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Nonessential Functions of Bacteriophage λ

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Seven plaque-forming biotin-transducing λ mutants (λ*bio*) have been used to characterize nonessential λ functions. Five of the λ*bios* have deletions in the center of the λ map extending to the right of the attachment region (att_λ), as predicted by the Campbell model, but the other two seem to be anomalous in this respect. The extent of the deletions establishes the order att_λ-*int*-(*exo*,*red*)-β-*cIII*, for markers in the λ center. The correlation in position of the *exo* and *red* markers suggest that the exonuclease protein is involved in genetic recombination, and the viability of $exo^-\beta^-$ λ*bios* shows that neither exonuclease nor β-protein is essential for λ growth. The two anomalous λ*bios* have defects in the *b2* region to the left of att_λ. The five standard λ*bios* are $b2^+$, yet they appear to have structural integration deficiencies in addition to their functional defects.

INTRODUCTION

Bacteriophage λ is a specialized transducing phage, able to transduce restricted regions of the *Escherichia coli* genome. This type of transduction was first observed for the galactose (*gal*) region (Morse *et al.*, 1956), which lies near the left end of the λ prophage map (Rothman, 1965). In the transducing particles (called λ*dg*) the *gal* genes are substituted for a block of phage DNA that codes for essential functions, and the transducing phages are therefore defective in phage growth (Campbell, 1961). Later Wollman (1963) observed transduction of the biotin (*bio*) region, which was subsequently shown to lie near the right end of the λ prophage map (Rothman, 1965).

The model proposed by Campbell (1962) accounts for deletion of the phage genome during the formation of a transducing particle and for the location of the deletions (see Fig. 1). In λ*dg* the deletions extend (on the vegetative map) from the attachment region (*att*) toward the A cistron for various distances (Campbell, 1961), and in phages transducing *bio*, from *att* toward the R cistron (Kayajanian, 1968). Some *bio* transducing phages are able to form plaques (Wollman, 1963), suggesting that there is a region just to the right of *att* in which there are no essential genes. Consistent with this, no conditional-lethal mutants have been found between *h* and *cIII* (Campbell, 1961). However, although by definition the plaque-forming λ*bio*s do not lack any essential functions, they do seem to be abnormal in at least one respect, for they are deficient in the ability to lysogenize (Wollman, 1963).

Study of several plaque-forming λ*bio*s, described here, has characterized some of the nonessential genes contained in the *att-cIII* region. These include genes for integration and recombination, as has been shown independently for the related hybrid phage $i^{\phi 80}h^{+\lambda}$ (Franklin, 1967), and genes for exonuclease (Radding *et al.*, 1967) and β-protein (Radding and Shreffler, 1966).

Five of the λ*bio*s conform to the predictions of the Campbell model, but two others are apparently more complicated.

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MATERIALS AND METHODS

Bacterial Strains

Bio⁻ strains. PA502 (*arg⁻his⁻gal⁻lac⁻-bio⁻pro⁻str^R sup⁺*), from Dr. F. Jacob, was crossed with Hfr Cavalli to give X5167 (*arg⁻his⁻gal⁻lac⁻bio⁻sup⁺*). X5167 was crossed with AR5 (Hfr P4X *mal⁻str^R rec⁻*; Signer and Weil, 1968), which carries the *rec⁻* allele described by Fuerst and Siminovitch (1965), to give QR8 (*arg⁻gal⁻lac⁻-bio⁻rec⁺*) and QR9 (*arg⁻gal⁻lac⁻bio⁻rec⁻str^R*). QR9 was transduced with P1 (grown on Hfr Hayes), with selection for UV resistance, to give QR23 (*arg⁻gal⁻lac⁻bio⁻-rec⁺str^R*); thus QR9 and QR23 are nearly isogenic. X5202 (*pro⁻pyr⁻lac⁻bio⁻str^R sup⁻*) was derived from AB2345 (*leu⁻pur⁻pro⁻-try⁻lac⁻gal⁻str^R sup⁺*), from Dr. F. Jacob, by several steps of mating with appropriate Hfr derivatives, followed by P1 transduction to introduce the *bio* marker from W602 (from Dr. E. Wollman).

Rec⁻ strains. AB2462, AB2463, AB2470 (Howard-Flanders and Theriot, 1966) and JC1569 (Clark *et al.*, 1966) were obtained from A. J. Clark.

Indicator strains. Permissive indicators were Q1, a *thi⁺* derivative of C600 (Appleyard, 1954), and Q5025, a $\lambda^R\lambda h^S$ derivative of C600. Nonpermissive indicator strains were 594 (Campbell, 1965a) and W3350 (Campbell, 1961).

Phage Strains

Except for λ*bio1* (see below), the phage strains used are derivatives of the wild-type λ of Kaiser (Kaiser, 1957), which will be called λ^+ in this paper. Mutant strains are those described by Signer and Weil (1968) and in addition λ*b2* (Kellenberger *et al.*, 1961), λ*sus029* (Campbell, 1961), λ*susN7N53* (from D. Hogness), and λ*int4* (Zissler, 1967). Other strains were constructed from these by recombination.

λ*ins80.* λ*h80.1* (Signer, 1964) and Hy4 ($h^+\lambda i^{80}$, Radding *et al.*, 1967), both of which attach at *att*80, were crossed to give an $h^\lambda i^\lambda$ phage. This phage transduces *try* but not *gal*. It also cures a (φ80) (φ80*dlac*) double lysogen of transducing phage, but not a (λi^{434}) ($\lambda i^{434} dg$) double lysogen. All the genome is presumably from λ except for a region between *h* and *c* responsible for attachment and curing specificity.

λ*bio11, 16, 64, 69, and 72.* C600 (λ) from the collection of S. E. Luria (A102) was induced with UV and divided before lysis into 16 cultures. λ*bio*s 64, 69, and 72 were isolated from three of the LFT lysates by transducing W602 at m.o.i. about one, purifying *bio⁺* transductants, making HFT lysates, and screening for plaque-forming λ*bio*. λ*bio*s 11 and 16 were isolated from two more LFT's by transducing QR8 at m.o.i. about 0.5, pooling 5–10 colonies to make a mixed HFT, and screening for plaque-forming λ*bio*s. These five λ*bio*s came from different LFT's and are therefore independent. No plaque-forming λ*bio*s were found by either method in the remaining 11 LFT lysates.

λ*bio1*. This phage was isolated by Wollman (1963) from a lysate of a different strain of λ, the strain carried by the lysogen Y10. This λ carries a small plaque marker, g^+, near *h*. We crossed the Wollman λ*bio* with λ*h susN7 c857* and selected a λ*bio* which was *h sus⁺* and which no longer carried the small plaque marker. The *h* allele was later removed by J. Weil in a cross with λ^+.

λ*bio7-20.* This is one of twelve plaque-forming λ*bio*s isolated by G. Kayajanian (Kayajanian, 1968).

Media

LB is 1% tryptone, 0.5% yeast extract, and 0.5% NaCl, adjusted to pH 7.5. EMBG agar is the EMB agar of Campbell (1957) with 0.05% glucose in place of the galactose. MM agar is 1.05% K_2HPO_4, 0.45% KH_2PO_4, 0.005% $MgSO_4$, 0.1% $(NH_4)_2SO_4$, 0.05% sodium citrate, 0.2% glucose, 0.1% Difco vitamin-free casamino acids, 0.001% arginine, 0.1 μg/ml thiamine-HCl, and 1.5% agar. λ broth is 1% tryptone and 0.25% yeast extract. λ agar and λ top agar contain in addition 1.2% and 0.65% agar, respectively.

Phage dilutions were made in SM (Weigle *et al.*, 1959).

Methods

Transduction experiments. Recipient bacteria were grown to about 1.5×10^9 in LB,

centrifuged and resuspended in 10^{-2} *M* $MgSO_4$. Transducing phage were added at m.o.i. 0.01 and helper phage at m.o.i. 5–10. After 20 min adsorption, the infected cells were diluted and spread on MM agar. In some experiments 0.1 unit avidin (Nutritional Biochemicals Corporation) was spread on each MM plate.

Single plaques were assayed for transducing activity by using a *bio*⁻ strain as indicator. The turbid centers of the plaques were picked, stabbed into MM agar, and incubated overnight. Growth was observed only when the phage was λ*bio*.

Bacteriophage crosses. Crosses were done by the method of Signer and Weil (1968).

Tests for normal integration. Phage were tested in two ways for the ability to integrate into the bacterial chromosome. One was the plaque assay of Signer (Signer, 1967). Under the condition of this assay, integration-deficient mutants—λ*b2*, λ*int*, and λ*bio*—produce colorless plaques; λ⁺ makes blue plaques. Plaques on ordinary plates were tested for the ability to integrate by picking the turbid centers to EMBG agar, either with (Gottesman and Yarmolinsky, 1968) or without (Zissler, 1967) 10^9 λ*b2cI* spread on the plate. The bacteria from plaques of integration-deficient mutants grow as red or mottled colonies; those from λ⁺ grow as white colonies.

Complementation experiments. QR9 (*bio*⁻*rec*⁻) was grown to about 2×10^8 per ml in λ broth + 0.2% maltose, centrifuged, and resuspended in 10^{-2} *M* $MgSO_4$. The cells were infected at m.o.i. about 5 for each phage used. After adsorption for 20 min, the adsorption mixture was diluted and plated on λ agar. After incubation for about 40 hours at 34°, the colonies were transferred with sterile toothpicks to EMBG-λ*b2cI* plates, one incubated at 40° and one at 34° (see *Test for immunity*).

Test for immunity. Colonies were tested for immunity by transferring them with sterile toothpicks to EMBG-λ*b2cI* plates (Gottesman and Yarmolinsky, 1968). When immunity at 40° was tested, the plates were prewarmed and kept on a 45° heated block during the picking of the colonies. Immune cells grew into a white colony; sensitive cells gave a very small red colony. At 40° λ*c857* lysogens often gave no growth, although sometimes there were red colonies of what must have been sensitive segregants.

Assays of exonuclease and β-protein. Lysogens were induced and assayed as described previously (Radding, 1966; Radding and Shreffler, 1966).

RESULTS

Integration

We shall refer to the five strains λ*bio1*, λ*bio11*, λ*bio69*, λ*bio72*, and λ*bio7-20* as the

TABLE 1

TRANSDUCTION OF *rec*⁻*bio*⁻ RECIPIENT[a]

Helper	Transductants per 10^6 PFU						
	Standard set λ*bio*s					Anomalous λ*bio*s	
	7–20	*69*	*72*	*11*	*1*	*16*	*64*
None	6	12	2	4	6	16	120
λ	1,600	6,000	8,000	4,000	3,000	4,000	300
λ*b2*	16,000	17,000	10,000	16,000	7,000	<1	8
λ*int*	<2	<2		2		<2	<2
λ*ins80*	50	500		600		70	400

[a] Transduction of QR9 (*rec*⁻*bio*⁻) as described in Materials and Methods. Transduction frequencies under 100 are based on fewer than 50 colonies. The table contains the results of four overlapping experiments: experiment 1, λ*bio*s 69, 11, and 64; experiments 2 and 3, λ*bio*s 7–20, 69, 16, and 64; experiment 4, λ*bio*s 69, 72, 11, 1, 16, and 64 with all helpers except λ*int* and λ*ins80*. The values which represent more than one experiment are weighted means, each transduction frequency being weighted by the number of colonies it was based on.

standard set to distinguish them from λ*bio16* and λ*bio64*, which will be shown to behave anomalously in several respects.

Integration of λ*bio* results in transduction, and therefore low frequencies of integration can readily be detected simply by selecting transductants. This type of transduction is best studied in a recombination-deficient (*rec*⁻) bacterial host, where transduction by recombination in the *bio* region, promoted by the host recombination system, is greatly depressed. Table 1 shows the transduction frequencies obtained with λ*bio*s in single infection of a *rec*⁻ host, and in mixed infection with one of the following helper phages: λ⁺, the integration-defective mutants λ*b2* or λ*int*, or the hybrid λ*ins80* which integrates at a different chromosomal region.

λ*bio*s of the standard set give very low transduction frequencies in single infection.[2] Helping with λ⁺ raises the frequency 100- to 1000-fold, showing that the integration defect of λ*bio* can be complemented by the wild-type. Since λ*b2* complements even better than λ⁺, the helper phage need not be able to integrate by itself. However, λ*int* does not complement at all, indicating that part of the complementation process is the provision of a functional Int system. A helper that attaches at a different site (λ*ins80*) complements, but less efficiently than wild-type.

The anomalous strains, λ*bio16* and λ*bio64*, differ in two ways. Neither is complemented by λ*b2*, and both transduce more efficiently in single infection than the strains in the standard set. In addition, λ*bio64* is complemented by λ⁺ less efficiently than any of the others.

[2] Infection with λ*bio* alone produces rare transductants which are usually λ-sensitive.

λ*bio7-20* produces the λ system (Red) which promotes general vegetative recombination (see *Functional Extent of the Deletions*). Although one might expect this recombination to result in transduction, the transduction frequency of λ*bio7-20* in single infection is no higher than that of the other λ*bio*s in the standard set, which are *red*⁻. It may be that the Red system is not expressed efficiently in infected cells destined for lysogeny (Gottesman and Yarmolinsky, 1968). Although the other *red*⁺ λ*bio*s (16 and 64) yield a higher level of transductants in single infection, these λ*bio*s are anomalous in several respects (see below).

TABLE 2

TRANSDUCTION OF *rec*⁺*bio*⁻ RECIPIENT[a]

Helper	Transductants per 10^6 PFU		
	Standard set λ*bio*s		Anomalous λ*bio*
	69	*11*	*64*
None	7,000	300	40,000
λ	50,000	60,000	10,000
λ*b2*	100,000	90,000	800
λ*int*	400	100	300
λ*ins80*	7,000	7,000	3,000

[a] Transduction of QR23 (*rec*⁺*bio*⁻) as described in Materials and Methods. Frequencies under 400 are based on fewer than 50 colonies.

Table 2 shows that, in a *rec*⁺ recipient, the pattern of transduction frequencies seen in the *rec*⁻ is superimposed upon a background of transduction promoted by the bacterial recombination system.

FUNCTIONAL EXTENT OF THE DELETIONS

The Campbell model predicts that any phage material deleted in λ*bio* should lie to the right of *h* on the λ map (Fig. 1). We have analyzed the phages both genetically and physiologically for their content of λ genes whose location in this region is known (*int*, *b2*, *red*, exonuclease, *cIII*, *N*) or suspected (β-protein).

1. int (Zissler, 1967; Gingery and Echols, 1967)[3]

The data discussed in the preceding section show that all the λ*bio*s can be complemented by λ⁺ but not by λ*int*. Table 3 shows that the three λ*bio*s tested fail to complement λ*int*, and that the *int*⁺ allele cannot be rescued from any of the λ*bio*s by recombination. In contrast, λ*b2* both complements λ*int* and recombines with it to give λ⁺ (Table 3). Therefore both functionally and genetically all the λ*bio*s are *int*⁻.

2. b2 (Jordan, 1965)

Table 4 shows that all the λ*bio*s in the standard set give normally integrating recombinants when crossed with λ*b2*, and

[3] References in the section headings refer to mapping.

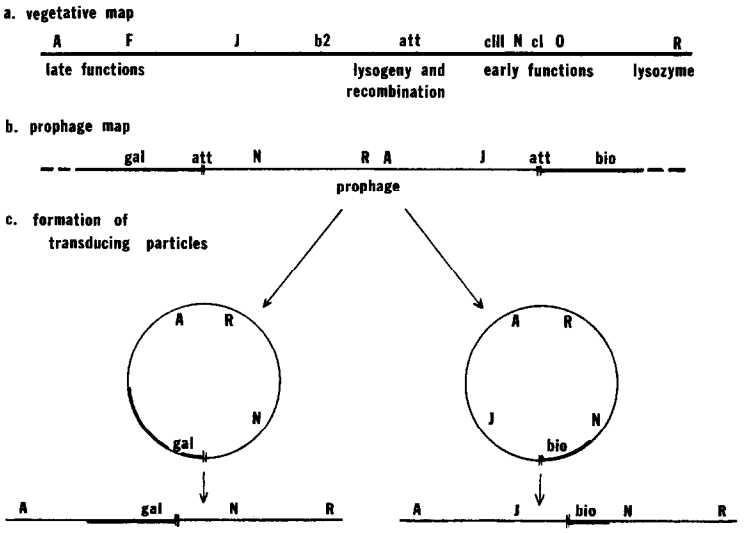


FIG. 1. Vegetative and prophage maps of λ and mechanism of formation of transducing particles according to Campbell (1962).

hence appear to be *b2*$^+$. However, λ*bio16* and λ*bio64* are again anomalous since they cannot be shown in this way to be *b2*$^+$.

TABLE 3

PRESENCE OF *int*[a]

	Cross with λ*int4*		Complementation of λ*int6*
	Frequency of normally integrating recombinants	Recombination	Frequency of lysogens
λ*bio7-20*	0/7400	<0.027	—
λ*bio69*	0/1650	<0.12	0/278
λ*bio72*	0/1930	<0.10	—
λ*bio11*	0/1340	<0.15	0/160
λ*bio1*	0/1800	<0.11	—
λ*bio16*	0/1880	<0.11	—
λ*bio64*	0/1450	<0.14	0/224
λ*b2*	56/2530	4.4	86/514

[a] Complementation of λ*int6*: QR9 (*bio*$^-$*rec*$^-$) was mixedly infected with λ*bio* and λ*c857 int6* and the survivors were tested for immunity as described in Materials and Methods. Crosses with λ*int4* were done with ultraviolet irradiation, and plated nonselectively in the plaque assay for integration (Signer, 1967). Since λ*bio*s integrate poorly (Table 1), rescue of the *int*$^+$ allele from λ*bio* was scored by the presence or absence of normally integrating recombinants. Recombination between the *bio* deletions and *int4* is expressed as the frequency of normally integrating recombinants times 2 times 100.

3. red (Echols et al., 1968; Signer and Weil, 1968)

The *red* genes are determinants for a phage-produced system promoting general vegetative recombination. λ*bio*s 16 and 64 (Signer and Weil, 1968) and 7-20 (Shulman, personal communication) are *red*$^+$, whereas λ*bio*s 1, 11, 69 and 72 are *red*$^-$ (Signer and Weil, 1968).

4. Exonuclease and β-protein (Radding et al., 1967; Szpirer, Thomas, and Radding, in preparation)

Table 5 shows that assays of crude extracts for exonuclease activity and β-protein antigenicity group the λ*bio*s into three classes: (a) *exo*$^+$β$^+$ (λ*bio*s 16, 64 and 7-20); (b) *exo*$^-$β$^+$ (λ*bio*s 69 and 72); and (c) *exo*$^-$β$^-$ (λ*bio*s 1 and 11). An example of the immunodiffusion assay for β-protein is shown in Fig. 2.

5. N and cIII (Eisen et al., 1966; Kaiser, 1957)

The fact that λ*bio*s form turbid plaques indicates that they have normal *cIII* and *N* genes. This is confirmed by the following two crosses:

(*a*) λ*bio11* (*exo*$^-$β$^-$) × λ*susN7N53*. The cross was done in a *sup*$^-$ host. The burst size of λ*susNN* was about 30, indicating complementation by λ*bio11* (cf. Table 5).

TABLE 4

PRESENCE OF *b2*[a]

	Crosses with λ*b2*		
	Frequency of normally integrating recombinants		Recombination
	Method 1	Method 2	
λ*bio7-20*	89/10,800	20/600	2
λ*bio69*	33/1,100		6
λ*bio72*	38/2,100		4
λ*bio11*	65/2,700	11/378	5
λ*bio1*	72/2,200		7
λ*bio16*	0/2,000	0/473	<0.3
λ*bio64*	0/2,400	0/480	<0.3
λ*int4*	54/1,850		6

[a] Ultraviolet-irradiated crosses with λ*b2* scored as in Table 3 (method 1) or by picking plaques to EMBG medium (method 2, see Materials and Methods).

TABLE 5

SYNTHESIS OF PROTEINS AND PHAGE[a]

Phage	After induction of lysogen			After infection
	Exonuclease units/mg protein	β-protein	Phage titer per ml	Average burst size
λ+	7-9	+	9.9×10^9	150
λ*bio7-20*	6.5	+	4.0×10^9	—
λ*bio69*	<0.5	+	1.2×10^8	—
λ*bio72*	<0.5	+	9.2×10^7	—
λ*bio11*	<0.2	−	1.1×10^8	16
λ*bio1*	<0.5	−	4.3×10^6	—
λ*susN7N53*	<0.2	−	$<10^2$	<0.05
λ*bio16*	8.6	+	2.0×10^9	—
λ*bio64*	4.1	+	2.0×10^9	—

[a] Lysogens of QR8, QR23, or W3350 grown to approximately 2×10^8 cells/ml were induced with mitomycin C at 6–15 μg/ml and assayed for exonuclease activity and β antigen as described previously (Radding and Shreffler, 1966). Infection experiments were done at multiplicity of infection of 10 with strain W3350.

Sus+ recombinants which plate on *rec*− (see Section 6 below) appeared at a frequency of 0.5%; five of these were tested and shown to be *exo*+β+ (two of them shown in Fig. 2).

(*b*) λ*bio1* × λ*cIII67*. The phage-infected cells were irradiated with ultraviolet light to increase recombination frequency. About 3% of the large plaques appearing on a *rec*− indicator (on which λ*bio1* does not plate efficiently, see below) were turbid.

6. Growth characteristics

Phage yield after induction of *rec*+ λ*bio* lysogens is shown in Table 5. Although part of the reduced yield observed for all the λ*bio*s is certainly due to the fact that they are *int*−, the yield appears to be less for

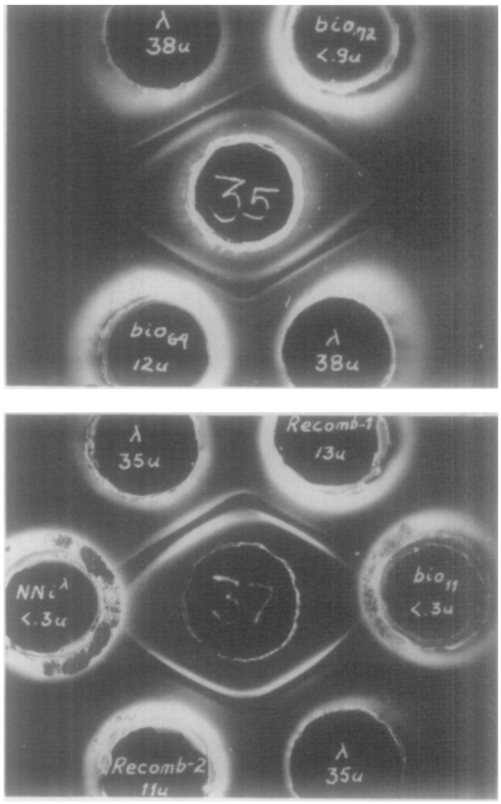


FIG. 2. Detection of exonuclease and β-protein by immunodiffusion (Radding and Shreffler, 1966). The central wells contained antiserum to exonuclease and β-protein; the peripheral wells contained crude extracts of induced lysogens. The numbers indicate the total units of exonuclease measured by enzymatic assay (see Table 5). The precipitin band farthest from the central well represents exonuclease, and the other precipitin band represents β-protein. Extracts of λT11 (Radding and Shreffler, 1966), marked λ, were used as a source of exonuclease and β-protein as antigenic standards. *Recomb-1* and *-2* are recombinants from the cross of λ*bio11* and λ*susN7N53*.

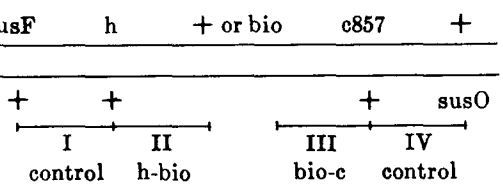
TABLE 6

SUMMARY OF PROPERTIES

λ*bio*	*b2*	*int*	*red*	*exo*	*β*	Plating on *rec*⁻
Standard						
1	+	−	−	−	−	−
11	+	−	−	−	−	−
72	+	−	−	−	+	+
69	+	−	−	−	+	+
7-20	+	−	+	+	+	+
Anomalous						
16	−	−	+	+	+	+
64	−	−	+	+	+	+

TABLE 7

GENETIC EXTENT OF *bio* DELETIONS[a]



Cross used for mapping

Phage	Single Exchanges in Region I	II	III	IV	Triples	Total	Sus⁺ frequency (%)
a. Raw data							
λ⁺	438	591		69	5	1103	3
λ*bio7-20*	441	334	307	82	18	1182	3
λ*bio69*	601	443	322	80	33	1497	3
λ*bio72*	605	448	305	98	31	1487	4
λ*bio11*	606	466	295	74	44	1485	3
λ*bio1*	518	396	223	66	24	1227	3

	II	III
b. Exchange frequencies normalized to sum of I + IV		
λ⁺	1.17	
λ*bio7-20*	0.64	0.59
λ*bio69*	0.65	0.47
λ*bio72*	0.64	0.43
λ*bio11*	0.69	0.43
λ*bio1*	0.68	0.38

[a] Crosses were of the type λ*susO* × λ*susF h cI*. The *susO*⁻ parent was always *int*⁻. The *susF*⁻*h cI* parent was either *int*⁻ or *bio*. *Sus*⁺ recombinants were selected by plating on X5202 (*bio*⁻*sup*⁻) at 34°. They were scored for internal markers by picking with toothpicks and transferring to MM agar at 34° (for *bio*), a lawn of Q5025 at 37° (for *h*), and a lawn of Q1 at 40° (for *cI*). Recombinants were scored as single exchanges in regions I, II, III, or IV (see figure) or triples. In each case part a of the Table lists the pooled data from four separate crosses.

The genetic size of an interval is proportional to the single exchange frequency for small frequencies (Bailey, 1951). We first compute the genetic size of intervals II and III by normalizing each cross to the same relative scale. The intervals *susF-h* (I) and *cI-susO* (IV) are control intervals which should correct for variation in absolute recombination frequency. As expected, the ratio I/IV in each cross is constant within experimental error. Therefore, part b of the Table presents exchange frequencies in intervals II and III, normalized in each case to the sum I + IV. A map based partly on these results is presented in Fig. 3.

those with larger deletions (compare Table 6 and next section), in agreement with plaque size on *rec*⁺ strains. Table 5 also compares growth after infection for λ*bio11* and λ*susN7N53*. Although both phages are *exo*⁻*β*⁻, it is clear that λ*susN7N53* is markedly defective and λ*bio1* is not.

λ*bio1* and λ*bio11* differ from the others in that they fail to plate efficiently on *recA*⁻ hosts [AB2462, AB2463, JC1569 (Howard-Flanders and Theriot, 1966), QR9; efficiency of plating $\leq 10^{-3}$, very tiny plaques]; they do plate on a *recB*⁻ host (AB2470, efficiency ≥ 0.5).

The results presented in this section are summarized in Table 6. Assuming the Campbell model, the data indicate the map order *att-int-*(*exo*, *red*)*-β-cIII*.

GENETIC EXTENT OF THE DELETIONS

The extent of each deletion in the standard set has been mapped in vegetative crosses. Since the integration (Int) system of λ promotes site-specific vegetative recombination in the attachment region (*att*) and would therefore distort genetic distances in the center of the λ map, mapping was done by crossing the λ*bio*s (which are all *int*⁻) against an *int*⁻ point mutation. The plan of the crosses and the results are presented in

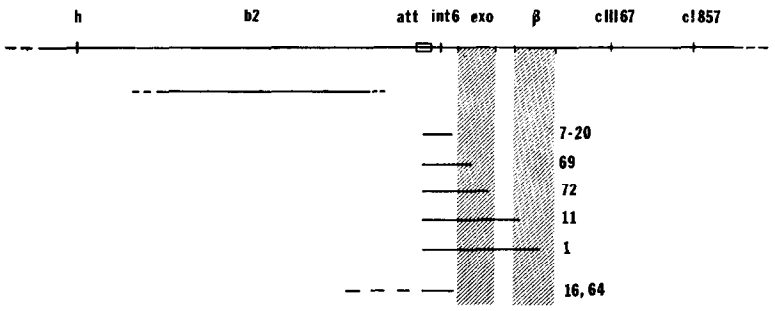


FIG. 3. Qualitative map of the middle region of the λ genome, based primarily on the results summarized in Table 6. The map order of *int*, *exo*, and *β* is based on Table 6 and the assumption that the standard set λ*bios* were formed according to the Campbell model. The position of *h*, *int6*, *cIII67*, and *cI857* are from Signer and Weil (1968). The lengths of the deletions in the λ*bios* are based on the results in Table 6 (which show that λ*bio72* must carry a shorter deletion than λ*bio11*) and the results in Table 7 (which suggest that the deletion in λ*bio69* is shorter than that in λ*bio72*, and that the deletion in λ*bio11* is shorter than that in λ*bio1*). The *b2* deletion has been placed to the left of all the *bio* deletions, in agreement with Table 4.

Table 7. Within the limits of measurement, all the deletions of the standard set have the same left end point. It is clear that the deletion in λ*bio7-20* is the shortest *bio* deletion. The fact that the total *h-c* recombination frequency is about the same in λ$^+$ and λ*bio7-20* suggests that little phage material is deleted in the latter, and therefore that the *int* gene is located close to att_λ. In the case of the remaining four λ*bio*s, there is some suggestion that the right end points may be arranged in the order 69-72-11-1, which is consistent with the order deduced from functional analysis (Table 6). However, the significance of the differences in frequency among these four is questionable, and these deletions are now being mapped with respect to loci closer than *c857*. We should point out that in crosses of the sort described here there is no way to estimate whether the nonhomology between the parental phages has any effect on recombination frequency in adjacent homologous regions.

A qualitative map of this region, derived from Tables 6 and 7, is shown in Fig. 3.

MECHANISM OF COMPLEMENTATION

An *int*$^-$ point mutant can be complemented by λ*b2* in trans; that is, λ*b2* can allow λ*int* to integrate without stably integrating itself (Zissler, 1967; Gingery and Echols, 1967; Gottesman and Yarmolinsky, 1968). In contrast, although λ*bio* can be complemented by λ*b2*, the complementation appears to act only in *cis*, as shown by the experiment presented in Table 8. Whereas two-thirds of the lysogenic survivors of mixed infection with λ*int* + λ*b2* are singly lysogenic for λ*int*, none of the lysogenic

TABLE 8

COMPLEMENTATION BY λ*b2* OF λ*int* AND λ*bio*[a]

Infection with		Immunity type of survivor		
		Immune		
		Thermostable	Thermoinducible	Sensitive
λ*bio69cI857* +	λ*b2c*$^+$	100	4	104
λ*int6cI857* +	λ*b2c*$^+$	21	40	249
—	λ*b2c*$^+$	0	0	53
λ*bio69cI857*	—	0	0	67
λ*int6cI857*	—	0	0	48

[a] Complementation was measured as described in Materials and Methods. The thermostable lysogenic survivors from λ*bioc857* + λ*b2c*$^+$ were mostly (97%) *bio*$^+$ and therefore must have been either doubly lysogenic for λ*bioc857* and λ*b2* or singly lysogenic for recombinant λ*bioc*$^+$. The four thermoinducible survivors from λ*bioc857* + λ*b2* are probably either doubly lysogenic for λ*bioc857* and recombinant λ*b2c857*, or singly lysogenic for recombinant λ*c857*. When tested for phage production they were found to be high yielders, in contrast with four thermoinducible survivors from λ*intc857* + λ*b2c*$^+$ which were low yielders (see Gottesman and Yarmolinsky, 1968; Fischer-Fantuzzi, 1967).

survivors from the mixed infection with λ*bio69* + λ*b2* appear to be singly lysogenic for λ*bio* (see legend to Table 8). Similar results have been obtained in a less rigorous way for λ*bio*s 1, 11 and 72. Among 10, 26, and 10 transductants (respectively) made with λ*bioc857* + λ*b2c*$^+$, all were immune at high temperature and therefore carried the c^+ allele of the helper phage.

We conclude that, although the λ*bio*s are *int*$^-$, they must have some other integration defect as well.

DISCUSSION

The Campbell Model

Our data bear on three predictions of the model. First, the biotin genes in λ*bio* should replace λ genes in the center of the vegetative map. Second, although the right end points of the deletion may differ among the λ*bio*s, the left end points should be the same. Third, only a single continuous block of phage genes should be deleted.

The λ*bio*s in the standard set appear to fit these predictions. Table 7 shows that the *bio* region lies between *h* and *c*. Within the accuracy of the measurement the left end point is the same for all the deletions,[4] whereas the right end points seem to be different. The mapping data are neither precise nor extensive enough to indicate whether each deletion removes only a single block of genes, although they are consistent with this notion.

The two anomalous λ*bio*s, which appear to be *b2*$^-$ in addition to *int*$^-$, were isolated from the same lysogenic clone as four of the standard set, and hence cannot have originated from abnormal insertion. If the Campbell model is correct, the same deletion cannot have removed both *b2* and *int*. A possible explanation is that the *b2* defect is due to a second mutation, which might also account for the unusual transduction properties. We have recently backcrossed λ*bio16* to λ$^+$ and isolated two new recombinant types, one very similar to λ*bio7-20* and the other very similar to λ*b2*. Further studies are in progress.

Mapping

Functional and genetic analysis indicate the map order *att-int-*(*exo, red*)*-β-cIII*, in agreement with other studies (Radding *et al.*, 1967; Szpirer, Thomas and Radding, in preparation).[5] Thus this portion of the map is concerned with integration and recombination, as found independently for the hybrid $i^{\phi 80}h^{+\lambda}$ by Franklin (1967). We note in addition that, assuming that standard λ*bio*s were formed according to the Campbell model, the point at which circular λ must be opened to give the prophage map must lie between *b2* and *int*, since all the standard λ*bio*s are *b2*$^+$*int*$^-$ (see also Fischer-Fantuzzi, 1967; Weil and Signer, 1968; Echols *et al.*, 1968).

Exonuclease and β-Protein

Although N mutants produce little or no exonuclease and β-protein, the *exo*$^-$*β*$^-$λ*bio*s confirm other evidence indicating that N is not the structural gene for either of these (Radding *et al.*, 1967; Szpirer, Thomas, and Radding, in preparation; Radding and Echols, 1968). The fact that this deficiency in N mutants is not due to polarity of the nonsense type (Radding and Echols, 1968) suggests that the N product regulates the synthesis of these proteins, as it might that of other λ proteins (Thomas, 1966; Protass and Korn, 1966; Skalka *et al.*, 1967). In any case, the fact that the *exo*$^-$*β*$^-$λ*bio*s form plaques shows clearly that these proteins are not essential for λ development.[6] There-

[4] The recombination frequencies from λ*b2* × λ*bio* (Table 4) are not relevant, since we now know that, at least for *bio11*, all the normally integrating recombinants arise through Int-promoted rather than general recombination (Kimball and Signer, unpublished).

[5] *Exo* is the structural gene for exonuclease (Radding *et al.*, 1967). Although *β* has not been shown to be the structural gene for the β-protein, this is a likely possibility since the two proteins are under joint regulatory control (Radding and Shreffler, 1966).

[6] At least not in a *rec*$^+$ host. Although the two λ*bios* (1 and 11) which are *β*$^-$ fail to grow in a *rec*$^-$ host, λ*red3* (Signer and Weil, 1968), which makes no detectable β-protein (Radding, unpublished), grows normally in *rec*$^-$. It is not yet clear whether this property is due to a gene which is present in λ*bios* 69 and 72 and absent in λ*bios* 1 and 11, or whether it is due to some other property of λ*bios* 1 and 11.

fore their absence is not the reason for the general defectiveness of N mutants, but rather another manifestation of it.

The original immunologic data (Radding and Shreffler, 1966) argued that exonuclease and β-protein were distinct proteins rather than different polymeric forms of the same protein. This conclusion is confirmed by the existence of both a λ-$\phi 80$ hybrid that is *exo*$^+\beta^-$ (Radding *et al.*, 1967) and λ*bio*s that are *exo*$^-\beta^+$ (Table 6). Both exonuclease and β-protein have been purified, free from detectable contamination by each other (Radding, unpublished).

The correlation between the presence of exonuclease and Red function among the λ*bio*s suggests that exonuclease might be involved in vegetative recombination. Recent biochemical studies of recombination-deficient (red) mutants have confirmed the role of exonuclease in recombination and have implicated the β-protein as a second recombination protein (Shulman, personal communication; Radding, unpublished).

Integration Deficiency of λ*bio*

All the λ*bio*s are *int*$^-$. Whereas λ*b2* complements λ*int* in *trans*, it appears to complement λ*bio* only in *cis*, suggesting that λ*bio* has an additional integration defect.

In integration λ*bio* behaves very much like λ*b2* and λ*dg*. All three attach poorly in single infection and can be complemented for integration only in *cis*, suggesting that a structural defect is involved (λ*dg*: Campbell, 1957; Weisberg, personal communication; λ*b2*: Campbell, 1965b; Gottesman, personal communication). It may be that the attachment regions in the phage and bacterial genomes are not identical (suggested independently by Guerrini, personal communication; see also Fischer-Fantuzzi, 1967). If so, then the attachment region at either end of the prophage, i.e., the ones contained in λ*dg* and λ*bio*,[7] might be different from both the phage and bacterial regions. Complementation of λ*dg*, λ*bio* and λ*b2* for integration might then reflect the provision of a suitable attachment region by the helper phage, as suggested by Guerrini (personal communication).

Let us consider three ways by which the helper might provide a suitable attachment region: (1) integration of helper phage followed by integration of λ*bio* at one of the *att* regions at either end of the prophage; (2) formation of a circular double-length molecule by joining of the single-stranded cohesive ends of helper and λ*bio*, followed by integration of the dimer to form a double lysogen; or (3) Int-promoted recombination between circular forms of helper and λ*bio*, followed by integration of this dimer. The dimers of (2) and (3) differ because, although each carries two *att* regions, those of (2) are parental whereas those of (3) are recombinant.

Since the Int system is site-specific (Signer and Beckwith, 1966; Echols *et al.*, 1968; Gottesman and Yarmolinsky, 1968; see also Weil and Signer, 1968), either (1) or (3) will explain why λ*ins80* helps less well than λ. Mechanism (2), on the other hand, would predict that λ and λ*ins80* would help equally well, although they would cause integration at different sites.

Neither (1) nor (2) explains why λ*b2* helps λ*bio*, since both mechanisms require λ*b2* to integrate efficiently by itself. However, λ*b2* and λ*bio* are recombined very efficiently by the Int system in vegetative crosses and one of the recombinants carries a normal *att* region (Signer, Weil, and Kimball, in preparation). Therefore, mechanism (3) can account for the helping because the circular dimer could integrate using the normal *att* region generated by recombination. Furthermore, since vegetative Int-promoted recombination between λ*b2* and λ*bio* is more efficient than that between λ and λ*bio* (Signer, Weil, and Kimball, in preparation), this mechanism would explain why λ*b2* helps more efficiently than λ (Table 1).

Thus, of the three hypotheses we have chosen to consider, mechanism (3) is the most plausible explanation. Weisberg and Gottesman (in preparation) have proposed models similar to (1) and (3) on the basis of their work with λ*dg*. We should point out that helping is at best an indirect reflection of events at the molecular level, and we may not be aware of all the relevant factors.

[7] Also possibly λ*b2*, which may be structurally similar to λ*dg* (see Hershey, 1967; Signer, 1968).

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