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λ*imm*λ·434: A Phage with a Hybrid Immunity Region

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The genomes of coliphages λ and λ*imm*434 are identical with the exception of the immunity region, which codes for the respective repressors and corresponding operators. Although previous genetic studies failed to detect any recombinations that might have occurred within the immunity regions of these two phages, electron-micrographic heteroduplex mapping demonstrated a short interval of homology between λ and λ*imm*434 DNA located in this otherwise heterologous region. We report the isolation of a recombinant which resulted from a cross-over event between λ and λ*imm*434 within the immunity region. This phage, denoted λ*imm*λ·434, carries the leftward promoter-operator as well as gene *rex* of λ and the rightward promoter-operator as well as gene *tof* (*cro*) of phage 434. Since the recombination occurred within the *c*I gene, phage λ*imm*λ·434 must carry a hybrid repressor gene.

INTRODUCTION

Coliphage λ, like other temperate phage, can establish and maintain a state of lysogeny because, among other things, it can express a phage-specific repressor (reviewed by Ptashne, 1971). The λ repressor encoded for by the *c*I gene binds only to λ DNA, which contains λ-specific operators. This specificity was shown in experiments using a hybrid phage, λ*imm*434, constructed by crossing λ and the closely related temperate coliphage 434 (Kaiser and Jacob, 1957). This hybrid phage derives only a small region, called the immunity region, from 434, with the rest of the phage genome being derived from λ (see Fig. 1). In addition to the *c*I gene, the immunity region includes: the two operators which bind repressor (Ptashne, 1971), the two promoters from which early major transcription initiates (Thomas, 1971), a regulatory function, *tof* or *cro* (Eisen and Ptashne, 1971), and in the case of λ an additional gene *rex* (Howard, 1967). Therefore, although λ*imm*434 contains primarily λ genetic material, the fact that it contains the immunity region of 434 means that it both carries the genetic information for synthesis of 434-specific repressor and is repressed by the 434 repressor. In addition to the repressor, the Cro (or Tof) regulatory function is also immunity specific (Pero, 1971). This regulatory function apparently acts in three ways to control the expression of the phage genome. By binding at o_L it reduces the expression of functions in the *N-int* operon (Sly, Rabideau, and Kolber, 1971), and also it appears to regulate expression of the *c*II-*O-P* and *c*I-*rex* genes (Echols, 1972).

The functional heterogeneity relating to the immunity regions of λ and λ*imm*434 reflects a structural heterogeneity, the corresponding DNA sequences in this region not being homologous. This was indicated by the fact that it was not possible to demonstrate recombinations between λ and λ*imm*434 occurring in the immunity region (Kaiser and Jacob, 1957; Thomas, 1964). Kaiser and

Jacob (1957) concluded, "Either the *c*I regions of λ and 434 fail to pair, or recombination occurs but the recombinants are inviable because of the hybrid character of their *c*I region." Indeed, more recent experimental evidence suggests that both conclusions are partially correct.

Westmoreland *et al.* (1969) employed electron microscopy to examine heteroduplex DNA molecules composed of the *l* strand of λ and the *r* strand of λ*imm*434 DNA and showed that over 94% of their genomes appear identical and only the immunity regions are grossly heterologous. They also found that there is a short interval of homology (estimated to equal or be less than 0.3% of λ length) located within this otherwise heterologous *imm*λ/*imm*434 region (see Fig. 3A). This suggested that recombination might occur within this short homology interval and that such a recombinant phage with a hybrid immunity region might be found, providing that a proper selection technique could be devised.

A phage carrying a hybrid immunity region would be expected to have unique characteristics. It should have one operator as well as the homologous promoter from each phage (see Fig. 2). Since it would carry the *cro* gene of one phage and the o_L of the other it should exhibit the Cro⁻ phenotype. Therefore, it might be expected that a hybrid phage formed by a recombination in the immunity region would grow poorly, because λ*cro*⁻ phages plate at a low frequency on normal hosts (Calef *et al.*, 1971). The poor viability of λ*cro*⁻ phages is due to the overproduction of the repressor and the uncontrolled expression of the *N-int* operon. However, if the recombination occurs within the *c*I genes, the resulting hybrid repressor would most probably be inactive. Hence, such a phage should act like a *c*I mutant. We report the isolation of a phage with these characteristics which results from a recombination within the immunity region between phages λ and λ*imm*434.

MATERIALS AND METHODS

Bacterial strains. K37 is strain 28 of Meselson; K100 is the same as N100 and is a *rec*A⁻ derivative of strain 28; N958 is a lysogen of strain 28 which carries a λ*sex*3*c*I857*x*13 prophage; all three were kindly supplied by M. Gottesman (Gottesman and Yarmolinsky, 1968).

Phage stocks. Both λ*susN*7*N*53*imm*434*cIt*1 and λ*imm*434*c*17*byp* (which was derived from λ*imm*434T and λN⁻*c*17*byp* of Butler and Echols, 1970) were constructed in the laboratory of D. I. F.; λ*imm*434T, λh80*susN*7*N*53*c*I857 and λ⁺⁺ were supplied by M. Gottesman; λ*r*32 was obtained from P. Brachet; λ*bio*N2-1*nin*5 from G. Kayajanian; λb519*imm*434 was constructed by M. F. (see Davidson and Szybalski, 1971).

Media. Tryptone broth (TB): 1% tryptone, 0.5% NaCl, 1.0 μg/ml thiamine hydrochloride, 10^{-2} *M* $MgSO_4$ and 0.2% maltose. Tryptone agar: 1% tryptone, 0.25% NaCl, 1.0 μg/ml thiamine hydrochloride, and 1.1% agar. Tryptone top agar: 1% tryptone, 0.5% NaCl and 0.7% agar. Phosphate buffer: 0.7% $Na_2HPO_4 \cdot 7H_2O$, 0.3% KH_2PO_4, 0.4% NaCl and 10^{-3} *M* $MgSO_4$.

Construction of lysogens. The method of Gottesman and Yarmolinsky (1968) was used.

Recombination. Infected cells were sedimented and resuspended in phosphate buffer, irradiated with ultraviolet light (9000 ergs/cm²), diluted into TB, incubated 90 min at 40°C, treated with chloroform, and then plated on the appropriate bacterial lawn.

Heteroduplex mapping. For most of the heteroduplex preparations, separated strands of phage DNA were fractionated by the poly(U,G)-CsCl gradient procedure (Szybalski *et al.*, 1971). The heteroduplexes were prepared by mixing the CsCl solutions of the *l* and *r* strands in carbonate-buffered 50% formamide, as originally described by Westmoreland, Szybalski, and Ris (1969). Grid preparation, uranium oxide shadow casting, electron microscopy, length measurements and their evaluation were described by Westmoreland *et al.* (1969) and Fiandt *et al.* (1971).

RESULTS

Basis for Selection of Phage with Hybrid Immunity Region

The limited amount of homology observed between the immunity regions of λ and λ*imm*434 suggests, *a priori*, that recombination within this heterologous region should

be a very rare event. Moreover, although such recombinant phage would have some unique features, there is no readily apparent characteristic that could be used in selecting for them, and as discussed, such a phage would be expected to exhibit the Tof$^-$ phenotype associated with a low plating efficiency. Therefore, it is not surprising that the progeny of such a recombinational event are difficult to detect (Kaiser and Jacob, 1957).

We have been able to select a phage recombinant with a hybrid immunity region (λ·434) using parental phages carrying specific mutations. In this genetic cross, the λ*imm*434 parent carries two mutations which map outside of the immunity region, *c*17 and *byp* (Hopkins, 1970; Butler and Echols, 1970). The presence of these two mutations permit λ to grow independent of the *N* function, a phage function which extends transcription of the λ genome and is normally needed for λ growth (Echols, 1971). The λ parent carries two mutations which map within the immunity region, *sex*3 and *x*13 (Eisen *et al.*, 1966; Roberts, 1969; Gottesman and Weisberg, 1971; Blattner *et al.*, 1972). These two mutations affect the two early promoters, p_L and p_R, respectively, and result in a lowered expression of functions located in the operons regulated by them. While phages carrying *x* mutations are not viable, many phages carrying *sex* mutations are viable. However, studies by F. R. Blattner and P. Twose (personal communication) suggest that it is not possible to construct plaque-forming, *N*-requiring λ derivatives which also carry the *sex*3 mutation. This reduced viability is most likely due to the profound effect of the *sex*3 mutation in reducing transcription initiating at p_L (S. Nakahishi, personal communication).

How did this cross permit selection of a phage with a hybrid immunity region? To answer this question we must look to the nature of the recombinants formed by a cross-over in the immunity region between λ*sex*3*c*I857*x*13 and λ*imm*434*c*17*byp*. Figure 2 shows the two possible recombinants formed by a single recombinational event. Recombinant "A," which has the left-hand immunity region (iL) from λ, including the *sex*3 mutation, and the right-hand immunity region (iR) from λ*imm*434, also carries the *c*17 and *byp* mutations. We expected such a phage to be viable for the following reasons: First, the *sex*3 mutation should compensate for the Tof$^-$ phenotype by reducing the unregulated transcription to the left of the immunity region. Second, the combination of the *c*17 and *byp* mutations should help overcome any undersupply of *N* product caused by the *sex*3 mutation by permitting *N*-independent transcription to the right of the immunity region. The product of the reciprocal recombinational event, recombinant "B," carries the right-hand immunity region (iR) from λ, which includes, in this case, the *x*13 mutation. Like the λ*sex*3*x*13 parent, this recombinant should be nonviable. The problem of selection resolves itself into finding conditions which will permit recombinant "A" to be distinguished from the viable parent, λ*imm*434*c*17*byp*.

One possible method to distinguish between the recombinant and parent phage is through the use of a 434 lysogen. The basis for such a selection is the observation that, although phage carrying both the *c*17 and *byp* mutations are not virulent, i.e., cannot grow on a lysogen carrying a homoimmune prophage, the addition of a *c*I mutation results in the phage becoming virulent (Hopkins, 1970; Sly *et al.*, 1971). Since the recombination should occur in the *c*I gene (Westmoreland *et al.*, 1969), we would expect a phage with a hybrid repressor gene to act like a *c*I$^-$ mutant and, therefore, recombinant "A" could be expected to be virulent and thus form plaques on the 434 lysogen. The virulence of recombinant "A" should be still further enhanced by its constitutive *N* expression in the lysogens with 434 immunity, since its λ-derived o_L operator is insensitive to the 434 repressor (This *N* expression would not be affected by Cro repression, although it would be reduced due to the *sex*3 mutation). On the other hand, the λ*imm*434-*c*17*byp* parent, which is *c*I$^+$ and has o_L of 434 specificity, is not virulent and does not form plaques on the 434 lysogen (Table 1).

Formation of Recombinants

In order to implement our strategy for selecting a phage with a hybrid immunity region, bacteria lysogenic for the defective prophage λ*sex*3*c*I857*x*13 were infected with

TABLE 1

GROWTH OF λA-4 ON VARIOUS BACTERIAL HOSTS[a]

Host	K37	K124	K159	K350	K100	K68	K334
*Rec*A genotype	+	+	+	+	−	−	−
Immunity of prophage	None	λ	434	434, λ	None	λ	434
Infecting phage							
λ	+	−	+	−	+	−	+
λ*imm*434	+	+	−	−	+	+	−
λA-4	+	+	+	−	+	−	+
λ*imm*434c17*byp*	+	+	−	−	0	0	0

[a] Bacteria were grown overnight in TB. Lawns of each bacteria were poured onto tryptone using top agar. Dilutions of each phage were then placed on each lawn. Plates were incubated overnight at 32°C. If obvious phage growth occurred, it was scored as +; if no phage growth occurred, it was scored as −; symbol 0 means that no experiments were done. The genotype of λA-4 is λ*sex*3*imm*λ·434c17*byp*. All Rec⁺ lysogens were derived from K37 and all Rec⁻ lysogens were derived from K100. K124 and K68 carry a λ⁺⁺ prophage, K159 and K334 carry a λ*imm*434T prophage, and K350 carries both λ⁺⁺ and λ*imm*434T prophages. Identical results were obtained with K350 and K259, which carries λh80*sus*N7N53cI857 and λ*sus*N7N53*imm*434cI*t*1 prophages.

λ*imm*434c17*byp* and phages that made plaques on a lysogen carrying a 434 prophage were isolated. Two classes of recombinant phage could be distinguished by testing their ability to grow on various bacteria. One class, thought to be λ*sex*3cI857*x*13c17*byp*, will be discussed in another paper (Friedman, Jolly, Mural, and Wilgus, manuscript in preparation). The other class of recombinant phage, exemplified by a phage called λA-4, showed growth characteristics (Table 1) which suggested that it carried a hybrid immunity region. This was inferred from the observation that although λA-4 grows in either a lysogen with immunity to λ or one with immunity to 434, it was unable to grow in a lysogen with immunity to both phages. Thus, λA-4 is affected by both the 434 and λ repressors, a fact which strongly suggests that this phage has a mixed or hybrid immunity region.

In order to confirm the position of each partial immunity region in the λA-4 hybrid, we took advantage of the fact that λ will only grow on a *rec*A⁻ bacterial host if, in addition to all the functions normally needed for vegetative growth, either of two normally nonessential functions, Red and Gam (Zissler, Signer, and Schaefer, 1971) are expressed. The genes encoding for these functions lie in the *N-int* operon, which is controlled by the o_L operator, the repressor binding site (Fig. 1). If the left-hand side (iL) of the immunity region of λA-4 is derived from λ, then the phages will have the o_L operator from λ (Fig. 2) and, therefore, in the presence of λ repressor will be unable to express functions encoded for by the *N-int* operon. Thus, the observations that λA-4 grows both in a *rec*A⁻ λ-free bacterium as well as in a *rec*A⁺(λ) lysogen, but not in a *rec*A⁻(λ) lysogen, indicates that the λ repressor keeps the *N-int* operon from being expressed (Table 1). This implies that the λ segment of the hybrid immunity region lies on the left-hand side of the λA-4 immunity region. Furthermore, λA-4 must then be able to overcome repression of *N* function since it forms plaques on *rec*A⁺(λ) lysogens. Therefore, if λA-4 carries the right hand immunity region of 434, it also must have inherited the *c*17 and *byp* mutations from the λ*imm*434 parent, which render it *N*-independent.

Heteroduplex Mapping of the Immunity Region of λA-4

Heteroduplex mapping of the DNA isolated from λA-4 confirmed our initial impression that the phage carries a hybrid immunity region. When λA-4 DNA was denatured and reannealed with λ DNA, electron microscopic examination revealed that there was homology in the left hand immunity region, but not in the right-hand immunity region. Schematic diagrams of representative electron micrographs are shown in Fig. 3. In Fig. 3A, the heteroduplex structure formed between λ and λ*imm*434 DNA strands is shown. The two regions of nonhomology, iL and iR, are clearly identifiable. On the other hand, the heteroduplex structure formed between λ and λA-4 DNA strands shows only one region of nonhomology (region iR; Fig. 3B). In order to orient and measure the DNA molecules, the λ DNA used carried an insertion of foreign DNA, *r*32 (Brachet, Eisen and Rambach, 1970) which is located immedi-

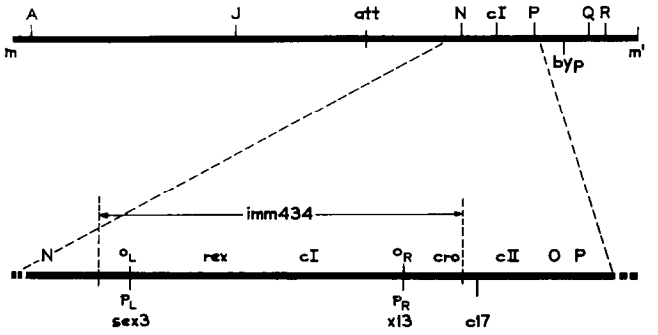

FIG. 1. Genetic map of λ and the enlargement of its immunity region. For gene designation see Davidson and Szybalski (1971).

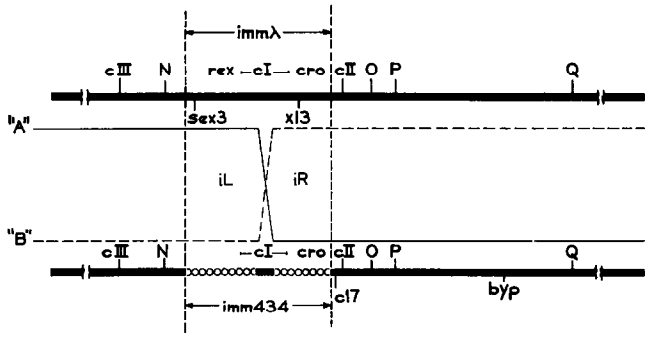

FIG. 2. Reciprocal products of recombination between λ*imm*434*c*17*byp* and λ*sex*3*c*I857*x*13 within the short homology interval in immunity region. DNA of λ is represented by heavy lines and the heterologous 434 DNA by the chain-like lines.

ately to the right of the immunity region (Blattner *et al.*, 1972; Fiandt and Szybalski, 1973). The single region of nonhomology found in λ and the λA-4 heteroduplex is region iR, since it is located immediately adjacent to the *r*32 insertion. On the other hand, in the heteroduplex between λb519*imm*434 and λA-4, the nonhomology corresponds to the region iL, as based on its size and position in respect to the b519 deletion and to the right the *m'* end of the λ molecule (Fig. 3C). These results definitely prove that in the λA-4 hybrid, the left-hand region iL of immunity is derived from λ and the right-hand region iR is that of phage 434. Moreover, the measurements of the heteroduplex between λ*bio*N2-1*nin*5 and λA-4 DNA, together with the earlier data of Fiandt and Szybalski (1973), permit us to place the right end of the λ·434 homology interval between the iL and iR regions at 77.7% from the left terminus of the mature λ DNA molecule (see Fig. 3). As measured in the λ/λ*imm*434 heteroduplexes, the length of this λ·434 homology interval, which is variable, approached 0.3% λ. The measured distances from the ends of the homology interval to the corresponding left and right termini of the *imm*434 region (3.9 and 1.4 % λ, respectively) agree well with the analogous but single-strand measurements (3.9 and 1.3% λ, respectively) of Westmoreland *et al.* (1969).

DISCUSSION

The experiments outlined in this paper demonstrate that we have isolated a phage with a hybrid immunity region, the left-hand side (iL) derived from λ and the right-hand side (iR) from 434. The heteroduplex studies confirm the conclusion that the recombination event which generated the λ*imm*λ·434 hybrid phage occurred within the interval of demonstrated λ·434 homology shared by the immunity regions of λ and

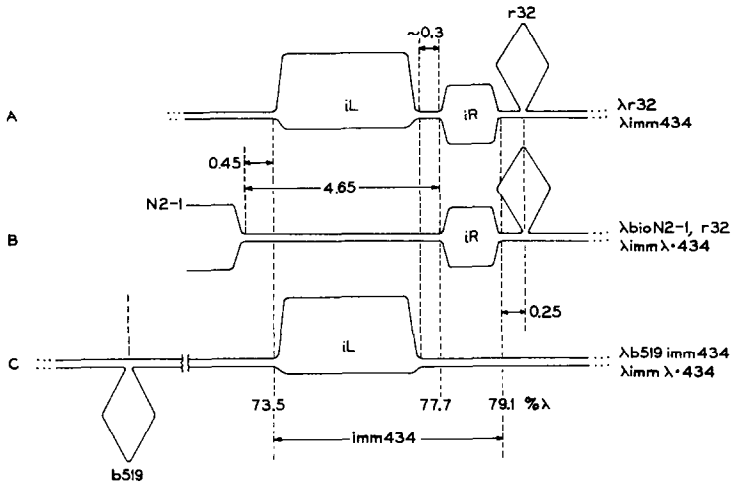

FIG. 3. Schematic representation of heteroduplex λ DNA molecules obtained by annealing *l* and *r* strands of the corresponding phage DNAs, indicated at the right margin. Drawing B is a composite of two heteroduplexes: λ*bio*N2-1*min*5/λ*imm*λ·434 and λ*r*32/λ*imm*λ·434. The size of intervals and the positions on the λ map, as measured from the left terminus of the mature λ DNA, are given in the percentage of λpapa genome length (Westmoreland *et al.*, 1969). For absolute measurements of the end points of *bio*N2-1 and *imm*434 substitutions (corrected to 73.05 and 73.5 %λ, respectively, according to the most recent data of M. Fiandt, E. H. Szybalski, and W. S.), *r*32 insertion and b516 decetion, see Fiandt and Szybalski (1973) and Davidson and Szybalski (1971).

λ*imm*434. Such a recombination event, then, defines an upper limit to the minimum number of nucleotides needed in a region of homology in order for recombination to occur between otherwise heterologous regions. Westmoreland, Szybalski, and Ris (1969) estimated the size of this short interval of homology to be at most 0.3 % of the λ DNA molecule. This corresponds to about 140 nucleotides (Davidson and Szybalski, 1971). However, this length appears to be an upper limit and the interval of homology might be shorter or consist of only partially homologous nucleotide sequences.

The properties of λA-4, the genotype of which is λ*sex*3*imm*λ·434*c*17*byp*, were already discussed and are summarized in Table 1. The ability of λA-4 to plate on the 434 lysogen might attest to the nonfunctionality or failure of synthesis of the hybrid repressor, but the constitutive expression of the N function also has to be considered when making comparison with the growth characteristics of λ*imm*434*c*17*byp* in a lysogen immune to phage 434. If λA-4 is truly virulent due to its *c*I⁻*c*17 phenotype, it should form plaques even if both its o_L and o_R operators are repressed, which is possible only in the (λ,434) double lysogen. However, λA-4 does not form plaques on this double lysogen. This might be caused by the *sex*3 mutation, which lowers the *N* expression, by the unexpected activity of the hybrid *imm*λ·434 repressor, or by the relatively high dose of λ or 434 repressors in the lysogens with double (λ,434) immunity. Since λ*c*I*c*17 virulence depends on the titration of repressor (Packman and Sly, 1968), it might be that the hybrid phage cannot titrate sufficient repressor to permit phage growth, especially since λ*imm*λ·434 carries only a single dose of each operator. Indeed, λ*c*I*c*17 is not virulent in multiple λ lysogens (Merril and Gottesman, personal communication), and the *sex*3 mutation might be expected to lower still further the virulence of the *c*I*c*17 phages, if the derepression is incomplete and they partially depend on the *N* product.

It should be possible to answer some of these questions after crossing out the *sex*, *c*17 and *byp* mutations from the λA-4. The *sex* mutation might be obligatory for tempering the lethality of the uncontrolled leftward transcription because of the Tof⁻ phe-

notype of λA-4, since the Tof function of phage 434 cannot control the o_L operator of λ (Pero, 1971). The *c*17 and *byp* mutations might also be obligatory since λ*sex*3 mutants do not plate.

Assessment of properties of the hybrid λ·434 repressor protein would be quite interesting in view of the functionality of other hybrid gene products of λ hybrid phages: the putative hybrid *O* product of φ80*hy*42 (Szpirer, 1972), hybrid *exo* product of φ80*hy*1 (Szpirer *et al.*, 1969) and the *c*II product of λ*imm*21*hy*1 (Liedke-Kulke and Kaiser, 1967).

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