SEGREGATION OF NEW LYSOGENIC TYPES DURING GROWTH OF A DOUBLY LYSOGENIC STRAIN DERIVED FROM ESCHERICHIA COLI K12

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THE experiments to be described below are most simply interpretable in terms of a new type of genetic recombination between temperate bacteriophages. This takes place between two related prophages during normal growth of a bacterial strain the cells of which carry them both. For the purpose of such a discussion it has been found necessary to refine and extend some terms in common use (JACOB, LWOFF, SIMINOVITCH and WOLLMAN 1953) and to add others.

TERMINOLOGY

When a bacterium transmits to its descendants the ability to produce bacteriophage under suitable physiological conditions, the cell is *lysogenic.* If all the descendants of the cell possess the same property, the lysogenicity is *stable:* if a significant proportion of them do not, it is *unstable.* Stable lysogenicity frequently becomes established in one or more descendants of an unstable cell. We think of this ability to release bacteriophage as determined by some specific component of the cell, called a *prophage.* Because a sensitive cell upon infection by a temperate phage may become lysogenic and later liberate identical phage, the prophage may be thought of as a stage in the life-cycle of the phage. If each cell of a lysogenic clone is potentially able to liberate a number of types of phage, we have a *poly-lysogenic* in which each cell carries several prophages. If only one prophage is carried and only one type of phage is liberated, the clone is *mono-lysogenic.*

Many hereditary character differences observed in the mature phage are transmitted as inherited differences during the growth of the corresponding clones of lysogenic bacteria. The lysogenic bacteria therefore contain hereditary determinants of these differences. In the absence of evidence to the contrary it is reasonable to suppose that these determinants are specific components, parts of the prophage. Such components will be called *prophage genes.* Where alternative forms of a phage phenotype are determined by alternative states of the corresponding prophage gene, these alternative states will here be called *prophage alleles,* by analogy with other systems.

Lysogenic clones will be described in which the prophage is present but there is a defect which almost entirely prevents production of infective phage. The defect has the following properties (*vide infra*): (1) It is reproduced

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from generation to generation in all cells of the clone. (2) It is associated with the prophage rather than with the bacterial residuum of the lysogenic complex. (3) It is not a general malformation of the whole prophage structure but is to some extent localized. Genes carried by a defective prophage in a double lysogenic can recombine with other genes during vegetative phage development and can segregate from the defect during bacterial growth. Moreover the defect by no means impairs the whole physiological activity of the prophage.

These properties will be taken as sufficient to establish the defect as due to a gene of the prophage although the property, in this instance, is not transmissible through the infective particle stage of the temperate bacteriophage life-cycle. In default of more detailed evidence the defect will here be ascribed to a particular allele of a prophage gene. However, the cause could equally be a small or large loss of material or a modification in part of the prophage structure or of its relation to the bacterial residuum. Any of these would be consistent with the data and interpretations to be presented below.

In some poly-lysogenic clones each cell is potentially able to liberate more than one unrelated type of phage. Other clones are found in which each cell is able to liberate more than one of several closely related types of phage which differ among themselves only in a limited number of genes. These two kinds of poly-lysogenic clone will be distinguished respectively as *allolysogenic* and *homolysogenic*.

The question arises, are the prophage genes in a homolysogenic cell organized into sets, each set associated with the liberation of one of the related types of phage? If this is the case, we may properly think of each set as the gene complement of a separate prophage, whether or not there also exist cellular components which determine the over-all capacity of the cell to liberate bacteriophage. Such a usage adheres to the idea of a prophage as a continuing form of one phage. All present evidence suggests that prophage genes in a homolysogenic are, in fact, organized into separate prophages. This description of lysogenicity will therefore be taken as a basis for discussion.

The possibility exists of the interchange of prophage genes between two related prophages carried in one cell. The new combinations of prophage genes so formed would represent new types of lysogenicity. This phenomenon will be called *prophage recombination* by analogy with other systems.

EXPERIMENTAL MATERIAL

Associated with the naturally occurring strain *Escherichia coli* K12 is a temperate bacteriophage lambda, to which some natural and artificially derived strains of *Escherichia coli* are sensitive (LEDERBERG and LEDERBERG 1953). Other strains, resistant to lambda but sensitive to the strong virulent mutant lambda-v2, have been designated immune-1 by LEDERBERG and LEDERBERG (1953). Two genetically different strains carrying the immune-1 property, which turned up in our experiments, have proved on further investigation to be lysogenic, although a defect in the prophage caused the phage yield to be

very small. Such strains will be called *defective lysogenics*. When one of these strains was superinfected with the weak virulent mutant lambda-v1, a homolysogenic clone arose. During growth this homolysogenic strain threw off several kinds of segregant clone which were mono- or poly-lysogenic and possessed combinations of prophage genes different from those in either the original homolysogenic or its parent defective lysogenic strain. These new combinations are interpreted as having arisen through prophage recombination.

MATERIALS AND METHODS

Bacteria. Bacteria used are listed in table 1, with the genotype and source or manner of derivation of each. The source strain Y-70 was kindly supplied by E. WITKIN. The sensitive strain K12S was kindly supplied by E. M. LEDERBERG. At that time it possessed a second nutritional requirement which was lost during subsequent growth.

Phages. Table 2 describes the lambda phages which have been used and their sources. Lambda-v2 was kindly supplied by E. M. LEDERBERG. The remaining mutants were isolated and kindly supplied by M. LIEB. All temperate phage stocks were repurified by the repeated picking of single plaques. High titre phage stocks were then made by ultraviolet induction of corresponding lysogenic strains.

Media. (a) Culture media as described by WEIGLE and DELBRÜCK (1951).

(b) Endo medium: commercial (Difco) Endo with the addition of 0.5% NaCl and approximately 0.001% $MgSO_4 \cdot 7\ H_2O$. The exact optimal quantity

TABLE 1

Description and sources of bacterial stocks.

Stock No.	Description	Source
Lysogenic		
E. coli K12 C6	$L^-T^-B^-_1\ Lac^-V^r_{1,5}$ (λ)	Y70 (WITKIN) by selective action of T1
E. coli K12 C60	$L^-T^-B^-_1\ Lac^-V^r_{1,5}$ (λw)	C6 by selective action of lambda-v1
E. coli K12 C655	$L^-T^-B^-_1\ Lac^-V^r_{1,5}$ (λw, λv1)	C60 by action of lambda-v1
Sensitive		
E. coli K12S	B^-_1	LEDERBERG (W1294)
E. coli K12 C600	$L^-T^-B^-_1\ Lac^-V^r_{1,5}$	C60 by 2 min. UV
E. coli K12 C47	$B^-_1\ Lac^-$	Recombinant in lysogenic × sensitive cross
E. coli 122	Wild type	London Nat'l Type Culture Collection

Other Strains: "/λ" derivatives of certain of above, derived by selective action of lambda-v2. These strains do not adsorb lambda or any of its mutants.

L^-: leucine requiring
T^-: threonine requiring
B^-_1: thiamine requiring
Lac^-: non-lactose fermenter
$V^r_{1,5}$: resistant to coliphages T1 and T5
(λw): Lysogenic for defective modification of lambda prophage

TABLE 2

Lambda phage stocks.

Stock	Description	Source
Lambda	Temperate; forms turbid plaques; unable to plate on Endo	Principal constituent of wild-type lambda stock
Lambda-1	Temperate; forms turbid plaques; able to plate on Endo	Mutant selected from wild-type stock
Lambda-v1	Weak virulent; forms clear plaques; able to plate on Endo	Spontaneous occurrence as mutant in lambda-1 stock
Lambda-v2	Strong virulent; forms clear plaques; able to plate on Endo; able to plate on bacterial strains lysogenic for lambda	LEDERBERG'S lambda-2

of magnesium to be added depends slightly on the conditions of autoclaving and storage of the medium. A supplement of 5 γ/ml thiamine hydrochloride is added if thiamine deficient strains are to be grown. This medium deteriorates after a few days' storage. It is best used after storage for 24 hours at 4°C.

Phage assays. Phage assays are made by the agar-layer technique described by ADAMS (1950).

Irradiations with ultraviolet. These are carried out as described by WEIGLE and DELBRÜCK (1951), but at an intensity of 1.5×10^3 ergs/sec/cm^2, sufficient to kill 99% of T2 phage in 8 secs. All irradiations are carried out either in buffer or on the surface of agar plates.

Replica and seeded plate techniques. (a) The velveteen replication method of LEDERBERG and LEDERBERG (1952) has been used in testing for presence and type of lysogenicity. Seeded plates are prepared by pouring on a 1% nutrient agar plate 2.5 ml of 1.5% nutrient agar to which has been added 0.3 ml of a fresh fully grown broth culture of indicator bacteria. The plates may then be stored at 4°C for several weeks without deterioration. Test colonies are transferred to the surface of the plate by a velveteen intermediate, and the plate is exposed to ultraviolet for 10–20 secs. before incubation. Lysogenic colonies are surrounded by a clear halo after 24 hours at 37°C. The type of lysogenicity may frequently be inferred from inspection of the halo.

(b) Endo medium as "host-range" indicator: ability to plate on Endo distinguishes the mutant lambda-1 from wild type lambda more critically than plaque size, the original criterion. Lambda-1 plates on Endo with a variable efficiency in the region 20–40%. Under the same conditions the plating efficiency for wild type lambda is about 0.001%, and those phage particles which form plaques subsequently breed true for this character. Lambda-v1 and lambda-v2 are both able to plate on Endo medium with efficiencies as high as that of lambda-1. Throughout the present work the slightly variable behavior of the indicator medium has been checked with the same two standard stocks of lambda and lambda-1. The choice of bacterial indicator strain is unimportant, and the *lac*$^-$ sensitive strain C47 has usually been employed purely for convenience in visibility. To determine the plating efficiency

of a given phage stock on the medium, parallel assay plates are made on Endo and nutrient agar plates. To test the ability of lysogenic colonies to produce phage capable of plating on Endo, parallel replicas are made using the two media. The same seeded plates as are used in replication tests may be used to study the Endo plating character of the phage in a single plaque or in the replica halo surrounding a given colony. The plaque or halo is stabbed with a platinum needle bent at 90° about 1 cm from the tip. The needle is first touched to a seeded Endo plate, then to the surface of a seeded nutrient plate, which serves to check the presence of adequate phage on the needle and may also be examined to score the phage as a clear or turbid plaque former.

EXPERIMENTAL RESULTS

Derivation and properties of a defective lysogenic. The defective lysogenic strain C60 was an apparently non-lysogenic survivor after superinfection of C6 (lysogenic for wild type lambda) with the weak virulent mutant lambda-v1, followed by 4 hours' growth in broth. The experiment was suggested by the fact that such superinfection is known in Shigella to give rise to some non-lysogenic survivors (BERTANI 1953). Wild type lambda, lambda-1, and lambda-v1 plate on C60 with efficiencies less than 10^{-8} whereas lambda-v2 plates on it with the same efficiency as on sensitive or lysogenic strains of *E. coli* K12. C60 was therefore initially classified as immune-1. The following properties show that it is a defective lysogenic: (1) The sensitivity to ultraviolet killing, shown in figure 1, is at least as great as that of the parent lysogenic strain C6 under the same conditions. It is much greater than the ultraviolet sensitivity of a derived lambda-sensitive strain (C600). C600 was, however, selected as a survivor of C60 following a very heavy exposure to ultraviolet (*vide infra*). (2) After ultraviolet irradiation, the optical density drops at the same time and to much the same extent as in the parental lysogenic strain (fig. 2). Under the microscope, a similar pattern of lysis is then observed in many cells of cultures of either strain. (3) When the cells have been grown in nutrient broth to a density of 3×10^8/ml, at most one bacterium in 10^5 yields infective wild type lambda phage following ultraviolet irradiation. The average number of infective phage particles liberated per yielder bacterium is low (10–20), and the proportions of yielders increases slowly with doses up to 25 secs. In normal lysogenic strains under similar conditions 200–400 phage per bacterial yielder are observed, with a maximum proportion of yielders at a rather lower dose (10–15 secs.).

KELLENBERGER (unpublished experiments; the author is indebted to DR. E. KELLENBERGER of Geneva for permission to quote these results) has shown in electron micrographs that most cells of C60 do not liberate any particles of a size comparable to that of infective phage during lysis after UV induction.

To determine whether the defect of the defective lysogenic system resided in the prophage or in the bacterium, a sensitive strain C600 was derived by exposure of C60 to 2 min. ultraviolet irradiation. 95% of the survivors were sensitive to temperate lambda. (This experiment and the derivation of strain

C600 were carried out by MR. A. D. KAISER, to whom the author is indebted for permission to quote the results.) All available lambda mutants form plaques with the same efficiency on K12S and on the sensitive strain thus selected, and temperate lambda mutants form stable lysogenics. The latter

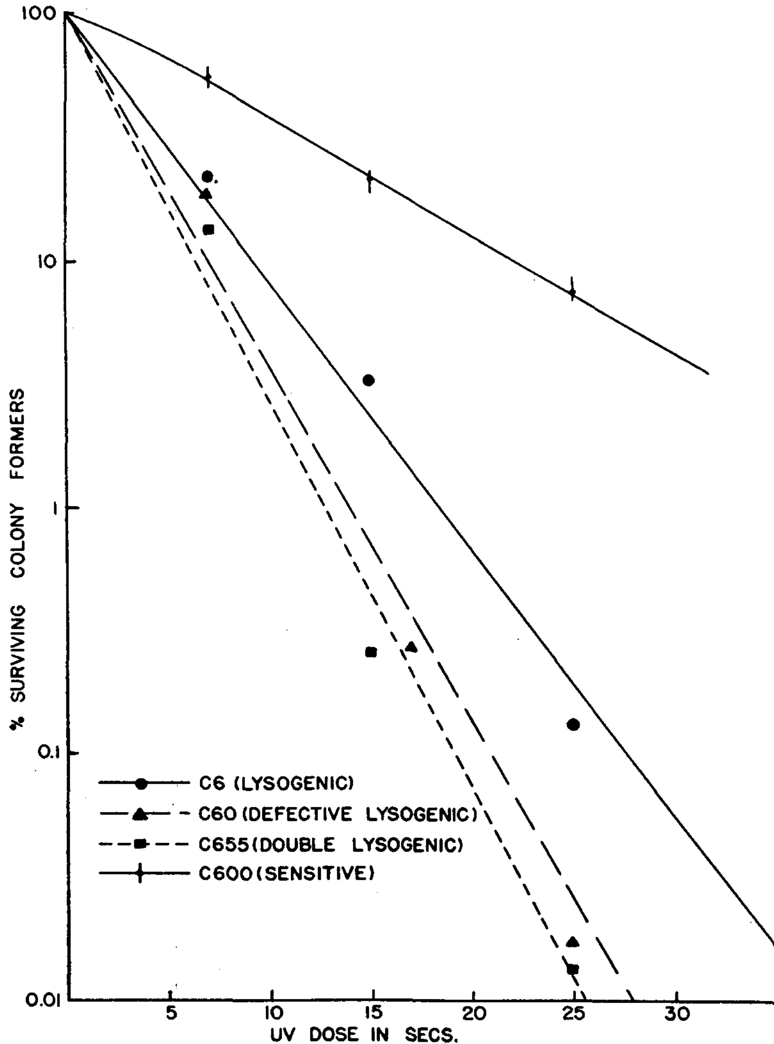

FIGURE 1.—UV killing of defective lysogenic strain C60 of *E. coli* K12 and related strains. Exp.: all strains were grown and irradiated as described in table 3. Assay for colony formers on nutrient plates 10 min. after irradiation.

strains are typical fully inducible lysogenics. When grown to 3×10^8 cells/ml and irradiated 10–15 secs. with ultraviolet 90% of the cells liberate phage in an average yield 200–400. It is concluded that in the defective lysogenic the defective element is the prophage. The phage liberated by C60 is wild type lambda, forming turbid plaques but unable to plate on Endo medium. Similar

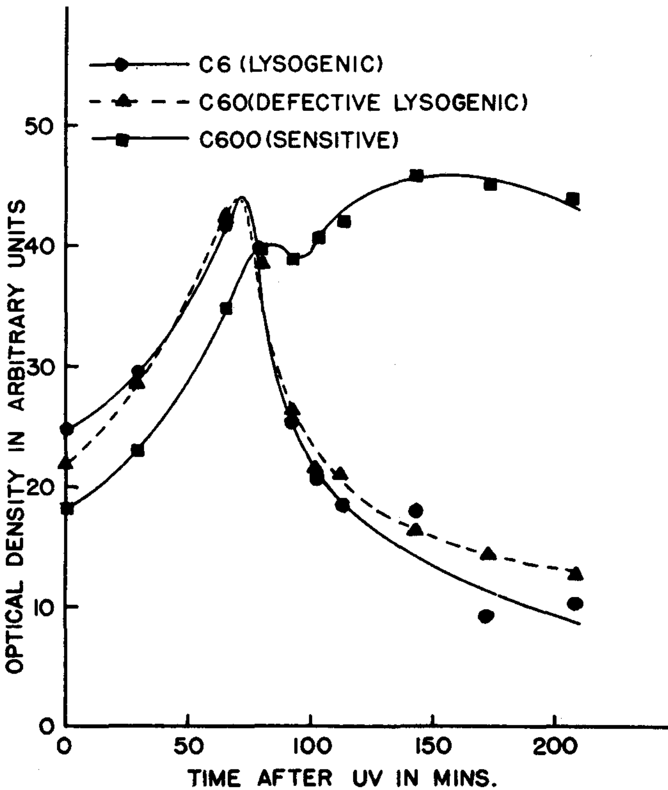

FIGURE 2.—UV induced mass lysis of defective lysogenic strain C60 of *E. coli* K12 and related strains. Exp.: cultures grown as described in table 3 were centrifuged and resuspended in an equal volume of buffer, then exposed to 15 secs. ultraviolet. They were diluted 1 : 1 with fresh broth at time zero and incubated at 37°C with aeration.

results have been observed with a defective lysogenic derived from wild type K12 by the action of 2 mins. ultraviolet irradiation.

Superinfection of defective lysogenic. When C60 is exposed to the weak virulent mutant lambda-v1 at a high multiplicity (10–20), the phage is adsorbed normally. About ⅔ of the cells are killed. If the infected cells are exposed to anti-lambda serum to remove free phage and so prevent reinfection, and then grown in aerated broth at 37°C for several hours, some libera-

TABLE 3

Classification of phage yield from poly-lysogenic C655.

Phage phenotype	+ +	+ cl	E +	E cl
No. observed in yield	8	20	14	17

E: able to plate on Endo — cl: clear plaque former
+: unable to plate on Endo — +: turbid plaque former

Expt.: 24-hr. broth culture diluted 1 : 200 in fresh broth and grown with aeration to $3 \cdot 10^8$ cells/ml. Diluted 1 : 100 in buffer and exposed 15 secs. to ultraviolet. Diluted 1 : 100 in fresh broth and incubated 2 hours at 37° C. Assay on nutrient plates.

tion of weak virulent lambda is observed during the first 2½ hours. After this time, few additional virulent lambda particles are produced. A very much larger number of temperate lambda are now liberated at an increasing rate at least up to 5 hours after the original infection. The surviving cells may be plated and the colonies tested for normal, i.e., non-defective, lysogenicity by replication. When plated immediately after infection, 4% of the survivors gave rise to halos on the replica plates and were classed as lysogenic. At the end of 5 hours' growth in broth, one colony out of 1200 tested was lysogenic. The pure strain (C655) derived from this colony turned out to be homolysogenic. Its behavior is discussed in the following section.

PROPERTIES OF HOMOLYSOGENIC STRAIN C655

Homolysogenicity. C655 was obtained in pure culture by three successive single colony isolations on nutrient agar plates. A stock culture was thereafter maintained on nutrient agar slants. To demonstrate the homolysogenicity a suitable broth culture was induced by ultraviolet, incubated at 37°C for 2 hours to allow the cells to lyse, and assayed for phage. Individual plaques picked at random from the assay plates were scored for clear or turbid plaque formation and for ability or inability to plate on Endo. Table 3 shows that four closely related types of phage were liberated by the induced culture in approximately equal numbers.

Effect of ultraviolet irradiation. Ultraviolet irradiations of C655 and related strains at various doses were carried out under identical conditions of growth. The bacteria were assayed on nutrient plates for surviving colony formers. The lethal effects of the ultraviolet on C655 and on the parent defective lysogenic strain C60 were found to be the same; the lethal effect of

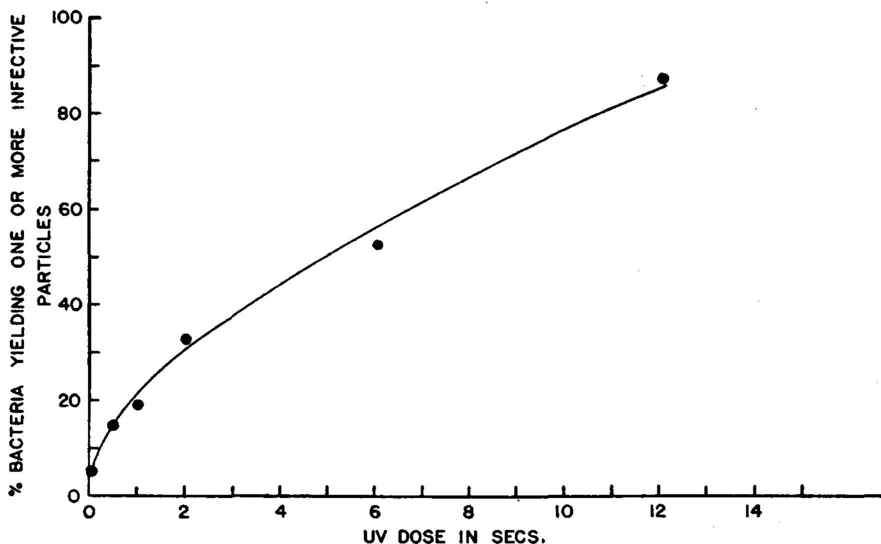

FIGURE 3.—UV induction of poly-lysogenic strain C655. Exp.: as in figure 1.

ultraviolet on the sensitive strain C600 derived from C60 was considerably smaller (fig. 1).

When a C655 culture is exposed to different doses of ultraviolet and assayed for infective centers by the agar layer technique, plaques arise either from induced cells, or from surviving colony formers if one or more spontaneous liberations of phage take place early in the colony. Under the microscope the latter type of plaque, even if turbid, is readily distinguishable by the presence at or near its center of the large single parental colony. By subtraction of this class the number of cells induced at each ultraviolet dose can be calculated. The ultraviolet induction curve of figure 3 was derived in this way. It is similar to the induction curve for wild type monolysogenics.

Yields from single induced C655 cells were examined after exposure to ultraviolet by dilution of a culture into separate tubes, each of which contained on the average 0.3 bacteria before burst. After 2 hours incubation at 37°C the contents of each tube was assayed for infective centers. 90% (27/30) of those tubes whose contents gave rise to plaques contained a mixture of clear and turbid plaque formers. The production of mixed phage yields is therefore a property of most individual ultraviolet-induced cells of C655.

Bacterial segregants from poly-lysogenic strain C655. A fresh broth culture of C655 containing 2×10^8 cells/ml was diluted and plated on nutrient agar. When the colonies were tested for lysogenicity by replication and 15 secs. ultraviolet induction, some appeared to be non-lysogenic and did not form halos, while others were distinguished by unusually turbid halos. A number of segregants of each type were obtained in pure culture by three successive single colony isolations and maintained as stocks on nutrient agar slants. The lysogenicity of each segregant was examined in the following manner. A fresh broth culture of 3×10^8 cells/ml was assayed by dilution and spreading on nutrient plates. A suitable aliquot was suspended in buffer, induced by exposure to 15 secs. ultraviolet, and incubated for 2 hours in broth at 37°C. After dilution the phage yield was assayed in parallel on nutrient plates and on Endo medium. The bacterial assay plates were replica-tested to see whether the strain threw off any further non-lysogenic segregants. Each segregant was now scored for its prophage content as follows:

Phage yield much greater than 1 per bacterium.	At least one non-defective prophage.
Phage yield much less than 1 per bacterium.	No non-defective prophage.
Phage yield all clear plaque formers.	No prophage gene for turbid plaque formation.
Phage yield all turbid plaque formers.	No prophage gene for clear plaque formation.
Phage yield mixed turbid and clear plaque formers.	Prophage genes for both turbid and clear plaque formation. Segregant is poly-lysogenic.
Less than 5% able to plate on Endo medium.	No prophage gene for ability to plate on Endo.

More than 5% able to plate on Endo medium.	At least 1 prophage gene for ability to plate on Endo.
Less than 1/10th as many apparently non-lysogenic segregants as from C655.	No defective prophage.

In the cases of some poly-lysogenics, individual phage particles from the yield were tested on Endo by the needle technique in order to show that the prophage allele for inability to plate on Endo medium was present as well as the prophage allele for ability to plate.

Table 4 summarizes the properties of the phage yields from segregant strains which have been investigated in this way and lists the totality of prophage genes present in each segregant. For convenience, the prophage genes at-

TABLE 4

Bacterial segregants from poly-lysogenic C655.

Type stock	Absolute number tested	Proportion in culture %	Phage types observed in yield	Presence of weak or non-lysogenic segregants	Prophage alleles present in stock
C655			++,+cl,E+,Ecl	+	E/+,cl/+,w/+
C655 t1	12	3.6	E+	<0.1%	E,+,+
C655 t7	2	0.6	++,E+	+	E/+,+,w/+
C655 t15	1	0.3	++,+cl,E+,Ecl	<0.1%	E/+,cl/+,+
C655 i1	5	1.3	++		+,+,w
C655 i2	7	1.8	E+		E,+,w
C655 i5	1	0.3	++,+cl,E+,Ecl		E/+,cl/+,w
C60			++		+,+,w
Lambda-v1			Ecl		E,cl,+

Phage types: notation as in table 3

Prophage alleles: **E: yields some phage able to plate on Endo**
+: yields some phage unable to plate on Endo

cl: yields some clear plaque formers
+: yields some turbid plaque formers

w: very small phage yield following UV induction
+: high phage yield following UV induction

tributed to the parental defective lysogenic and to the super-infecting phage are also listed in the table. It will be seen that all the segregant bacteria are derived from the poly-lysogenic parent C655 by the loss of one or more prophage alleles. The presence of the mutant bacterial characters listed in table 1 for strain C6 has been confirmed for each of the bacterial strains in table 4. Between the observation of bacterial segregants and the last previous single colony isolation, strain C655 divided approximately 70 times. The relative frequencies of appearance of different types of segregants might therefore have been subject to modification by selection. If, however, the bacterial segregants arise by any kind of random event, the majority of them will still be distributed in small clones of less than 50 cells. The effect of selection will therefore be small. Table 4 then shows that simultaneous loss of an allele from

each of three prophage genes is a decidedly more probable event than loss of one.

A similar general pattern of mixed phage yields and of bacterial segregants has been observed using a lambda resistant derivative (C655/λ) of C655 unable to adsorb lambda phage. Such resistant strains may be selected as survivors following exposure to the strong virulent mutant lambda-v2.

DISCUSSION

Defective lysogenics. LWOFF and SIMINOVITCH (1951) have described a defective lysogenic strain of *B. megatherium* which is formally very similar in its properties to C60. Further analysis by SIMINOVITCH (1951) has shown that the defect in their strain involves an inability to resume manufacture of DNA after this process has been interrupted by ultraviolet induction. The existence of defective lysogenics in the system lambda–*E. coli* K12 has also been recognized by LEDERBERG and LEDERBERG (1953).

From each of our defective lysogenics, normal sensitives can be produced by a single massive dose of ultraviolet: the defect in the lysogenic complex is lost at the same time as the prophage. This suggests that the defect is connected with the specific prophage structure, rather than with the bacterial residuum of the complex.

JACOB (1952) has obtained a number of lysogenic strains which differ very widely in their ability to produce phage, by infection of a sensitive strain of *Pseudomonas pyocyanea.* Without further analysis it is not certain whether these variations are due to genetic heterogeneity in the bacteria or the phage used, or in the relations between them. JACOB's results differ from ours in the high frequency of occurrence of the defective strains, in the existence of a continuous gradation of intermediate degrees of defect, and in the fact that the strains were prepared by infection. It may therefore be questioned whether the same underlying cause is responsible.

By superinfection of the defective lysogenic C60, a homolysogenic has been derived which is phenotypically non-defective, but from which both defective and non-defective lysogenics segregate. Both the defective and non-defective properties of lysogenicity are therefore represented in the homolysogenic. The same homolysogenic also contains two alleles of each of two prophage genes, one controlling plaque type and the other Endo plating ability. From it, mono-lysogenics segregate which have lost one allele of each gene. In all cases so far examined, these mono-lysogenics have also lost either the defective or the non-defective property of the homo-lysogenic. It is also observed that most but not all of the segregants which have lost either the defective or the non-defective property have also lost one allele of each of the two prophage genes and are mono-lysogenic. This correlated reduction is consistent with the idea that the defect in the parental strain C60 is due to an allele of a prophage gene.

Superinfection. For the phage P2 active on Shigella, BERTANI (1953) has described the initial multiplication of a weak virulent phage which superinfects

a related lysogenic, followed by a large yield of recombinant types of phage. Our results are consistent with these. Bertani found some sensitive bacterial survivors of the superinfection process, as well as unstable lysogenics for the superinfecting phage.

Poly-lysogenicity and prophage interaction. The release of four related bacteriophages by induced C655 requires that the strain be doubly homolysogenic. A higher multiplicity for the poly-lysogenicity need not be assumed, since phage recombination does occur in the vegetative phase in this system (A. D. KAISER personal communication). In the double lysogenic one prophage carries defective lysogenicity while the other does not. Most cells of a culture are phenotypically non-defective: they yield infective particles after a suitable dose of ultraviolet irradiation. Physiological interaction between the developing prophages therefore leads to a " dominance " of the non-defective characteristic.

Prophage recombination. During growth of our poly-lysogenic, bacterial clones segregate which have lost one or more prophage alleles. A striking feature of the segregation is that in the majority of the segregants one allele of each of three prophage genes has been lost and the segregant is mono-lysogenic. Three different types of mono-lysogenic segregant have been observed. A minority of segregants remain poly-lysogenic but have lost a single allele of one prophage gene. Three different types of such poly-lysogenic segregant have been observed. No segregants have yet appeared in which two prophage alleles have been lost.

Among the bacterial segregants a sufficient number of different kinds occur to make it certain that some at least contain new combinations of prophage genes, i.e. combinations not present in either prophage of the parent cell of the clone. The same is true of segregants from the derived stock C655/λ which cannot adsorb lambda phage. The phenomenon is therefore not a consequence of successive cycles of prophage loss and superinfection (BERTANI loc. cit.) coupled with intervening phage recombination in the vegetative phase. While there is as yet not enough evidence for the definitive exclusion of all other alternatives, the appearance of new combinations of prophage genes during growth of a polylysogenic clone strongly suggests the following as the simplest interpretation. There exists a process, formally akin to somatic crossing over, by means of which the genes of two related prophages carried in one cell can recombine. This process, here called prophage recombination, gives rise to segregant poly-lysogenics which, as compared to the parent strain, have lost one or more prophage alleles. It is possible to account for all the observed segregants in this manner, since present experimental methods cannot distinguish a mono-lysogenic from a " homozygous " poly-lysogenic. However, because of the high frequency with which mono-lysogenic segregants occur, it is equally likely that another process, the loss of one prophage, also takes place during the growth of the poly-lysogenic.

SUMMARY

1. Two genotypically different strains of defective lysogenic *Escherichia coli* have been selected from derivatives of *Escherichia coli* K12 and shown to carry a defective lambda prophage.

2. By superinfection of one of these strains with a weak virulent mutant, lambda-v1, a poly-lysogenic strain has arisen. This carries a total of six recognizable prophage alleles. Some properties of the poly-lysogenic are described.

3. During growth of this poly-lysogenic, segregant bacterial clones arise in which one or three prophage alleles have been lost, leading to new types of mono- or poly-lysogenesis. It is suggested that this phenomenon results from a recombination of prophage genes during growth.

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