Repression of λ -Associated Enzyme Synthesis After λ_{vir} Superinfection of Lysogenic Hosts

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ABSTRACT

LISIO, ARNOLD L. (National Institutes of Health, Bethesda, Md.), AND ARTHUR WEISSBACH. Repression of λ -associated enzyme synthesis after λ_{vir} superinfection of lysogenic hosts. *J. Bacteriol.* 90:661-666. 1965.—Phage λ_{vir} is a multiple mutant of λ which is capable of overcoming the immunity of a host lysogenic for λ , and initiating normal vegetative replication of the superinfecting phage genome. Superinfection of *Escherichia coli* K-112 (λ_{22}) with λ_{vir} results in a normal phage yield, lysis time, and H^3 -thymine incorporation compared with infection of the sensitive host, K-112 (S). However, the production of the λ phage-specific early protein, λ -exonuclease, after superinfection of *E. coli* K-112 (λ_{22}) with λ_{vir} is only 25 to 50% of that obtained from corresponding infection of a nonlysogenic host, *E. coli* K-112 (S). This repression of λ -exonuclease synthesis is dependent on the C_1 cistron of the prophage and is overcome if the lysogenic host cells are induced prior to superinfection. The data are interpreted as evidence for partial repression of λ_{vir} by the host immunity.

The vegetative replication of a superinfecting bacteriophage is repressed in a host lysogenic for a homologous phage; the lysogenic host is immune (Lwoff, 1953). Upon superinfection of *Escherichia coli* lysogenic for λ with the homologous phage, the synthesis of an early phage-directed enzyme, λ -exonuclease, necessary for phage deoxyribonucleic acid (DNA) replication (Radding, 1964) is repressed (Lisio and Weissbach, 1965), and replication of the superinfecting genome DNA does not take place (Wolf and Meselson, 1963). Recently, it was demonstrated that this repression of the superinfecting phage occurs at the level of DNA transcription to form messenger ribonucleic acid (mRNA, Sly, Echols, and Adler, 1965). The immunity of the host bacterium to superinfection is determined by the C_1 cistron located in the "C region" of the λ prophage (Kaiser and Jacob, 1957; Bode and Kaiser, 1965). This host immunity can be overcome by lysogenic induction or superinfection with the immunity-insensitive mutant, λ_{vir} .

Phage λ_{vir} is a multiple mutant of phage λ (Jacob and Wollman, 1954); no single mutation which results in virulence is known. One of the mutations in λ_{vir} is in the C_1 region. This region has been implicated as the structural gene for synthesis of a phage-specific immunity-repressor (Kaiser and Jacob, 1957); it is this postulated repressor which maintains the prophage state

and confers host immunity to superinfecting homologous phage (Jacob and Monod, 1961).

Experiments designed to elucidate the stage at which host immunity repressed the homologous superinfecting phage demonstrated that the synthesis of λ -exonuclease, an early phage-specific enzyme (Korn and Weissbach, 1963), was completely repressed (Lisio and Weissbach, 1965). It has been found that λ_{vir} infection of a sensitive host, *E. coli* K-112(S), and a lysogenic host, *E. coli* K-112(λ_{22}), results in identical total DNA synthesis, lysis times, and phage yields. However, the synthesis of λ -exonuclease in the lysogenic host is markedly inhibited. The purpose of this report is to demonstrate the partial repression of synthesis of the early enzyme, λ -exonuclease, when λ_{vir} superinfects host cells lysogenic for λ . The other mutations in λ_{vir} are presumed to involve a repressor-sensitive, or "operator," region of the phage genome, rendering it insensitive to the immunity-repressor substance (Jacob and Wollman, 1961). Phage λ_{vir} is thus able to overcome the immunity of a lysogenic host because it is insensitive to the postulated specific repressor.

MATERIALS AND METHODS

Bacterial strains. *E. coli* K-112(S), sensitive and nonlysogenic (Wollman, 1953), was kindly provided by Francois Jacob. The following lysogenic derivatives of K-112 were obtained by isolating a

single lysogenic colony after infecting K-112(S) with the appropriate phage. K-112(λ_{22}) (Fry, 1959), a wild-type λ lysogen, was obtained from F. Jacob. K-112(λ_{ind}^-) (Jacob and Campbell, 1959), a noninducible lysogen which contains a mutation in the C_I cistron of λ , was obtained from A. D. Kaiser. K-112(λ^{i434}) (Kaiser and Jacob, 1957), prophage λ^{i434} , obtained by multiple crosses of 434 with λ , contains the C_I cistron of phage 434, but is otherwise isogenic with λ (also referred to as λ_{imm434}). Phage λ^{i434} was kindly provided by A. D. Kaiser. C600(434) (Jacob and Wollman, 1961) was obtained from F. Jacob and given to us by M. B. Yarmolinsky. The inducible prophage is related to phage λ but with different immunity characteristics.

Phage strains. Phage λ_{vir} (Jacob and Wollman, 1954) was a gift of A. D. Kaiser. The λ_{vir} stock was prepared by infecting K-112(S) in Tryptone broth (Difco) with phages isolated from a single plaque and allowing lysis to occur. The phages were concentrated by centrifugation at $5,000 \times g$ for 10 min, followed by centrifugation for 1 hr at $44,000 \times g$; the phage-containing pellet was suspended in a medium containing 0.01 M tris-(hydroxymethyl)aminomethane (Tris)-HCl buffer (pH 7.4), 0.01 M $MgSO_4$, 20 g of Cs_2SO_4 per 100 ml, and stored at 4°C.

Phage λ^{i434} was prepared by ultraviolet (UV) induction of *E. coli* K-112(λ^{i434}) in synthetic medium and concentrating the phage as described for λ_{vir} .

Bacterial cultures were prepared in standard Tryptone broth (Kaiser and Hogness, 1960) containing 5×10^{-3} M $MgSO_4$ to facilitate phage adsorption. Conditions for culture growth and phage infection and harvesting of cells have been described; under these conditions 90 to 100% of the cells are infected (Lisio and Weissbach, 1965). The procedures for preparation of extracts and λ -exonuclease assay have been described (Korn and Weissbach, 1963). Experiments requiring UV irradiation were carried out in synthetic medium (Korn and Weissbach, 1962) containing 5×10^{-3} M $MgSO_4$.

RESULTS

When λ_{vir} infects the nonlysogenic host K-112(S), or superinfects the lysogenic host K-112(λ_{22}), the times of lysis (Fig. 1) and phage yields (2.7×10^{10} phages per milliliter of lysate) were identical. The rates of H^3 -thymine incorporation into DNA after λ_{vir} infection were also identical (Fig. 2).

The amounts of λ -exonuclease produced 30 min after λ_{vir} superinfection of several different *E. coli* lysogens in Tryptone broth are presented in Table 1. The expected large amount of λ -exonuclease was produced when the sensitive, nonlysogenic host K-112S is infected with λ_{vir} , or when a host carrying the prophage 434, C600(434), is superinfected with λ_{vir} . Phage

FIG. 1. Times of lysis of *Escherichia coli* K-112(S), ●, and K-112(λ_{22}), ○, after infection with λ_{vir} . The experimental conditions were as described in Table 1. Phage yields were assayed according to the method of Adams (1959). The yields from K-112(S) and K-112(λ_{22}) were both 2.7×10^{10} phages per milliliter of lysate. All plaques examined were clear.

434 endows its lysogenized host with immunity specific for phage 434 and not for λ phage. In contrast, the superinfection of hosts lysogenic for λ , K-112(λ_{22}) and K-112(λ_{ind}^-), results in a markedly diminished λ -exonuclease synthesis. However, λ_{vir} superinfection of a host carrying the heteroimmune prophage λ^{i434} , K-112(λ^{i434}), results in a normal amount of λ -exonuclease. Phage λ^{i434} is isogenic with λ except for the C_I cistron of phage 434; thus, prophage λ^{i434} confers on the host K-112(λ^{i434}) immunity to phage 434 but not phage λ (Kaiser and Jacob, 1957).

λ -Exonuclease assays of mixed extracts obtained from λ_{vir} infection of K-112(S) and K-112(λ_{22}) were additive, indicating that the decreased λ -exonuclease activity of extracts obtained from λ -lysogenic cells was not due to an inhibitor present in the lysogenic host.

To determine whether the repression of λ -exonuclease synthesis was present throughout the latent period, we determined the kinetics of λ -exonuclease synthesis after λ_{vir} infection of

FIG. 2. Incorporation of H^3 -thymine into DNA after λ_{vir} infection of *Escherichia coli* K-112(S), ●, and K-112(λ_{22}), ○. K-112(S) or K-112(λ_{22}) were grown to a cell concentration of 2×10^8 cells per milliliter in Tryptone broth containing 5×10^{-3} M $MgSO_4$. The λ_{vir} phages (multiplicity of input, 4 to 5) and 0.5 mc of H^3 -thymine (6.7 c/mM, New England Nuclear Corp., Boston, Mass.) were added at zero-time. Samples (10 ml) were taken at 10-min intervals and chilled rapidly in an ice-water mixture. Isolation of the DNA was carried out by use of a modification of the method of Schmidt and Thannhauser (1945) which has been described previously (Korn and Weissbach, 1962).

the nonlysogenic host, K-112(S), and after superinfection of the lysogenic host, K-112(λ_{22}) (Fig. 3). The content of λ -exonuclease is maximal 20 min after infection of both host cells. The amount of λ -exonuclease synthesized after λ_{vir} infection of K-112(S) was always two to three times the values obtained after superinfection of K-112(λ_{22}).

The exposure of inducible lysogenic bacteria to UV light results in the induction of vegetative multiplication of the phage presumably by altering the concentration or structure of the immunity-repressor substance (Jacob and Monod, 1961). The irradiation of K-112(λ_{22}) grown in synthetic medium with UV light sufficient to induce all of the cells released the repression of λ -exonuclease synthesis subsequent to

λ_{vir} superinfection (Fig. 3). The amount of enzyme produced when λ_{vir} superinfects the lysogenically induced host was comparable to the amount obtained with λ_{vir} infection of the nonlysogenic K-112(S).

The lysogen K-112(λ_{ind}^-) was noninducible by UV light. This property arises from a mutation in the C_I region of the λ prophage, presumably resulting in production of a radiation-resistant repressor (Jacob and Campbell, 1959). In contrast to the inducible lysogen K-112(λ_{22}), UV irradiation of K-112(λ_{ind}^-) appears to release only partially the repression of λ -exonuclease synthesis upon superinfection with λ_{vir} (Fig. 4).

The above experiments were performed with λ_{vir} phages produced in the nonlysogenic host K-112(S). The λ -bacteriophage particles are known to carry a "host-specificity" determined by the bacterial strain on which they were produced (Arber and Dussoix, 1962). To exclude the possibility that a host-controlled modification of the superinfecting λ_{vir} was responsible for the repression of λ -exonuclease synthesis, K-112(S) and K-112(λ_{22}) were infected with λ_{vir} prepared by infection of K-112(λ_{22}) rather than K-112(S). The amounts of λ -exonuclease produced were identical to the corresponding values given in Table 1.

Further experiments were carried out by superinfecting several different *E. coli* lysogens with the temperate phage λ^{i434} (Kaiser and Jacob, 1957). The repression of exonuclease synthesis

TABLE 1. Increase in deoxyribonuclease activity of crude extracts after infection with λ_{vir} *

Culture	Activity (units/mg of protein)
<i>Escherichia coli</i> K-112(S)	$6.1 \pm 0.7^\dagger$
<i>Escherichia coli</i> K-112 (λ_{22})	$1.6 \pm 0.2^\dagger$
<i>Escherichia coli</i> C600(434)	$6.7 \pm 0.3^\dagger$
<i>Escherichia coli</i> K-112 (λ^{i434})	$6.2 \pm 0.4^\dagger$
<i>Escherichia coli</i> K-112 (λ_{ind}^-)	1.1

* Cultures were grown to early log phase (2×10^8 cells per ml) in 500 ml of Tryptone broth, containing 5×10^{-3} M $MgSO_4$, with shaking at 37 C in a 2-liter flask. Phage λ_{vir} was added at a multiplicity of 4 to 5 phage per cell. Cells were harvested and prepared for enzyme assay 30 min after phage infection. Enzyme assays were carried out at pH 10.0 to measure the activity of λ -exonuclease. A unit of enzymatic activity is defined as 0.018 μ mole of base solubilized in 15 min at pH 10.0. Protein was determined by the method of Lowry et al. (1951).

† Average of three separate experiments plus or minus standard deviation.

was dependent upon the immunity characteristics of the host prophage, and repression was complete when phage λ^{i434} infected a host carrying the homologous prophage (Table 2). This is identical to the result of infecting K-112(λ_{22}) with λ_{22} (Lisio and Weissbach, 1965).

FIG. 3. Increase in λ -exonuclease activity in crude extracts of *Escherichia coli* K-112(S) and K-112(λ_{22}) after λ_{vir} infection in synthetic medium. Phages were added at zero-time. K-112(S) infected with λ_{vir} , ●; K-112(λ_{22}) superinfected with λ_{vir} , ○; K-112(λ_{22}) lysogenically induced with UV light and immediately superinfected with λ_{vir} , ⊙; K-112(λ_{22}) lysogenically induced with UV light only, ▲. The experimental procedures were as described in Table 1.

TABLE 2. Increase in deoxyribonuclease activity of crude extracts after infection with phage λ^{i434} *

Culture	Activity (units/mg of protein)
<i>Escherichia coli</i> K-112(S)	4.6
<i>Escherichia coli</i> K-112 (λ_{22})	3.2
<i>Escherichia coli</i> K-112 (λ_{ind^-})	3.5
<i>Escherichia coli</i> K-112 (λ^{i434})	0.07

* Experiments were carried out in Tryptone broth, containing 5×10^{-3} M $MgSO_4$, as described in Table 1. The cells were harvested 30 min after phage infection, and crude extracts were prepared for enzyme determination.

FIG. 4. Increase in λ -exonuclease in crude extracts of K-112(λ_{ind^-}) grown in synthetic medium. After λ_{vir} superinfection only, ●; UV irradiation followed immediately by λ_{vir} superinfection, ○; and UV irradiation only, ■. The experimental conditions were as described in Table 1.

DISCUSSION

The immunity of a bacterium lysogenic for λ to superinfecting homologous phage is controlled by the C_I cistron of the prophage linkage group. It has been postulated that the C_I cistron controls the synthesis of a phage-specific repressor substance which confers immunity on the host (Kaiser and Jacob, 1957; Jacob and Wollman, 1961). The immunity-repressor substance actind at an *operator* locus of the phage genome would prevent expression of the phage genome and synthesis of early proteins necessary for phage replication (Jacob and Monod, 1961).

Phage λ_{vir} is a polygenic mutant of λ and appears to result from the summation of a mutation in the C_I cistron and of mutations at other loci; these other loci may be operator loci responsible for the sensitivity of the λ -genome to the immunity-repressor substance (Jacob and Wollman, 1961). Though the superinfecting λ_{vir} undergoes a normal vegetative cycle with the production of normal phage yields in hosts lysogenic for λ , the synthesis of an early protein, λ -exonuclease, is repressed, as shown in this

paper. This repression appears to be determined by the C_I cistron of the λ prophage, since λ_{vir} superinfection of K-112(λ^{i434}), a host lysogenized by a phage isogenic with λ except for the C_I region derived from phage 434 (Kaiser and Jacob, 1957), results in normal λ -exonuclease synthesis.

The repression of λ -exonuclease synthesis is relieved by prior irradiation with UV light sufficient to induce the host cells. This result is presumably due to a change in the concentration or structure of the immunity-repressor substance produced by the C_I cistron (Jacob and Monod, 1961). This is supported by the finding that prior irradiation of a host cell carrying the noninducible prophage λ_{ind}^- , a radiation-resistant C_I mutant of λ (Jacob and Campbell, 1959), results in only partial derepression of λ -exonuclease synthesis subsequent to λ_{vir} superinfection. These results support the idea that the immunity repressor in cells lysogenic for λ_{ind}^- is not destroyed by the same UV irradiation which is capable of lifting the repression in cells lysogenic for λ .

Thus, sensitivity to immunity-repressor may be partial or complete. That insensitivity to immunity may not be absolute is also demonstrated by the isolation of phage P2- vir_6 , which behaves as immunity-insensitive in singly lysogenic strains but not in doubly lysogenic strains (Thomas and Bertani, 1964).

The function and control of synthesis of the early phage protein, λ -exonuclease, is unknown. During thymineless induction of K-12(λ)thy⁻, λ -exonuclease synthesis continues until thymine is added back to the culture, at which point synthesis of the enzyme ceases; this indicates that control of early protein synthesis may be related in some way to the onset of viral DNA synthesis (Korn and Weissbach, 1964). It has also been found that defective lysogens unable to synthesize λ -exonuclease are unable to synthesize phage DNA (Radding, 1964). Also pertinent to this is the demonstration that though the defective mutant of λ , λ_{TH} , produces excessive amounts of λ -exonuclease after lysogenic induction, it is unable to synthesize phage DNA (Radding, 1964).

Whatever the function of the λ -exonuclease, the data presented in this paper indicate that the amount of λ -exonuclease produced during the vegetative replication of λ is far in excess of that needed for normal phage DNA synthesis and phage yield. Though the synthesis of the enzyme during λ_{vir} infection is repressed about 75% in cells lysogenic for λ , synthesis of DNA and other phage components is apparently normal.

It has recently been demonstrated that the repression of superinfecting wild-type λ phage or λ_{vir} apparently occurs at the level of messenger RNA synthesis (Sly et al., 1965). The work reported in this paper is consistent with those findings and supports the general model for the regulation of protein synthesis applied to the control of λ proposed by Jacob and Monod (1961). Phage λ_{vir} can be visualized as an operator constitutive mutant of λ in which the operator shows a decreased sensitivity to repressor. This is analogous to the O^c mutants of the *lac* operon of *E. coli* (Jacob and Monod, 1961).

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