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A New Locus of *Escherichia coli* That Determines Sensitivity to Bacteriophage φX174

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A new gene designated *phxB*, necessary for adsorption of φX174 to the cell surface of *Escherichia coli*, is located between *gal* and *aroG* on the *E. coli* chromosome.

Bacteriophage φX174 infects *Escherichia coli* strain C but not strain K-12. The difference is due to the absence of *phx*, a gene required for φX174 adsorption, in *E. coli* K-12. The *phx* gene is located near *xyl* at 79 min of the map of *E. coli* chromosome (1, 9). In this paper we describe another locus which is also required for adsorption of φX174. The gene(s) is closely linked to *gal* (at 17 min) and is present in both strains C and K-12. We propose that the new locus be designated *phxB* while the former (the original *phx*) is named *phxA*.

When *E. coli* C (Gal$^+$, φX174 sensitive) lysogenic for λcI857*xis*1 was plated on eosin-methylene blue galactose agar plates and incubated at 42°C, white-colored Gal$^-$ colonies were produced at high frequencies; with 11 independent lysogens, 11 to 19% of survivors were Gal$^-$. Since the λ strain used is defective in excision capacity, it is likely that portions of the bacterial genome adjacent to *att*λ are excised together with λ prophage. This notion was supported by the finding that among Gal$^+$ and Gal$^-$ survivors there were many Bio$^-$ and/or UR-sensitive mutants (Table 1). It has been shown that *bio* and *uvrB* genes are present at a region close to *att*λ, on the opposite side of the region that *gal* occupies (1).

It was unexpected, however, that most of Gal$^-$ mutants were resistant to φX174 though they remained sensitive to other phages (λ, P1, and T-phages). As shown in Table 1, 82% of Gal$^-$ survivors were φX174 resistant. A few partially resistant mutants, on which φX174 produced turbid plaques, were also found. In contrast, no φX174-resistant mutant was found among Gal$^+$ survivors. It was shown, moreover, that all the φX174-resistant Gal$^-$ mutants were Aro$^+$ and TolPAB$^+$. These results can be explained by the idea that a locus that determines sensitivity to φX174 is present between *gal* and *aroG* (Fig. 1).

The above notion was supported by the following experiments. (i) When φX174-resistant Gal$^-$ mutants were infected with P1 phages grown on *E. coli* K-12, all the Gal$^+$ transductants (e.g., strain RY544) were found to be sensitive to φX174. (ii) Introduction of F$_8$*gal*, an F prime plasmid carrying *gal* and the adjacent region, into φX174-resistant Gal$^-$ strains produced φX174-sensitive Gal$^+$ cells. (iii) When λ*gal8 bio69*, which carries the whole *gal* operon but not *aroG* (see Fig. 1), was applied to φX174-resistant Gal$^-$ recipients, all of the Gal$^+$ transductants exhibited the φX174-sensitive character. It seems, therefore, that the gene order in this region is *aroG-phxB-gal-att*λ.

Figure 2 shows kinetics of adsorption of φX174 to various strains. No adsorption of phage occurred with strain RY528 and strain C600, whereas a rapid adsorption took place with strain C. Thus, the normal function of *phxB* gene is required for adsorption of phage to the cell surface. The finding that the replicative form (double-stranded) of φX174 can produce phages in $CaCl_2$-treated cells of RY528 as well as of strain C also supports this view. It has been shown that attachment of φX174 to cell surface takes place in the lipopolysaccharide of the cell outer membrane (5, 6). Mutants in lipopolysaccharide biosynthesis alter colonial morphology and often sensitivity to phages (8, 10). However, *phxB* strains in this report exhibited normal

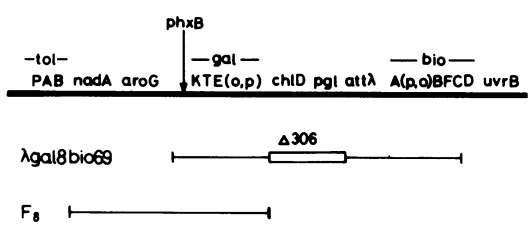

FIG. 1. *A genetic map near att*λ. *The data were taken from Bachmann et al. (1). The regions covered by* λ*gal8 bio69 (7) and F*$_8$*gal (11) are also presented.* Δ *denotes deletion.*

TABLE 1. *Properties of survivors derived from heat-induced E. coli C (λcI857xis1)*[a]

Selected markers	Unselected markers			No. of isolates
	φX174	Bio	UV	
E. coli C wild type	S	+	R	
Gal+	S	+	R	25
	S	−	S	23
Gal−	S	−	S	5
	R	+	R	8
	R	−	R	6
	R	−	S	18
	R/S	−	S	2

[a] Eleven independent strains of *E. coli* C lysogenic for λcI857*xis*1, which were altered to be resistant to λ (by selection with λ*vir*), were cultivated in broth to full growth, and a 0.2-ml portion of each culture was plated on EMB-galactose agar plates and incubated at 42°C for 24 h. Red-colored Gal+ and white-colored Gal− colonies were picked (about four colonies per lysogen) and purified. The Gal+ or Gal− character was verified by testing growth ability on minimal galactose agar supplemented with biotin. Each Gal+ or Gal− strain was then tested for unselected markers as follows; φX174 sensitivity (S) or resistance (R) by cross-streak test with phage φX174 on broth agar; Bio+ (ability to grow without added biotin) by streaking on minimal glucose agar plate supplemented with or without 0.1 μg of biotin per ml; UV (wild-type level of UV resistance or sensitivity) by spreading on broth agar and irradiating at 12 J/m^2 (Toshiba 15W germicidal lamp). R/S denotes partially φX174-resistant phenotype.

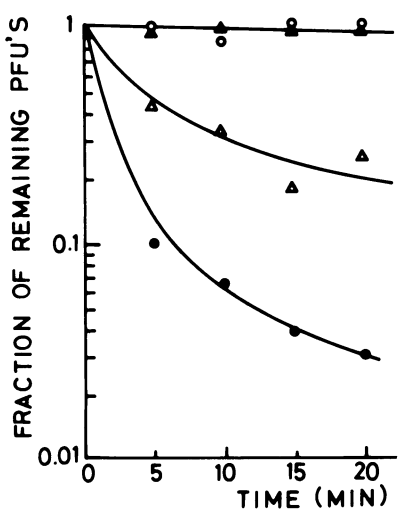

FIG. 2. *Kinetics of adsorption of φX174 to E. coli strains. Bacteria were grown in KC broth, harvested during exponential phase, and suspended at 10^9 cells per ml in the same broth containing 3 mM KCN. Prewarmed cell and phage suspensions were mixed to give a multiplicity of infection of 2 phage per cell, and the mixture was kept at 37°C. At times indicated, 0.1 ml of the mixture was withdrawn and added to 9.9 ml of a minimal medium saturated with chloroform. Appropriately diluted suspension was plated with E. coli C to titer number of unadsorbed phage (plaque-forming units [PFU]). (●) E. coli strain C (phxA+ phxB+); (○) strain RY528 [a deletion mutant derived from strain C; Δ(phxB-gal-uvrB)]; (▲) strain C600 (a derivative of strain K-12; phxA− phxB+); (△) strain RY530 (partially φX174-resistant strain derived from strain C).*

TABLE 2. *Adsorption of φX174 to E. coli with or without induction of the gal operon*[a]

Carbon source	Induction of *gal* operon	Strain		
		C	RY544	RY528
Glucose	−	1.4	1.8	65
Glycerol	−	3.3	2.0	93
Glycerol + D-fucose	+	3.4	1.6	NT[b]

[a] Overnight culture from each strain was centrifuged, washed once, resuspended in M9 minimal medium, and added to M9 minimal medium supplemented with 2.5 mM $CaCl_2$, 0.1 μg of biotin per ml, 1 μg of thiamine per ml, 400 μg of Casamino Acids per ml, and either 0.6% glucose or 1% glycerol with or without 5 mM D-fucose. After incubation at 37°C with shaking, the exponentially growing culture was centrifuged and resuspended in starvation buffer (3) at 10^9 cells per ml. φX174 was added at a multiplicity of infection of 2 and incubated at 37°C for 20 min. Other procedures were as described in Fig. 2. Data express percentage of remaining plaque-forming units. RY544 is a derivative of RY528, to which the *gal* region of *E. coli* K-12 was transferred by P1 transduction. Properties of other strains are described in the legend to Fig. 2.

[b] NT, Not tested.

colony form and normal growth rate as compared with parent strain C. Strain RY530, a partially φX174-resistant mutant, exhibited an intermediate adsorption curve (Fig. 2). Efficiency of transfection of strain RY530 with φX174 replicative form was four times higher than that of strain C. Although the nature of the mutation has not been elucidated, there is a possibility that the partial resistance is due to an independent alteration of another gene.

The rates of adsorption of φX174 to sensitive strains (strain C and RY544) were not affected by the states of expression of *gal* operon (Table 2). Cells grown in M9 minimal medium supplemented with glucose or glycerol, in which the *gal* operon is repressed, or cells grown in M9

minimal medium supplemented with glycerol and D-fucose, in which the *gal* operon is induced (2, 4), adsorbed ϕX174 equally well. Thus, the function of *phxB* is independent of the *gal* operon.

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