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Gene Responsible for Superinfection Exclusion of Heteroimmune Corynebacteriophage

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Wild-type β and γ corynebacteriophages are heteroimmune and infect lysogens of each other productively. Unlike their wild-type counterparts, the *bin* mutants of each phage are excluded in lysogens carrying the heteroimmune phage. The wild-type phages overcome exclusion by means of the *bin* gene product which appears to act as an antirepressor. When repression is lifted, exclusion of *bin* mutants is abolished (N. Groman and M. Rabin, J. Virol. **28**:28–33, 1978; J. Virol. **36**:526–532, 1980). It has not been clear whether the excluding compound is the immune repressor itself or one whose synthesis is positively regulated by repressor. We have isolated β exclusion mutants (*xcl*) that as prophage exhibited normal immune repression but no longer excluded γ-*bin* mutants. Furthermore, we have shown that an *xcl* phage with an active immune repressor acted in *trans* to continue the positive regulation of exclusion by a second *xcl*$^+$ prophage whose immune repressor was inactivated. From these results it was concluded that there is a gene distinct from the *imm* gene which is directly or indirectly responsible for exclusion. The *xcl* gene, mapped in prophage crosses, was located between *imm* and *bin*, that is, in the regulatory region of the phage genome. The simplest hypothesis compatible with the established observations is that β immune repressor regulates the expression of the *xcl* and *bin* genes, the former positively and the latter negatively. It is likely that an analogous regulatory model applies to γ phage since it has already been shown that both β and γ have *bin* alleles.

Corynebacteriophages β and γ are heteroimmune; that is, they superinfect lysogens of one another productively. However, certain mutants of γ phage (*bin* mutants) are excluded in β lysogens, and exclusion is presumably due to an inhibitor synthesized by β prophage (3, 4). Mutants of β phage analogous to the *bin* mutants of γ, that is, which fail to plaque productively on C7 (γ), have also been isolated. The data on γ-*bin* exclusion suggest that the excluding compound is either β immune repressor itself or an unknown compound synthesized during the prophage state which is positively regulated by immune repressor. In a cross between β-*bin* and γ-*bin* phages, a recombinant was isolated which retained the immune specificity of β prophage but lacked the ability to exclude γ-*bin* (4). This suggested that the genes for β immune repression and exclusion are distinct. We have now isolated exclusion-deficient (*xcl*) β mutants. The gene for exclusion has been mapped and is distinct from the *imm* gene for β repressor.

MATERIALS AND METHODS

Bacteria and phage. Bacterial strains C7, C7/β, C7 (β), and C7 (γ) were taken from our stock collection. The phages used were wild-type β and γ, two heat-inducible mutants, β-*tsr*-3 and β-*h' tsr*-3 (1), and γ-*bin*-6 and γ-*bin*-10 which are excluded by C7 (β) (3). Phages β-*bin*-104, -106, -109, and -110 are excluded by C7 (γ). Phage β-*xcl bin*-6 is a recombinant between wild-type β and γ-*bin*-6 which is excluded by C7 (γ) and which as prophage has lost the ability to exclude γ-*bin* phages (4). The immunity relationships between the various phages and their pertinent host ranges are illustrated in Table 1. In addition to the bacterial strains listed above, many single and double lysogens of various phages were employed, and these will be described in the appropriate experiments.

The *bin* marker designation was originally given to mutants of γ phage which were excluded by C7 (β) lysogens (3). Subsequently, it was shown that β phage contained an allele for the *bin* gene and that β mutants could be isolated which were excluded by C7 (γ). Since the evidence indicated that these phages are mutant in a β allele of *bin* (4), we have extended the *bin* genotype designation to these mutants as well. For convenience, all *bin* mutations of γ origin are numbered from 1 to 99, whereas those of β origin are numbered from 100 to 199. The β or γ prefix attached to phage designations indicates the immunity of the phage.

Media and methods. All phage techniques, including phage assays and activity tests, isolation of lysogens, tests for lysogeny, and production of phage stocks by UV induction, were described previously (5), as was the tryptose yeast extract medium employed in these

TABLE 1. Host range and immune specificity of some corynebacteriophages

Bacterial strain	Host range and immune specificity[a] of phage				
	β	β-*bin*[b]	β-*h'*	γ	γ-*bin*
C7	+	+	+	+	+
C7 (β)	−	−	−	+	−
C7 (β-*xcl*)[b]	−	−	−	+	+
C7 (γ)	+	−	+	−	−
C7/β	−	−	+	+	+

[a] Symbols: +, active; −, inactive.
[b] Phage β-*xcl bin* gave the same responses.

procedures. The procedure for mutagenization of phage with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was also described (6). Plaque counts were generally performed at 30°C, and, unless otherwise stated, all other incubations were carried out at 37°C. The restrictive temperature for heat-inducible β-*tsr* mutants was 38°C.

Selection of *xcl* mutants. Strain C7 (β) and subsequently C7 (β-*bin*) were mutagenized with nitrosoguanidine and plated to ensure development of well-isolated colonies. We first tried the "nibbling" procedure described by Susskind et al. (8) to identify *xcl* clones. Mutagenized cells were plated on tryptose yeast extract agar plates seeded with various dilutions of γ-*bin*-6, and after 24 and 48 h of incubation the plates were searched for nibbled colonies. This technique was not successful, and a modified procedure was employed. Mutagenized cells were diluted, plated, and incubated at 30°C for 24 to 36 hours. From plates with well-isolated colonies, samples of individual colonies were "streaked" with a sterile toothpick to a tryptose yeast extract plate previously seeded with 10^7 γ-*bin*-6 phage. Each streak was approximately 5 mm in length, and about 50 colonies could be tested on one plate. Strain C7 (β-*xcl bin*-6) was employed as a positive control. Clones with an *xcl* phenotype were easily detected by the extensive lysis they underwent.

Isolation of homoimmune double lysogens. Homoimmune double lysogens carrying both an *xcl* and *xcl*$^+$ phage were produced by superinfecting a monolysogen carrying an *xcl*$^+$ heat-inducible (*tsr*) mutant strain with a heat-stable *xcl* phage at the restrictive, that is, inducing temperature. Under these conditions, the superinfecting phage which produces a heat-stable repressor can rescue some of the *tsr*-carrying cells by forming a double lysogen. An agar base plate was overlaid with soft agar containing ca. 10^7 cells of a *tsr*-carrying monolysogen. The heat-stable phage (ca. 5×10^8/ml) was spotted at two or three points on the overlay, and the plates were incubated overnight at room temperature to allow double lysogens to form and stabilize. The plates were then incubated at 38°C for an additional 24 h during which the *tsr*-carrying monolysogens induced, leaving a clear plate with a small number of colonies in the area where the superinfecting phage had been spotted. These colonies were then streaked for purification and tested to determine whether they were double lysogens, revertant heat-resistant monolysogens, or monolysogens in which heat-insensitive superinfecting phage had displaced the original prophage.

Purified clones were tested as follows to establish double lysogeny with a *tsr* and non-*tsr* phage. Individual colonies of the isolate were grown to the logarithmic phase in tryptose yeast extract medium containing 0.2% Tween-80 (to prevent readsorption of phage). The cells were washed free of phage, suspended in Tween-broth, and induced with UV light. After incubation for 3 h at 35°C, the cells were removed by centrifugation and the supernatant was filter sterilized. The sterile filtrate was diluted, plated with C7 indicator, and incubated overnight at 38°C. If a significant proportion of the plaques were both clear (*tsr*) and turbid (non-*tsr*), then the strain was a double lysogen. Subsequently, these strains were tested for their exclusion trait by testing their sensitivity to γ-*bin* phage.

Genetic techniques. The orientation of prophage markers in a double lysogen was determined as previously described (2). The method depends on determining the proportionate representation of various classes of phage, that is, parental classes (P1 and P2) and recombinant classes (R1 and R2) in the UV-induced lysate. The markers in the predominant R1 class provide the essential clues to the orientation of the prophages.

The gene for exclusion was mapped by prophage crosses. Phage from UV-induced homoimmune double lysogens carrying β-*tsr xcl*$^+$ *bin*$^+$ and β-*tsr*$^+$ *xcl bin* phages was plated on C7 for isolated plaques at 38°C, the restrictive temperature. The phenotypes of four phages, β-*tsr bin*$^+$, β-*tsr bin*, β-*tsr*$^+$ *bin*$^+$, and β-*tsr*$^+$ *bin*, could be distinguished. The *tsr* phages produced clear plaques, and the non-*tsr* phages produced turbid plaques at 38°C. The *bin*$^+$ phages plaqued on C7 (γ), and the *bin* phages did not. The orientation of the phage markers was established as noted above. Since the ability to exclude is only expressed in the prophage state, a C7 lysogen was isolated for each phage to be tested for its exclusion trait. In mapping the *xcl* gene, we noted that the characteristics of recombinant phage β-*xcl bin*-6 (4) strongly suggested that a gene for exclusion was located between the *imm* and *bin* genes on the β prophage map. Therefore, from these crosses, attempts were made to isolate C7 lysogens of all progeny which exhibited recombination between the parental *imm* and *bin* markers. In the crosses between homoimmune β phages, we assumed that the *tsr*$^+$ and *tsr* markers distinguished the *imm* alleles of the two prophages.

RESULTS

Isolation of nonexcluding (*xcl*) β mutants. In our initial efforts to isolate *xcl* β mutants, strain C7 (β) was mutagenized with nitrosoguanidine, and surviving colonies were tested as described in Materials and Methods for acquisition of sensitivity to γ-*bin* phage. No sensitive isolates were found in a screen of over 1,830 colonies ($P < 0.05\%$). Since we expected to find such mutants at a frequency similar to that for other mutations (ca. 0.2%), it occurred to us that something might be interfering with their isolation. We noted that the single *xcl* strain available, a recombinant of β wild type and γ-*bin*

phages (4), was also *bin*. This suggested that prior mutation in the *bin* gene might enhance the isolation of *xcl* mutants.

Strains of C7 (β-*bin*) carrying β-*bin*-104, -106, -109, or -110 were mutagenized, and surviving clones were checked for their sensitivity to γ-*bin*. Mutant clones exhibiting the *xcl* phenotype were isolated from all four strains at an overall frequency of 0.5%. The characteristics of these strains and their phages are given in Table 1. Two of the mutant isolates, one carrying β-*bin*-104, the other carrying β-*bin*-109, produced phages which retained the *bin* trait, that is, were excluded on C7 (γ). A new C7 lysogen carrying β-*bin*-104 also exhibited the *xcl* phenotype, but the β-*bin*-109 phage produced by the *xcl* mutant clone could not be relysogenized. The *xcl* clones carrying β-*bin*-106 and β-*bin*-110 did not produce any phage at all. Thus, three of the four *xcl* clones were defective in some step in the lysogenic cycle, either failing to excise or produce infective phage or failing to integrate normally at a detectable frequency. The mutagenization of C7 (β-*bin*-104) was repeated a second time, and again an *xcl* mutant phage was isolated which exhibited normal lytic and lysogenizing properties.

These results clearly show that the isolation of *xcl* phages was facilitated by a prior *bin* mutation. This suggests that the *xcl* gene product plays some role in regulating the *bin* gene product, which directly or indirectly produces antirepressor action (4) and appears to explain the need for a preceding *bin* mutation. Hypothetically a *bin*$^+$ prophage mutated to the *xcl* state would induce, and therefore *bin*$^+$ *xcl* lysogens would not be isolated. However, as seen later, a paradox remains for we have isolated *xcl bin*$^+$ recombinants of β phage which readily and stably lysogenize C7. A second point of interest in these results is that three of the four *xcl* clones harbored phage which was defective in some step in the lysogenic cycle. There are two possible explanations for this concurrence. The first is that the *xcl* gene product is involved in lysogeny, and the second is that the *xcl* gene is located close to other genes concerned with lysogeny, and these effects on lysogeny are due to multiple mutations produced by nitrosoguanidine.

Exclusion in double lysogens. The isolation of *xcl* mutants that exhibit normal β repression strongly suggests, but does not prove, that the excluding inhibitor is distinct from β repressor. For example, repressor could be bifunctional, and an altered repressor could have lost one function while retaining the other. However, further proof of this distinction was obtained by examining the ability of certain homoimmune double lysogens to exclude γ-*bin*. Each of the double lysogens carried one heat-inducible phage (β-*tsr*-3 or β-*h' tsr*-3) and one nonexcluding phage (β-*xcl bin*) which produces a heat-stable repressor. A double lysogen carrying both of these phages is stable at the restrictive temperature due, presumably, to the presence of the heat-stable repressor.

The responses of monolysogens and double lysogens to superinfection are given in Table 2. The crucial observation is that at the restrictive temperature γ-*bin* was not excluded in monolysogens carrying either the β-*tsr*-3 or the β-*xcl* phages, whereas in double lysogens carrying both of these phages exclusion occurred. Thus, the combination of phages was able to exclude γ-*bin* under restrictive conditions (that is, at 38°C), whereas the repressor of neither phage could accomplish this. We conclude that at 38°C the heat-stable repressor acts in *trans* to maintain exclusion by the β-*tsr*-3 phage. These results demonstrate that β repressor itself is not the excluding compound but that it positively regulates the production of the excluding compound as previously postulated. The results also show that exclusion is dominant to nonexclusion.

Mapping the *xcl* gene. The gene for exclusion (*xcl*) was mapped by prophage crosses. The rationale for the cross is given in Fig. 1. Double

TABLE 2. Exclusion of γ-*bin* by selected double lysogens[a]

Bacterial strain	Superinfecting phage			
	γ		γ-*bin*	
	30°C	38°C	30°C	38°C
C7 (β-*h' tsr*-3) C7 (β-*tsr*-3)	+	+	−	+[b]
C7 (β-*xcl bin*-106) C7 (β-*xcl bin*-104) C7 (β-*xcl bin*-109)	+	+	+	+
C7 (β-*h'tsr*-3) (β-*xcl bin*-106) C7 (β-*tsr*-3) (β-*xcl bin*-104) C7 (β-*tsr*-3) (β-*xcl bin*-109)	+	+	−	−

[a] Agar overlays were seeded with the bacterial strains. Two dilutions of each phage were spotted on a pair of plates containing each bacterial strain, and one plate was incubated at 30°C and the other at 38°C. Symbols: +, indicates production of infectious centers and no exclusion; −, indicates lack of productive infection and hence exclusion.

[b] The spot test described above cannot be used at 38°C with the two *tsr* strains since they are induced at this temperature and the agar overlay is totally cleared. To test for infectivity, a broth culture of these two strains was infected with γ-*bin* at 38°C at a low multiplicity of infection, and the number of γ-*bin* infectious centers was determined as a measure of productive infections.

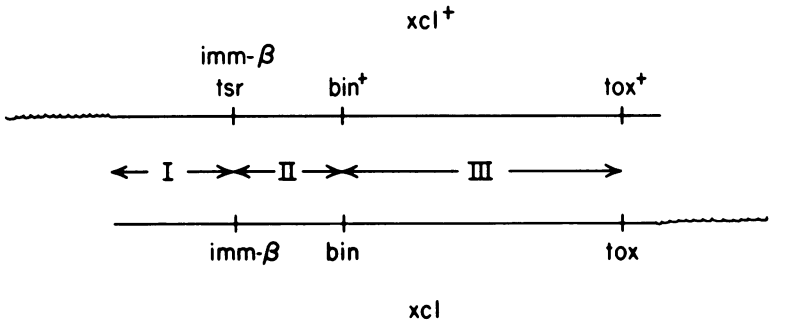

FIG. 1. Prophage mapping of the *xcl* gene. In the cross shown, the two prophages are in tandem and their DNAs are continuous with the bacterial DNA (wavy line). In each cross, one parent prophage is xcl^+ and the other is *xcl*. The prophage genomes are shown in an offset position to make it easier to visualize the cross. The orientation of the prophage markers and identification of the various classes of phage are described in the text. Assuming single crossing-over events, the following is predicted for the recombinants generated from the above cross. If the exclusion gene is in region I or if it is in region II but tightly linked to the *imm* gene, then all β-*tsr* recombinants will be xcl^+ and all of the β recombinants will be *xcl*, a finding that will not identify the region in which *xcl* is located. However, if *xcl* is in region II and not tightly linked to either *imm* or *bin*, then *xcl* recombinants could be found in either class of *imm-bin* recombinants and this would place the *xcl* gene in region II. Finally, if *xcl* is located in region III and is not tightly linked to *bin* or *tox*, then *xcl* recombinants could be found in either class of *bin-tox* recombinants and this would place the *xcl* genes in region III. As noted in the text, to test a phage for its exclusion phenotype, a lysogen must first be isolated. This technically limits the numbers of phage which can be tested for *xcl*.

lysogens containing an appropriately marked xcl^+ and *xcl* β phage were UV induced, and the resulting phage progeny were plaqued and then characterized (Table 3). Of the 400 plaques tested, a total of 17 contained recombinant phage produced by crossing-over in the region between the immunity gene (*imm*) and the antirepressor gene (*bin*). Since previous data (4) suggested that *xcl* might be located between *imm* and *bin*, we examined the exclusion phenotype of these recombinants by isolating C7 lysogens of as many as possible and testing them for sensitivity to γ-*bin* phage. Of the seven β-bin^+ recombinants isolated from the first lysogen, three were xcl^+, two were *xcl*, and two could not be relysogenized. Of the nine β-bin^+ recombinants isolated from the second double lysogen, six were xcl^+, and three were nonlysogenizable. The single β-*tsr bin* recombinant was xcl^+. In a third cross involving double lysogen C7 (β-*tsr*-3 *h' tox*$^+$) (β-*xcl bin*), the phenotype of 243 phage progeny was tested. One recombinant was isolated in which the crossing-over event occurred between the *imm* and *bin* genes. It had the genotype β-xcl^+ bin^+ tox^+. Assuming that all of the recombinants examined for their exclusion trait were produced by single crossing-over events, it can be concluded from these results (Fig. 1) that the gene for exclusion is located between the *imm* and *bin* genes. This location was previously suggested by the characteristics of β-*bin*-106, a *bin xcl* recombinant produced in a mating of γ-bin^+ and β-*bin* (4). As an added check on the location of the *xcl* gene, we examined the possibility that *xcl* was actually located to the right of *bin* and that *xcl* recombinants were products of a double crossing-over event. C7 lysogens of 10 β-*bin* tox^+ (R1 class) recombinants from the cross in the third experiment were tested for exclusion, and as expected all were negative. In addition, four R1 class phages, from each of the first two crosses, were similarly tested, and as expected those from the first cross were *xcl* and those from the second were xcl^+. All of these results were consonant with the conclusion that *xcl* lies between *imm* and *bin* and not to the right of *bin*.

The results of crosses from the two prophage

TABLE 3. Prophage mapping of the exclusion (*xcl*) gene

Expt no.	Bacterial strain[a]	No. of phage progeny			
		β-tsr^+ *bin*	β-*tsr* bin^+	β-tsr^+ bin^+	β-*tsr bin*
1	C7 (β_1) (β_2)	77	20	3	0
2	C7 (β_1) (β_2)	59	37	4	0
1	C7 (β_2) (β_1)	44	48	7	1
2	C7 (β_2) (β_1)	44	54	2	0

[a] The prophages are: β_1 = β-*tsr* xcl^+ bin^+; β_2 = β-tsr^+ *xcl bin*. Orientation of prophages in the double lysogen is given by the order of the phages. The double lysogens were UV-induced, and the phage progeny were plaqued and then characterized as described in the text.

orientations were in full accord with the proposed position of the *xcl* gene, but two aspects of the data derived from double lysogen C7 $(\beta_2)(\beta_1)$ need to be noted. First, we would have predicted that the major recombinant class resulting from crossing-over between *imm* and *bin* in this strain would be β-*tsr bin*, whereas it was the β-*tsr bin*$^+$ class. The second anomaly was that all of the phages within this latter class were *xcl*$^+$, not the mixture of *xcl*$^+$ and *xcl* recombinants which were isolated from the first double lysogen, C7 $(\beta_1)(\beta_2)$.

Relationship of the β and γ *bin* genes. In making various deductions and inferences regarding the behavior of the β-*bin* and γ-*bin* phages, we have assumed that the *bin* genes in these phages are allelic. For example, in isolating β-*xcl* mutants we have used β-*bin* mutants assuming they were mutant in the same gene as the γ-*bin* mutants. Similarly, in mapping the *xcl* gene, we have assumed that the *bin* gene of β is located in the same relative site with respect to *imm* as the *bin* gene of γ. Although mapping of the β-*bin* gene would provide some estimate of its position relative to the γ-*bin* gene, there are so many factors that affect map distances that at best one could only expect the result to be an approximation.

We have approached the problem of genetic identity through an examination of the frequency of recombination between the *bin* mutants of β and γ. We already know (4) that when γ-*bin* is plaqued on C7 (β), γ recombinants which are *bin*$^+$ are formed. This proves that β and γ carry *bin* alleles but does not prove that the β-*bin* mutants we have employed are mutant in that particular gene. We have reasoned that if the β-*bin* and γ-*bin* mutations are in the same gene, that the frequency of γ-*bin*$^+$ recombinants resulting from plaquing γ-*bin* on C7 lysogens carrying various β-*bin* mutants should be no greater and in all probability should be less than that from platings on C7 (β) where the β-phage carries a wild-type *bin* gene. The results of these tests (Table 4) show that the frequency of recombination is greater with wild-type β than with any of the β-*bin* mutants. The differences range from 6- to 100-fold greater and were observed with two different γ-*bin* mutants. The relative shift in frequencies as between the two γ-*bin* mutants is to be expected assuming these two phages have mutated at different sites. In theory, this approach could be used to map the location of the *bin* mutations relative to one another, but more work would be required to establish the degree of refinement necessary to that purpose. The results obtained are consonant with the interpretation that the *bin* mutations of both β and γ are in the same gene.

We have assumed in the above discussion that efficiency of plating measurements reflect recombination between superinfecting phage and prophage rather than the back mutation rate of the γ-*bin* phages to wild type. As noted above, we have previously shown that when γ-*bin* is plaqued on C7 (β), the phages producing these plaques are recombinants (4). In the present experiment, we have verified that phage from plaques on the various C7 (β-*bin*) indicators have also acquired the γ wild-type phenotype, but verifying that recombination has occurred is technically too demanding to be carried out in this larger series of experiments. However, the fact that the efficiency of plating measurements of each γ-*bin* phage differed so greatly on the various C7 (β-*bin*) strains, all of which have a common C7 background, supports the view that we are measuring recombination and not back mutation rate.

TABLE 4. Test for identity of the *bin* genes of β and γ

Bacterial indicator	Phage relative EOP[a]	
	γ-*bin*-6	γ-*bin*-10
C7	1	1
C7 (β)	7.5×10^{-5}	2.5×10^{-5}
C7 (β-*bin*-104)	9×10^{-6}	5.4×10^{-7}
C7 (β-*bin*-106)	8×10^{-7}	8.1×10^{-7}
C7 (β-*bin*-109)	1.2×10^{-5}	1.6×10^{-6}
C7 (β-*bin*-110)	8.6×10^{-6}	2.1×10^{-6}

[a] EOP, efficiency of plating.

DISCUSSION

The phenomenon of exclusion in heteroimmune corynebacteriophages β and γ was revealed by the isolation of mutants (*bin*) which, unlike their wild-type counterparts, failed to plaque on lysogens carrying the heteroimmune bacteriophage (3). Subsequently, it was shown that the *bin* gene in wild-type phage codes for antirepressor action (4). Lowering the repressor level lifts exclusion and permits the superinfecting phage to complete a productive cycle. Mutation in *bin*, leading to loss of an active or functional level of antirepressor, prevents the *bin* mutant from lifting exclusion. The *bin* mutants appear to initiate the vegetative cycle but cannot complete it. Thus, directly or indirectly, the immune repressor regulates the *bin* gene negatively, a requirement for the establishment and maintenance of its lysogenic state, and regulates exclusion positively.

Up to the present time it has not been clear whether the compound that excludes the *bin* mutants was the immune repressor or some other gene product. Two pieces of evidence presented in this paper support the conclusion that the inhibitor responsible for exclusion is

distinct from the immune repressor. One is the isolation of *xcl* mutants in which the immunity response is unaffected. The second is the restoration of excluding ability in double lysogens in which the repressor of neither is sufficient to maintain exclusion. The only ways in which repressor might still be associated with exclusion is (i) if repressor monomers from each phage, each of which is now inactive in exclusion, combined to form an active excluding oligomer, or (ii) if induced inactive immune repressor combined with the product of the *xcl* gene to produce exclusion.

At the present time, the simplest hypothesis with respect to these interactions is that β immune repressor regulates the *xcl* gene positively and the *bin* gene negatively. It is likely that a similar pattern of regulation exists in γ phage as well, although we do not know whether the *xcl* genes for β and γ are allelic. The possibility that the *xcl* gene product has a role in regulating the *bin* gene is also supported by the fact that some *xcl* phages fail to lysogenize, although some *xcl* phages do lysogenize. An alternative interpretation of this finding is that the product of the exclusion gene has a reciprocal regulatory role on immune repressor synthesis.

One observation for which an answer cannot be formulated at the present time is the failure to isolate β-*xcl* *bin*$^+$ mutants from a C7 (β-*xcl*$^+$ *bin*$^+$) lysogen. On the surface, this failure is not unreasonable. It suggests that the *xcl* gene product regulates the *bin* gene and that in C7 (β-*xcl* *bin*$^+$) mutants the antirepressor action of the *bin*$^+$ gene is so great that these lysogens cannot survive due to induction. However, in recombination experiments between homoimmune β strains, we were able to isolate β-*xcl* *bin*$^+$ recombinants that established and maintained lysogeny. It is as if there is a difference in survival if phage is already integrated as opposed to phage going through the infective stage and establishing lysogeny, that is, as if the regulatory circuits have the potential to adjust to the latter which includes the establishment as well as maintenance stages of lysogenization but cannot adjust when the maintenance stage has already been set.

A second set of puzzling observations relates to the results of the second prophage cross (Table 3). One explanation suggested for these results is that the lysogen employed in the cross was a triple lysogen rather than a double lysogen. A triple lysogen C7 (β-*tsr* *xcl*$^+$ *bin*$^+$) (β-*tsr*$^+$*xcl* *bin*) (β-*tsr* *xcl* $^+$ *bin*$^+$) would account for the near equivalence of the two major classes of phage progeny and, if one assumed a preferential recombination between the first two phages, for the predominance of β-*tsr*$^+$ *bin*$^+$ recombinants in the minor classes. Postulating a triple lysogen would not change expectations for the segregation of the exclusion gene, but as already noted the results from this cross are compatible with the proposed location of *xcl* and are equally compatible whether a double or triple lysogen was involved.

We have previously discussed the physiological similarities between superinfection exclusion by corynephage β and superinfection exclusion in the *sieB*, *rex*, and *old* systems of phages P22, λ, and P2, respectively (4). The similarity of β exclusion to the *sieB* exclusion of P22 was further reinforced by our present finding of a separate gene for exclusion. Thus, in both systems there is an excluding gene (*xcl*, *sieB*) and an escape gene (*bin*, *esc*). An additional point of common interest is the observation by Susskind and Botstein (7) that lysogens carrying phage λ *imm*^{434}T exclude P22 more efficiently than those carrying the wild-type allele. The mutant phage overproduces repressor, and this would fit with our proposal that immune repressor is a positive regulator of exclusion in the corynephages.

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LITERATURE CITED

1. **Groman, N., and W. Laird.** 1977. Heat-inducible mutants of corynebacteriophages. J. Virol. **23**:587–591.
2. **Groman, N., and W. Laird.** 1977. Bacteriophage production by doubly lysogenic *Corynebacterium diphtheriae*. J. Virol. **23**:592–598.
3. **Groman, N., and M. Rabin.** 1978. Control of corynebacteriophage reproduction by heteroimmune repressor. J. Virol. **28**:28–33.
4. **Groman, N., and M. Rabin.** 1980. Superinfection exclusion by heteroimmune corynebacteriophages. J. Virol. **36**:526–532.
5. **Laird, W., and N. Groman.** 1976. Prophage map of converting corynebacteriophage beta. J. Virol. **19**:209–218.
6. **Laird, W., and N. Groman.** 1976. Isolation and characterization of *tox* mutants of corynebacteriophage beta. J. Virol. **19**:220–227.
7. **Susskind, M. M., and D. Botstein.** 1980. Superinfection exclusion by prophage in lysogens of *Salmonella typhimurium*. Virology **100**:212–216.
8. **Susskind, M. M., A. Wright, and D. Botstein.** 1974. Superinfection exclusion by P22 prophage in lysogens of *Salmonella typhimurium*. Virology **62**:367–384.