

A New Locus of *Escherichia coli* That Determines Sensitivity to Bacteriophage ϕ X174

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A new gene designated *phxB*, necessary for adsorption of ϕ X174 to the cell surface of *Escherichia coli*, is located between *gal* and *aroG* on the *E. coli* chromosome.

Bacteriophage ϕ X174 infects *Escherichia coli* strain C but not strain K-12. The difference is due to the absence of *phx*, a gene required for ϕ X174 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 ϕ X174. 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⁺, ϕ X174 sensitive) lysogenic for λ cl857 x isl 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 ϕ X174 though they remained sensitive to other phages (λ , P1, and T-phages). As shown in Table 1, 82% of Gal⁻ survivors were ϕ X174 resistant. A few partially resistant mutants, on which ϕ X174 produced turbid plaques, were also found. In contrast, no ϕ X174-resistant mutant was found among Gal⁺ survivors. It was shown, moreover, that all the ϕ X174-resistant Gal⁻ mutants were Aro⁺ and TolPAB⁺. These results can be explained by the idea that a locus that determines sensitivity to ϕ X174 is present between *gal* and *aroG* (Fig. 1).

The above notion was supported by the following experiments. (i) When ϕ X174-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 ϕ X174. (ii) Introduction of F₂*gal*, an F prime plasmid carrying *gal* and the adjacent region, into ϕ X174-resistant Gal⁻ strains produced ϕ X174-sensitive Gal⁺ cells. (iii) When λ *gal* δ *bio69*, which carries the whole *gal* operon but not *aroG* (see Fig. 1), was applied to ϕ X174-resistant Gal⁻ recipients, all of the Gal⁺ transductants exhibited the ϕ X174-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 ϕ X174 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 ϕ X174 can produce phages in CaCl₂-treated cells of RY528 as well as of strain C also supports this view. It has been shown that attachment of ϕ X174 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

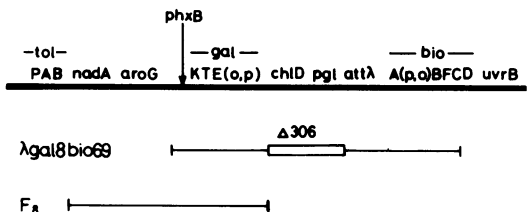


FIG. 1. A genetic map near *att* λ . The data were taken from Bachmann et al. (1). The regions covered by λ *gal* δ *bio69* (7) and F₂*gal* (11) are also presented. Δ denotes deletion.

TABLE 1. Properties of survivors derived from heat-induced *E. coli* C (λ I857xis1)^a

Selected markers	Unselected markers			No. of isolates
	ϕ X174	Bio	UV	
<i>E. coli</i> 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 λ I857xis1, 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; ϕ X174 sensitivity (S) or resistance (R) by cross-streak test with phage ϕ X174 on broth agar; Bio⁺ (ability to grow without added biotin) by streaking on minimal glucose agar plate supplemented with or without 0.1 μ g of biotin per ml; UV (wild-type level of UV resistance or sensitivity) by spreading on broth agar and irradiating at 12 J/m² (Toshiba 15W germicidal lamp). R/S denotes partially ϕ X174-resistant phenotype.

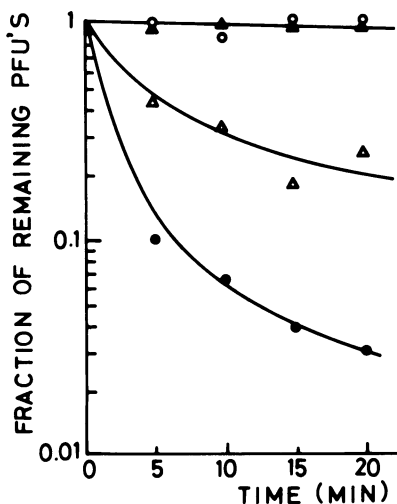


FIG. 2. Kinetics of adsorption of ϕ X174 to *E. coli* strains. Bacteria were grown in KC broth, harvested during exponential phase, and suspended at 10⁸ cells

TABLE 2. Adsorption of ϕ X174 to *E. coli* with or without induction of the *gal* operon^a

Carbon source	Induction of <i>gal</i> 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 CaCl₂, 0.1 μ g of biotin per ml, 1 μ g of thiamine per ml, 400 μ g 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 10⁹ cells per ml. ϕ X174 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 ϕ X174-resistant mutant, exhibited an intermediate adsorption curve (Fig. 2). Efficiency of transfection of strain RY530 with ϕ X174 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 ϕ X174 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

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 ϕ X174-resistant strain derived from strain C).

minimal medium supplemented with glycerol and D-fucose, in which the *gal* operon is induced (2, 4), adsorbed ϕ X174 equally well. Thus, the function of *phxB* is independent of the *gal* operon.

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