

## Host Specificity of DNA Produced by *Escherichia Coli*

### I. Host controlled modification of bacteriophage $\lambda$

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Lambda bacteriophage particles carry a "host specificity" determined by the bacterial strains on which they were produced. Upon infection of a different bacterial host (1) the phage DNA may be either accepted or rejected on the basis of this specificity, (2) if accepted, the phage multiplies and progeny phage are produced. Those progeny to which the parental phage DNA molecule is transferred, in either conserved or semi-conserved form, also receive the parental phage host specificity. All progeny containing only newly synthesized DNA receive only the specificity of the new bacterial host. It is concluded that host specificity is carried on the bacteriophage DNA.

Phage P1, present in a bacterial cell as either prophage or vegetative phage, imparts to  $\lambda$  DNA multiplying in the same cell a host specificity over and above that determined by the host itself. Such P1-induced specificity can be impressed equally well onto replicating and non-replicating  $\lambda$  DNA.

### Introduction

Non-mutational changes of host range properties upon growth on new bacterial host strains have been found for many bacteriophages (see Luria, 1953). For bacteriophage  $\lambda$  such a modification occurs if *Escherichia coli* C is used as host instead of the usual *E. coli* K12 (Bertani & Weigle, 1953; Weigle & Bertani, 1953): phage  $\lambda$ , adapted to *E. coli* C, grows on *E. coli* K12 with a probability of only about  $10^{-4}$ . Other systems producing host controlled modification in phage  $\lambda$  will be described in the present paper.

The reproduction of phage  $\lambda$  in a new sensitive host strain will be shown to be submitted to two successive host control mechanisms (Arber & Dussoix, 1961): (1) the infecting phage DNA is either recognized as incompatible with the host and degraded, or is accepted; (2) if fully accepted, it multiplies and its DNA replicas receive "host specificity", i.e. the particular non-heritable stamp given by the host. The present study will be concerned with the establishment of this host specificity.

Host controlled modification of phage DNA can be governed by the genetic material of the bacterial host cell. In some instances the controlling loci have been mapped on the bacterial chromosome (Zinder, 1960), while in other cases they are known to be contained in the genome of an unrelated prophage. Prophage P1, for example, induces modifications of phages T1, T3, T7 and P2 (Lederberg, 1957) and, as will be shown here, of phage  $\lambda$ . This modifying action will be shown to occur not only when  $\lambda$  phage multiplies in cells lysogenic for P1, but also following P1 superinfection of non-lysogenic cells in which  $\lambda$  is multiplying. The same holds true for phages T1 and P2 as found independently by Christensen (1961).

### Materials and Methods

*Notation.* The "host specificity" of a phage will be represented as follows: the symbol of the phage and of its genotype (if relevant) will be followed by the name of the bacterial host strain from which the phage acquired its host specificity, phage and bacterial symbols being separated by a point. For example,  $\lambda$ c-K means: phage lambda, clear plaque type mutant, having the host specificity imparted by *E. coli* K12. Several host-specific characters may be found in one and the same phage particle, but we will give only the ones considered in the particular experiment. Classification of a phage stock with respect to host specificity is possible both by consideration of its history and by determination of its efficiency of plating on various hosts.

*Bacterial strains.* The following strains of *E. coli* and some derivatives of them have been used.

(a) *E. coli* K12. The K12 strains referred to in this paper are W3110, W3350 (see Arber, 1960a) and C600 (Appleyard, 1954). All of these strains were used as hosts for growth of phage  $\lambda$ ; only C600 was used as a plating indicator strain.

(b) *E. coli* B. Be (Cohen, 1959) and its derivative no. 251, a hybrid derived by transduction and carrying the *mal*<sup>+</sup>- $\lambda$ <sup>s</sup> region from K12 (Arber & Lutasté-Dorolle, 1961).

(c) *E. coli* C (Bertani & Weigle, 1953).

#### *Bacteriophages*

(a) Phage  $\lambda$ <sup>++</sup>, wild type (Kaiser, 1957) and its mutants *c* (clear plaque, Jacob & Wollman, 1954), *mi* (minute, Kaiser, 1955) and *b*<sub>2</sub> (buoyant density mutant, Kellenberger, Zichichi & Weigle, 1960).

(b) Phage P1 adapted to K12 (see Arber, 1960b).

#### *Media*

(a) Tryptone broth: 1% Difco Bactotryptone, 0.5% NaCl, pH 6.9 to 7.1; for solid medium completed with 1.5% agar.

(b) Solid LB broth (Bertani, 1951), used for growth of phage P1.

(c) Synthetic medium M9: 0.7% Na<sub>2</sub>HPO<sub>4</sub>, 0.3% KH<sub>2</sub>PO<sub>4</sub>, 0.1% NH<sub>4</sub>Cl, 0.05% NaCl, 10<sup>-4</sup> M-CaCl<sub>2</sub>, 10<sup>-3</sup> M-MgSO<sub>4</sub>, 0.4% glucose, 2 × 10<sup>-6</sup> M-Fe<sup>3+</sup> citrate, pH 7.0, completed if desired with 1% Bactocasamino acids (= M9a).

(d) H medium, a glycerol-lactate medium (Stent & Fuerst, 1955) with phosphorus supplied only by 0.05% casamino acids, pH 7.2.

(e) Adsorption medium for phage  $\lambda$ : 0.01 M-MgSO<sub>4</sub>.

(f) Dilution media: tryptone broth or phosphate buffer: 0.7% Na<sub>2</sub>HPO<sub>4</sub>, 0.4% NaCl, 0.3% KH<sub>2</sub>PO<sub>4</sub>, 0.02% MgSO<sub>4</sub>, pH 6.9.

(g) Tris buffer: 0.05 M-tris, 0.5% NaCl, 0.1% NH<sub>4</sub>Cl, 10<sup>-3</sup> M-MgSO<sub>4</sub>, completed with a 10<sup>-4</sup> dilution of phosphate buffer.

(h) 3XD medium: 0.45% KH<sub>2</sub>PO<sub>4</sub>, 1.05% Na<sub>2</sub>HPO<sub>4</sub>, 0.3% NH<sub>4</sub>Cl, 0.03% MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.0% casamino acids, 3% glycerol, 0.003% gelatin, 3 × 10<sup>-4</sup> M-CaCl<sub>2</sub>.

Preparation of <sup>32</sup>P-containing H medium: <sup>32</sup>P of the desired activity and casamino acids (half of which had been rendered P-free) were evaporated to dryness and then resuspended in the casamino acid-free H medium. Preparation of deuterated medium: M9 salt mixture (except CaCl<sub>2</sub>) and casamino acids were evaporated to dryness, resuspended in 99.7% D<sub>2</sub>O and completed with CaCl<sub>2</sub> and glucose.

The general phage techniques are described by Adams (1950) and those particular to  $\lambda$  by Arber (1958, 1960a). The CsCl density gradient centrifugation technique is described by Meselson, Stahl & Vinograd (1957) and by Weigle, Meselson & Paigen (1959). For all density gradients the swinging bucket rotor SW 39 of the Spinco preparative centrifuge was run for 16 hr at 22,000 rev./min. A hole was punched in the bottom of each tube and each drop was collected in a separate tube containing 1 ml. of tryptone broth.

As the lysogenic condition is not stable in many K12(P1) strains only young cultures grown from recently re-isolated single colonies were used in our experiments. These conditions ensured a proportion of non-lysogenic subunits of less than 10<sup>-3</sup>.

## Results

*Definition of systems giving host controlled modification of  $\lambda$* 

The efficiencies of plating (e.o.p.) of some variants of phage  $\lambda$  are given in Table 1 for different host strains. The e.o.p. on the host strains on which the phage stock was produced is put equal to 1. For  $\lambda$ -K this procedure is justified, since the plaque-forming titer on K12 and the number of  $\lambda$  particles, as determined by electron microscopy, are approximately equal (Kellenberger & Arber, 1957). The similarity of the titers found in lysates of  $\lambda$ -K(P1),  $\lambda$ -B and  $\lambda$ -C and those in  $\lambda$ -K lysates suggests that the former lysates also do not contain many more  $\lambda$  particles than plaque-formers on their competent host.

TABLE 1

*Efficiency of plating of phage  $\lambda$  variants on different host strains*

Phage variant	Efficiency of plating on host strains			
	K12	K12(P1)	Be 251	C
$\lambda$ -K	1	$2 \times 10^{-5}$	$10^{-4}$	1
$\lambda$ -K(P1)	1	1	$10^{-4}$	1
$\lambda$ -B	$4 \times 10^{-4}$	$7 \times 10^{-7}$	1	1
$\lambda$ -C	$4 \times 10^{-4}$	$4 \times 10^{-7}$	$2 \times 10^{-4}$	1

Indicator bacteria were grown in aerated tryptone broth to about  $4 \times 10^8$  cells/ml. and then starved in 0.01 M-MgSO<sub>4</sub>. Pre-adsorption was for 15 min at 37°C. For K12, either C600 or W3110 was used.

The physiological condition of the recipient bacteria does not greatly influence the e.o.p., although small fluctuations are found if the age of the bacteria or the medium are varied. Treatment of the infecting phage stock with DNase does not change the e.o.p.

Hosts containing several factors known to restrict the acceptance of a given phage result in a lower e.o.p. than hosts in which only one restricting factor is present, e.g. K12(P1) accepts phages  $\lambda$ -C and  $\lambda$ -B at a frequency of only  $10^{-6}$  to  $10^{-7}$ , while K12 non-lysogenic for P1 accepts the same phages at a frequency of  $10^{-3}$  to  $10^{-4}$ .

*Modification of phage  $\lambda$  upon vegetative multiplication in a new host*

Phage  $\lambda$ -K(P1) is accepted without restriction in the non-lysogenic host K12. The injected phage genomes multiply vegetatively and after the usual  $\lambda$  latent period the bacteria liberate active progeny phages with about the same burst size as if  $\lambda$ -K were used for infection. The great majority of the progeny phages show host controlled modification, i.e. are  $\lambda$ -K. A low proportion of  $\lambda$ -K(P1) is found, however, since plating of the lysate with the indicator strain K12(P1) gives a number of plaques distinctly higher than attributable to the usual e.o.p. of  $\lambda$ -K. This reappearance of apparently non modified  $\lambda$ -K(P1) was studied in a number of one-cycle growth experiments in which non-lysogenic K12 was infected with  $\lambda$ -K(P1) and the resulting infective centers and subsequent lysates were assayed for total plaque formers by plating with K12 bacteria and for  $\lambda$ -K(P1) by plating with K12(P1). In Table 2 it may be seen that bacteria infected with single  $\lambda$ -K(P1) particles in such an experiment

TABLE 2  
*Efficiency of vegetative multiplication of  $\lambda$ -K(P1) on K12*

Exp.	Strain medium	M.o.i.	Free $\lambda$ -K(P1) ml. after washing	Infective centers ml. (plaque titer before lysis)		Progeny phage ml.		Burst size	
				on K12	on K12(P1)	on K12	on K12(P1)	Total	$\lambda$ -K(P1)
1	H	1.0	$2.0 \times 10^2$	$4.2 \times 10^7$	$2.9 \times 10^7$	$3.8 \times 10^8$	$1.7 \times 10^7$	80	0.6
2	H	1.3	$2.5 \times 10^2$	$3.4 \times 10^7$	$2.9 \times 10^7$	$8.8 \times 10^8$	$3.8 \times 10^7$	260	1.3
3	H	0.7	$10^3$	$5.0 \times 10^7$	$3.3 \times 10^7$	$1.1 \times 10^{10}$	$4.9 \times 10^7$	220	1.9
4	H	1.0	$2.0 \times 10^2$	$4.0 \times 10^7$	$2.8 \times 10^7$	$4.0 \times 10^8$	$2.0 \times 10^7$	100	0.7
5	H	0.75	$2.3 \times 10^2$	$4.8 \times 10^7$	$3.2 \times 10^7$	$7.4 \times 10^8$	$2.6 \times 10^7$	154	0.8
6	H	0.2	—	$8.0 \times 10^8$	$1.0 \times 10^8$	$3.6 \times 10^7$	$1.2 \times 10^8$	120	1.2
7	H	0.12	—	$8.0 \times 10^8$	$3.1 \times 10^8$	$8.0 \times 10^8$	$2.3 \times 10^8$	100	0.7

lysate of  $\lambda$ -K(P1) generally did not need of contaminating P1 phages by treatment with anti-P1 serum, was adsorbed for 10 min to starved K12 (non-lysogenic for P1). The mixture was diluted in aerated growth medium to assure a complete injection of the phage genomes, then treated with 0.01 M-MgSO<sub>4</sub> and finally washed three times by low speed centrifugation. After resuspension in fresh growth medium, free phage was assayed by the plaque method on K12 and K12(P1) indicator bacteria. The same procedure was used for the determination of total plaque formers. The same indicators were used to plate the lysates. Burst size was defined as the number of liberated phages per infective center on the respective indicator. M.o.i. = multiplicity of infection.

have a very high probability (about 0.3 to almost 1, varying with different experimental conditions) of liberating at least one infective  $\lambda$ -K(P1), i.e. of forming a plaque on K12(P1). The average  $\lambda$ -K(P1) burst size per productive bacterium is only about one, whereas the burst size of  $\lambda$ -K is, as normally, between 100 and 250. This finding suggests that the  $\lambda$ -K(P1) progeny phage found after one cycle of growth on K12 are composed of material transferred from the parent  $\lambda$ -K(P1) phages.

#### Joint transfer of host specificity and DNA

Since it is known that  $\lambda$  parental phage DNA may be transferred to the progeny in one cycle of growth (Kellenberger, Zichichi & Weigle, 1961; Meselson & Weigle, 1961) the question arises as to whether transferred DNA and transferred host specificity are found in the same or in different phage progeny particles. Two types of experiments were designed to answer this question.

#### (a) Inactivation of parental and transferred $\lambda$ -K(P1) by decay of incorporated $^{32}P$

Heavily  $^{32}P$ -labeled  $\lambda$ -K(P1) were prepared, either by infection of K12(P1) with  $\lambda$ -K(P1) or by u.v.-induction of K12(P1) ( $\lambda$ )/ $\lambda$ , in H medium containing  $^{32}P$  at a specific activity between 200 and 800 mc/mg. Phages thus obtained and stored at 4°C in 3XD medium show an exponential inactivation due to the decay of the  $^{32}P$  incorporated in the DNA (Hershey, Kamen, Kennedy & Gest, 1951). Aliquots of the  $^{32}P$ -labeled  $\lambda$ -K(P1) stocks were allowed to grow for one cycle in unlabeled non-lysogenic K12 in unlabeled medium. The lysates thus obtained were also stored at 4°C in 3XD medium and assayed from day to day. Two typical experiments are represented in Fig. 1. The stock used for the experiment of Fig. 1(a) apparently contained a low proportion of the original parental phage which had not adsorbed and therefore remained unlabeled and stable to  $^{32}P$  decay, and possibly also contained some progeny phages with a significantly lower than average labeling because of transferred parental  $^{31}P$  atoms. The stock used for the experiment of Fig. 1(b) was obtained by induction of lysogenic cells and contained no detectable amount of non-labeled transferred prophage material, although the bacteria had been put in  $^{32}P$  medium only after induction.

The results of these experiments, done at low multiplicity of infection (m.o.i.) (Table 3), are as follows:

(1) The total phage progeny after one growth cycle of  $^{32}P$ -labeled  $\lambda$ -K(P1) phage on K12 non-labeled cells shows no detectable inactivation as a function of storage time, as is expected, since the bulk of the phage DNA is newly synthesized material.

(2) All progeny  $\lambda$ -K(P1) phages (plaque-formers on K12(P1)) are sensitive to  $^{32}P$  decay. Hence the phages with transferred host specificity all contain transferred parental phosphorus. We conclude that the substances providing host specificity to the phage are physically linked to the DNA molecule.

(3) For the transferred  $\lambda$ -K(P1) progeny the rate of  $^{32}P$  decay inactivation is about half that of the parental  $\lambda$ -K(P1): 45 and 46% for the experiments plotted in Figs. 1(a) and 1(b) respectively. Other experiments, not described here in detail, gave  $^{32}P$  decay sensitivities of the transferred  $\lambda$ -K(P1) ranging from 40 to 50% of that of the parents. The most likely explanation of this finding is that the half sensitive, and thus half labeled, "hybrid" DNA results from replication according to the Watson-Crick model (Watson & Crick, 1953). One  $^{32}P$ -labeled parental DNA strand would thus be

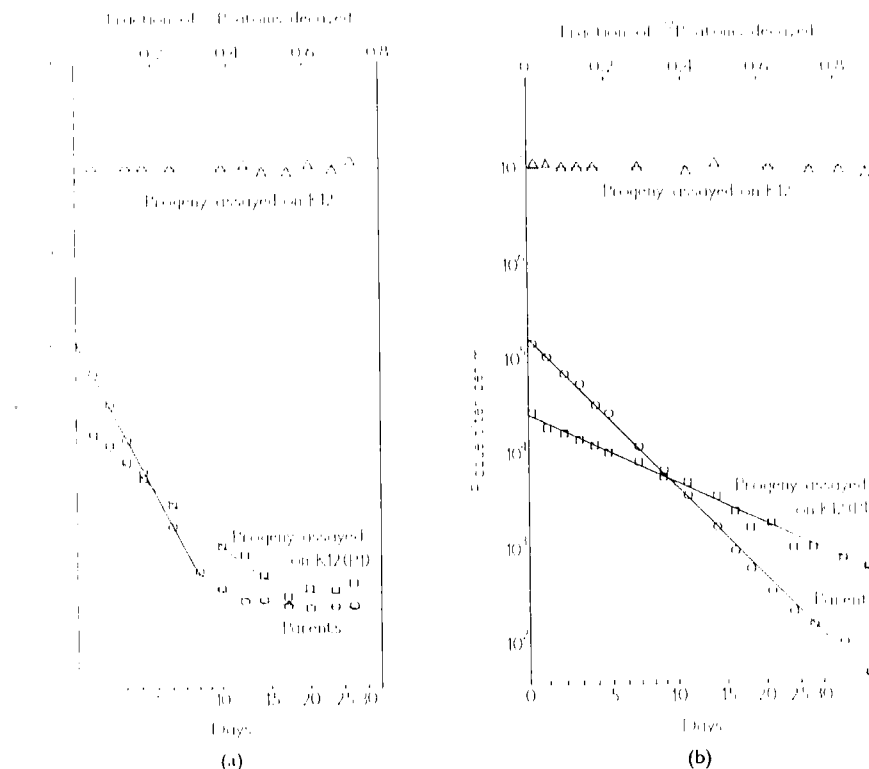


Fig. 1. Joint transfer of host specificity and sensitivity to inactivation by disintegration of  $^{32}P$  incorporated in the DNA. Heavily  $^{32}P$ -labeled stocks of  $\lambda$ -K(P1) were prepared in H medium either by the multiplication of  $\lambda$ eb<sub>2</sub>-K(P1) in strain W3350(P1) (experiment a) or by u.v. induction of strain W110(P1) ( $\lambda$ )/ $\lambda$  (experiment b). The chloroformed lysates were diluted immediately in 3XD medium. Aliquots were stored at 4°C and assayed from day to day. Both indicators K12 and K12(P1) give the same plaque titers (○). Other aliquots were treated with anti-P1 serum and then allowed to grow for one lytic cycle in non- $^{32}P$ -labeled, non-lysogenic K12 bacterium (strain W110(P1)) in non-labeled tryptone medium (supplemented with  $4 \times 10^{-3}\%$  gelatin and 0.2% glucose) as described in Tables 2 and 3. Dilutions of the lysates in 3XD were also stored at 4°C and assayed from day to day on K12 (△) and K12(P1) (□). Titers obtained on K12(P1) were corrected for the o.e.p. of  $\lambda$ -K. Titers of  $^{32}P$ -labeled parent phage (○) were adjusted for the plot, so that the initial titer coincides with the number of bacteria which were productive in the one cycle of growth.

TABLE 3  
One cycle growth of  $^{32}P$ -labeled  $\lambda$ -K(P1) in non-labeled K12  
(experiments plotted in Fig. 1)

Time (days)	Phage stock	M.o.i.	Plaque titer before lysis		Plaque titer immediately after lysis		Burst size
			on K12	on K12(P1)	on K12	on K12(P1)	
0	W3350(P1)	0.008	$1.1 \times 10^9$	$1.7 \times 10^8$	$6.1 \times 10^7$	$1.6 \times 10^6$	53
0	W110(P1)	0.006	$1.5 \times 10^8$	$5.3 \times 10^7$	$1.7 \times 10^7$	$5.8 \times 10^5$	30

transferred, associated with a newly synthesized unlabeled daughter strand, to each  $\lambda$ -K(P1) progeny phage. Although the parental DNA strand serves as template one or more times in the course of vegetative phage multiplication the parental host specificity does not separate from it.

That the transferred  $\lambda$ -K(P1) is inactivated at slightly less than half the inactivation rate of the parental phage may be due to breakage and reunion recombination between labeled parental DNA strands and unlabeled progeny strands. As will be seen later some such recombinants can indeed be found.

(b) *Density labeling of parental  $\lambda$ -K(P1) with deuterium*

Preliminary experiments showed that differently modified variants of phage  $\lambda$  (tested  $\lambda$ -K,  $\lambda$ -K(P1) and  $\lambda$ -B) have the same buoyant density in CsCl, within the precision of the determination by the drop-collecting method, whereas many genetic mutants are known which differ in their density from the reference type (Kellenberger *et al.*, 1960).

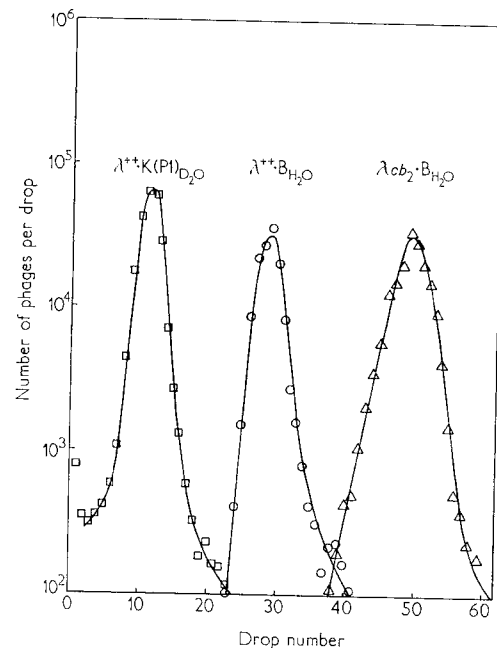


Fig. 2. *Density distribution of deuterated  $\lambda^{++}$ -K(P1), after centrifugation in CsCl density gradient. Deuterated  $\lambda^{++}$ -K(P1) was obtained by u.v.-induction of strain W3350( $\lambda$ ) (P1)/ $\lambda$ , which had been grown for many generations in M9a medium prepared with 99.7%  $D_2O$ .  $H_2O$ -grown  $\lambda^{++}$ -B and  $\lambda cb_2$ -B were added as density references and they were assayed on B (strain Be 251).*

In the experiments to be described here phage  $\lambda$ -K(P1) was grown in a synthetic M9a medium prepared with 99.7%  $D_2O$  instead of normal water. Such "heavy" deuterium-labeled phages formed, upon centrifugation in a CsCl density gradient, a band as sharp as normal ( $H_2O$  grown) phages (Fig. 2). We conclude that our deuterated phage stocks were composed of uniformly dense phage particles. The exact density was not measured but was compared with the densities ( $\rho$ ) of phage  $\lambda b_2$  and  $\lambda b_2^+$  grown with  $H_2O$  and having  $\rho = 1.491 \text{ g/cm}^3$  and  $1.508 \text{ g/cm}^3$  respectively (Kellenberger *et al.*, 1960). In the centrifugation of heavy  $\lambda cb_2$  obtained by several

lytic growth steps on K12(P1), the normal  $\lambda b_2$  and  $\lambda b_2^+$  control bands were separated by 20 drops and the deuterated  $\lambda b_2$  formed a band 3 drops on the heavier side of the  $\lambda b_2^+$  control. In the centrifugation of heavy  $\lambda b_2^+$  (Fig. 2), obtained by u.v. induction of strain W3350(P1) ( $\lambda$ )/ $\lambda$ , the distance between the  $H_2O$  grown  $\lambda b_2$  and  $\lambda b_2^+$  controls was again 20 drops and the deuterated  $\lambda b_2^+$  banded at a distance of 17 drops on the heavier side of the  $\lambda b_2^+$  control.†

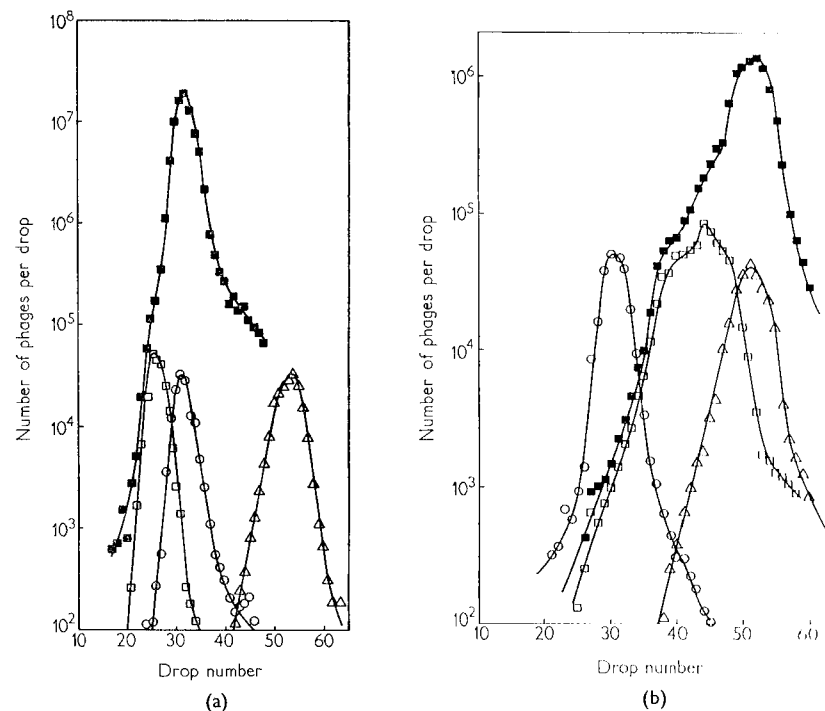


Fig. 3. *Density distribution of lysates obtained by one cycle growth of deuterated  $\lambda$ -K(P1) on normal, non lysogenic K12 (strain W3110).*

(a) Lysate of experiment 23/5a (see Table 4); m.o.i. = 0.019. Aliquot centrifuged: 0.9 ml. of lysate, 82 drops collected.

□,  $\lambda^{++}$ -K(P1), assayed on K12(P1). ■, total  $\lambda^{++}$ , assayed on K12. ○, density reference  $\lambda^{++}$ -B. △, density reference  $\lambda cb_2$ -B.

(b) Lysate of experiment 23/3d (see Table 4); m.o.i. = 28. Aliquot centrifuged: 0.06 ml. of lysate, 78 drops collected.

□,  $\lambda cb_2$ -K(P1), assayed on K12(P1). ■, total  $\lambda cb_2$ , assayed on K12. ○, density reference  $\lambda^{++}$ -B. △, density reference  $\lambda cb_2$ -B.

The deuterated  $\lambda$ -K(P1) phage stocks were allowed to grow for one cycle of multiplication on non lysogenic K12 bacteria in medium prepared with  $H_2O$  (Table 4). The rate of transfer of the host specificity was as found for normal, non deuterated  $\lambda$ -K(P1). The one step lysates were centrifuged in a CsCl density gradient and the collected fractions were assayed for total phage and phage with K(P1) host specificity. Two typical distributions are reproduced in Fig. 3.

† The u.v. induced stock of deuterated  $\lambda^{++}$  contained 1.5% phage type mutants, the stock of deuterated  $\lambda b_2$  obtained by infection contained less than 0.1% phage type mutants.

TABLE 4  
One-cycle growth of deuterated  $\lambda$ -K(P1) on K12 in  $H_2O$  medium

Exp.	Phage $\lambda$ -K(P1)	Growth medium	M.o.i.	Free $\lambda$ -K(P1)/ml. after washing	Infective centers/ml. (plaque titer before lysis)			Progeny phage/ml.		Burst size	
					on K12	on K12(P1)	$\frac{\lambda\text{-K(P1)}}{\text{total } \lambda}$	on K12	on K12(P1)	Total	$\lambda$ -K(P1)
23 3a	$\lambda^{++}$	M9a	0.014	$4.0 \times 10^2$	$1.4 \times 10^6$	$4.4 \times 10^5$	0.31	$5.6 \times 10^7$	$1.9 \times 10^5$	40	0.43
	$\lambda cb_2$	M9a	0.022	$4.0 \times 10^2$	$1.5 \times 10^6$	$6.0 \times 10^5$	0.40	$2.3 \times 10^8$	$5.0 \times 10^5$	153	0.83
	$\lambda cb_2$	M9a	7.1	$1.4 \times 10^3$	$1.8 \times 10^6$	$1.2 \times 10^6$	0.66	$2.8 \times 10^8$	$5.0 \times 10^6$	156	4.2
	$\lambda cb_2$	M9a	28	$6.0 \times 10^2$	$1.5 \times 10^6$	$1.7 \times 10^6$	1	$1.8 \times 10^8$	$1.2 \times 10^7$	120	7.1
23 5a	$\lambda^{++}$	H	0.019	$5.0 \times 10^2$	$1.8 \times 10^6$	$6.4 \times 10^5$	0.36	$7.0 \times 10^7$	$2.7 \times 10^5$	39	0.42
	$\lambda cb_2$	H	0.015	$8.0 \times 10^2$	$2.0 \times 10^6$	$8.2 \times 10^5$	0.41	$1.6 \times 10^8$	$5.0 \times 10^5$	80	0.61
	$\lambda cb_2$	H	2.8	$2.7 \times 10^3$	$6.4 \times 10^7$	$4.2 \times 10^7$	0.66	$7.3 \times 10^8$	$8.9 \times 10^7$	114	2.1

Deuterated  $\lambda$ -K(P1) phage stocks, treated with anti-P1 serum, were adsorbed to starved K12 bacteria (strain W3110). The complexes were then washed three times and, after resuspension in fresh growth medium, assayed for free  $\lambda$ -K(P1) and infective centers. The lysates were chloroformed for 15 min after suspension in growth medium.

If the one cycle growth was initiated by an infection at low multiplicity, (1) all the transferred  $\lambda$ -K(P1) phages banded sharply at a density a few drops heavier than the reference carrying the same genotype, with a density corresponding to a transfer of roughly one-quarter of the initial "extra" density of the deuterated parent; (2) the majority of the  $\lambda$ -K banded sharply at the reference density. After infection at multiplicities higher than one, the band of the transferred  $\lambda$ -K(P1) appeared enlarged on its dense side and, with increasing multiplicity of infection, a second peak became more and more important (Fig. 3(b)). The maxima of the enlarged double band of  $\lambda$ -K(P1) were situated at about 25% and 50% of the initial distance separating the deuterated  $\lambda$ -K(P1) parent from the corresponding reference type. Qualitatively the same behavior was found for both  $\lambda b_2^+$ -K(P1) and  $\lambda b_2$ -K(P1) phage.

From these experiments we conclude that

(1) none of the phages having the transferred parental host specificity is composed uniquely of newly synthesized material,

(2) after low multiplicity of infection the transferred  $\lambda$ -K(P1) contain about half of the parental DNA, corresponding to about one-quarter of the parental "extra" density, since about half of this density is attributable to the protein coats. The parental DNA would thus be semi-conserved, probably having been replicated according to the Watson-Crick model.

(3) With high multiplicity of infection the DNA of some of the transferred  $\lambda$ -K(P1) is fully conserved, i.e. transferred intact, only the protein coat being new, non-deuterated. These findings are in agreement with similar observations made by Meselson & Weigle (1961) in transfer experiments using  $\lambda$  phage labeled with  $^{13}C^{15}N$ .

The question arises whether it is necessary for conservation of the parental host specificity that at least one complete parental DNA strand is transferred, or if a *partial transfer* of certain genome regions is sufficient. In order to answer this question, deuterated  $\lambda b_2 cmi^+$ -K(P1) was crossed with normal  $\lambda b_2 c^+ mi^-$ -K in a non-lysogenic K12 host in normal tryptone medium (Table 5). The lysate was then assayed on K12(P1) for the  $\lambda$ -K(P1) phages and on K12 for the total phage titer. As seen in Table 5, both parental types participated in vegetative multiplication and gave rise to recombinants at usual frequencies. But among the progeny phage particles, essentially only  $\lambda$ -K(P1) parental *cmi*<sup>+</sup> and some *cmi* recombinants were able to grow on K12(P1). The frequencies of phages showing transferred host specificity were 1.3% for  $\lambda cmi^+$  and 0.55% for  $\lambda cmi$  genotypes. Hence the host specificity governed by P1 can be transferred by somewhat less than the whole  $\lambda$  genome. In our experiment a small proportion of  $\lambda c^+ mi^+$ -K(P1) was found and most of such plaques were mottled, typical for *c/c*<sup>+</sup> heterozygotes. Some of them were further tested and their heterozygous nature was confirmed; none of them was *mi*, nor were they *mi/mi*<sup>-</sup> heterozygotes. The few phages of the  $\lambda$ -K parental genotype (*c*<sup>-</sup>*mi*) which are found by assay on K12(P1) can be attributed to the rate of  $\lambda$ -K plaque formers on K(P1).

A CsCl density gradient centrifugation of the lysate of the cross described in Table 5 revealed that all the  $\lambda$ -K(P1), including the *cmi* recombinants and the few *c/c*<sup>+</sup>*mi*<sup>+</sup> heterozygotes, had a density corresponding approximately either to semi-conserved DNA transfer or, less frequently, to conserved DNA transfer, i.e. only

† A deuterated phage stock was used in this experiment in order to permit an analysis of the lysate by density gradient centrifugation. The results of the cross are, however, not influenced by the deuterium present in one of the parents, as has been shown by the same cross done with non-deuterated phages.

phages with deuterated parental material were able to grow on K12(P1). On the other hand, deuterium label was found among the phages restricted on K12(P1), eliminating the possibility that parental  $\lambda$ -K(P1) could not exchange material with  $\lambda$ -K genomes because of the difference in host specificity.

TABLE 5

*Cross of deuterated  $\lambda b_2 c m i^+$ -K(P1) with normal  $\lambda b_2 c^+ m i$ -K on non-lysogenic K12 (strain W3110)*

	Genotypes			
	Parental		Recombinants	
	$c m i^+$	$c^+ m i$	$c m i$	$c^+ m i^+$
Multiplicity of infection	4.3	3.6		
Free phage/ml. after adsorption and washing	$8.0 \times 10^4$	$3.0 \times 10^5$		
Infective centers/ml. before lysis, assayed on	$3.3 \times 10^7$ $2.4 \times 10^7$	(35% mottled) (0.6% mottled)		
Fraction of infective centers yielding $\lambda$ -K(P1)			0.73	
Progeny phage/ml., plated on K12	$8.5 \times 10^8$	$3.4 \times 10^8$	$3.3 \times 10^7$	$1.5 \times 10^7$
Fraction of recombinants per parental $\lambda c m i^+$ -K			3.9%	1.8%
Progeny phage/ml., plated on K12(P1)	$1.1 \times 10^7$	(10 <sup>4</sup> )†	$1.8 \times 10^5$	$7.5 \times 10^3$ ‡
Fraction of recombinants per parental $\lambda c m i^+$ -K(P1)			1.6%	0.07%

† Corresponds to the spontaneous modification rate of the  $3.4 \times 10^8$  phages  $\lambda c^+ m i$ -K/ml.

‡ Mostly heterozygotes  $c/c^+$ .

#### *Production of $\lambda$ -K(P1) variants induced by superinfection with P1 of a K12- $\lambda$ complex*

In the preceding sections, we studied the modification of host specificity in the progeny of  $\lambda$ -K(P1) which had infected a non-lysogenic K12 strain. The reverse experiment, to study the modification of  $\lambda$ -K multiplying in K12(P1), cannot be done in the same way since by virtue of the host specificity the infecting  $\lambda$ -K DNA is recognized and subsequently degraded (Dussoix & Arber, 1962). This restricted acceptance can, however, be surmounted if, instead of an already established P1-lysogenic acceptor K12(P1), a K12 recently infected with P1 is used or if phage P1 is introduced only after adsorption of  $\lambda$  on K12.

These superinfection experiments have been done by infecting K12 bacteria of strain W3350, grown in tryptone broth to  $10^9$  cells/ml. and subsequently starved in adsorption medium, with phage  $\lambda c$ -K. After 10 minutes of adsorption at 37°C, fresh tryptone broth was added and the complexes were aerated at a concentration of  $4 \times 10^8$  cells/ml. At various times aliquots were superinfected with phage P1 (grown on W3350). Good adsorption was insured by adding 1/500 M-CaCl<sub>2</sub> to the bacteria 10 minutes before the infection with P1. For P1-superinfections simultaneously, before, or shortly after the infection with  $\lambda$ , this general plan had to be slightly modified. After adsorption of both  $\lambda$  and P1 the non-adsorbed phages were measured and the infected bacteria were diluted in tryptone broth to a concentration of about  $10^6$  cells/ml. Except in the cases of very late superinfection with P1, infective centers were then measured on both K12 and K12(P1) indicators. At 59 to 60 minutes after the infection with  $\lambda$  all the

$\lambda$ -producing bacteria had lysed, as revealed by periodically measuring intra- and extracellular  $\lambda$  phage. The lysates were chloroformed at 60 to 75 minutes after  $\lambda$  infection and assayed on K12 and K12(P1) indicators for the variant types of  $\lambda$  progeny. Non-lysogenic bacteria infected with both  $\lambda$  and P1 phages produce principally  $\lambda$  phage particles with a latent period characteristic for the K12- $\lambda$  complex. In our experimental conditions very few P1 progeny phages are found,  $10^{-3}$  to  $10^{-4}$  as many as  $\lambda$ .

TABLE 6

*Induced modification of host specificity by superinfection with phage P1 of K12 bacteria infected with  $\lambda$  phage*

Time of addition of superinfecting P1 (min)	Proportion $\lambda$ -K(P1) total $\lambda$		
	Infective centers before lysis	Lysates chloroformed	
		at 60 min	at 75 min
0	0.97	0.77	0.74
10	0.50	0.17	0.15
15	0.80	0.33	0.45
20	0.91	0.24	0.39
25	0.52	0.22	0.30
30	0.13	0.083	0.18
35	0.025	0.005	0.11
40	0.019	$2.6 \times 10^{-5}$	0.02
45	0.022	$4.1 \times 10^{-5}$	0.007
Without superinfection	$2 \times 10^{-3}$	$3 \times 10^{-5}$	

Infection of K12 (strain W3350) with phage  $\lambda c b_2$ -K at  $t = 0$  min (m.o.i. = 0.6). Superinfection with P1 at various times (m.o.i. = 5-4). For procedure see text. First appearance of intracellular  $\lambda$  phage particles, liberated by chloroforming the culture, at 32 min. First appearance of extracellular  $\lambda$  phage particles (latent period) at 40 min. Massive liberation of first  $\lambda$  progeny completed at 50 min.

The results of a typical experiment are represented in Table 6. *Simultaneous infection* with  $\lambda$  and P1 usually induces the P1-specific modifications in almost all  $\lambda$ -producing complexes. In the lysates the fraction of phages showing the host specificity typical for K12(P1) is generally between 50% and almost 100% of the total phage output. As the superinfection with P1 is *delayed* the fraction of modified phages decreases. For the first 25 minutes irregular variations between 5 and 100% modifications were found, depending more, perhaps, on the metabolic conditions than on the effective time of superinfection. As the first intracellular  $\lambda$  phage particles appear the possibility of modification drops rapidly and disappears almost completely with the beginning of lysis. It should be pointed out that the actual time of P1 penetration into the K12- $\lambda$  complex is a few minutes after the measured P1 addition (see Table 6), since adsorption and injection are not immediate. In the experiments shown in Table 6 the one-step lysates were chloroformed 20 or more minutes after the occurrence of lysis, i.e. very late P1 superinfections induced some detectable modifications. This might be due to a minor fraction of bacteria retarded in the production of  $\lambda$  or to a late-infected bacteria producing a second generation of  $\lambda$ .

Higher frequencies of modification are found if a high multiplicity of P1 is used for superinfection. After simultaneous infection with  $\lambda$ -K and P1, maximum modification may already be attained with a multiplicity of 5 P1 phages per bacterium. At very low P1 multiplicity the  $\lambda$ -K(P1) producing centers increase linearly with multiplicity, and it is possible to calculate the probability of modification occurring in a cell infected by a single P1. In one particular experiment this probability was 34%, but it may depend on the experimental conditions. The multiplicity of  $\lambda$ -K does not seem to influence the frequency of modification.

These experiments show that the conversion of a  $\lambda$ -K-producing complex into a  $\lambda$ -K(P1)-producing complex occurs soon after the infection with phage P1. This conversion certainly does not depend on the presence of  $\lambda$  at the time of P1 infection, but may occur also if P1 is introduced into the K12 cells previous to the infection with  $\lambda$ . Experiments designed to explore this situation indicated that, indeed, late superinfection with  $\lambda$ -K of K12-P1 complexes is possible for a relatively long period, and that a high proportion of the  $\lambda$  progeny phages are thereby modified into  $\lambda$ -K(P1). These results suggest that the control of host specificity by the presence of P1 can be dissociated into (1) the control over the *acceptance* of non-adapted DNA and (2) the control over the *modification* of non-adapted DNA. The first control mechanism seems to be established only slowly, perhaps in parallel to the establishment of P1 lysogeny. The control of modification, however, is established rapidly, as already shown, and is also induced by the virulent mutants of P1 which do not yield stable lysogenics. More experiments are necessary to explore the connections between the establishment of these two control mechanisms on the one hand, and the steps leading to either vegetative multiplication or establishment of P1 lysogeny on the other hand.

The results presented in Table 6 suggest that  $\lambda$ -producing cells which are converted by superinfection with P1 may liberate mixed bursts of both non-modified  $\lambda$ -K and modified  $\lambda$ -K(P1) variant types, since the relative proportions of plaque formers on K12(P1) are smaller in the lysates than if the productive bacteria are plated before their lysis. The mixed nature of host specificity types liberated by individual bacteria has, indeed, been confirmed by single burst experiments.

It is of interest to know if the  $\lambda$ -K(P1) phage particles contain only genomes which were newly synthesized after the conversion by superinfection with P1, or if the new host specificity may be given even to finished, non-replicating phage genomes. We first tried to answer this question with genetic experiments of the following type: K12 was infected with  $\lambda$  and, after various times of incubation, superinfected with P1 and—simultaneously or after a short delay—with a second, genetically distinct type of  $\lambda$ . After lysis had occurred, the liberated phages were classified for their genotypes and their host-specificity types. The proportions of modification diminished as superinfection was retarded, but for all superinfection times the relative amount of modified  $\lambda$ -K(P1) among the genotypes of the first infecting parent and the superinfecting parent was roughly equal. The possibility that P1 host specificity may be given to a non-replicating DNA is thus suggested, although the experiments are not conclusive. Indeed, only very late superinfection at a moment where finished DNA is already withdrawn from the vegetative pool could give sure results, but by that time superinfection with a second  $\lambda$  is no longer successful, i.e. the superinfecting  $\lambda$  does not participate in the vegetative growth (Séchaud, 1960).

In order to surmount these difficulties, we designed another experiment, based on the fact that, after infection at high multiplicity, conserved phage DNA can be

transferred to the progeny, apparently without active participation in the vegetative pool. Non-lysogenic K12 bacteria (strain W3110) were simultaneously infected with deuterated  $\lambda cb_2$ -K (m.o.i. = 20) and normal P1 (m.o.i. = 6.7). After 15 minutes of adsorption, the complexes were washed and then incubated in tryptone broth. After 3 minutes, free phage titer and titer of productive centers were measured (Table 7).

TABLE 7  
*Modification of  $\lambda$ -K into  $\lambda$ -K(P1) by superinfection with phage P1*

K12 bacteria (strain W3110), grown to a concentration of  $10^9$  cells/ml. and starved in 0.01 M-MgSO<sub>4</sub>, were simultaneously infected with deuterated  $\lambda cb_2$ -K and normal P1.

M.o.i. of deuterated $\lambda cb_2$ -K	20
M.o.i. of P1	6.7
Free $\lambda$ -K after adsorption and washing	$1.8 \times 10^6$ /ml.
Infective centers/ml. (plaque titer before lysis) assayed on	
{ K12	$9.3 \times 10^6$
{ K12(P1)	$8.7 \times 10^6 = 94\%$
Progeny phage/ml. assayed on	
{ K12	$1.1 \times 10^9$
{ K12(P1)	$9.4 \times 10^8 = 85\%$
Total burst size	118

This lysate was centrifuged in CsCl density gradient, see Fig. 4.

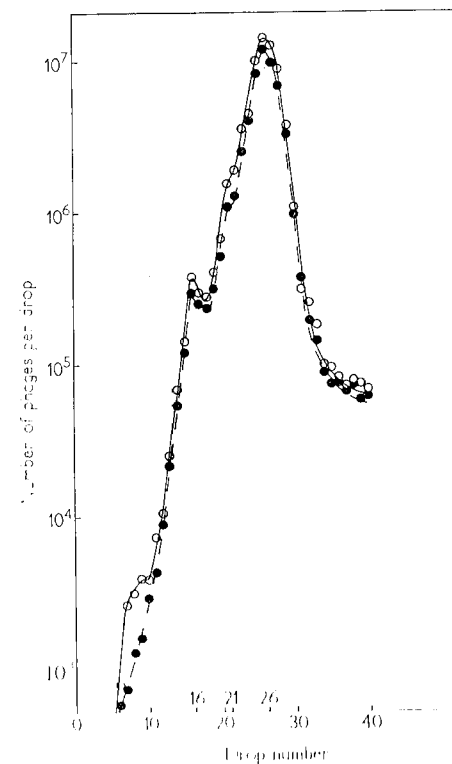


FIG. 4. Density distribution of the lysate obtained by one-step growth of deuterated  $\lambda cb_2$ -K (m.o.i. = 20) on non-lysogenic K12 (strain W3110) simultaneously infected with P1 (m.o.i. = 6.7). The lysate was centrifuged; 0.05 ml. of lysate, i.e.  $5 \times 10^7$   $\lambda$  particles; 78 drops collected. Phages conserved on K12 (○) and K12(P1) (●). Phages with conserved (drop 16), newly synthesized (drop 26) DNA are modified in equal proportions. A few parental phages (●) which had not adsorbed and were not removed by washing (Table 7) are found at drop 30.

The lysate, chloroformed after 60 minutes of incubation, was assayed on K12 and K12(P1) and an aliquot was analysed by density gradient centrifugation (Fig. 4). It may be seen that not only phages containing newly synthesized DNA (either entirely new or half new-half conserved) showed the modified host specificity character, but also phages with fully conserved, transferred DNA were modified in about equal proportion as the total lysate. Similar results were obtained in two other experiments using  $\lambda cb_2^+$ ·K at m.o.i. of 25 and 12.5 respectively. These findings allow the conclusion that replication is not necessary for modifying  $\lambda$ ·K into  $\lambda$ ·K(P1) after conversion of the productive host cell by superinfection with phage P1.

### Discussion

Many bacterial viruses show a pronounced specificity in respect to their host range. Several independent factors may restrict the number of hosts which can be infected successfully by a phage.

(1) The first condition for phage reproduction is its specific adsorption to the host. Tests for resistance to phage adsorption are easily performed and indicate the absence of appropriate phage receptors on the bacterial cell wall. It is possible to surmount this barrier either by isolation of bacterial mutants which can synthesize such phage receptors, or by isolation of phage mutants able to adsorb to the existing sites.

(2) After adsorption of a phage particle, its DNA must penetrate into the host (injection). Very little is known to date about aberrations which might occur at this step of infection, and whether such aberrations might be governed by bacterial or phage properties or both.

(3) As the phage DNA enters the bacterial host cell its "host specificity" is recognized by some at present unknown means. If the specificity is incompatible with the host, the DNA may be at least partially degraded. Such control over acceptance of DNA of foreign host specificity seems to be a widespread phenomenon and will be discussed in more detail in another article (Dussoix & Arber, 1962). This type of specificity is here shown to have its site on the DNA molecule, despite its non-mutational nature (Bertani & Weigle, 1953).

(4) Many processes are known or could be imagined which inhibit the replication of fully injected and non-degraded phage DNA, or which do not allow one of the other phage specific syntheses necessary for the production and liberation of new, infective phage particles. Such inhibitions may be governed either by bacterial or phage genes or by both, rendering the phage-bacterium complex incompetent. Examples are provided by the  $\lambda sus$  mutants (Campbell, 1959, 1961) and the T4 amber mutants (Epstein, personal communication) which grow on certain bacterial strains, but not on others. Many defective phages (Appleyard, 1954) are known, for which no competent host has been found and which can be reproduced only with the help of an active, superinfecting phage (Arber & Kellenberger, 1958).

Foreign DNA introduced into a new host is not necessarily degraded, and can in fact be fully accepted even though it differs in host specificity from phage DNA that is produced by the host cells in question. Full acceptance is found either (a) in a few cells of a bacterial population otherwise showing restriction, or (b) in the total population of certain bacterial hosts, as in the case described here in which *B. coli* C accepts phages A·K and A·B as well as the adapted A·C. Such extended host range systems, which accept phage DNA of a foreign host specificity, enable us to study the process by which the new specificity is imparted during the growth of progeny particles.

The fate of the host specificity of  $\lambda$ ·K(P1) was investigated in one cycle growth experiments on *E. coli* K12, which fully accepts  $\lambda$ ·K(P1) DNA. Roughly as many progeny phage particles were found to carry the parental host specificity as had been used for infection, and all other phages were  $\lambda$ ·K, carrying the host specificity of the new host K12. Host specificity of  $\lambda$ ·K(P1) DNA is thus transferred to the progeny but cannot be replicated. Material linkage of transferred host specificity with transferred parental DNA could be demonstrated in experiments using two different techniques of labeling: (1) incorporation of enough  $^{32}\text{P}$  to destroy the activity of the phage in the course of radioactive disintegration, (2) incorporation of deuterium to give the labeled phage a higher than normal density. These labeling experiments gave direct evidence that the transferred  $\lambda$ ·K(P1) host specificity does not separate from the parental DNA molecule with which it was originally associated even in the course of DNA replication during vegetative phage growth. Indeed, all progeny phages, which were able successfully to infect K12(P1), were, in the case of  $^{32}\text{P}$  labeling, still sensitive to disintegration of the  $^{32}\text{P}$  contained in the backbone of the transferred DNA strand (as seen, in the case of deuterium labeling, at least partially heavy due to the label retained). The DNA of phage  $\lambda$  is believed to be two-stranded. When such DNA infects a host cell at low multiplicity we assume that the two parental strands separate, and replication and synthesis of new DNA occurs. This hypothesis predicts the formation of hybrid DNA molecules, carrying one parental and one new strand. Indeed, the phage particles obtained (in low m.o.i. experiments) which show joint transfer of parental DNA material and host specificity are found to be such hybrids. In the case of  $^{32}\text{P}$  labeling, they are inactivated by decay of incorporated  $^{32}\text{P}$  at about half the rate of the parents; in the case of deuterium label, they show a density intermediate between that corresponding to conserved DNA transfer and that of non-deuterated phage particles.

It is interesting to note that another sort of information concerning the sensitivity of DNA to decay of incorporated  $^{32}\text{P}$  comes from the labeling experiments. This sensitivity seems directly proportional to the number of  $^{32}\text{P}$  atoms present in the DNA—whether they are distributed over both strands or contained in only one of the strands of a hybrid DNA molecule. The inactivation by  $^{32}\text{P}$  decay thus cannot be attributed to disintegrations occurring relatively close to each other, one on one strand and the other on the second strand, but must instead be a one hit event.

The occurrence of phages with hybrid DNA molecules, which behave in respect to their host specificity like the parental  $\lambda$ ·K(P1) phages, suggests that the specific characters given by the K12(P1) host are "dominant", i.e. phage DNA carrying the host specificity on one strand only is accepted and phage replication occurs. Genetic recombinants between the transferred  $\lambda$ ·K(P1) parent and a superinfecting  $\lambda$ ·K genome can be found for certain markers (e.g. the *mi* marker) without loss of the  $\lambda$ ·K(P1) host specificity. When recombination involves the loss of longer sections of the parental genome, however, the  $\lambda$ ·K(P1) host specificity is also lost.

After infection of K12 with a high multiplicity of  $\lambda$ ·K(P1) phages some of the parental DNA molecules are transferred into the progeny without active replication. The conserved DNA is, however, found in new protein coats. After superinfection with genetically marked  $\lambda$ ·K, such complexes formed by infection of K12 with a high multiplicity of  $\lambda$ ·K(P1) also liberate some recombinants showing almost fully conserved DNA. This confirms the similar findings of Meselson & Weigle (1961), who interpreted such recombinants as having been formed by a breakage and reunion

mechanism. These recombinants, as well as those which are about semi-conserved, lose the original host specificity if by recombination they lose more than a small part of the parental genome.

It would be interesting to know if one parental DNA molecule may give rise to two or only one hybrid progeny molecule carrying the parental host specificity. In the present investigation, no experiment was designed to answer this question. Its resolution may be quite difficult, since transfer of  $^{32}\text{P}$  label into the progeny DNA is known to be incomplete and so probably also is the transfer of parental host specificity. Part of the phage DNA, parental and replica, is indeed not yet assembled into finished phage particles at the moment of lysis, and is thus lost. Furthermore one does not know if parental  $\lambda\cdot\text{K}(\text{P1})$  DNA strands, conserved or semi-conserved, appearing in new infective phage particles, obligatorily contain their parental host specificity, or if they can lose it even without recombination with genomes showing the new host specificity. We could not find a definite answer to this question either, since many genetic recombinants in unmarked regions of the genome behave genotypically like the parental type and may have nevertheless lost their host specificity by recombination.

We have discussed the modification of  $\lambda\cdot\text{K}(\text{P1})$  into  $\lambda\cdot\text{K}$  during growth on K12. In the opposite modification, from  $\lambda\cdot\text{K}$  to  $\lambda\cdot\text{K}(\text{P1})$ , which is easily induced by superinfection of the K12- $\lambda\cdot\text{K}$  complex with phage P1, the appearance of phages with conserved DNA but nevertheless modified  $\lambda\cdot\text{K}(\text{P1})$  host specificity is clearly shown by the data summarized in Fig. 4, which show that the proportion of modified progeny phages stays constant for the density gradient fractions representing  $\lambda$  with conserved, semi-conserved and completely new DNA. The presence of phage P1 can thus induce the modification of  $\lambda$  DNA even when the DNA is not replicating and when it is transferred as a conserved parental molecule.

The possibility that such modified, conserved genomes could be in reality "almost conserved" breakage and reunion recombinants formed between the heavy parent and a vegetative copy having acquired the new host specificity is excluded (1) by the high frequency of conserved particles showing modified host specificity and (2) by the fact that a large part of the  $\lambda\cdot\text{K}(\text{P1})$  DNA is needed to confer the K(P1) host specificity to a  $\lambda$  genome.

The rapid onset of modification of  $\lambda\cdot\text{K}$  to  $\lambda\cdot\text{K}(\text{P1})$  after superinfection of the K12- $\lambda$  complex with phage P1 is not paralleled by a rapid establishment of the control mechanism over acceptance of  $\lambda\cdot\text{K}$  DNA. Indeed K12-P1 complexes may still be successfully infected with  $\lambda\cdot\text{K}$  for a relatively long period after P1 infection. This asynchronous appearance of the two effects induced by the presence of phage P1 may be interpreted in two ways: (1) control over modification of  $\lambda\cdot\text{K}$  into  $\lambda\cdot\text{K}(\text{P1})$  and control over acceptance of non-modified  $\lambda\cdot\text{K}$  DNA are established by at least partly independent steps; or (2) establishment of the "modification" mechanism occurs at a low level of some P1-induced substance, whereas a much higher level of this substance is needed to establish the "acceptance" mechanism.

Nothing is known about the chemical nature of host specificity, about the way it is produced by the host, nor about the way it is recognized upon infection of a new host. This specificity could be due to the presence or absence, induction or repression of particular substances. The host specificity imparted to  $\lambda$  DNA in the presence of phage P1, for example, could be a substance which is produced under the direction of P1 and gets attached to the  $\lambda$  DNA. Upon multiplication of such DNA in K12 cells an

P1-specific substance would be produced because of the absence of a P1 genome, and all new DNA replicas would thus lack the parental P1 host specificity.

Tentative models for the explanation of the phenomena in relation to host specificity should be able to account for the following points established by our experimental data. (1) Every host cell produces DNA of its own host specificity: e.g.  $\lambda$  DNA produced by *E. coli* K12 carries the host specificity characteristic for K12;  $\lambda$  DNA produced by *E. coli* K12(P1) carries the host specificity characteristic for K12 and that characteristic for the presence of phage P1;  $\lambda$  DNA produced by *E. coli* B(P1) carries the host specificity characteristic for B and for the presence of phage P1, and so on. Only further studies will reveal if all these different host specificities are related in their nature, and to what extent they are additive rather than substitutive. (2) Host specific characters are closely associated with DNA and can be transferred with it in growth involving active participation in replication (semi-conserved transfer). New replicas, however, do not carry the parental host specificity unless the new host has the same specificity. (3) Genetic recombinants formed between  $\lambda$  genomes of different host specificities may or may not preserve the parental host specificity, probably depending on both the length and the location of the gene region involved. (4) Conversion of the host cell in respect to its specificity during  $\lambda$  replication, as by superinfection of a K12- $\lambda$  complex with P1, can induce modification of both replicating and non-replicating  $\lambda$  DNA.

Host specificity may play a very important role in viral infections as suggested by the fact that host controlled modification is a widely spread phenomenon. It should be remembered here that our system is only a representative case and that for any other system some aspects of host specificity might be different. Luria & Human (1957) described, for example, a case where a modified phage seems to be no longer accepted by its own host. Further studies will have to be made in order to know if all the observed facts may be explained by any single model.

On the experimental level, host specificity gives us a useful biological label to follow the parental phage DNA molecule in phage infections.

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#### REFERENCES

- ARBER, W. (1950). In *Methods in Medical Research*, ed. by J. H. Comroe, vol. 2, p. 147. Chicago: The Year Book Publishers.
- ARBER, W. & K. (1954). *Genetics*, **39**, 440.
- ARBER, W. (1958). *Arch. Sci., Geneva*, **11**, 259.
- ARBER, W. (1960a). *Virology*, **11**, 250.
- ARBER, W. (1960b). *Virology*, **11**, 273.
- ARBER, W. & Dussoix, D. (1961). Abstracts of the Int. Biophys. Cong., Stockholm, p. 291.
- ARBER, W. & Kellenberger, G. (1958). *Virology*, **5**, 458.
- ARBER, W. & Lafaste-Dorolle, C. (1961). *Path. Microbiol.* **24**, 1012.
- BURTON, G. (1951). *J. Bact.* **62**, 293.
- BURTON, G. & Weigle, J. J. (1953). *J. Bact.* **65**, 113.
- CAMPBELL, A. (1959). *Virology*, **9**, 293.
- CAMPBELL, A. (1961). *Virology*, **11**, 77.
- CAMPBELL, J. R. (1961). *Virology*, **11**, 10.

- Cohen, D. (1959). *Virology*, **7**, 112.  
 Dussoix, D. & Arber, W. (1962). *J. Mol. Biol.* **5**, 37.  
 Hershey, A. D., Kamen, M. D., Kennedy, J. W. & Gest, H. (1951). *J. Gen. Physiol.* **34**, 305.  
 Jacob, F. & Wollman, E. L. (1954). *Ann. Inst. Pasteur*, **87**, 653.  
 Kaiser, A. D. (1955). *Virology*, **1**, 424.  
 Kaiser, A. D. (1957). *Virology*, **3**, 42.  
 Kellenberger, E. & Arber, W. (1957). *Virology*, **3**, 245.  
 Kellenberger, G., Zichichi, M. L. & Weigle, J. (1960). *Nature*, **187**, 161.  
 Kellenberger, G., Zichichi, M. L. & Weigle, J. J. (1961). *Proc. Nat. Acad. Sci., Wash.* **47**, 869.  
 Lederberg, S. (1957). *Virology*, **3**, 496.  
 Luria, S. E. (1953). *Cold Spr. Harb. Symp. Quant. Biol.* **18**, 237.  
 Luria, S. E. & Human, M. L. (1952). *J. Bact.* **64**, 557.  
 Meselson, M., Stahl, F. W. & Vinograd, J. (1957). *Proc. Nat. Acad. Sci., Wash.* **43**, 581.  
 Meselson, M. & Weigle, J. J. (1961). *Proc. Nat. Acad. Sci., Wash.* **47**, 857.  
 Séchaud, J. (1960). *Arch. Sci., Geneva*, **13**, 427.  
 Stent, G. S. & Fuerst, C. R. (1955). *J. Gen. Physiol.* **38**, 441.  
 Watson, J. D. & Crick, F. H. C. (1953). *Nature*, **171**, 964.  
 Weigle, J. J. & Bertani, G. (1953). *Ann. Inst. Pasteur*, **84**, 175.  
 Weigle, J., Meselson, M. & Paigen, K. (1959). *J. Mol. Biol.* **1**, 379.  
 Zinder, N. D. (1960). *Science*, **131**, 813.

## Host Specificity of DNA Produced by *Escherichia Coli*

### II. Control over acceptance of DNA from infecting phage $\lambda$

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DNA of  $\lambda$ -K ( $\lambda$  phage grown on *E. coli* strain K12) is shown to be degraded upon infection of the new host strains *E. coli* K12(P1) or *E. coli* B. This breakdown begins shortly after phage attachment and successful DNA injection.  $^{32}$ P label from the  $\lambda$ -K DNA submitted to this degradation appears partly in acid-soluble components (organic and inorganic) and partly in acid-insoluble compounds. The host cell survives such an infection and permits diffusion of a fraction of the degradation products into the medium, while probably retaining another fraction.

Genetic markers from  $\lambda$ -K are rescued in K12(P1) host cells infected with both restricted  $\lambda$ -K and unrestricted  $\lambda$ -K(P1). Since DNA breakdown competes in time with the rescue, the probability of marker rescue is high if the unrestricted phage infects first and low if the restricted phage infects first. Only closely linked markers have a good chance to be rescued together.

The host specificity imparted to phage DNA by the bacterial strain on which it was produced is thought to be responsible for its recognition as incompatible with a new host strain. Bacterial mutants are described which, despite the presence of prophage P1, accept infecting  $\lambda$ -K at relatively high rates.

### Introduction

"Host specificity" of phage DNA has been shown (1) to be determined by the host in which the phage growth occurred and (2) to be closely associated with the DNA molecule of the phage genome (Arber & Dussoix, 1962). If phage  $\lambda$ -K, grown on the bacterial host *Escherichia coli* K12, is used to infect a new host strain, two alternative results are possible according to the nature of the strain used.

(1) The infecting phage DNA is accepted by the host, is allowed to multiply vegetatively, and mature phages are produced. In the case of host *E. coli* C and phage  $\lambda$ -K, most of the progeny phages differ from their parents in that they are no longer able to grow on K12 (Bertani & Weigle, 1953). They have now the host specificity typical of *E. coli* C, which therefore is said to have produced a "host-related modification" of  $\lambda$ . Only the phages containing transferred parental DNA material also preserve the parental host specificity and can thus grow on K12 (Arber & Dussoix, 1962).

(2) Phage  $\lambda$ -K adsorbed to certain new hosts, e.g. the lysogenic *E. coli* K12(P1), grows only exceptionally (efficiency of plating in our example  $\approx 10^{-6}$ ). No explanation for this failure of reproduction was found until recently although Leshchere (1957) presented evidence that  $^{32}$ P of the DNA from restricted phage T1 attached to *E. coli* B(P1) enters the host cell and later diffuses out into the medium. We have found the same behavior for DNA of  $\lambda$ -K infecting K12(P1) cells and shown that this excretion is a consequence of the breakdown of phage DNA (Arber & Dussoix,