*THE OPERATORS CONTROLLED BY THE λ PHAGE REPRESSOR**

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The ability of the *coli*-phage λ to lysogenize its host is an example of the control of gene functioning by repressors.[1] In the lysogenic state, the phage genes are switched off by the λ phage repressor. This repressor, a protein coded for by the C_I gene of the phage, binds specifically to λ DNA *in vitro*, and this implies that the repressor blocks gene expression *in vivo* by preventing transcription from DNA to RNA.[2]

The sites to which the repressor binds (the operators) lie within a segment of the λ DNA molecule called the immunity region.[2, 3] This region extends over only a few per cent of the total length of the chromosome, including the C_I gene. It is flanked by genes belonging to two operons, from which transcription proceeds outwards from the immunity region (Fig. 1) as follows: One operon,

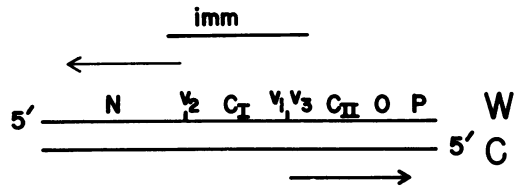

FIG. 1.—A schematic representation of a portion of the λ genome. The line labeled *imm* shows the extent of the immunity region. The arrows show the directions of transcription from the DNA strands labeled *W* and *C*.

which contains gene *N*, is transcribed from the strand labeled *W* in Figure 1. The other operon, containing genes C_{II} and *O*, is transcribed from the *C* strand.[4–6] Various genetic experiments suggest that the repressor directly blocks one or both of these operons and that other phage genes function only if supplied with the products of the genes which are under direct repressor control.[7] It has also been suggested that in addition to controlling certain phage genes, the repressor directly prevents replication of λ DNA.[8]

The experiments discussed in this paper utilize a mutant of λ called λ *virulent* (λ*vir*) to determine which phage functions are under direct repressor control. We will first show that this phage bears mutations which decrease the affinity of its DNA for repressor *in vitro*. Evidence will then be presented to show that the λ repressor binds to at least two distinct sites in the immunity region and independently controls the adjacent operons. We will also discuss the possible mechanisms by which the repressor controls λ DNA replication.

Phage λ*vir*.—In 1954, Jacob and Wollman[9] described a multiple mutant of λ that grows on λ lysogens, that is, in the presence of λ repressor. This phage

(λ*vir*) contains three mutations in its immunity region: the mutation v_2 is located on the left between genes N and C_I, and the two mutations v_1 and v_3 are located on the right between genes C_I and C_{II} (see Fig. 1). Phages bearing the mutations v_2, v_2v_3, v_1v_3, and v_3 have been isolated. Attempts to isolate a phage bearing v_1 without v_3 have not succeeded. Phage strains and the techniques used to isolate and map these mutations are described by Jacob and Wollman[9] and by Ptashne and Hopkins.[10]

Repressor Binding.—In 1961, Jacob and Monod[1] suggested that the mutations in λ*vir* render its operators insensitive to the λ repressor. The experiment shown in Figure 2 supports this suggestion: DNA isolated from phage λ*vir* binds

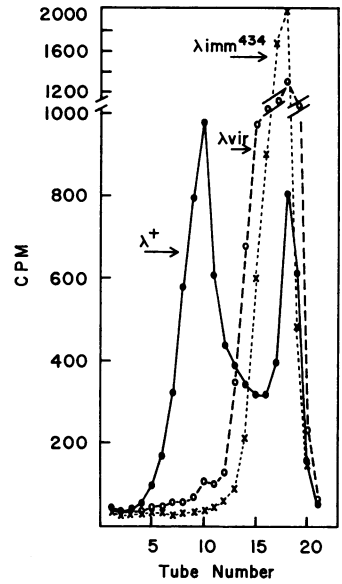

FIG. 2.—Binding of λ repressor to DNA from the phages λ, λ*imm*^{434}, and λ*vir*. Equal amounts of C^{14}-leucine-labeled λ*ind*$^-$ repressor[2, 15] were sedimented with 50 μg of phage DNA in three separate sucrose gradients, as described previously.[15]

repressor much less well than does wild-type DNA. Only at DNA concentrations about tenfold higher than that necessary to reveal repressor binding to wild type DNA does the extent of binding approach that observed with wild-type DNA. Figure 2 also shows, as reported previously,[2] that no binding to DNA isolated from phage λ*imm*^{434} is detected; this phage differs from λ only in its immunity region, and it is completely insensitive to the λ repressor *in vivo*.[3, 11]

The experiment described in Figure 3 shows that the v_2 mutation and the double mutation v_1v_3 separately decrease the affinity of DNA for the repressor. For a fixed amount of DNA, these mutant forms bind about half as much repressor as does λ wild type. In this and the following experiment, DNA concentrations were used at which binding to λ*vir* DNA is negligible. DNA carrying the mutation v_3 or the double mutation v_2v_3 also binds repressor less well than does wild-type DNA, as may be seen in Figure 4. The observed order of binding, wild type > v_3 > v_2v_3 > *vir*, suggests that both the v_1 and v_3 mutations, as well as the v_2 mutation, decrease the affinity of DNA for the repressor.

In Vivo Effects of the Components of λ*vir.*—We have tested the isolated genetic

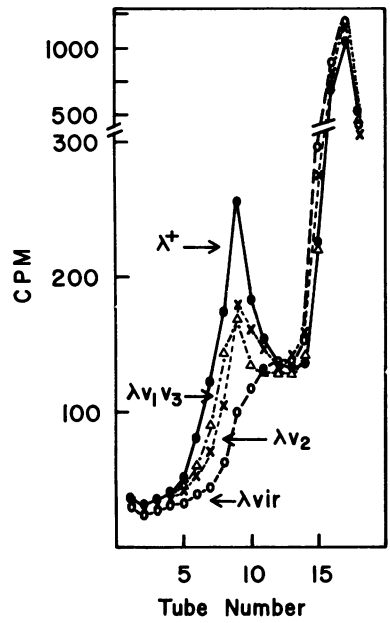

FIG. 3.—Binding of λ repressor to DNA isolated from the phages λ, λ*vir*, λv_2, and λv_1v_3.

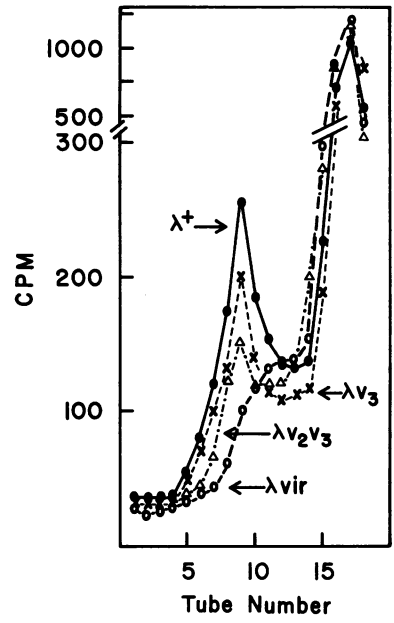

FIG. 4.—Binding of λ repressor to DNA from phages λ, λ*vir*, λv_3, and λv_2v_3.

Phage DNA, 1.5 μg, was mixed with 0.1 ml of H^3-leucine-labeled λ*ind*$^-$ repressor in a total volume of 0.3 ml containing 0.01 *M* Tris, pH 7.4, 0.01 *M* EDTA, and 10^{-4} *M* Cleland's reagent. After 5 min incubation at 37°C, the mixtures were layered on 7.5–30% glycerol gradients in the same buffer, containing 0.025 *M* KCl and 1 mg/ml bovine serum albumin as carrier. The gradients were centrifuged at 60,000 rpm for 1.4 hr in an International SB405 rotor at 6°C, collected directly into Bray's scintillation fluid,[16] and assayed for radioactivity. The main difference between the procedure used here and in Fig. 2 is that the lower salt concentration in the gradient allowed the use of smaller amounts of DNA.[2]

components of λ*vir* for the ability to synthesize the products of genes *N* or *O* in the presence of repressor. The experiment consists of infecting a λ lysogen with the λ phage to be tested, splitting the culture into two parts, and adding separately λ*imm*^{434} phages bearing a mutation in *either* gene *N* or gene *O*. Phage λ*imm*^{434} progeny will be produced only if the λ phage provides the missing *N* or *O* gene product. Table 1 shows that in the presence of repressor, λv_2 synthesizes detectable levels of the product of gene *N* but not of gene *O*, whereas the opposite is true for λv_3. When this experiment is performed with λv_1v_3, both the *N* and *O* gene products are made in sufficient quantities to produce a large number of phage in both test cases. However, it will be shown in the next section that λv_1v_3 replicates extensively under these conditions. We believe it likely that the functioning of the *N* gene is due to escape synthesis as the gene dosage exhausts the repressor supply.

The experiments presented thus far show that the v_2 and v_3 mutations are analogous to the o^c mutations of the *lac* operon.[1, 12] Each mutation decreases the affinity of DNA for the repressor and renders the adjacent operon constitu-

TABLE 1. *Constitutivity of phages λv₂ and λv₃ for the N and O gene products, respectively.*

Infecting Phage		Phage in Lysate		
λ	λ*imm*^{434}	λ	λ*imm*^{434}	λ*imm*^{434} test / λ*imm*^{434} control
*C*Ι*sus34*	+ *susN*$_7$	1.3×10^3	1.7×10^3	—
"	+ *susO*$_{29}$	1.5×10^4	4.7×10^2	—
v$_2$*C*Ι*sus34*	+ *susN*$_7$	2.9×10^4	3.4×10^5	200
"	+ *susO*$_{29}$	1.6×10^4	2.3×10^2	0.5
v$_3$*C*Ι	+ *susN*$_7$	7.2×10^3	3.4×10^3	2
"	+ *susO*$_{29}$	2.9×10^5	2.7×10^4	58

E. coli strain W3350 (λ*ind*$^-$) was grown in tryptone broth plus 0.2% maltose and 0.01 *M* $MgSO_4$ to a concentration of 2×10^8 cells/ml, washed, and resuspended in 0.01 *M* $MgSO_4$ at a concentration of 10^9 cells/ml. In each experiment, the cells were simultaneously infected with the λ phage to be tested at a multiplicity of 7 phages/cell and with either λ*imm*^{434}*susN*$_7$ or λ*imm*^{434}*susO*$_{29}$ at a multiplicity of 0.5 phage/cell. After 10 min adsorption at 37°C, the infected cells were aerated at a concentration of 2×10^4 cells/ml for 1 hr at 37°C in tryptone broth containing maltose and 10^{-3} *M* $MgSO_4$.

The cultures were then chilled, chloroformed, and assayed for phage on the *E. coli* strains C600(λ) and C600(λ*imm*^{434}). The λ*v*$_2$ phage bears the *C*Ι amber mutation *C*Ι*sus*^{34}, and phage λ*v*$_3$ bears a spontaneous *C*Ι mutation designated simply *C*Ι. The results are expressed as the ratio of the number of λ*imm*^{434} phages produced in each case to that produced when the infecting λ phage is λ*C*Ι*sus34*.

tive. We conclude that the λ repressor acts on at least two separate operators, independently controlling the operons located on either side of the immunity region.

DNA Replication.—Thomas and Bertani[8] first noticed that co-infection of a λ lysogen with λ and λ*imm*^{434} results in the production of a large excess of λ*imm*^{434} phages among the progeny. The low number of λ phages is accounted for by the fact that under these conditions the λ DNA undergoes only limited replication.[13] Since the λ*imm*^{434} phages provide all the gene products known to be required for DNA replication, Thomas and Bertani suggested that in addition to controlling the functioning of certain phage genes, the λ repressor directly prevents λ DNA replication.

We have repeated this experiment using λ wild type, λ*v*$_2$, λ*v*$_3$, and λ*v*$_1$*v*$_3$. Table 2

TABLE 2. *Loss of replication inhibition by λv₁v₃.*

Bacterium	Phage λ*imm*^{434}*C*Ι*sus60* plus λ	Phage/Bacterium in Lysate: λ	λ*imm*^{434}	λ/λ*imm*^{434}
1. W3350 (λ*ind*$^-$)	+ *C*Ι*sus34*	13	270	0.05
"	+ *v*$_2$*C*Ι*sus34*	16	270	0.06
"	+ *v*$_3$*C*Ι	28	280	0.09
"	+ *v*$_1$*v*$_3$	120	75	1.6
"	+ *v*$_1$*v*$_3$*susO*$_{29}$*susP*$_3$	70	80	0.9
2. W3350	+ *C*Ι*sus34*	75	85	0.9
"	+ *v*$_2$*C*Ι*sus34*	80	100	0.8
"	+ *v*$_3$*C*Ι	85	110	0.8
"	+ *v*$_1$*v*$_3$	100	85	1.2
"	+ *v*$_1$*v*$_3$*susO*$_{29}$*susP*$_3$	70	130	0.5

E. coli strains W3350 (λ*ind*$^-$) and W3350 were grown, washed, and resuspended as described in Table 1 and were then infected in each experiment with phage λ*imm*^{434} and the λ phage to be tested at a multiplicity of infection of 5 phages/cell of each type. After 1.5 hr growth as described in Table 1, the cultures were chloroformed and assayed for phage on strain C600 (λ*imm*^{434}) and C600 (λ). The λ*imm*^{434} phage bears in its *C*Ι gene the amber mutation *C*Ι*sus60*. The results are expressed as the ratio of λ to λ*imm*^{434} phage in each lysate. The experiment with W3350 (2), as a control, shows that the asymmetries among the progeny from the infected lysogenic cells largely disappear when the same phages are grown in a nonlysogenic host.

shows that of the λ phages tested only $\lambda v_1 v_3$ replicates extensively in a λ lysogen co-infected with phage λimm^{434}. Note also that this property is not significantly altered by the introduction of mutations in genes *O* and *P* that code for enzymes required for DNA replication, and this shows that the ability of $\lambda v_1 v_3$ to replicate here is not accounted for simply by the production of the *O* and *P* gene products by this phage.

We may consider two explanations for the fact that the v_1 mutation, in combination with the v_3 mutation, relieves replication inhibition by the repressor. (1) The mutation v_1 might be located in a repressor binding site in the immunity region, distinct from the *N* and C_{II}-*O* operators, at which DNA replication is initiated; and v_1 mutation would then prevent the repressor from attaching and inhibiting DNA replication. According to this explanation, v_3 would play no role in relieving replication inhibition. (2) Mutation v_1 might be a second mutation in the operator already mutated by v_3 that increases the constitutivity of the right-hand operon; the ability of $\lambda v_1 v_3$ to replicate in the presence of repressor would then be due to some secondary effect of this increased transcription. For example, this operon might contain a gene, as yet undetected, whose product is nondiffusible and is required for DNA replication. It seems likely that some form of the second hypothesis is correct since there are two other mutations (namely, *C17* and ri^c) located outside and to the right of the immunity region, each of which renders the phage insensitive to replication by the repressor.[14]

Summary.—The phage λ *virulent* which grows in the presence of the λ repressor is shown to bear mutations in its operators which decrease the affinity of its DNA for the repressor *in vitro*. Physical and genetic experiments with this mutant and its derivatives show that the λ repressor binds to at least two separate operators, independently controlling two separate operons.

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