvC*$^+$ *argH*$^+$	F-plasmid isolated as described previously (16)
pAN111	*pyrE*$^+$ *phoT32 ilvC*$^+$ *argH*$^+$	F-plasmid isolated as described previously (16)
pAN119	*pyrE*$^+$ *pstA2 ilvC*$^+$ *argH*$^+$	F-plasmid isolated as described previously (16)
pAN36	Cm^r Tc^s *uncDC*$^+$ *phoS*$^+$ *phoT*$^+$ *pstB*$^+$ *pstA*$^+$	10

[a] Chromosomal gene nomenclature according to Bachmann and Low (4); plasmid gene nomenclature according to Novick et al. (25).

[b] Strains obtained from the Coli Genetic Stock Center.

Media and growth of organisms. The mineral salts minimal medium and additions used were as described previously (16). The low P_i medium used was that described by Gerdes and Rosenberg (13).

Cells used for the preparation of membrane and periplasmic-cytoplasmic fractions were grown in 14-liter fermentors (New Brunswick Scientific Co., New Brunswick, N.J.) essentially as described previously (8).

Cell fractionation. The procedure used for preparation of cell membranes was as described previously (32). The periplasmic-cytoplasmic fraction was the supernatant obtained after the ultracentrifugation of the cell membranes. The membranes were washed in low-ionic-strength buffer in the presence of *p*-aminobenzamidine, as described previously (32).

Measurement of phosphate uptake. Cells grown to stationary phase were washed twice in a phosphate-free medium containing 0.05 M triethanolamine hydrochloride; 0.015 M KCl; 0.01 M $(NH_4)_2SO_4$; and 0.001 M $MgSO_4$, pH 6.5. The cells were suspended in the same medium at an absorbance (A_{660}) of 0.35, supplemented with 0.002 M glucose and other nutritional supplements, and shaken for 2 h at 37°C. They were then centrifuged and resuspended in the same medium with glucose added to 0.01 M. The cells were stored for up to 3 h at 4°C until required. Samples (2.5 ml) were shaken for 5 min at 37°C to equilibrate, and $^{32}P_i$ was added to the appropriate concentration (see Table 2, footnote *a*). Samples (0.5 ml) of the suspension were transferred at intervals onto membrane filters and washed automatically in an apparatus described elsewhere (28). Rates were expressed in terms of nanomoles of P_i per milligram of dry weight per minute, using the experimentally derived factor A_{660} of 1.0 ≡ 0.43 mg of dry weight per ml. Assay of transport specifically through the Pst system in strains carrying both the Pst and the Pit system was carried out at 0.5 μM external $^{32}P_i$. At this concentration, the Pst system (K_m = 0.2 μM) could be satisfactorily assayed without interference from the Pit system (K_m = 25 μM), which, at 0.5 μM P_i, retained less than 3% of its normal activity as measured at 50 μM P_i.

Analysis of plasmid DNA. Plasmid DNA was digested by the appropriate restriction endonuclease, and DNA fragment sizes were determined as described previously (9).

Polyacrylamide gel electrophoresis. Proteins were separated by two-dimensional gel electrophoresis as described previously (26, 27) and were stained with Coomassie blue R250.

Assay of alkaline phosphatase. Samples of the periplasmic-cytoplasmic fractions containing about 0.2 mg of protein were incubated at 30°C in 0.16 M Tris-hydrochloride buffer, pH 9.0, containing 1 mM zinc acetate and 20 mM *p*-nitrophenyl phosphate. Samples (0.5 ml) were transferred at 1-min intervals to 4.5 ml of 2 M NaOH, and absorbance was measured at 410 nm.

Determination of protein. Protein concentrations were determined by using the Folin phenol reagent (22), with bovine serum albumin (fraction V; Sigma Chemical Co., St. Louis, Mo.) as the standard.

Assay of Mg-ATPase. Assays for Mg-ATPase activity were carried out as described previously (16).

RESULTS

Characterization of the *pst-401* allele. A mutant strain carrying a mutation in an *unc* gene was isolated after mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (7). The mutation was transduced with the *ilv* genes into strain AN346 to give strain AN1117. A preliminary characterization of strain AN1117 indicated that membranes lacked ATPase activity but that ATPase activity was present in the periplasmic-cytoplasmic fraction. However, this activity was not inhibited by antibody prepared against F_1-ATPase, even though cytoplasmic ATPase activity from another *unc* mutant strain was completely inhibited by the same amount of antibody. It appeared likely that the ATPase activity could have been due to alkaline phosphatase and that, in addition to the mutations in an *unc* gene, strain AN1117 carried a mutation in one of the genes involved in the Pst phosphate transport system (36). These genes map close to the *unc* genes on the *E. coli* chromosome, and mutations affecting the Pst phosphate transport system cause the derepression of a number of periplasmic proteins, including alkaline phosphatase.

Phosphate uptake rates in strain AN1117 were compared with those in strains AN1088 and AN710, which carried only the Pst or the Pit system, respectively (Table 2). It can be seen that strain AN1117 displayed an uptake of the same type as that of strain AN710, which carries only the Pit system and which operated satisfactorily at 50 μM P_i but not at 0.5 μM P_i. This is contrasted with strain AN1088, which carries the Pst system only and which retained, at 0.5 μM P_i, 55% of the rate it displayed at 50 μM P_i. It thus appeared that strain AN1117 did not possess a normally operative Pst system, and the mutant allele was designated *pst-401*.

Genetic complementation between the *pst-401* allele and other mutant alleles affecting the Pst system. A number of strains

TABLE 2. *Comparison of rates of phosphate uptake in strains AN1117, AN1088, and AN710*[a]

Strain	System present	Uptake (nmol of P_i per mg/min)	
		50 μM P_i	0.5 μM P_i
AN1117		72.7	3.1
AN710	Pit	35.0	1.0
AN1088	Pst	66.1	36.6

[a] Uptakes were measured as described in the text. Cell densities were at A_{660} = 0.4 with 50 μM $^{32}P_i$. With 0.5 μM $^{32}P_i$, a higher specific radioactivity and a cell density of A_{660} = 0.04 were used to measure initial rates. Suspensions were sampled at 15- and 10-s intervals, respectively.

carrying mutations affecting the Pst system and including the alleles *phoT32*, *phoS28*, *phoS21*, and *pst-2* were obtained from the Coli Genetic Stock Center (CGSC, New Haven, Conn.). Each of the alleles was transferred by transduction with the *ilv* genes into the female strain AN346, and *recA* derivatives of each were prepared as described previously (15). Each of the mutant alleles, as well as the *pst-401* allele, were transferred to F-plasmids by the method described previously (17). A complete set of partial diploid strains was prepared in which one mutant allele was on the plasmid and a different mutant allele was on the chromosome. Each of the partial diploid strains was grown in minimal medium (containing 10 mM P_i), and the cells were broken by passage through a Sorvall-Ribi cell fractionator (Ivan Sorvall, Inc., Norwalk, Conn.). After removal of the cell debris, the extract was fractionated by ultracentrifugation to give a membrane fraction and a periplasmic-cytoplasmic fraction. Complementation between the various mutant alleles was assumed to occur when the periplasmic-cytoplasmic fraction had little or no alkaline phosphatase activity.

There appeared to be three complementation groups represented by the alleles *pst-2*, *pst-401*, and *phoT32* (Table 3). On this basis, it is proposed to designate *pst-2* as *pstA2* and *pst-401* as *pstB401*. The *pstA2* allele may exert a low level of polarity, since a majority of the partial diploid strains carrying the *pstA2* allele had a low level of alkaline phosphatase activity (Table 3). There is little doubt, however, that the *pstA2* allele represents a distinct complementation group from the *pstB401* and *phoT32* alleles. The *phoS28* allele appears to be a polar mutant affecting both the *phoT* and *pstB* genes but not the *pstA* gene. The polar effect is not complete since some complementation of the *phoT32* and *pstB401* alleles does occur (Table 3). The *phoS21* allele appears to be a polar mutation in the *pstA* gene and to affect also the *phoT* and *pstB* genes (Table 3).

Analysis of cell fractions by two-dimensional gel electrophoresis. Phosphate starvation leads to the accumulation of four proteins in the periplasmic space of *E. coli* cells (see above). Each of these four proteins can be identified by their molecular weight on a two-dimensional gel electrophoretogram of the periplasmic-cytoplasmic fraction from normal *E. coli* cells grown under conditions of P_i starvation (Fig. 1). These four proteins were not present on a two-dimensional gel electrophoretogram of the periplasmic-cytoplasmic fraction from the same strain grown under conditions of excess P_i (Fig. 1). The identities of alkaline phosphatase and phosphate-binding protein were confirmed by coelectrophoresis with alkaline phosphatase obtained commercially (Worthington Diagnostics, Freehold, N.J.) and with a purified phosphate-binding protein (13), respectively. A similar pattern was obtained with the various Pst mutants, even when grown under conditions of excess P_i, with two exceptions: (i) an additional protein was formed with a slightly higher apparent isoelectric point and a lower molecular weight than those of alkaline phosphatase (Fig. 1), and (ii) the phosphate-binding protein was formed only at a low level in the strains carrying either the *phoS21* or the *phoS28* allele (see Fig. 1). No phosphate-binding protein of altered charge was detected in any of the mutants examined.

The additional protein near the alkaline phosphatase on the electrophoretograms appears to be a processed form of alkaline phosphatase itself. Both proteins were absent from the periplasmic-cytoplasmic fraction obtained from CGSC strain 5506, which carries a deletion of the *phoA* region of the *E. coli* chromosome (data not shown). The absence of this processed al-

TABLE 3. *Alkaline phosphatase activity in periplasmic-cytoplasmic fraction from partial diploid or haploid strains*

Recipient strains (mutant allele on chromosome)	Alkaline phosphatase activities of recipient strains	Activities (μmol of *p*-nitrophenol formed per min/mg of protein) of partial diploid strains: Donor strains (mutant allele on F-plasmid)				
		AN1237 (*pstB401*)	AN1703 (*phoS28*)	AN1704 (*phoS21*)	AN1702 (*phoT32*)	AN1708 (*pstA2*)
AN1403 (*pstB401*)	2.28	ND[a]	0.51	0.20	<0.10	<0.10
AN1697 (*phoS28*)	0.82	0.43	ND	0.53	0.45	0.15
AN1698 (*phoS21*)	1.23	0.33	0.61	ND	0.42	0.72
AN1696 (*phoT32*)	1.28	<0.10	0.54	0.33	1.30	0.16
AN1685 (*pstA2*)	0.81	0.14	0.16	0.79	0.16	ND
AN1664 (normal)	1.78[b]	<0.10	<0.10	<0.10	<0.10	<0.10

[a] ND, Not determined.

[b] This activity was obtained when cells were grown on limiting P_i medium (13). All other activities were obtained when cells were grown on excess P_i medium (16).

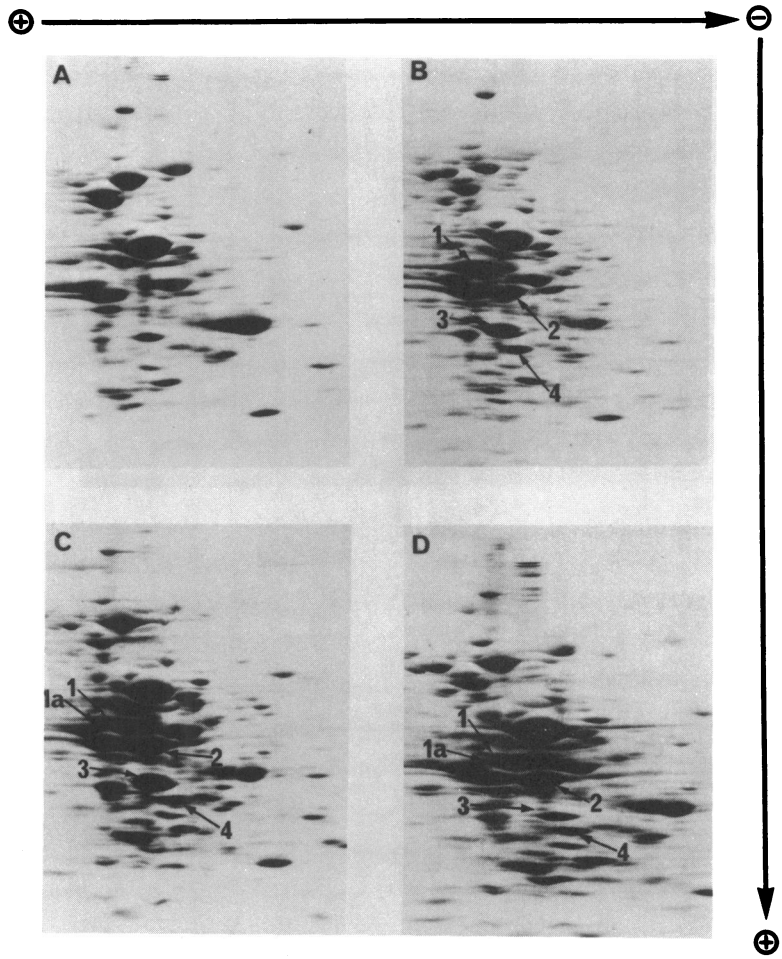

FIG. 1. *Two-dimensional gel electrophoresis of the periplasmic-cytoplasmic fraction from normal and Pst mutant strains of E. coli. Samples of the periplasmic-cytoplasmic fractions were mixed with an equal volume of lysis buffer (26), and 50 μl was used for electrophoresis. In the first dimension, ampholines of pH 5 to 7 and pH 3.5 to 10 were present at 1.2% (wt/vol) and 0.8%, respectively. In the second dimension, an acrylamide gradient of 10.5 to 24.5% (wt/vol) was used. Only the relevant portions of the gel are shown. (A) Periplasmic-cytoplasmic fraction from strain AN1664 grown under conditions of excess P_i; (B) periplasmic-cytoplasmic fraction from strain AN1664 grown under conditions of low P_i concentration; (C) periplasmic-cytoplasmic fraction from strain AN1685 (pstA2) grown under conditions of excess P_i; (D) periplasmic-cytoplasmic fraction from strain AN1697 (phoS28) grown under conditions of excess P_i. The labeled arrows refer to particular proteins identified either by coelectrophoresis with authentic samples or by comparison with published molecular weights (2, 3, 34). 1, Alkaline phosphatase; 1a, processed alkaline phosphatase; 2, α-glycerophosphate-binding protein; 3, phosphate-binding protein; 4, protein GP4 (3) of unknown function.*

kaline phosphatase in the normal cells grown under conditions of P_i starvation may be related to the low level of Zn^{2+} in the P_i starvation medium. Thus, alkaline phosphatase specific activity in the periplasmic-cytoplasmic fraction from such cells was only 0.14 μmol/min per mg of protein without Zn^{2+} added to the assay system, compared with 1.78 μmol/min per mg of protein with the addition of Zn^{2+}. The addition of Zn^{2+} to the assay system had no effect on the alkaline phosphatase activity in the Pst mutant strains grown in the medium containing excess P_i. When Zn^{2+} was added to the phosphate starvation medium, the processed alkaline phosphatase was also present in the normal cells, and the addition of Zn^{2+} to the assay medium had no effect (data not shown).

Inner membrane preparations from each of the Pst mutants were also examined by two-dimensional gel electrophoresis. Four additional proteins were membrane associated in the Pst mutants, as compared with membranes from a normal strain (Fig. 2).

The alkaline phosphatase that coelectropho-

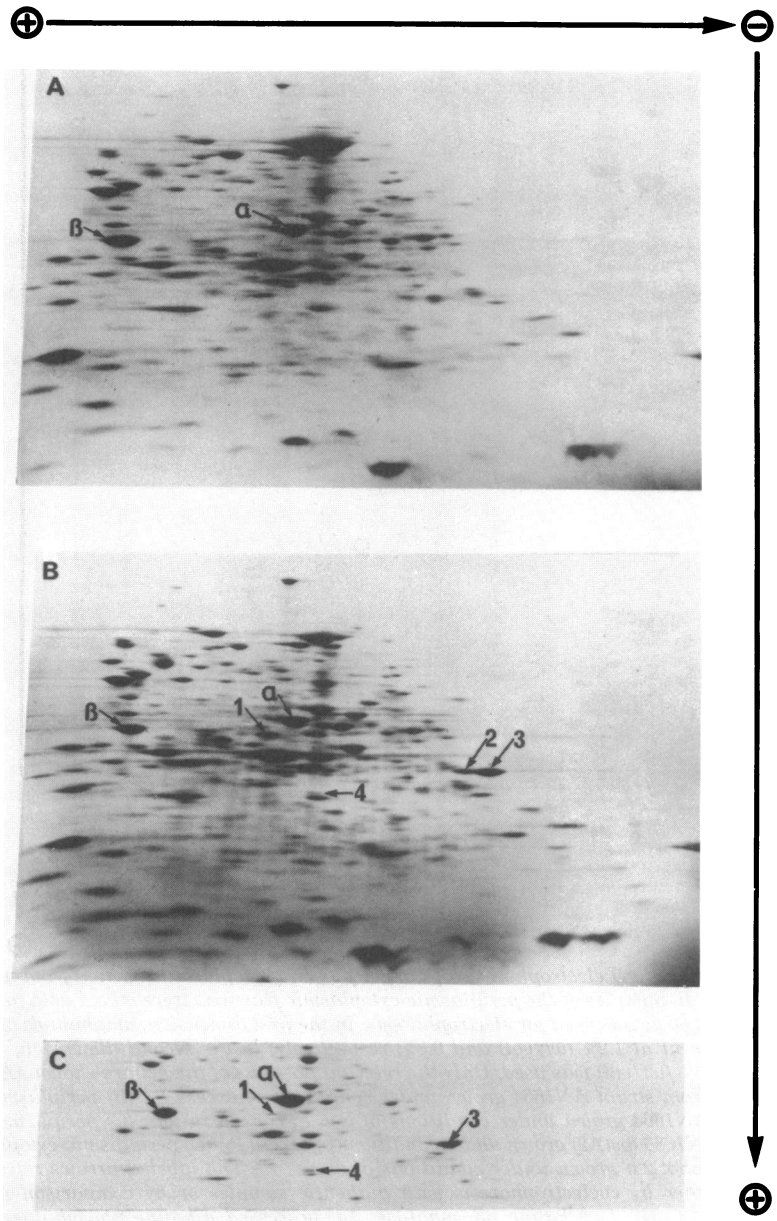

FIG. 2. *Two-dimensional gel electrophoresis of the inner membrane from normal and Pst mutant strains of E. coli. Conditions of electrophoresis were as described in the legend to Fig. 1. (A) Membranes from the normal strain AN1664; (B) membranes from strain AN1697 (phoS28); (C) membranes from strain AN1685 (pstA2). Only the relevant portion of this gel is shown. The proteins labeled α and β are the α- and β-subunits of the membrane-bound ATPase (32). The proteins labeled 1 and 4 were identified by coelectrophoresis as alkaline phosphatase and the phosphate-binding protein, respectively. Proteins 2 and 3 have molecular weights of about 40,000 and are referred to in the text.*

resed with Worthington alkaline phosphatase and the phosphate-binding protein accounted for two of the four additional proteins. The processed alkaline phosphatase was not associated with the inner membrane. The other two proteins had molecular weights of about 40,000 and apparent isoelectric points of about 7.5 and 7.6. The protein with a lower isoelectric point was absent from membranes of strains carrying the *pstA2* or the *phoS21* allele (see Fig. 2). It

was concluded (see above) that the *phoS21* allele is a polar mutation in the *pstA* gene.

Order of genes coding for components of the Pst transport system. The plasmid pAN36, derived from the amplifiable plasmid pACYC184, has been shown previously to carry the *uncD* and *uncC* genes (10). Transformation by plasmid pAN36 of each of the strains carrying the various mutations affecting that Pst transport system was carried out, and the transformants were assayed for alkaline phosphatase activity. No activity was obtained in any of the transformants, indicating that plasmid pAN36 carried the normal genes corresponding to the *pstA2*, *pstB401*, *phoT32*, *phoS21*, and *phoS28* mutant alleles.

A plasmid (pAN127) was constructed from pAN36 by the introduction of a large deletion after digestion with the restriction endonuclease *Pst*I and transformation with linear DNA (33; Fig. 3). This plasmid was used to transform each of the Pst system mutants, and the resultant transformants were assayed for alkaline phosphatase activity. Complementation was obtained between pAN127 and the *pstA2*, *pstB401*, or *phoT32* mutant allele, but not between pAN127 and the *phoS28* or *phoS21* allele. The periplasmic-cytoplasmic fraction from strain AN1861 (pAN127/*phoS28*) was examined by two-dimensional gel electrophoresis, and whereas both alkaline phosphatases, the glycerol-3-phosphate-binding protein, and the protein GP4 could be identified, little or no phosphate-binding protein was formed (data not shown). The gene coding for the phosphate-binding protein is, therefore, not the *pstA*, *pstB*, or *phoT* gene and is closer to the *unc* genes than are these genes.

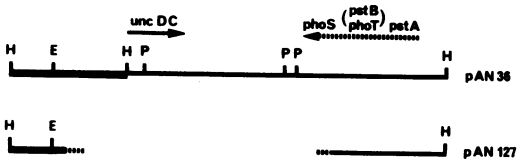

FIG. 3. *Restriction endonuclease cleavage maps and locations of some genes for plasmids pAN36 and pAN127. The vector portion (pACYC184) of the plasmids is indicated by the heavy line. The restriction endonuclease sites are indicated as follows: H, HindIII; E, EcoRI; P, PstI. Plasmid pAN36 is about 15 kilobases in length (9), and plasmid pAN127 (see text) is about 6.5 kilobases in length. The locations of the ends of the deletion are uncertain and are indicated by the broken lines at the bottom. The length of the DNA corresponding to the phoS, pstB, phoT, and pstA genes is unknown and is represented by the dotted arrow.*

DISCUSSION

The various mutant alleles affecting the Pst phosphate transport system were divided into three complementation groups. The *pstA2*, *pstB401*, and *phoT32* alleles characterized the three groups. The *phoS28* and *phoS21* alleles appeared to be polar mutations. The *phoS21* allele affected all three genes, whereas the *phoS28* allele affected only the *pstB401* and *phoT32* alleles. The classical characteristic of the R2a (*phoS*) mutant (12, 36) is its lack of the phosphate-binding protein. If the gene coding for the phosphate-binding protein is part of an operon, then any polar mutation between the promotor and that gene would be classified as a *phoS* mutant, but such mutants may well be affected in other elements of the Pst system. This proposition explains, and gains support from, the once surprising observation (14) that the purified phosphate-binding protein is only slightly effective in the reconstitution of phosphate transport in spheroplasts of several *phoS* mutants. The purified phosphate-binding protein was fully effective in spheroplasts of parent cells from which the periplasmic proteins were lost as a result of spheroplast formation. The polarity of the *phoS* mutants also explains the lack of complementation between *phoS* and *phoT* mutants obtained by Levitz et al. (21).

From the data obtained with plasmid pAN127, it is clear that the phosphate-binding protein is not coded for by the *pstA*, *pstB*, or *phoT* genes. If the *phoS* gene is defined as the gene coding for the phosphate-binding protein, then the *phoS28* and *phoS21* alleles should be redefined as *pho-28* and *pho-21*, as neither of these mutations occur in the *phoS* gene. The order of the genes coding for components of the Pst phosphate transport system would therefore be *pstA*-(*pstB*-*phoT*)-*phoS*, with the *pstA* gene being promotor proximal and the direction of transcription being opposite to that in the *unc* operon (see Fig. 3). The relative order of the *pstB* and *phoT* genes was not determined.

The function of the particular proteins coded for by the four genes proposed, apart from the *phoS* gene, is unknown. The two 40,000-molecular-weight proteins found in membranes from the strains carrying the alleles *pstB401*, *pho-28*, and *phoT32* probably vary by only a single charge. It seems possible that one may be a modified form of the other, and the modification does not occur in the strains carrying the *pstA2* or *pho-21* alleles, where only one of the 40,000-molecular-weight proteins was found. The outer membrane protein e also has a molecular weight of about 40,000, and the relationship between this protein and those of similar molecular

weights in the inner membrane remains to be determined.

The processing of alkaline phosphatase by outer membrane preparations to a smaller molecular weight has been reported previously by Inouye and Beckwith (19) and by Chang et al. (6). It would appear from the present work that this processing requires the alkaline phosphatase to be in the Zn^{2+}- and Mg^{2+}-containing dimeric form and that, in mutant strains grown in the appropriate media, the lower-molecular-weight form is the predominant species.

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LITERATURE CITED

1. **Aono, H., and N. Otsuji.** 1968. Genetic mapping of regulator gene *phoS* for alkaline phosphatase in *Escherichia coli*. J. Bacteriol. **95**:1182–1183.
2. **Argast, M., and W. Boos.** 1979. Purification and properties of *sn*-glycerol-3-phosphate-binding protein of *Escherichia coli*. J. Biol. Chem. **254**:10931–10935.
3. **Argast, M., and W. Boos.** 1980. Co-regulation in *Escherichia coli* of a novel transport system for *sn*-glycerol-3-phosphate and outer membrane protein Ic (e, E) with alkaline phosphatase and phosphate-binding protein. J. Bacteriol. **143**:142–150.
4. **Bachmann, B. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K, edition 6. Microbiol. Rev. **44**:1–56.
5. **Butlin, J. D., G. B. Cox, and F. Gibson.** 1973. Oxidative phosphorylation in *Escherichia coli* K12: the genetic and biochemical characterization of a strain carrying a mutation in the *uncB* gene. Biochim. Biophys. Acta **292**:366–375.
6. **Chang, C. N., H. Inouye, P. Model, and J. Beckwith.** 1980. Processing of alkaline phosphatase precursor to the mature enzyme by an *Escherichia coli* inner membrane preparation. J. Bacteriol. **142**:726–728.
7. **Cox, G. B., and J. A. Downie.** 1979. Isolation and characterization of mutants of *Escherichia coli* K12 affected in oxidative phosphorylation or quinone biosynthesis. Methods Enzymol. **56**:106–117.
8. **Cox, G. B., N. A. Newton, F. Gibson, A. M. Snoswell, and J. A. Hamilton.** 1970. The function of ubiquinone in *Escherichia coli*. Biochem. J. **117**:551–562.
9. **Downie, J. A., G. B. Cox, L. Langman, G. Ash, M. Becker, and F. Gibson.** 1981. Three genes coding for subunits of the membrane sector (F_0) of the *Escherichia coli* adenosine triphosphatase complex. J. Bacteriol. **145**:200–210.
10. **Downie, J. A., L. Langman, G. B. Cox, C. Yanofsky, and F. Gibson.** 1980. Subunits of the adenosine triphosphatase complex translated in vitro from the *Escherichia coli unc* operon. J. Bacteriol. **143**:8–17.
11. **Echols, H., A. Garen, S. Garen, and A. Torriani.** 1961. Genetic control of repression of alkaline phosphatase in *E. coli*. J. Mol. Biol. **3**:425–438.
12. **Garen, A., and N. Otsuji.** 1964. Isolation of a protein specified by a regulator gene. J. Mol. Biol. **8**:841–852.
13. **Gerdes, R. G., and H. Rosenberg.** 1974. The relationship between the phosphate-binding protein and a regulator gene product from *Escherichia coli*. Biochim. Biophys. Acta **351**:77–86.
14. **Gerdes, R. G., K. P. Strickland, and H. Rosenberg.** 1977. Restoration of phosphate transport by the phosphate-binding protein of *Escherichia coli*. J. Bacteriol. **131**:512–518.
15. **Gibson, F., G. B. Cox, J. A. Downie, and J. Radik.** 1977. Partial diploids of *Escherichia coli* carrying normal and mutant *unc* alleles affecting oxidative phosphorylation. Biochem. J. **162**:665–670.
16. **Gibson, F., G. B. Cox, J. A. Downie, and J. Radik.** 1977. A mutation affecting a second component of the F_0 portion of the magnesium ion-stimulated adenosine triphosphatase of *Escherichia coli* K12. The *uncC424* allele. Biochem. J. **164**:193–198.
17. **Gibson, F., J. A. Downie, G. B. Cox, and J. Radik.** 1978. Mu-induced polarity in the *unc* operon of *Escherichia coli*. J. Bacteriol. **134**:728–736.
18. **Horiuchi, T., S. Horiuchi, and D. Mizuno.** 1959. A possible negative feedback phenomenon controlling formation of alkaline phosphatase in *Escherichia coli*. Nature (London) **183**:1529–1531.
19. **Inouye, H., and J. Beckwith.** 1977. Synthesis and processing of an *Escherichia coli* alkaline phosphatase precursor *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **74**:1440–1444.
20. **Lederberg, E. M., and S. N. Cohen.** 1974. Transformation of *Salmonella typhimurium* by plasmid deoxyribonucleic acid. J. Bacteriol. **119**:1072–1074.
21. **Levitz, R., R. Bittan, and E. Yagil.** 1981. Complementation tests between alkaline phosphatase-constitutive mutants (*phoS* and *phoT*) of *Escherichia coli*. J. Bacteriol. **145**:1432–1435.
22. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
23. **Medveczky, N., and H. Rosenberg.** 1970. The phosphate-binding protein of *Escherichia coli*. Biochim. Biophys. Acta **211**:158–168.
24. **Morris, H., M. J. Schlesinger, M. Bracha, and E. Yagil.** 1974. Pleiotropic effects of mutations involved in the regulation of *Escherichia coli* K-12 alkaline phosphatase. J. Bacteriol. **119**:583–592.
25. **Novick, R. P., R. C. Clowes, S. N. Cohen, R. Curtiss III, N. Datta, and S. Falkow.** 1976. Uniform nomenclature for bacterial plasmids: a proposal. Bacteriol. Rev. **40**:168–189.
26. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**:4007–4021.
27. **O'Farrell, P. Z., H. M. Goodman, and P. H. O'Farrell.** 1977. High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell **12**:1133–1142.
28. **Rosenberg, H.** 1979. Transport of iron into bacterial cells. Methods Enzymol. **56**:388–394.
29. **Rosenberg, H., R. G. Gerdes, and F. M. Harold.** 1979. Energy coupling to the transport of inorganic phosphate in *Escherichia coli* K12. Biochem. J. **178**:133–137.
30. **Russell, L. M., and H. Rosenberg.** 1979. Linked transport of phosphate, potassium ions and protons in *Escherichia coli*. Biochem. J. **184**:13–21.
31. **Selker, E., K. Brown, and C. Yanofsky.** 1977. Mitomycin C-induced expression of *trpA* of *Salmonella typhimurium* inserted into the plasmid ColE1. J. Bacteriol. **129**:388–394.
32. **Senior, A. E., D. R. H. Fayle, J. A. Downie, F. Gibson, and G. B. Cox.** 1979. Properties of membranes from mutant strains of *Escherichia coli* in which the β-subunit of the adenosine triphosphatase is abnormal. Biochem. J. **180**:111–118.
33. **Takahashi, H., M. Shimizu, H. Saito, and Y. Ikeda.** 1979. Studies of viable T4 bacteriophage containing cytosine-substituted DNA (T4dC phage). II. Cleavage of T4dC DNA by endonuclease *SalI* and *BamH1*. Mol. Gen. Genet. **168**:49–53.
34. **Tommassen, J., and B. Lugtenberg.** 1980. Outer membrane protein e of *Escherichia coli* K-12 is co-regulated

with alkaline phosphatase. J. Bacteriol. **143**:151–157.
35. **Torriani, A.** 1960. Influence of inorganic phosphate in the formation of phosphatases by *Escherichia coli*. Biochim. Biophys. Acta **38**:460–470.
36. **Willsky, G. R., R. L. Bennett, and M. H. Malamy.** 1973. Inorganic phosphate transport in *Escherichia coli*: involvement of two genes which play a role in alkaline phosphatase regulation. J. Bacteriol. **113**:529–539.
37. **Yagil, E., N. Silberstein, and R. G. Gerdes.** 1976. Coregulation of the phosphate-binding protein and alkaline phosphatase synthesis in *Escherichia coli*. J. Bacteriol. **127**:656–659.
38. **Zuckier, G., and A. Torriani.** 1981. Genetic and physiological tests of three phosphate-specific transport mutants of *Escherichia coli*. J. Bacteriol. **145**:1249–1256.