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Studies of the Lysogenic Response as a Function of Multiplicity of Infection of Lambdoid Bacteriophages

Mark Floyd Fedyk
Eastern Illinois University

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STUDIES OF THE LYSOGENIC RESPONSE AS A FUNCTION OF MULTIPLICITY OF INFECTION OF LAMBDOID BACTERIOPHAGES

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BY

Mark Floyd Fedyk

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STUDIES OF THE LYSOGENIC RESPONSE AS A FUNCTION OF MULTIPLICITY OF INFECTION OF LAMBDOID BACTERIOPHAGES

BY

MARK FLOYD FEDYK

B. S. in Zoology, Eastern Illinois University, 1979

ABSTRACT OF A THESIS

Submitted in partial fulfillment of the requirements for the degree of Master of Science in Zoology at the Graduate School of Eastern Illinois University

CHARLESTON, ILLINOIS
1979

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Mark F. Fedyk

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ABSTRACT

The lambdoid bacteriophages λ imm$^{\lambda}$, λ imm^{434} and λ cI are ideal subjects for the study of gene product interactions at the molecular level due to their closely related genetic makeup. Previous work in this laboratory has indicated the possibility that phage λ imm is sensitive to phage λ imm^{434} cI gene product and that phage λ imm^{434} is sensitive to phage λ imm$^{\lambda}$ cI gene product. The purpose of this research was to determine if the frequency of lysogeny for phage λ imm$^{\lambda}$ is affected by the multiplicity of coinfection with the λ imm^{434} phage. By comparing the graph of the frequency of lysogeny versus multiplicity of infection for λ imm$^{\lambda}$ alone with the graph of the frequency of lysogeny versus multiplicity of infection for λ imm$^{\lambda}$ coinfected with λ imm^{434} it is possible to determine whether the lysogenic response of λ imm$^{\lambda}$ utilizes the cI gene product of phage λ imm^{434}. The results of this study demonstrate clearly that λ imm^{434} has no multiplicity of infection - dependent effect upon the ability of λ imm$^{\lambda}$ to enter the lysogenic state. The combined results of the present study and previous work in this laboratory suggest that although the cI gene of one phage does appear to influence the reproduction of the other, the effect does not involve the primary function of cI, that is, initiation of lysogeny. This suggests that the cI gene could possibly have other functions besides producing repressor protein.

INTRODUCTION

Lambda is classified as a temperate phage due to its ability to follow two distinct pathways of growth. During lytic growth, which is exemplified by both temperate and virulent phage, the lambda chromosome is replicated (approximately 100 times) and packaged into mature virus particles by newly synthesized viral proteins. The particles are then released as a result of cell lysis. The second pathway, lysogenic growth, is brought about by two phage proteins. One of these proteins catalyzes the insertion of the lambda chromosome into the bacterial host chromosome, and the other is a repressor protein that inhibits further expression of the lambda genes. The resultant cell which contains the combination of host and viral DNA together in one chromosome is termed a lysogen. The process by which a lysogen is created is called lysogenization, and the inserted lambda chromosome is referred to as a prophage.

Previous experiments in this laboratory have indicated a possibility that phage λ imm$^{\lambda}$ is sensitive to phage λimm^{434} repressor. Thus the presence of λ imm^{434} increases the probability for phage λ imm$^{\lambda}$ to establish a lysogenic state. The λ imm^{434} cI gene which produces the repressor protein is functionally analogous to, but structurally different from the cI gene of λ imm$^{\lambda}$ (Kaiser and Jacob 1957). The purpose of this study is to determine if the frequency of lysogeny for phage λ imm$^{\lambda}$ is affected by the multiplicity of infection (m.o.i.) of the coinfecting phage λimm^{434}.

REVIEW OF LITERATURE

Phage Life Cycle

Lambdoid bacteriophages are classified as temperate phages; that is, they can either be transmitted from cell to cell by infection or passed from mother to daughter within a cell line. The latent phage genome in such a cell line is called a prophage and the bacterial cells harboring the prophage are termed lysogenic. The presence of the phage genome within a lysogenic culture can be detected by the spontaneous liberation of phage from a small fraction of the cell population in which viral development is spontaneously activated.

The λ virion is composed of an icosahedral head 54.0 nm long, and a tail that terminates in a single fiber (Figure 1). The DNA, contained in the head, has a unique nucleotide sequence and is double-stranded throughout its 46.5 kb length, except for the 12 bases at the 5' termini of the polynucleotide chains. The 12-base-long cohesive ends are single-stranded, and the single strands at opposite ends are complementary to each other. Thus DNA extracted from virions can establish an equilibrium between linear molecules and doubly nicked rings. At high concentrations, end-to-end aggregates can also form (Luria et al. 1978).

Adsorption of λ virions to the cell surface requires specific interaction between the tail fiber protein (product of the λ J gene) and a cellular protein that also determines chemotactic response to the disaccharide sugar maltose. Some bacterial mutations to λ resistance alter this protein, whereas others inactivate the regulatory genes controlling maltose utilization (Luria et al. 1978). After injection, intracellular λ DNA molecules form closed circles whose nicks have been covalently joined by the host enzyme, polynucleotide ligase.

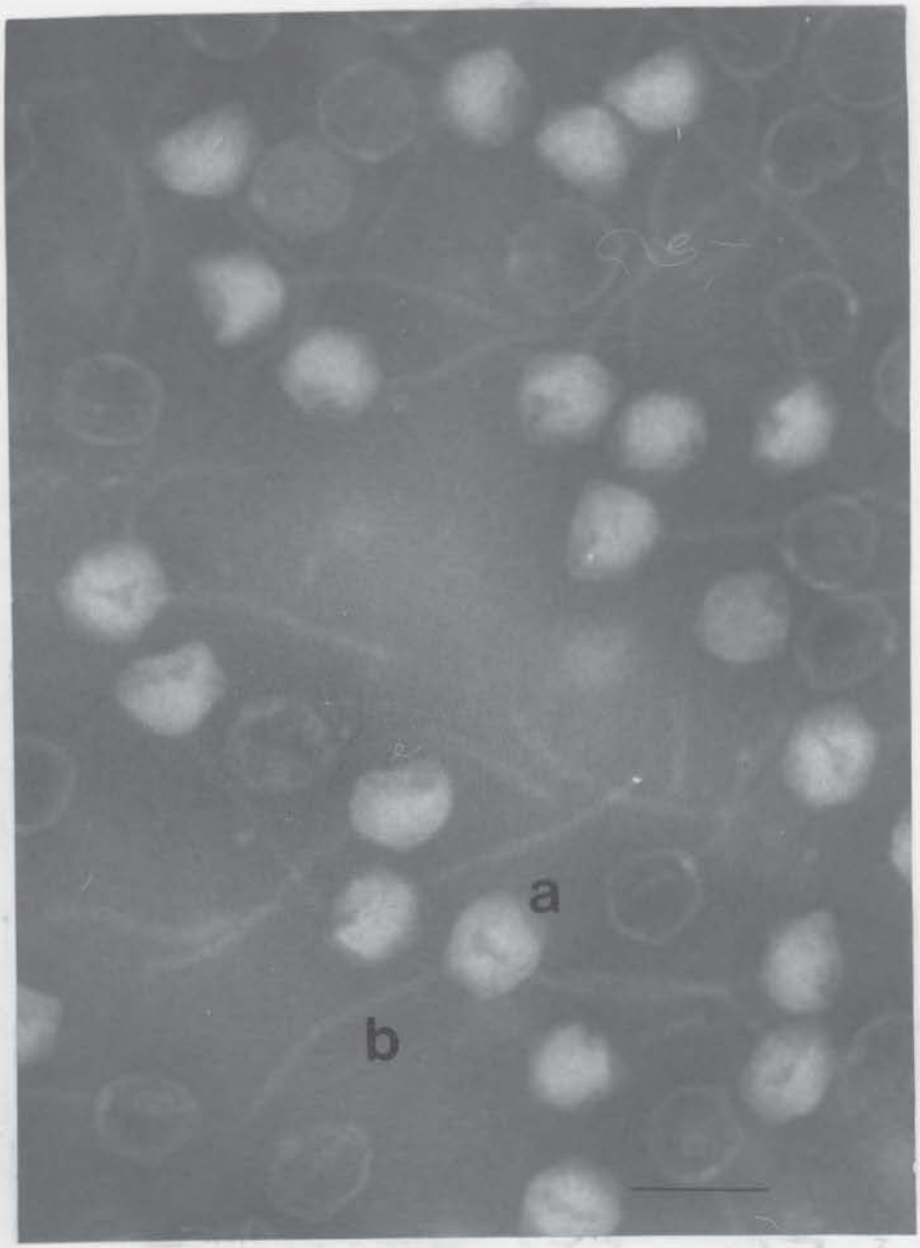

Figure 1. Particles of lambda phage negatively stained with phosphotungstate acid. This phage has a polyhedral head (a) to one vertex of which is attached a flexible tail (b). The hollow appearance of some of the phage heads can be attributed to the loss of their DNA. The bar represents 95.0 nm.

Gene Regulation of the Lytic Cycle

As soon as the λ DNA cohesive ends have joined, host DNA-dependent RNA polymerase can be utilized for transcription of the viral genome (Hershey 1971). Three phases of transcription occur during the lytic cycle: immediate-early, delayed-early, and late (Hershey 1971).

The immediate-early messenger RNAs are transcribed from the N and cro genes, starting at the left (pL) and right (pR) promoter sites, and ending at the left (tL) and right (tR) termination sites (Figure 2). The transcriptions are in opposite directions and thus on different strands since mRNA synthesis only occurs in a 5' to 3' direction.

Expression of the delayed-early genes requires the N gene product. These transcriptions also originate at pL and pR. A second set of termination sites has been proposed, tR2 and tL2, for this second set of transcriptions (Goodenough 1978). The N protein is thought to modify the DNA-dependent RNA polymerase so that it overrides tL and tR allowing the second set of transcriptions to ensue (Hershey 1971). Binding the cro gene product in this region blocks transcription from pL and pR. However, because this protein is active as an oligomer, time is required for active cro gene product to accumulate. Thus enough mRNA from the second set of transcriptions will usually have already been synthesized to allow for the third set of transcriptions. In those cases where the cro gene product blocks the delayed-early transcription before enough mRNA has been made to further lytic growth, the lysogenic response can result; this will be discussed later.

The two λ DNA synthesis genes, O and P, are needed for phage DNA replication. Mutants of gene N are deficient in DNA synthesis because genes O and P are rarely transcribed in the absence of N gene

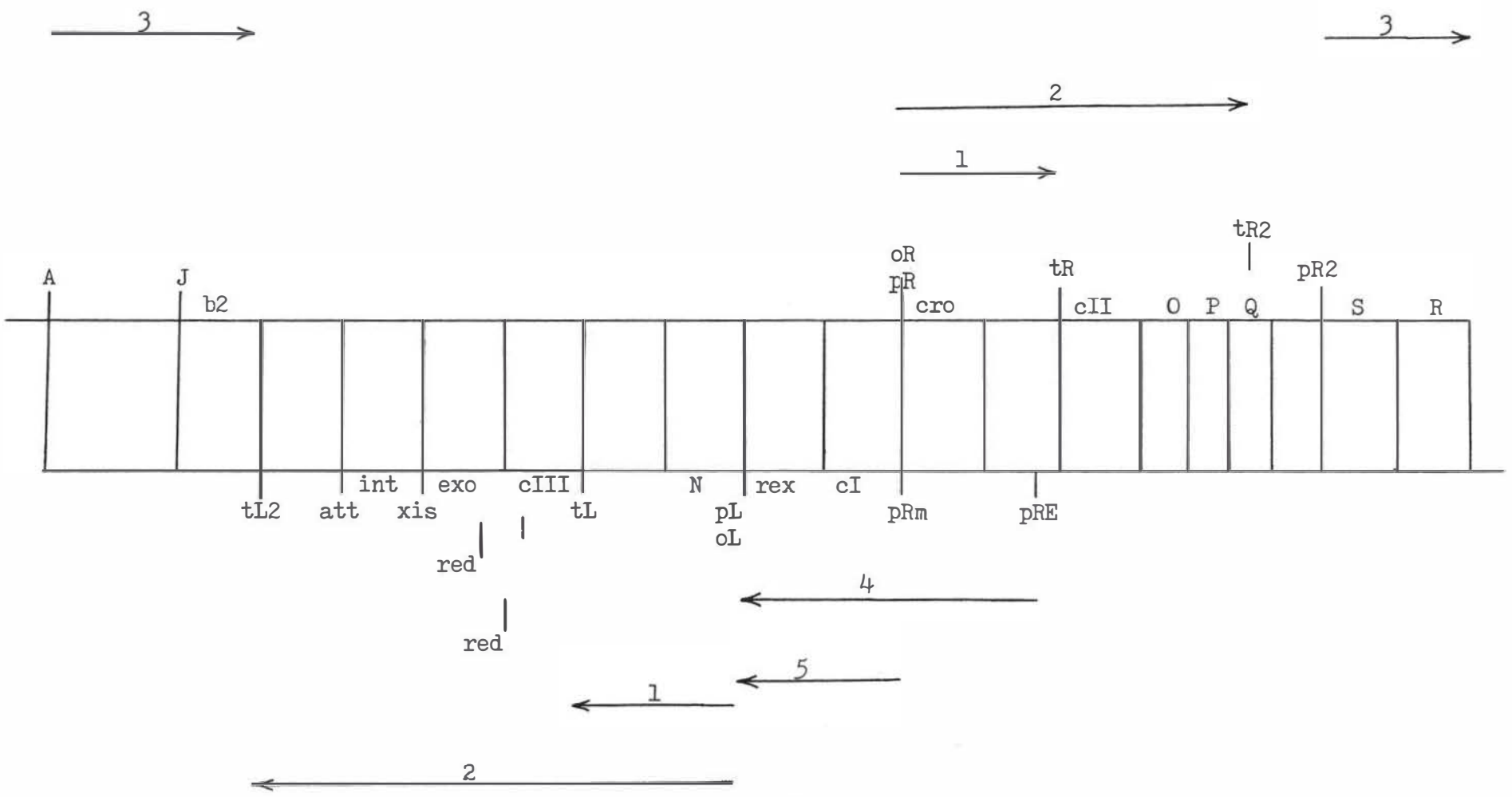

Figure 2. Lambda genetic map. Arrows 1, 2 and 3 correspond to the three sets of transcription described in the lytic cycle. Arrows 4 and 5 correspond to transcriptions described in the lysogenic response. Promoters (pRE, pRM, pL, pR and pR2), terminators (tL, tL2, tR and tR2) and operators (oL and oR) are indicated.

function (Hershey 1971). The O and P genes as such have no direct controlling effect; if the O and P transcribed proteins stimulate DNA replication, a pool of λ DNA is accumulated. Transcription can also take place from these copies, so indirectly, O and P genes have an effect on what makes up the molecular environment of the λ chromosome (Court, Green and Echols 1975).

The third set of transcriptions (late) begins at approximately ten minutes following infection (Oppenheim et al. 1977). The O gene product allows transcription from all of the late genes (S, R and A through J) by possibly acting as a RNA polymerase sigma factor and thus allowing transcription from the late promoter site (pR2) (Campbell 1971). This mRNA is transcribed rightward from pR2. Since the λ DNA is now circularized, transcription continues from gene S through the cohesive ends and then proceeds through genes A, W, B, C, D, E, F, Z, U, V, G, I, H, M, L, K and J. The genes A, W, B, C, D, E and F are needed for the production of phage head protein. Mutants defective in any of these genes fail to make active heads, but do produce approximately normal numbers of tails (Hershey 1971). The genes Z, U, V, G, I, H, M, L and J are positioned just to the right of the head genes and are needed for tail synthesis.

Liberation of λ from the cell at the end of the productive cycle requires the S and R gene products. The S gene product appears to digest or damage the cytoplasmic membrane (Skalka 1977; Luria et al. 1978). The R gene product is an enzyme, endolysin, which digests the rigid mucopolypeptide layer of the cell envelope. Endolysin differs from a true lysozyme in that it causes the hydrolysis of the peptide bond

between the D-amino acids, whereas lysozymes split bonds between adjacent N-acetylglucosamine residues (Hershey 1971: Luria et al. 1978). Mutations that inactivate gene S allow intracellular phage development to continue for several hours, resulting in very high yields of phage per infected cell.

Gene Regulation of the Lysogenic State

The events described up to this point take place in those cells that produce mature virus. It is implicit in the temperate nature of λ that only a portion of infected cells enter the productive cycle, whereas other cells survive and multiply as lysogenic bacteria. In a lysogen, the prophage is inserted into the bacterial chromosome and phage repressor protein prevents transcription of most of the viral genome. Both the establishment of repression and insertion into the bacterial chromosome are specifically controlled by λ genes (Luria et al. 1978).

The lambdoid phage promotes its own insertion (Campbell 1971). The int gene product allows for insertion of the entire λ chromosome between the gal and bio genes of the Escherichia coli chromosome at the att (attachment) site. The attachment sites for this insertion are represented as P.P' (on the phage chromosome) and B.B' (on the bacterial chromosome) (Gottesman and Weisberg 1971). The int - promoted recombination occurs by reciprocal exchange between the two participating chromosomes at a unique crossover locus within each attachment site (Gottesman and Weisberg 1971). Symbolically, the crossover locus is represented by the dot, so that P,P', and B and B' represent the nucleotide

sequences of the portion of the attachment sites which flank the cross-over point. The recombination resulting in prophage insertion can then be written as P.P'+B.B' → B.P'+P.B', where B.P' and P.B' represent the recombinant attachment sites located at the left and right prophage ends respectively (Gottesman and Weisberg 1971). The integration process is diagrammed in Figure 3.

The cII and cIII gene products (which were transcribed along with the other delayed-early genes) activate the promoter called pINT which governs the integration genes int, exo, redα, redβ, and γ, and allows the infecting genome to integrate into the host chromosome as a stable prophage (Goodenough 1978). Once the λ genome is integrated, the cII and cIII gene products also activate cI gene transcription, by unknown mechanisms, from a promoter known as pRE (promoter for repressor establishment). The pRE site lies in the L (left) strand of the λ chromosome and is located approximately a thousand nucleotides to the right of the cI gene. Transcription from this promoter proceeds in a leftward fashion and covers a portion of the antisense strand of the cro gene before the cI and rex genes are copied. The resultant transcript thus has a long "leader" of RNA (Goodenough 1978). Translation of this mRNA results in the production of a large amount of λ repressor protein in addition to the rex gene product, which in some unknown fashion, prevents λ lysogens from being lysed by infecting T4 phages carrying a mutation in the rII gene (Hershey 1971).

As repressor accumulates, it binds to pL and pR thus stopping further transcription of the genes for the reproductive cycle. In so doing, repressor also blocks transcription of the cII and cIII

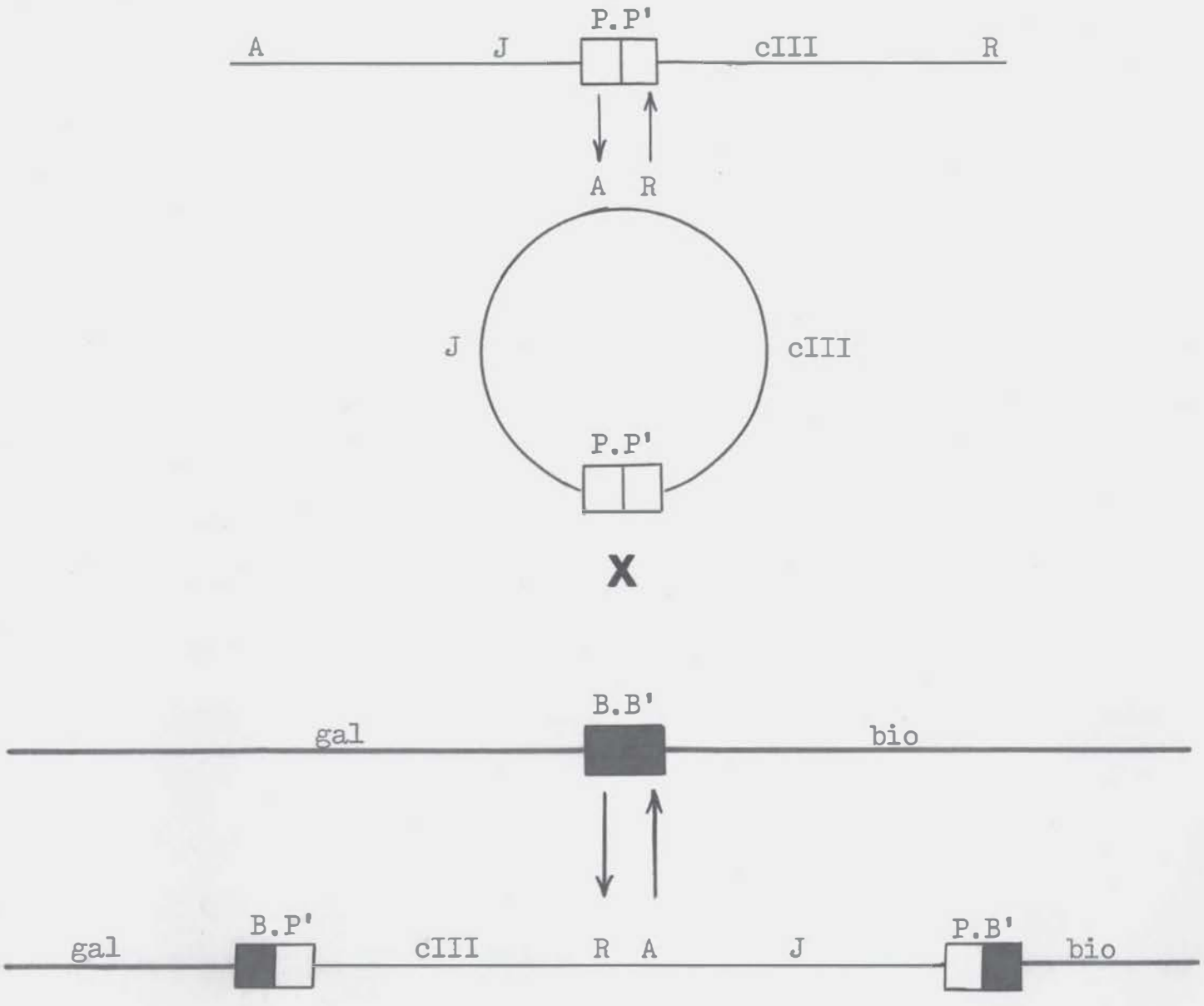

Figure 3. Prophage insertion and excision. The light lines represent the phage and prophage chromosomes, and the heavy lines the bacterial chromosome. The rectangles represent the attachment sites. The markers J, cIII and R represent phage genes. Bacterial genes gal and bio are required for the utilization of galactose and the synthesis of biotin, respectively. The distances between markers are arbitary. From Hershey (1971).

genes, whose products are therefore eventually not available to induce transcription of the cI gene. The cI gene continues to be transcribed, however, because of a second promoter, called "promoter for repressor maintenance", pRM, located next to pR. This second transcription of the cI gene occurs late (60 minutes) in the growth cycle, starting at pRM and proceeding leftward through the cI gene locus. Transcription from pRM is repressible by high concentrations of repressor (Ptashne 1971), whereas low concentrations of repressor may stimulate transcription. By an unknown mechanism, repressor seems to thus participate directly in its own maintenance in the form of autoregulation and continues on to maintain the lysogenic state. The state of lysogeny is usually stable; however, spontaneous lysis does occur at a frequency of about 10^{-5} per cell generation. Induction of the λ prophage is the result of a stimulus which causes reduction in repressor activity and exision of the prophage by a site specific endonuclease (xis gene product). Upon excision, the λ genome assumes the circle structure (by connecting its cohesive ends with host ligase) and follows the lytic pathway (Nash 1977).

Lysis versus Lysogeny

Whether a phage follows the lytic or lysogenic pathway is dependent upon a delicate balance of cro, cII/cIII and cI gene products. The cII/cIII gene products are required for cI gene transcription and thus, in this case, are positive regulators. The cro gene product has a dual role in the pathway decision. It inhibits cII/cIII transcription by binding near pL and pR and in this way negatively controls repressor

production. However, by blocking oL and oR, the delayed - early transcription of gene Q is also depressed, so that lysogeny is indirectly favored. The cro and cI proteins have been shown to act at overlapping, but non-identical sites (Reichardt 1975b). The cI protein has an affinity for λ DNA 100 times greater than the cro gene product. This indicates the lysis - lysogeny decision is a function of the intracellular concentrations of cro and cI gene products which appear to be competing for the same site (Takeda, Folkmanis and Echols 1977). The lytic response may also be favored because transcription from one strand may be affected by that occuring on the complementary strand. Transcription of the cro gene could then be inhibiting cI gene transcription and thus favoring the lytic pathway (Ptashne 1971; Reichardt 1975a).

The Lambda Mutants

The restriction of lambda repressor to lambda lysogens suggests that repressor is encoded by a phage gene (Weisberg et al. 1977). Mutants which were unable to synthesize active repressor, and therefore defective in lysogeny were isolated quite early. These mutants were distinguished by their clear plaque phenotype. Wild - type lambda makes a turbid plaque on a lawn of bacterial cells growing on an agar plate. The turbidity is due to the survival and growth of repressor containing cells (lysogens) within the plaque. A phage that cannot make repressor kills all of the cells that it infects, and no lysogenic cells appear in the center of the plaques.

The clear (c) mutants have been classified into four groups - cI,

cII, cIII and cY - by complementation tests, map location and the nature and severity of the lysogenization defect (Kaiser 1957; Brachet and Thomas 1969). Complementation between clear mutants is demonstrated by coinfecting cells with, for example, λ cII and λ cIII, neither of which efficiently lysogenizes by itself. The doubly infected cells are lysogenized well by both of the clear mutants together (Weisberg et al. 1977).

The behavior of the cI mutants indicates that the cI gene encodes the lambda repressor: (1) Lambda cII, cIII and cY mutants lysogenize with a reduced but measurable frequency (10^{-1} to 10^{-5} per infected cell) (Kaiser 1957); cI never lysogenizes. (2) Although lambda cII, cIII or cY single lysogens are easily obtained by complementation, complementation of lambda cI mutants never results in the formation single lambda cI lysogens. Cells bearing a cI prophage always carry a second cI^+ prophage as well (Jacob and Monod 1961). (3) Lambda cII, cIII or cY lysogens are indistinguishable from wild-type lysogens: They do not synthesize lytic lambda functions and they are immune to superinfection by lambda. Thus these mutants, unlike cI, are defective in the establishment of lysogeny but not in the maintenance of it (Kaiser 1957).

The Lambda Hybrid Phage - λ imm^{434}

The temperate phage 434 is closely related to lambda, but grows on lambda lysogens following superinfection. By repeated crosses with lambda, a hybrid phage called λ imm^{434} has been constructed (Kaiser and Jacob 1957). This phage is nearly isogenic with lambda but retains the

immunity specificity of 434; i.e., it is sensitive to 434 but not to lambda repressor, and λ *imm*^{434} lysogens are immune to 434 but not to lambda (Weisberg *et al.* 1977). Crosses between λ *imm*^{434} *cI*$^{+}$ and λ *cI* yield neither λ *imm*^{434} *cI* nor λ *cI*$^{+}$. This failure to separate *cI* from *imm* by recombination suggests that the genetic determinants of 434 immunity include a *434* - *cI* gene functionally analogous to, but structurally different from the *cI* gene of lambda (Kaiser and Jacob 1957). In addition, since the two phages are insensitive to each others repressor, the operator sites of the two repressors must also be different and located within the immunity region (Jacob and Monod 1961). Subsequent electronmicroscopic observations of heteroduplexes between the DNAs of lambda and λ *imm*^{434} have shown a region of nonhomology between 73.6 and 79.1 on the physical map of the lambda chromosome (Davidson and Szybalski 1971). As predicted, this region contains the gene *cI* (Fiandt *et al.* 1971) and the operator sites (Hopkins and Ptashne 1971).

MATERIALS AND METHODS

Media

Bacterial hosts were grown in K medium (0.075 M sodium - potassium phosphate buffer, pH 7.0, 0.02 M NH_4Cl, 0.005 M $MgSO_4$, 0.006 M NaCl and 1.5% casamino acids) supplemented with 2.4 mg/ml of maltose (KM medium).

The nutrient broth (TB) contains 1.0% Bacto - tryptone and 0.5% NaCl. Agar derivatives include TB soft agar (0.7% agar), TB plate agar (1.0% agar), and TB slant agar (2.0% agar).

Phage stocks were stored in TMB medium, which consists of 0.01 M tris-HCl buffer, pH 7.5, 0.01 M $MgSO_4$ and 0.1 mg/ml bovine plasma albumin (BPA). One drop of chloroform was put into each stock tube in order to deter bacterial contamination. All dilutions were made in TMB. During phage absorption the bacteria were suspended in TM, which is TMB with the BPA being deleted.

Bacterial Strains

Escherichia coli K12 W3104 and its derivatives were used for extraction of phage, phage assays and testing for the frequency of lysogeny. E. coli strain K12 and its lysogenic derivatives (W3104 λ imm^{434} and W3104 λ imm$^{\lambda}$) were obtained from H. Echols.

Maintenance of Bacteria

Bacterial cultures were grown every three days from refrigerated single cell isolates stored on TB slant agar. These cultures provided innocula for bacterial hosts of phage growth and lysogeny, and for the assay of phage titers. This method of maintaining W3104 guarantees

a minimum of bacterial growth and thereby constrains genetic change in the bacterial population during the course of the experiments.

Bacteriophages

Phage λ imm$^{\lambda}$ forms homogeneously sized plaques which have turbid centers when plated on W3104. This is a wild-type phage which can be extracted by ultraviolet irradiation from W3104 λ imm$^{\lambda}$. Phage λimm^{434} also forms homogeneously sized plaques which have turbid centers when plated on W3104. The λ imm^{434} phage contains the immunity region of phage 434 in an otherwise λ genome. This hybrid phage is extracted by ultraviolet irradiation of W3104 λ imm^{434}. The λcI mutant contains a mutation in the gene which codes for the λ repressor protein.

Phage Extraction

The wild-type phages (λ imm$^{\lambda}$ and λ imm^{434}) were obtained in the manner outlined below:

1. Bacterial cells containing the integrated prophage were starved in TM medium for 5 hours at 37° C.

2. A 5.0 ml innoculum from the above stock was placed into 15.0 mls of KM medium.

3. This culture was grown to 5.0 x 10^8 cells/ml, as determined by the precalibrated Bausch & Lomb Spectronic 20 absorbance (600 nm) reading, at 37° C in a New Brunswick Gyrotory water bath shaker, and then chilled to 4.0° C in order to stop all bacterial growth and cell division.

4. The entire culture was centrifuged at 4.0° C for 15 minutes at 500 rpm. The lysate was discarded and the bacterial pellet was resuspended in TM medium at 4.0° C.

5. Aliquotes of 20.0 mls were placed under an Ultraviolet Products Inc. Mineralight UVSL 13 for 90 seconds (times for ultraviolet irradiation are dependent on the strength of the lamp and its distance from the culture).

6. Cultures were again chilled to 4.0° C.

7. Tryptone broth (10x concentrated) was added to these cultures to provide an abundance of nutrients. They were then transfered to the gyrotory bath at 37°C and the lytic cycle was followed using the Bausch & Lomb Spectronic 20.

8. After completion of the lytic cycle, cultures were centrifuged at 4.0° C for 15 minutes at 5000 rpm to separate out bacterial debris. A high speed centrifugation (120 minutes at 15000 rpm) was performed to remove the phage from the maltose medium, so that they could be resuspended in TMB and chloroform for cold storage. These and all other separations were performed using a Sorvall Superspeed RC2-B centrifuge which has a centrifugal radius of 4.25 inches.

Phage λ cI was obtained from H. Echols. A procedure similar to that outlined above was employed to prepare a λ cI stock, except instead of using ultraviolet irradiation, the stock was prepared by collection of the lysate from a liquid culture of λ cI - infected W3104.

Phage Assays

To determine phage titers, 0.5 ml samples of the lysate for λ imm$^{\lambda}$, λ imm^{434} and λ cI were taken through a sequential dilution series using tenfold dilutions (0.5 ml sample into 4.5 mls TMB) until appropriate concentrations for plating were obtained.

Plating was conducted according to the soft agar overlay method

(Adams 1959). To 0.2 ml samples of W3104 were added 0.2 ml aliquots of the appropriately diluted phage samples. These were then incubated for 20 minutes at 37° C. The phage samples and the assay bacteria were mixed with 3.0 mls of TB soft agar and plated on thick fresh TB agar plates. The plates were incubated overnight at 37° C. The number of plaques on each plate multiplied by the dilution factor gives the phage titer.

Lysogeny Testing

Tests for lysogeny were divided into three consecutive parts: (1) The determination of how many W3104 cells infected with λ imm$^{\lambda}$ at a m.o.i. of three actually become lysogenic. (2) The construction of a graph showing the relationship between the frequency of lysogeny and m.o.i. (3) The determination of the effect of varying λ imm^{434} m.o.i. in combination with a fixed λ imm$^{\lambda}$ m.o.i. on the potential of W3104 cells to become lysogenic for λ imm$^{\lambda}$. The procedures of these tests are outlined below:

1. A stock culture of W3104 cells suspended in KM medium was grown at 37°C to a concentration of 5.0×10^8 cells/ml (600 nm absorbance reading) and then chilled to 4.0° C.

2. The above culture was centrifuged at 5000 rpm for 15 minutes at 4.0° C. The lysate was discarded and the bacterial pellet was resuspended in TM at one-half its original volume, giving a final concentration of 1.0×10^9 cells/ml.

3. Phage stocks were diluted in TM to the appropriate concentrations for m.o.i.s of 3, 5, 7, 10, 15, and 20 pertaining to a concentration of 1.0×10^9 cells/ml for W3104.

4. One milliliter of the desired concentration of phage was added to 1.0 ml of W3104 cells (0.5 ml of λ imm$^{\lambda}$ and λ imm^{434} phages in the case of coinfection experiments). This brought the W3104 concentration back to 5.0×10^8 cells/ml and yet retained the desired m.o.i. of infecting phage.

5. These 2.0 mls cultures of combined phage and W3104 cells were incubated at 37° C (no shaking) for an adsorption time of 35 minutes. In the case of coinfection experiments, 2.0 mls of λ cI phage was added after 20 minutes. Addition of λ cI was necessary to kill any λ imm^{434} lysogens.

6. After adsorption had been allowed to take place, the cultures were chilled to 4.0° C and the appropiate dilutions were made, followed by plating using the soft agar overlay technique. Following overnight incubation, the number of colonies was determined. Nearly all colonies were lysogenic, as shown by a preliminary experiment in which each surviving colony from the assay plates was grown in 1.0 ml of TB at 37° C and then streaked across a fresh plate which had a lawn of W3104 cells on it. If the streak produced a clearing (plaques) on the W3104 lawn, then the colony was judged lysogenic originally, since the formation of plaques is due to spontaneous lysis. Over 80 percent of the colonies were found to be lysogenic, as judged by this assay.

EXPERIMENTAL RESULTS

Figure 4a is a graph of standard values for the m.o.i. - dependent frequency of lysogeny for λ imm$^{\lambda}$ alone as determined by this experimenter. The percent cells lysogenized as a function of the average phage input is a standard method used by phage biologists to measure the frequency of lysogeny. This graph was determined by procedures described in lysogeny testing, steps 1 through 7, in the materials and methods section of this paper. The results shown in Figure 4a are comparable with those prepared by Kourilsky (1973) for several other lambda phages.

Figure 4b represents the frequency of lysogeny for λ imm$^{\lambda}$ coinfected with λ imm^{434}. To determine the effect produced by the coinfection of the λ imm^{434} phage into the λ imm$^{\lambda}$ - W3104 system, experiments were conducted using a constant m.o.i. of 4.6 for λ imm$^{\lambda}$. To this m.o.i. were added λ imm^{434} m.o.i.'s of 4, 6, 10, 15, and 20. The results, as shown in Figure 4b, indicate that λ imm^{434} does not contribute any measurable m.o.i. - dependent increase to the frequency of lysogeny of λ imm$^{\lambda}$. In fact, a slight downward slope of the curve is noted. This result will be explained in the discussion. Figure 4c is an extension of Figure 4b along the abscissa. Figure 4c shows that between m.o.i.s of about 7 and 15 for λ imm^{434} the m.o.i. for λ imm$^{\lambda}$ does indeed remain constant at 4.6 (point at which Figure 4a intersects Figure 4c).

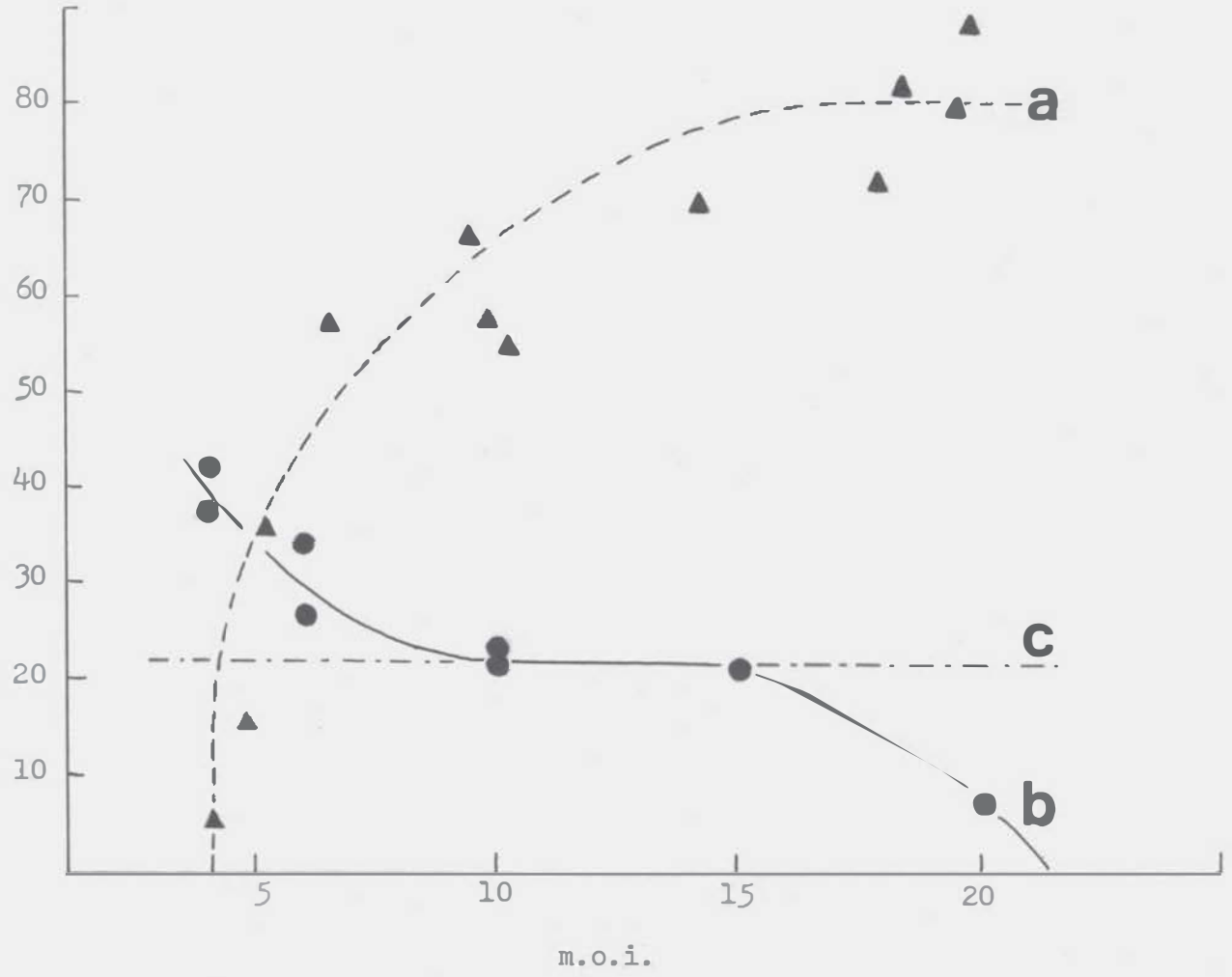

Figure 4. (a) Lysogenization of exponentially growing cells by $\lambda \frac{imm^{\lambda}}{imm}_{434}$ (b) Effect of coinfection of $\lambda \frac{imm}{imm}^{\lambda}$ and $\lambda \frac{imm}{imm}$ on the yields of $\lambda \frac{imm}{imm}^{\lambda}$. The $\lambda \frac{imm}{imm}^{\lambda}$ m.o.i. was held constant at 4.6. (c) Point of intersection of line (c) with (a) occurs at a λ m.o.i. of 4.6.

DISCUSSION

The results of this study demonstrate clearly that λ imm^{434} has no m.o.i. - dependent effect upon the ability of λ imm$^{\lambda}$ to establish the lysogenic state, as shown by the section of Figure 4b between m.o.i.s of about 7 and 15. The finding that λ imm^{434} has no m.o.i. - dependent effect is not unexpected since it is well known that the primary lysogenic functions of λ imm$^{\lambda}$ and λ imm^{434} are immunity specific. This means that the repressor function of the λ imm^{434} cI gene does not stimulate λ imm$^{\lambda}$ lysogeny, since the two phages are heteroimmune to each other (Thomas and Bertani 1964; Brooks 1965).

The portion of Figure 4b between m.o.i.s 15 and 20 shows a downward slope of the total curvilinear response. This can be attributed to lysis of the cells from without during the initial stages of infection. This occurs at m.o.i.s of 25 or greater (Kourlisky 1973). Due to the high numbers of phage attachments to the W3104 cell, the cell membrane is disrupted and the bacterial DNA "leaks" out causing the death of the cell. The m.o.i.s shown on the abscissa of Figure 4b are those only for λ imm^{434}. To these m.o.i.s were also added m.o.i.s of 4.6 for λ imm$^{\lambda}$ and 5.0 for λ cI. This means that at a m.o.i. of 15 for λ imm^{434}, the total m.o.i. for all three phages is actually 24.6 which would cause lysis from without.

From Figure 4b, it is apparent that low m.o.i.s of λ imm^{434} exert a general enhancing effect on lysogeny by λ. This is particularly marked at m.o.i.s less than 5. It has been the experience of lambda workers in general (and in this lab) that results obtained for phage yields at low m.o.i.s differ extensively from results obtained at higher

multiplicities. We are at present completely unable to explain this result. This finding does not, however, detract from the overall conclusion that λ imm^{434} does not exert a m.o.i. - enhancing effect on λ lysogeny.

Previous studies in this laboratory have concerned measurements of phage yields as a function of m.o.i. of the heteroimmune phages (Baumgardner, Elseth and Simmons in preparation). These results indicated the following:

(1) λ imm$^{\lambda}$ alone had a m.o.i. - dependent depressing effect on its own phage yields.

(2) λ imm^{434} alone had a m.o.i. - dependent depressing effect on its own phage yields.

(3) λ imm$^{\lambda}$ had a m.o.i. - dependent depressing effect on λ imm^{434} phage yields.

(4) λ imm^{434} had a m.o.i. - dependent depressing effect on λ imm$^{\lambda}$ phage yields.

(5) λ imm$^{\lambda}$ cI mutant has lost its m.o.i. - dependent depressing effect on λ imm^{434} phage yields.

The above results indicate that the depression of phage yield as a function of the m.o.i. of a heteroimmune phage is due to the cI gene. Figure 4b shows that λ imm$^{\lambda}$ is not able to utilize λ imm^{434} cI^{+} repressor for a m.o.i. - dependent increase in its own frequency of lysogeny. The combined results of Baumgardner, Elseth and Simmons (in preparation) and this experimenter suggest that the λ imm$^{\lambda}$ cI^{+} gene is responsible for a depression in λ imm^{434} phage yields and the λ imm^{434} cI^{+} gene is responsible for depression in

λ imm λ phage yields, but the depression is not due to the lysogenic response caused by the cI gene. This strongly suggests that the cI gene could possibly have other functions besides producing repressor. As no specific role for cI other than specification of repressor has been reported in the lambda literature, this is an interesting result. Unfortunately, time-considerations prevented further investigation of this problem at the present time.

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