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Construction from Mu d1 (*lac* Ap^r) Lysogens of Lambda Bacteriophage Bearing Promoter-*lac* Fusions: Isolation of λ p*pheA*-*lac*

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Bacteriophage Mu d1 (*lac* Apr^r) was used to obtain strains of *Escherichia coli* K-12 in which the *lac* genes are expressed from the promoter of *pheA*, the structural gene for the enzyme chorismate mutase P-prephenate dehydratase. A derivative of bacteriophage lambda which carries the *pheA-lac* fusion was prepared; the method used is generally applicable for the construction, from Mu d1 lysogens, of specialized transducing lambda phage carrying the promoter-*lac* fusions. A restriction enzyme cleavage map of λ p*pheA-lac* for the enzymes *Hin*dIII and *Pst*I is presented.

Bacteriophage Mu d1 (*lac* Ap^r) of Casadaban and Cohen (6) has been widely used to obtain *lac* operon fusions to aid in the study of the regulation of expression of a variety of operons in *Escherichia coli*. In this report, we describe a general method for the isolation, from a Mu d1 lysogen, of specialized transducing lambda derivatives carrying the promoter-*lac* fusion; we applied this method to the preparation of λ p*pheA-lac* phage from a strain in which Mu d1 (*lac* Ap^r) had been used to fuse the *lac* genes to the promoter of *pheA*, the structural gene for the enzyme chorismate mutase P-prephenate dehydratase (EC 5.4.99.5/4.2.1.51) (13). As discussed in this and in the accompanying paper (14), λ p*pheA-lac* offered several advantages over the original *pheA*::Mu d1 (*lac* Ap^r) fusion in elucidation of the mechanisms of transcriptional control of the *pheA* operon.

MATERIALS AND METHODS

Bacterial strains and phage. The bacterial strains used were all derivatives of *Escherichia coli* K-12 and are listed in Table 1.

λp1(209) was obtained from M. Casadaban (5). λ *vir*, λ *c*I *h*80 Δ*int*, λ *c*I *b*2, Mu-1, and Mu *cts* from our laboratory stocks. λ p*pheA-lac* was constructed for this study (see Fig. 2).

Chemicals. The chemicals used were obtained commercially and not further purified. 5-Bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) and *o*-nitrophenyl-β-D-galactoside were obtained from Sigma Chemical Co., St. Louis, Mo. Barium prephenate, prepared by the method of Cotton and Gibson (9), was a gift from B. E. Davidson.

Growth media. Unless otherwise specified, the minimal medium used was half-strength medium 56, as described by Monod et al. (23), supplemented with 0.2% glucose or other carbon sources as indicated, thiamine (10 μg/ml), and appropriate growth factors. The nutrient media used were Luria broth and nutrient agar (Oxoid Ltd., London). MacConkey agar was also from Oxoid. When the minimal medium was supplemented with repressing concentrations of the aromatic amino acids and vitamins (end products), these were added in the following concentrations: L-phenylalanine, 10^{-3} M; L-tyrosine, 10^{-3} M; L-tryptophan, 5×10^{-4} M; shikimic acid, 10^{-4} M; *p*-aminobenzoic acid, 10^{-6} M; *p*-hydroxybenzoic acid, 4×10^{-6} M; and 2,3-dihydroxybenzoic acid, 5×10^{-5} M.

Oxoid 3 agar was routinely used in the preparation of plates except for minimal plates with carbon sources other than glucose, for which the purer Oxoid 1 agar was used.

Tetracycline was used at a final concentration of 5 μg/ml in minimal medium and 15 μg/ml in nutrient medium; ampicillin, at a final concentration of 25 μg/ml; rifampin, at 100 μg/ml; and X-gal, at 40 μg/ml.

P1 *kc* transduction and conjugation. Transduction and conjugation of P1 *kc* were carried out by the methods previously described (24, 25).

Preparation of Mu d1 (*lac* Ap^r) lysate (from MAL103) and its use in mutagenesis. The method for preparing the Mu lysate described by Casadaban and Cohen (6) was followed.

Preparation of λ lysates. λ lysates were prepared by UV induction of lysogens or by lytic propagation in soft agar overlays on nutrient plates, as described by Miller (22).

Selection for λ lysogens. λ lysogens were obtained by spotting the phage lysate on a lawn of the sensitive bacterial strain; colonies from the center of the cleared areas were purified and tested for lysogeny by demonstration of their resistance to λ *c*I *b*2 and sensitivity to λ *vir* phage.

In an alternate method based on that described by Debarbouille and Schwartz (11), sensitive cells grown in Luria broth supplemented with 0.4% maltose were harvested in midexponential phase and mixed with λ phage at a multiplicity of infection of 0.1 to 0.5; after adsorption for 30 min at 37°C and further expression for 30 min, an excess of λ *c*I *h*80 Δ*int* or λ *c*I *b*2 was

TABLE 1. List of *E. coli* K-12 strains used

Strain	Genotype[a]	Origin or reference
MAL103	[Mu cts d1 (*lac* Ap^r)] [Mu cts] Δ(*proAB-lacIPOZYA*)XIII *rpsL*	M. Casadaban (6)
CA7027	HfrH Δ*lacU169 thi*	J. Beckwith
JP2867	*ilv-1 his-29* Δ*lacU169*	From JP2140 (3) by conjugation[b]
JP2869	*pheA pheAo351 rpoB361* Δ*lacU169*	C. S. Cobbett (unpublished data)
JP2999	*tyrA4 rpoB361* Δ*lacU169*	From JP2869 by P1 *kc* transduction
JP3036	Δ*lacU169 pheA370*::Mu d1 (*lac* Ap^r)·+ *rpoB361*	This work
JP3037	Δ*lacU169 pheA371*::Mu d1 (*lac* Ap^r)·− *rpoB361*	This work
JP3141	JP2999 (λ p*pheA-lac*)	This work
JP3145	JP3141 *aroB351 rpsL recA56 srl-1300*::Tn*10*	From JP3141 by P1 *kc* transduction
JP1638	*proC14 tyrR366 aroL476 rpsL*	R. DeFeyter (unpublished data)
JP2934	W3110 *trpR lacZU118 trpE trpA9605* Val^r Azi^r	C. Yanofsky
JP3311	*trpR thi* Δ*lacU169* Val^r Azi^r	From JP2934 through several intermediate strains
JP3312	JP3311 (λ p*pheA-lac*)	This work
JP3314	JP3311 (λ p*pheA-lac*)	This work
JP3317	JP3312 *pheR372 zjd-351*::Tn*10*[c]	By P1 *kc* transduction
JP3341	JP3314 *pheR372 zjd-351*::Tn*10*[c]	By P1 *kc* transduction

[a] The nomenclature for genetic symbols follows that described by Bachmann and Low (1) and for transpositional insertions described by Kleckner et al. (17). The orientation of Mu d1 (*lac* Ap^r) insertions in *pheA* is indicated as + or −, depending on whether the Mu *S* end is clockwise or anticlockwise, respectively, from the Mu *c* end on the *E. coli* genetic map (4). Allele numbers are indicated where they are known. Fermentation markers are not described.

[b] Δ*lacU169* was introduced into the different strains by one of two methods: (i) by conjugation with CA7027 or (ii) in two steps if the strain was originally Lac$^-$: transduction to Lac$^+$ Pro$^-$ by P1 *kc* (JP1638) and then to Pro$^+$ Δ*lac* by P1 *kc* (JP3037).

[c] *pheR372 zjd-351*::Tn*10* is described in the accompanying paper (14).

added and the mixture was spread on appropriate selection plates.

Isolation of phage λ DNA. Phage lysates were prepared in bulk by lytic propagation as described above, and the phage were pelleted by centrifugation at 90,000 × *g* for 1 h. The pellet was suspended in a small volume of 0.1 M Tris-hydrochoride buffer (pH 9.0), and the protein was removed by repeated extractions with phenol. The DNA was then obtained by ethanol precipitation at −70°C.

Restriction endonuclease digests. DNA was digested with the enzymes *Hin*dIII and *Pst*I and analyzed by standard methods of gel electrophoresis under the conditions previously described (10).

Chemostat experiments. *aroB* strains were used to effect conditions of phenylalanine limitation in continuous culture in the chemostat. The minimal medium used was supplemented with 10^{-4} M L-tyrosine, 10^{-4} M L-tryptophan, 2×10^{-5} M L-phenylalanine, and 10^{-6} M aromatic vitamins (2). This medium was sufficient to maintain a biomass of 2×10^8 to 3×10^8 cells per ml in the culture vessel, with phenylalanine being the limiting factor in growth. (The latter fact was established retrospectively during each run by demonstrating a linear increase in the biomass with an increase in the L-phenylalanine concentration in the fresh medium flowing into the culture vessel.) The volume of the culture was 500 ml, and the dilution rate was set at 0.1 to 0.2 per h. A period of five to six generations was allowed for the cells to achieve steady state before samples of 200 to 400 ml were taken directly from the culture vessel for enzyme assays.

Growth of cells for enzyme assays and preparation of cell extracts. Cells were harvested from 200-ml cultures at a turbidity of 160 Klett units; they were washed twice in chilled 0.9% saline and suspended in 4 ml of 0.1 M sodium phosphate buffer (pH 7.0). Cell breakage was achieved by using a French pressure cell at 20,000 lb/in^2. Cell extracts were obtained by centrifugation at 20,000 × *g* for 20 min. These extracts were always assayed for activity within 1 h.

Assay of prephenate dehydratase. The method used for the prephenate dehydratase assay was based on that described by Gething et al. (12) with the following modifications. The reaction mixture contained 500 μg of bovine serum albumin per ml, and the mixture was incubated at 37°C for 30 min before the reaction was terminated. The amount of phenylpyruvate formed was calculated with a molar extinction coefficient at 320 nm of 1.75×10^4 (9).

Other enzyme assays. The assay conditions for anthranilate synthetase have been described previously (25). Tryptophan synthetase was assayed by the method of Yanofsky (28). β-Galactosidase was assayed with toluene-treated cells as described by Miller (22), and the activity is expressed in the units described therein.

Protein estimations. Protein concentrations in cell extracts were determined by the method of Lowry et al. (21).

Enzyme activity. All enzyme activities other than those of β-galactosidase are expressed in international units. Specific activities are expressed as milliunits per milligram of protein in the cell extracts.

RESULTS

Isolation of Mu d1 (*lac* Ap^r) insertions in *pheA*. The method for the selection of *pheA*::Mu d1 (*lac* Ap^r) lysogens made use of the high cotransduction frequency between the *tyrA* and *pheA* genes (25). Strain JP2867 was mutagenized with Mu d1 (*lac* Ap^r) as described above, and Ap^r colonies were selected on nutrient agar plates

incubated at 30°C. Approximately 5,000 clones were pooled, and a P1 *kc* lysate prepared on the pool of cells was used to transduce JP2999 to Tyr^+ Ap^r, thereby selecting for strains with Mu d1 (*lac* Ap^r) insertions near *tyrA*. The transductants obtained were then scored for the Phe^- phenotype, by which those in which Mu d1 (*lac* Ap^r) had been inserted in *pheA* could be identified.

The initial Phe^- strains so obtained were all multiple lysogens for Mu d1 (*lac* Ap^r) in that they continued to be immune to Mu, resistant to ampicillin, and temperature sensitive even after transduction back to Phe^+. P1 *kc* lysates prepared on these strains were used to transduce JP2999 to Tyr^+, and the transductants were screened for the ones that had integrated *pheA*::Mu d1 (*lac* Ap^r). Even in this step, only 50% of the Phe^- Ap^r transductants obtained were single lysogens for Mu d1 (*lac* Ap^r), indicating that this phage, although defective, was capable of at least limited replication on zygotic induction in a sensitive recipient cell.

Two *pheA*::Mu d1 (*lac* Ap^r) lysogens, JP3036 and JP3037, were saved, and chromosome mobilization studies [with F(Ts) *lac*; data not shown] established that the *lac* genes were disposed on the chromosome in anticlockwise and clockwise orientations, respectively, in the two strains. The direction of *pheA* transcription is clockwise (30), and this suggested that in JP3037 the *lac* genes were being transcribed from the *pheA* promoter (*pheAp*).

This hypothesis was confirmed by growing JP3037 under phenylalanine limitation in the chemostat. As the strain was Phe^-, an *aroB* mutation was not necessary. Under these conditions, the β-galactosidase activity was derepressed fourfold (Table 2), indicating that the *lacZ* gene was being expressed from *pheAp* and was under phenylalanine-specific control. Derepression of *lacZ* could not, however, be demonstrated when the cells were starved for phenylalanine in batch culture, under conditions which had previously been shown to produce a threefold derepression of prephenate dehydratase activity (15); under these conditions, the activity of β-galactosidase actually showed a progressive decrease with an increasing period of starvation (data not shown). As expected, there was no derepression of β-galactosidase in JP3036 when it was limited for phenylalanine in the chemostat (Table 2).

TABLE 2. Specific activities of β-galactosidase and prephenate dehydratase in strains grown under phenylalanine limitation in a chemostat

Strain	Relevant genotype[a]	Sp act[b]	
		β-Galactosidase (U)	Prephenate dehydratase (mU/mg of protein)
JP3036	*pheA*::Mu d1 (*lac* Ap^r)·+[b]	12 (14)	—[c]
JP3037	*pheA*::Mu d1 (*lac* Ap^r)·−	420 (115)	—
JP3145	*aroB recA* (λ p*pheA-lac*)	480 (115)	75 (7)

[a] See Table 1, footnote *a*.
[b] Values in parentheses indicate the activities obtained when the strains were assayed simultaneously after growth in repressing concentrations of the aromatic end products. The units for β-galactosidase activity are those described by Miller (22).
[c] —, None detected.

Construction and characterization of λ p*pheA-lac*. λ p*pheA-lac* was constructed from JP3037 in two steps (Fig. 1 and 2). This method is different from the one employed by Komeda and Iino (18) in obtaining λ *hag-lac* phage from a *hag*::Mu d1 (*lac* Ap^r) strain.

In the first step, λp1(209) was used to lysogenize JP3037. λp1(209) is deleted for the λ *att* site and can integrate into the chromosome only by *recA*-dependent homologous recombination. In JP3037, integration could occur by (i) *lac* homology, (ii) Mu *c* end homology, or (iii) chromosomal *trp* homology (such lysogens would be Trp^-) (Fig. 1). Of these, only i and ii would give lysogens where λ could be crossed out by transducing the strains to Phe^+

The second step involved the induction of the above lysogens by UV irradiation and the use of the λ lysates so obtained to select for Lac^+ transductants in a Δ*lac* recipient. When induced, the predominant particles formed from lysogens of both classes i and ii would be λp1(209) by a homologous excision event, and these would be Lac^-. λp1(209) can, of course, integrate by *trp* homology to give Lac^+ lysogens, but these would be identical to the class iii lysogens discussed above and, being Trp^-, would not grow on lactose-minimal plates not supplemented with tryptophan. Lysogens of class i would also be expected to give, at low frequency, λ particles formed by imprecise excision that would carry *pheAp* (Fig. 2b); these particles would then give Lac^+ transductants that on subsequent UV induction would yield high-frequency transducing Lac^+ lysates. Lysogens of class ii, in which integration had occurred by Mu *c* homology, would not be expected to give Lac^+ transducing phage particles by any single imprecise excision event.

As there was no simple means of differentiating the lysogens of class i from those of class ii, 15 independent lysogens belonging to one or the other of these classes were UV induced, and the lysates were used to obtain Lac^+ transductants

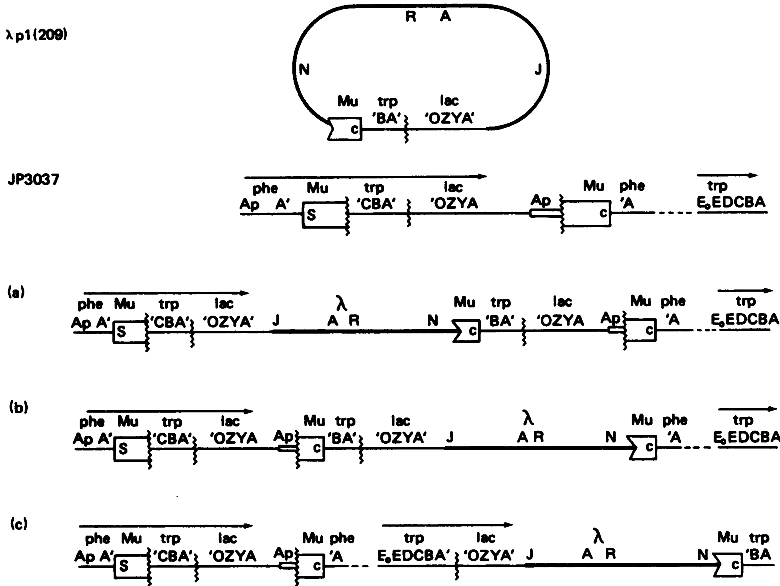

FIG. 1. Three possible modes of *recA*-mediated recombination of λp1(209) into JP3037 [*pheA371*::Mu d1 (*lac* Ap^r)]: (a) by *lac*$^+$ *trp'BA'* (in Mu d1) homology; (b) by Mu *c* homology; and (c) by chromosomal *trp* homology. In c, the *lac* genes are transcribed from *trpEop*, and *trpA* is not expressed. Not drawn to scale. Direction of transcription is indicated by arrows. A prime next to a genetic symbol indicates that it is deleted or interrupted on the side on which the prime is written.

in JP2999; the selection was effected on lactose-minimal plates. One of the lysates yielded Lac$^+$ transductants that, on subsequent UV induction, produced high-frequency transducing Lac$^+$ lysates; one of these was chosen for further investigation and shown to be λ p*pheA-lac* phage (see below). Nine of the fifteen original lysates gave no Lac$^+$ transductants, and the Lac$^+$ transductants obtained from the other five lysates failed to yield high-frequency transducing phage on subsequent UV induction. This unusual result was not investigated further.

That the *lac* genes in λ p*pheA-lac* lysogens were under the control of *pheAp* was reconfirmed by enzyme assays on an *aroB* derivative (JP3145) grown under conditions of phenylalanine limitation in the chemostat (Table 2). The β-galactosidase activity was derepressed four- to fivefold, as in JP3037, whereas prephenate dehydratase activity was derepressed 10- to 12-fold, as was reported previously (2). The reason for this discrepancy in the relative degree of derepression of the two enzymes is unclear; mutations that alter the regulation of *pheA* expression, described in the accompanying paper (14), also produced similar discrepancies in the extent to which they derepressed the activities of β-galactosidase and prephenate dehydratase in λ p*pheA-lac* lysogens. One possibility is that not all of the observed β-galactosidase activity in λ p*pheA-lac* fusion strains represents expression from *pheAp*, but that part of it may represent transcriptional expression from a constitutive promoter between *pheAp* and the start of the *lacZ* gene. An alternative explanation for the chemostat results is that β-galactosidase is unstable and is more rapidly turned over during growth-limiting conditions.

λ p*pheA-lac* lysogens were not Mu immune, indicating that the phage did not carry an intact Mu *c* gene; the phage was Spi$^+$ (it did not plaque on a P2 lysogen), and this localized the approximate point of excision on the right to that point shown in Fig. 2a. The exact point of excision on the left, beyond *pheAp*, has not been determined.

Notwithstanding the fact that the expression of the *lac* genes from a derepressed *pheAp* is quite substantial (14), plaques of λ p*pheA-lac* on a lawn of a Δ*lac* strain were only very faintly blue on X-gal indicator plates. This was an unexpected result, because other workers who have isolated promoter-*lac* fusions on λ have reported high levels of β-galactosidase expression during the lytic growth cycle of the phage (5, 18, 26, 27); however, in each of those instances the phage itself had initially been identified by its ability to form blue plaques on X-gal plates. Perhaps the intensity of the blue color of the plaques depends not so much upon the expression of *lacZ* from the promoter to which it has been fused as it does upon read-through

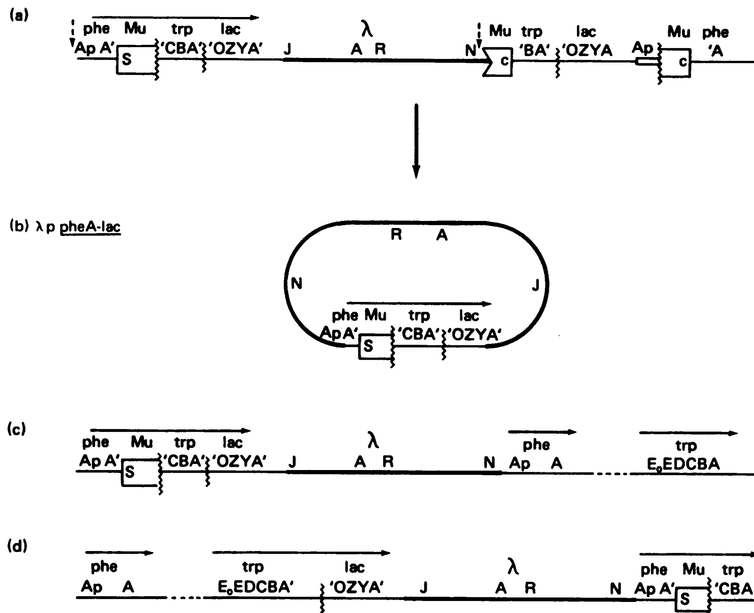

FIG. 2. Construction of λ p*pheA-lac*. (a) λpl(209) lysogen of JP3037 by lac^+ *trp'BA'* homologous integration (see Fig. 1a). (b) λ p*pheA-lac* obtained by imprecise excision on induction of the lysogen in a. Approximate points of excision are shown by broken-line arrows. (c and d) Lysogens obtained on integration of λ p*pheA-lac* by *pheA'* and *trp'CBA'* homology, respectively. Overhead arrows indicate the direction of transcription. In d, the *lac* genes are transcribed from *trpEop*, and *trpA* is transcribed from *pheAp*.

transcription from the powerful promoter p_L of λ itself. This would suggest then that in λ p*pheA-lac*, such read-through transcription is prevented by the presence of one or more termination signals in the substituted region upstream from the *lacZ* gene.

Restriction endonuclease cleavage map of λ p*pheA-lac*. The restriction endonuclease cleavage maps of λ p*pheA-lac* for the enzymes *Hin*dIII and *Pst*I and of λpl(209) (19) are shown in Fig. 3. The results showed that λ p*pheA-lac* lost one *Hin*dIII and one *Pst*I site located in the *c* end of Mu (16) and gained one new *Hin*dIII site within the *trp'CBA'* segment (7) and one *Pst*I site known to be located 160 nucleotides upstream from the start point of transcription of the *pheA* gene (31).

Integration of λ p*pheA-lac* by *trp'CBA'* homology. λ p*pheA-lac* integrated into the chromosome by homologous recombination at the *pheA-tyrA*

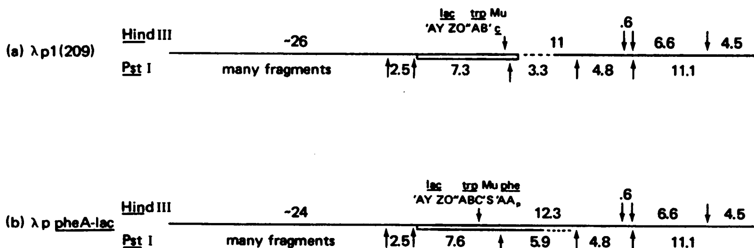

FIG. 3. *Hin*dIII and *Pst*I restriction endonuclease cleavage maps of (a) λpl(209) and (b) λ p*pheA-lac*. Open bars indicate regions of substituted DNA in the two phages. Cleavage sites for *Hin*dIII and *Pst*I are represented by vertical arrows above and below, respectively. The corresponding fragment sizes are indicated in kilobases. The gene organization within the substituted DNA segments is also shown. The precise extent of substituted DNA upstream from *pheAop* has not been established. The ordering of cleavage sites was achieved by reference to Leathers et al. (19) for *Hin*dIII sites in λpl(209) and to Cobbett and Pittard (8) for *Pst*I fragments of wild-type λ; the presence of one *Hin*dIII and one *Pst*I site in the Mu *c* region has been reported by Kahmann et al. (16). The positions of the *Hin*dIII site in the *trp'CBA'* segment and the *Pst*I site in the *pheAop* region of λ p*pheA-lac* were derived from the physical maps of pMC81 (7) and pKB45 (31), respectively.

locus (Fig. 2c), and this was demonstrated in JP3141, which is a λ *ppheA-lac* lysogen and also carries the *tyrA4* mutation. After transduction of the strain to Tyr^+, a large percentage of the transductants had become λ sensitive and Lac^-, indicating that λ *ppheA-lac* had been crossed out in the process. Another possibility is for λ *ppheA-lac* to integrate by *trp* homology (Fig. 2d); in these lysogens the *lac* genes would be under *trp* promoter (*trpEp*) control, and the strains would continue to be Trp^+, with the *trpA* gene being expressed from *pheAp*. The frequencies of these two different modes of integration were screened in the Δ*lac trpR* strain JP3311. Most (85%) of the lysogens obtained had a low level of β-galactosidase activity (100 U), which was consistent with expression from a repressed *pheAp*. The introduction of the mutation *pheR372* (16) into one of these lysogens (JP3312) resulted in a derepression of β-galactosidase activity (Table 3, JP3317). The other 15% of the lysogens obtained in JP3311 had a high β-galactosidase activity (1,000 U), which was consistent with the hypothesis that integration had occurred by *trp* homology and that the *lac* genes were being expressed from the derepressed *trpEp*. In one of these latter strains (JP3314) this was confirmed by assays of anthranilate synthetase (*trpE*) and tryptophan synthetase (*trpA* and *trpB*) (Table 3). Thus, whereas the former was elevated to the level expected in *trpR* strains, the latter was decreased to 5% of the activity seen in JP3312; furthermore, the tryptophan synthetase activity was now derepressed on the introduction of the *pheR* mutation (Table 3, JP3341), which is entirely in agreement with the model in which the *trpA* gene is now being expressed from *pheAp*. Thus, it would appear that λ *ppheA-lac* can integrate into the chromosome either by *pheA'* or by *trp* homology, the former process being approximately six times more frequent than the latter.

Instability of λ *ppheA-lac* lysogens. Lysogens of λ *ppheA-lac* were unstable in that they tended, at frequencies of between 4 and 10%, to give rise to colonies with one more or one less copy of the λ *ppheA-lac* phage integrated in the chromosome. Thus, a strain with one copy of λ *ppheA-lac* per chromosome would produce Lac^- λ^s colonies at a frequency of 4 to 10%; it would also produce at a similar frequency stronger Lac^+ colonies in which the β-galactosidase activity, still under the control of *pheAp* as demonstrated by chemostat studies (data not shown), would be twice that in the original strain. The magnitude of such instability was greater in the lysogens where λ *ppheA-lac* had integrated by *pheA'* homology than in those where the integration had occurred by *trp'CBA'* homology. The instability itself could be suppressed by making the strains *recA*, indicating that the underlying mechanism is RecA dependent and probably involves homologous "looping-out" or duplication of the λ *ppheA-lac* phage.

TABLE 3. Enzyme specific activities in *trpR* strains lysogenic for λ *ppheA-lac*[a]

Strain	Relevant genotype[b]	Sp act			
		Anthranilate synthetase (mU/mg)	Tryptophan synthetase (mU/mg)	Prephenate dehydratase (mU/mg)	β-Galactosidase (U)[c]
JP3312	*trpR* (λ *ppheA-lac*) (integrated by *pheA'* homology)	4.0	80	8	100
JP3317	JP3312 *pheR372*	4.0	75	86	605
JP3314	*trpR* (λ *ppheA-lac*) (integrated by *trp'CBA'* homology)	4.2	4.5	8	925
JP3341	JP3314 *pheR372*	4.1	20	90	990

[a] All strains were grown in repressing concentrations of the aromatic end products.
[b] See Table 1, footnote *a*.
[c] Units for β-galactosidase activity are those described by Miller (22).

DISCUSSION

Our studies with Mu d1 (*lac* Ap^r)-generated *pheA-lac* operon fusions showed that this defective bacteriophage underwent replication and transposition when transduced by P1 into a Mu-sensitive recipient cell. Consequently, it is possible that ampicillin-resistant strains obtained even after low multiplicities of infection with Mu d1 (*lac* Ap^r) may harbor more than one prophage; in studies of gene regulation by this system, therefore, it is necessary to verify that there is only one copy of the phage integrated in the genome.

Furthermore, the transposability of Mu d1 (*lac* Ap^r) rendered even single lysogens unsuitable for direct use in selecting for regulatory mutants with increased β-galactosidase activity. Thus, attempts to obtain mutants altered in the regulation of *pheA* in the *pheA*::Mu d1 (*lac* Ap^r) strain JP3037 with the lactose analog phenyl-β-D-galactoside were unsuccessful because the colonies that were obtained (at frequencies of 10^{-3} to 10^{-4} per plated cell had all undergone transposition of the Mu d1 (*lac* Ap^r) phage, and the *lac* genes were being expressed from additional strong promoters (unpublished data). Lee

et al. (20) reported the similar ability of Mu d1 (*lac* Ap^r) to transpose itself in *Salmonella typhimurium*.

On the other hand, the preparation from Mu d1 lysogens of bacteriophage λ derivatives carrying the promoter-*lac* fusions offers a method by which such *lac* operon fusions can subsequently be used for the isolation of regulatory mutants. Thus, λ p*pheA-lac* lysogens that have been made *recA* are quite stable and have been used reliably for the selection in phenyl-β-D-galactoside of *pheR* mutants (14).

The use of strains lysogenic for λ bearing the promoter-*lac* fusion also permits ready differentiation of regulatory mutations acting in *cis* from those acting in *trans*. Because such lysogens are essentially diploid for the regulatory region of interest, *trans*-acting mutations would be expected to alter not only β-galactosidase activity but also the expression of the wild-type gene; *cis*-acting mutations, on the other hand, would alter only β-galactosidase activity, and the expression of the wild-type gene would remain unchanged. This feature of λ p*pheA-lac* lysogens has been exploited to advantage in screening for *trans*-acting mutations that increase the expression of the *pheA* operon (14).

Strains with λ integrated by *trp'CBA'* homology could also, under appropriate conditions, be used to select for regulator "up-mutants," if the parent lysogen is Trp$^-$ because of the absence of expression of *trpA* from the promoter of interest, or for "down-mutants" by using the analog 5-methylanthranilic acid (29).

The method described for the preparation of λ p*pheA-lac* may be generally applicable for the isolation from Mu d1 lysogens of the promoter-*lac* fusions on λ phage. It has also been used successfully in obtaining λ p*tyrP-lac* transducing phage from *tyrP*::Mu d1 (*lac* Ap^r) lysogens (P. Kasian, personal communication).

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