JOURNAL OF BACTERIOLOGY, Sept. 1984, p. 979–985
0021-9193/84/090979-07$02.00/0
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Vol. 159, No. 3

Regulation of the Phosphate Regulon in *Escherichia coli* K-12: Regulation of the Negative Regulatory Gene *phoU* and Identification of the Gene Product

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Received 16 December 1983/Accepted 29 May 1984

The *phoU* gene is one of the negative regulatory genes of the *pho* regulon of *Escherichia coli*. The DNA fragment carrying *phoU* has been cloned on pBR322 (Amemura et al., J. Bacteriol. 152:692–701, 1982). Further subcloning, Tn*1000* insertion inactivation, and complementation tests localized the *phoU* gene within a 1.1-kilobase region on the cloned DNA fragment. The gene product of *phoU* was identified by the maxicell method as a protein with an approximate molecular weight of 27,000. A hybrid plasmid that contains a *phoU'*-*lac'Z* fused gene was constructed in vitro. This plasmid enabled us to study *phoU* gene expression by measuring the β-galactosidase level in the cells. The plasmid was introduced into various regulatory mutants related to the *pho* regulon, and *phoU* gene expression in these strains was studied under limited and excess phosphate conditions. It was found that *phoU* is expressed at a higher level when the cells are cultured under the excess phosphate condition. The higher *phoU* expression was observed in a *phoB* mutant and a *phoR-phoM* double mutant. The implications of these findings for the regulation of *pho* genes are discussed.

The genes belonging to the phosphate (*pho*) regulon, as represented by *phoA* (structural gene for alkaline phosphatase; EC 3.1.3.1), are derepressed by phosphate limitation. They are under very complex genetic control. Their expression is positively regulated by *phoB*, *phoM*, and *phoR*, and negatively regulated by *phoR*, *phoS*, *phoT* (*pstA*), *pstB*, and *phoU* (1, 6, 16, 18, 19, 20). The *phoS*, *phoT*, *pstB*, and *phoU* genes are clustered at 84 min on the *Escherichia coli* genetic map (2, 6, 7, 17). We have cloned the DNA fragment carrying these genes on pBR322 and shown that *phoS*, *phoT* (*pstA*), and *phoU* are different cistrons (1). As the *phoS* and *phoT* genes are involved in phosphate transport (12, 16, 20, 22), they may regulate *pho* regulon by influencing the level of a hypothetical effector, such as a phosphate derivative (21). Since *phoU* is located next to the *phoS-phoT* region, whose functions are required for phosphate transport, the gene may also be involved in phosphate metabolism.

In our previous report (14), we presented evidence supporting the hypothesis that *phoA* transcription is positively regulated by the *phoB* gene product, and *phoB* gene expression, in turn, is regulated positively by the *phoR* and *phoM* gene products and negatively by the *phoR*, *phoS*, *phoT*, and *phoU* gene products, directly or indirectly. It was also shown that *phoB* as well as *phoA* is derepressed by phosphate limitation. In these studies, we constructed a *phoB'*-*lac'Z* fused gene which enabled us to quantify the levels of *phoB* expression by measuring the β-galactosidase activity in cells carrying the fused gene. To unravel the complex regulatory system of the *pho* regulon and to elucidate the role of *phoU* in the regulation, we studied the genetic and physiological regulation of *phoU* expression itself. In the present work, we describe the construction of a plasmid carrying the *phoU'-lac'Z* fused gene and the results of studies of the regulation of *phoU* expression, and we discuss the implications of our findings in terms of the regulatory mechanism of the *pho* regulon. Furthermore, we localized the *phoU* gene on the cloned DNA fragment by means of Tn*1000*(γδ) transposon insertion inactivation. We also identified the *phoU* gene product by using the maxicell method.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. Strain ANCC41 was constructed by mating ANCC4 cells with JC10240 cells. From the exconjugant colonies, which were resistant to tetracycline and streptomycin, a clone showing the same sensitivity to UV irradiation as JC10240 (*recA*⁻) was selected.

Media. T broth and T agar plates were described previously (1). Synthetic medium was Tris-glucose, supplemented with either excess (640 μM) or limited (64 μM) phosphate (as KH_2PO_4) (11). When needed, ampicillin, kanamycin, streptomycin, or tetracycline was added to the medium at a concentration of 100, 25, 100, or 10 μg/ml, respectively.

Enzyme assays. The method of scoring alkaline phosphatase-synthesizing colonies on T agar plates or on Tris-glucose plates was described previously (1). For assay of the enzyme activities, cultures grown in excess phosphate medium were diluted 100-fold into fresh Tris-glucose medium with either excess or limited phosphate. They were incubated at 37°C with shaking for 12 h. The alkaline phosphatase assay was carried out as described previously (1), and the β-galactosidase assay was carried out as described by Miller (9). β-Galactosidase activity was expressed as micromoles of *o*-nitrophenol liberated per minute per milligram of cellular protein.

DNA manipulations. Methods of plasmid DNA preparation, restriction endonuclease digestion, DNA agarose and polyacrylamide gel electrophoresis, ligation of DNA fragments with T4 DNA ligase, and transformation of cells with plasmid DNA were described previously (1).

Tn*1000*(γδ) insertion and labeling of plasmid-coded proteins in maxicells. The procedures for Tn*1000* insertion into

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TABLE 1. Bacterial strains and plasmids

Strain	Characteristics	Source or reference
E. coli		
ANC24	F^- *leu lacY trp his argG rpsL ilv met thi*	1
ANCC2	F^- *leu phoR68 trp his argG rpsL ilv met thi*	1
ANCC4	F^- *leu lacY purE trp his argG rpsL phoU35 met thi*	1
ANCC41	F^- *leu lacY purE trp his recA56 srlB10*::Tn*10 argG rpsL phoU35 met thi*	JC10240 × ANCC4
ANCC75	F^- *leu lacY purE trp his argG rpsL phoS64 met thi*	1
ANCC90	F^- *leu lacY purE trp his argG rpsL phoT9 met thi*	1
BW521	*lac phoR68 phoM451 rpsL thi*	B. L. Wanner
CSH26	F^- *ara* Δ(*pro-lac*) *met thi*	9
CSR603	F^- *phr-1 recA1 uvrA6*	13
JC10240	Hfr (PO45) *thr-300 srlB10*::Tn*10 recA56 relA1 rpsE300 ilv-318 thi-1*	13
LEP1	F^- *proC34 phoB23 purE42 trpE38 thi-1 lacZ73 lacI22 xyl-5 mtl-1 azi-6 rpsL109 tonA23? tsx-67 supE44?*	B. J. Bachmann
LC169	F′ *proA*$^+$*B*$^+$ *lac*$^+$ Δ(*pro-lac*)	Y. Nishimura
MG1063	F^+ *recA1*	13
Plasmids		
pACYC177	Ap^r Km^r	5
pAU1	pACYC177 derivative carrying the *phoU*$^+$ gene, Km^r	This paper
pMC1403	Vector for cloning of transcriptional and translational signals, Ap^r	4
pMF3	mini-F, Ap^r	8
pSN547	pBR322 derivative carrying the *phoU*$^+$ gene, Ap^r Tc^r	1

plasmids and labeling of plasmid-coded proteins in maxicells were described by Sancar et al. (13).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A 5-ml culture was incubated overnight, and the cells were harvested, washed once with 1 ml of buffer containing 10 mM Tris-hydrochloride (pH 7.2) and 30 mM NaCl, and resuspended in 0.1 ml of the same buffer. The same volume of sample buffer (10) was added, and the cell suspension was heated at 90°C for 5 min. Portions of the samples were applied to a 7.5% polyacrylamide gel containing 1% sodium dodecyl sulfate, and gel electrophoresis was carried out as described previously (10). The gels were stained with Coomassie brilliant blue R-250 and destained as described previously (10).

RESULTS

Complementation of the *phoU35* mutation by the low-copy number recombinant plasmid pSN5490. In our previous work (1), we cloned an *E. coli* chromosomal fragment which complemented *phoU35* (formerly designated *phoT35*) but not *phoS64*, *pstA*, and *phoT9* mutations. However, we observed a few cases in which particular DNA fragments, other than those carrying the *phoR*$^+$ gene, suppressed alkaline phosphatase synthesis in *phoR* mutants when they were borne on multicopy plasmids, but not when they were on a low-copy number plasmid (1). Therefore, to provide further evidence that the cloned DNA fragment carries the authentic *phoU* gene, we recloned the DNA fragment on mini-F plasmid pMF3 and tested whether the recombinant plasmid complemented the *phoU35* mutation.

pSN547, a hybrid plasmid which carries the *phoU* gene on a pBR322 vector (Fig. 1), was described previously (1). pSN547 and mini-F plasmid pMF3 were digested with *Eco*RI, and the digested mixture was ligated by using T4 ligase. ANCC4(*phoU35*) cells were transformed with the DNA mixture. All of the transformant colonies, which were ampicillin resistant and tetracycline sensitive, showed a phosphate-repressible phenotype for alkaline phosphatase synthesis (PhoU$^+$). The plasmid DNA (pSN5490) was isolated from one of the transformants and was confirmed to consist of pMF3 and the 4.3-kilobase (kb) DNA fragment derived from pSN547 by restriction enzyme analysis.

pSN5490 and pMF3 (as a control) were introduced into a series of nearly isogenic *pho* mutant cells by transformation to examine the complementability of these mutations. pSN5490 complemented *phoU35* but not *phoS64* and *phoT9* (Table 2), as indicated by the repressibility of alkaline phosphatase synthesis under excess phosphate. Therefore, we concluded that the chromosomal DNA fragment carried on pSN5490 contains the authentic *phoU* gene.

Fine mapping of the *phoU* gene by Tn*1000* insertional inactivation. To further localize the *phoU* gene on the cloned DNA fragment, transposon Tn*1000*(γδ) was inserted into pAU1, which was constructed by subcloning the 2.4-kb *Hpa*I fragment of pSN547 on the *Hinc*II site of pACYC177 (Fig. 2a). Since pAU1 could complement the *phoU35* mutant, the *phoU* gene is located within the 2.4-kb *Hpa*I fragment.

Strain MG1063(F^+ *recA1*) harboring pAU1(*phoU*$^+$ Km^r) was mated with ANCC41(*phoU35 recA56* Tc^r Sm^r), and

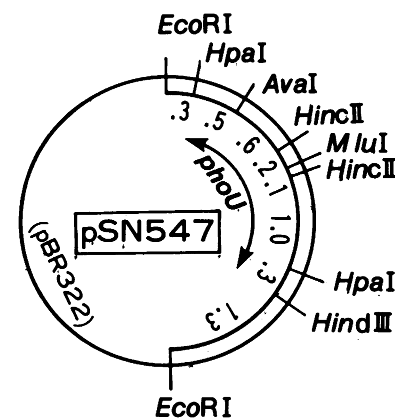

FIG. 1. Restriction map of the cloned *phoU* gene on pBR322 (pSN547) (1).

TABLE 2. Complementation of the *phoU* mutation by cloned wild-type (wt) allele on pMF3 (pSN5490)

Strain	Plasmid	Alkaline phosphatase activity[a] in medium with:	
		Excess Pi	Limited Pi
ANC24 (WT)	pMF3	0.009 ± 0.003	3.41 ± 0.70
	pSN5490	0.006 ± 0.002	2.45 ± 0.23
ANCC4 (*phoU35*)	pMF3	2.14 ± 0.08	7.32 ± 0.38
	pSN5490	0.011 ± 0.004	4.47 ± 0.83
ANCC75 (*phoS64*)	pMF3	2.57 ± 0.14	5.88 ± 0.52
	pSN5490	2.20 ± 0.14	3.09 ± 0.24
ANCC90 (*phoT9*)	pMF3	1.65 ± 0.11	5.56 ± 0.23
	pSN5490	1.42 ± 0.20	3.17 ± 0.61

[a] Values are expressed as micromoles of *p*-nitrophenol liberated per minute per milligram of cellular protein, and are the averages of four experiments.

Km^r, Tc^r, Sm^r exconjugants were selected on T broth plates. The exconjugants were classified as $PhoU^+$ or $PhoU^-$ according to the repressibility of alkaline phosphatase synthesis, as examined by spraying the colonies formed on T broth plates with a chromogenic substrate. All of the plasmids isolated from the exconjugants possessed a 5.7-kb Tn*1000* insertion in pAU1. The sites of Tn*1000* insertion were determined by digesting the isolated plasmids with *Bam*HI, *Mlu*I, and *Eco*RI restriction enzymes, followed by agarose gel electrophoresis. The insertions within the 1.1-kb region covering the *Mlu*I and two *Hinc*II sites on the 2.4-kb DNA fragment inactivated the *phoU* gene, whereas those outside the region did not affect the ability of the plasmid to complement the *phoU35* mutant (Fig. 2b and c). These results indicate that 1.1 kb is the minimal size and 1.5 kb is the maximal size of the functional *phoU* gene.

Identification of the *phoU* gene product. To identify the product of the *phoU* gene, the proteins encoded by the plasmids were compared in extracts of the maxicells. pAU1 codes for proteins of molecular weight (MW) 29,000 and 27,000 (Fig. 3). Since the 29,000-MW protein is the product of the Km^r gene, the 27,000-MW protein is likely to be the *phoU* gene product. This is further supported by the fact that the 27,000-MW protein is missing in the extracts of CSR603 cells harboring the $PhoU^-$::Tn*1000* insertion plasmids, although it is observed in extracts of the cell harboring the $PhoU^+$::Tn*1000* insertion plasmids.

Construction of *phoU'-lac'Z* fusion gene. To study the transcriptional direction and regulation of the *phoU* gene, a *phoU'-lac'Z* gene fusion was constructed by in vitro recombination. Plasmid pMC1403 was used to identify and clone the DNA fragment that initiates transcription and translation internally and yields transcriptional and translational readthrough into *lacZ* (4). From the results of our previous work (1) and also from the present work, the *Mlu*I site on pSN547 probably splits the *phoU* gene within the coding region.

The construction and structure of plasmids containing a *phoU'-lac'Z* fusion gene are illustrated in Fig. 4. pSN547 was digested with *Mlu*I, and the staggered ends were filled with T4 DNA polymerase. Then the plasmid DNA was digested with *Eco*RI, and the resulting DNA fragments were purified by electroelution. pMC1403 was double-digested with *Eco*RI and *Sma*I, and the larger fragment was purified by electroelution. The 1.6- and 2.7-kb *Eco*RI-*Mlu*I fragments from pSN547 were ligated with the digested pMC1403. CSH26(*pro-lac* deletion) cells were transformed with each of the ligated DNA mixtures. Approximately 20% of the transformant colonies with the 1.6-kb DNA fragment showed a Lac^+ phenotype, whereas none of the 2,240 transformant colonies with the 2.7-kb DNA fragment showed a Lac^+ phenotype when examined on lactose-MacConkey plates.

Plasmids were prepared from the six Lac^+ colonies, and their restriction patterns were examined. It was found that

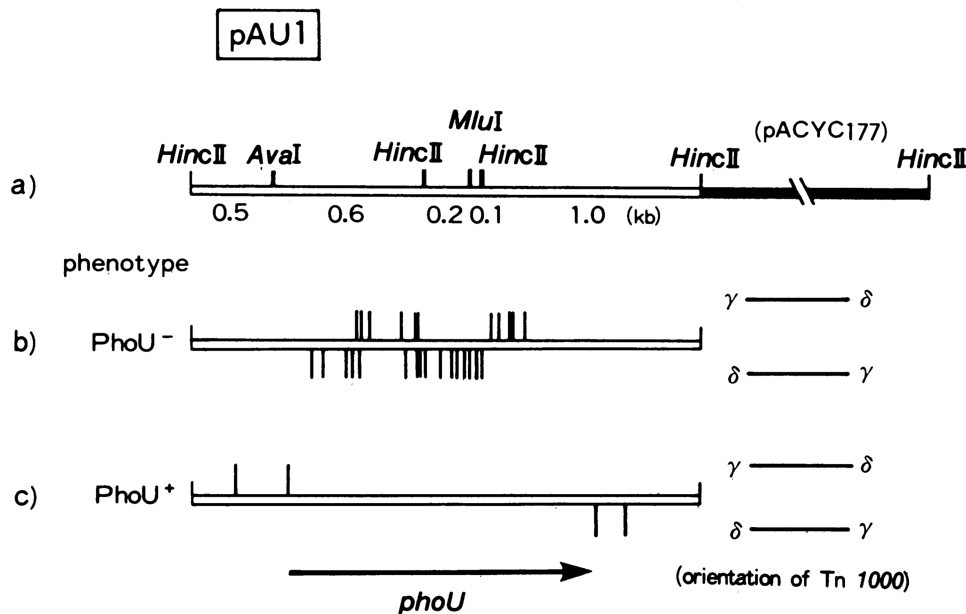

FIG. 2. Restriction map of the DNA fragment including the *phoU* gene on pACYC177 (pAU1) (a). (b and c) Distribution of the Tn*1000*(γδ) transposon inserted into pAU1, resulting in $PhoU^-$ (b) or $PhoU^+$ (c). The orientation of the *phoU* gene was deduced from the experiments with the *phoU'-lac'Z* fusion gene (see the text).

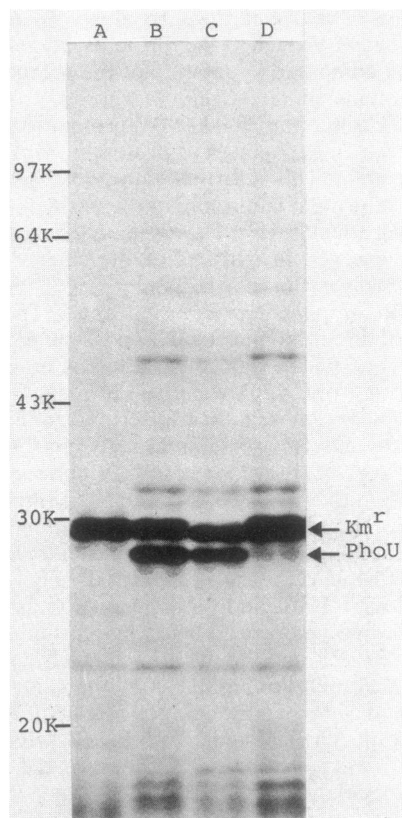

FIG. 3. [^{35}S]methionine-labeled proteins produced in maxicells by pAU1(*phoU*$^+$) and its derivatives. Lanes: A, pACYC177; B, pAU1(PhoU$^+$); C, pAU1-5(PhoU$^+$); D, pAU1-101(PhoU$^-$). The host strain was CSR603 (*recA1 uvrA6 phr-1*) for all plasmids. Proteins were separated by electrophoresis on a 10% polyacrylamide gel containing sodium dodecyl sulfate and visualized by fluorography. Phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), and soybean trypsin inhibitor (20,100) were used as molecular weight standards.

all of the Lac$^+$ plasmids (e.g., pSN5473) carried the 1.6-kb chromosomal fragment on pMC1403. These results suggest that pSN5473 carries a *phoU'-lac'Z* fusion gene that synthesizes active, fused β-galactosidase, as a product of transcriptional and translational read-through from the *phoU* gene. The translational reading frame of the *phoU'-lac'Z* gene is in-phase; when the plasmid was digested with *Bam*HI and the staggered region was either removed by S1 nuclease or filled with T4 DNA polymerase before ligation, which produced a −1 or +1 coding frame shift, the treated plasmids became Lac$^-$. The *phoU* promoter does not seem to be present on the 2.7-kb *Eco*RI-*Mlu*I fragment on pSN547, since plasmids isolated from the Lac$^-$ transformant (pSN5474), which carry the 2.7-kb fragment on pMC1403, did not give Lac$^+$ plasmids, even after the coding frame was shifted by +1 or −1 at the *Bam*HI site by the procedure described above. These results, together with the results obtained in the Tn*1000* insertion inactivation, suggest that (i) the transcriptional and translational initiation signals of *phoU* reside within the 1.3-kb *Hpa*I-*Mlu*I fragment on pSN547, and (ii) the transcriptional and translational direction is from the *Hpa*I to the *Mlu*I site, corresponding to a counterclockwise direction on the *E. coli* genetic map (Fig. 2).

The product of the *phoU'-lac'Z* fused gene was examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the cell extracts. The fused gene product identified in the extract of CSH26 cells carrying pSN5473 had a molecular weight of 125,000, which is larger than native β-galactosidase (116,000) by ca. 10,000 daltons, equivalent to 270 base pairs of DNA (Fig. 5).

Since pSN5473 is a multicopy plasmid whose replicon was derived from pBR322, the regulation of gene expression may be different from that in the single-gene state. To minimize this effect, the DNA fragment carrying the *phoU'-lac'Z* gene was transferred to a low-copy number mini-F plasmid vector, pMF3. The resultant plasmid was pSN5475, whose *Eco*RI-*Sal*I fragment was derived from pSN5473 (Fig. 4).

Regulation of *phoU* gene expression. To examine the effects of phosphate and various *pho* regulatory mutations on the regulation of *phoU* expression, we introduced pSN5475 into a *pho*$^+$ strain as well as *pho* regulatory mutants. pMF3 was also introduced into these strains to construct control strains without the fused gene. The levels of *phoU* expression were studied by measuring the β-galactosidase activity of the fused protein in the cells carrying the plasmid under excess and limited phosphate conditions. The levels of alkaline phosphatase were also measured in the same strains to monitor the degree of phosphate limitation under the growth conditions and also to check the properties of the *pho* mutants. The cells were grown in excess or limited phosphate medium for 12 h before measurement of the enzyme levels. The level of alkaline phosphatase was confirmed to be characteristic of each mutant strain under the culture conditions. Not much difference in the levels of *phoU* expression, as measured by the level of β-galactosidase, was observed in the various *pho* strains when the cells were grown in the limited-phosphate medium (Table 3). The levels of *phoU* expression in all strains examined were higher under the excess phosphate condition than under the limited phosphate condition. In particular, the levels in the *phoB*, *phoU*, and *phoR-phoM* mutants were two to five times higher under the excess phosphate condition than under the limited phosphate condition.

DISCUSSION

In this report, we provided further evidence to show that the chromosomal fragment, which was cloned together with the *phoS* and *phoT* genes and complemented the *phoU* mutation on a multicopy plasmid, carried the authentic *phoU* gene. We did this by showing that the fragment cloned on a low-copy number vector also complemented the *phoU* mutation. The size and the location of the *phoU* gene on the cloned chromosomal fragment were more accurately determined by Tn*1000* insertional inactivation (Fig. 2). The minimal and maximal sizes of the functional *phoU* gene were defined to be approximately 1.0 and 1.5 kb, respectively. The maxicell experiment showed that the *phoU* gene codes for a protein with MW 27,000 (Fig. 3). Therefore, the protein coding region of the *phoU* gene is ca. 0.75 kb, and the actual size of the *phoU* gene may be closer to the minimal size defined by the transposon insertion experiment.

The results presented here, together with our previous reports (1, 7) and our unpublished data, suggest that the gene order in this region is *phoS-phoT(pstA)-pstB-phoU* in the counterclockwise direction on the *E. coli* genetic map (2). There exist at least three transcriptional units, *phoS*, *phoT-pstB*, and *phoU*, and each has a counterclockwise direction of transcription on the genetic map (7, 17).

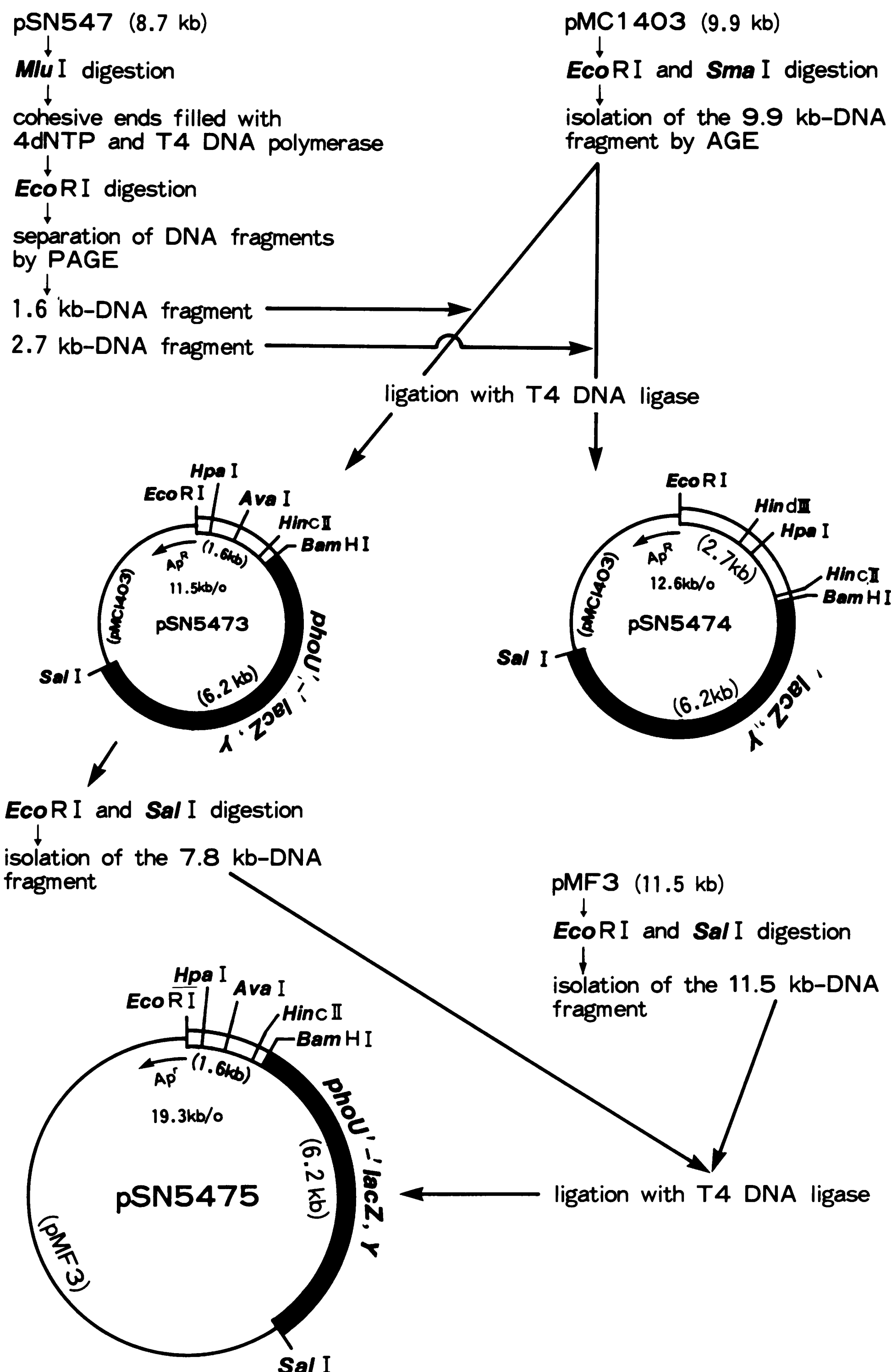

FIG. 4. Construction of recombinant plasmids carrying a *phoU'-lac'Z* fusion gene. Abbreviations: 4dNTP, four kinds of deoxyribonucleoside triphosphate; PAGE, polyacrylamide gel electrophoresis; AGE, agarose gel electrophoresis.

In the previous report (14), we presented evidence that the *phoB* protein positively regulates the level of *phoA* expression, and that *phoB* expression, in turn, is physiologically regulated by phosphate and genetically regulated by the *pho* regulatory genes, including *phoU*.

The genes (*phoS*, *phoT*, and *pstB*) involved in the highly specific phosphate transport system (12, 16, 20) are contiguous and adjacent to *phoU*. All of them function as negative regulatory genes of the *pho* regulon, and all of them except for *phoU* are involved in the phosphate transport system

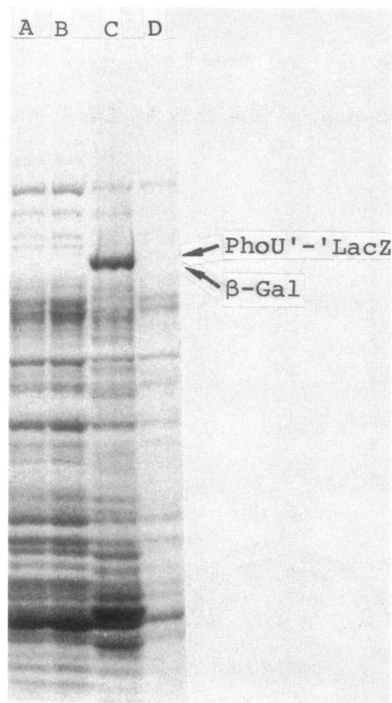

FIG. 5. Identification of the *phoU'-'lacZ* fusion gene product by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cells of strain CSH26 carrying either pMC1403 (lane A) or pSN5473 (lane B) were grown in T broth. Cells of strain LC169 were grown in T broth containing either isopropylthiogalactoside (lane C) or 0.2% glucose (lane D).

(22). We postulated that *phoU* encodes a repressor, or a subunit of repressor for *phoB*, or one of the enzymes for converting phosphate to the hypothetical effector (corepressor), which activates a repressor for *phoB* (14).

The physiological and genetic regulation of *phoU* gene expression was studied by using a fused gene. The results obtained were rather ambiguous. It was observed that the highest level of β-galactosidase was obtained during the early stationary phase of growth, and the level declined gradually during the stationary phase (data not shown). In general, higher expression of *phoU* was observed in a wild-type strain, as well as in various *pho* regulatory mutants under the excess phosphate condition, than under the limited phosphate condition (Table 3). The levels were highest in *phoB* and a *phoR-phoM* mutant grown under the excess phosphate condition. These physiological and genetic conditions decrease the expression of *phoA* and *phoB* (14). Since *phoU* is one of the negative regulatory genes for the *pho* regulon, the highest levels of *phoU* expression may result in a higher level of the repressor or may act as an activator of the repressor for *phoB* in the cells. The higher level of *phoU* expression in a *phoU* mutant may suggest that the *phoU* gene is under autogenous regulation by its product. Since the observed differences in the levels of *phoU* expression of the various *pho* mutants were not great, these differences may be coincidental and may have no biological significance. The data about the regulation of *phoU* may become more meaningful when they are combined with other findings in the future.

The *phoU'-lac'Z* fusion gene encodes a hybrid protein which consists of the amino-terminal part of the PhoU protein and β-galactosidase that lacks seven amino-terminal amino acids. As the localization of the wild-type *phoU* product is unknown, the possibility that this product is secreted cannot be excluded. The product of the hybrid gene then could have serious export problems, as was reported previously in the cases of *lamB'-lac'Z* (15) and *malE'-lac'Z* (3). This might have influenced the maximum level of the hybrid protein synthesis, and the results shown in Table 3 may not truly reflect the amount of the gene expression. However, we think this possibility is very unlikely for the following reasons. CSH26 cells carrying pSN5473, a multicopy plasmid containing the *phoU'-lac'Z* fusion gene, produced ca. 10 times more of the hybrid protein than did the cells carrying pSN5475 (data not shown). Yet the former strain grew as fast as the latter strain or as the strain without the fusion gene, and all three strains showed the same colony-forming ability after full growth (data not shown).

Since the cells seem to tolerate the synthesis of the hybrid protein to the level produced in CSH26-pSN5473, the synthesis at ca. one-tenth of the levels shown in Table 3 should not interfere with normal cell physiology and should not

TABLE 3. Effect of phosphate and the pho^- mutations on regulation of *phoU* expression

Strain	Plasmid	β-Galactosidase activity[b]		Alkaline phosphatase activity[c]	
		Excess Pi	Limited Pi	Excess Pi	Limited Pi
ANC24 (WT)[a]	pMF3	0.011 ± 0.002	0.007 ± 0.002	0.008 ± 0.002	3.71 ± 0.008
	pSN5475	0.192 ± 0.004	0.103 ± 0.002	0.005 ± 0.001	5.12 ± 1.98
LEP1 (*phoB32*)	pMF3	<0.001	<0.001	<0.001	0.016 ± 0.006
	pSN5475	0.491 ± 0.013	0.093 ± 0.004	<0.001	0.005 ± 0.005
ANCC2 (*phoR68*)	pMF3	0.008 ± 0.002	0.003 ± 0.001	1.08 ± 0.09	0.53 ± 0.01
	pSN5475	0.193 ± 0.013	0.138 ± 0.008	0.96 ± 0.02	0.50 ± 0.02
ANCC75 (*phoS64*)	pMF3	0.003 ± 0.001	0.002 ± 0.001	2.69 ± 0.08	2.45 ± 0.05
	pSN5475	0.184 ± 0.010	0.109 ± 0.027	2.30 ± 0.08	2.45 ± 0.22
ANCC90 (*phoT9*)	pMF3	0.012 ± 0.001	0.006 ± 0.002	1.37 ± 0.61	5.93 ± 0.81
	pSN5475	0.169 ± 0.02	0.155 ± 0.016	2.34 ± 0.12	7.01 ± 1.37
ANCC4 (*phoU35*)	pMF3	0.004 ± 0.001	<0.001	1.78 ± 0.28	5.81 ± 0.57
	pSN5475	0.372 ± 0.016	0.168 ± 0.008	1.72 ± 0.05	5.89 ± 0.22
BW521 (*phoR-phoM*)	pMF3	<0.001	<0.001	0.011 ± 0.001	0.023 ± 0.001
	pSN5475	0.406 ± 0.028	0.147 ± 0.007	0.032 ± 0.002	0.042 ± 0.006

[a] WT, Wild type.
[b] Expressed as micromoles of *o*-nitrophenol liberated per minute per milligram of cellular protein.
[c] Expressed as micromoles of *p*-nitrophenol liberated per minute per milligram of cellular protein.

affect the results of *phoU* gene expression as examined by using the fusion gene.

ACKNOWLEDGMENTS

We are grateful to B. L. Wanner and B. J. Bachmann for kindly supplying the bacterial strains and to A. Torriani for valuable discussion. We also thank M. R. Moynihan for correcting the English of this manuscript.

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