VIROLOGY 36, 30–41 (1968)

Studies on the Genetics of Biotin-Transducing, Defective Variants of Bacteriophage λ[1]

GARY KAYAJANIAN[2]

Department of Biology, University of Rochester, Rochester, New York 14627, and Department of Human Genetics, University of Michigan, Ann Arbor, Michigan 48104

Accepted May 23, 1968

A collection of λbio transducing particles were isolated and the phage and bacterial extent of their genomes characterized. λbio transducing phages transduce variable amounts of bacterial genome, and have densities that differ from one independent HFT to another. Some of the transducing particles are able to form plaques (λpb), others are not (λdb); the λdbs have phage defects of various extents. The nature of the λdb particles conforms to the structural predictions of the Campbell model. Some of the properties of λbio particles and (λdb) lysogens are described and discussed.

INTRODUCTION

Phage λ acts as a transducing agent for host markers located very close to its attachment site: for a cluster of galactose genes (Morse *et al.*, 1956a, b; Campbell, 1959), an aromatic marker (Wallace and Pittard, 1967), and a series of loci responsible for the synthesis of biotin (Rothman, 1965; del Campillo-Campbell *et al.*, 1967).

We undertook to isolate and characterize a number of biotin transducing variants of λ for a number of reasons. First, to test the validity of the Campbell model, which delimits in a very precise manner the genetic and physical quality of the genomes of transducing λ variants. Some of these predictions are explicit: namely, the DNA in transducing particles should represent a contiguous block of genetic and physical material drawn from a lysogenic bacterial chromosome. Implicit in the model is the requirement of the vegetative ends in all genomes able to go through a lysogenic cycle. Second, one might expect not to find certain classes of transducing genomes that could be formed *à la Campbell* but would be unable to survive as single defective lysogens for reasons of physiology. Genetic material in the arm of λ that might be deleted in defective biotin transducing genomes would be concerned with functions of λ occurring early in the cellular development of phage λ. λc_I genomes are unable to establish a stable relation with their host, but would λdb genomes missing c_I and (according to Campbell) other early regions of λ be able to form a stable lysogenic complex with its host? Third, we were interested in ordering a large number of biotin mutants of *Escherichia coli* (del Campillo-Campbell *et al.*, 1967) and a large number of λ mutants for which Campbellian λbio genomes would be valuable.

MATERIALS AND METHODS

Media

Synthetic medium with 1% glucose, 0.4% 2,3,5-triphenyltetrazolium chloride, and supplements of leucine and thiamine served as our biotin transduction medium (Kayajanian and Campbell, 1966). Cells not requiring exogenous biotin for growth (bio^+) grow to form dark red colonies on a white

[1] Supported by United States Public Health Service Grants E-2862 and PO 1 GM15419-01.

[2] Present address: Department of Biochemistry, Stanford University School of Medicine, Palo Alto, California 94304.

TABLE 1
BACTERIAL STRAINS

Strain	*Properties*[a]	*Function*
C600	gal$^+$, bio$^+$, leu$^-$, thi$^-$, pm$^+$	λ assay
C600 (λ)	gal$^+$, bio$^+$, leu$^-$, thi$^-$, lysogenic	Source of gal and bio LFT particles
W3350	gal$^-$ (kinaseless and transferaseless) bio$^+$, pm$^-$	Recipient in gal transduction *sus*$^+$ assay
W3805	gal$^-$ (epimeraseless) bio$^+$, pm$^-$	"
W602	gal$^-$ (transferaseless), bio$^-$, pm$^-$, designated bio$^-$0	Recipient in bio and gal transduction
AC 2, 3, 4, 12, 17, 18, 19, 23, 24	gal$^+$, pm$^-$, bio$^-$ nitrosoguanidine treated derivatives of a gal$^+$ bio$^+$ segregant of *E. coli* strain 15-68 (Campbell, 1965; del Campillo-Campbell, Kayajanian, Campbell and Adhya, 1967), designated bio$^-$ 2, 3, 4, etc.	Recipient in bio transduction

[a] Notation: gal$^-$ = unable to utilize galactose as a carbon source; bio$^-$ = requires biotin for growth; leu$^-$ = requires leucine for growth; thi$^-$ = requires thiamine for growth; pm$^+$ = permissive host for *sus* mutants; pm$^-$ = nonpermissive host for *sus* mutants.

TABLE 2
PHAGE STRAINS

Strains	Function
λ.W3350	Helper phage for gal transductions on W3350
λ.bio$^-$0	Helper phage for bio and gal transductions on bio$^-$0
λc_{71}	λc_I with λ's density
$\lambda c_{I\text{-}47} sus8$	Recombinant between $\lambda c_{I\text{-}47}$ and *sus*8, for marker rescue
$\lambda c_{II\text{-}68} sus8$	Recombinant between $\lambda c_{II\text{-}68}$[a] and *sus*8, for marker rescue
$\lambda c_{It2} sus29$[a]	For marker rescue
$\lambda c_{Itu51} sus29$[a]	For marker rescue
$\lambda c_{It} sus29$[a]	For marker rescue
$\lambda c_{Itu46} sus29$[a]	For marker rescue
$\lambda c_{Itu50} sus29$[a]	For marker rescue
λcb2	A clear plaque density mutant with an estimated DNA content of 86.5% of our λ's
λdg_4^-	A density marker λdg (Kayajanian and Campbell, 1966)
λdg_1^+	A gal$^+$ transducing phage, missing phage markers from *sus* A to *sus* J
λsus A_{11}, B_{10}, J_6, N_7, N_{53}, O_8, O_{29}, O_{125}, P_3, P_{72}, P_{116}, Q_{21}, Q_{73}, Q_{117}, R_5, R_{16}, R_{54}, R_{60}	For marker rescue

[a] Kindly sent by Dr. M. Lieb.

background of a biotin-requiring (bio$^-$) strain on this medium. The medium used to detect biotin segregation was Tris-buffered synthetic medium with 0.4% glucose, 7×10^{-4} *M* $NaHCO_3$, 1 mg/liter thiamine, 20 mg/liter leucine, about 5×10^{-2} μg/liter biotin, and eosin Y and methylene blue as in EMB agar. On this medium bio$^+$ colonies are large with prominent dark centers and thick white rims; bio$^-$ colonies are smaller and are either entirely dark or have a very thin white rim surrounding the dark center. Bio variable colonies are of two types: one looks like bio$^+$ colonies; the other is highly sectored. Segregation for the gal and bio markers can be observed simultaneously on the segregation medium if galactose replaces glucose in the recipe.

The composition of soft, 1% and 2% tryptone agar and broth and buffer has been described previously (Kayajanian and Campbell, 1966).

Methods

The bacterial and phage strains used in our experiments are listed in Tables 1 and 2. LFT biotin transducing activity was assayed on bio$^-$ 0 cells (with helper phage), which were plated on minimal medium with glucose. The dark red transductants were streaked on the segregation medium, and a well isolated bio$^+$ colony was streaked

again. If this second plate contained at least one bio$^-$ or sectored colony from among 300 well isolated colonies, the transduced cell was classified as heterogenotic (het); if not, it was designated a stable bio$^+$ transductant.

λbio particles were characterized as plaque-forming (λpb) if the lysogenic centers of individual plaques made from an HFT lysate consisted of bio$^+$ transductants. Induction of these lysogens produces particles almost all of which transduce the lysogenic cells in the center of plaques they make on bio$^-$0. In practice λpb plaques are distinguished from λ plaques by their pink color on a bio$^-$ host on transduction medium; λ plaques do not have this hue. Isolation of defective lysogens among bio$^-$ cells transduced by other HFT lysates allowed the classification of non-plaque-forming, bio transducing particles (λdb). We isolated λdb lysogens after the procedures of Campbell (1959) for λdg lysogens. These defective lysogens were picked as bio$^+$ colonies which when induced and spotted with the mutant phage λ*sus*J6, on a nonpermissive (pm$^-$) strain produced a region of lysis. Neither the phage λ*sus*J6 nor the induced lysogen spotted alone on a pm$^-$ background produced this lysis. The phage content of a λdb genome was determined by *sus*$^+$ marker rescue from the defective lysogen. The presence of superinfection immunity in the lysogen was assayed by plating dilute λ lysates on backgrounds of the defective lysogens. When appropriate, the presence or absence of some c_I^+ and c_{II}^+ alleles in the λdb genomes was assayed by infecting the induced λdb lysogen with λc_I*sus*0 or λc_{II}*sus*0 and plating the phage yield after 110 minutes on a pm$^-$ strain. Turbid plaques on the pm$^-$ host (at 43° for c_{Its} mutants) indicate the presence in the λdb genome of the c$^+$ allele of the infecting phage mutant. The identity of bio$^+$ alleles present in each λbio isolate was determined by spotting about 0.05 ml of an HFT lysate on lawns of the different bio$^-$ mutants spread on the transduction medium (del Campillo-Campbell *et al.*, 1967).

Phage lysates were made by the induction of lysogens or by the infection of sensitive cells in equal volumes of broth and buffer (Kayajanian and Campbell, 1966).

Density gradient experiments were performed as described in Kayajanian and Campbell (1966).

RESULTS

Of 420 bio$^+$ transductants picked from an LFT lysate, 72 or 17.2% were hets. The hets were the sole source of our HFT biotin transducing lysates. No (0/39) nonsegregating, λ lysogenic biotin transductants produced a lysate with a biotin transducing activity greater than 5×10^3/ml. Of nearly 100 hets examined, all but one had transducing titers greater than 6×10^4/ml. Most of the HFT titers were about 10^6 to 10^7 transductants/ml.

Our HFT lysates had disappointingly low transducing titers. We were concerned that somehow our assay for transducing particles was not very efficient. It is possible that the presence of some phage function(s) missing in λbio might be provided by normal λ. But the presence of λ as helper does not markedly alter the efficiency of transduction by λbio phage particles (Table 3).

Yet the measure of transduction frequency is not equivalent to the number of transducing particles. A lysate made by the induction of the single lysogen bio$^-$0 (λpb M72-3) yields a transducing activity on bio$^-$0 of 1.3×10^6/ml and a plaque-forming activity on the same host of 1.6×10^8 (the lysogenic centers of nearly all of the λpb plaques contain bio$^+$ cells). So the efficiency of transduction is 7.8×10^{-3} of the plaque-forming activity of λpbM72-3.

For λdb's, ultraviolet (UV) absorption constitutes an independent assay of the

TABLE 3

EFFECT OF λ HELPER ON TRANSDUCTION FREQUENCY OF THREE λbio's

λbio	Phage end point	Frequency of transduction	
		No helper	Moi of helper
—	—	—	2.3
M7-20	λpb	3.8×10^5	1.2×10^5
M3-29	N/c_I	2.2×10^5	1.8×10^5
M29-7	O/P	6.5×10^4	2.3×10^5

TABLE 4

ULTRAVIOLET ABSORPTION OF λdb M3-29 AND λdb M55-3

Number of cells ml at induction	PFU per ml	Observed transducing titer/ml	Fraction number	Absorbance units 260 mμ per ml	λ titer per ml	Observed transducing titer	PFU/OD units	Titer of potential transducing particles	Observed transductants/potential transductant particles	Yield of potential λdb/ml	Burst	
											λdb	λ+
λdb M3-29												
5.2×10^7	1.4×10^9	3.2×10^6	2	0.308	6.0×10^6	6.6×10^7		1.3×10^{10}	5.0×10^{-3}	6.4×10^8	12	26
			4	0.414	1.6×10^{10}	$<1.5 \times 10^5$	3.8×10^{10}	—	—	—	—	—
			5	0.282	1.5×10^{10}	$<1.5 \times 10^5$	5.1×10^{10}	—	—	—	—	—
λdb M55-3												
7.9×10^6	7.0×10^8	2.7×10^6	4′	0.152	1.4×10^{10}	$<1.2 \times 10^6$	9.0×10^{10}	—	—	—	—	—
			5′	0.227	2.4×10^{10}	$<1.2 \times 10^6$	1.1×10^{11}	—	—		—	—
			8′	0.125	1.8×10^8	4.2×10^7		1.3×10^{10}	3.2×10^{-3}	6.2×10^8	84	88

number of transducing particles (Smith, 1968). Phage induced from bio$^-$0 (λdb M3-29) (λ) and from bio$^-$0 (λdb M55-3) (λ) were concentrated by pelleting, treated with RNase and DNase, and fractionated in a CsCl density gradient. The UV absorption at 260 mμ of three fractions from these gradients, two primarily rich in λ, the other predomniantly λdb, was measured. These data (Table 4), corrected for the relative amounts of DNA in the transducing the plaque-forming phage [as judged by phage density measurements (Table 7)] and for the UV-absorbing residue from the original lysates, reveal approximately the relative efficiency of transduction by λdb M3-29 and M55-3 as 5.0×10^{-3} ($6.6 \times 10^7/1.3 \times 10^{10}$) and 3.2×10^{-3} ($4.2 \times 10^7/1.3 \times 10^{10}$).

The plaque-forming and transducing activity in the lysate harvested from the induction of bio$^-$0 (λdb M3-29) (at 5.2×10^7 cells/ml) were, respectively, 1.4×10^9/ml and 3.2×10^6/ml. So the yield of λdb (M3-29) particles in this lysate is 6.4×10^8 ($3.2 \times 10^6/5.0 \times 10^{-3}$). If the plaque-forming activity is a good measure of the number of λ particles, the yield of transducing particles is about one-half the number of λ particles. The approximate burst size of λ particles per induced cell is 26 (1.4×10^9 PFU/ml/5.2×10^7 cells/ml); the λdb burst is about 12. In a second experiment, the induction of bio$^-$0 (λdb M55-3) (λ) (at 7.9×10^6 cells/ml) yielded a lysate with a plaque-forming titer of 7.0×10^8 and a transducing titer of 2.7×10^6/ml. The λ burst then is 88 (7.0×10^8 PFU/ml/7.9×10^6 cells/ml). The λdb burst is nearly at the λ level (84). We do not find transducing particles in induction of λdb defective lysogens. So it appears that λdb genomes multiply with λ help in the induced double lysogen.

Genetic Extent of λdb Genomes

We assayed for the presence or absence of certain phage and bacterial markers in the genomes of a number of our λbio transducing phages. For some of our assays, we made defective lysogens from bio transducing HFT lysates. The ability of defective lysogens to contribute certain sus$^+$ or c$^+$ alleles but not others, revealed which sus$^+$ or c$^+$ markers were carried by each λdb genome. The ability or inability of phage λ to plate on defective lysogens indicated the absence or presence in the λdb genome of an intact and functioning c_I region. The ability or inability of λbio lysates to transduce bio$^-$ mutants other than the one on which they were originally selected, revealed the extent of the bacterial chromosome carried inside the transducing phages. The extent of a number of λbio genomes is described in Table 5. As in the case of λdg, each λbio genome represents a contiguous block of phage and bacterial genes from a λ lysogenic bacterial chromosome. Loss of the bio$^+$ phenotype from λdb lysogens, presumably by segregation, was nearly always accompanied by loss of the phage markers associated with it in the transducing genome.

The variety of phage and bacterial markers in the λbio genomes permits us to map the right arm of the λ chromosome and to order our collection of bio$^-$ mutants. Three of the 26λdb genomes (R30h–2a, M38-5, E5a-20) are clearly broken within the phage cistrons O and P and can be used to order the various *sus* O and P mutants:

$$N_{7,53} - O_{8,29} - O_{125} - P_{72} - P_3 - P_{116} - Q, R$$

The pattern of transduction of the λbio lysates on different bio$^-$ mutants, orders the bacterial mutants into seven classes:

$$\lambda - 0,4,24 - 2,17 - 12 - 3 - 23 - 18 - 19$$

(del Campillo-Campbell *et al.*, 1967).

Those λdb genomes which gave *sus*$^+$ marker rescue for *sus*O, but not for *sus*N were also tested for immunity against superinfection by λ and for c_I^+ and c_{II}^+ marker rescue with c_I*sus*O and c_{II}*sus*O (Table 6). Of eleven λdb genomes examined, one (R24-2) gives marker rescue for none of the clear mutants tested, is not immune to superinfection by phage λ, and thus seems to be broken either within the c_{II} cistron or between c_{II} and the O cistron. Four (M3-29, R24-5, R3h-1, M16-3) give marker rescue for all of the c_I and c_{II} mutants tested, and are immune to superinfection, and thus seem

TABLE 5

PHAGE AND BACTERIAL END POINTS OF SOME λbio TRANSDUCING GENOMES[a]

bio genome	$N_{7,53}$	i^{λ}	c_{I47}	c_{III50}	c_{II68}	$O_{8,29}$	O_{125}	P_{72}	P_3	P_{116}	$Q_{21,73,117}$ $R_{5,16,64,69}$	A_{11} J_6	bio⁻ 0,4, 24	bio⁻ 2,17	bio⁻ 12	bio⁻ 3	bio⁻ 23	bio⁻ 18	bio⁻ 19
M108-1	+	+	N	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	−
M72-3	+	+	N	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	−
M66-1	+	+	N	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	−
M47-1	+	+	N	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	−
M38-3	+	+	N	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	−
M22-5	+	+	N	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	−
M18-4	+	+	N	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	−
M9-11	+	+	N	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	−
M9-21	+	+	N	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	−
M15-1	+	+	N	N	N	+	+	+	+	+	+	+	+	+	+	+	+	−	−
M7-20	+	+	N	N	N	+	+	+	+	+	+	+	+	+	+	+	+	−	−
M5-5	+	+	N	N	N	+	+	+	+	+	+	+	N	N	N	N	N	N	N
M3-29	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
R24-5[b]	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
R3h-1[b]	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
M16-3	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
M30-7	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
M29-6	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
M38-1	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
M42-2	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
M40-3	−	−	−	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
M37-1	−	−	−	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
R24-2	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
M20-5	−	N	N	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+
R30h-2a	−	N	N	N	N	−	+	+	+	+	+	+	+	+	+	+	+	+	+
M36-4	−	N	N	N	N	−	−	+	+	+	+	+	+	+	+	+	+	+	+
M30-2	−	N	N	N	N	−	−	+	+	+	+	+	+	+	+	+	+	+	+
M29-7	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
R40h-1b	−	N	N	N	N	−	−	+	+	+	+	+	+	+	+	+	+	+	+
R36-1	−	N	N	N	N	−	−	+	+	+	+	+	+	+	+	+	+	+	+
M86	−	N	N	N	N	−	−	+	+	+	+	+	+	+	+	+	+	+	−
M36-2	−	N	N	N	N	−	−	+	+	+	+	+	N	N	N	N	N	N	N
M38-5	−	N	N	N	N	−	−	−	+	+	+	+	+	+	+	+	+	−	−
E5a-20	−	N	N	N	N	−	−	−	−	+	+	+	+	+	+	+	+	−	−
M39-2	−	N	N	N	N	−	−	−	−	−	+	+	+	+	+	+	+	+	+
R24-3	−	N	N	N	N	−	−	−	−	−	+	+	+	+	+	+	+	+	+
M39-6	−	N	N	N	N	−	−	−	−	−	+	+	N	N	N	N	N	N	N
M55-3	−	N	N	N	N	−	−	−	−	−	−	+	+	+	+	+	+	−	−

[a] Bacterial and phage end points were determined as indicated in the Methods section. + indicates the presence of a given marker; −, the absence; N, not tested. The first 12 genomes listed were from λpb particles; their ability to generate immunity was tested. The ability of λpbs to generate plaques on W3350 was interpreted to mean that *sus*N⁺, O⁺, P⁺, Q⁺, R⁺, A⁺, J⁺ were present in the phage genome. More complete data on c⁺ marker rescue from λdbs are given in Table 6.

[b] All λdb genomes tested for marker rescue are in bio⁻0, except for these two, which are in bio⁻3.

to end between the c_I region and the N cistron. One of these four excludes $T4_{rII}$ mutants; the other three do not (Dove, personal communication). The remaining 6 λdbs (M30-7, M29-6, M38-1, M42-2, M40-3, M37-1) are not immune to superinfection, give marker rescue for c_{II} and some c_I mutants, but not for other c_I mutants, and are thus broken within the c_I region; two λdb genomes are broken within c_IB; the

TABLE 6

c^+ MARKER RESCUE BY *csusO* FROM λdb DEFECTIVE LYSOGENS; PROPORTION OF TURBID *sus*$^+$ TO CLEAR *sus*$^+$ PLAQUES

Defective lysogen	Infecting strain						
	c_IA47 *sus*8	c_IAt2 *sus*29	c_IAtu51 *sus*29	c_IBtu46 *sus*29	c_IBt1 *sus*29	c_IBtu50 *sus*29	c_{II}68 *sus*8
R3h-1	0.280	—	—	—	—	—	4.6
R24-5	0.305	—	—	—	—	—	2.8
M3-29	0.094	0.184	0.286	—	1.038	0.620	2.8
M16-3	0.184	—	—	—	—	—	2.3
M29-6	<0.013	<0.0026	<0.0020	<0.0020	0.108	0.046	2.3
M30-7	<0.003	<0.0030	<0.0044	0.267	0.144	0.447	2.1
M38-1	<0.002	<0.0021	<0.0033	0.091	0.184	0.346	1.8
M43-2	<0.024	<0.019	<0.0011	0.029	0.044	0.478	1.8
M37-1	<0.001	<0.0013	<0.0013	<0.0030	<0.0009	0.127	1.3
M40-3	<0.005	<0.0018	<0.0006	<0.0041	<0.0017	0.29	1.0
R24-2	<0.003	<0.0009	<0.0055	<0.0070	<0.0016	<0.0032	<0.04
M29-7	<0.010	—	—	—	—	—	<0.004

other four may extend into c_IA. In general the c_I and c_{II} mutants can be ordered with respect to each other by deletion mapping (c_{I47}, c_{It2}, c_{Itu51} — c_{It1}, c_{Itu46} — c_{Itu50}, c_{II68}) and to a lesser degree, by differences in the proportion of turbid to clear plaques among *sus*$^+$ recombinants (c_{I47} — c_{It2} — c_{Itu51} — c_{Itu46}, c_{It1} — c_{Itu50} — c_{II68}).

There is always the potential problem that independently isolated HFT lysates may have more than one kind of bio transducing particle. Let us dismiss from consideration the existence of LFT particles in HFT lysates as Adler and Templeton (1963) have described them for λdg lysates. To perform our marker rescue experiments, defective lysogens were made from λdb HFT lysates. Often more than one defective lysogen were isolated from a single HFT lysate. All these lysogens contained the same block of phage genes. We determined the densities of ten different λ bios in CsCl. Transducing activity in each case was confined to a narrow part of the gradient in a manner which suggested a single physical transducing class of particles.

Our CsCl gradient measurements (Table 7) reveal that the density of one λbio may differ from that of another. The plaque-forming and transducing activity in pure λpb lysates are associated with particles of the same density. Assuming the protein content of λ and all the λbio particles to be equal, the DNA contribution to the mass of bio transducing genomes (selected on bio$^-$O) ranges from 80% to 115% that of λ's (Table 7). By subtracting the DNA content of λdb M37-1 from that of λdb M29-6, a minimum size of one-seventh the DNA content of λ can be assigned to the bio region of the *E. coli* chromosome.

Segregation of λdb Lysogens

Biotin transductants were characterized as hets by their ability to segregate bio$^-$ subclones on subculturing. About one-sixth of all LFT and two-thirds of all HFT biotin transductants are hets (Table 8). Although it is possible to distinguish sectored (bio variable) clones from either bio$^+$ or bio$^-$ colonies on our segregation medium, hets were characterized not so much by visible sectoring of bio$^-$ (λbio) (λ) colonies, but by bio$^-$ subclones arising from streaking bio$^-$ (λbio) (λ) lysogens. Visibly sectored colonies rarely if ever were encountered in streaking bio$^-$ (λbio) (λ) double lysogens or most bio$^-$ (λbio) single lysogens. However, single lysogens of the six λdbs whose genomes terminate within c_I (designated λdbc_I) have very different segregation behavior from all our other strains. They give rise on streaking to a majority of bio$^-$ colonies and a minority of highly sectored bio variable colonies. These bio variable colonies in turn give rise on further restreaking to a majority of bio$^-$

TABLE 7

DENSITY OF VARIOUS λbio's: DNA MASS OF THESE PARTICLES RELATIVE TO THAT OF λ[a]

Bio strain	Phage end point	Bacterial end point	Position of λ maxima (tube No.)	Position of λcb2 maxima (tube No.)	Position of λbio maxima		DNA mass relative to λ's (=1.00)
					Transduction	Plaque	
M66-1	λpb	18/19	12	21	7	8	1.06
M108-1	λpb	18/19	9	19	9	9	1.00
M7-20	λpb	23/18	11	22	11	11	1.00
M55-3	R/A	23/18	10	20	20	—	0.87
M38-5	$P_{72}/P_{3.116}$	18/19	14	27	31	—	0.81
M37-1	Within c_I	0, 4, 24/2, 17	4	14	17	—	0.82
M40-3	Within c_I	2, 17/12	42	59	64	—	0.82
M42-2	Within c_I	3/23	42	59	52	—	0.92
M29-6	Within c_I	18/19	14	24	17	—	0.96
M3-29	N/i^{λ}, c_{47}	Beyond 19	42	59	20	—	1.15

[a] The density of 10 different λbio particles relative to that of λ and λcb2 was determined by placing the three phage types together in a CsCl density gradient. λcb2 was added to HFT λdb lysates, which contain λ and the transducing particles; both λc_{I-71} and λcb2 were added to HFT λpb lysates made lytically from a single plaque. Transducing activity was assayed on bio⁻0, except in one experiment (with phage types M40-3, M42-2, and M3-29) where bio⁻19 and bio⁻3 were also hosts. Turbid and clear plaques were assayed on C600; turbid plaques in λpb lysates were from λpb particles. Transducing and turbid plaque maxima for λpb lysates are listed by tube number; λ and λcb2 maxima are listed in the table too. The DNA content of the λcb2 phage is 86.6% that of λ. The density of c_{I71} is indistinguishable from λ's. The mass difference between λbio and λ was calculated according to the method described in Kayajanian and Campbell (1966).

TABLE 8

PROPORTION OF HETEROGENETIC CLONES IN LFT AND HFT BIOTIN TRANSDUCTION

Experiment	Induced strain	Nature of lysate	Number of biotin transduced clones analyzed	Percentage of heterogenotic clones among transductants on bio⁻0
1	C600 (λ)	LFT	420	17.6
2	C600 (λ)	LFT[a]	52	19.2
	bio⁻0 (λdbM39-2) (λ)	HFT[a]	18	66.7
	bio⁻0 (λpbM72-3) (λ)	HFT[a]	16	62.5
3	bio⁻0 (λdb20-5) (λ)	HFT	15	60%
4	bio⁻0 (λdb30-2) (λ)	HFT	12	75%

[a] Transduction for the three lysates were done on the same sample of cells with the same multiplicity of infection of "helper" phage.

colonies and a minority of bio variable colonies. The bio⁻ colonies do not show marker rescue of *sus* markers, while the bio variable clones do. Rare bio⁺ colonies which appear do not segregate and do not give marker rescue of any *sus* markers.

When grown in broth, the number of cells giving rise to bio variable colonies increases, but the proportion of these cells decreases significantly. Double (λdbc_I) (λ) lysogens as well as single lysogens of other λdbs do not exhibit any marked change in the proportion of bio⁺ to bio⁻ cells during growth (Table 9). The visual appearance of pronounced sectoring on our segregation medium may be the result of fast conversion of bio⁺ cells to bio⁻ cells, or slower conversion coupled with a killing of bio⁺ cells, or fast conversion coupled with killing of bio⁺ cells. (This slower conversion might be at the rate at which other single lysogens segregated bio⁻ cells.) If the second or third explanation were correct, then there should be a greater fraction of dead cells in the fast segregating (λdbc_I) than in the slow segregating (λdb) cultures. Dead *E. coli* take up methylene

TABLE 9

FRACTION OF bio⁻ CELLS IN bio VARIABLE COLONIES

Strain	λdb phage end point	Fraction of bio⁻ cells in a bio variable clone at time=0	Fraction of bio⁻ cells in a bio variable culture after *n* generations of growth[a]	
			Fraction	(*n*)
bio⁻0 (λcb M29-6)	Within c_I	81/123 = 66%	587/620 = 94.7%	6.8
bio⁻0 (λdb M30-7)	Within c_I	145/175 = 83%	249/251 = 99.2%	19.6
bio⁻0 (λdb M37-1)	Within c_I	219/259 = 85%	955/959 = 99.6%	21.0
bio⁻0 (λdb M38-1)	Within c_I	1132/1256 = 90%		
bio⁻0 (λdb M40-3)	Within c_I	207/256 = 81%	565/601 = 94.0%	6.1
bio⁻0 (λdb M42-2)	Within c_I	303/340 = 89%	888/917 = 97.3%	20.5
bio⁻0 (λdb M30-7) (λ)	Within c_I	10/295 = 3.4%	4/77 = 5.2%	18.0
bio⁻0 (λdb M37-1) (λ)	Within c_I	11/580 = 1.9%	1/33 = 3.0%	15.9
bio⁻0 (λdb M55-3)	Between R and A	1/39 = 2.6%	10/202 = 5.0%	23.2
bio⁻0 (λdb M29-7)	Between O and P	2/309 = 0.6%	1/88 = 1.1%	14.8
bio⁻0 (λdb M3-29)	Between N and c_I	2/89 = 2.2%	16/252 = 6.3%	22.8
Reconstruction bio⁻O (λdb M3-29) +bio⁻O	—	32/72 = 44.4%	44/89 = 49.4%	21.6

[a] Broth tubes are inoculated with isolated bio⁺ or bio^v colonies from freshly streaked plates. The cultures are diluted into warmed broth while growing at 37°. Initially, during the course of growth, and after *n* generations of growth, samples of the culture are assayed for the proportion of bio⁺ and/or bio^v colonies to bio⁻ colonies on our segregation medium.

blue which stains structures inside the cell; live *E. coli* exclude the dye. By this test, cultures of the λdb_{cI} single lysogens have a higher proportion of dead cells (55 dead/607 total = 9.1%) than other λdb lysogens or bio⁻O (λ) control cultures (21/574 = 3.7%). Since the colonies arising from bio⁻ (λdb_{cI}) lysogens contain at least two classes of cells, one of which is slower dying bio⁻, the proportion of dead cells arising from the other classes may be higher than the figure quoted above. So part of the appearance of fast segregation in a growing culture of bio⁻ (λdb_{cI}) lysogens is the result of killing of λdb lysogens.

Position of Attachment of λdb Genomes

We have asked, as has Fuerst (1966), whether a λbio genome attaches to the bacterial chromosome at or near the normal λ insertion site. If a λpb attaches at or near the normal attachment site, induction of a λpb lysogen should result in the formation of LFT particles able to transduce gal markers or other bio markers. In fact, induction of the λpb lysogen bio⁻O (λpb M72-3) results in the production of a lysate with HFT bio transducing activity on bio⁻O, LFT gal transducing activity on W3805 and LFT bio transducing activity on bio⁻19. HFT lysates were made from three of the gal transductants; the gal transducing particles in these lysates transduce bio at high frequency. These bio-gal transducing particles were defective as phages, missing a block of phage genes which includes *sus*J and extends in various lengths through the tail and into the head region of λ's genome. These particles do not transduce bio_{19}^-. It is reasonable to conclude, therefore, that the λpb lysogenized either at the normal attachment site or near it, but not to the right of the bio region.

A number of gal⁻ bio⁻ (λdg) (λdb) double lysogens were constructed to determine where the two genomes will attach to the host chromosome. The gal⁻ bio⁻ (λdb) single lysogen was made by the low multiplicity transduction of bio⁻O with λdb M55-3 (missing phage cistrons N through R). This gal⁻ bio⁻ (λdb) lysogen was then transduced at a low multiplicity of infection by $\lambda dg0_{1\text{-}26}$ (missing phage cistrons A through J). Two independent gal transductants capable of segregating gal⁻ subclones, but yielding low titers of plaque forming particles on induction were studied further. We asked whether

the gal⁻ segregants from the double lysogen were still bio⁺ and whether they retained cistron P and B markers (cistron P is associated with the gal⁺ in λdg; B, with the bio⁺ in λdb). A number of independently arising gal⁻ segregants from each of the double lysogens were tested for the presence of B and P cistrons and the bio⁺ phenotype. We also assayed for the presence of B and P cistrons and the gal phenotype in bio⁻ segregants of these double lysogens. These results are summarized in Table 10.

We have assumed that the formation of gal⁻ segregants results from the lysogenic host chromosome recombining with itself in a region of gal⁺–gal⁻ homology, and the subsequent loss of the small excised chromosomal fragment. Any marker between the two gal markers and therefore on this fragment would be lost too and not recoverable from the gal⁻ segregant. The same assumptions apply for the study of bio⁻ segregants. Gal⁻ segregants from double lysogen strain No. 5 have lost the P cistron, but neither the B cistron nor the bio⁺ phenotype. Bio⁻ segregants from strain No. 5 are gal⁺ and have lost cistron B but not P. Gal⁻ segregants from strain No. 14 are bio⁻ and have lost both B and P markers. Bio⁻ segregants from strain No. 14 are gal⁻ and have lost cistron B and P genes. The only gene order possible for strain No. 5 is

gal⁻ (λdg) (λdb) bio⁻

where the prophages are in their normal orientation. The gene orders possible for strain No. 14 are:

gal⁻ (λdb) (λdg) bio⁻

and

gal⁻ (λdb) bio⁻ (λdg)

where the prophages are in their normal orientation. So, the λdb genome resides between the gal and bio markers of the *E. coli* host, and probably was inserted at the λ attachment site or in a region of homology with the host genome.

A subclone was isolated from strain No. 14 which would not give marker rescue for *sus*B or *sus*P markers, but still segregated gal⁻ and bio⁻ daughter cells. For this segregant to arise from strain No. 14, the prophage order in that double lysogen would have to be

gal⁻ (bio⁺ A J) (N R gal⁺) bio⁻

and the subclone from it is probably

gal⁻ (bio⁺ gal⁺) bio⁻.

In strains 5 and 14 both λdg and λdb genomes reside between the gal and bio markers of the host chromosome. The presumptive gal⁻ (bio⁺ gal⁺) bio⁻ strain was easily lysogenized by phage λ, and the (λ) (λdbg) double lysogen produced an HFT lysate where gal and bio transducing activity were associated with particles of the same low density. Joint transduction frequencies were no less than one-third of the individual transduction frequencies. The DNA content of the λdbg genome is less than the DNA mass of the λdb by about as much as the λdg's is less than λ's (Kayajanian, unpublished results).

TABLE 10

ANALYSIS OF gal⁻ AND bio⁻ SEGREGANTS FROM TWO gal⁻ bio⁻ (λ db) (λ dg) DOUBLE LYSOGENS

Strain No.	Residual genotype of gal⁻ segregants				Residual genotypes of bio⁻ segregants		
	Number examined	B⁻ P⁻ bio⁻	B⁺ P⁻ bio⁺	B⁺ P⁻ bio⁻	Number examined	B⁻ P⁻ gal⁻	B⁻ P⁺ gal⁺
5	15	1	12	2	2	0	2
14	7	6[a]	1	0	6	5	1

[a] A sister clone of one of these segregants was B⁺ P⁺ bio⁻.

DISCUSSION

λbio transducing particles derived from an HFT lysate are identical in their content of phage markers and form a Gaussian distribution with a slight tail in a CsCl density gradient. HFT lysates are developed from independent transductants made from an LFT lysate. λbio particles derived from HFT lysates may and generally do differ from one lysate to another in the content of phage and bacterial markers and in their density. As in the case of λdg, the gene content in λbio genomes represents a contiguous stretch of hereditary material lifted from the lysogenic bacterial chromosome. In general, the denser

a λbio particle is, the larger its phage genome and/or its bacterial component (Tables 5 and 7).

The variability in end points of λdb genomes suggests an independent origin for at least those λdb genomes in LFT lysates that are capable of lysogenizing their bio⁻ hosts. The difference in the ability of LFT and HFT lysates to transduce by lysogeny, rather than just transducing, suggests a difference between λbio particles in an LFT lysate and in all the HFT lysates so far studied. It is possible, therefore, that a significant fraction of biotin transducing particles in LFT lysates may be structurally very different from those which we describe in HFT lysates and may be unable to transduce by lysogeny as a result of these structural differences. These structural differences may be, among other things, the absence of λ's cohesive ends. The variability that we observe in the end points of λdb genomes may not be as great as really exists for another reason. Some of the λdb genomes capable of being maintained as reasonably stable lysogens in the presence of λ as (λ) (λdb) lysogens (which give rise to HFT lysates) may be quite unable to be stably maintained as single (λdb) lysogens. Some λdb genomes may not be stable even as (λ) (λdb) lysogens. We were rather surprised when none of eleven λdb genomes having phage end points between cistrons N and O were broken between c_I and c_{II}, but six were broken within c_I, for we suspected the c_I cistron to be smaller than the c_I to c_{II} intercistronic distance.

All of our λdb genomes contain the phage markers from the J through A cistrons, though they need not contain cistron R markers. Symmetrically, all the λdg genomes we have encountered (Campbell, 1959, 1962; Kayajanian and Campbell, 1966) contain the phage markers from the N through R cistrons, though they do not necessarily contain cistron A markers. In the absence of actually performing specific assays for the presence of the vegetative single-stranded ends of λ (located between R and A) in all λdb and λdg particles in HFT lysates, we would like to argue that the method of selection and the measurable genetic end points of λdb and λdg genomes themselves support the presence of λ's single-stranded ends in λdbs missing cistron R markers and λdg's missing cistron A markers. Despite the fact that λdg defective lysogens are characterized by the presence of λ immunity and the absence of plaque-forming ability, these genomes always contain the unselected markers in cistrons O through R. Similarly, λdb defective lysogens are characterized by the presence of a J cistron marker and absence of plaque-forming ability, but always contain the unselected markers from cistrons A through J. In view of the role in circularization and, therefore, in lysogenization attributed to the single-stranded ends of λ, we consider the data above a strong indication that all λdg and λdb genomes capable of forming defective lysogens do contain the vegetative ends of λ.

Assuming this, then $\lambda dg0_{1\text{-}26}$ (missing A through J) and λdb M55-3 (missing N through R) both contain the vegetative ends of phage λ. Both also contain an insertion region involved in the recombinational act in lysogenization. The λdbg prophage recombinant between these two, carrying gal and bio in place of both phage arms of λ, should contain only the insertion and vegetative regions that are of phage origin. The ability of λdbg to undergo a lysogenic cycle with λ's help suggests that the structural requirement for undergoing such a circuit are satisfied by the presence of the prophage and vegetative ends of λ.

Defective lysogens of the six λdb genomes whose phage end points fall within the λ immunity region give rise to highly sectored colonies on our segregation medium. Lysogens of more defective and less defective λdb genomes do not give this appearance. Neither do (λ) (λdb_{cI}) double lysogens. We suppose that bacterial control maintains the more defective λdbs as prophages. It seems reasonable to assume that the function of part of the λ phage genome located between c_I and the O cistron is responsible for the altered phenotype of the (λdb_{cI}) lysogens. Lysogens of less complete λdb genomes segregate more slowly; so these genomes would not have the wherewithal to manifest a fast segregation phenotype. Lysogens of more complete λdb genomes would manage

to control the expression of the phenotype. We note that all the λdb genomes more complete than (λdb_{cI}) contain an intact immunity region which is recognized as such a control region.

The growth of bio$^-$ (λdb_{cI}) cells to form highly sectored colonies is the result, at least, of segregation of bio$^-$ subclones from parental cells. That practically every colony arising from bio$^-$ (λdb_{cI}) lysogens is very highly sectored indicates that independent segregational events occur frequently—more frequently, than in other bio$^-$ (λdb) or gal$^-$ (λdg) lysogens. This greater rate of conversion of lysogenic to nonlysogenic cells may by itself explain the very large fraction of bio$^-$ cells in a sectored colony and the pronounced increase in the proportion of bio$^-$ cells in a culture starting from a bio$^-$ (λdb_{cI}) colony. Segregation alone will account for highly sectored colonies, whereas differential growth rates will not.

What about the killing? It may be an irrelevance to our other observations or a byproduct of segregation which expresses itself at least one generation after the segregational event. We have imagined the segregational event to be the excision of the λdb_{cI} genome from the lysogenic host chromosome. If the effect of the excision were to damage the host chromosome (in a polynucleate cell) so it would not function, then all the bio$^-$ segregants should be killed, and we would not see segregation in bio$^-$ (λdb_{cI}) colonies. If the killing function were physiological, expressed some time after the segregational event by a nonreplicating, nonrepressible excision fragment, then the daughter cell that would die would be the one containing the fragment. Sometimes this cell would be bio$^+$, and sometimes bio$^-$. O-P killing (Packman and Sly, 1968), resulting from the inability of phage genomes to control O and P function could account for our dead cells. The absence of an N gene in our λdb_{cI} genome could delay the killing for more than a cell division so we would be able to see segregation.

ACKNOWLEDGMENT

The author is grateful to the Drs. Campbell for looking after him.

REFERENCES

ADLER, J., and TEMPLETON, B. (1963). The amount of galactose genetic material in λdg bacteriophage with different densities. *J. Mol. Biol.* **7**, 710–720.

CAMPBELL, A. (1959). Ordering of genetic sites in bacteriophage λ by the use of galactose-transducing defective phages. *Virology* **9**, 293–305.

CAMPBELL, A. (1962). Distribution of genetic types of transducing λ phages. *Genetics* **48**, 409–421.

DEL CAMPILLO-CAMPBELL, A., KAYAJANIAN, G., CAMPBELL, A., and ADHYA, S. (1967). Biotin mutants of *Escherichia coli* K12. *J. Bacteriol.* **94**, 2065–2066.

FUERST, C. R. (1966). Defective biotin-transducing mutants of bacteriophage Lambda. *Virology* **30**, 581–583.

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

MORSE, M. L., LEDERBERG, E. M., and LEDERBERG, J. (1956a). Transduction in *Escherichia coli* K12. *Genetics* **41**, 142–156.

MORSE, M. L., LEDERBERG, E. M., and LEDERBERG, J. (1956b). Transductional heterogenotes in *Escherichia coli*. *Genetics* **41**, 758–779.

PACKMAN, S., and SLY, W. S. (1968). Constitutive λDNA replication by λc_{17}, a regulatory mutant related to virulence. *Virology* **34**, 778–789.

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

SMITH, H. O. (1968). Defective phage formation by lysogens of integration deficient phage P22 mutants. *Virology* **34**, 203–223.

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