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STUDIES ON DEFECTIVE LYSOGENY DUE TO CHROMOSOMAL DELETION IN *ESCHERICHIA COLI*

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SUMMARY Spontaneous deletions extending to the attachment site of phage $\phi 80$ were selected from several strains of *Escherichia coli* (C, K-12, φ) by colicine B treatment. These deletions arising in bacteria lysogenic for $\phi 80$ (or $\lambda .80$) cause defective lysogeny in most cases; they extend into the phage genome for variable distances.

Lysis occurs in many defective lysogens after UV-irradiation. The lysates do not usually contain any active phage particle. Preliminary examination of the lysates has shown the release of coat material. A rare defective lysogen was found to produce a few (10^{-6}) infectious particles, which are unable to lysogenize.

INTRODUCTION

Among mutations conferring cross-resistance to colicine B and phage T1 in *Escherichia coli*, some are deletions extending to the tryptophan operon on one side and covering the attachment site of Matsushiro's phage $\phi 80$ on the other side. In strains initially lysogenic for $\phi 80$, these deletions affect the ability to produce this phage (GRATIA, 1964). This genetic peculiarity has been considered in respect to the mode of integration of the prophage in the bacterial chromosome. The results obtained independently in two laboratories (FRANKLIN *et al.*, 1965; GRATIA, 1965) are in agreement with the linear insertion model proposed by CAMPBELL (1962). This paper gives some additional data concerning the occurrence and the characteristics of this prophage's cure or defective lysogeny due to chromosomal deletion in several strains of *Escherichia coli*.

MATERIALS AND METHODS

1. *Media*

Buffer saline: Difco Hemagglutination Buffer

Broth: Difco Broth for current manipulations, and enriched broth (G-broth) for cultures: Difco Beaf extract, 3 g; yeast extract, 2 g; Bactopeptone, 5 g; glucose, 1 g; buffered saline, 1 liter.

1 The results of this study were presented at the National Congress of Medical Microbiology held at Bucharest on September 17, 1965.

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Solid medium : Agar Broth Difco ; in some cases, this medium was enriched with yeast extract (2 g/l). For studies with phages and for testing lysogeny, the agar concentration in this medium was reduced to 1 per cent.

Synthetic medium : Simmonds citrate agar containing glucose (2 g/l), plus supplements when required by the strain.

2. *Bacterial strains*

Strains of *E. coli* C, φ, some K-12 derivatives (HCA 23 (1) ; PA409 (2)), 28 TAM.1 carrying the episome *F'col try* (3), and also the B colicinogenic strain CA18 were used.

3. *Bacteriophages*

Besides T1, the temperate phages λ, ϕ80 and the following derivatives were used :

1) The hybrid phage λ.80 (2) with the markers imm^{λ} (immunity of λ) and h^{+80} (host range of ϕ80) and the same location on the host chromosome as ϕ80 (SIGNER, 1964) ;

2) A mutant of this phage, $\lambda.80h$, carrying a host range mutation h^{80} (also a clear mutant was used.).

3) A phage carrying imm^{80} and h^{80} from a cross between λ.80h and ϕ80

4) A phage carrying the markers imm^{80}, h^{80} and *mi* from λ.80h crossed with the phage λmi (minute plaques) (2)

Transduction experiments were performed with phage P1*kc*. Some fo the phage techniques used are given by ADAMS (1959), JACOB and WOLLMAN (1956) and MATSUSHIRO (1963). Some techniques are also described in the following paragraphs.

4. *Selection of colicine B-resistant mutants*

Among the mutants resistant to colicine B, only those which are also resistant to phage T1 have been considered in this work. With colicine B alone mutants must be selected at a high concentration of this material. B-colicinogenic bacteria (about 1000) are plated by the agar-layer method on nutrient agar medium containing yeast extract. After 50–56 hours incubation at 37°, the thin agar-layer with bacteria is removed with water. A layer of 6 ml of agar medium is poured on to the plates and then the plates are sterilized with chloroform vapor. They are then stored to allow diffusion of the colicine. From cultures of a given lysogen, aliquots were spreaded on colicine plates. These had been smeared with anti-ϕ80 immune serum to eliminate free phage from the background for replica plating must be made on sensitive bacteria as follows. The resistant colonies appearing after 2 days incubation were replicated on plates (1) containing synthetic medium without tryptophan for checking the nutritional requirements, and also on plates (2) covered with ϕ80-sensitive bacteria for checking lysogeny. After replication on plates (2), bacteria were exposed to a low dose of UV-light. The distinction between the colonies was clear-cut ; either they gave rise to large plaques and were judged to be truely lysogenic or they did not show any trace of lysis at all.

5. *UV-induction of lysis*

The ability of mutants to lyse on UV-irradiation was studied on bacteria resuspended in buffer and exposed to UV-light (time of exposure : 45 seconds, corresponding to a dose of about 500 ergs/mm^2 (germicidal mercury vapor lamp)). When greater accuracy was required, the treatment was done at low temperature. For studies on the kinetics of lysis, the irradiated suspension in buffer was mixed with an equal volume of G-broth and incubated at 37° with aeration. The suspension was examined at intervals in a " Vitatron " colorimeter (662 μ).

6. *Marker-rescue experiments*

The bacteria presumed to be defective lysogenic for ϕ80 were irradiated in $MgSO_4$ M/100 and superinfected with genetically marked phages (see " Bacteriophages " : 2. and 4.). After contact of 1–2 $\times 10^8$ bacteria with 5–10 $\times 10^8$ plaque forming particles in 1 ml of medium at 37° for 15–20 minutes, the unadsorbed phage was treated with antiphage serum and removed by centrifugation. After suitable dilution to prevent readsorption of phage progeny on intact cells, the induced superinfected bacteria were incubated in broth for 2 hours. The lysates sterilized with chloroform were tested on indicator strain for eventual rescue of markers carried by the defective prophage.

RESULTS

1. *Genetic relationships between mutations of resistance to colicine B and lysogeny for ϕ80.*

As mentioned previously (GRATIA, 1964),

(1) From YANOFSKY C. ; ((2) JACOB F. ; (3) FREDERICQ P. ; (4) MATSUSHIRO A.

one locus governing sensitivity to colicine B is closely linked to the tryptophan operon and to the site of attachment of prophage $\phi 80$. Transductions were performed with phage P1*kc* among strains of *E. coli* K-12 marked by *cys*, *try*, lysogeny for $\phi 80$ and, in some experiments, resistance to colicine B. The results are presented in Table 1 indicating the frequencies of the various recombinants cys^+ or try^+. It is clear from experiment n°1 that the less frequent classes must arise from a single crossing-over between the marker *try* and the $\phi 80$ attachment site att^{80}. Either the donor or recipient is marked by a mutation of resistance in experiments n°2 and n°3. Rare recombinants for resistance and lysogeny (2 IV and 3 III) cannot result from multiple crossing-over but from a single crossing-over between segment *try-b*(or bt_1) and att^{80}. From the table the order is conclusively as follows:

$$cys \ldots anth—try—b(bt_1)—att^{80}$$

This order is in agreement with the previous results for segment *cys—anth—try* (YANOFSKY and LENNOX, 1959) and for segment *anth-try-*att^{80} (MATSUSHIRO, 1963). The main point in this study is the location of the resistance markers between *try* and att^{80}.

Among the various non-reverting mutations of resistance, presumably deletions of variable extent, only those of the most complex phenotype of resistance (cross-resistance to colicines B, I and V and phage T1) may extend far on this chromosomal segment. Either they reach, on the left, the tryptophan operon or they cover, on the right, the prophage attachment site att^{80}. This last case is shown in three ways: (a) failure of recombination between the resistance marker and lysogeny; (b) failure of true lysogenization by a phage carrying a suitable

TABLE 1 *The co-transduction of nutritional, resistance and non lysogenic* $(\phi 80)^-$ *markers with phage P 1 kc. Analysis of the recombinant classes*

Exp no	Donor (1)	Recipient (1)	Number Recomb. scored	Select Marker	Classes	%	Crossing--over (*)
1	*anth* $(\phi 80)^-$	*cys ; try* $(\phi 80)^+$	50	try^+	I. cys^+ *anth* $(\phi 80)^-$	42	1 ; 6
					II. *cys anth* $(\phi 80)^-$	21	2 ; 6
					III. cys^+ *anth* $(\phi 80)^+$	21	1 ; 4–5
					IV. *cys anth* $(\phi 80)^+$	8	2 ; 4–5
					V. *cys* $anth^+$ $(\phi 80)^-$	6	3 ; 6
					VI. *cys* $anth^+$ $(\phi 80)^+$	2	3 ; 4–5
2	b $(\phi 80)^-$	*cys ; try* $(\phi 80)^+$	130	cys^+	I. *try* b^+ $(\phi 80)^+$	50	1 ; 2
					II. try^+ *b* $(\phi 80)^-$	42	1 ; 6
					III. try^+ b^+ $(\phi 80)^+$	6	1 ; 4
					IV. try^+ *b* $(\phi 80)^+$	2	1 ; 5
3	$(\phi 80)^-$	*(cys) ; try* $(\phi 80)^+$; bt_1	190	try^+	I. bt_1^+ $(\phi 80)^-$	69	1–2 ; 6
					II. bt_1 $(\phi 80)^+$	28	1–2 ; 4
					III. bt_1^+ $(\phi 80)^+$	3	1–2 ; 5

(*) *cys* —1— *anth* —2— *try* —3— *b ; bt*$_1$ —4— —5— att^{80} —6—

(1) The mutations marking parents are: *try* (*try A23*): tryptophan dependence; *anth* (*try4–1*): response to anthranilic acid; *cys* (*cys B*) cystein dependence; *b*: partial resistance to colicine B: bt_1: cross-resistance to colicine B and phage T.1. b^+ and bt_1^+ are the alleles of sensitivity.

host-range marker, i.e. h^{80} (P. 2: Bacteriophages 2. and 3.); (c) loss of ability to produce ϕ80 in strains initially lysogenic for this phage. According to the extent of the deletion, the mutations of resistance to B, I, and T1 arising in try^+ bacteria lysogenic for ϕ80 (or λ.80) are classified into the following 4 classes.

(I) resistance and persistence of ability to produce active phage: bt_1,ly^+

(II) resistance and tryptophan requirement, persistance of active phage production: bt_1try,ly^+;

(III) resistance and loss of ability to produce active phage: bt_1,ly^-;

(IV) resistance, tryptophan requirement and loss of lysogenic character: bt_1try,ly^-.

The relative frequencies of occurrence of these 4 classes have been examined using the replica plating method. The mutant colonies have been selected by use of a high concentration of colicine B. Mutants from strains K-12, C and φ have been assigned to class I, II, III and IV by checking their ability to replicate in the absence of tryptophan and to lyse sensitive indicator (Table 2). When *E. coli* C and HCA23 are used, about 70 per cent of the mutants are of class I (bt_1ly^+); class II are rare (3 per cent); 27 per cent are non-phage producers, 18 per cent are of class III and 9 per cent of class IV. The results with PA409 are different, since all except one of the mutants are of class I. In strain ϕ, on the contrary, more than 90 per cent are non phage producers, i.e. chiefly class III and some class IV. It has been verified that the lysogenic character is very stable in the wildtype and mutant ly^+ bacteria of this strain. Therefore, some diversity appears in the genetic organization in the various strains of *E. coli*, though the order try-bt_1-att^{80} must be valid in all cases.

2. *Persistence of immunity or other prophage markers in some defective lysogenic mutants* bt_1,ly^- and bt_1try,ly^-

Mutants may lose their ability to produce infectious phage particles without losing the corresponding immunity. Since the mutants studied here are resistant to ϕ80 or λ.80 (because of the cross-resistance to T1 and to ϕ80; MATSUSHIRO and HASEGAWA, 1961), immunity

TABLE 2 *Loss of ability to produce active phage and loss of immunity in mutants highly resistant to colicine B derived from strains coli C, K-12 and φ lysogenic for ϕ80 or λ.80*

Strain	Number of mutants (1)	% Mutants of class				Loss of immunity in mutants ly^-	
		I bt_1, ly^+	II bt_1, try, ly^+	III bt_1, ly^-	IV bt_1try, ly^-	III	IV
C (ϕ80)	399 (20)	75.7	2.0	14.3	8.0	1/14	1/15
C (λ.80)	193 (10)	63.1	4.1	23.5	9.3	0/8	0/7
HCA23 (ϕ80) (2)	412 (12)	70.5	3.3	16.7	9.5	1/8	1/5
PA409 mal^+ (λ.80) (3)	850 (12)	99.9	0	0.1	0	—	—
φ (ϕ80)	86 (6)	8.1	0	79.1	12.8	5/9	3/3
φ (λ.80)	202 (6)	13.8	0	78.8	7.4	6/11	2/2

(1) The number of subclones used is indicated in parenthesis.
(2) The initial strain is indol dependent and mutants considered in classes II and IV do not respond to indol, but only to tryptophan.
(3) Only one mutant is non phage producer; it has maintained a single try^- mutation from the initial strain and immunity of λ.

had to be studied against a phage imm^{80} h^{80}. When mutants were derived from a strain lysogenic for the hybrid phage λ.80 with markers imm^{λ} and h^{+80}, the sensitivity test was performed with phages λ and λ.80h. Immunity was examined with diploids formed by the acquisition of an F′ episome carrying the wild-type alleles of the deleted region (FREDERICQ, 1963) which are able to adsorb φ80 or λ.80.

The results presented in the second part of table 1 show that the phage immunity character still persists in a variable proportion of mutants depending on the initial strain. In *E. coli* C, loss of immunity was observed in only 2 of 44 mutants bt_1,ly^- and bt_1,*try*,ly^- of independant origin; 42 still carry some phage genome and are therefore defective lysogens. In *E. coli* φ, on the contrary, more than 50 per cent of the mutants (bt_1,ly^- or bt_1,*try*,ly^-) lost immunity.

As the deletion seems to affect a segment of prophage of variable length, one may expect different sets of phage genes to appear in the phage progeny after superinfection with a genetically marked phage. Results of such marker rescue experiments are presented in Table 3. As expected, there is a gradient in the markers recovered according to the mutant studied: h^{+80}, host range of φ80; mi^+, large plaques; imm^{80}, immunity of φ80; c^+, turbid plaques. The loss concerns either h^{+80} and mi^+ or h^{+80} and imm^{80} (or c^+ indissociably linked to imm^{80}), indicating the following order for the lysogenic strain

$$try—bt_1—h—mi—(c\,;\ imm)$$

as represented schematically in Fig. 1.

3. *Physiological properties of some defective lysogens after UV-treatment: replication of the defective genome, lysis and release of phage material*

As a defective prophage carries a deletion, the question arises as to how the properties determined by the normal prophage are maintained in the defective lysogens. As shown in the superinfection experiments (Table 3), the recombination frequencies are about the same (1.5 per cent between imm^{80} (from the induced prophage φ80) and h^{80} (from the superinfecting phage λ.80hc), whether the induced prophage is defective or not. This result indicates that the replication of the defective genome proceeds normally after induction, at least in the mutants studied in these experiments.

In *E. coli* C, the deletion rarely reaches the immunity gene located on the right side; it affects only a part of the prophage genome in most mutants. Correspondingly, more than 50 per cent are sensitive to UV-light. On the contrary, in *E. coli* φ, where the deletion is often extensive, most mutants are resistant to UV-light. Several mutants derived from *E. coli* C lysogenic for φ80 or λ.80, including some which had lost their lysogenic character, were studied with respect to (1) the UV dose-effect on the survival; (2) the kinetics of lysis; (3) the final defective material released.

The graphs I in Fig. 2 show results with strains (a) *E. coli* C; (b) a mutant of C initially non-lysogenic carrying a deletion (bt_1try); (c) C (λ.80); (d) C (φ80). From curve b, the mutant is slightly more sensitive than the wild-type. The sensitivities of C (φ80) and C (λ.80) are not exactly the same. The graphs II show the dose-effect in 3 mutants derived from C (λ.80): L38 non defective; L9 defective but producing rare infectious particles (cf. n°4); L8 producing no active phage at all. They are alike and resemble the curve of C (λ.80) in spite of the variable genetic defect. This, however, is not exactly the same as the case with mutants derived from C (φ80). Their survival curves are different and also differ from that of the wild-type (III). The mutants L7 and L57 respectively lysogenic and defective lysogenic for φ80 are very sensitive to UV-light but their survival curve has an irregular shape in contrast with the normal one which is straight. Strain L1 appears to be more sensitive than C (φ80) (curve d). It is worth noting that curves c and d are parallel, showing that different doses are required for the minimum effect with L1 and L57 but that

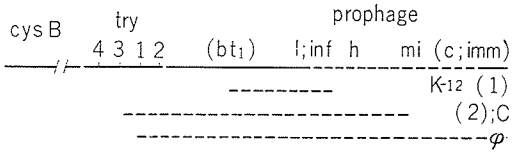

FIGURE 1 Diagrammatic representation of the chromosomal segment with *cys* gene (cystein synthesis) and *try* (tryptophan) cluster genes, and prophage $\phi 80$.
Deletions cover locus bt_1 (sensitivity to colicine B and to phage T1), some extending to *try* or to $\phi 80$ prophage. The phage markers are *h* (host range), *mi* (minute plaques), *c* (clear plaques) and *imm* (immunity). *l* means linkage between prophage and chromosome; *inf* is the most proximal gene necessary for the infectious character. The extent of the deletions varies in the different strains: (K-12 (1), PA409, K-12 (2), HCA23).

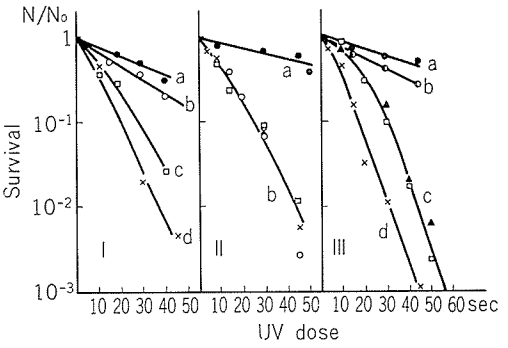

FIGURE 2 Ultraviolet survival curves of *Escherichia coli* C:
I: ●, wild type (a); ○, mutant derivative carrying mutation bt_1try (b); lysogenic derivative $(\lambda.80)^+$ (c); ×, lysogenic $(\phi 80)^+$ (d).
II: ●, wild type (a); ○, mutant derivative L38 still lysogenic for $\lambda.80$ (b); defective lysogenic derivatives L8 (×) and L9 (□) (b).
III: ●, wild type (a); ○, mutant derivative L50 having lost lysogeny and immunity (b); △ mutant derivative L7 still lysogenic for $\phi 80$ (c); defective lysogenic derivatives: □, L57 (c) and ×, L1 (d).

the lethality increases with the dose at the same rate in both cases. L50 shows the same resistance as the initially non-lysogenic mutant, in agreement with the fact that it has no recoverable marker including immunity which marks the distal end of the integrated prophage.

The kinetics of lysis in each strain were followed after induction at the optimal dose. Lysis is quite similar in induced C ($\lambda.80$) and defective lysogenic derivatives (Fig. 3a), but variations are observed with mutants derived from C ($\phi 80$) (Fig. 3b) and reflect differences in the number of lysed bacteria. The latent period is normal: about 80 minutes for $\lambda.80$ and defective $\lambda.80d$ as for λ; about 135 minutes for $\phi 80$ and defective $\phi 80d$.

TABLE 3 *Superinfection of induced normal and defective lysogenic bacteria (E. coli C)*

Strain	Induced prophage	Markers of superinfecting phage	Markers rescue (1)	% Recombination h^{80}—imm^{80} (2)
C ($\phi 80$)	$\phi 80$	h^{80}; *c* imm^{λ}		1.60
L7	$\phi 80$	,,		1.90
L1	$\phi 80$ d1	h^{80}; *c* imm^{λ}	h^{+80} c^+ imm^{80}	1.45
L57	$\phi 80$ d2	,,	c^+ imm^{80}	1.56
L57	$\phi 80$ d2	h^{80}; *mi*; imm^{λ}	mi^+; imm^{80}	
L40	$\phi 80$ d3	,,	imm^{80}	
L50	$\phi 80$ d4	h^{80}; *c imm*	—	

(1) h^{+80} checked in c^+ phage after passage on *E. coli* C by the replica plating method using strain C/B try (h^{+80}-resistant)
(2) % plaques appearing on strain L8 (h^{+80} resistant; λ-immune) among those counted on C/B try (h^{+80}-resistant).

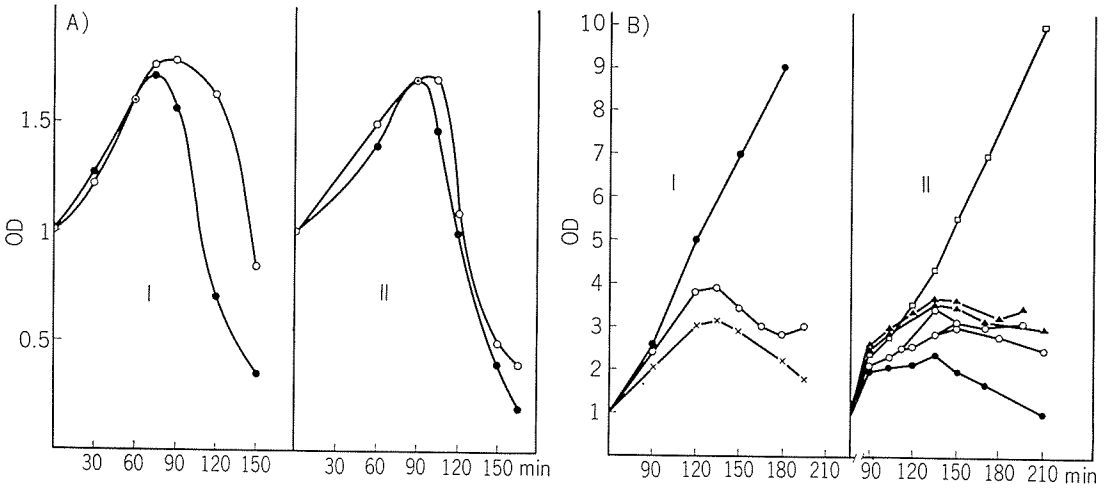

FIGURE 3 Lysis of lysogenic and defective lysogenic derivatives of *E. coli* C and K-12 (OD, variation of optical density: final to initial; (A): 45 sec. irradiation; (B): 30 sec. irrad.)
A) I: ●, PA409 lysogenic for λ.80; ○, defective derivative L104.
II: ●, C lysogenic for λ.80; ○, defective lysogenic derivative L9.
B) I: ●, wild-type strain C; ○, C lysogenic for φ80; ×, idem, but 45 sec. irradiation.
II: ●, defective lysogenic derivative L1 (φ80 dl); ○, L40 (φ80 d3) (2 irradiated samples); ▲, 15 (2 irradiated samples); □, L50.

It is therefore reasonable to suppose that in the UV-sensitive defective lysogens deletion does not prevent the synthesis of phage material responsible for lysis and that some material can eventually be detected.

Among 6 lysates examined with the electron microscope, 5 contained detectable particles which were not all alike. Indeed, in the case of L9, defective lysogenic for λ.80 but producing a few infectious particles (cf. n°4), the preparations showed full heads and, sometimes a separate tail but no complete particles like those observed with a normal lysogenic strain. The lysates of L1 ($\phi 80 d_1$), L8 ($\lambda .80 d_1$) and L104 ($\lambda .80 d_3$) contain empty heads. In the case of L40 ($\phi 80 d_3$), no particles have yet been found in the lysates.

On the other hand, the lysate of induced L8 bacteria has been analysed with respect to the presence of antigenic material detectable by its antiphage-serum blocking power. Diluted samples of the lysate were incubated with anti-λ.80 serum for 30 min. at 37°. Then the serum was tested for its antiphage activity. As shown in the Table 4, treated sera had less activity than untreated sera. Phage tail material is therefore synthesized in this strain.

4. *A defective lysogen producing a small amount of active phage*

The resistant mutants ly^- of our collection (101 of independant origin) do not produce any infectious phage particles. An exception is the strain L9, defective lysogenic for λ.80 with deletion bt_1try,ly^-, which produces plaque-forming-particles with a frequency of about 10^{-6} of normal. After UV-induction, L9 lyses normally and produces the normal amount of noninfectious particles which are detectable with an electron microscope (cf. n°3). The active phage released has the same characters as the initial phage λ.80 with respect to the host range, immunity and plaque morphology. However, it is not absolutely identical since it is unable to lysogenize even though it forms turbid plaques.

TABLE 4 *Serum-blocking power of lysate L8 on the antiphage activity of the immune serum against λ.80*

Experiment n°		Dilution of serum	Titer of phage	Dilution of lysate	K* untreated serum		K* treated serum	
1		1 : 100	5.5×10^6	1 : 20	16.3		7.1	
				1 : 50			9.2	
2	a	1 : 200	4.2×10^6	1 : 50	13.8	13.2	8.5	9.2
	b	1 : 200	2.0×10^6	1 : 50	12.7		9.9	
3		1 : 200	4.3×10^6	1 : 100	13.8		10.5	

* Calculated from the time corresponding to 90% inactivation of the virus.

The kinetics of phage production was followed. A suitable aliquot of a fresh suspension of L9 bacteria in buffer was irradiated and added to broth for incubation. At intervals, samples were plated with sensitive bacteria and the infective centers counted. For comparison, a diluted sample of induced wild-type bacteria C (λ.80) was incubated and examined in the same manner. The results presented in Fig. 4 show that the induced bacteria of the mutant strain L9 produced phage after a normal latent period. However, the relative number of infective centers increased slightly. Also analysis of the individual productions showed that the average output was low (Table 5). Indeed, the tubes with one productive bacterium contained a very variable number of plaque-forming-particles. This result indicates that in the defective progeny of a few induced bacteria a fraction of variable size consists of infectious particles and these may arise at random during vegetative development.

Unlike the case studied by JACOB and WOLLMAN (1956) with the strain P14, defective lysogenic for λ by reversible mutation d_1, the restoration of active phage in L9 occurs spontaneously (Table 6). UV treatment stimulates this process but at the same time it induces phage development in the initial strain (Table 6) and lysis in both strains (Fig. 3a II). Secondary irradiation during the latent period does not increase the formation of active centers. The mechanism involved is therefore unrelated to UV-treatment which only induces the vegetative state.

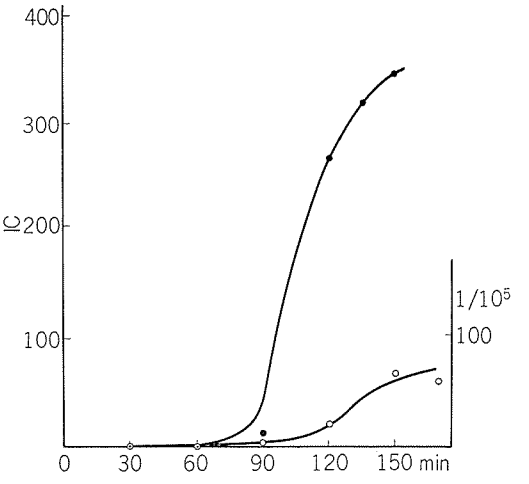

FIGURE 4 Variation in the relative number of infective centers (IC) after UV-induction of strain C lysogenic for λ.80 (●) and of its defective lysogenic derivative L9 producing a few amount of infectious particles (○). (Scale reduced $1/10^5$ for L9 since 10^5 times more bacteria were used). Before incubation, the irradiated suspension of the normal lysogenic bacteria was diluted (1×10^2 bact. per ml incubated) to avoid readsorption of progeny phage on unlysed bacteria. Phage produced by a heavy sespension of mutant bacteria L9 is not adsorbed on cells which lack the receptors. Infective centers were counted after 30 min to 180 min incubation.

TABLE 5 *Individual production of active phage by bacteria L9. The distribution of infectious phage n individual tubes*

Experiment	N°1	N°2	N°3	
Number of irradiated bacteria/tube	3.9×10^{4}	3.4×10^{5}	(A) 1.1×10^{4}	
			(B) 5.5×10^{4}	
Infective centers /irrad. bact.	1.6×10^{-5}	2.3×10^{-5}	1.1×10^{-5}	
Number of tubes containing plaque forming units	4/30	9/50	(A) 7/20	(B) 14/20
Average output	—	11.9	43.4*	38.2*
Number of p.f.u. in each tube	1	1	1	1
	5	1	1	1
	39	2	11	2
	120	2	26	2
		3	59	2
		5	78	4
		14	128	14
		30		16
		49		36
				69
				74
				152
				163

* The true value is almost certainly lower, since one or more tubes must contain two or more bursts.

TABLE 6 *Effect of UV-irradiation on the production of bacteriophage by lysogenic strain C* (λ.80) *and mutant derivative L9*

Strain	Number of bacteria irradiated	Number of infective centers				
		without UV	with UV (dose in seconds) 15	30	45	60
C (λ.80)	215×10^{3}	140×10^{2}	100×10^{3}	131×10^{3}	162×10^{3}	114×10^{3}
L9	204×10^{5}	22	37×10^{1}	96×10^{1}	161×10^{1}	91×10^{1}

DISCUSSION

Mutants of *E. coli* which are not resistant to colicine B may carry a deletion which reaches or covers the attachment site of a prophage, ϕ80. Such deletions are detected only among mutants which are highly resistant to colicine B. If they arise in a lysogenic strain, they may cause the loss of the ability to produce mature phage affecting the prophage genome most often partially as shown by marker rescue experiments. Most mutants with immunity are induced to lyse after UV-treatment and eventually release phage material. They may yield

phage material detectable by electron microscopy or by the serological method. Rarely they produce active phage particles; these have lost the ability to lysogenize.

This phenomenon gives very valid confirmation for the linear insertion of the prophage into the continuity of the bacterial chromosome proposed by CAMPBELL (1962). The deletions appear to be of more or less extent in the phage genome of $\phi 80$ (or a hybrid phage $\lambda.80$) extending from a proximal end (in respect to *try*) adjacent to a chromosomal locus called bt_1. Independently, FRANKLIN *et al.* have analyzed a set of T1-resistant " small colony " mutants from the K-12 strain lysogenic for a hybrid phage (imm^{80} $h^{+\lambda}$). Furthermore they performed extensive marker rescue experiments with the *sus* mutant of λ. Their results agree with those of ROTHMAN (1965) and also with our present results and lead to the same conclusion that the integrated prophage is linearily inserted into the continuity of the bacterial chromosome.

The immunity gene appears to be located at the distal end (in respect to *try*) and, therefore, when a deletion reaches *imm*, as we often observed with lysogenic derivatives of the strain φ, it causes the complete (or nearly complete) disappearance of the prophage.

The variable extent of the deletions from the integrated prophage starting from a given point, adjacent to the locus bt_1, should give evidence of critical functions. As indicated by the results of superinfection experiments, the synthesis of genetic material proceeds normally. Possibly the replication starts at the distal end so that the deletions would not affect this process. It is worth comparing the effect of deletion on the synthesis and the structure of phage components in several defective lysogens. Our preliminary studies in this field has already shown differences between the strains: incomplete particles but full heads; empty heads; no particles.

The inability of the active phage to lysogenize resulting from the defective lysogen L9 is interesting. It is suggested that a deletion covers the linkage point *l* between the bacterial chromosome and the prophage so that the vegetative phage would maintain a lesion inhibiting further integration. FRANKLIN *et al.* have also observed the inability of a phage produced in subnormal amount ($1/10^3$) to lysogenize and these authors have suggested an analogy with the λb^2 type described by KELLENBERGER *et al.* (1961). However, the relationship between the case of their phage and the case of L9 which yields much less active phage cannot yet be determined.

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