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Evidence that HT mutant strains of bacteriophage P22 retain an altered form of substrate specificity in the formation of transducing particles in *Salmonella typhimurium*

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SUMMARY

The effects of two different deletions of the tryptophan operon on the cotransduction linkage of the nearby *cysB* and *pyrF* markers were studied using three sets of donor lysates, each produced by a different HT mutant P22 phage strain. Each *trp* operon deletion (present in both donor and recipient to preserve homology) caused changes in the cotransduction frequencies. This indicated that the HT mutant phage encapsulating mechanism, whose ability to discriminate phage DNA from host-cell DNA is absent or diminished, could still distinguish among nucleotide sequences in selecting bacterial chromosome sites at which to initiate transducing particle formation. The three HT mutant phage strains each produced different sets of cotransduction linkage values, indicating that this aspect of substrate specificity was altered differently and uniquely by each HT mutation.

1. INTRODUCTION

Genetic markers from different regions of the *Salmonella typhimurium* chromosome are transduced by bacteriophage P22 with greatly differing efficiencies, apparently because the encapsulation of the host cell DNA into transducing particles does not occur randomly and with equal frequency at all locations on the chromosome (Zinder, 1955; Ozeki, 1959; Schmieger, 1972; Chelala & Margolin, 1974). The data suggest that specific local structural nuances determine the probability of a site on the bacterial chromosome serving as the substrate in the process which initiates encapsulation of host cell DNA by phage P22.

Schmieger's discovery of phage P22 HT mutants with highly increased frequency of transducing-particle formation produced evidence for the loss of a specificity determining factor (apparently associated with a phage nuclease) which normally had permitted the encapsulating mechanism to distinguish between phage DNA and host-cell DNA (Schmieger, 1972; Raj & Schmieger, 1974). Presumably the mutations diminished or eliminated a strong preference of the phage encapsulating mechanism to make an initial scission at a specific nucleotide sequence (Schmieger, 1972) at a particular site on the phage chromosome (Tye, Huberman & Botstein,

1974). Schmieger (1972) also noted that markers showing distinctly differing transduction efficiencies with wild-type phage donor lysates were all transduced at very high and relatively similar frequencies by HT mutant donor lysates. This suggested that the HT mutations also eliminated or reduced the specificity which discriminated among different nucleotide sequences on the host-cell chromosome so as to identify preferred encapsulation start points for transducing particle formation.

We recently reported that the presence of host-cell chromosomal deletions caused distinctly changed phage P22-mediated cotransduction linkages for pairs of nearby genetic markers, apparently due to effects upon the formation of transducing particles (Chelala & Margolin, 1974). We interpreted the changed linkages as having resulted from the deletions altering the positions of the genetic markers relative to nearby specific nucleotide sequences which serve as high probability start points for a sequential encapsulation process leading to formation of a series of transducing particles. These effects of host-cell chromosome deletions could, if our interpretation is correct, serve as a sensitive test for the existence of specificity in the interactions between the encapsulating mechanism of the phage and the host-cell DNA.We therefore examined the effects of such bacterial chromosome deletions on cotransduction frequencies when using three different HT mutant P22 phage strains to produce the donor lysates.

2. MATERIALS AND METHODS

(i) *Bacterial strains and the transduction-mediated crosses*

All bacterial strains used are derivatives of *S. typhimurium* LT2. Table 1 shows the three transduction-mediated crosses performed with each phage strain. All three crosses are alike with respect to recipient and donor genotypes except for the state of the tryptophan operon. In crosses 2 and 3, which involve *trp* operon deletions,

Table 1. *The three crosses carried out with lysates produced with the various phage strains being studied*

Cross number	Recipient strain	Donor strain
1	PM92 (*cysB529 leu-500*)	PM456 (*pyrF146 leu-500*)
2	PM563 (*trpΔOABEDC101 cysB529 leu-500*)	PM454(*trpΔOABEDC101 pyrF146 leu-500*)
3*	PM565 (*trpΔBED164 cysB529 leu-500*)	PM658 (*trpΔBED164 pyrF146 leu-500*)

* This cross was not done with an HT 104/2 phage lysate.

the same deletion was present in both recipient and donor so that homology was maintained for that portion of the bacterial chromosome. In each cross, selection was for *cysB*$^+$ and cotransduction of the *pyrF146* marker was identified by replica-plating. The origins of the strains and the mutations were described previously (Chelala & Margolin, 1974). In subsequent references to deletions in the text or tables the multiple gene-letter designations of the *trp* operon deletions are omitted (*trpΔ101* and *trpΔ164*) to improve visual clarity. The extents of deletions *trpΔ101* (Chelala & Margolin, 1974) and *trpΔ164* (Blume & Balbinder, 1966) and their positions relative to the two linked loci *cysB* and *pyrF* are shown in Fig. 1.

In some of the transductions the recipient strains were used in the form of phage-immune P22 lysogens because two of the HT mutant phage strains used to produce donor lysates were derived from a clear-plaque mutant phage strain. Using normal phage-sensitive recipient bacterial strains with such donor phage lysates results in extensive killing of recipient cells and very poor transductant clone survival.

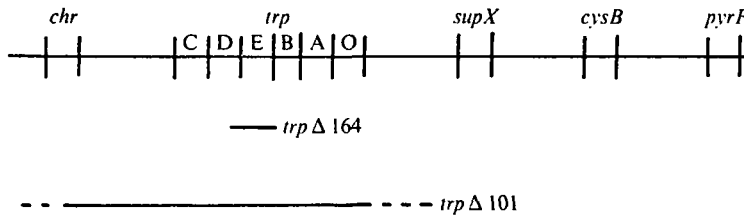

Fig. 1. Details of the *chr* to *pyrF* region of the chromosome, showing the extents of the two *trp* operon deletions used in the experiments.

(ii) *Bacteriophage strains used to produce donor lysates*

Donor lysates were produced for all three crosses using five different phage strains: (1) the wild-type P22 phage, (2) strain H5, a clear-plaque mutant of P22 isolated and originally designated as v2 by Zinder (1958), resulting from a c_2^- mutation which makes it unable to lysogenize (Levine, 1957), and (3) the three different HT (high transducing ability) phage mutant strains isolated (Schmieger, 1971) and kindly provided by Dr H. Schmieger. The HT mutant phage strains used were HT 104/2 (Schmieger, 1971, 1972), a turbid-plaque strain derived from wild-type P22 phage and HT 12/4 (Schmieger, 1971, 1972) and HT 13/4 (Schmieger, 1971), both clear-plaque strains derived from the H5 clear-plaque mutant strain.

(iii) *Media and transduction procedures*

The media and general transduction procedures have been described previously (Margolin, 1973; Chelala & Margolin, 1974). A multiplicity of infection of approximately 15 was used for all transductions. In every transduction-mediated cross the initial plating was on minimal agar medium (Margolin, 1963) supplemented with leucine, tryptophan and uracil, but not cysteine, to select for the *cysB*$^+$ transductions. The *cysB*$^+$ colonies which arose were then replica-plated onto agar medium lacking uracil to determine the percentage of *cysB*$^+$ transductant clones which had also gained the donor *pyrF146* mutant allele.

3. RESULTS

(i) *Linkages obtained with wild-type P22 phage and in control transductions*

Using wild-type phage P22 donor lysates, the cotransduction linkage of *cysB*$^+$ to *pyrF146* found with no *trp* deletion present (39·5%) was dramatically decreased in the presence of *trpΔ101* (19·2%). In contrast, when *trpΔ164* was present the cotransduction linkage was sharply increased (47·5%). These previously published

data (Chelala & Margolin, 1974) are shown in Expts. 1–3 of Table 2. However, two new factors would be present in the cotransduction studies with the two HT mutant clear-plaque phage strains (HT 12/4 and HT 13/4); the use of lysogenic recipient strains and the presence of the c_2^- mutation causing the clear-plaque phenotype of the donor phage lysates. We therefore carried out control experiments to determine whether either of these two factors affected cotransduction linkage of *cysB*$^+$ with *pyrF146* or altered the effect of the presence of the *trp* deletions.

Table 2. *Cotransduction linkages of* cysB$^+$ *with* pyrF146 *obtained with wild-type phage P22 donor lysates and either phage-sensitive (non-lysogenic) or phage-immune (lysogenic) recipient strains in the absence and in the presence of* trp *operon deletions*

Experiment number*	State of recipient	*trp* deletion present in both donor and recipient†	% cotransduction‡ ± standard error
1	Non-lysogenic	None	39·5 ± 0·4 (12072) §
2	Non-lysogenic	*trpΔ101*	19·2 ± 0·9 (1914)
3	Non-lysogenic	*trpΔ164*	47·5 ± 0·6 (3862)
4	Lysogenic	None	36·0 ± 0·8 (1298)
5	Lysogenic	*trpΔ101*	13·6 ± 0·9 (1495)
6	Lysogenic	*trpΔ164*	50·6 ± 1·6 (1006)

* The data of Expts 1, 2 and 3 are taken from a previous paper (Chelala & Margolin, 1974).

† Recipient's non-*trp* markers: *cysB529 leu-500*. Donor's non-*trp* markers: *pyrF146 leu-500*.

‡ In all transductions the selection was for *cysB*$^+$. The donor's *pyrF146* mutation was the unselected marker detected by replica-plating.

§ Figures within parentheses show total number of transductant colonies tested for each cross.

The data of Expts 4–6 in Table 2 present the cotransduction frequencies obtained when the same donor lysates (wild-type P22 phage) were used but the recipient strains were previously lysogenized with phage P22 and were, therefore, phage immune. In such crosses the efficiency of transduction is reduced by about 90% (Lenny & Margolin, unpublished data). However, we observed little change in the cotransduction linkage of *cysB*$^+$ with *pyrF146* or in the effect of the presence of the *trp* deletions. Compare, in Table 2, the cotransduction frequencies of Expts 1–3 (non-lysogenic recipients) to those of Expts 4–6 (lysogenic recipients), respectively. The small differences may barely border on significance if tested statistically, but we hesitate to consider them significant without extensive repeats of the transductions.

When clear-plaque mutant strain H5 is used to produce donor lysates, the c_2^- mutation in the phage does not affect the cotransduction frequencies of the *cysB*$^+$ and *pyrF146* markers (Table 3). These linkages obtained with the H5 phage donor lysates and the lysogenic recipient strains are essentially identical to those in Table 2 obtained with the wild-type P22 (c_2^+) donor lysates and lysogenic recipients (experiments 4, 5 and 6). Thus, the data of Tables 2 and 3 demonstrate that neither the use of a lysogenic recipient nor the presence of a c_2^- mutation in the phage used to produce a donor lysate caused any significant change in the cotransduction frequencies or in the effects of the presence of *trp* deletions.

Table 3. *Test for the possible effects of the use of the H5 clear-plaque mutant phage strain* (c_2^- *mutation*) *donor lysates for determining the cotransduction frequencies of the* cysB+ *and* pyrF146 *markers using lysogenic recipient cells and in the presence and absence of* trp *operon deletions*

trp deletions present in both donor and recipient*	% cotransduction† ± standard error
None	35·7 ± 1·2 (1604)‡
trpΔ101	14·0 ± 0·9 (1583)
trpΔ164	51·1 ± 1·2 (1663)

* Recipient's non-*trp* markers: *cysB529 leu-500*. Donor's non-*trp* markers: *pyrF146 leu-500*.

† In all transductions the selection was for *cysB*+. The donor's *pyrF146* mutation was the unselected marker detected by replica-plating. All donor lysates were produced with the H5 phage strain and all recipient strains were P22 lysogens.

‡ Figures within parentheses show total number of transductant colonies tested for each cross.

Table 4. *Use of HT 104/2 mutant phage donor lysates to test for any possible effect on cotransduction frequencies of the* cysB+ *and* pyrF146 *markers in the absence and in the presence of the* trp-101 *deletion**

Experiment number	State of recipients	*trp* deletion in both donor and recipient†	% cotransduction‡ ± standard error
1	Non-lysogenic	None	30·9 ± 0·9 (2626) §
2	Non-lysogenic	*trpΔ101*	16·3 ± 0·8 (2080)
3	Lysogenic	None	28·2 ± 1·0 (2094)
4	Lysogenic	*trpΔ101*	13·3 ± 1·1 (972)

* The HT 104/2 mutant phage strain is temperate (c_2+).

† Recipient's non-*trp* markers: *cysB529 leu-500*. Donor's non-*trp* markers: *pyrF146 leu-500*.

‡ In all transductions the selection was for *cysB*+. The donor's *pyrF146* mutation was the unselected marker detected by replica-plating.

§ Figures within parentheses show total number of transductant colonies tested for each cross.

(ii) *Linkages obtained with phage strain HT 104/2*

Phage strain HT 104/2 donor lysates were used to examine the *cysB*+ to *pyrF146* cotransduction linkage in the absence of any *trp* deletion and in the presence of *trpΔ101*. Although Schmieger (personal communication) found that the HT 104/2 mutation tends to revert to a form producing low transducing-frequency lysates, our HT 104/2 donor lysates still exhibited a five- to tenfold improved transducing ability relative to wild-type phage lysates. The HT 104/2 cotransduction linkages obtained with both non-lysogenic and lysogenic recipient cells (Table 4) confirm our previous finding (Table 2) that use of lysogenic recipient strains causes very little change in cotransduction linkages; in Table 4 the percentages of cotransduction do not differ significantly between Expts 1 and 3 nor between 2 and 4. However, with HT 104/2 the cotransduction linkage in the absence of any *trp* deletion (Expts 1 and 3 of Table 4) is distinctly reduced to 30·9% and 28·2% compared to the 39·5% and 36·0% found previously with wild-type P22 phage (Expts 1 and 4 of Table 2). Surprisingly the cotransduction linkage in the presence of *trpΔ101* was essentially

the same with either wild-type P22 or HT 104/2 mutant phage lysates (compare data of Expts 2 and 5 in Table 2 with those of Expts 2 and 4 in Table 4). Consequently, with HT 104/2 phage the extent of the change in linkage resulting from the presence of *trpΔ101* is quite different from that observed when using wild-type phage lysates.

Table 5. *Use of HT 13/4 mutant phage donor lysates to test for any possible effect on cotransduction frequencies of the* cysB$^+$ *and* pyrF146 *markers in the absence and in the presence of* trp *deletions**

Experiment number	*trp* deletion present in both donor and recipient†	% cotransduction‡ ± standard error
1	None	32·7 ± 1·1 (1834)§
2	*trpΔ101*	47·5 ± 1·1 (1989)
3	*trpΔ164*	47·0 ± 1·3 (1425)
4‖	None	30·0 ± 1·4 (1097)
5‖	*trpΔ101*	45·4 ± 1·2 (1602)

* The HT 13/4 mutant phage strain was obtained in the H5 clear-plaque mutant phage P22 strain (c_2^- mutation) and therefore all recipients used for this transduction were necessarily phage-immune P22 lysogens.

† Recipient's non-*trp* markers: *cysB529 leu-500*. Donor's non-*trp* markers: *pyrF146 leu-500*.

‡ In all transductions the selection was for *cysB*$^+$. The donor's *pyrF146* mutation was the unselected marker detected by replica-plating.

§ Figures within parentheses show total number of transductant colonies tested for each cross.

‖ Expts 4 and 5 are repeats of Expts 1 and 2, respectively, except that new, independent donor phage lysate preparations were used.

(iii) *Linkages obtained with phage strain HT 13/4*

The *cysB*$^+$ to *pyrF146* cotransduction linkages obtained with the HT 13/4 lysates are shown in Table 5, Expts 1–3. They are best compared with the data from the equivalent three crosses in Table 3 since both sets of experiments involve clear-plaque donor lysates and lysogenic recipient strains. With no *trp* operon deletion the 'wild-type' (no HT mutation) donor lysate produced a cotransduction frequency of 35·7 % (Table 3) and the HT 13/4 mutant phage lysate produced a very similar frequency of 32·7 % (Table 5). With *trpΔ101* present the 'wild-type' phage lysate gave a sharply reduced cotransduction linkage of 14·0 % whereas the HT 13/4 lysate produced a sharply increased linkage of 47·5 %. Tables 3 and 5 show that in the presence of *trpΔ164* the two types of donor phage lysates gave rather similar cotransduction percentages.

The HT 104/2 mutant phage lysate had distinctly altered the linkage with no *trp* deletion present but produced essentially the same linkage as wild-type phage donor lysates with *trpΔ101* present. In contrast the HT 13/4 mutant phage lysate dramatically altered the effect of *trpΔ101* without causing much change in the absence of a *trp* deletion or the presence of *trpΔ164*. It seemed possible that production of HT phage donor lysates might be sensitive to effects of uncontrolled variables (i.e. minor temperature or aeration-rate fluctuations) during formation of transducing particles, such that each new lysate produced a different pattern of cotransduction linkages. Therefore, two new HT 13/4 donor lysates were made and used

for experiments 4 and 5 in Table 5, which are simply repeats of the crosses done in Expts 1 and 2. The cotransduction linkages in the two sets of experiments are essentially identical, indicating that the differences in linkage we had found are determined by different HT mutations borne by each phage strain rather than variations between individual lysates.

Table 6. *Use of HT 12/4 mutant phage donor lysates to test for any possible effect on cotransduction frequencies of the* cysB$^+$ *and* pyrF146 *markers in the absence and in the presence of* trp *deletions**

trp deletion present in both donor and recipient†	% cotransduction‡ ± standard error
None	16·8 ± 0·9 (1607)§
trpΔ101	54·3 ± 1·5 (1097)
trpΔ164	26·6 ± 1·2 (1303)

* The HT 12/4 mutant phage strain was obtained in the H5 clear-plaque mutant phage P22 strain (c_2^- mutation) and therefore all recipients used for this transduction were necessarily phage-immune P22 lysogens.

† Recipient's non-*trp* markers: *cysB529 leu-500*. Donor's non-*trp* markers: *pyrF146 leu-500*.

‡ In all transductions the selection was for *cysB*$^+$. The donor's *pyrF146* mutation was the unselected marker detected by replica-plating.

§ Figures within parentheses show total number of transductant colonies tested for each cross.

(iv) *Linkages obtained with phage strain HT 12/4*

Table 6 presents data obtained by use of the HT 12/4 phage mutant to produce donor lysates. The percentages of cotransduction can be compared with those of Tables 3–5 for the equivalent crosses. The HT 12/4 mutation sharply changes the linkages in all three crosses. The cotransduction frequency is changed with no *trp* deletion present, and the effects of the presence of *trpΔ101* and *trpΔ164* are each distinctly altered. These results further support our conclusion that each HT mutation has its own characteristic interaction with the effects of host chromosome deletions on the cotransduction linkage of nearby markers.

4. DISCUSSION

The HT mutant phage have lost a specificity factor which had allowed the encapsulating mechanism of wild-type phage to distinguish phage DNA from bacterial DNA (Schmieger, 1972). We have shown that in transductions utilizing HT mutant P22 phage donor lysates the presence of nearby host chromosome deletions can dramatically alter the cotransduction frequency of a pair of linked markers. The strong effects of the *trp* deletions which we observed with HT phage lysates should, according to our model (Chelala & Margolin, 1974), have resulted from altered positions of the linked markers with respect to specific sites on the bacterial chromosome. Therefore, our findings indicate that during transducing-particle formation an HT mutant phage encapsulating mechanism still retains some aspect of specificity in its interactions with bacterial chromosomes. It is evident, however, that the specificity is altered and the greatly differing patterns of linkage

values found with the three HT mutant phage strains indicate that each HT mutation alters the specificity uniquely. The HT mutations, which occur in a gene specifying a phage nuclease (Raj, Raj & Schmieger, 1974), apparently affect the two aspects of specificity, eliminating or diminishing one and altering the other. Probably both aspects of specificity are, as suggested by Schmieger (1972), brought into play at one of the earliest steps in the process of encapsulation of DNA by a phage head – an initial double-stranded scission in the DNA.

Schmieger & Backhaus (1976) had previously studied the effect of an HT mutation on cotransduction linkage and kindly made their unpublished results available to us. They found that an HT mutant phage donor lysate produced cotransduction frequencies quite different from those obtained with wild-type phage lysates. They also noted a striking pattern of changes related to the marker used for selection, suggesting to them that the HT mutation had caused a loss or alteration of specificity. We feel that our evidence for an altered rather than lost specificity of HT phage interactions with the bacterial chromosome strongly supports a similar explanation for their results.

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