Deletion Map of the *Escherichia coli* Structural Gene for Alkaline Phosphatase, *phoA*

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Lambda transducing phages containing portions of the *phoA* gene have been isolated and used to construct a deletion map of the *phoA* gene. The isolation of a plaque-forming lambda transducing phage carrying the entire *phoA* gene is also described. Two new methods for screening or selection of mutants that have altered levels of alkaline phosphatase activity are reported.

The structural gene *phoA*, which codes for the enzyme alkaline phosphatase, is located between 8 and 9 min on the *Escherichia coli* linkage map (1). A genetic map of the *phoA* gene has been previously presented, based mainly on two-factor crosses (7). Four-factor crosses with mutations in the *phoA* gene and the nearby *lac* and *proC* genes have also been used to order mutations in the *phoA* gene relative to the nearby *proC* gene (10). In this paper, we report a more accurate deletion map of *phoA*, using λ transducing phages that contain varying lengths of the *phoA* gene.

MATERIALS AND METHODS

Media and chemicals. The following media were used: M63 minimal medium, LB broth, F minimal top agar, TYE rich medium, and H top agar (9). When it was necessary to use a medium in which the phosphate concentration could be varied, Tris minimal medium was used (3). Tris–low-phosphate medium contains KH_2PO_4 at 10^{-4} M. Tris–high-phosphate medium contains KH_2PO_4 at 10^{-2} M. Minimal medium agar contains the given sugar at 0.2% concentration and amino acids at 20 μg/ml. Liquid minimal medium contains 0.4% glucose and amino acids at 20 μg/ml. Streptomycin was added where indicated to a concentration of 114 μg/ml.

The concentration of 5-bromo-4-chloro-3-indolylphosphate (XP) and 5-bromo-4-chloro-3-β-D-galactoside (XG) was 40 μg/ml. The use of the indicator dye XP is the same as that described for XG (6), except that XP is a substrate for the enzyme alkaline phosphatase.

Bacteria and phage strains. The bacterial strains and phage used are listed in Table 1. Phage Mu(*cts*) (8) was obtained by a temperature induction of a Mu(*cts*) lysogen. λ *cI857 rex*::Tn*5 Oam29 Pam80 b221* was kindly provided by M. Lichten.

Direct selection for Mu(*cts*) insertions in the *phoA* gene. Strain Xph26 contains a *glpD* mutation, which affects the ability of strains containing the *phoA*⁺ gene to grow in the presence of β-glycerophos-

phate. *E. coli* is unable to transport β-glycerophosphate. However, in the presence of alkaline phosphatase, β-glycerophosphate is cleaved in the periplasm, yielding glycerol and inorganic phosphate. Glycerol enters cells and subsequently is phosphorylated to yield α-glycerophosphate. α-Glycerophosphate is converted by α-glycerophosphate dehydrogenase to dihydroxyacetone phosphate, which enter the glycolytic pathway. A mutation in the *glpD* gene which codes for α-glycerophosphate dehydrogenase leads to accumulation of α-glycerophosphate inside cells under these growth conditions, which is toxic (5). However, a *glpD* strain can escape death in the presence of β-glycerophosphate if it acquires a second mutation which eliminates its ability to produce alkaline phosphatase. In such a strain β-glycerophosphate is not metabolized at all and remains outside the cytoplasm.

Mu insertions in strain XPh26 were generated (4), and a few thousand were spread on Tris minimal agar containing β-glycerophosphate, KH_2PO_4 (10^{-4} M), sodium succinate as a carbon source, and XP. After 2 days at 30°C, XPh26 forms small blue colonies on this medium, whereas mutants reduced in expression of the *phoA* gene form large white colonies. Since strains containing regulatory mutations that reduce alkaline phosphatase activity, such as those in the *phoB* gene, also appear as pale blue or white colonies on these plates, the white colonies were screened further by cross-streaking against a φ80 p*phoA*⁺ phage (16) on Tris-glucose-XP agar. Mutants which were complemented by the φ80 p*phoA*⁺ phage gave rise to dark blue colonies on these plates and were presumed to be *phoA*.

Isolation of *phoA*::Tn*5* insertions. MC4100 was infected with bacteriophage λ *cI857 rex*::Tn*5 Oam20 Pam80 b221* to obtain Tn*5* insertions in *phoA* (2). Cells representing 2×10^6 independent transposition events were scraped into L broth and grown overnight. A P1*vir* lysate was grown on this culture and used to transduce MPh4 to *proC*⁺ and kanamycin resistance on media containing XP. Since *proC* is cotransducible with *phoA*, we were able to enrich for Tn*5* insertions in the *phoA* gene by screening among *proC*⁺ transductants. Seven out of 200 *pro*⁺ kanamycin-resistant transductants appeared as white on the indicator media, indicating loss of ability to make alkaline phosphatase. Six of the seven transductants were comple-

TABLE 1. *Bacterial strains and phages used*[a]

Strain	Genotype or bacterial genes carried	Origin
Bacteria		
XPh26	F⁻ *lacZ524 phoA⁺ proC⁺ phoB⁺ phoR glpD rpsL*	J. Beckwith
XPh63.1	F⁻ Δ*lac-169* Δ(*phoA-proC*) *phoB⁺ phoR trp rpsL*	J. Beckwith
MC4100	F⁻ *araD149* Δ*lac-169 rpsL relA thi*	(4)
MPh4	F⁻ *araD139* Δ*lac-169 rpsL relA thi proC*:: Tn5	J. Beckwith
XPh4	F⁻ *lacZ524* Δ*phoA20 phoB⁺ phoR rpsL*	(3)
HfrC *phoA*	Hfr Cavalli *phoA* mutants from collection of A. Garen	(7)
MPh21	Lac⁺ fusion derived from Mu(cts) insertion in *proC* (MC 4100), lysogenized with λp1.209	See Text
Phage		
λp1.209	::(+Mu′) *trp′BA′* Δ*W209 lac′OZYA′*	(4)
φ80p *phoA*	*phoA*	(16)
XPh17 (φ80 *phoA⁺ proC⁺*)	*phoA⁺ proC⁺*	(3)
λ *cIh80* Δ(*int9*)		(4)
Mu(cts)		(8)
λ *cI857 rex*::Tn*t Oam29 Pam80 b221*		Constructed by M. Lichten

[a] Abbreviations are those of Bachmann and Low (1).

mented for *phoA* by the λ pp*hoA* phage. Revertants of these six *phoA* mutants of *phoA⁺* also lost the kanamycin resistance phenotype, indicating that these six mutants are indeed *phoA*::Tn5.

Introduction of the various *phoA* mutations into MPh4. Hfr strains carrying various *phoA* mutations were crossed with MPh4 (*proC*), and *pro⁺* recombinants were selected. The recombinants were purified on Tris-glucose-low phosphate-XP plates to detect those which had also received the closely linked *phoA* mutation. The *phoA*::Mu(cts) insertions were transferred from strain XPh26 to strain MPh4, using P1 cotransduction with *proC*.

Isolation of *proC*::Mu(cts) in MC4100 and of Lac⁺ fusions. A penicillin enrichment for proline auxotrophs was performed on a collection of Mu insertions in MC4100. The *pro* auxotrophs were characterized to determine whether the Mu(cts) insertion was in the *proC* gene by determining whether a φ80 *proC⁺* phage would complement the defect. Strains containing Mu insertions in *proC* were used to obtain Lac⁺ fusions, using the Casadaban technique (4). Strain MPh21 is one of these Lac⁺ fusion isolates. Glycyl proline was used as a proline source in the selection plates containing lactose, since proline itself is rapidly broken down by the bacteria in the absence of a strongly catabolite-repressing carbon source. The Lac⁺ fusion strains were induced with UV in order to obtain λ transducing phages.

Isolation of transducing phages containing the entire *phoA* gene or only portions of the *phoA* gene. Dilutions of the phage lysates were adsorbed to strain XPh4, and the mixture was spread on Tris-glucose-low phosphate-XP plates. Blue plaques were obtained from the UV-induced lysate of strain MPh21, which was isolated as a Lac⁺ thermoresistant derivative of MC4100 *proC*::Mu(cts). These plaques were

derived from λ transducing phages which carried the entire *phoA* gene.

The lysate of MPh21 was also examined to identify transducing phages that carried only portions of the *phoA* gene. Dilutions of the phage lysate were adsorbed to strain XPh63.1 (*phoA proC*), and the adsorption mixture was spread on Tris-glucose-XP agar containing high phosphate to select for *proC⁺* transductants and simultaneously screen for absence of the complete *phoA* gene.

After 2 days, any white colonies were purified and induced with UV (9), and the lysates were tested for the presence of λ phage containing a part of the *phoA* gene. Drops of individual lysates were spotted on a lawn of a strain carrying the *phoA* mutation, *S10*, on Tris-glucose-XP agar containing no added phosphate. The *S10* mutation lies at the end of the *phoA* gene closest to the *proC* gene (10). Those lysates which yielded blue recombinants in this test were considered to contain a phage which carried part of the *phoA* gene.

Mapping the *phoA* gene: a new selective technique for *phoA⁺* recombinants. A 0.2-ml amount of an exponentially growing culture of MPh4 containing a *phoA* mutation was spread on Tris-glucose-XP agar containing no added phosphate. A drop of the phage lysate to be tested was spotted on the lawn of cells. A single plate could accommodate 16 such drops. The plates were then incubated at 30°C for 5 to 6 days. The only phosphate source available to the cells was that released from the breakdown of XP by alkaline phosphatase; hence, *phoA⁺* recombinants appeared as large blue colonies in the spots. As a result, we were able to use this medium as a direct selective medium for *phoA⁺* colonies. Lysates that gave four or fewer blue colonies with *phoA* mutations were then tested in a more sensitive recombination test to distin-

guish between true recombinants or revertants. Strains carrying the *phoA* mutations in a *proC* background were lysogenized with the various λ transducing phages. Purified lysogens were grown up overnight in 5 ml of broth, and 0.1 ml of the suspension was then plated on Tris-minimal glucose-XP agar. This allows the accumulation and multiplication of recombinants. The method is sensitive enough to detect recombination frequencies of 10^{-8}. Strains containing point mutations, deletions, or Mu(cts) or Tn5 insertions were analyzed in this way.

RESULTS

We have used the general approach described by St. Girons and Margarita (12) and Silhavy et al. (15) to obtain a set of transducing phages which carry different amounts of the *phoA* gene.

Isolation and characterization of λ pphoA⁺. To generate λ transducing phages carrying the entire *phoA* gene, we first obtained Lac⁺ fusions derived from a Mu insertion in the nearby *proC* gene, using the technique of Casadaban (4). The Casadaban technique for generating fusions results in the positioning of a λ phage close to the *lac* genes. Thus, UV-induced lysates from the Lac⁺ fusion strains could contain λ particles which, as a result of aberrant excision events, include the entire *phoA* gene.

Lac⁺ fusion derivatives of MC4100 were derived from four independent Mu insertions in the *proC* gene. The Lac⁺ isolates were induced with UV light, and the phage lysates were tested for the presence of λ phages carrying the entire *phoA* gene. Plaques from these lysates were examined on XPh4 (*phoA phoB⁺*) on Tris-minimal glucose agar containing XP (a phosphatase indicator). Lambda particles carrying the entire *phoA* gene form blue plaques on a lawn of XPh4 on this medium.

The lysate from Lac⁺ isolate MPh21, obtained from MC4100 *proC*::Mu(cts)2, yielded blue plaques in this test. The titer of phage released was 10^7 phage/ml. The low yield of total phage is expected since homologous regions of DNA used in the integration of λ into the chromosome have been deleted. Ten percent of these phages carried the entire *phoA* gene. Surprisingly, at least one of the λ pphoA⁺ phage examined also carried a functional *proC* gene. This phage could complement a *proC* mutant at 37°C but not at 30°C. It may be that in the construction of this strain a fragment of the *proC* gene making an active product at 37°C was incorporated onto the λ phage.

We do not know to which promoter the *lacZ* gene is fused in MPh21. It may be fused either to a Mu promoter or to a promoter of a gene that is beyond the *proC* gene. That MPh21 does not contain a *proC-lac* fusion is suggested by

preliminary results which indicate that fusions of *lacZ* to *proC* can be obtained only when *lac* is inserted in the chromosome in the opposite orientation from that in strain MPh21 (A. Sarthy and J. Beckwith, unpublished data).

Isolation of λ phoA (deletion) phages and construction of a fine structure genetic map of the *phoA* gene. We anticipated that the phage lysate that contained the λ pphoA⁺ phage would also contain a series of λ phages carrying only portions of the *phoA* gene. These phages could then be used to construct a fine-structure genetic map of the *phoA* gene. Since the λ pphoA⁺ phage also carried the *proC* gene, we screened the λ proC⁺ phages from the phage lysate of MPh21 for those which carried part of the *phoA* gene, as described in Materials and Methods. From this screening, we obtained 13 phages which carried part but not all of the *phoA* gene. These phages were crossed with various *phoA* mutations, including point mutations, Tn5 insertions, and Mu insertions. The results of these crosses yield the deletion map of the *phoA* gene presented in Fig. 1.

Of the mapping phages used in this study which were tested, XPh202, -203, -211, -247, -250, -279, -290, and -296 were plaque-forming λ phages, whereas XPh207 and -231 were defective (S. Goldberg, personal communication). In characterizing these phages, we demonstrated that one of them, XPh203, contains an internal deletion of the *phoA* gene.

DISCUSSION

We have isolated a series of λ transducing phages which contain various segments of the *phoA* gene. The technique for obtaining these phages involves the use of a specialized λ transducing phage to first generate fusions of the *lac* operon to a promoter of a gene located near the *phoA* gene. Thus, the λ phage is positioned close to the *phoA* gene. Since the regions of homology used in the insertion of λ in this region have been removed by the fusion event, only aberrant excisions can generate λ phages. These λ phages occasionally carry either the entire *phoA* gene or portions of it. The technique described here is based on the approach developed in this laboratory for mapping of the *thr* and *malB* operons (12, 15). The dissimilarity in this technique is that we did not isolate λ transducing phages carrying the *lac* operon fusion itself, but those containing a functional *proC* gene along with regions of *phoA*.

These phages have allowed us to construct an unambiguous fine-structure map of the *phoA* gene. Our results indicate that some mutations in the previously published map (7) are incor-
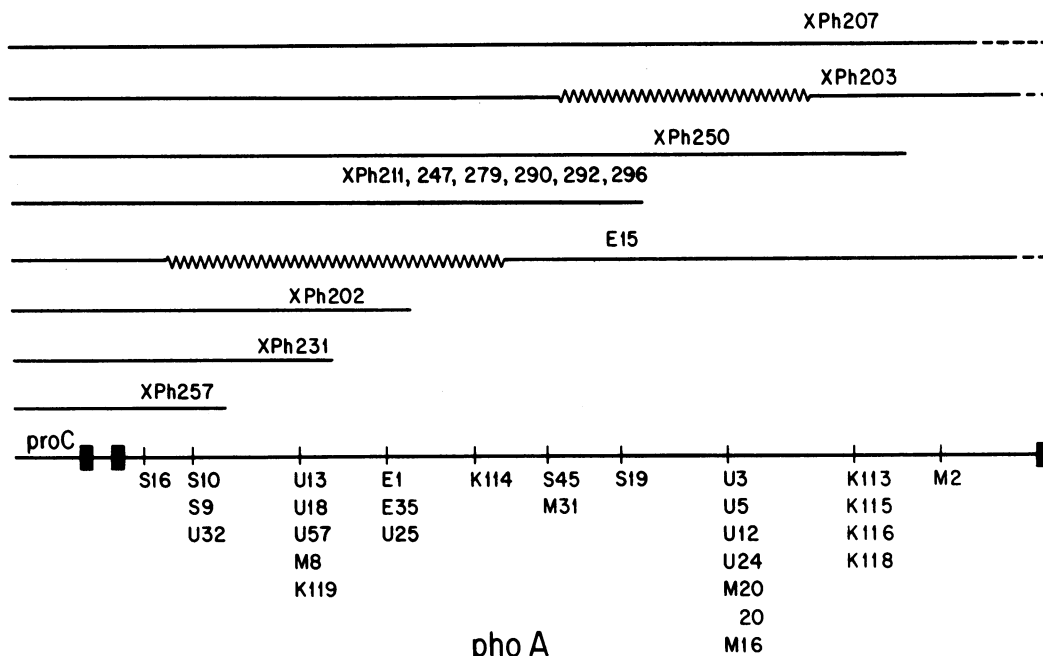
FIG. 1. *Deletion map of various mutations and insertions in the phoA gene. Heavy lines above the map represent the amount of phoA DNA carried by each phage. A wavy line represents a deletion of the material. Each phage also carries the proC gene. The endpoint at one end of the phoA gene present in XPh203 and XPh207 is not known and is represented by a dashed line. The phage carrying the E15 deletion was constructed by H. Inouye. phoA Δ20 has been described by Brickman and Beckwith (3). All of the other mutations either have been described by Fan et al. (7) or are from A. Garen's collection of phoA mutants. phoA mutations labeled M are Mu insertions, and those labeled K are Tn5 insertions.*

rectly positioned. The errors in the previous map may be due to the use of two-factor crosses, which do not always give an accurate map order (11). A deletion in the *phoA* gene, *E15*, which was originally characterized as spanning one end of the *phoA* gene (7), can be seen from this map to be an internal deletion in *phoA*, since it does recombine with a mutation in *phoA* which is close to the *proC* gene.

The λ transducing phages containing different segments of *phoA* have been used to map various Mu(cts) insertions in *phoA*. Using the Casadaban technique (4), we have isolated *lacZ* fusions to the *phoA* operon, starting with strains containing Mu(cts) insertions in *phoA*. The map presented here has allowed us to characterize the *phoA* region contained in *phoA-lacZ* fusions isolated on λ transducing phages (13). In addition, the accurate map of the *phoA* gene has allowed us to obtain evidence to establish that the direction of transcription of the *phoA* gene on the *E. coli* chromosome which was previously proposed is incorrect (14).

Finally, the λ p*phoA*⁺ phage described here provides a means of screening for mutants that affect the regulation and expression of *phoA*. It

is also being used to characterize *phoA* DNA corresponding to the promoter of the *phoA* gene and amino-terminal segment of alkaline phosphatase (H. Inouye, unpublished data).

We wish to point out that two new selective techniques for the genetic analysis of the alkaline phosphatase synthesis are reported in this paper: (i) a technique to screen for strains containing mutations which reduce expression of alkaline phosphatase synthesis (the *glpD* selection) and (ii) a positive selection for mutants that increase the expression of the *phoA* gene (use of XP-containing media).

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