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Multistep transduction of tryptophan (*trp*) genes in *Escherichia coli*

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1. INTRODUCTION

Inducible temperate bacteriophage ϕ80 is capable of specialized transduction of tryptophan (*trp*) markers in *Escherichia coli* (Matsushiro, 1963). Some of the ϕ80 transducing particles are still able to form plaques and are designated with the symbol *pt* (plaque-forming transducing; Matsushiro, Sato & Kida, 1964; Gratia, 1967*a*; Deeb, Okamoto & Hall, 1967). Upon infection of T_1r Tryp$^-$ deletion mutants with *pt* phages, the transduced genes complement the corresponding deleted segments of the tryptophan operon (Taylor & Yanofsky, 1966*a*; Deeb *et al.* 1967). This paper presents results, previously reported in an abstract (Gratia, 1967*b*), on infection of deletion mutants lacking most or all tryptophan genes by *pt* phages transducing only segments of the tryptophan operon; when they infect such mutants, *pt* phages carrying overlapping *trp* segments can co-operate to allow normal synthesis of tryptophan. Successive transductions lead to the formation of polylysogenic di-, tri- and probably also tetraploid heterogenotes.

2. MATERIAL AND METHODS

A schematic representation of the genetic regions of the ϕ80 *pt* phages and host chromosome which are relevant to the present study is presented in Fig. 1.

Media. The liquid media are as described by Matsushiro *et al.* (1964): T_1 buffer; synthetic medium; LB broth. The solid media are: Difco agar; LB agar; Simmons citrate agar supplemented with 0·2% glucose and 0·1% acid hydrolysate of casein (NBC).

Abbreviations: bt_1, locus governing adsorption of the colicines B, I and V and phages T_1 and ϕ80; att^{80}, attachment site of phage ϕ80 on the bacterial chromosome; *trp A*, *B*, tryptophan synthetase genes (mutation in *trp A* causes indole requirement (Ind-) and in *trp B* tryptophan requirement (Tryp$^-$)); *trp C*, indoleglycerol-phosphate synthetase gene (mutant phenotype: Ind-); *trp D*, phosphoryl transferase gene (mutant phenotype: Ind-); *trp E*, anthranilate synthetase gene (mutant phenotype: Ant-, anthranilate requirement); O_t, tryptophan operator gene; Δ*trp CBA*, deletion of variable extent stretching from bt_1, covering *trp A* and ending in either *trp B* or *trp C*; other deletions end in *trp D*, *trp E* or further as

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determined by genetic recombination with phage P_1; i^{80}, i^{λ} and h^{+80} mean respectively immunity of ϕ 80, λ and wild-type host range of ϕ 80.

Strains. Derivatives of *E. coli* C and K 12 were used. Their characteristics are recorded in Table 1. Many strains carry a prophage in the attachment site of phage ϕ 80 (att^{80}), either ϕ 80 or a hybrid phage with the same location and host range (h^{+80}) as ϕ 80 but having the immunity of lambda ($\lambda hy80_1$; Signer, 1964) and designated here λ 80. They carry either a point-mutation in the *trp* operon or a deletion of variable extent from *trp A* to *trp C* or further. Deletion mutants were all

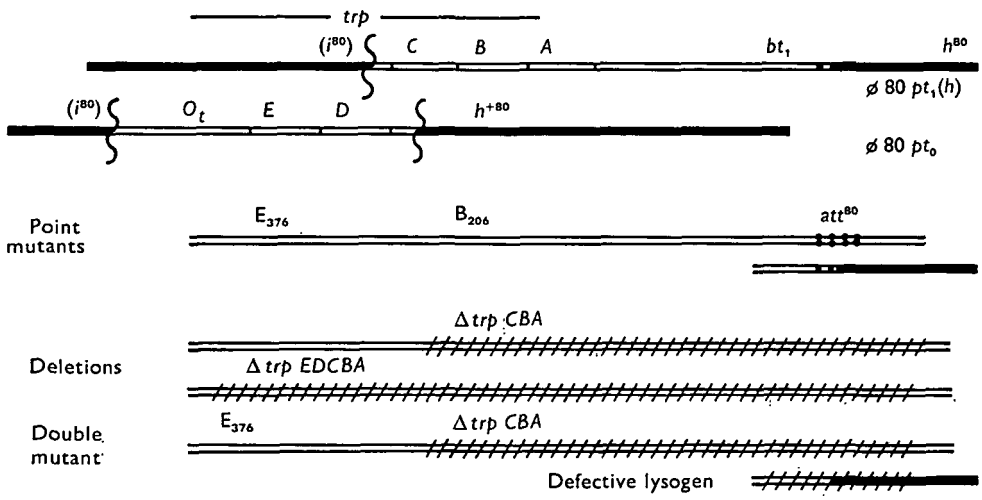

Fig. 1. Schematic representation of the genetic structure of phages ϕ 80 $pt_1(h)$ and ϕ 80 pt_0 and host chromosomes (according to Yanofsky & Lennox (1959), Gratia (1964), Franklin *et al.* (1965), Sato & Matsushiro (1965)). The phage genome is shown in black and the positions of deletions of the phage genome are indicated by undulating lines. (i^{80}) is the presumed location of the immunity determinant. The presence of the bt_1 locus in ϕ 80 pt_1 and its absence in ϕ 80 pt_0 are established in the present work. E_{376} and B_{206} are point mutations in strains W3102.376 and W3102.206 respectively. Deleted regions in the host chromosome, including the attachment site of ϕ 80 (att^{80}) in the non-lysogenic recipient, or proximal part of the prophage genome herewith integrated, are shown by hatching.

obtained among mutants selected for cross-resistance to colicine B and phage T 1 as deletion always covers the bt_1 locus governing sensitivity to these agents (Yanofsky & Lennox, 1959; Gratia, 1964). Usually deletion also affects the attachment site of ϕ80 or part of the prophage genome herewith integrated (Gratia, 1964; Franklin, Dove & Yanofsky, 1965).

Transducing ϕ80 pt *phages.* The *pt* phages most used in the present work are those described by Sato & Matsushiro (1965) and kindly provided by these authors: ϕ 80 pt_0 carrying the *trp* $(O_tED)^+$ segment; ϕ 80 pt_1 carrying *trp* $(CBA)^+$. To infect deletion mutants, which are all resistant to ϕ80 (Matsushiro, 1963), the derivative ϕ 80 pt_1 (*h*) carrying a host-range mutation (h^{80}) inherited from recombination with ϕ80 *h* (Franklin *et al.* 1965) was used (ϕ80 *h* was obtained from N. Franklin through Dr Matsushiro). Other *pt* phages are of the *ptd* type produced

by defective lysogens carrying a terminal deletion of the integrated prophage genome (Gratia, 1967*a*), namely $\phi 80$ ptd_{28} (i^{80}, h^{80}, *trp* $(CBA)^+$), $\phi 80$ ptd_{68} (i^{80}, h^{+80}, *trp* $(DCBA)^+$), $\lambda 80$ ptd_9 (i^{λ}, h^{+80}, *trp* E^+). The techniques are as described by Matsushiro *et al.* (1964).

Table 1. *Tryp⁻ strains of* Escherichia coli: *point-mutants and deletion mutants*

(1) Point mutants of *E. coli* K 12

Strain and source*	Tryp phenotype	Genotype
W 3623 (M)	Ind-	*trp A*
W 3102.206 (M)	Tryp⁻	*trp B; also* ($\phi 80$) or ($\lambda 80$)
T 24 (M)	Ind-	*trp C* (λ)
T 16 (M)	Ind-	*trp D* (λ)
T 15	Ant-	*trp E* (λ)
W 3102.376 (M)	Ant-	*trp E; also* ($\phi 80$) *or* ($\lambda 80$)
HCA 23 (Y)	Ind-	*trp A, cys B, his* (λ; P_1); *also* ($\phi 80$)
T 25.16	Ant-	*trp E*, etc. (cf. HCA 23)

(2 A) Deletion mutants of *E. coli* K 12†

G 32, G 35	Tryp⁻	Δ*trp CBA*
G 31, G 38, G 39	Tryp⁻	Δ*trp DCBA*
G 33/S	Tryp⁻	Δ*trp EDCBA*
G 48	Tryp⁻	Δ*trp CBA* + *trp E*
H 8	Tryp⁻	Δ*trp CBA* + *trp E* ($\phi 80$)
H 35	Tryp⁻	Δ*trp CBA* + *trp E* ($\phi 80$d)
H 23	Tryp⁻	Δ*trp DCBA trp E* ($\phi 80$)
H 7, H 19, H 25, H 35	Tryp⁻	Δ*trp DCBA* + *trp E* ($\phi 80$d) (or *EDCBA*)
Md 73	Tryp⁻	Δ*trp CBA* ($\phi 80$ lost by deletion)
Md 19	Tryp⁻	Δ*trp EDCBA* ($\phi 80$d)
2.1/6	Tryp⁻	Δ*trp DCBA* (λ)
L 96	Tryp⁻	Δ*trp CBA* ($\phi 80$d) (λ; P_2)

(2 B) Deletion-mutants of *E. coli* C

C/B, trp	Tryp⁻	Δ*trp CBA*
L 7	Tryp⁻	Δ*trp...CBA*
L 1	Tryp⁻	Δ*trp...CBA* ($\phi 80$d)
L 8	Tryp⁻	Δ*trp CBA* ($\lambda 80$d)
L 9	Tryp⁻	Δ*trp DCBA* ($\lambda 80$d)

* (M), (Y): strains kindly provided by Dr Matsushiro and Dr Yanofsky; other strains derive from them by mutation or genetic recombination.

† Mutants of independent origin, mostly derived from W 3102.

3. RESULTS

(i) *Transduction of the* trp $(CBA)^+$ *segment by* $\phi 80$ pt_1 *and related phages*

Tryp⁻ bacteria carrying either point mutation or deletion in the *trp* operon were infected by $\phi 80$ $pt_1(h)$. In some cases the recipients carried $\phi 80$ or the hybrid phage $\lambda 80$, which has λ-immunity. The results varied depending on the case, as follows (Table 2).

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Table 2. *Transduction of the* trp $(CBA)^+$–bt_1^+ *segment by* $\phi 80$ pt_1(h)

	Recipient		Prophage in *att*^{80}*	Dilution of phage sample used†	Number of transduct. colonies‡	Relative transduct. rate (%)§	Phenotype		Other recipients giving similar results
(1)	W 102.206	*trp B*	—	10^{-4}	169	100	Tryp$^+$	T_1^s	W 3623; HCA 23; T 24
(2)	G 32	Δ*trp CBA*	—	10^{-4}	152	90	Tryp$^+$	T_1^s	G 35; Md 73; *C/B, try*
(3)	2.1/6	Δ*trp DCBA*	—	10^{-4}	208	120	Ind-	T_1^s‖	G 31; G 38; G 39; G 43
(4)	W 3102.206 (ϕ80)	*trp B*	ϕ80	10^{-2}	75	0·45	Tryp$^+$	T_1^s	HCA_{23} (80)
(5)	W 3102.206 (λ80)	*trp B*	λ80	10^{-3}	152	9	Tryp$^+$	T_1^s	
(6)	Md 19	Δ*trp EDCBA*	ϕ80 d	10^{-2}	70	0·41	Ind-	T_1^s	L 1; L 7; L 96
(7)	L 9	Δ*trp DCBA*	λ80 d	10^{-3}	105	6	Ind-	T_1^s	L 8
(8)	G 48	Δ*trp CBA* + *trp E*	—	10^{-4}	150	89	Ant-	T_1^s	
(9)	H 8	Δ*trp CBA* + *trp E*	ϕ80	10^{-2}	68	0·40	Ant-	T_1^s	H 35
(10)	H 7	Δ*trp DCBA* + *trp E*	ϕ80 d	10^{-2}	97	0·57	Ind-	T_1^s	H19; H 23; H 25; H 55

* ϕ80 or ϕ80 d: ϕ80-immunity; λ80 or λ80 d: λ-immunity.

† 0·1 ml. of dilution 10^{-n} + 0·4 ml. Tryp$^-$ bacteria in T_1 buffer 30 min at 37°.

‡ Counted after 48 h incubation on minimal medium slightly enriched with acid hydrolysate of casein, and supplemented with either indole or anthranilic acid when required.

§ The number of transductants was referred to the number of *trp* B^+ colonies from the non-lysogenic recipient (line 1). Experiments were run 2 or 3 times with highly reproducible results.

‖ Unstable or abortive transductants growing in conditions of continuous reinfection on medium supplemented with indole; partial sensitivity to T_1.

(*a*) *Non-lysogenic recipients.* (1) When the recipient carried a point-mutation in *trp A*, *trp B* or *trp C* (Table 2, line 1), almost every particle of $\phi 80$ pt_1 (or -$pt_1(h)$) gave rise to a regular round-shaped colony of trp^+ bacteria. Around each colony a lytic halo was more or less apparent in the background of the surrounding bacteria. (2) With recipients carrying a deletion extending from *trp A* to *trp B* or *trp C* ($\Delta trp\ CBA$), $\phi 80$ $pt_1(h)$ particles gave rise to a similar type of trp^+ colony (line 2). (3) With recipients carrying a longer deletion which overreaches *trp C*, the *trp D* gene was not complemented by $\phi 80$ $pt_1(h)$ and transductants appeared only on medium supplemented with indole (line 3). (4) With recipients carrying $\Delta trp\ CBA$ and in addition a *trp E* point mutation, the pt_1 transductants still required anthanilic acid (line 8).

When *trp* point mutants were used as recipients, the transductants were either trp^+ recombinants or diploid trp/trp^+ heterogenotes or trp^+/trp^+ homogenotes; they were more or less stably lysogenic and sometimes retained their $Tryp^+$ character after being cured of $\phi 80$ pt_1. But when deletion mutants were used, transductants (150 derived from three recipients were checked) were all diploid heterogenotes which did not recombine the transduced trp^+ alleles, as shown by (i) the persistence of lysogenicity for a *pt* phage and (ii) the segregation of cured $Tryp^-$ bacteria (detectable among survivors of u.v.-treated populations, or by their resistance to phage T_1 as described below). The persistence of the prophage in the transduced deletion mutants strikingly differs according to the case. Indeed, transductants derived from recipients lacking a short segment with *trp B* and *trp A* were stable diploids and segregated very few haploid cells (lines 2 and 8). On the contrary, transductants derived from recipients lacking a larger segment over-reaching *trp C* were very unstable and rather abortive, and were able to grow on indole without tryptophan only under conditions of continuous reinfection (line 3).

(*b*) *Lysogenic or defective lysogenic recipients carrying a prophage in* att^{80}. (1) With recipients carrying a point mutation, $\phi 80$ pt_1 gave rise to colonies of the usual type but at a reduced rate, i.e. 10^{-1} per adsorbed phage if the prophage of the recipient had the immunity of lambda and 10^{-2} or even less if immunity was of $\phi 80$ (lines 4–5). (2) With recipients carrying a deletion the results were similar but, if the deletion over-reached *trp C*, again the transductants were indole-requiring. Now, in all lysogenic or defective lysogenic recipients carrying a prophage adjacent to the deletion, the transducing prophage was stably maintained whatever the extent of the deletion in the *trp* operon. Those which carried a long deletion and were not entirely complemented were stable indole-requiring diploids (lines 6, 7 and 10). The rate of transduction to deletion mutants, as to point mutants, was reduced; the reduction was the same whether or not deletion affected the integrated prophage (except in the case of Md 73, where the immunity determinant, which is distal (Franklin *et al.* 1965), is deleted; here the rate was normal either because of elimination of i^{80} or complete disappearance of the prophage). The transduction rate of λ-lysogens was normal (lines 1 and 3: strains T 24 and 2.1/6).

Phage $\phi 80$ pt_1 transduced the wild-type gene bt_1^+ conferring sensitivity to

phage T_1 and $\phi 80$ (as seen by susceptibility to hetero-immune h^{+80} phages, such as $\lambda 80$ or $\phi 81$), and to colicines B, I and V. In newly transduced strains the ability to adsorb $\phi 80$ particles was detectable only after 12–15 h incubation and then increased gradually for a similar period of time. During exponential growth, stable transductants lost the transducing prophage with a frequency of $5–10/10^4$ cells and the segregants re-expressed their resistance character after a certain delay. Another phage $\phi 80$ ptd_{28}, produced by a defective lysogen and transducing also segment *trp* $(CBA)^+$, gave results similar to those already described, except that it did not transduce the sensitivity determinant as it carries a deletion involving the bt_1 locus. As described previously for the resistant mutants (Gratia, 1967*c*), these apparently resistant transductants were, however, able to adsorb $\phi 80$ particles to a very small extent.

(ii) *'Supertransduction' of* pt_1 *heterogenotes by* $\phi 80$ pt_0 *or related phages*

The trains carrying long deletions and stably transduced to tryptophan independence by $\phi 80$ $pt_1(h)$ are still indole-requiring, because the transducing prophage carries only the *trp* $(CBA)^+$ segment. They were superinfected with $\phi 80$ pt_0 (Table 3,

Table 3. *Transduction or supertransduction of the* trp $(ED)^+$ *segment by* $\phi 80$ pt_0

	Initial recipient			Final recipient			Relative transduction rate (%)‡
				1st exogenote*	2nd exogenote†	Phenotype	
(1)	W 3102.376	*trp E*		—	—	Ant- T_1^s	100
(2)	W 3102.376 ($\phi 80$)	*trp E*	$\phi 80$	—	—	Ant- T_1^s	12
(3)	W 3102.376 ($\lambda 80$)	*trp E*	$\lambda 80$	—	—	Ant- T_1^s	14
(4)	L 9	Δ*trp DCBA*	$\lambda 80$d	*trp* $(CBA)^+$ bt_1^+	—	Ind- T_1^s	0·2
(5)	Md 19	Δ*trp EDCBA*	$\phi 80$d	*trp* $(CBA)^+$ bt_1^+	—	Ind- T_1^s	0·2
(6)	—	—		*trp* $(CBA)^+$	—	Ind- T_1^r	0·001
(7)	—	—		*trp* $(CBA)^+$ bt_1^+	*trp* $(DCBA)^+$	Ant- T_1^s	4
(8)	G 48	Δ*trp CBA* + *trp E*		*trp* $(CBA)^+$ bt_1^+	—	Ant- T_1^s	3
(9)	—	—		*trp* $(CBA)^+$	—	Ant- T_1^r	0·001
(10)	H 7	Δ*trp DCBA* + *trp E*	Ø80d	*trp* $(CBA)^+$ bt_1^+	*trp* $(DCBA)^+$	Ant- T_1^s	5

* Prophage $\phi 80$ pt_1 (h), or $\phi 80$ ptd_{28}. † Prophage $\phi 80$ ptd_{68}. ‡ Cf. Table 2.

lines 4–5) and plated on minimal medium; trp^+ colonies appeared. The same result was obtained when indole-requiring diploids carrying a Δ*trp DCBA* deletion were superinfected with a *−ptd* phage (ptd_{68}, p. 83) complementing *trp D* without *trp E*. But when recipients carrying a longer deletion were used, *trp E* was still not complemented by this *ptd* phage and the transductants expressed the anthranilate requirement. Such second-step heterogenotes were finally used as recipients for a transduction by $\phi 80$ pt_0 carrying the *trp* $(ED)^+$ genes or a *−ptd* phage carrying *trp* E^+ and were then transduced to complete tryptophane independence by acquiring the ability to synthetize anthranilic acid (line 7). In the same way, double

mutants carrying Δ*trp DCBA* and *trp E* point mutation have been infected with transducing phages in three steps and again supertransduced *trp*$^+$ heterogenotes were obtained (line 10).

The rate of transduction by $\phi 80\ pt_0$ was reduced when lysogenic recipients were used, either point mutants or deletion mutants. This reduction varied according to the recipient but, unlike $\phi 80\ pt_1$, $\phi 80\ pt_0$ transduced as efficiently recipients carrying homo- or heteroimmune prophage in att^{80}. Surprisingly, the rate of transduction to second-step transductants was higher than to the first-step transductants even derived from the same strain (Table 3; lines 5, 7).

Table 4. *Plaque-forming transducing phage yield of u.v.-induced diploid and triploid* pt-*heterogenotes*

Strain	Endogenote	Exogenotes	*pt* phage* produced	Number of *pt* units $\times 10^8$
M (1)	Δ*trp EDCBA* i^{80-}	*trp* $(CBA) + i^{80}h^{80}$	*trp* $(CAB)^+$ $i^{80}h^{80}$	1·4
M (1, 0)	Δ*trp EDCBA* i^{80-}	*trp* $(CBA) + i^{80}h^{80}$	*trp* $(CBA)^+$ $i^{80}h^{80}$	1·14
			trp $(CBA)^+$ $i^{80}h^{80}$	0·06†
		trp $(ED) + i^{80}h^{+80}$	*trp* $(ED)^+$ $i^{80}h^{+80}$	1·66
			trp $(ED)^+$ $i^{80}h^{80}$	0·18

* When plated on minimal medium, phages transducing *trp* $(CBA)^+$ or *trp* $(ED)^+$ grew on *trp B*- and *trp E*-sensitive bacteria respectively; after 24 h incubation the *h* marker was checked by the replica plating method using resistant bacteria plated on LB agar.

† 3/6 formed mottled plaques on mixed resistant and sensitive indicators.

Unlike $\phi 80\ pt_1$, $\phi 80\ pt_0$ did not transduce the bt_1 gene. Therefore, when transductants carrying $\phi 80\ ptd28$ and still apparently resistant to the phages T_1, $\phi 80$ (or derivatives with the same host-range h^{+80}) and the colicines B, I and V were supertransduced (at a very reduced rate) by $\phi 80\ pt_0$, they maintained their undissociable characters of resistance (lines 6, 9).

When the recipient bacteria carried a point mutation in *trp E*, transduction by $\phi 80\ pt_0$ resulted either in heterogenotes or in recombinants which maintained their Tryp$^+$ character even after curing of the transducing prophage. When recipients carried long deletions, supertransduction resulted in the formation of stable triploids and even also probably tetraploids. Very rare segregants were found being cured of one or two of the transducing prophages and expressing the tryptophane or indole requirement. After u.v. induction, triploid heterogenotes yielded *pt* phage particles similar to the infecting phages; namely, the $\phi 80\ pt_1(h)$ type marked by host range h^{80} and transducing the *trp* $(CBA)^+$ segment, and the $\phi 80\ pt_0$ type marked by h^{+80} and transducing *trp* $(ED)^+$. But they also produced recombinant phages: 5 % of the pt_1 type but having inherited h^{+80} (or heterozygous type segregating h^{+80} and h^{80} particles giving mottled plaques on mixed indicators) and 10 % of the pt_0 type but having inherited h^{80} (Table 4). No recombinant phage transducing the complete *trp* operon has been found yet. A third-step transductant produced, in addition to a normal amount of $\phi 80\ pt_0$ type,

a lower amount of particles transducing *trp* $(CBA)^+$ and bt_1^+ like $\phi 80$ $pt_1(h)$ but defective in its plaque-forming ability. In addition, plaque-forming non-transducing phage was produced in high amount by each heterogenote tested.

DISCUSSION

Transduction of *trp* genes by phages $\phi 80$ *pt* to *trp* point mutants results in complementation and eventually in recombination in the *trp* operon; part of the transductants are non-lysogenic haploid recombinants. Transduction to deletion mutants results only in complementation: the cured cells are Tryp$^-$. (In unpublished experiments, we fail to obtain any evidence of recombination of *trp* genes within a Δtrp deletion.) Taylor & Yanofsky (1966*a*) and Deeb *et al.* (1967) reported similar observations.

The deletions studied here include bt_1 and extend into the *trp* operon from *trp A* to *trp C* or further. When $\phi 80$ *pt* phages, carrying the *trp* $(CBA)^+$ segment, infect such mutants, they transduce the ability to synthesize tryptophan (1) in the absence of precursor if the deletion does not overreach *trp C*; (2) in the presence of indole if the deletion affects also the *trp D* gene; (3) in the presence of either indole or anthranilic acid if the recipient is a double mutant carrying a deletion in the *trp CBA* segment and in addition a point mutation in *trp E*. Diploids partially complemented, which then express an intermediate phenotype, were used as recipients for a *supertransduction* to complete tryptophan independence by *pt* phages carrying the missing *trp* genes.

This multistep transduction reveals that the *trp* genes need not be linked to complement together a long deletion. Indeed, supertransductants yield *pt* phages carrying the initial *trp* segments, suggesting that the *trp* genes have not been integrated into a reconstructed operon. P_1-transductions have given further evidence of this and this question will be discussed in detail elsewhere.

Transduction to deletion mutants does not always result in the formation of stable diploids. The difference between stable and unstable transduction seems to be correlated with the variable alteration of the bacterial chromosome in the region where the prophage is expected to insert by a single crossing-over event. This hypothesis is supported by the following observations: (1) in strains carrying no prophage in the attachment site of $\phi 80$ (att^{80}), stable transduction by $\phi 80$ $pt_1(h)$ (or another phage like $-ptd_{28}$ transducing *trp* $(CBA)^+$) is observed in all strains if the deletion affects only a part of the *trp* $(CBA)^+$ segment, but never if the deletion extends further; (2) when a prophage, defective or not, is adjacent to the deletion, then stable transduction always occurs whatever the extent of the deletion in the *trp* operon. In the first case, one may presume that transduction requires partial integrity of the *trp* segment to allow pairing, crossing-over and insertion at that eventual site. If the deletion is too large, integration is prevented and transduction cannot be more than a transient partial complementation. In the second case, transduction to lysogenic (or defective lysogenic) recipients results in the insertion of the $\phi 80$ *pt* prophages into the pre-existing phage genome integrated in att^{80}. This second situation, highly suggested by the results of Calef, Marchelli & Guerrini

(1965) on superinfection double lysogens of phage λ and by observations of Signer (1965) and Taylor & Yanofsky (1966*a*) on hybrid $\lambda\phi 80$ phages, is quite clearly indicated by results of P_1 transductions (Gratia, in preparation).

Therefore, the effect of the presence of the preintegrated prophage is to facilitate, rather than hinder, transduction. However, the prophage depressed further prophage integration at each step of transduction. Taylor & Yanofsky (1966*b*) present a case in which it is clearly shown that the transduction frequency by a $\phi 80$ *pt* phage with segment *trp* $(BA)^+$ infecting lysogenic *trp B* strain is decreased if the pre-existing prophage has the same immunity as the transducing phage. Our data agree with this observation concerning phages transducing *trp C*, *B*, *A* genes (i.e. $\phi/80\ pt_1$ and $-ptd_{28}$) but not $\phi 80\ pt_0$ and other phages transducing distal genes. It is worth considering that *pt* phages transducing proximal genes do not contain the tryptophan operator while $\phi 80\ pt_0$ does, according to Sato & Matsushiro (1964). The latter show that when $\phi 80\ pt_0$ is used to transduce, then the transduced *trp* genes are under normal control by repressors of the tryptophan pathway, while the transduced *trp* genes, free from their normal operator in $\phi 80\ pt_1$, are repressed by the $\phi 80$ immunity substance. But this observation which concerns enzymes and biochemical events studied during a short period of time does not allow a clear and complete understanding of our results based on the appearance of colonies after long incubation.

SUMMARY

Plaque-forming transducing (*pt*) particles of bacteriophage $\phi 80$ have been used to transduce genes governing tryptophan synthesis. Main interest was devoted to strains which carry deletions extending for varying distances into the tryptophan (*trp*) operon, covering *trp A* and ending in *trp B*, *trp C* or further. If the deletion does not over-reach *trp C*, infection by *pt* particles carrying the *trp* $(CBA)^+$ segment gives rise to stable partial diploids which express the complete tryptophan independence. If the deletion is further extended, transductants are not entirely complemented and appear only on medium containing indole. If the recipients do not carry any prophage in the attachment site of $\phi 80$ (att^{80}) the indole-requiring transductants are very unstable; if the recipients do carry such prophage, even defective, all transductants are very stable. Stable indole-requiring diploids can be superinfected by other *pt* phages carrying the complementary genes of the tryptophan operon, giving rise to supertransduced indole-independent bacteria. Transduction to complete independence of tryptophan or any precursor has also been achieved in three steps. Supertransductants yield after u.v. induction *pt* phages carrying the initial *trp* segments and segregate rare cells with incomplete ability to synthesize tryptophan. They are therefore polylysogenic polyploid heterogenotes carrying overlapping segments of the *trp* operon linked to prophage genomes.

When recipients are carrying a prophage in att^{80}, transductants appear at a low frequency. The reduction is particularly pronounced with $\phi 80$ *pt* transducing segment *trp* $(CBA)^+$ to immune bacteria.

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