ISOLATION OF PLAQUE-FORMING, GALACTOSE-TRANSDUCING STRAINS OF PHAGE LAMBDA[1]

MICHAEL FEISS,[2] SANKAR ADYHA,[3] AND DONALD L. COURT[4]

Department of Biological Sciences, Stanford University, Stanford, California 94305

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ABSTRACT

Plaque-forming, galactose-transducing lambda strains have been isolated from lysogens in which bacterial genes have been removed from between the galactose operon and the prophage by deletion mutation.——A second class has been isolated starting with a lysogenic strain which carries a deletion of the genes to the right of the galactose operon and part of the prophage. This strain was lysogenized with a second lambda phage to yield a lysogen from which galactose-transducing, plaque-forming phages were obtained. These plaque-forming phages were found to be genetically unstable, due to a duplication of part of the lambda chromosome. The genetic instability of these partial diploid strains is due to homologous genetic recombination between the two identical copies of the phage DNA comprising the duplication. The galactose operon and the duplication of phage DNA carried by these strains is located between the phage lambda *P* and *Q* genes.

THE bacteriophage lambda chromosome integrates into the *E. coli* chromosome as a specific site. In lysates obtained from lysogenic cultures, transducing variants are found at low frequency. These variants carry bacterial genes, but only those that are closely linked to the prophage. Each of these transducing variants appears to result from a single non-homologous recombination event which excises a DNA segment from the lysogenic chromosome. This segment includes bacterial genes and phage genes; the sequence of genes in the lysogenic chromosome is maintained in transducing variants. The only requirement for phage material carried by transducing variants seems to be that the cohesive end site of the phage be present (KAYAJANIAN and CAMPBELL 1966; SATO and CAMPBELL 1970). Whether such transducing variants need be defective (unable to form plaques) or not seems to be determined by a limitation on the size of a transducing fragment (KAYAJANIAN and CAMPBELL 1966). This size limitation is in all probability the capacity of the phage lambda capsid. Thus, among galactose-transducing variants of lambda, isolated from a wild-type lysogen, the amount of bacterial DNA is so large that some essential phage genes must be

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[2] Present address and reprint address: Department of Microbiology, College of Medicine, University of Iowa, Iowa City, Iowa 52240.

[3] Present address: Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014.

[4] Present address: Department of Molecular Biology, University of California, Berkeley, California 94720.

discarded to produce molecules that can be packaged into mature phage particles. These galactose-transducing strains have been the subject of much research and a phage from this class will be referred to as a λ*dgal*: the *d* indicates the phage is defective, *gal* denotes the ability to transduce galactose markers. The phage material which is replaced by bacterial genes in λ*dgal* strains is a contiguous block from the right prophage end (as drawn in Figure 1a) and includes the *b* region and essential genes. The amount of essential material deleted varies from isolate to isolate but always includes genes *K*, *I* and *J*. A typical λ*dgal* has the following vegetative gene order: *A gal chlD pgl N cI O P Q S R*.

Recently, methods have been developed which allow selection of deletion mutations on either side of lambda (ADHYA *et al.* 1968; SHAPIRO and ADHYA 1969). Such deletions can alter the proximity and sequence of bacterial genes and lambda prophage genes. We describe here non-defective (plaque-forming) galactose-transducing variants obtained from two types of deletion lysogens. The first contains a deletion which removes bacterial DNA between the galactose genes and the prophage. In the second type, a deletion obtained in a lambda lysogen removes bacterial DNA between the galactose operon and the prophage and also removes part of the prophage. A second phage chromosome inserted in tandem into this latter type yields upon induction galactose-transducing variants. In these, the galactose genes are located in a different position than in λ*dgal* strains. These strains are plaque-forming and, in addition, contain duplications of phage DNA, a result consistent with the proposed structure of the deletion lysogen. Our studies indicate a vegetative gene order of: *A J N cI O P gal Q S R*.

MATERIALS AND METHODS

Strains: These are listed in Table 1.

Media: Tryptone broth, tryptone broth agar (1.0% agar), and tryptone soft agar (0.65% agar) were used for growth and titration of bacteria and phage; eosin methylene blue plus galactose agar (EMBG) was used for selection of transductants able to utilize galactose (Gal+) and testing the Gal character; these complex media have been described by CAMPBELL (1957 and 1961). Eosin methylene blue agar lacking sugar (EMBO) was used during selection of the lysogen SA307(λ*cI857b221*). Synthetic agar, appropriately supplemented, was used for biotin, nicotinic acid, and *aroG* transduction. P1*kc* transduction was carried out as described by ROTHMAN (1965). The test for the *pgl* character has been described by ADHYA and SCHWARTZ (1971).

Transductions: Gal⁻ bacteria (W3805, W3350, etc.) were grown to 4×10^8/ml, mixed with phage lysates, allowed to absorb and plated on indicator agar. Transduction of *aroG* was tested as described by WALLACE and PITTARD (1967), using AB3250. Transduction of *nicA*, using GG30, was as described by SATO and CAMPBELL (1970).

Preparation of lysates: Induction was carried out by heating growing cultures of λ*cI857* lysogens at 43°C for 15 min. After heating, aeration was continued at 37°C until lysis.

Preparation of strains: Phage recombinants were prepared by standard crossing techniques (PARKINSON 1968). Strains carrying the *red270* allele were detected by their inability to form plaques on p3478 (H. SHIZUYA pers. communication); strains containing *Nam53* were detected by their ability to plate on C600 and 112-12 and inability to plate on R594. Strains were made *recA1* by mating with KL16-99, followed by selection of *his*+ streptomycin-resistant recombinants. The *recA1* allele was scored by spotting suspensions of recombinants on EMBG agar and treating with ultraviolet (UV) light. The UV dose chosen (300 ergs/mm²) allowed spots of *rec*+ bacteria to grow on incubation but completely killed *recA1* bacteria. The *b221* deletion was

TABLE 1A

E. coli *K12 strains*

Strain	Description	Source or reference
SA500	*his, str,* lysogenic for λ*cI857*	This laboratory
W3805	*galE16*	E. LEDERBERG
W3350	*galK2, galT1*	This laboratory
R594	W3350 *str*	This laboratory
138–2	*galK138*	BUTTIN
MSA151	*galT151*	SHAPIRO and ADHYA (1969)
MSA79	*galT79*	SHAPIRO and ADHYA (1969)
MS226	*galT226*	SHAPIRO and ADHYA (1969)
MS60	*galT60*	SHAPIRO and ADHYA (1969)
W3101	*galT1*	SHAPIRO and ADHYA (1969)
R865	W3350 (λ*imm*^{434})	This laboratory
SA307	(*chlD . . . P*)Δ, *str, his*	This laboratory
C600	*leu, thr, thi, suII+*	This laboratory
C246	(*galT . . . bioD*)Δ, *str, his, pro*	P. CLEARY
KL16–99	*Hfr, thi, recA1*; transfers *recA1* early	LOW (1968)
SA472	(*aroG . . . chlA*)Δ	This laboratory
112–12	*gal, cys, su112–12+*	THOMAS *et al.* (1967)
GG30	(*nicA . . . chlD*)Δ, *his, str*	This laboratory
p3478	W3110 *thy, polA1*	DELUCIA and CAIRNS (1969)
AB3250	*thi, his, pro, arg, ilv, gal, aroG*	WALLACE and PITTARD (1967)

his = histidine requirement; *str* = resistant to streptomycin; *cI857* = heat-sensitive λ repressor mutation; *galK,T,E* = inability to ferment galactose, mutations in kinase, transferase, epimerase, respectively; *chlD* and *chlA* = mutations conferring resistance to anaerobic growth on chlorate; *imm*^{434} = immunity region of phage 434 recombined into λ; *I* = genes of λ; *Iam2* = particular amber mutation in I gene of λ; *suII+* = amber suppressor; *su112–12+* = ochre suppressor; *polA* = absence of DNA polymerase; *aroG* = requirement for tryptophan when grown in presence of tyrosine and phenylalanine; *nicA* = requirement for nicotinic acid. Δ symbolizes a deletion of the region in parentheses. Other genotypic symbols as proposed by TAYLOR (1970).

TABLE 1B

Phage strain

Strain	Description	Source or reference
λ*cI857*		This laboratory
λ*c26b221*	DNA content = 0.78*	D. BERG: DAVIS and DAVIDSON (1968)
P1*kc*		P. CLEARY
λ*cI857red270*	*am* mutation in β cistron	D. SCANDELLA
λ*cI857Nam7am53*		E. SIGNER
λ*imm*^{434}	DNA content = 0.97*	This laboratory
λ*b2imm*^{434}*c*	DNA content = 0.85*	This laboratory
λ*imm*^{434}*b221*	DNA content = 0.75*	This laboratory
λ*cI857Nam7am53nin5*		This laboratory

* λ+ DNA content = 1.00; these phages used as DNA content standards in CsCl gradients. Values of DNA content for the strains are from DAVIS and PARKINSON (1971).

scored by measuring the stability of phage particles to incubation in the presence of a chelating agent. As shown by PARKINSON and HUSKEY (1971), λ*b221* is inactivated by chelating agents very much more slowly than λ+. SA307(λ*cI857b221*) was prepared by spotting λ*cI857b221* on a lawn of SA307 at 30°C and the resulting spot of lysis was streaked at 30°C on EMBO agar seeded with about 10^9 λ*c26b221* as described by GOTTESMAN and YARMOLINSKY (1968). Clones of immune cells were purified. An isolate yielding only turbid plaque-formers (at 30°C) was selected and designated SA307 (λ*cI857b221*).

Isolation of bacterial deletions: Using techniques described previously (ADHYA *et al.* 1968), *chl* bacterial mutants of SA500 were isolated and screened for deletions entering adjacent genes. Five deletion mutants were used in this study. Four are shown in Figure 1b: SA306, SA322, SA317, SA326. The fifth, SA307, is shown in Figure 2.

CsCl gradients: 4.8 ml of a CsCl solution in 0.01 M $MgSO_4$ were mixed with phage in a nitrocellulose tube, overlayed with mineral oil and spun for 20 hr at 29K rpm in the Spinco SW39 rotor at 20°C. Fifty to 100 fractions were collected into 0.01 M $MgSO_4$ and analyzed for phage titers. Densities and DNA contents were calculated from gradients in which two marker phages were present (most often λ*imm*^{434} and λ*b2imm*^{434}*c*) using the equation of DAVIS and DAVIDSON (1968).

RESULTS

Isolation of plaque-forming λgal: Strains SA322 and SA306 carry independently isolated deletions of the *chlD* and *Pgl* genes (Figure 1b). The λ*cI857* prophage in strain SA322 has not been altered by the deletion as judged by inducibility, phage yield, and buoyant density of phage released. Lysates of SA322 and SA306 were used to infect W3805 at a multiplicity of 10 and plated on EMBG plates to select transductants. Gal+ colonies appeared at a frequency of 10^{-5} per

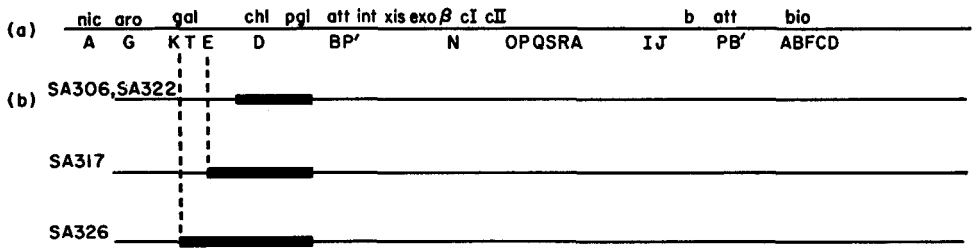

FIGURE 1.—Orientation of prophage λ and neighboring genes. *nicA* = nicotinic acid biosynthesis; *aroG* = 3-deoxy-D-arabino-heptulosonic acid-7-phosphate synthetase (tyrosine-repressible enzyme); *galK* = galactokinase; *galT* = galactose-1-phosphate uridyl-transferase; *galE* = uridine diphosphogalactose 4-epimerase, the operator-promoter proximal gene in the galactose operon; *chlA,chlD* = reduction of chlorate, nitrate; *pgl* = 6-phosphogluconolactonase; *bioA, B,F,C,D* = biotin biosynthesis. Capital letters on the prophage map indicate cistrons defined by nonsense (*am*) mutations. Not all lambda cistrons are indicated. There are 15 essential genes between the *A* and *I* genes of lambda. The termini of the prophage map are delineated by the two hybrid attachment regions *attBP′* on the left and *attPB′* at the right (see GUERRINI 1969). If the prophage were to be cured, the bacterial attachment site alone would remain (*attBB′*). *int* = a function necessary for site specific integration and excision, *xis* is required only for excision. *exo* and *β* are cistrons comprising *red*, the phage generalized recombination system. *cI* specifies the λ immunity substance or repressor, *cII* is necessary for the establishment of lysogeny. *b* is a region consisting of about 17% of the length of the λ chromosome which contains no essential functions (SZYBALSKI 1970). (b). *chlD* type deletion strains used for isolation of non-defective galactose-transducing variants of λ. The heavy bars indicate the extent of deletions.

TABLE 2

Gal *content of non-defective* gal-*transducing phages*

Frequency of occurrence among *Gals*	*Gal* strain numbers	Galactose markers present							DNA content*
		K-2	T-79	T-226	T-151	T-1	T-60	E-16	
1/21	8	c	c	c	c	c	c	c	0.97
16/21	1,2,5,9,11,12, 13,14,16,17,18, 19,20,21,22,25	+	c	c	c	c	c	c	0.95 (#25)
2/21	24,27	—	c	c	c	c	c	c	
1/21	28	—	—	+	+	+	+	c	
1/21	26	—	—	—	+	+	+	c	
—	317	+	+	+	+	+	+	—	
—	326	+	+	+	—	—	—	—	

Galactose markers were assayed by spot test for transducing activity of diluted λ*gal* lysates. Markers were ordered previously by SHAPIRO and ADHYA (1969). c = complementation of the gene in question; + = allele present by recombination; — = allele absent by recombination.
* DNA content of λ+ = 1.00.

plaque-forming particle; 37 transductants were purified by restreaking. Thirty-five of these segregated Gal⁻ clones, and on heat induction gave high frequency transducing (HFT) lysates for Gal. Twenty-one of these HFT lysates, when plated on EMBG plates for single plaques on W3805 at 30°C produced both normal turbid plaques and (at a frequency of about 0.05 to 0.2) plaques with a central Gal⁺ turbid area. All the Gal⁺ plaques when purified gave lysates containing only phages that can transduce W3805. Various of these phage variants differed in density from the parental phage when banded in CsCl gradients (Table 2 and unpublished). The *gal* endpoints, determined by transduction of various *gal* point mutants, vary among isolates (Table 2).

We have also isolated plaque-forming λ*gal* phages carrying the distal gene (kinase) of the galactose operon. In these experiments hosts were used in which the proximal part of the galactose operon, together with the *pgl* and *chlD* genes, was deleted (strains SA326 and SA317; Figure 1b).

λgal *segregation and lysogeny:* According to the CAMPBELL (1962) model of transducing phage genesis, λ*gal* particles should carry the "left" prophage attachment region, *attBP'*. Since some of the "*b*" region is not essential for phage growth, and since the densities of some plaque-forming λ*gal* particles are lower than the parental (Table 2), we assume that the λ*gal* particles must have lost some phage genetic material from the "right" prophage end—presumably the "*b*" region. One property of such a phage is that it lysogenizes at extremely low frequencies (GUERRINI 1969). We will show next that this is also true for the λ*gal* studied.

When the Gal⁺ center of a plaque on W3805 on EMBG was streaked out, most of the colonies were Gal⁻ and only a few weakly Gal⁺; these on restreaking yielded more Gal⁻ colonies. The Gal⁻ cells were non-lysogenic (i.e., had lost the transducing phage). Since the Gal⁺ cells continually segregate non-lysogenic colonies (at very high frequencies), we consider them abortive lysogens.

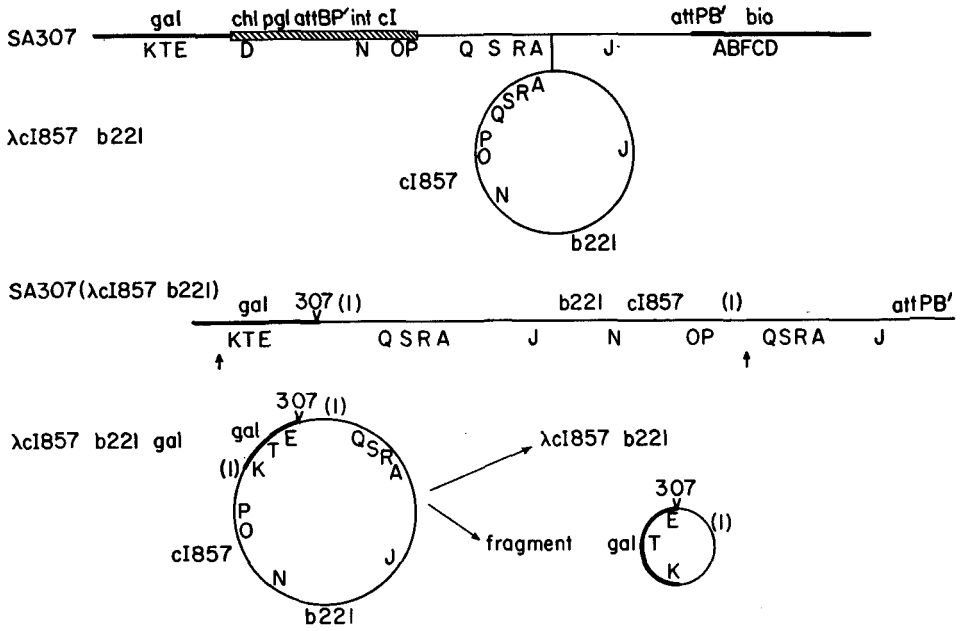

FIGURE 2.—Lysogenization of SA307 by λ*cI857b221*. The deletion of SA307 is indicated by the hatched bar in the diagram of SA307; thereafter this deletion is noted by a check with the strain number: 307. Heavy lines denote bacterial genetic material. The numbers (*1*) indicate identical stretches of λ DNA between *P* and *Q*. Abnormal excision of the prophage (at the arrows) with the left break point to the left of *gal* and the right break point to the right of the region (*1*) generates a galactose-transducing phage which is diploid for the region (*1*). Recombination in the duplicated region, if reciprocal, would generate the hypothetical fragment shown and λ*cI857b221*.

Stabilized Gal$^+$ transductants can be isolated by coinfection with both the λ*gal* and a λ$^+$ or λ*imm*^{434} helper phage. These stable lysogens segregate Gal$^-$ cells at a frequency (10^{-3}) comparable to that observed with lysogens of ordinary λ*dgal*.

A λ*gal* single lysogen can be isolated by selection for Gal$^+$ on minimal galactose medium, or by plating on EMBG agar in the presence of antiserum to prevent lytic phage growth.

To summarize in part, a λ*gal* phage does not form a stable lysogen except in rare instances detectable by strong selection. It readily lysogenizes with a helper phage.

Transducing particles from SA307 (λcI857b221): SATO and CAMPBELL (1970) reported a system for isolating lambda transducing variants containing deletions of specific lambda genes. They used a deletion mutant of a lambda lysogen in which the deletion removed *chlD* and *pgl* and part of the prophage. This strain was then lysogenized with a second phage and from the resulting lysogen transducing particles were isolated. Such a system should in theory also yield plaque-forming transducing phages if the maximum capacity of the phage capsid need

not be exceeded. We have used this system to look for plaque-forming transducing phages. The bacterial strain used was SA307 which contains a deletion removing *chlD* and *pgl* and prophage genes including the *P* gene, as shown in Figure 2. The galactose operon in SA307 appears unaltered by the deletion. SA307 was lysogenized with *λcI857b221*. The *b221* marker is a large deletion of non-essential DNA and was used to increase the amount of relevant bacterial and phage DNA which might be carried by transducing particles.

A lysate of SA307(*λcI857b221*) was prepared by heat induction of a culture in tryptone broth. The lysate was used as a donor (at a multiplicity of 10) to transduce the Gal⁻ strain R594 to Gal⁺. R594 is *galK2galT1*, so a transducing phage must contain the wild-type *galK2⁺galT1⁺* alleles. Since the normal integration system is missing in strains carrying the *b221* deletion, integration should require homologous recombination between bacterial DNA of the transducing particle and the recipient chromosome.

The frequency of transduction of the lysate was about 1×10^{-7} transductions per plaque-forming unit. Thirty-one transductants were examined. Of these, 8 released no plaque-forming phages and were not examined further. The remaining 23 transductants were heat-induced to prepare lysates. The lysates were diluted and plated at 30°C on R594 on EMBG agar. All 23 lysates yielded some plaques which contained a Gal⁺ center. When Gal⁺ clones derived from some of these Gal⁺ plaques were purified and induced, the resulting lysates again contained galactose-transducing plaque-forming phages when assayed on R594 on EMBG plates at 30°C. Phage isolated from plaques containing Gal⁺ centers were purified by single plaque isolation and high titer lysates prepared from confluent lysis plates. Such lysates contained mostly plaque-forming phage particles which could transduce R594 to Gal⁺. Therefore it was concluded that plaque-forming galactose-transducing particles had been isolated. Several properties of four such strains are presented in Table 3. Two of the four strains studied make somewhat smaller plaques than *λcI857b221*. The strains were also tested for ability to transduce all of the galactose operon by using as a recipient a strain, SA472, containing a deletion of the whole operon. Isolates *λcI857b221galq1* and *λcI857b221galq6* must be broken in the *galK* gene, since they contain the wild-

TABLE 3

Properties of galactose-transducing phages from SA307 (λcI857b221)

Property	Phage strain *λcI857-b221galq1*	*λcI857-b221galq4*	*λcI857-b221galaroGq5*	*λcI857-b221galq6*
Plaque size	Small	Normal	Small	Normal
Complete galactose operon present	—	+	+	—
Frequency of non-transducing segregants	12%	1%	30%	2%
DNA content*	0.89	0.85	1.00	0.89

* λ⁺ = 1.00; *λb221* = 0.78.

type *galK2*$^+$ allele but don't transduce SA472 to Gal$^+$. The two isolates capable of transducing all of *gal* were tested for the ability to transduce the adjacent bacterial genes *aroG* and *nicA*. Isolate λ*cI857b221galq5*, hereafter designated λ*cI857b221galaroGq5*, transduced the *aroG* marker; λ*cI857b221galq4* did not. Neither strain transduced *nicA*. The letter *q* which is included in the designation of these isolates serves to distinguish them from other galactose-transducing strains of λ from which they differ in a number of respects. The letter *q* was chosen because the *Q* gene is the first λ gene to the right of the *gal* insertion in these strains, as shown below.

Such plaque-forming, galactose-transducing strains of λ presumably contain all the genes essential for the growth of wild-type λ. Therefore the abnormal excision events giving rise to the transducing strains must have occurred to the right of the *P* gene in the prophage contained in SA307 (λ*cI857b221*) (see Figure 2).

Plate lysates (containing about 10^{11} plaque-forming units) derived from single plaques of galactose-transducing strains contained a fraction, from 1% to 30% (Table 3) of the plaque-forming particles which did not transduce the recipient R594 to Gal$^+$. Repeated cycles of purification of galactose-transducing, plaque-forming phage resulted in similar lysates which contained a fraction of non-transducing particles. It was postulated at this point that these phage strains are genetically unstable because of a duplication of phage genetic material bracketing the bacterial genes carried by the phage strains. Such a structure can be generated by a single abnormal excision event in SA307(λ*cI857b221*) if the right-hand break occurs to the right of the genetic site between *P* and *Q* defined by the SA307 deletion. The origin of this structure is indicated in Figure 2, where (1) denotes the phage material in the duplication and the arrows indicate the sites of breakage in the excision event. Such a phage strain is expected to be unstable because homologous recombination can promote crossing over between the two homologous copies of phage genetic material comprising the duplication. Such recombination results in a chromosome which has lost the galactose genes and the duplication.

The fraction of non-transducing segregants is presumably a function of the rate of recombination in the duplication and the growth rate of the segregants relative to the growth rate of the galactose-transducing particles.

The DNA content of the four strains was determined by CsCl centrifugation with appropriate marker strains. The DNA content of these strains, as noted in Table 3, varies from 0.85 for λ*cI857b221galq4* to 1.00 for λ*cI857b221galaroGq5*, where λ$^+$ has a DNA content of 1.00 and the parental phage, λ*cI857b221*, has a DNA content of 0.78 (Davis and Davidson 1968).

As mentioned above, recombination between the two segments of DNA comprising the duplication results in loss of the bacterial genes and the duplication—thus the non-transducing segregants should be identical to the parental phage, λ*cI857b221*. This prediction was tested by banding a lysate of λ*cI857b221galq4* (DNA content = 0.85) in CsCl with the marker phage λ*imm*^{434}*b221* (DNA content = 0.75). The non-transducing segregants were estimated to have a DNA

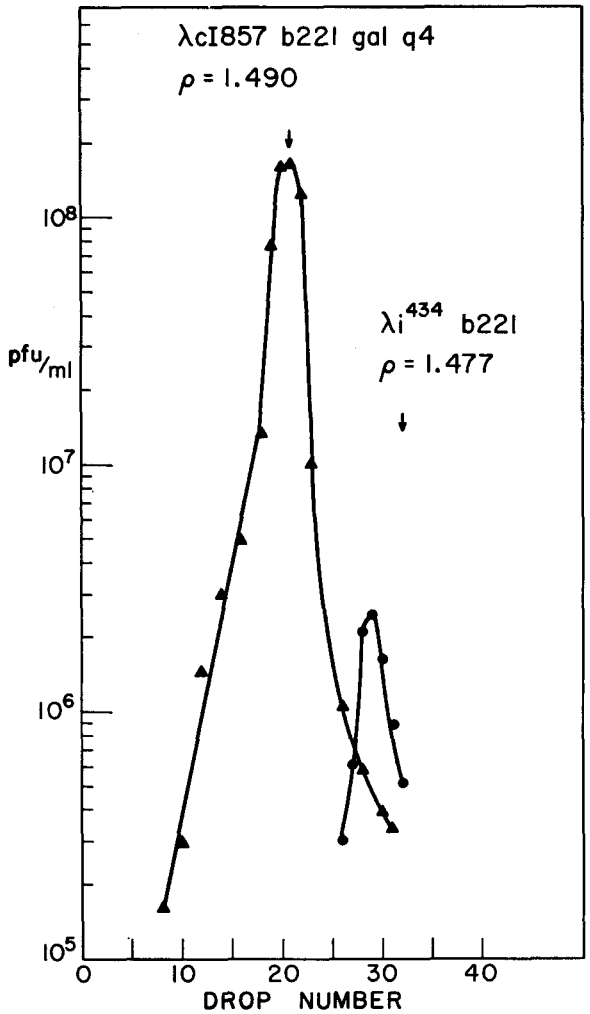

FIGURE 3.—CsCl gradient analysis of non-transducing segregants in a lysate of λ*cI857b221galq4:* ▲-▲-▲ = λ*cI857b221galq4*; ●-●-● = non-transducing segregants; the arrow marks the position of the marker phage λ*imm*^{434}*b221*.

content of 0.78 which agrees exactly with the DNA content of the parental phage λ*cI857b221* (see Figure 3).

Lysogens of λcI857b221galq *strains:* Lysogens of the four phage duplication strains were prepared using as a host the strain C246 which contains a deletion extending from *gal* (the break in *gal* is either in *galT* or *galE*) through most of the biotin operon. This strain was transduced to Gal$^+$ by the four galactose-transducing strains. Lysogenization of C246 by these phage strains was infrequent. λ*cI857b221galq4*, for example, was classed as integration defective in the test of GOTTESMAN and YARMOLINSKY (1968). Lysogens of λ*cI857b221galq4* and λ*cI857b221galaroGq5* were selected by the techniques described above for prepa-

ration of lysogens of the galactose-transducing strains from SA322 and SA306. Rare transductants were easily found with *λcI857b221galq1* and *λcI857b221-galq6* since transduction required recombination between the two fragments of the galactose operon carried by the phages and C246. C246 was chosen because the *gal* mutation it carries, being a large deletion, cannot be picked up by the transducing phages and thus cannot segregate from the host.

These C246 lysogens are useful for obtaining information about the phage strains they carry, as discussed below. We sought first to show for one of the lysogens, C246(*λcI857b221galq4*), that the transducing phage was integrated into the bacterial chromosome by crossing over between the galactose genes carried by the phage and the homologous part of the galactose operon present in C246. This sort of integration is diagrammed in Figure 5, which shows the proposed structure of a C246(*λcI857b221galq*) lysogen. A P1 transduction experiment has been performed to test this proposed structure, and thus eliminate other possibilities, such as plasmid formation (SIGNER 1969) and integration at sites in the C246 chromosome other than *gal*. Also note that the structure drawn for a C246(*λcI857b221galq*) lysogen is dependent on the presumed structure of the *λcI857b221galq* phage strains. The P1 transduction experiment (diagrammed in Figure 4) also tests the order of lambda genes in the prophage which is a test of the proposed structure of the *λcI857b221galq* phages.

The donor strain used is W3350(*λIam2*); it is Gal$^-$ and Bio$^+$ and contains the defective prophage *λIam2*. As shown in Figure 4, the wild-type prophage gene order in W3350(*λIam2*) differs from the gene order of the *λcI857b221galq4* prophage in the recipient. The recipient is Gal$^+$ and Bio$^-$. Gal$^+$Bio$^+$ recombinants are expected to arise in several ways in this cross. Since the P1 transducing phage carries a linear fragment of donor DNA, a double crossover is required for integration of donor genetic material into the recipient chromosome. For the recipient to become Bio$^+$ the C246 deletion must be crossed out; this requires the rightmost crossover indicated in Figure 4. There are four possibilities for the second crossover; these are indicated in Figure 4. Of these four possible crossover types, two yield non-defective lysogens (recombinant strains which upon induction pro-

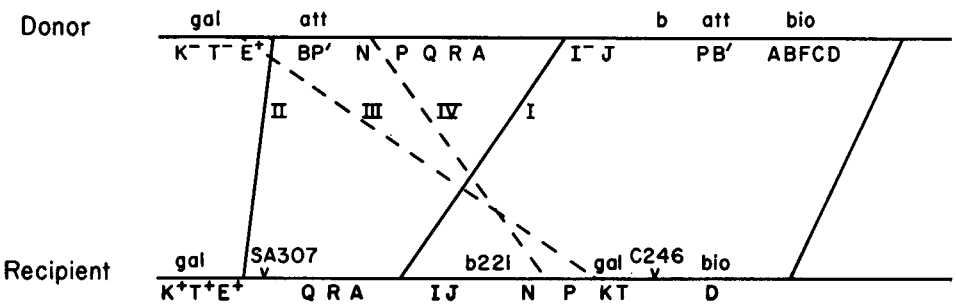

FIGURE 4.—P1*kc* transduction of recipient C246(*λcI857b221galq4*) to Bio$^+$ by donor W3350 (*λIam2*). To generate the Bio$^+$Gal$^+$ transductant the rightmost crossover is necessary. There are four possibilities for the second crossover; crossover types I and II yield defective lysogens, types III and IV yield non-defective lysogens.

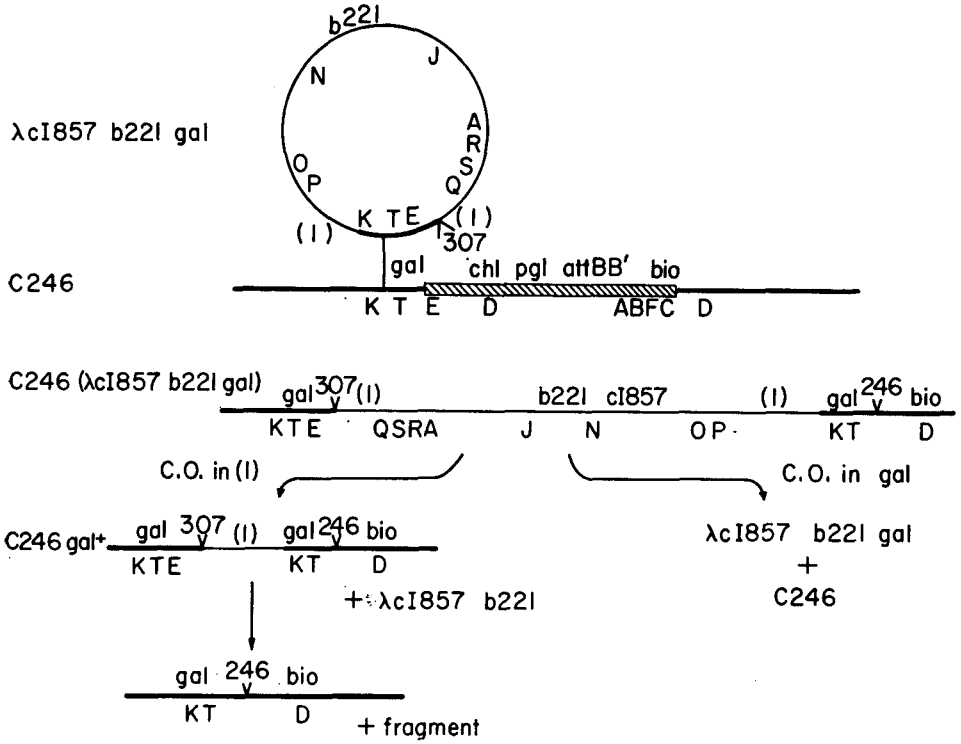

FIGURE 5.—Lysogenization of C246 by λ*cI857b221galq* isolates. The deletion in C246 is shown for the diagram of C246 as a hatched bar; thereafter the C246 deletion is noted by a check with the strain number: 246. Heavy lines denote bacterial genetic material. Segregants which have lost the prophage can arise in two ways. Crossing over in the *gal* regions, noted as c.o. in gal, is expected to yield a *gal* bacterial strain identical to C246. Recombination in the phage duplication, noted as c.o. in (*1*), is expected to yield a bacterial strain that is a *gal/gal*+ heterozygote and which contains one copy of the phage genetic material comprising the duplication. The *gal/gal*+ heterozygote is expected to give *gal* segregants identical to C246.

duce plaque-forming phage particles). These two types are indicated by dashed lines in Figure 4 and are labeled III and IV. The other two crossover types yield defective lysogens and are indicated by solid lines and are labeled I and II. For technical reasons we have only studied Gal$^+$ Bio$^+$ recombinants that are defective lysogens. The expected crossovers of type I yield defective lysogens containing lambda genes from *Q* to *J* (as in SA307) which are either I^+ or *Iam2*. Type II crossovers yield lysogens containing λ*Iam2*. Both classes of recombinants are expected only if λ*cI857b221galq4* has integrated at *gal* in C246. Recombinants of the sort described for type I crossovers are only expected if the presumed structure for λ*cI857b221galq4* is correct.

Bacteriophage P1*kc* was grown on W3350(λ*Iam2*) to produce a donor lysate. C246(λ*cI857b221galq4*) was infected with this lysate at a multiplicity of 1. The infected cells were then plated on minimal galactose agar supplemented with amino acids, along with streptomycin to select Gal$^+$Bio$^+$ recombinants. Bio$^+$

transductants were purified and 12/35 were found to be defective lysogens. The defective lysogens were of the type expected—of 12 examined, one was a type II recombinant containing λ*Iam2* and ten were type I recombinants. Three of the type I recombinants were *Iam2*$^+$ and seven were *Iam2*. The remaining transductant carried no phage genes and perhaps arose by transduction of a non-immune recipient cell from which the λ*cI857b221* prophage has segregated, resulting in excision and segregation of the λ*Iam2* prophage from the donor fragment. The fact that the two predicted types of Bio$^+$ transductants were obtained strongly supports the structure of C246 (λ*cI857b221galq4*) indicated in Figure 4.

Integration of λ*cI857b221galq1* and λ*cI857b221galq6* in the galactose region of C246 is very likely since generation of Gal$^+$ cell requires crossing over between the two fragments of the galactose operon carried by the phage and bacterial chromosomes.

Several properties of the four lysogens are presented in Table 4. As shown in Figure 5, the lysogens have two duplications; the duplication of phage material and a duplication of bacterial genes including the galactose region. The lysogens are thus *gal*$^+$/*gal* heterozygotes and should segregate *gal* cells. Cultures of the lysogens plated on EMBG agar at 30°C contain *gal* segregants. Such *gal* segregants have also become non-lysogenic, as expected from the structure indicated in Figure 5.

Segregants which have lost the λ*cI857b221* prophage can also be selected by plating a culture (grown at 30°C to allow segregation) at 43°C with anti-λ serum to prevent reinfection. Cells carrying the prophage are killed when the phage is induced by heating. Two types of segregants are expected; recombination within the duplication of phage DNA will generate a *gal*$^+$/*gal* cell line, whereas recombination within the duplication of bacterial genes will generate a *gal* cell as shown in Figure 5. The total frequency of cured segregants and the percentage of segregants that are Gal$^+$ and Gal$^-$ are presented in Table 4. In cultures of each of the four lysogens both Gal$^+$ and Gal$^-$ segregants were obtained. The Gal$^+$ segregants still contain the duplication of galactose material and one copy of the

TABLE 4

Properties of C246 lysogens of transducing phages from SA307 (λcI857b221)

Property	Lysogen C246(λ*cI857-b221galq1*)	C246(λ*cI857-b221galq4*)	C246(λ*cI857-b221galaroGq5*)	C246(λ*cI857-b221galq6*)
Gal$^-$ segregants	5/729	3/604	8/667	1/157
Prophage loss in Gal$^-$ segregants	not tested	7/7	5/5	2/2
Survival at 43°C	1.1%	0.2%	1.0%	0.2%
Gal$^+$ survivors at 43°C	73%	3%	9%	35%
Gal$^-$ survivors at 43°C	27%	97%	91%	65%
Segregation of Gal$^+$ survivors*	3/1131	6/600	9/726	2/1900

* Includes sectored clones.

TABLE 5

Properties of C246(λcI857b221galq4): *effect of* rec *and* red *mutations*

Property	Lysogen			
	C246(λ*cI857-b221galq4*)	C246(λ*cI857-b221red270galq4*)	C246*recA*(λ*cI857-b221galq4*)	C246*recA*(λ*cI857-221red270galq4*)
Gal⁻ segregation at 30°C	2/1550	4/910	0/2000	0/1750
Survival at 43°C	0.2%	0.5%	7×10^{-5}%	5×10^{-5}%
Gal+ survivors at 43°C	1.4%	3.0%	14%	20%
Gal⁻ survivors at 43°C	98.6%	97%	86%	80%

phage material originally duplicated. These *gal*$^+$/*gal* heterozygotes are expected to be genetically unstable, segregating *gal* clones. As noted in Table 4 a Gal$^+$ survivor from each lysogen was unstable. Since the Gal$^+$ survivors of C246 lysogens still contain one copy of the phage material present in the duplication one can ask if the duplication contains any known λ genetic markers. This is done for the Gal$^+$ survivors of C246(λ*I857b221galq6*). If any phage markers are carried one expects that *Q* gene markers would be most likely to be present. However, no *Q* markers could be rescued.

Role of generalized recombination systems in segregation properties of lysogens: The effect of mutations in the bacterial (*rec*) and phage (*red*) generalized recombination systems on segregation properties of a C246 lysogen of one of the transducing strains: λ*cI857b221galq4* is shown in Table 5. The phage *red* system has little if any role in the production of segregants: C246(λ*cI857b221red270galq4*) has about the same survival frequency as C246(λ*cI857b221galq4*) when plated at 43°C, and the percentages of Gal$^+$ and Gal$^-$ survivors are not significantly changed. This result is expected since the phage *red* system is under the control of the λ repressor and is therefore not expressed during growth of a lysogenic culture at 30°C. A dramatic effect is observed when the bacterial *rec* system is inactive. With C246*recA1*(λ*cI857b221galq4*) survival decreased by a factor of 10^4 to 7×10^{-5}%, and an increased fraction of Gal$^+$ survivors (14%) was obtained. These results are independent of the state of the *red* system, since similar values were obtained with C246*recA1*(λ*cI857b221red270galq4*). Survivors obtained from *rec* lysogens could occur by a number of mechanisms: (1) reversion of the *rec* mutation, followed by segregation, (2) deletions, (3) phage mutations allowing survival of the lysogen, (4) residual recombination activity present in *recA* lysogens which allows a low rate of segregation. Both the Gal$^+$ and Gal$^-$ survivors obtained from *rec* lysogens are still *rec*, as determined by the test of UV sensitivity, indicating that the survivors are not *rec*$^+$ revertants. The *rec* character was further tested for two Gal$^-$ survivors by matings with KL16-99, followed by selection for Gal$^+$ *str* recombinants. Under conditions in which C246 gave a fraction of 2.8×10^{-3} gal$^+$ recombinants, the fraction of the two survivors and for C246*recA1* was less than 10^{-6}. Thus, within the limits of this test, the survivors are quite defective in recombination. The possibility of deletion formation was tested by spotting λ*cI857gal28* (see Table 2) on the Gal$^-$ survivors: if the Gal$^-$ survivors occur by deletion mutations, one expects variable

endpoints in the galactose region. Thus some survivors should not be transduced by λ*cI857gal28* which contains the *galE* gene and part of the *galT* gene. In fact, 24/24 (in two independent groups of 12) Gal⁻ survivors of C246*recA1* (λ*cI857b-221galq4*) were transduced to Gal⁺ by λ*cI857gal28*. This transduction result indicates that the C246 deletion endpoint remains in the survivors and that deletions are not the source of the Gal⁻ survivors of *recA* lysogenes. In sum, these tests indicate that a residual generalized recombination activity remains in the *recA* lysogens.

The increased fraction of Gal⁺ survivors in *recA* derivatives of C246 (λ*cI857b-221galq4*) is possibly due to a significant fraction of cells which survive the 43°C treatment because the prophage has lost (by mutation) the ability to kill the host at 43°C, but this possibility has not been tested.

The duplication strains of λ described here have the *E. coli* galactose operon situated between the λ *P* and *Q* genes. One might expect such an addition to have a radical effect on normal phage development. Transcription of the region *cII·O·P·Q·S·R* proceeds in a rightward direction (KOURILSKY *et al.* 1968; TAYLOR, HRADECNA and SZYBALSKI 1967), whereas the galactose operon is transcribed leftwards (GUHA, TABACZYNSKI and SZYBALSKI 1968). The opposite direction of transcription of the galactose operon is expected to interfere (LEVINTHAL and MIKAIDO 1969) with transcription from the *P* gene to the *Q* gene (if it occurs). Such speculation raises the question of how transcription of the *Q* gene is accomplished in strains carrying *galq* additions. Although this question remains open, we have compared the *galq4* addition with another chromosomal abnormality, *nin5*, which maps between *P* and *Q* and alters normal lambda transcription. The *nin5* mutation, isolated and described as *pf* by COURT and SATO (1969), is a deletion of 5.4% of the λ chromosome (COURT 1970). The *nin5* mutation renders strains of λ *N*-independent; that is, able to grow in the absence of *N* gene product. λ*cI857b221Nam53galq4* was prepared, and its ability to grow in the non-permissive host R594 was compared with control strains.

R594 was infected at a multiplicity of 2.5 phage/cell. The yield of phage/infected cell was determined 90 min after infection. The yield of λ*cI857b221galq4* was 84 ;the yield of λ*cI857Nam7am53nin5* was 24. Both λ*cI857b221Nam53-galq4* and λ*cI857Nam7am53* gave yields of less than one phage/cell, indicating that the *galq4* addition does not confer the *nin* character.

DISCUSSION

Isolation of plaque-forming λ*gal* strains has been described. We note that these strains have already been useful in genetic studies (SIGNER 1969; ECHOLS 1970) and galactose transformation of mammalian cells (MERRILL, GEIER and PETRICCIANI 1971).

The transducing phages isolated from SA307 (λ*cI857b221*) carry additions which contain various amounts of bacterial and phage genetic material. The smallest addition, that carried by λ*cI857b221galq4*, is estimated to be 7% of the λ chromosome length. The minimum size of the galactose operon can be esti-

mated from the sum of the number of amino acid residues in the three galactose enzymes. This sum is 1088 (WILSON and HOGNESS 1969a,b; SAITO, OZUTSUMI and KURAHASHI 1967). The sum of 1088 × 3 base pairs per amino acid equals 3264 base pairs as the minimum size of the galactose operon. 3264 base pairs ÷ 50,000 base pairs per λ^+ chromosome gives an estimate for the galactose operon of 6.5% of the length of the λ^+ chromosome. Thus the size of the *galq4* addition agrees well with the minimum size of the galactose operon. One expects that the size of the duplication of phage DNA in this strain is at most 1% of the λ^+ length. The genetic data are consistent with this expectation; only 3% of the non-lysogenic survivors of C246(*λcI857b221galq4*) are Gal^+ and arose by recombination in the phage duplication.

λcI857b221galq1 and *λcI857b221galq6* both contain 11% additions. Both are broken in the galactose operon between the amino terminal end of the *galK* gene and the *galK2* site. They contain less bacterial DNA than *λcI857b221galq4*, which contains the complete galactose operon. Since the *galq6* and *galq1* additions are both estimated to be larger than the *galq4* addition, the excess DNA must be phage DNA comprising the duplication. The difference is a minimum estimate of the size of the duplications carried by *λcI857b221galq1* and *λcI857b221galq6*; for both strains this estimate is 11% − 7% = 4%.

As discussed above, the Gal^+ survivors of C246 lysogens of these duplication phages contain the addition which includes the phage DNA in the duplication. This phage DNA is at least 4% of the λ^+ chromosome length in the case of the *galq6* addition. Since no *Q* gene markers could be rescued from the Gal^+ survivors of C246(*λcI857b221galq6*) the *Q* gene of λ is not included in the phage duplication carried by *λcI857b221galq6*. This means that the deletion mutation in SA307 has left intact a stretch of prophage DNA between the *P* and *Q* genes equal in length to at least 4% of the λ^+ length. Recent studies have resulted in a physical map of the vegetative lambda chromosome (DAVIDSON and SZYBALSKI 1971). The lambda chromosome length is defined as 100% and the end of the chromosome near the *A* gene as 0% and the end near the *R* gene as 100%. On this map a site in the *Q* gene is at 91.7 and a site in the *P* gene is at 81.4. The SA307 deletion endpoint is at least 4% to the left of the *Q* gene and thus must be at or to the left of 87.7. *nin5* deletes DNA from 83.8 to 89.2. COURT (1970) was unable to recover the nin^+ allele from SA307, so the SA307 deletion endpoint probably does not map to the left of 83.8.

It has been noted by SATO and CAMPBELL (1970), that use of lysogens containing deletions which penetrate the prophage can allow isolation of specific phage deletions. Here it is shown that the same procedure yields duplication strains. The technique for isolation of duplication strains is expected to be generally applicable to other parts of the λ chromosome. This is likely to be useful in situations where duplication of a particular region or structure is desired. Note that the duplication strains contain complex additions, including bacterial genetic material. While detailed information concerning the structure of duplication strains can be obtained, changes in phenotypic properties of strains carrying additions become hard to interpret. Such changes can be the result of any or several

of the elements comprising the addition: the duplication, bacterial transcription units including promoters and stop signals, and entirely new base sequences created by the deletion and abnormal excision event.

Other examples of partial diploid strains of bacteriophage exist. WEIL *et al.* (1965) have described tandem duplication strains of T4 in which duplications were specifically selected in the *rII* region of the T4 chromosome. BERG (1970) has described tandem duplication strains of λ formed by recombination between λ and the λ-derived plasmid λ*dv*. BELLETT, BUSSE and BALDWIN (1971) have presented evidence that tandem duplication strains can be detected in stocks of a λ-*θ*80 hybrid phage. ANDOH and OZEKI (1968) isolated an *su3*$^+$-transducing derivative of *θ*80 which was genetically unstable. This phage segregated non-transducing variants which also contained less DNA than the transducing particles. It seems likely, as ANDOH and OZEKI suggested, that *θ*80*su3*$^+$ phage has a structure analogous to the structure of the partial diploid galactose-transducing phages described here.

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LITERATURE CITED

ADHYA, S., P. CLEARY and A. CAMPBELL, 1968 A deletion analysis of prophage lambda and adjacent genetic regions. Proc. Nat. Acad. Sci. U.S. **61**: 956–962.

ADHYA, S. and M. SCHWARTZ, 1971 Phosphoglucomutase mutants of *Escherichia coli* K-12. J. Bacteriol. **108**: in press.

ANDOH, T. and H. OZEKI, 1968 Suppressor gene su3$^+$ of *E. coli*, a structural gene for tyrosine TRNA. Proc. Nat. Acad. Sci. U.S. **59**: 792–799.

BELLETT, A. J. D., H. G. BUSSE and R. L. BALDWIN, 1971 Tandem genetic duplications in a derivative of phage lambda. pp. 501–514. In: *The Bacteriophage Lambda*. Edited by A. D. HERSHEY. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

BERG, D. E., 1970 Plasmid mutants of bacteriophage lambda. Genetics **64**: s6.

CAMPBELL, A., 1957 Transduction and segregation in *Escherichia coli*. Virology **4**: 366–384. ——, 1961 Sensitive mutants of bacteriophage lambda. Virology **14**: 22–32. ——, 1962 Episomes. Advan. in Genetics **11**: 101–145.

COURT, D., 1970 Studies of bacteriophage lambda: I. Helping effect in lambda *gal* transduction, II. Regulation of lambda development: The isolation of two new regulatory gene mutants of Lambda. Ph.D. Thesis, University of Rochester.

COURT, D. and K. SATO, 1969 Studies of novel transducing variants of lambda: dispensability of genes *N* and *Q*. Virology **39**: 348–352.

DAVIDSON, N. and W. SZYBALSKI, 1971 Physical and chemical characteristics of lambda DNA. pp. 45–82. In: *The Bacteriophage Lambda*. Edited by A. D. HERSHEY. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

DAVIS, R. W. and N. DAVIDSON, 1968 Electron-microscopic visualization of deletion mutations. Proc. Nat. Acad. Sci. U.S. **60**: 243–250.

DAVIS, R. W. and J. S. PARKINSON, 1971 Deletion mutants of bacteriophage lambda. III. Physical structure of *att*$^\phi$. J. Mol. Biol. **56**: 403–423.

DE LUCIA, P. and J. CAIRNS, 1969 Isolation of an *E. coli* strain with a mutation affecting DNA polymerase. Nature **224:** 1164–1166.

ECHOLS, H., 1970 Integrative and excisive recombination by bacteriophage λ: Evidence for an excision-specific recombination protein. J. Mol. Biol. **47:** 575–583.

FEISS, M. and A. CAMPBELL, 1970 Isolation and characterization of partial diploid strains of lambda. Genetics **64:** s19.

GOTTESMAN, M. E. and M. B. YARMOLINSKY, 1968 Integration-negative mutants of bacteriophage lambda. J. Mol. Biol. **31:** 487–505.

GUERRINI, F., 1969 On the asymmetry of lambda integration sites. J. Mol. Biol. **46:** 523–542.

GUHA, A., M. TABACZYNSKI and W. SZYBALSKI, 1968 Orientation of transcription for the galactose operon as determined by hybridization of *gal* mRNA with the separated DNA strands of coliphage lambda *dg*. J. Mol. Biol. **35:** 207–213.

KAYAJANIAN, G. and A. CAMPBELL, 1966 The relationship between heritable physical and genetic properties of selected gal$^-$ and gal$^+$ transducing lambda *dg*. Virology **30:** 482–492.

KOURILSKY, P., L. MARCAUD, P. SHELDRICK, D. LUZZATI and F. GROS, 1968 Studies on the messenger RNA of bacteriophage lambda. I. Various species synthesized early after induction of the prophage. Proc. Nat. Acad. Sci. U.S. **60:** 1013–1020.

LEVINTHAL, M. and H. NIKAIDO, 1969 Consequences of deletion mutations joining two operons of opposite polarity. J. Mol. Biol. **42:** 511–520.

LOW, B., 1968 Formation of merodiploids in matings with a class of rec$^-$ recipient strains of *Escherichia coli* K12. Proc. Nat. Acad. Sci. U.S. **60:** 160–167.

MERRILL, C. M., M. GEIER and J. PETRICCIANI, 1971 Bacterial gene expression in mammalian cells. Nature **233:** 398–400.

PARKINSON, J. S., 1968 Genetics of the left arm of the chromosome of bacteriophage lambda. Genetics **59:** 311–325. ——, 1971 Deletion mutants of bacteriophage lambda. II. Genetics properties of *att*-defective mutants. J. Mol. Biol. **56:** 385–401.

PARKINSON, J. S. and R. J. HUSKEY, 1971 Deletion mutants of bacteriophage lambda. I. Isolation and initial characterization. J. Mol. Biol. **56:** 369–384.

ROTHMAN, J., 1965 Transduction studies on the relation between the prophage and host chromosome. J. Mol. Biol. **12:** 892–912.

SAITO, S., M. OZUTSUMI and K. KURAHASHI, 1967 Galactose-1-phosphate uridylyltransferase of *Escherichia coli*, II. Further purification and characterization. J. Biol. Chem. **242:** 2362–2368.

SATO, K. and A. CAMPBELL, 1970 Specialized transduction of galactose by lambda phage from a deletion lysogen. Virology **41:** 474–487.

SHAPIRO, J. A. and S. L. ADHYA, 1969 The galactose operon of *E. coli* K12. II. A deletion analysis of operon structure and polarity. Genetics **62:** 249–264.

SIGNER, E. R., 1969 Plasmid formation: a new mode of lysogeny by phage λ. Nature **233:** 158–160.

TAYLOR, A. L., 1970 Current linkage map of *Escherichia coli*. Bacteriol. Rev. **32:** 155–175.

TAYLOR, K., Z. HRADECNA and W. SZYBALSKY, 1967 Asymmetric distribution of the transcribing regions on the complementary strands of coliphage lambda DNA. Proc. Nat. Acad. Sci. U.S. **57:** 1618–1635.

THOMAS, R., C. LEURS, C. DAMBLY, D. PARMENTIER, L. LAMBERT, P. BRACHET, N. LEFEBVRE, S. MOUSSET, J. PROCHERET, J. SZPIRER and D. WAUTERS, 1967 Isolation and characterization of new *sus* (amber) mutants of bacteriophage lambda. Mutation Res. **4:** 735–741.

SZYBALSKI, W., 1970 Genetic and molecular map of *Escherichia coli* bacteriophage lambda (λ). Handbook of Biochemistry, 2nd Ed. I-35-I-38. The Chemical Rubber Co., Cleveland, Ohio.

WALLACE, B. J. and J. PITTARD, 1967 Genetic and biochemical analysis of the isoenzymes concerned in the first reaction of aromatic biosynthesis in *Escherichia coli*. J. Bacteriol. **93**: 237–244.

WEIL, J., B. TERZAGHI and J. CRASEMANN, 1965 Partial diploidy in phage T4. Genetics **52**: 683–693.

WILSON, D. B. and D. S. HOGNESS, 1969 The enzymes of the galactose operon in *Escherichia coli*. II. The subunits of uridine diphosphogalactose 4-epimerase. J. Biol. Chem. **244**: 2132–2136. ——, 1969 The enzymes of the galactose operon in *Escherichia coli*. III. The size and composition of galactokinase. J. Biol. Chem. **244**: 2137–2142.