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Specialized Transduction of Tetracycline Resistance by Phage P22 in *Salmonella typhimurium*

I. Transduction of R Factor 222 by Phage P22

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Transduction of R factor 222, which carries four drug resistance markers (sul^R, str^R, cam^R, tet^R), by phage P22 in *Salmonella typhimurium* results in the segregation of the drug resistance markers into tet^R and the other three. The tet^R marker was found to be integrated at *pro*A near the P22 prophage attachment site of the recipient chromosome, confirming the previous result of Dubnau and Stocker (1964), who used an independently isolated R factor with drug resistance markers similar to those of 222. The sul^R, str^R, and cam^R markers were found to be integrated together elsewhere on the recipient chromosome.

High-frequency-transducing (HFT) lysates could be obtained from some of the tet^R but not from any of the $sul^R \cdot str^R \cdot cam^R$ transductants after UV induction. The transduction of tet^R by an HFT lysate was multiplicity dependent; coinfection with wild-type P22 phage abolished this multiplicity dependence and restored the transduction frequency to the maximum level attained by the HFT lysate alone at high multiplicity. The integration of the tet^R marker in the proline region of the *Salmonella* chromosome thus appears to be due to P22-mediated specialized transduction.

INTRODUCTION

R factors are transmissible plasmids carrying multiple drug resistance markers (see Watanabe, 1963), which can be transduced by phage P1 in *Escherichia coli* and by phage P22 in *Salmonella typhimurium* (Watanabe and Fukasawa, 1961b). The patterns of transduction of R factors in these two systems are considerably different; often the entire R factor can be transduced en bloc by phage P1, whereas generally only portions of the R factor are transduced by phage P22. The difference in the patterns of transduction of R factors is dependent on the transducing phage rather than on the bacterial species employed (Okada and Watanabe, 1968). In transduction of the R factor 222 (one of our standard R factors) by phage P22 in *S. typhimurium*, the drug resistance markers [resistance to sulfanilamide (sul^R), streptomycin (str^R), chloramphenicol (cam^R), and tetracycline (tet^R)] are invariably segregated into tet^R and the other three markers (Watanabe and Fukasawa, 1961b); transduction by P22 also separates the drug resistance markers from the RTF (resistance transfer factor)—that part of the R factor which promotes transfer of the plasmid from cell to cell (Watanabe and Fukasawa, 1961b). The P22-transduced tet^R marker was found to be integrated into the chromosome of recipient bacteria by Dubnau and Stocker (1964) using an independently isolated R factor with drug resistance markers similar to those of 222.

In this paper we ask whether specialized transduction is responsible for the transduction of R factor 222 by phage P22 in *Salmonella typhimurium*. If it were, we

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would expect the transduced drug resistance marker to map at the chromosomal attachment site of the P22 prophage and the transductants to produce high-frequency-transducing (HFT) lysates. We have done bacterial crosses which show that the tet^R marker from 222 is integrated at *pro*A near the P22 prophage attachment site, but that the other drug resistance markers transduced by P22 are integrated together into the chromosome of recipient bacteria close to the galactose (*gal*) region. We were able to find HFT lysates for tet^R, but not for any of the other drug resistance markers.

A tet^R transductant that gave rise to HFT lysates was chosen for further study. The properties of the HFT lysate are discussed in more detail in the following paper (Chan *et al.*, 1973) along with data relevant to the mechanism of specialized transduction of tet^R by P22.

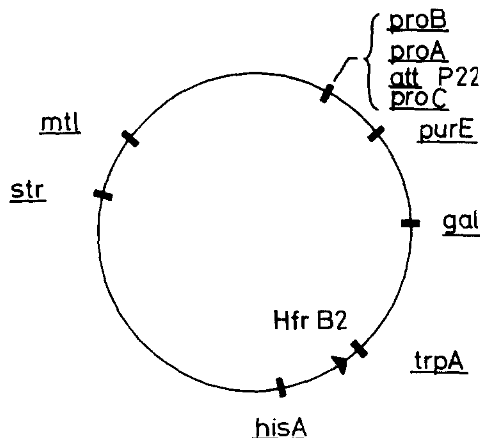

FIG. 1. A simplified chromosome map of *Salmonella typhimurium* with the origin and direction of chromosome transfer of Hfr B2. *pro*, proline; *att*, attachment site; *pur*, purine; *gal*, galactose; *trp*, tryptophan; *his*, histidine; *str*, streptomycin; *mtl*, mannitol. Modified from Sanderson (1970).

MATERIALS AND METHODS

Media. Tokyo: These were the same as those described in a previous paper (Watanabe and Fukasawa, 1961a). In addition, tryptone broth (pH 6.9) containing 1% (w/v) Bacto-tryptone (Difco) and 0.5% (w/v) NaCl was used.

Cambridge: LB broth, buffered saline and green indicator plates with tetracycline hydrochloride are described in Chan *et al.* (1973).

Strains of bacteria, phage, and R factors. The following strains of *Salmonella typhimurium* LT-2 were used: wild type (DB21 in Cambridge (Botstein and Matz, 1970)); *pro*A36; DB46 (HfrB2 his^-gal^-) (Fig. 1 shows the origin and direction of transfer of HfrB2); DB47 (rec^-) (Botstein and Matz, 1970); and TR92 (F^-trpA *pur*E *his*A mtl^- str^r).

DB5218 ($F'lac^+pro\text{AB}^+/F^-pro\text{AB47}rec^-$) is *S. typhimurium* LT-7 strain *pro*AB47 with a rec^- allele from LT-2 strain DB43 (HfrB2 $rec^-met\text{A}^-str^r$) [see Botstein and Matz (1970) for the method of construction] and a $F'lac^+pro^+$ episome from *E. coli* strain E5013, kindly supplied by E. Signer.

The phage used were P22 wild type, P22 *int*3 (Smith and Levine, 1967), and P22 *mnt* (Gough, 1968).

The R factors used were 222 and S-a. S-a has recently been found to have a kanamycin resistance marker (kan^R) in addition to the sul^R, str^R, and cam^R markers which were already reported (Watanabe *et al.*, 1968).

Bacterial crosses. The procedure for conjugal transfer of R factors was described previously (Watanabe and Fukasawa, 1961a).

The filter method of Sanderson and Demerec (1965) was used in Hfr by F^- crosses. The Hfr B2 strain DB46 is sensitive to streptomycin and was selected against in the recombination experiments with this drug. The resistance to streptomycin conferred by the str^R allele from the R factor is at a much lower level and does not interfere.

Transduction by P22. Tokyo: The procedure for transduction of R factors in *S. typhimurium* LT-2 by phage P22 was described in a previous paper (Watanabe and Fukasawa, 1961b).

Cambridge: For the transduction of tet^R by an HFT lysate, exponentially growing cells in LB broth were centrifuged and resuspended in buffered saline to 6×10^8 cells/ml. Equal volumes of the HFT lysate and of the recipient cells were mixed, allowed to adsorb for 15 min at 37°, diluted,

and plated on green indicator plates supplemented with 25 μg/ml of tetracycline hydrochloride. The plates were incubated overnight at 37°.

UV induction of P22 lysogens. Tokyo: Drug-resistant transductants and other lysogenic bacterial strains of *S. typhimurium* LT-2 were grown in tryptone broth to about 1×10^8 cells/ml with gentle shaking at 37°, and 6 ml of each culture were irradiated in a petri dish with a germicidal lamp (National, 60 watts) in the dark for 20 sec from a distance of 30 cm. The UV-irradiated cultures were aerated at 37° in the dark for 2.5 hr. The partially lysed cultures were centrifuged at 8000 rpm for 3 min and the supernatants were treated with chloroform and used as lysates.

Cambridge: The procedure for UV induction is described in Chan *et al.* (1973).

RESULTS

The Effect of the Bacterial rec *Function and the Phage* int *Function on the Frequencies of Transduction of R Factors 222 and S-a by Phage P22 in* S. typhimurium *LT-2*

In an attempt to determine some of the phage and host functions which are required for the successful transduction of an R factor, we have used an integration-deficient (int^-) (Smith and Levine, 1967) mutant of P22 as a transducing phage and a recombination-deficient (rec^-) (Wing *et al.*, 1968) bacterial host as a recipient for transduction. In addition, we have compared the P22-mediated transduction of R factors 222 and S-a because S-a, unlike 222, is small enough to be incorporated en bloc into a transducing particle of P22 (Watanabe *et al.*, 1968). As shown in Table 1, the frequencies of transduction of S-a were independent of the phage *int* gene and the bacterial *rec* gene, whereas the frequencies of transduction of 222 were lower when an int^- mutant phage or a rec^- recipient was used. No drug-resistant transductants could be found when 222 was transduced by an int^- phage to a rec^- recipient.

Conjugal Transferability of the Transduced Drug Resistance Markers of R Factor 222

None of the drug-resistant transductants obtained by transduction of R factor 222 by P22 was able to transfer its drug resistance marker by causing conjugation. These results confirm our previous observations with 222 (Watanabe and Fukasawa, 1961b) and can be contrasted with our previous finding that all drug-resistant transductants obtained by transduction of R factor S-a by P22 were able to transfer their drug resistance markers by conjugation (Watanabe *et al.*, 1968). The loss of conjugal transferability and the dependence of transduction on *rec* and *int* function mentioned above suggest that the tet^R and the sul^R, str^R, cam^R markers of 222 may become integrated into the recipient chromosome after transduction by P22.

TABLE 1

FREQUENCIES OF TRANSDUCTION OF R FACTORS 222 (sul^R, str^R, cam^R, tet^R) AND S-a (sul^R, str^R, cam^R, kan^R) BY P22 WILD TYPE (int^+) AND P22 *int*3 IN *Salmonella typhimurium* LT-2 WILD-TYPE (rec^+) AND rec^- (DB47)[a]

Donor:	P22 int^+ grown on LT-2 rec^+ (222)		P22 *int3* grown on LT-2 rec^+ (222)		P22 int^+ grown on LT-2 rec^+ (S-a)	P22 *int3* grown on LT-2 rec^+ (S-a)
Recipient:	Selected by Cm[b]	Selected by Tc[b]	Selected by Cm	Selected by Tc	Selected by Cm	Selected by Cm
LT-2 rec^+	1.2×10^{-7}	4.3×10^{-9}	2.7×10^{-9}	7.1×10^{-10}	3.2×10^{-6}	2.9×10^{-6}
LT-2 rec^-	2.1×10^{-9}	1.9×10^{-10}	$<1.7 \times 10^{-10}$	$<1.7 \times 10^{-10}$	4.4×10^{-6}	3.5×10^{-6}

[a] Frequencies of transduction are expressed per plaque-forming unit.

[b] Cm, chloramphenicol; Tc, tetracycline.

Mapping of the Drug Resistance Markers in the Transductants by Bacterial Crosses

If the drug resistance markers of 222 are integrated into the recipient chromosome, it should be possible to demonstrate linkage of the drug resistance markers to bacterial genes in bacterial crosses. Lysogenic drug-resistant transductants obtained by transduction of R factor 222 by phage P22 at a high multiplicity of infection to *pro*A36 as recipient were mated with DB46 (HfrB2), and *pro*$^+$ recombinants were selected and tested for their genetic constitution. The transduced *tet*^{R} marker and the P22 prophage are closely linked to the *pro*A locus (Table 2) which is known to be next to the chromosomal site of the P22 prophage (S. M. Smith and Stocker, 1962; H. O. Smith, 1968). In contrast, the transduced *sul*^{R}·*str*^{R}·*cam*^{R} markers are not closely linked to the *pro*A locus (Table 3). It is possible that the P22 prophage in the *sul*^{R}·*str*^{R}·*cam*^{R} transductants may be located at some site other than the normal prophage site.

In order to determine more precisely the chromosomal location of the transduced *sul*^{R}·*str*^{R}·*cam*^{R} markers, the R factor 222 was first transduced to strain TR92 and the *sul*^{R}·*str*^{R}·*cam*^{R} transductants obtained were mated with the Hfr DB46. The results with two such transductants (Nos. 5 and 47) are shown in Table 4. Each cross was plated for selected markers as indicated; the order of markers was determined by examining the linkage of unselected markers to the selected markers. The results in Table 4 suggest that the *sul*^{R}·*str*^{R}·*cam*^{R} markers are integrated into the chromosome between *gal* and *trp*A (see Fig. 1) in transductant No. 5 and may be integrated between *pur*E and *gal* (see Fig. 1) in transductant No. 47.

TABLE 2

MATING BETWEEN *Salmonella typhimurium* DB46 (Hfr B2) AND *tet*^{R} TRANSDUCTANTS OBTAINED BY PHAGE P22 IN *S. typhimurium pro*A36

Strain of *tet*^{R} transductant	Markers of *pro*$^+$ recombinants[a]			
	tet^R P22$^+$	*tet*^{R} P22$^-$	*tet*^{S} P22$^+$	*tet*^{S} P22$^-$
TC-1	0	0	0	88
TC-2	0	1	0	97

[a] P22$^+$ and P22$^-$ indicate the presence and absence of prophage P22, respectively, which was checked by the ability and inability to liberate infectious P22 particles upon UV irradiation.

Screening for Drug-Resistant Transductants That Produce HFT Lysates

Drug-resistant transductants were made by transduction for *cam*^{R} or *tet*^{R} at high multiplicity of infection of a drug-sensitive LT-2 *rec*$^+$ recipient with P22 *int*$^+$ grown lytically on LT-2 *rec*$^+$ (222).

We could not find HFT lysates for the *sul*^{R}·*str*^{R}·*cam*^{R} markers using 27 independently isolated *sul*^{R}·*str*^{R}·*cam*^{R} transductants; all but one of these transductants liberated infectious phage particles upon UV irradiation.

On the other hand, three out of 26 independently isolated *tet*^{R} transductants gave rise to HFT lysates upon UV irradiation. These HFT lysates had low titers (10^4 to 10^5 PFU/ml) and high efficiencies of trans-

TABLE 3

MATING BETWEEN *Salmonella typhimurium* DB46 AND *sul*^{R}.*str*^{R}.*cam*^{R} TRANSDUCTANTS OBTAINED BY PHAGE P22 IN *S. typhimurium pro*A36

Strain of *sul*^{R}.*str*^{R}.*cam*^{R} transductant	Markers of *pro*$^+$ recombinants[a]			
	sul^R.*str*^{R}.*cam*^{R} P22$^+$	*sul*^{R}.*str*^{R}.*cam*^{R} P22$^-$	*sul*^{S}.*str*^{S}.*cam*^{S} P22$^+$	*sul*^{S}.*str*^{S}.*cam*^{S} P22$^-$
CM-1	48	33	0	1
CM-2	88	0	0	0
CM-3	58	0	30	0

[a] P22$^+$ and P22$^-$ indicate the presence and absence of prophage P22, respectively, which was checked by the ability and inability to liberate infectious P22 particles upon UV irradiation.

TABLE 4

MATINGS WITH $sul^R.str^R.cam^R$ TRANSDUCTANTS

	str	pur	gal	trp	cam	Genotype of recombinants (%)	
Donor:	S	+	−	+	S	No. 5	No. 47
Recipient:	R	−	+	−	R		
pur^+str^R selected	(R)[a]	(+)	+	−	R	65	39
	(R)	(+)	−	−	R	32	20
	(R)	(+)	+	−	S	0	28
	(R)	(+)	−	−	S	2	13
	(R)	(+)	+ or −	+	S or R	1	0
						(88)[b]	(100)[b]
trp^+str^R selected	(R)	−	+	(+)	R	31	29
	(R)	+	+	(+)	R	3	15
	(R)	−	−	(+)	R	3	2
	(R)	+	−	(+)	R	10	18
	(R)	−	+	(+)	S	23	7
	(R)	+	+	(+)	S	6	7
	(R)	−	−	(+)	S	3	1
	(R)	+	−	(+)	S	21	21
						(100)[b]	(100)[b]
$trp^+pur^+str^R$ selected	(R)	(+)	+	(+)	R	9	21
	(R)	(+)	−	(+)	R	15	31
	(R)	(+)	+	(+)	S	15	15
	(R)	(+)	−	(+)	S	61	33
						(100)[b]	(98)[b]

[a] Parentheses indicate selected markers.
[b] Total colonies examined.

duction (1.0 tet^R transductants/PFU). One of the tet^R HFT-producing transductants, strain DB5000, was selected for further study. The other 23 tet^R transductants which did not give rise to HFT lysates contained a wild-type P22 prophage and were not studied further.

Transduction of the Ability to Make HFT Lysates

The tet^R transductants made from a DB 5000 HFT lysate were irradiated with UV to see if HFT lysates could be obtained successively. As shown in Table 5, many of the tet^R transductants obtained from an HFT lysate for tet^R again give rise to HFT lysates upon UV induction. This process could be repeated indefinitely. We conclude that the ability to produce HFT lysates can be transduced from one cell to another by the HFT lysate. We propose that the

TABLE 5

tet^R TRANSDUCTION BY UV-INDUCED LYSATES OF tet^R TRANSDUCTANTS DERIVED FROM AN HFT LYSATE

Strain of tet^R transductant[a]	Titer of PFU[b] (per ml)	Frequency of tet^R transductants (per PFU)
TC-10-11	0	0
TC-10-12	1.2×10^4	1.5×10^0
TC-10-13	9.7×10^3	1.1×10^0
TC-10-14	1.8×10^4	1.0×10^0
TC-10-15	2.7×10^4	1.2×10^0
TC-10-A	2.4×10^4	1.2×10^0
TC-10-B	9.3×10^4	5.4×10^{-1}
TC-10-C	3.0×10^4	9.0×10^{-1}
TC-10-D	1.5×10^4	8.8×10^{-1}
TC-10-E	9.2×10^4	8.5×10^{-1}

[a] The first five transductants were obtained in one experiment and the following five transductants in the other experiment.
[b] PFU, plaque-forming unit.

phage entity responsible for producing HFT lysates in strain DB5000 be called P22 Tc-10.

Mapping of tet^{R} *Transductants Derived from a DB5000 Lysate*

The map location of the *tet*^{R} marker in transductants derived from a DB5000 lysate was determined by crosses using strain DB5218 (F′*lac*$^{+}$*pro*AB^{+}/F^{-}*pro*AB47 *rec*$^{-}$). In strain DB5218, the P22 prophage attachment site is carried on an F′*lac*$^{+}$*pro*$^{+}$ episome from *E. coli* (Roth and Hoppe, 1972); the chromosomal P22 prophage attachment site is removed by the *pro*AB47 deletion (Smith and Levine, 1965). A *tet*^{R} transductant of DB5218 that gave rise to HFT lysates and was therefore carrying P22Tc-10 was made from a DB5000 lysate. In order to prove that the Tc-10 prophage was on the episome, DB5218 (P22 Tc-10) was mated with *E. coli* strain X5096 which contains a deletion of the *lac-pro* region. (*E. coli* strains cannot adsorb P22 particles.) All the *E. coli* recombinants selected for *lac*$^{+}$*pro*$^{+}$ were also *tet*^{R}; likewise all the *E. coli* recombinants selected for *tet*^{R} were also *lac*$^{+}$*pro*$^{+}$. These results show that the transduced *tet*^{R} marker obtained from a DB5000 lysate is integrated at the P22 prophage attachment site on the F′*lac*$^{+}$*pro*$^{+}$ episome.

The Multiplicity Dependence of tet^{R} *Transduction by a DB5000 HFT Lysate*

In the accompanying paper (Chan *et al.*, 1973), we show that UV-induced lysates of DB5000 contain at least 10^{4}-fold more defective particles (as determined by absorbance at 260 nm of purified particles) than normal plaque-forming phage. Therefore, the multiplicity of infection (m.o.i.) in experiments with a DB5000 lysate will be given in terms of the actual number of particles determined from absorbance measurements.

The efficiency of transduction of *tet*^{R} by a DB5000 HFT lysate increases as the multiplicity of infection increases, as shown in Fig. 2a. This result implies that transduction is much less likely in singly infected cells than in multiply infected ones.

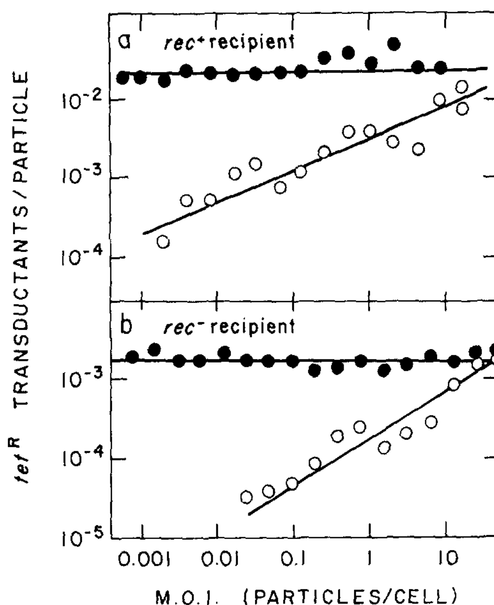

Fig. 2. Efficiency of *tet*^{R} transduction as a function of multiplicity of infection in a *rec*$^{+}$ and a *rec*$^{-}$ recipient. The particle titer was estimated by assuming that the titer of defective particles as determined by absorbance at 260 nm is 16 times the titer on DB147 (see Chan *et al.*, 1973). DB21 was the *rec*$^{+}$ recipient, and DB47 was the *rec*$^{-}$ recipient. The m.o.i. of P22 c^{+} helper phage was 6 in *rec*$^{+}$ and 17 in *rec*$^{-}$. ●, DB5000 lysate + P22 c^{+} helper. ○, DB5000 lysate alone.

If one coinfects with wild-type nontransducing phage, the efficiency of transduction does not change with the multiplicity of DB5000 particles. The level of transduction remains constant at about 0.02–0.04 stable transductants per input particle. This is the maximum level attained by the HFT lysate alone at high multiplicity. Thus, whatever factor accounts for the increase in transduction efficiency at high multiplicity can be provided by ordinary wild-type phage.

In order to ascertain the role of host-specified recombination systems on the multiplicity-dependence of transduction, the experiments were repeated in a *rec*$^{-}$ host (Fig. 2b). The results are similar: the DB5000 lysate alone shows a marked dependence of transduction on the m.o.i. which is totally abolished by coinfection with a wild-type helper phage. However, the absolute level of transduction is reduced 10-fold in all cases.

We made a preliminary attempt to identify some of the phage functions involved in the ability of wild-type phage to help transduction by single particles in the HFT lysate. Phage defective in prophage integration (*int*$^-$; Smith and Levine, 1967) and phage defective in the maintenance of lysogeny (*mnt*$^-$; Gough, 1968) were compared with wild-type phage as helpers. As shown in Table 6, P22 *mnt*$^-$ helps as well as P22 wild type; P22 *int*$^-$ also appears to help, though not as efficiently as P22 wild type. It should be noted that the particles in the DB5000 HFT lysate are *int*$^+$ and *mnt*$^+$; in order definitively to establish the role of these genes in transduction, it will be necessary to construct P22Tc-10 *int*$^-$ and P22Tc-10 *mnt*$^-$ lysogens.

To summarize, our results suggest that many, if not all, of the particles in a DB5000 lysate can transduce *tet*^{R} if helped either by another particle or by wild-type phage. This helping is affected by alterations in the *int* gene of the phage and possibly by the *rec* gene of the host.

Minute tet^R *Transductants*

When LT-2 cells infected with a DB5000 lysate were plated on nutrient agar containing 25 μg/ml of tetracycline hydrochloride, two kinds of colonies were seen—large normal-sized colonies and minute colonies. The minute colonies were about 100 times more frequent than the large colonies. We suspect that the minute colonies are abortive transductants, because no such colonies developed when uninfected cells were plated on tetracycline-containing medium. This idea is consistent with our observation that the addition of helper phage increases the number of (large) *tet*^{R} transductants (Fig. 2), but decreases the number of minute transductants, since the helper phage has presumably converted many of the abortive (minute) transductants to complete (large) transductants.

TABLE 6

HELPER PHAGE IMPROVES TRANSDUCTION BY A DB5000 LYSATE[a]

Helper	Transduction frequency (*tet*^{R} colonies/particle)
—	2.8×10^{-4}
P22 c^+	110.0×10^{-4}
P22 *mnt*	100.0×10^{-4}
P22 *int*	19.0×10^{-4}

[a] DB5000 lysate was added at an m.o.i. of 1.35×10^{-2} particles/cell. Helper phage were added at an m.o.i. of 2. The particle titer was estimated by assuming that the titer of defective particles is five times the titer obtained by the marker rescue assay (see Chan *et al.*, 1973).

DISCUSSION

It was previously reported that the transduction of R factor 222 by phage P22 in *S. typhimurium* results in the segregation of the drug resistance markers and the loss of their conjugal transferability (Watanabe and Fukasawa, 1961b). We show here that the transduced drug resistance markers from 222 are integrated into the chromosome of the recipient bacteria. These unusual patterns of transduction of 222 by P22 as compared with transduction of the same R factor by phage P1 can be ascribed to the small size of P22. Unlike P1, P22 cannot incorporate both the drug resistance markers and the RTF (resistance transfer factor) (see Watanabe, 1963) into the same transducing particle. The *sul*^{R}, *str*^{R}, and *cam*^{R} markers are closely linked to each other and thus are usually transduced together. The *tet*^{R} marker, however, is not located close enough to the other drug resistance markers on the R factor genome to be picked up with them by P22. The drug resistance markers that are transduced separately from the RTF are no longer independent replicons and become abortive unless they are integrated into the recipient chromosome (Watanabe and Ogata, 1970). In contrast, R factor S-a is small enough to be incorporated en bloc into a transducing particle of P22 as reported previously (Watanabe *et al.*, 1968); this conclusion is confirmed by our present finding that S-a can be transduced very efficiently to a *rec*$^-$ recipient by an *int*$^-$ phage.

Our results show that not only the *tet*^{R} marker but also the *sul*^{R}·*str*^{R}·*cam*^{R} markers together are integrated into the recipient

chromosome. The integration of the *tet*^{R} marker at the *pro* region is most likely due to specialized transduction by P22, but the mechanism of integration of the other three markers near the *gal* region is not yet known.

R factors can also be transduced by epsilon phages in *Salmonella anatum* and *Salmonella newington* and the patterns of transduction of the drug resistance markers of an R factor carrying four drug resistance markers are very similar to those in the transduction system of *S. typhimurium* and phage P22 (Harada *et al.*, 1963). In other words, the *sul*^{R}, *str*^{R}, *cam*^{R}, and *tet*^{R} markers are invariably segregated into *tet*^{R} and the other three; none of the drug-resistant transductants obtained was able to transfer its drug resistance marker by causing conjugation. Unlike the drug resistance markers on ordinary R factors, the drug resistance markers in these transductants could not be eliminated by treatment with acriflavine, suggesting integration of the drug resistance markers into the bacterial chromosome. Furthermore, it was found that UV-induced lysates of *tet*^{R} transductants obtained by phage ϵ_{15} caused high-frequency transduction of *tet*^{R} (Kameda *et al.*, 1965), although the nature of the transducing phage was not studied in detail.

Because of the similarities in the patterns of transduction of R factors by P22 and ϵ_{15}, we asked whether HFT lysates were possible in the system of transduction of 222 by P22 in *S. typhimurium*. HFT lysates for *tet*^{R} were obtained by UV induction of three out of 26 independently isolated *tet*^{R} transductants, but no HFT lysates for *sul*^{R}·*str*^{R}·*cam*^{R} were obtained by UV induction of 27 independently isolated *sul*^{R}·*str*^{R}·*cam*^{R} transductants. However, as we point out below and in the accompanying paper (Chan *et al.*, 1973), high-frequency transduction of *tet*^{R} by P22 seems to be fundamentally different from the high-frequency transduction of *tet*^{R} by ϵ_{15} described by Kameda *et al.* (1965).

One of the *tet*^{R} transductants that produced HFT lysates after UV induction, DB5000, was selected for further study. Although most of our conclusions about specialized transduction of *tet*^{R} by P22 are based on experiments with a DB5000 HFT lysate, the other two independently isolated *tet*^{R} transductants that produce HFT lysates appear to be similar to DB5000 in all respects.

The major features of specialized transduction of *tet*^{R} with a DB5000 HFT lysate may be summarized: (1) Transduction results in the insertion of *tet*^{R} at or near the P22 prophage attachment site; (2) transductants usually can in turn be induced to yield HFT lysates; (3) transduction for *tet*^{R} is multiplicity dependent—at low multiplicities of infection, the transduction frequency is nearly 100 times lower than the frequency obtained at high multiplicities of infection. Coinfection with a wild-type phage abolishes this multiplicity dependence and restores the transduction frequency to the maximum level attained by the DB-5000 lysate alone at high multiplicity. This multiplicity dependence distinguishes our HFT lysate from the ϵ_{15} HFT lysate (Kameda *et al.*, 1965). The significance of this multiplicity dependence for transduction is discussed in more detail in the following paper (Chan *et al.*, 1973) together with other data which suggest a specific mechanism for the specialized transduction of *tet*^{R} by P22.

Transduction of *tet*^{R} by a DB5000 lysate also takes place in a *rec*$^-$ recipient; the absolute frequency of transduction, with and without helper phage, is reduced by 10-fold, suggesting that the bacterial *rec* system, though not absolutely essential, may function in the transduction process. We interpret this result as indicating that transduction by the *HFT* lysate requires general recombination. This requirement can be met either by the host *rec* function or the phage *erf* function (Botstein and Matz, 1970); in our experiments the phages were all *erf*$^+$. Similarly, the reduced ability of a P22 *int*$^-$ phage to help the DB5000 lysate in transduction suggests that the phage *int* function may also participate in the transduction process. Since the P22Tc-10 prophage in DB5000 is *int*$^+$, it will be necessary to construct a P22Tc-10 *int*$^-$ HFT lysate in order unequivocally to establish the role of the *int* function in transduction.

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