BIKEN JOURNAL Vol. 10, 55-63, 1967

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

II. LYSOGENIC HETEROGENOTES

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(Received March 23, 1967)

SUMMARY Lysogenic heterogenotes carrying a defective transducing $\phi 80$ prophage in addition to the normal $\phi 80$ prophage have been studied with respect to the effect of deletions selected by colicine B treatment. In agreement with results of P1 transduction experiments, the examination of deletion mutants suggests that the defective transducing prophage is integrated on the right side of the bacterial chromosome (in respect to the *try* operon) over essential genes of the normal $\phi 80$ prophage and might be inserted into this genome itself.

Lysis occurs in defective lysogens after UV-irradiation or mitomycin C treatment. Their lysates rarely contain a high yield of detectable transducing particles and often do not contain any. When a few plaque forming particles are found (i.e. 1×10^{-6} of the normal yield), most of these are themselves able to transduce *try*. The production and the characteristics of such *pt* phage are unrelated to the eventual presence of a defective transducing prophage. The possible origin of this phage is suggested.

INTRODUCTION

Chromosomal deletions in various strains of *Escherichia coli* cause defective lysogeny for $\phi 80$ or a related phage located in att^{80} (GRATIA, 1964, 1966; FRANKLIN *et al.*, 1965). Such deletions cover the adjacent locus bt_1 which governs adsorption of colicine B and phage T1 so that defective lysogens were isolated after colicine B treatment or from among small colony *T1-r* mutants. The analysis of such deletion lysogens revealed that the prophage is linearly inserted in att^{80} and that the determinant of immunity is the last marked gene reached by deletion (FRANKLIN *et al.*, 1965, GRATIA, 1966).

The present study concerns the effect of deletions arising in lysogenic heterogenotes carrying the normal $\phi 80$ prophage and in addition a defective prophage transducing the whole *try* operon ($\phi 80dt_0$, MATSUSHIRO *et al.*, 1964) or the suppressor su_c ($\phi 80dsu_c$, SIGNER *et al.*, 1965). A large number of mutants were analysed with respect to modification of

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various characters related to the presence of the normal $\phi 80$ and defective transducing prophages. The characters examined were active phage production, immunity, and the transduced marker. Further, the transducing ability of lysates was examined and information obtained on the problem of transduction, as reported in a previous note (GRATIA, 1967).

MATERIALS AND METHODS

1. *Media*

T1-buffer

Synthetic medium (MATSUSHIRO *et al.*, 1964) or Simmons citrate agar with 0.2% glucose was used with (T^+), and without (T^-) tryptophan (100 μg/ml); with 1.5% Bacto-agar (solid agar) or with 0.6% Bacto-agar (soft agar). In some experiments, glucose was replaced by lactose (0.6%).

LB- or tryptone-broth Difco

Nutrient-agar (Eiken, Jap). Difco-Agar was also used mainly to differentiate mutants and wild-type bacteria, mutants forming small colonies on it.

Lambda-agar; polypeptone (Daigo-Eiyo, Jap.), 10 g; NaCl, 2.5 g; agar, 12 g; distilled water 1 l; used with 0.5% agar for plating phage with bacteria.

2. *Bacterial strains*

The following strains of *E. coli* K–12 were used: (1) a try_B (or *try*) and several try_E (or *anth*) point-mutants of W 3102; (2) a lysogenic heterogenote carrying $\phi 80$ and $\phi 80\ dt_0$; (3) M 7067 carrying $\phi 80$ and $\phi 80$ *dsu* and mutation *lac*0° suppressed by the transduced suppressor su_c; (4) a recombinant from W 3102 carrying almost irreversible mutation of try_B and the *lac*0° mutation. These strains were kindly supplied by Drs. Matsushiro, Oiwa and Otsuji and by Dr. Beckwith through Dr. Matsushiro. Other strains were prepared from them.

3. *Bacteriophage lysates*

Besides the phage stocks described in the preceding paper (GRATIA, 1966) a host-range h^{80} mutant of $\phi 80$ and a hybrid $imm^{80}h^{+\lambda}$ (FRANKLIN *et al.*, 1965) were used. These 2 phages were obtained from lysogenic strains kindly provided by Dr. N. Franklin. Lysates of the $\phi 80$ transductional heterogenotes mentioned above were prepared as described below. Transduction experiments were performed with phage P1*kc*.

4. *Isolation and characterization of col B-r mutants*

The procedure described previously (GRATIA, 1966) was slightly modified as follows: colonies of resistant mutants were picked up, transplanted into T^+ agar and, after 18–24 hr incubation, they were replicated on indicator plates for checking their a) tryptophan requirement; b) anthranilic acid requirement; c) active phage production; d) suppression of *lac*-mutation. They were also plated on T^+ agar smeared with phage T1 to test cross-resistance to colicine B and this phage. Reisolated mutants were examined for eventual production of small amounts of particles which could form plaques or transduce. Immunity tests were performed with different homo-immune phages carrying an *h* marker (hetero-immune phages with the same *h* marker were used as controls of adsorption).

5. *Preparation and analysis of the lysates*

Inducible strains were treated with UV-rays or with mitomycin C in T^+ medium. LB medium was added and ca. 1×10^8 bacteria were incubated with aeration for 3–4 hours. Lysates were sterilized with chloroform and centrifuged at low speed. Samples were mixed with heavy suspensions of the recipient bacteria in T1 buffer. After 30 min incubation at 37°C. the mixtures were poured with soft agar on lambda-agar for active phage titration and on selective medium for the try^+, $anth^+$ or lac^+ (i.e. su^+) transductants. Recipient bacteria alone were used as control for reversion.

RESULTS

1. *Linkage relationships between bacterial and phage genes in $\phi 80$-transductional heterogenotes*

The analysis of the segregation patterns of λ-lysogenic heterogenotes (CAMPBELL, 1963) and the results of P1-transduction with λ-double lysogens (CALFF *et al.*, 1965) are interpreted to indicate that the double lysogens are formed by linear insertion of both prophages into the bacterial chromosome, one after the other or one into the other. With $\phi 80$-lysogenic heterogenotes carrying the transducing prophage $\phi 80 dt_0$ with the whole tryptophn operon (MATSUSHIRO *et al.*, 1964) and the normal $\phi 80$ prophage, we looked for similar linkage relationships between the 2 prophages

and chromosomal markers using P1 transduction as follows.

In these experiments, 2 donors were used, which were both tryptophan independent and carried the prophage $\phi 80$. One (donor 1) was *anth*$^+$ at the usual locus while the second (donor 2) carried both alleles, *anth*$^-$ being on the chromosome and *anth*$^+$ on the persisting transduced fragment. The recipient was marked either by *try*$^-$ mutation or by *cys*$^-$ and *anth*$^-$ mutations and carried a homoimmune derivative of $\phi 80$ with the host-range h^{80} or h^+ marker. The *try*$^+$ or *cys*$^+$ recombinants were selected on synthetic medium supplemented with anthranilic acid and scored for the markers anthranilate-independence and host-range of the phage produced.

In experiments I and II of Table 1, ca. 40% *cys*$^+$ recombinants inherited the *anth*$^+$ marker of donor 1 while only 1% at most receive the same marker from donor 2. The co-transduction of the *anth*$^+$ allele and the *cys*$^+$ gene from this donor is therefore much reduced, indicating another position for *anth*$^+$, at a distance from the usual locus. Fifteen of the *cys*$^+$ recombinants inherited the h^{+80} marker of $\phi 80$ prophage from both donors, but linkage relationships between this marker and nutritional markers differed according to the donor used. In donor 1, the order appeared to be as reported (GRATIA, 1966), i.e. *cys—anth —try—h* while, in donor 2, the position of the *anth*$^+$ and h^{+80} markers with respect to the *cys*$^+$ marker seems to be inverted i.e. *cys—h*

TABLE 1 *The co-transduction of nutritional markers of the chromosome or the transducing prophage $\phi 80\, dt_0$ and of the host-range (h) marker of the phage produced*

Exp.	Donor 1	Donor 2	Acceptor	Selected Marker (SM)	Non Selected Marker (NSM)	Co-transduction SM-NSM among 100 recomb.
I	+ ; $\phi 80$ (h^{+80})		*cys anth* $\phi 80$ (h^{80})	*cys*$^+$	*anth*$^+$	36
					h^{+80}	15
					anth$^+$ h^{+80}	15
			„ „ $\phi 80.\lambda$ ($h^{+\lambda}$)	„	*anth*$^+$	44
					h^{+80}	16
					anth$^+$ h^{+80}	15
II		*anth*$^-$; $\phi 80$; $\phi 80\, dt_0$ (*anth*$^+$)	*cys anth* $\phi 80$ (h^{80})	*cys*$^+$	*anth*$^+$	0 (<1) (1)
					h^{+80}	15
					anth$^+$ h^{+80}	0
			„ „ $\phi 80.\lambda$ ($h^{+\lambda}$)	„	*anth*$^+$	1
					h^{+80}	14
					anth$^+$ h^{+80}	1
III	+ ; $\phi 80$ (h^{+80})		*try* $\phi 80$ (h^{80})	*try*$^+$	h^{+80}	61
			„ $\phi 80.\lambda$ ($h^{+\lambda}$)	„	„	68
IV		*anth*$^-$; $\phi 80$; $\phi 80\, dt_0$ (*anth*$^+$)	*try* $\phi 80$ (h^{80})	*try*$^+$	*anth*$^+$	18
					h^{+80}	67
					anth$^+$ h^{+80}	6
			„ $\phi 80.\lambda$ ($h^{+\lambda}$)	„	*ahth*$^+$	30
					h^{+80}	55
					anth$^+$ h^{+80}	13

(1) selected *cys*$^+$ *anth*$^+$ colonies appeared with a frequency of 0.9% among the *cys*$^+$ recombinants.

—*anth*. Indeed, the fact that in experiment II rare *anth*$^+$ recombinants were found among the recombinants which inherited the host-range h^{+80} marker strongly suggests that the transduced *anth*$^+$ allele is located on the " right " side with respect to *cys*.*

In the experiments III and IV, the h^{+80} allele is cotransuduced with the selected *try*$^+$ marker with a frequency of ca. 60% from both donors 1 and 2. In experiment IV, 18-30% of the try$^+$ recombinants inherited the *anth*$^+$ gene mostly through by a cross-over between the chromosomal *anth*$^+$ and *try*$^+$ markers, but also, among those with the h^{+80} marker, some would arise by co-transduction of the *try*$^+$ marker of the donor chromosome and the *anth*$^+$ gene carried by $\phi 80dt_0$. Indeed, one of these *try*$^+$ *anth*$^+$ h^{+80} transductants inherited the ability to segregate *anth*$^-$ cells, indicating that it received the whole segment with the following presumed sequence of markers: *anth*$^-$ *try*$^+$ h^{+80} *anth*$^+$.

None of the P1 transductants was found to have inherited the HFT property of donor 2. This observation can be related to the linear configuration of the dt_0 genome since the segments transferred by P1 would in that case contain only part of this prophage. The observation of a recombinant which can segregate is unusual indicating that the diploid structure is not always reproducible and that most strains which receive a long segment may integrate the wild-type alleles separately in their respective loci.

$\phi 80dt_0$ carries the partial prophage genome of $\phi 80$ including the h^{+80} marker. As this marker is transduced with the same frequency whether the donor carries the $\phi 80$ prophage only or both prophages, this marker is presumed to be derived from the normal $\phi 80$ prophage in most cases.

Therefore, the linear insertion of $\phi 80dt_0$ on the right side of the normal $\phi 80$ genome—or part of it—appears to be highly possible and is considered in the following study.

2. *Characterization of the deletion mutants*

Several derivatives of *E. coli* K-12, mostly strain W 3102 lysogenic for $\phi 80$, were used either singly carrying $\phi 80$ or carrying $\phi 80$ and also the $\phi 80dt_0$ transducing segment *anth-try* or $\phi 80dsu$ transducing su_c without *try*. Numerous mutants which were highly resistant to colicine B were isolated and characterized for the markers concerned; *try*$^+$, independence of tryptophan in the absence or presence of a precursor; *anth*$^+$, independence of anthranilic acid; *su*$^+$, suppressor of mutation *lac*0°; *ly*$^+$ active phage production; imm^{80}, $\phi 80$-immunity.

As shown in Table 2, mutants were classified into 4 categories previously described (GRATIA, 1966). In lysogenic heterogenotes as in single lysogens, some deletions affect *try* genes (bt_1try) or suppress active phage production (*ly*$^-$). They do not necessarily affect the transduced *anth*$^+$ or *su*$^+$ marker. Most mutants of *anth*$^+$/*anth*$^-$ heterogenotes are still tryptophan-independent and active phage producer; some are anthranilate-requiring whether they carry an irreversible mutation (deletion) or a reversible one in the bt_1 locus. They correspond to class I (bt_1, *ly*$^+$). Very rare mutants of class II retain the ability to produce active phage particles but are tryptophan-requiring through deletion on the left side in the *try* operon (bt_1try, *ly*$^+$). They are no longer complemented by the transduced *try*$^+$ genes. Some class III mutants which have lost the ability to produce active phage by deletion extending into the $\phi 80$ genome (bt_1, *ly*$^-$) still keep $\phi 80$ dt_0 as revealed by their prototrophic character, their eventual ability to segregate *anth*$^-$ cells (some are very stable) or to produce (in variable amount according to the strain) particles transducing the *anth*$^+$ gene (cf. 3). In the mutants of class IV, long deletions affect *try* genes on one side and the integrated $\phi 80$ prophage on the other.

From the results of the P1-transduction

* From now, as in Fig. 1 of the preceding paper, the chromosome is oriented from " left " with *cys* to " right " with $\phi 80$.

experiments reported above, the transduced *anth*$^+$ marker is not located in the *try* operon but at some distance from it on the " right " side over the *h* locus in the proximal part (in respect to *try*) of the integrated genome of ϕ80. The various categories of mutants result from deletions of variable extent in a non-modified segment *try*—bt_1—att^{80}. The *anth*$^-$ mutants of class I and mutants of class II have presumably lost the transduced genes (or $\phi 80 dt_0$) spontaneously but the *anth*$^-$ mutants of the classes III and IV have deletions on the right side which eventually affect the transduced *try*$^+$ and *anth*$^+$ genes.

These deletions hardly reach the immunity gene which belongs to the normal ϕ80 prophage (as shown in other experiments). The rare mutants which are no longer immune to ϕ80 have apparently lost both prophages. Therefore, the immunity gene which marks the distal end of the ϕ80 prophage might be located further away through linear insertion of $\phi 80 dt_0$ into this genome.

The same characteristics seems to occur in *su*$^+$/*su*$^-$ heterogenotes. From strain M 7067, 512 mutants from 10 subclones were also classified into 4 classes, by scoring, with respect to the *try* and *ly* markers (Table 2). The transduced *su*$^+$ marker persists in most mutants of each class but though the percentage of *su*$^-$ cells in almost the same for mutants of class I and III (18–19%) it is higher for mutants of class IV due to long deletions (31%) This suggests that the *su*$^+$ marker is located over essential genes and can be reached by long deletions only. Here also, the ϕ80-immunity gene has disappeared in rare mutants which have not apparently kept any prophage.

3. *Transduction properties and characteristics of defective lysogenic heterogenotes*

In single lysogens carrying deletion extending to ϕ80, the LFT property has only been found in strains producing infectious particles (FRANKLIN *et al.*, 1965 ; GRATIA, 1967). In lysogenic heterogenotes, the same appears to be true, since the HFT is reduced to various extents in different strains and seems to be correlated with the production of infectious phage (Table 3).

On the other hand, a few defective lysogens in which the ϕ80 genome is probably not essentially affected by deletion have acquired a particular property of transduction. Indeed, these mutants produce few infectious particles (about 10^{-6} less than normal) and, in

TABLE 2 *Classes of col B^r mutants derived from single lysogens or lysogenic heterogenotes*

Strain	Transduced Marker	Number of Mutants (1)	% Mutants of Class I bt_1, ly^+ (2)	II bt_1 *try*, ly^+	III bt_1, ly^- (2)	IV bt_1 *try*, ly^- (2)	Loss of Immunity in ly^- Mut
W3102 (ϕ80) *anth*$^-$ or$^+$		379(18)	84.4	1.1	10.6	3.9	
anth$^-$ (80.λ)	—	25(3)	92.0	0	8.0	0	1/ 15
„ (λ.80)		133(5)	97.0	0	3.0	0	
W3102 *anth*$^-$ —1		200(19)	69.0(34 a^-)	2.5	16.5(45 a^-)	12.0	
(ϕ80 ; $\phi 80\, dt_0$) —2	*anth*$^+$	72(6)	94.5(32 a^-)	0	4.2(1/3 a^-)	1.3	1/ 85
(3) —3		211(8)	93.0(62 a^-)	2.3	16.0(85 a^-)	18.7	
M7067	su_c^+	512(10)	43.6(19 su^-)	2.5(1/13)	32.2(18 su^-)	21.7(33 su^-) (4)	4/106

(1) The number of subclones used is indicated in parenthesis.
(2) a^- ; su^- : percentage of mutants of one class reexpressing the *anth*$^-$ or *su*$^-$ character.
(3) 3 lysogenic heterogenotes derived from distinct *try* E point-mutants.
(4) The percentage of *su*$^-$ cell is higher for mutants of class IV in 8 subcultures ; it is unlikely to be due to the accumulation of one type of mutant.

TABLE 3 *Transducing properties of some lysogenic heterogenotes carrying normal or defective φ80 prophage*

	Strain	Mutation	Infectious non-transduc. particles	Defective transducing particles		Non-defective transducing particles		
				anth$^+$	*su*$_c^+$	*anth*$^+$	*try*$^+$	*su*$^+$
1	W3102 (φ80)/B	bt_1	0.4×10^{10}	—	—	—	—	—
2	,,	bt_1, *ly*$^-$	1×10^6	—	—	—	—	—
3	,, (*anth*$^+$)	,,	$\pm1\times10^3$	—	—	1×10^4		—
4	,, (*anth*$^-$)	,,	$\pm1\times10^3$	—	—	—	—	—
5	M3102 (φ80 ; φ80 dt_0)	—	0.7×10^{10}	1.7×10^7	—	—	—	—
6	(φ80 ; φ80 *dsu*)	—	0.7×10^{10}	—	1×10^4	—	—	—
7	W3102 *anth*$^-$ (φ80 ; φ80 dt_0)/B	bt_1, *ly*$^+$ *rev* (1)	0.5×10^{10}	1×10^7	—	0	0	
8	,, (H68)	bt_1, *ly*$^-$	$\pm0.5\times10^6$	1.8×10^3	—	0	2.2×10^5	—
9	,,	,,	$\pm0.5\times10^2$	0	—	0	0	—
10	M7067/B (M6)	bt_1, *ly*$^-$	?	—	0.6×10^3	1×10^4		0
11	W3102 *lac* 0° (φ80 ; φ80 *dsu*)/B	bt_1, *ly*$^-$	1.5×10^5	—	2.5×10^3	0	0	0
12	,,	bt_1, *ly*$^-$	0.5×10^6	—	0 or 2 $\times10^2$(2)	0	0	0
13	,,	bt_1 *try*, *ly*$^-$	?	—	0 or 2 $\times10^2$(2)	0.5×10^3	0	0
14	,,	,,	0	—	,,	0	0	0

(1) reversible mutation.
(2) high number of revertant colonies.

most cases, these nondefective particles are themselves able to transduce a segment of the bacterial chromosome including *try* genes (*φ80ptd;* GARTIA, 1967). *φ80ptd* most often transduces all the *try* genes unaffected by deletion including *anth*, which is the most distal with respect to *att*^{80}. The *ptd* particles never transduce *anth*$^+$ from lysogenic heterogenotes with an *anth*$^-$ mutation on the chromosome and the allelic *anth*$^+$ gene carried by *φ80dt*$_0$. Among mutants of φ80 lysogenic *su*$^+$/*su*$^-$ heterogenotes, some produce a subnormal amount of infectious phage (10^{-3} of the normal yield) and in addition *φ80dsu* particles (Table 3, n° 11); other mutants which produce less active phage (n° 10, 13; 10^{-6}) usually produce no detectable *φ80dsu* particles but may yield *ptd* phage particles transducing *try*, not *su*$_c$. The production and the characteristics of this plaque-forming transducing phage therefore appear unrelated to the *φ80dt*$_0$ or *φ80dsu* prophage and moreover are unchanged in haploid segregants.

The deletion lysogen H68 which is *anth*$^+$ *try*$^+$ stable and produces few infectious or transducing particles has been studied in some detail (Tables 3 and 4). Its lysate contains ca. 10^5 plaque-forming units which are of at least 2 types, the *pt* type, which can transduce *try*$^+$ but not *anth*$^+$ and the non-transducing *p* type. The phage of type *pt* gives rise to large colonies surrounded by partial growth of the recipient bacteria and a lytic halo is more or less apparent; *p* phage particles form tiny plaques of 1-2 mm in diameter in the background of the recipient bacteria slightly growing on the medium used, but no additional growth occurs. The lysate also contains *dt* particles which can mostly transduce *anth*$^+$ as the initial *dt*$_0$ type does, but the

TABLE 4 *Production of detectable particles by induced H68 bacteria* (45 sec irradiation; plating with *str-r* recipient bacteria carrying *try*-mutation on synthetic T⁻ agar slightly enriched; the induced bacteria are counterselected by application of a very thin layer of streptomycin-agar after 6 hr incubation). Analysis of the individual production after dilution, repartition in a series of tubes, 4 hr incubation at 37°C and plating with recipient bacteria on synthetic agar slightly enriched and supplemented with streptomycin. Particles are detected by their ability to form plaques (*p*, plaque-forming; *pt*, plaque-forming transducing) or to give rise to colonies (*dt*).

Number of irradiated bacteria		0.93×10^8 (1.2% survival)			
Number of infective center	type *p* type *pt*	7.5×10^4 4.3×10^4			
Number of colonies (*dt*)		2.7×10^3 { 19 non lysogenic 4 lysogenic			
Nubmer of irrad. bact. per tube		1.9×10^3			
Number of tubes contain. detect. partic.		12/60 *p* 7	*pt* 4	*p; pt* 1	*dt* 0
Number of plaque-forming *p* or *pt*/tube		1 6 7 9 12 18 37	1 3 4 94	4; 1	

amount of these phage is ca. 10^{-3} of lysates less than in lysates prepared from wild-type strains or non-defective lysogens with reversible bt_1 mutation. The rate of transduction is somewhat increased in irradiated recipients which are lysogenic for $\phi80$ or after mixed infection with helper phage $\phi80$ (1×10^9/ml). This helping effect is about the same as that found with a diluted lysate of wild-type bacteria (cf. MATSUSHIRO *et al.*, 1964). When 10^8 induced bacteria are plated on λ-agar with sensitive bacteria, ca. 10^5 infective centers are formed. If they are plated on slightly enriched T⁻ agar (their growth is inhibited), different types of plaques are again apparent indicating that each type comes from a independent bacterium; a few colonies also appear, 19 on the 23 checked being non-lysogenic and resulting from infection by *dt* particles only. The individual production of detectable phage particles of the various types has been analysed: 12 tubes which were each inoculated with one productive bacterium, after lysis 7 contained a variable but low number of plaque-forming-units of type *p* only, 4 contained *pt* particles and 1 contained both types; no colonies due to the *dt* particles appear. Therefore, a few induced bacteria produce particles of type *p*, *pt* or *dt* but none (almost) produces these 3 types in the same time. Therefore, even though the reduced number of *dt* particles produced was somewhat in parallel with the reduction of the infectious phage yield, the particles were not necessarily due to the bacteria producing plaque-forming phage.

DISCUSSION

Lysogenic heterogenotes carrying the normal $\phi80$ and defective transduceing $\phi80dt_0$ or *dsu*- prophages were compared with single lysogens with respect to the effect of deletions arising in the region of the $\phi80$ attachment site. It appears that the presence of $\phi80dt_0$ or $\phi80$ *dsu* not modify the respective position of the chromosomal genes and prophage genes including essential genetic determinants of active phage production. In agreement with results of P1 transduction experiments, a preliminary analysis of deletion mutants indicates that the transducing prophage is integrated on the " right " side of the *try*—bt_1—att^{80} segment and suggests its possible insertion into the genome of the normal $\phi80$ prophage itself, between essential genes for phage production close to the *h* locus and the immunity gene marking the distal end of $\phi80$. A similar conclusion was reached by CALFF

et al. (1965) in interpreting their results on double λ-lysogens studied by P1-transduction. The observations of SIGNER (1966) with prophage *λdg* and the TAYLOR and YANOFSKY (1966) with *ϕ80pt* suggest an interaction with a hybrid helper phage in att^{80} or in att^{λ} that these authors interpreted as also representing an intra-prophage insertion at the sites of genetic homology.

Thus far, analysis of the transducing properties of defective lysogens indicates that the production of transducing particles is somewhat related to the degree of defectivity of the normal *ϕ80* prophage. In a culture of the defective lysogen H68, the low production of dt_0 particles is not necessarily due to rare bacteria which produce infectious particles but merely to bacteria in which presumably detachment and some vegetative development occur.

The transducing ability of a very slight amount of released active phage (1×10^{-5} of normal) is due to the *ϕ80* genome which is slightly affected by deletion and is not related to the eventual presence of a defective transducing prophage like $\phi80dt_0$ or *dsu*. This plaque-forming transducing deletion (*ptd*) phage transduces a segment of the bacterial chromosome located on the other side of the deletion. This segment most often carries the whole *try* operon or, naturally, the genes unaffected by deletion. Some *ϕ80ptd* producers also yield non-transducing phage, which is still infectious, and analysis of individual production (of Table 4) shows that this type of particles is not (or not usually) produced by the same bacteria in which *ptd* particles are formed. It seems that the *ϕ80* prophage still carries the genetic information for active phage production but that, owing to deletion of one end of the integrated genome, circularization and detachment occur at low frequency by means of rare crossing-over, either very near the deletion in some bacteria or well over the deletion in others where the prophage incorporates a segment of the bacterial chromosome carrying *try* genes.

ACKNOWLEDGEMENTS

This work mostly done in Japan was supported by the Japan Society for Promotion of Science, the Fonds National Belge de la Recherche Scientifique and the Fondation Francqui of Belgium. I am indebted to Dr. AMANO T. for kind hospitality in his Institute and to Dr. MATSUSHIRO A. who made everything available for the realization and publication of this work. Part of it was performed in Liège; I wish to thank Dr. FREDERICQ P. for hospitality and financial aid during this period.

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