SEGREGATION OF LAMBDA LYSOGENICITY DURING BACTERIAL RECOMBINATION IN ESCHERICHIA COLI K12

R. K. APPLEYARD[1]

Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, California

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A CLOSE connection is known to exist between lysogenicity for the temperate bacteriophage lambda and the genetic apparatus of *Escherichia coli* K12. LEDERBERG and LEDERBERG (1953) have shown that lambda lysogenicity is closely linked to the galactose marker gal_4 in recombination from lysogenic × non-lysogenic crosses and in segregation from suitable heterozygous strains. WOLLMAN (1953) has demonstrated a close linkage between lambda lysogenicity and a galactose marker independently isolated by MONOD.

WOLLMAN has pointed out the possibility that lambda lysogenicity and the galactose marker differ from other genetic markers in that they are never transferred from the genetic donor to the genetic acceptor and thus do not play any part in genetic recombination. Our current picture of recombination in *E. coli* K12 is that it is asymmetric (HAYES 1952), the asymmetry being under the control of an infective agent denoted by F (LEDERBERG, CAVALLI and LEDERBERG 1952). In the simplest type of cross most of the genotype of any given recombinant comes from the F^- parent (LEDERBERG, CAVALLI and LEDERBERG *loc. cit.*). The genic contribution of the F^+ parent (gene donor) is small: individual characters which are not linked to a nutritional deficiency in the F^- parent appear from the F^+ parent in only a small fraction of the prototrophic recombinants. This appears to be the situation for both gal_4 and lysogenicity. However, when a strain A, gal^-F^+, is mixed with a strain B, gal^+F^-, under suitable conditions " transduction " of the F occurs and F^+ B cells rapidly begin to appear in the culture. Moreover, under suitable physiological conditions certain F^+ strains are known to behave phenotypically like F^- strains. It is therefore possible that on a mixed plate during a cross of A (gal^-F^+) × B (gal^+F^-) both A (gal^-F^-) and B (gal^+F^+) cells appear. Recombinations between these classes of cells could give rise to gal^- recombinants in which the gal^- gene was never transferred from an F^+ to an F^- cell in the normal recombination process. But in contrast to the direct recombinants which are genotypically predominantly like strain B, these gal^- recombinants arising by such an F inversion process should have genotypes which closely resemble that of strain A in respect of unselected characters. The possibility raised by WOLLMAN is therefore susceptible of experimental control in $F^+ \times F^-$ crosses if another unselected marker not linked to lysogenicity and gal_4 is available.

[1] Present address: Atomic Energy of Canada Limited, Chalk River, Ontario, Canada.

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The experiments of LEDERBERG and LEDERBERG and of WOLLMAN restrict the possible ways in which we may suppose lambda lysogenicity to be organized in *E. coli* K12. However, we cannot be sure on the basis of lysogenic × non-lysogenic crosses alone whether lysogenicity consists of a specific allelic state of a bacterial gene, or of an additional genetic component attached to the bacterial genetic apparatus, or of many cytoplasmic particles under the partial or overriding control of a bacterial gene.

It is now possible to make artificial lysogenics for lambda mutants distinguished by their plaque morphology, as well as double lysogenics which release two different kinds of phage. If lysogenic × lysogenic crosses are made between strains which carry two such mutants, we can see whether each phage remains coupled to the galactose allele with which it is associated in the parent. If doubly lysogenic strains are crossed with non-lysogenics, we can see whether double lysogenicity is similarly associated with galactose, and whether it segregates in the same way as single lysogenicity. It will be shown below that the results of these experiments suggest that lambda lysogenicity in *E. coli* K12 consists of a single genetic component, or prophage, attached to the bacterial genetic apparatus.

MATERIALS AND METHODS

Bacteria. All bacteria used in the genetic experiments have been derived from a sensitive F^- strain C602 and a sensitive F^+ strain C112, which carry the genetic markers indicated (for bacterial genetic notation see LEDERBERG 1947):

C602: F^-; $(LTB_1)^-$, S^r, lac^-, $V_6{}^r$, $V_{1,5}{}^r$
C112: F^+; C^-H^-, gal^-, $V_{1,5}{}^r$

C602 is a streptomycin resistant T6 resistant derivative of a lambda sensitive strain C600, which has been described elsewhere (APPLEYARD 1954). C112 carries the galactose marker of MONOD. It was very kindly supplied by E. WOLLMAN and is identical with his strain 112-12. Each singly lysogenic derivative of these strains was made by incubation at 37°C with a high concentration of the appropriate lambda mutant on nutrient plates. Doubly lysogenic derivatives were made by 24-hour exposure to mixed phage in high concentration (2×10^9/ml of each mutant) in nutrient broth at 37°C.

Before using any of these strains in genetic experiments, derivatives resistant to infection with lambda (/λ or $V_\lambda{}^r$) were selected. (These strains phenotypically resemble the $Lp_2{}^r$ strains of LEDERBERG and LEDERBERG (1953). The responsible gene in our strains is called V_λ, since it may not be identical with the LEDERBERGS' locus Lp_2.) This was done by incubation on nutrient plates with a high concentration of the strong virulent mutant lambda-v2. The use of lambda resistant strains in crosses of the type here intended has the advantage of preventing distortion of the results through infection after recombination.

Table 1 lists the bacterial strains used in the genetic experiments. ( ) denotes lysogenicity for the phages inside the brackets.

TABLE 1

*Bacterial strains used in genetic recombination.**

Source Strains (Sensitive to lambda)	
C602: F^-; $(LTB_1)^-S^r$, *lac*$^-$ V_6^r, $V_{1,5}{}^r$	C112: F^+; C^-, H^-, *gal*$^-$, $V_{1,5}{}^r$
Derivatives	
C602/λ	C112/λ
C602 (λs)/λ	C112 (λs)/λ
C602 (λcl)/λ	C112 (λcl)/λ
C602 (λs, λcl)/λ	C112 (λs, λcl)/λ

*Genetic notation as in LEDERBERG, 1947.

Phages. Wild-type lambda is a typical small turbid plaque-forming temperate phage found in lysates of UV-induced wild-type *E. coli* K12. A pure line was obtained by three successive isolations of small plaques. The artificial lysogenics for lambda-s listed in table 1 were all made by infection with samples from a single high-titre stock, prepared by UV induction of a prototrophic artificially lysogenic strain.

Lambda-cl appeared as a true-breeding small clear plaque-forming mutant of lambda during experiments on the UV induction of a prototrophic defective lysogenic (APPLEYARD 1954). The artificial lysogenics for lambda-cl listed in table 1 also were made by infection with samples from a single high-titre stock very kindly supplied by M. LIEB.

Media and techniques. Stocks were grown, manipulated, and scored in nutrient broth or on nutrient plates as described elsewhere (APPLEYARD 1954). Scoring for lysogenicity was carried out by streaking or by the LEDERBERG replica technique on plates seeded with *E. coli,* strain C (BERTANI and WEIGLE 1953).

Additional media were used in bacterial crosses: liquid minimal medium: M9 + 0.5% glucose + 5γ/ml thiamine hydrochloride; minimal indicator medium: LEDERBERG's EMS medium (LEDERBERG 1949) supplemented with 5 γ/ml thiamine hydrochloride and containing 0.5% of the sugar under test; complete indicator medium: LEDERBERG's EMB medium (LEDERBERG 1949) containing 0.5% of the sugar under test; Difco " Endo " medium containing lactose. Nutritional deficiencies were scored by streaking on EMS glucose with appropriate growth supplements.

Bacterial crosses. All bacterial crosses were made on glucose minimal indicator medium and all prototrophic recombinants were first scored for galactose fermentation and lysogenicity. Some or all prototrophic recombinants in each class of interest were then purified by streaking for single colonies, rescored for prototrophy, galactose fermentation and lysogenicity, and scored for lactose fermentation, resistance to phage T6 and resistance to streptomycin.

Bacterial strains to be crossed were grown at 37°C with aeration in yeast extract medium for 24 hours. They were then diluted 1 : 200 into fresh medium and reincubated under the same conditions for 2¾ hours. The cultures then contained about 8.10^8 cells/ml and were at the end of the logarith-

mic phase of growth. This condition has been found (VOGT unpublished) to be optimal for recombination. The bacteria were centrifuged, suspended in buffer, recentrifuged and resuspended in buffer at twice the original concentrations. Equal quantities of the two suspensions were mixed and 0.2–0.3 ml aliquots poured with 3–3.5 ml EMS glucose agar to form a top layer on an EMS glucose plate. Control platings were made of equal aliquots of the separate bacterial suspensions and the separate suspensions were assayed by diluting and spreading on the surfaces of nutrient plates.

Using the velveteen replica technique of LEDERBERG and LEDERBERG (1952), colonies appearing on the surface of the EMS glucose plates were transferred to EMS galactose plates, and after 24 hours' incubation were scored for galactose fermentation. The EMS galactose plates were further replica tested against plates seeded with *E. coli* strain C and scored for lysogenicity. All or a suitable proportion (20 or more if possible) of the recombinants in each class of interest were then picked with a needle, transferred to 3 ml broth and at once streaked thinly with a loop on EMB galactose medium. In this way the selected recombinants are re-scored for galactose, and isolated colonies can be picked, eliminating possible mixtures. The single colonies picked were transferred to tubes containing liquid minimal medium. After 24 hours' incubation at 37°C each was restreaked as follows:

(1) Against T6 on Endo medium (score V_6 and *Lac*)
(2) On EMS galactose (check prototrophy and galactose fermentation)
(3) Against streptomycin on nutrient agar (score streptomycin resistance)
(4) On "C" plates with 15 secs. UV induction (score lysogenicity).

When necessary, as in all cases of suspected double lysogenics, a final check on lysogenicity was made by picking phage from the centre of the streak described in (4) above and replating on strain C to observe the individual phage plaques.

Resistance to lambda was scored by cross-streaking on nutrient plates with the strong virulent mutant lambda-v2.

Doubtful characters were rechecked throughout by a second streak test made from the minimal growth tube.

EXPERIMENTAL RESULTS

Bacterial markers. In the absence of lysogenicity *gal, S, lac,* V_6, and V_λ segregated as shown in table 2. No linkage was observed between any pair of characters except *lac* and V_6 (table 3). In the presence of lysogenicity no linkage has been observed between *S, lac* or V_6, on the one hand, and *gal* or lysogenicity on the other, in any of the crosses indicated in tables 2–7.

Abnormal behavior of F^- lambda resistant non-lysogenic strain C602/λ. Streptomycin resistance segregates abnormally in the cross C602/λ × C112 (table 2), 60–80% of recombinants being S^s like the F^+ parent and 96% being $V_\lambda{}^s$ like the F^+ parent. In C602 × C112 the normal behavior is observed, only 10% of the recombinants being S^s. The same abnormal segregation of *S* has been observed whenever C602/λ has been used as the F^- parent

TABLE 2

Segregation in absence of lysogenicity.

F^-; $(LTB_1)^-S^r$ $lac^-V_6{}^r$ × F^+; $(CH)^-gal^-$

Cross	% prototrophs resembling F^+ parent for					% gal^- prototrophs resembling F^+ parent for S^s
	lac^+	$V_6{}^s$	S^s	$V_\lambda{}^s$	gal^-	
C602/λ × C112 at 37°	40 ± 7	42 ± 7	64 ±7	94 ±4	2.1 ±0.5	65 ±9
at 25°	42 ± 7	44 ± 7	82 ±6	98 ±2	14.3 ±1.6	64 ±6
C602 × C112 at 25°	45 ±11	35 ±10	10 ±7	....	3.1 ±0.7	10 ±7

in a cross, but not otherwise. A lethal mutation at a locus close to V_λ would cause the abnormality observed. C602/λ was therefore grown for several hours in minimal medium (M9 + glucose) so as to use up all growth factors and aliquots containing about 100 cells were spread on the surface of minimal plates (M9 + glucose, EMS − galactose) supplemented with leucine, threonine and thiamin. No colonies were formed, whereas the other derivatives of C602 formed colonies under these conditions. It was concluded that C602/λ carried a mutation for another growth factor in addition to leucine, threonine and thiamin. The abnormal segregation of S and V_λ is attributed to this mutation.

Non-allelic $V_\lambda{}^r$ mutations. Whenever lambda resistant lysogenic F^+ strains are crossed with lambda resistant non-lysogenic F^- strains, the possibility exists that the mutations to lambda resistance of the two strains are at different loci. As a result lambda-sensitive prototrophic recombinants would be found. A possible error could then arise through transfer of lysogenicity from F^+ parent to lambda sensitive recombinants by external infection after recombination. To eliminate any possible error, in crosses of this kind samples of all significant classes of recombinants were scored for lambda resistance. No lambda-sensitive recombinants were found in any cross except those involving the F+ double lysogenic C112 (λs, λcl)/λ. When this strain was crossed with the standard F^- non-lysogenic C602/λ, 50% of the recombinants were lambda sensitive. The same result was found with an independently occurring λ-resistant derivative of C112 (λs, λcl). It is concluded that the most common mutation to lambda resistance in C112 (λs, λcl) strains occurred in a gene not allelic with the gene which mutated to $V_\lambda{}^r$ in our other

TABLE 3

Linkage of V_6 with lac in absence of lysogenicity.

F^-; $(LTB_1)^-S^r$ $lac^-V_6{}^r$ × F^+; $(CH)^-$ gal^-

Cross	% prototrophs			
	$lac^+V_6{}^s$	$lac^+V_6{}^r$	$lac^-V_6{}^s$	$lac^-V_6{}^r$
C602/λ × C112 at 37°	34 ± 7	6 ± 3	8 ± 4	52 ± 7
at 25°	40 ± 7	2 ± 2	4 ± 3	54 ± 7
C602 × C112 at 25°	40 ±11	5 ± 5	0(0/20)	55 ±11

TABLE 4

Comparison of lambda-cI and lambda-s in lysogenic × non-lysogenic crosses at 25°C.

Cross	F^- parent $(LTB_1)^- S^r lac^- V_6^r$	F^+ parent $(CH)^-$	% prototrophs resembling F^+ parent for				% prototrophs like F^+ parent for S^s	
			—	*lys*	*gal*	*lys + gal*	In class like F^- parent for *gal*$^+$	In class like F^+ parent for *gal*$^-$
C602/λ × C112 (λs)/λ	*gal*$^+$ sens	*gal*$^-$ (λs)	99.1 ± 0.4	0(0/2000)	0.1 (1)	0.6 ± 0.2	55 ± 11	62.5 ± 17
C602/λ × C112 (λcI)/λ	*gal*$^+$ sens	*gal*$^-$ (λcI)	98.1 ± 0.3	0.1 (1)	0.2 (1)	1.0 ± 0.3	73 ± 10	57 ± 13
C602 (λs)/λ × C112/λ	*gal*$^+$ (λs)	*gal*$^-$ sens	94.2 ± 0.5	0.4 (2)	0.2 (1)	5.2 ± 0.5	5 ± 5	3 ± 3
C602 (λcI)/λ × C112/λ	*gal*$^+$ (λcI)	*gal*$^-$ sens	93.4 ± 0.5	0.8 ± 0.3	0(0/2000)	5.8 ± 0.5	5 ± 5	3 ± 3

TABLE 5

Effect of temperature on transfer of lysogenicity and of gal from F^+ parent to prototrophs.

Cross	F^- parent $(LTB_1)^- S^r$ $lac^- V_6^r$	F^+ parent $(CH)^-$	% prototrophs like F^+ parent for lys		% prototrophs like F^+ parent for gal	
			25°C	37°C	25°C	37°C
C602/λ × C112 (λs)/λ	*gal*$^+$ sens	*gal*$^-$ (λs)	0.6 ± 0.2	0.12 ± 0.07	0.7 ± 0.2	0.16 ± 0.07
C602/λ × C112 (λcI)/λ	*gal*$^+$ sens	*gal*$^-$ (λcI)	1.1 ± 0.3	0.07 (1)	1.2 ± 0.3	0.07 (1)
C602 (λs)/λ × C112/λ	*gal*$^+$ (λs)	*gal*$^-$ sens	5.6 ± 0.5	1.8 ± 0.6	5.4 ± 0.5	1.5 ± 0.5
C602 (λcI)/λ × C112/λ	*gal*$^+$ (λcI)	*gal*$^-$ sens	6.6 ± 0.6	3.7 ± 0.4	5.8 ± 0.5	3.5 ± 0.4

strains. This fact will be further discussed in connection with the crosses of F^+ double lysogenic $\times$ F^- non-lysogenic.

Comparison of lambda phages. In lysogenic $\times$ sensitive crosses lambda-s and lambda-cl behave alike. Lysogenicity is closely linked to galactose: both are rarely transferred from the F^+ parent to the prototrophic recombinants (table 4). This transfer from the F^+ parent is to a considerable degree suppressed when the crosses are carried out at 37°C instead of at 25°C (table 5).

Crosses of lysogenic $\times$ lysogenic (table 6). When F^+ and F^- lysogenics, distinguished by carrying lambda-s and lambda-cl, are crossed, no double lysogenics or sensitives are found. Each mutant appears in the recombinants strongly but not absolutely linked to the galactose marker with which it entered the cross. Each is transferred about as frequently from the F^+ parent to the recombinants as is lysogenicity in F^+ lysogenic $\times$ F^- sensitive crosses.

The last two columns of table 6 show that 90–95% of recombinants are S^r like the F^- parent whether they are lysogenic like the F^+ parent or the F^-. It is therefore unlikely that lysogenicity is transferred in these crosses by F inversion, in accordance with the possibility suggested by WOLLMAN. Such an F inversion process would result in 90% S^s among those recombinants lysogenic like the F^+ parent.

Crosses of double lysogenics. When a double lysogenic is crossed with a non-lysogenic strain, the number of classes to be scored is increased by the possible appearance of single lysogenicity. Table 7 shows the behavior of double lysogenicity in such crosses.

If double lysogenicity is carried by the F^- parent, linkage with galactose is preserved. The small fraction of singly lysogenic recombinants seen is not significant, in view of the tendency of double lysogenics to throw off singly lysogenic clones during growth (APPLEYARD 1954). It has already been shown (table 4) that when F^- single lysogenics recombine with F^+ sensitive cells over 90% of the recombinants resemble the F^- parent for gal and lysogenicity. In the crosses of F^-gal^+ double lysogenic with F^+gal^- non-lysogenic, gal^- non-lysogenic recombinants are about as frequent as singly lysogenic recombinants. Most of the gal^- non-lysogenic recombinants can therefore not arise from F^- single lysogenic $\times$ F^+ non-lysogenic recombinations, but must result from recombinations between F^- double lysogenic and F^+ non-lysogenic cells.

If double lysogenicity is in the F^+ parent, the genetic transfer of lysogenicity to the prototrophic recombinants is almost completely suppressed. The transfer of the galactose marker from the F^+ parent is also much reduced. A few singly lysogenic gal^- prototrophs have been observed. These are attributed to recombinations involving single lysogenics which have arisen during the growth of the doubly lysogenic F^+ culture. Nearly all the singly lysogenic recombinants observed showed " unstable " lysogenicity, repeatedly throwing off non-lysogenic clones during growth. Pure (stable) lysogenic strains were derived from 11 of these by 4–5 single colony isolations using LEDERBERG's indirect selection technique. All were gal^+ and sensitive to lambda-v2 ($V_\lambda{}^s$). Such " unstable " lysogenics probably arise by external

TABLE 6

Segregation of lysogenicity and gal in lysogenic × lysogenic crosses at 25°C.

Cross	F^- parent $(LTB_1)^-S^r$ $lac^-V_6{}^r$	F^+ parent $(CH)^-$	% prototrophs resembling F^+ parent for				% prototrophs resembling F^+ parent for S^s within class	
			——	*gal*	*lys*	*gal* + *lys*	like F^- for *gal* and *lys*	like F^+ for *gal* and *lys*
C602 (λcl)/λ × C112 (λs)/λ	gal$^+$ (λcl)	gal$^-$ (λs)	97.7 ± 0.3	0.2 ± 0.1	Small, not scored	2.1 ± 0.3	10 ± 7	5 ± 4
C602 (λs)/λ × C112 (λcl)/λ	gal$^+$ (λs)	gal$^-$ (λcl)	96.8 ± 0.3	0.7 ± 0.25	Small, not scored	2.5 ± 0.3	5 ± 5	5 ± 4

TABLE 7

Segregation of double lysogenicity in crosses with sensitivity at 25°C.

Cross	F^- parent $(LTB_1)^-S^r$ $lac^-V_6{}^r$	F^+ parent $(CH)^-$	% prototrophs			
			gal$^+$ (λs,λcl)	*gal*$^-$ (λs,λcl)	*gal*$^+$ sens	*gal*$^-$ sens
C602 (λs,λcl)/λ × C112/λ	gal$^+$ (λs,λcl)	gal$^-$ sens	94.9	0.1 (1)	0	3.6 ± 0.6
C602/λ × C112 (λs,λcl)/λ	gal$^+$ sens	gal$^-$ (λs,λcl)	0	0.03 (1)	98.6	0.14 ± 0.07

Cross	*gal*$^+$ (λs)	*gal*$^-$ (λs)	*gal*$^+$ (λcl)	*gal*$^-$ (λcl)	% like F^+ parent for S^s in class	
					like F^- parent for *gal*$^+$	like F^+ parent for *gal*$^-$
C602 (λs,λcl)/λ × C112/λ	Not scored	Not scored	1.5	0	5 ± 5	0(0/39)
C602/λ × C112 (λs,λcl)/λ	0.3 (u.s.)	0	0.8 (u.s.)	0.1 (1)	70 ± 10	100 (5/5)

u.s. = unstable.

infection. They have only been observed in the crosses of F^+ double lysogenic $\times$ F^- non-lysogenic in which 50% of the prototrophic recombinants are lambda-sensitive and therefore liable to become lysogenic by external infection.

The total number of prototrophic recombinants observed in all crosses using the same F^- parent (C602/λ) was approximately the same, whether double, single or no lysogenicity was present in the F^+ parent. Throughout all other crosses discussed the number of prototrophic recombinants observed was also constant, but about ten times as great as in crosses involving C602/λ. This observation is in agreement with the hypothesis that C602/λ carries a lethal mutation not present in our other F^- strains. Within experimental error the segregation ratios of *S*, *lac* and V_6 remain constant (except as noted earlier for *S*) throughout all the crosses of this series.

DISCUSSION

Both LEDERBERG and LEDERBERG (1953) and WOLLMAN (1953) have already shown that lambda lysogenicity is allelic to non-lysogenicity and is closely linked to galactose in lysogenic $\times$ non-lysogenic crosses. They have concluded that there is a genic component of lysogenicity. The present experiments extend these studies. They show that in recombination between strains which carry distinguishable lambda mutants neither non-lysogenics, nor double lysogenics able to release both kinds of phage, are formed. The phage character which differentiates the two mutants turns out to be closely linked to galactose. Moreover when a doubly lysogenic gal^+F^- strain is crossed with a non-lysogenic gal^-F^+ strain, non-lysogenicity and gal^- are

TABLE 8

Transfer of gal⁻ from lysogenic and non-lysogenic F⁺ parents to prototrophic recombinants in crosses at 25°C with the same non-lysogenic F⁻ parent (C602/λ).

Lysogenicity of F^+ parent	% gal^- recombinants
None	14.3 ± 1.6
Single	0.95 ± 0.2
Double	0.14 ± 0.07

closely associated in the small percentage of recombinants in which either appears. Double lysogenicity is therefore linked to galactose just as is single lysogenicity.

The experiments also indicate (e.g., table 8) that when otherwise genetically similar F^+ strains are crossed with F^- strains transfer of lysogenicity status and galactose character from F^+ parent to recombinants is greatly reduced if single rather than non-lysogenicity is to be transferred, and still further reduced in the case of double lysogenicity. In the case of single lysogenicity this transfer is much reduced if the temperature is increased, suggesting a parallel with the work of LIEB (1953) on establishment of lysogenicity after infection.

No evidence has been found that lambda mutant characters are carried by

any hypothetical cytoplasmic particles under genic control. Further discussion will therefore be limited to the genic component of lysogenicity.

The recombination data demonstrate that this is a complex entity, for not only does it control ability or inability to release phage under suitable circumstances, but also embodies determinants of individual hereditary characteristics of the phage concerned ("prophage genes," APPLEYARD 1954). It is therefore far from a simple bacterial gene, but so far as the recombination data are concerned could be equally a complex of bacterial genes, one controlling each phage character, or an additional cellular component, the prophage, attached to the bacterial genetic apparatus at a site which could correspond to a simple bacterial gene.

It is pertinent here to suggest some other reasons for preferring the second kind of hypothesis (cf. also LWOFF 1953):

(1) It provides a more satisfactory basis for the explanation of prophage recombination (APPLEYARD 1954). The attached prophages in a double lysogenic can lie side by side. They have sufficient free ends to make interpretation even of the existing very limited data much easier.

(2) It is general experience (cf. also APPLEYARD 1954) that when a cell is cured of its lysogenicity, for example by prolonged exposure to ultraviolet light, it usually reverts to full sensitivity: it can be reinfected and lysogenized quite normally. On the basis of the gene complex hypothesis we would conclude that simultaneous reverse mutations of all the genes involved was more frequent than reverse mutation of only some of them. By contrast we would expect an additional component to become detached and lost as a single unit.

On this basis our experiments show that the genic component of lambda lysogenicity is a complex entity embodying individual prophage genes. We suggest that this entity is an additional cellular component, the prophage, attached to the bacterial genetic apparatus at or near the galactose loci of LEDERBERG and LEDERBERG ("gal_4") and WOLLMAN.

SUMMARY

Bacterial crosses between lysogenic derivatives of *E. coli* K12 carrying different lambda mutants, and between doubly lysogenic and non-lysogenic derivatives are described. The results are consistent with the view that lysogenicity consists of an additional cellular component or prophage which is attached to the bacterial genetic apparatus at a specific locus.

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