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

II. Properties of a High-Frequency-Transducing Lysate

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A high-frequency-transducing (HFT) lysate for tetracycline resistance (tet^R) was obtained by inducing an unusual tet^R P22 lysogen which had been made by transducing *Salmonella typhimurium* LT-2 to tet^R with P22 phage grown on an LT-2 strain carrying the R factor 222. The HFT lysate contains defective P22 particles, called P22 Tc-10, which cannot grow or lysogenize (i.e., transduce tet^R) upon single infection. However, two defective P22 Tc-10 particles can cooperate with each other for growth or for lysogeny (i.e., transduction of tet^R). "Wild-type" phages which can grow on single infection and which no longer transduce tet^R can be found at a frequency of 5×10^{-5} PFU/particle in the HFT lysate. Five percent of these "wild-type" phages are also integration deficient. At high multiplicities of infection the tet^R transductants made with an HFT lysate appear to be similar to a P22 Tc-10 lysogen (i.e., they are immune to P22 and yield HFT lysates upon induction); at low multiplicities of infection, many of the tet^R transductants are tet^R P22 prophage deletions.

To account for these observations, a model is proposed in which the genes for tet^R are inserted into the intact P22 genome in the P22 Tc-10 prophage. The consequences of such an insertion are discussed with regard to the structure of phage P22 DNA and the topological requirements for successful DNA replication, phage growth, and lysogenization.

INTRODUCTION

The transduction of R factor 222, which carries the markers for resistance to chloramphenicol (cam^R), sulfanilamide (sul^R), streptomycin (str^R), and tetracycline (tet^R), by phage P22 in *Salmonella typhimurium* invariably yields tet^R transductants which lack the other three drug resistance markers. These transductants can no longer transfer the tet^R marker by causing conjugation (Watanabe and Fukasawa, 1961). In the preceding paper (Watanabe *et al.*, 1973) we have shown that the tet^R marker in these transductants maps at or near the P22 prophage attachment site, and that some of these tet^R transductants produce high-frequency-transducing (HFT) lysates for tet^R after induction with ultraviolet light. In studying one of these HFT lysate-producing strains, DB5000, we found that the transduction of tet^R was multiplicity dependent and could be improved, at low multiplicity of the transducing particles, by the addition of P22 helper phage.

This paper presents a detailed study of the HFT lysate. Apparently, the tet^R genes are contained in specialized transducing particles (i.e., particles which contain DNA molecules carrying phage genes as well as the tet^R genes). P22-mediated specialized transduction differs in several fundamental ways from specialized transduction mediated by the coliphage λ. We suggest that these differences can be accounted for by the fact that the DNA of P22 is circularly permuted and

terminally repetitious (Rhoades *et al.*, 1968) whereas the DNA of λ is a unique linear sequence (Davidson and Szybalski, 1971).

MATERIALS AND METHODS

Bacterial strains. All bacterial strains are derivatives of *Salmonella typhimurium* LT-2. The nonlysogenic prototroph DB21 (*su*$^-$) and the amber suppressor strain DB74 (su^+_{am}19*cys*A1348*his*C527) have been described previously (Botstein and Matz, 1970).

The isolation of DB5000 (P22Tc-10), a *tet*^{R} transductant that produces HFT lysates for *tet*^{R} upon induction was described in the accompanying paper (Watanabe *et al.*, 1973). Strain DB5204 (P22Tc-10$tsc_2^{30}m_3$*sie*A1*sie*B1) was constructed by a standard cross (Botstein *et al.*, 1972) between P22$tsc_2^{30}m_3$*sie*A1*sie*B1 and a UV-induced lysate of DB5000. The progeny from this cross were used to transduce strain DB21 to *tet*^{R} at 25°. The transductants were tested for their exclusion properties at 25° by a streak test (Susskind *et al.*, 1971) and for their ability to produce an HFT lysate for *tet*^{R} upon heating to 40°. A heat-inducible, *sie*$^-$, HFT-yielding transductant was purified and induced to give the P22 Tc-10 tsc_2^{30}*sie*A1*sie*B1 stock.

The *tet*^{R} P22 prophage deletion strains DB5005, DB5010, DB5057, DB5062, and DB5201 are described in Chan and Botstein (1972) along with the *pro*$^-$ P22 prophage deletion strains DB123, DB136, and DB147.

DB88 and DB124 (Chan and Botstein, 1972) are *su*$^-$ lysogens of the P22-related heteroimmune phage L.

Phage strains. The following P22 strains, derived from the wild-type strain of Levine (1957), were used: c^+, the wild-type phage; c_2^5, a clear-plaque mutant which does not lysogenize (Levine and Curtiss, 1961); *vir*B3, a virulent mutant (Bronson and Levine, 1971); *int*3, an integration-deficient mutant (Smith and Levine, 1967); *mnt*1, a mutant which is unable to maintain lysogeny (Gough, 1968); m_3*sie*A1*sie*B1tsc^{30} [*sie*A1 and *sie*B1 are mutations which remove the prophage superinfection exclusion system (Susskind *et al.*, 1971); tsc_2^{30} makes the prophage heat-inducible], kindly given to us by M. Gough; 5$^-$*am*N8, 12$^-$*am*N11c_2, 9$^-$*am*N110c_2, and 1$^-$*am*N10c_2, *amber* mutants in genes 5, 12, 9, and 1, respectively (Botstein *et al.*, 1972). The plaque-morphology markers m_3 and h_{21} have been described previously (Levine and Curtiss, 1961).

LcII$_{101}$ (Chan and Botstein, 1972) is a clear plaque mutant of the heteroimmune phage L (Bezdek and Amati, 1967).

Media. LB broth, lambda agar, and soft top agar are described in Ebel-Tsipis and Botstein (1971), as is buffered saline. Mineral medium containing amino acids (M9CAA) is described by Smith and Levine (1964).

Green indicator agar contains (per liter of medium): 8 g of tryptone, 1 g of yeast extract, 5 g of NaCl, 15 g of agar, 21 ml of 40% (w/v) glucose, 25 ml of 2.5% (w/v) Alizarin Yellow G (Matheson, Coleman and Bell), and 3.3 ml of 2% (w/v) Aniline Blue (water soluble) (Fisher Scientific) in distilled water. When desired, a freshly prepared solution of tetracycline-HCl (Calbiochem) was added to the green indicator agar just before pouring to give a final concentration of 25 μg/ml. The tetracycline solution was prepared by weighing the required amount aseptically and dissolving it in a small volume of sterile water.

Antiserum. Anti-phage P22 serum was prepared from a rabbit according to Ebel-Tsipis and Botstein (1971).

Preparation of HFT lysates. Lysogens carrying P22 Tc-10 prophage were grown in M9CAA to exponential phase and induced with ultraviolet light (150 ergs/mm^2) or, in the case of P22 Tc-10tsc_2^{30} strains, by incubation at 40° until lysis. In most cases, phage particles were purified on CsCl gradients as described by Botstein (1968). Since induced lysates are deficient in base-plates, lysates were routinely treated with purified base-plate parts (Israel *et al.*, 1967; Israel, 1967) before the final purification step.

As shown below, a DB5000 HFT lysate contains many defective particles which cannot be titered by an ordinary plaque assay. The highest and therefore the most accurate estimate of the defective particle titer was obtained by measuring the absorbance at 260 nm of the CsCl-purified lysate. HFT lysates were routinely titered on DB147, a *pro*$^-$ P22 prophage deletion, and the titer of defective particles was estimated using the rela-

tion between the titer on DB147 and the titer by absorbance at 260 nm (Table 1). The plaques on DB147 are much easier to see if after adsorption of the phage an equal number of sensitive (DB53) cells are added before plating.

Transduction for tet^R with an HFT lysate. The procedure for transduction was described in the accompanying paper (Watanabe *et al.*, 1973). We observed that the recipient cells become very sensitive to manipulation after adsorption of the phage. Therefore most of the transductions were done by mixing growing cells (without centrifugation) with the transducing particles, incubating briefly, and diluting in isothermal medium. Appropriate dilutions were spread or plated in soft agar on green indicator or LB plates containing 25 μg/ml tetracycline.

Marker rescue. The marker rescue assay, which is a modification of the one used by Smith (1968) to measure defective particles produced by induction of an *int*$^-$ lysogen, measures the ability of the DB5000 HFT lysate to donate *am*$^+$ markers to *am*$^-$ phage growing in an *su*$^-$ cell. The *su*$^-$ strain DB21 was grown with shaking in M9-CAA at 37° to about 3×10^8 cells/ml. The cells were centrifuged and resuspended in buffered saline to 6×10^8 cells/ml. Amber mutants in genes 12, 1, and 9 were used as tester phage. The infection was initiated by mixing 0.2 ml of a dilution of a DB5000 lysate and 0.2 ml of an *amber* tester phage at an m.o.i. of 5 with 0.5 ml of the resuspended DB21. The phage were allowed to adsorb for 15 min at 37°; then 0.9 ml of anti-P22 serum (Ebel-Tsipis and Botstein, 1971) at $K = 10$ min^{-1} was added. After 15 min, the mixture of infected cells was diluted in isothermal M9CAA and plated on DB21. Unadsorbed phage were estimated by treating a portion of the mixture with $CHCl_3$ before plating.

TABLE 1

DIFFERENT METHODS OF TITERING A CsCl-PURIFIED DB5000 LYSATE

Method	Titer (units/ml)	Relative titer
Optical density at 260 nm	1.36×10^{11}	1.0
Rescue of *su*$^-$ cells infected with 12$^-$*am*N11*c*$_2$	2.8×10^{10}	2.1×10^{-1}
Plaques on a P22 prophage deletion (DB147)	8.6×10^9	6.3×10^{-2}
tet^R transductants[a]	3.6×10^9	2.6×10^{-2}
Large plaques on DB21	6.8×10^6	5.0×10^{-5}

[a] Assayed with wild-type helper phage.

Reconstruction experiments in which an amber mutant (5$^-$*am*N8) was used in place of the DB5000 lysate show that the efficiency (observed infective centers on DB21/expected infective centers) of this assay was 50% when 12$^-$*am*N11*c*$_2$ and 9$^-$*am*N110*c*$_2$ were used as tester phage, and 18% when 1$^-$*am*N10*c*$_2$ was used as a tester. The lower efficiency with 1$^-$*am*N10*c*$_2$ is probably due to the smaller recombination frequency (see Fig. 4; also Botstein *et al.*, 1972) between genes 5 and 1 as compared with the recombination frequency between gene 5 and genes 9 and 12.

Streak tests. Streak tests for immunity and exclusion using P22*c*$_2$ and P22 *vir*B3 at 10^{10} phage/ml were performed as described by Susskind *et al.* (1971). A similar streak test was developed to identify *tet*^{R} P22 prophage deletions, which are indistinguishable from nonlysogenic cells by immunity, using the fact that amber mutants can grow on *su*$^-$ prophage deletions if the corresponding *am*$^+$ allele has not been deleted (Chan and Botstein, 1972). Nonimmune clones (sensitive to P22 *c*$_2$) that were also *tet*^{R} were confirmed as being *tet*^{R} prophage deletions by their sensitivity to suspensions of P22 12$^-$*am*N11*c*$_2$ (10^9 phage/ml), since virtually all *tet*^{R} prophage deletions retain gene 12 (Fig. 4). This test will not detect those rare prophage deletions deleted for gene 12.

Spot complementation tests for lysogeny. P22*mnt* mutants are indistinguishable from P22*int* mutants by the spot test of Smith and Levine (1967). Our presumptive *int* mutants were therefore further characterized by complementation for lysogeny against P22*int*3, P22*mnt*, and P22*c*$_2$. Suspensions (10^7 phage/ml) of the two phages to be complemented were spotted together on the surface of a lawn of DB21 on a λ plate. After incubation

at 37° for 16–24 hr, cells from the turbid centers of spots on the complementation plate were streaked for single colonies on green indicator plates. About 10 isolated colonies were tested for immunity by the streak test as described above. Complementation was scored as positive if any stable, immune colonies were found; about 30 colonies were tested before the test was considered negative.

Detection of cured cells. All *tet*^{R} P22 prophage deletion strains are resistant to the heteroimmune phage L because they retain the *sie*B exclusion system (Susskind *et al.*, 1971). Any cell which has lost its *tet*^{R} deletion prophage will have lost *sie*B$^+$ and therefore be sensitive to phage L. The frequency of cured, sensitive cells in a liquid culture of a *tet*^{R} prophage deletion was determined by using the nibbled colony test of Susskind *et al.* (1971) with LcII$_{101}$ as tester phage.

Superinfection of tet^R *P22 prophage deletions.* The P22 *tet*^{R} prophage deletions isolated so far are not immune (Chan and Botstein, 1972) and are therefore easily superinfected by wild-type P22. The superinfection was done on a plate by spotting a suspension of phage (10^7 phage/ml) on a lawn of a *tet*^{R} prophage deletion strain. The plates were incubated overnight at 37° and cells from within the spot of phage growth were incubated overnight at 37° and cells from within the spot of phage growth were purified by streaking on green indicator plates for isolated colonies. Stably lysogenized colonies were large and light green, whereas unstably lysogenized colonies were small and dark green (Smith and Levine, 1967).

Overnight stocks of P22 (Botstein and Matz, 1970) grown on *tet*^{R} P22 prophage deletion strains were used to transduce the *tet*^{R} prophage deletions from one strain to another.

RESULTS

Plaque-Forming Properties of a DB5000 Lysate

A high-frequency-transducing (HFT) lysate of DB5000 makes large plaques and small plaques when plated on a sensitive indicator. The large plaques are about the same size as wild-type P22 plaques and plate as a linear function of the concentration (Fig. 1). In contrast, the small plaques (which are often more numerous) vary from pinhole size to one-third normal size and plate as a function of the square of the concentration (Fig. 1), suggesting that small plaques arise initially from double infections. The turbid centers of small plaques, but not those of large plaques, contain *tet*^{R} transductants.

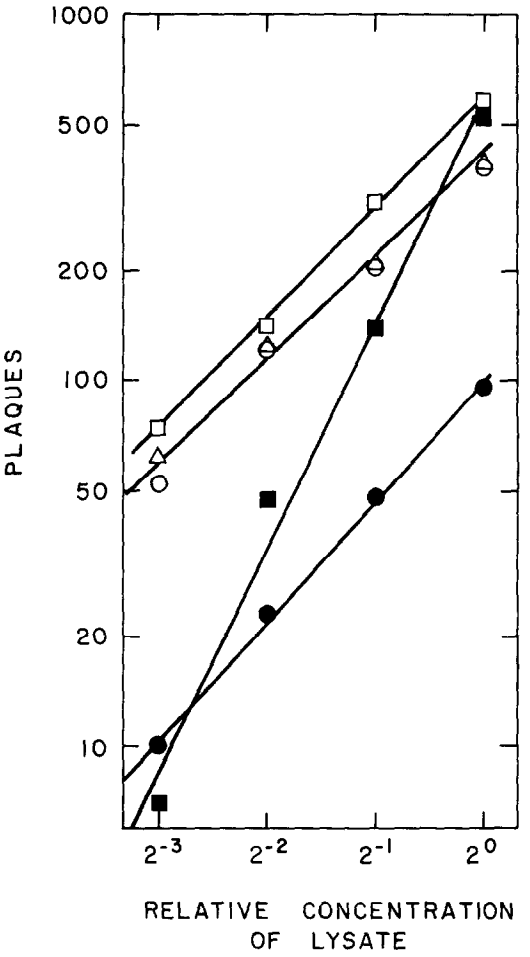
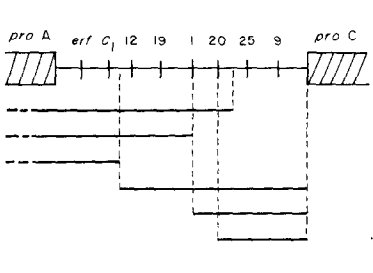


FIG. 1. Concentration dependence of the plaque assay. □, DB5000 lysate on DB147; △, DB5000 lysate on DB88; ○, DB5000 lysate on DB21, big plaques; ■, DB5000 lysate on DB21, small plaques; ●, P 22 c^+ on DB21. DB21 is nonlysogenic; DB88 is lysogenic for the heteroimmune phage L; DB147 is a *pro*$^-$ P22 prophage deletion. For each case, the data from an arbitrary set of serial dilutions is shown.



INDICATOR STRAIN	e.o.p. P22 c^+	e.o.p. DB 5000 LYSATE
DB 136	0.8	160.
DB 123	0.7	840.
DB 147	0.9	1740.
DB 5201	1.0	95.
DB 5062	0.9	93.
DB 5057	0.9	310.
DB 124	1.0	1200.

FIG. 2. Efficiency of plating (e.o.p.) of a DB5000 lysate on P22 prophage deletion strains and on a heteroimmune lysogen. e.o.p. = titer on indicated strain/titer of big plaques on a nonlysogen (DB21). Deletions are represented by the solid line. DB124 is lysogenic for the heteroimmune phage L. DB136, DB123, DB147 are *pro*⁻ P22 prophage deletions. DB5201, DB5062, DB5057 are *tet*^{R} P22 prophage deletion strains.

Defective Particles in a DB5000 Lysate

The nonlinear plating behavior of small plaques and the finding that a DB5000 lysate contained more transducing particles than large plaque-forming units (Watanabe *et al.*, 1973) suggested that defective particles were present in a DB5000 lysate. We have been able to assay these defective particles in several ways.

Under nonpermissive conditions, conditional-lethal mutants of P22 will form plaques at efficiencies of up to 10^{-1} on P22 prophage deletions and on a heteroimmune lysogen (Chan and Botstein, 1972). Figure 2 shows the efficiency of plating of a DB5000 lysate on various P22 prophage deletion strains and on a heteroimmune lysogen. The DB5000 lysate can plate up to 1000 times more efficiently on a prophage deletion strain than on a nonlysogenic strain. Moreover, the plaques which appear on a heteroimmune lysogen (DB88) and on a P22 prophage deletion (DB147) plate as a linear function of the concentration in contrast to the small plaques on a nonlysogenic indicator (DB21) which plate as a function of the square of the concentration (Fig. 1).

A less stringent assay for defective particles is measurement of the ability to supply an *am*$^+$ allele to *su*⁻ cells infected with *am*⁻ phage. A DB5000 lysate was assayed for its ability to donate the *am*$^+$ allele to *su*⁻ cells infected with amber mutants in genes 1, 9, and 12 (Fig. 3). The locations of genes 1, 9, and 12 are shown in the map in Fig. 4. The number of infective centers is directly proportional to the amount of DB5000 lysate added (Fig. 3).

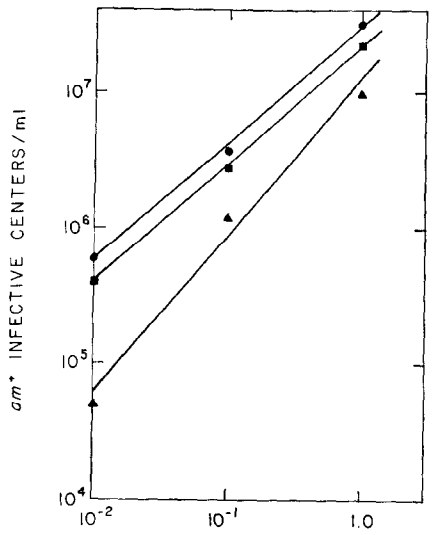


FIG. 3. Rescue of *amber*-infected *su*⁻ cells by superinfection with a DB5000 lysate. ●, DB21 infected with 12⁻*am*N11c_2 at m.o.i. = 3; ■, DB21 infected with 1⁻*am*N10c_2 at m.o.i. = 4.5; ▲, DB21 infected with 9⁻*am*N110c_2 at m.o.i. = 6.5.

Finally, the number of particles in a purified lysate may be estimated physically by measuring the absorbance at 260 nm (Smith, 1968). The results of titering a CsCl-purified DB5000 lysate by each of the methods described above are shown in Table 1. For comparison, the titer of *tet*^{R} transductants under optimal conditions (Watanabe *et al.*, 1973) is included. As expected, the highest estimate of particle titer is given by the absorbance method.

There appear to be 20,000 times as many particles in the HFT lysate as there are large

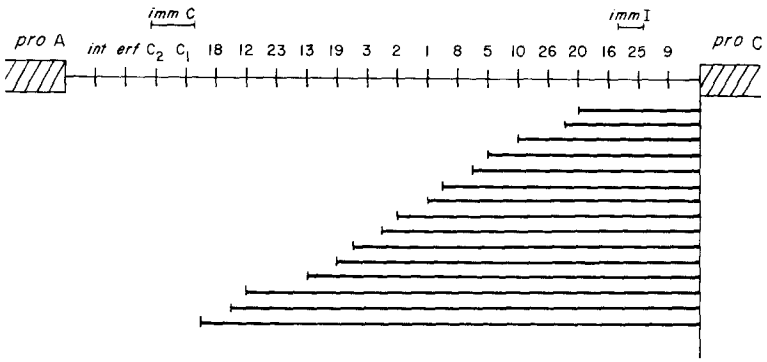


FIG. 4. Prophage of P22 showing extent of *tet*^{R} prophage deletions. Each line represents the material deleted in an individual strain. These deletions are described in more detail in Chan and Botstein (1972) and in Botstein *et al.*, (1972).

plaque-forming units. We conclude from the rescue test that many, if not all, of the defective particles contain P22 genes. However, no particular essential region of the genome is missing from all the defective particles, since overlapping prophage deletions (e.g., DB123 and DB5062) (see Fig. 2) each can support growth of a substantial number of the defective particles.

Preliminary CsCl equilibrium density gradients indicate that the defective particles do not differ significantly in density from the wild type.

Effect of Anti-P22 Serum on the HFT Lysate

In order to be sure that the various activities shown in Table 1 are related to DNA in P22 capsids, we assayed the inactivation of transduction and of plaque-formation on a prophage deletion strain by antiserum directed against wild-type P22 phage. Both activities of the HFT lysate are inactivated by anti-P22 serum at approximately the same rate as wild-type P22.

Cooperative Growth of Defective P22 Tc-10 Particles

The observation that the small plaques in the HFT lysate appear with the square of the amount of lysate plated suggested that two defective particles might cooperate and be able to grow whereas a single particle is, in general, unable to grow. This possibility was supported by the observation that no essential region of the genome is absent from all of the defective particles.

In order to test this idea directly, cells were infected with a range of multiplicities of defective particles or of wild-type but otherwise isogenic phage (control) and the yield of progeny measured after a single cycle of growth. Both phages used cannot lysogenize at the experimental temperature. As shown in Table 2, the defective P22 Tc-10 particles could not grow when the particle multiplicity was less than one particle per cell whereas the control grew well. On the other hand, at high multiplicity, the yield of defective particles was large and comparable to the yield of the control phage.

It should be emphasized that this experiment shows not only that the defective particles can multiply and produce more defective particles after lytic infection of more than one per cell, but also that the transducing activity is correspondingly multiplied. This result supports the idea that the defective particles contain both the *tet*^{R} genes and phage genes, and that these particles are in reality specialized transducing phages.

The data shown in Table 2 also suggest that almost all of the defective particles can cooperate to grow; i.e., ability to cooperate is not restricted to those particles which can plate on a prophage deletion strain. This conclusion derives from the observation that at a particle multiplicity of 1.5 the growth is substantial; at this particle multiplicity the number of prophage-deletion-plaque-forming units per cell is only 0.1, and the number doubly infected would be 0.01.

TABLE 2

COOPERATIVE GROWTH OF DEFECTIVE Tc-10 PARTICLES[a]

M.o.i.		Burst size			
PFU[b]	Particles[c]	Tc-10 lysate			Wild-type lysate (PFU)
		tet^R transductants[d]	PFU[b]	Particles[c]	
0.001	0.015	<0.01	0.012	0.18	120
0.01	0.15	0.02	0.057	0.85	130
0.1	1.5	0.7	0.85	13	105
1.0	15	22	18	270	292
5.0	75	32	13	195	390
10	150	19	13	195	285
20	300	34	22	147	295

[a] Strain DB53 cells growing exponentially in LB broth at 37° at a density of 2×10^8 cells/ml were infected with the indicated numbers of phage particles. The Tc-10 phage was P22 Tc-10 *tsc*$_2$^{30}*sie*Al and the control was P22 *tsc*$_2$^{30}*sie*Al. After adsorption the infected cells were diluted 100-fold into LB at 37° and incubated for a total of 60 min. Unadsorbed phage were negligible.

[b] PFU: for Tc-10 means titer on DB147/DB53 indicator as described in Methods; for wild-type means titer in DB53.

[c] These numbers, which apply only to the Tc-10 lysate, were obtained by multiplying the PFU by 15 (see Table 1).

[d] *tet*^{R} transduction was assayed by coinfecting with wild-type helper phage (m.o.i. = 7) as described in the Materials and Methods.

Cooperative Nature of tet^R *Transduction by Defective P22Tc-10 Particles*

The data of the preceding section show that the defective particles in a P22Tc-10 lysate can go through a single cycle of growth, but only at multiplicities of infection greater than one particle per cell. In the preceding paper (Watanabe *et al.*, 1973) we showed that transduction by P22Tc-10 particles is also dependent upon the multiplicity of infection. This multiplicity dependence of *tet*^{R} is shown in Fig. 5. At multiplicities of infection less than one particle per cell, the number of *tet*^{R} transductants increases as the square of the particle concentration. The number of *tet*^{R} transductants bcomes directly proportional to the particle concentration when the multiplicity is greater than one defective particle per cell. On the other hand, when a nontransducing, nondefective helper phage is added at high multiplicity, transduction by the defective P22Tc-10 particles is directly proportional to the number of particles added regardless of the multiplicity.

We interpret these results to mean that specialized transduction of *tet*^{R} (i.e., lysog-

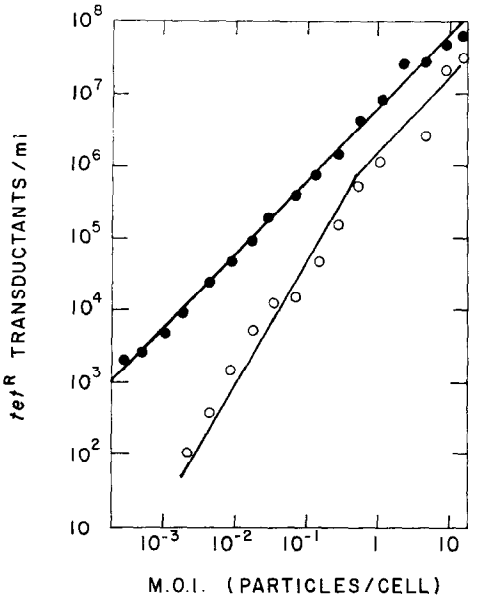


FIG. 5. Effect of multiplicity on *tet*^{R} transduction. The data are based on the experiment described in Fig. 2a of the preceding paper (Watanabe *et al.*, 1973). DB21 was the recipient strain. ●, DB5000 lysate + P22 c^+ helper at an m.o.i. of 6; ○, DB5000 lysate alone.

eny), like lytic growth, requires simultaneous infection by two defective P22Tc-10 particles. The inability of single P22Tc-10 particles to lysogenize can be overcome either by coinfection with another P22Tc-10 particle or by coinfection with a wild-type (helper) phage.

The Origin of the Large-Plaque-Forming Phage in the HFT Lysates

The results presented thus far suggest that the agent responsible for transduction is a defective specialized transducing particle (P22 Tc-10) which contains genes specifying tetracycline resistance. These particles are only defective on single infection, however. Two particles can cooperate either to grow lytically or to lysogenize and produce a tet^R transductant. The nature of the defect is apparently not the loss of particular genes from each of the particles, since overlapping prophage deletions can help the particles to grow. One way of explaining these properties is to assume that a P22 Tc-10 prophage is too large to fit into a phage head because of the insertion into the genome of the genes for tetracycline-resistance. As a result the particles made after induction might consist of random portions of the genome of this oversize prophage. Such particles could recombine with each other or with the genome of a prophage deletion in order to reconstitute a complete oversize genome and thus be able to grow or lysogenize.

It was observed above that HFT lysates contain a small proportion of apparently normal phage which make large plaques linearly with lysate concentration but which do not transduce tet^R, judged by the fact that the centers of these plaques do not contain tet^R transductants, whereas the centers of the small plaques do contain transductants. In the framework of our hypothesis (that the tet^R genes are inserted into the P22 Tc-10 prophage genome, making it too large for a P22 head) these large-plaque-forming phage might represent deletions of the inserted tet^R genes. Such deletions would return the prophage to a size which can be entirely encapsulated. Occasionally such deletion events might also remove neighboring nonessential genes. We therefore examined 96 large-plaque-forming phages from a DB5000 lysate for *int* function. The *int* function is not required for vegetative growth (Smith and Levine, 1967). We found that five out of the 96 large-plaque phages tested from a UV-induced DB5000 lysate were int^-, whereas none out of 100 phages tested from a UV-induced lysate of a wild type P22 lysogen were int^-. These large-plaque phages were classified as int^- because they failed to complement a known int^- phage for lysogeny. In addition, they also appeared to be structurally unable to integrate. A normal point mutation such as *int*3 can be complemented to form a lysogen by P22c_2 which itself cannot lysogenize (Smith, 1968). However, Table 3 shows that complementation of our int^- large-plaque phages by P22c_2 gave only int^+ recombinant lysogens or double lysogens.

This result suggests that loss of the tet^R region is occasionally accompanied by the loss of the *int* gene and a part of the P22 prophage attachment site. The fact that apparently normal, nondefective, plaque-forming phages occasionally arise from a P22 Tc-10 lysogen fortifies the previous conclusion that the P22 Tc-10 prophage is not missing any essential P22 gene.

TABLE 3

ANALYSIS OF LYSOGENS FORMED BY COMPLEMENTATION OF int^- LARGE-PLAQUE PHAGES WITH P22 $m_3c_2h_{21}$

Complementation pair[a]	Lysogen type		
	No. of int^- lysogens[b]	No. of int^+ recombinant lysogens[c]	No. of double lysogens[d]
BP1 × $m_3c_2h_{21}$	0	4	2
BP3 × $m_3c_2h_{21}$	0	2	1
BP4B × $m_3c_2h_{21}$	0	4	1

[a] BP1, BP3, and BP4B are int^- large-plaque-forming phages carrying wild-type plaque morphology markers (m^+, c^+, h^+).

[b] Lysogens which release little or no phage upon UV induction.

[c] Lysogens which give a good phage yield. Eight out of ten lysogens in this class were also recombinant with respect to the input phages for a plaque-morphology marker.

[d] Lysogens that give a good phage yield and are heterozygous for at least one plaque-morphology marker, after two successive purifications from a single colony.

Nature of tet^R *Transductants*

The kind of *tet*^{R} transductants obtained with a DB5000 lysate depends on the multiplicity of infection (Table 4). At multiplicities greater than one, all the *tet*^{R} transductants appear to be similar to DB5000. That is, they are immune to phage and give rise to HFT lysates after induction. At multiplicities less than one, we begin to find nonimmune *tet*^{R} transductants in addition to the immune transductants. Most of these nonimmune transductants are P22 prophage deletions. The mapping of these deletions has been described in Chan and Botstein (1972). Figure 4 shows the extent of the *tet*^{R} prophage deletions which have been characterized so far. All the deletions have lost a common block of genes beginning at the *pro*C side of the prophage. (Occasionally, we find nonimmune *tet*^{R} transductants which lack all P22 genes tested. These transductants have not been studied further.)

When helper phage is used in the transduction, all the *tet*^{R} transductants appear to be similar to DB5000 regardless of the multiplicity of infection of the DB5000 lysate.

TABLE 4

NATURE OF *tet*^{R} TRANSDUCTANTS AS A FUNCTION OF MULTIPLICITY OF INFECTION WITH A DB5000 LYSATE[a]

Multiplicity of infection (particles/cell)[b]	No. of *tet*^{R} P22 prophage deletions[c]	No. of P22 Tc-10 lysogens[d]
6.5×10	0	56
6.5	0	55
6.5×10^{-1}	10	44
6.5×10^{-2}	32	19
6.5×10^{-3}	38	11
6.5×10^{-4}	10	0

[a] Tenfold dilutions of a DB5000 lysate were used to transduce DB21. *Tet*^{R} transductants were picked and tested for sensitivity to P22 c_2 and P22 12$^-$ *am*N11c_2.

[b] 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 Table 1).

[c] Sensitive to P22c_2 and to P22 12$^-$*am*N11c_2.

[d] Resistant to P22c_2 and to P22 12$^-$*am*N11c_2.

Properties of the tet^R *P22 Prophage Deletions*

Colonies which have spontaneously lost their *tet*^{R} P22 prophage deletion can be detected at frequencies of 10^{-1} to 10^{-4} in liquid cultures grown in the absence of tetracycline. The exact frequency depends on the length of the deletion and on whether or not the culture was grown from a freshly isolated colony. Cured colonies may also be found among the survivors of superinfection with P22 *int*$^+$ or P22 *int*$^-$.

The *tet*^{R} prophage deletions can be transduced to *tet*^{S} recipient cells by a wild-type P22 stock grown on a *tet*^{R} prophage deletion strain. The transduction frequency of *tet*^{R} by such a stock is lower than that found with an HFT lysate, but higher than that of the original lysate grown on the R factor 222. The stable, nonimmune transductants obtained in this way appear to be identical to the parental *tet*^{R} prophage deletion. However, it is possible to get *tet*^{R} transductants which have deleted a larger portion of the prophage genome than the parental deletion; the *tet*^{R} prophage deletion in strain DB5201 (which ends between c_1 and gene 18) was derived from the one in strain DB5005 (which ends between gene 1 and gene 8) in this way (see Fig. 4, and Chan and Botstein, 1972).

Double lysogens (defined as clones which are both immune and *tet*^{R}) of P22c^+ and a *tet*^{R} P22 prophage deletion appear to be very unstable. This is illustrated by two observations: A *tet*^{R} prophage deletion which is superinfected by P22c^+ gives unstable *tet*^{R}, immune colonies which rapidly segregate *tet*^{R} prophage deletions, wild-type P22 lysogens, and cured cells in the absence of tetracycline. Similarly, when a wild-type P22 stock grown on a *tet*^{R} prophage deletion strain is used to transduce sensitive cells to *tet*^{R} at high multiplicity of infection, most of the *tet*^{R} transductants are immune and unstable, rapidly segregating the three types of clones described above.

The Role of UV Irradiation in the Formation of Prophage Deletions

A heat-inducible Tc-10 lysogen (DB5204) was isolated from a cross between a DB5000 lysate and P22 *tsc*$_2^{30}$*sie*A1*sie*B1. DB5204 was heat induced and the lysate was examined

and found to behave like a UV-induced DB5000 lysate in every respect except one: no *tet*^{R} prophage deletions were found after transduction at low m.o.i. Nonimmune, *tet*^{R} transductants were found at low m.o.i., but they contained no detectable phage genes. However, using a UV-induced DB5204 lysate, *tet*^{R} prophage deletions were found readily after transduction at low m.o.i. This result suggests that irradiation with UV stimulates the formation of prophage deletions and also implies that the production of prophage deletions is not an essential feature of transduction by an HFT lysate.

DISCUSSION

In the preceding paper (Watanabe *et al.*, 1973) we described the origin of high-frequency-transducing (HFT) lysates capable of transducing genes specifying resistance to tetracycline (*tet*^{R}). This paper concerns itself with the nature of the phage particles which are responsible for the transduction in these HFT lysates. Our results indicate that the HFT lysates contain P22 particles (called P22 Tc-10) which contain the *tet*^{R} genes as well as phage genes. These P22 Tc-10 particles are defective; they will not grow or lysogenize upon single infection of a sensitive host. However, two particles can apparently cooperate and grow normally or lysogenize (producing a *tet*^{R} transductant) after infection at multiplicities of more than one particle per cell. Induction of a P22 Tc-10 lysogen results in a normal-size burst of P22 Tc-10 particles.

The Insertion Model for P22 Tc-10

We believe that the unusual properties of a P22 Tc-10 HFT lysate can best be explained by a model in which the genes for *tet*^{R} are inserted into the intact prophage to produce a P22 Tc-10 lysogen such as DB5000. The consequences of such an insertion can be understood only in the context of the structure of phage P22 DNA and the topological requirements for successful DNA replication, phage growth, and lysogenization.

Phage P22 DNA is circularly permuted and terminally repetitious (Rhoades *et al.*, 1968). The properties of recombination-deficient (*erf*) mutants of phage P22 suggest that circularization of the infecting phage DNA molecule by recombination within the terminal repetition is essential to growth and lysogenization (Botstein and Matz, 1970).

The insertion model assumes that in a P22 Tc-10 lysogen (DB5000) the *tet*^{R} genes are inserted into intact P22 prophage genomes. This insertion is further assumed to make the genome too large to fit into a phage head. Upon induction the oversize prophage circularizes by the *int* system, and thus can replicate. A long linear repeating polymer of the genome is produced (Botstein and Levine, 1968; Botstein and Matz, 1970) which is cut into "headfuls" (Streisinger *et al.*, 1967). A normal-size burst of particles can thus be produced; however, these particles will be defective because the insertion of DNA containing the *tet*^{R} gene renders the phage genome too large to fit into a single phage head. The DNA in these particles is therefore missing the terminal repetition and possibly some adjacent phage genes. However, the molecules are still circularly permuted, and thus particles are each missing different regions. Phage particles without terminally repetitious DNA sequences will not be able to grow or lysogenize (and, therefore, to transduce *tet*^{R}) on single infection since they will not be able to circularize their DNA. However, in a double infection, the DNA molecules from two particles will be able to form a circle by recombining with each other since their DNA sequences are circularly permuted.

This model can explain the major properties of the P22 Tc-10 lysogens and the defective particles derived from them.

Cooperativity of growth. The results show that an HFT lysate contains a large number of defective particles which cannot grow on single infection. However, these particles can grow on multiple infection, or after induction. They can also grow on P22 prophage-deletion lysogens; no particular region of the genome need be present.

These observations are explained in terms of the model as follows: The defective particles are derived from an oversize but otherwise complete prophage which contains the *tet*^{R} genes inserted. The DNA in the particles

is still permuted, thus the missing region (i.e., the terminal repetition and, possibly, some adjacent genes) will be different in different individual particles. Any circumstance which can restore the missing material to some of these particles will serve to allow growth. Double infection is such a circumstance, since the permutation means that in general the DNA of two particles chosen at random will not have the same ends; such a pair of molecules will be able to recombine to make a larger molecule which has all of the information from the original P22 Tc-10 prophage and which will be repetitious at its ends. Thus there is no barrier to growth upon double infection. Similarly, a prophage deletion strain contains a segment of the phage genome which, by recombination, can restore terminal repetition to a large proportion of the defective particles; namely, those whose end falls within the region of the map which remains in the prophage deletion strain. Again, because of the permutation of ends, no particular region of the chromosome is necessary; even in a very long deletion a large number of the defective particles will have ends falling within the nondeleted region.

Cooperativity of lysogenization. The results here are simply that at low multiplicities of infection, the frequency of transduction increases with the square of the particle multiplicity; at high multiplicity transduction is linear with multiplicity. Addition of wild-type nontransducing (helper) phage greatly increases the transduction efficiency and makes it linear with the P22 Tc-10 particle multiplicity.

The model explains this result essentially as above. Circularization by recombination inside the terminal repetition is apparently essential to lysogeny (Botstein and Matz, 1970). Transduction by P22 Tc-10 occurs by lysogeny. The P22 Tc-10 particles have no terminal repetition; however, two permuted molecules can recombine to produce a circular molecule and thus lysogenize. The ability of normal phages to help transduction derives from the ability of their DNA molecules to recombine with the P22 Tc-10 and thus to create the possibility of the production of a circular P22 Tc-10 molecule which can be integrated.

The appearance of rare wild-type phage in HFT lysates. The appearance of wild-type phage in HFT lysates is easily explained by the loss of the *tet*^{R} insertion. If this loss occurs in the prophage state, then upon induction a normal-size circular genome is produced. The resultant genome (including terminal redundancy) fits into the phage head and is thus no longer defective. This result emphasizes the fact that the P22 Tc-10 prophage is not missing genes; the particles are defective *only* because the insertion makes the genome too large for the head.

The occasional loss of nonessential parts of the genome (*int* and *att*) along with loss of *tet*^{R} among the "wild-type" revertants of P22 Tc-10 strongly supports the conclusion that these phages are deletions of the *tet*^{R} region and not wild-type phage which are somehow involved as helpers in transduction. The frequency of 5×10^{-5} which we observed for the occurrence of "wild-type" revertants is not unreasonable in view of the fact that Parkinson and Huskey (1971) calculated a frequency of 10^{-4} for the occurrence of deletions in a UV-induced lysate of a λ lysogen.

The nature of tet^R *P22 prophage deletions.* Transduction at very low multiplicity with P22 Tc-10 results in the appearance of transductants which are missing blocks of phage genes. This result is also explained by the insertion model. Circularization is required for lysogeny and thus for transduction. P22 particles have no terminal repetition and hence cannot circularize. However, late phage functions are not essential for lysogeny (Smith and Levine, 1967; Botstein *et al.*, 1972) and thus they can be deleted before encapsulation, thus restoring terminal repetition. This mode of transduction should be, and is, the primary mode at very low multiplicity of infection.

Our insertion model is summarized in Fig. 6. The P22 Tc-10 prophage is shown at the left; the structure of the intracellular intermediate DNA is shown for three cases; no deletion, deletion of *tet*^{R}, and deletion of phage late genes. The properties of particles matured in the three cases are listed at the right of Fig. 6.

Several testable predictions concerning the physical nature of defective P22 Tc-10 par-

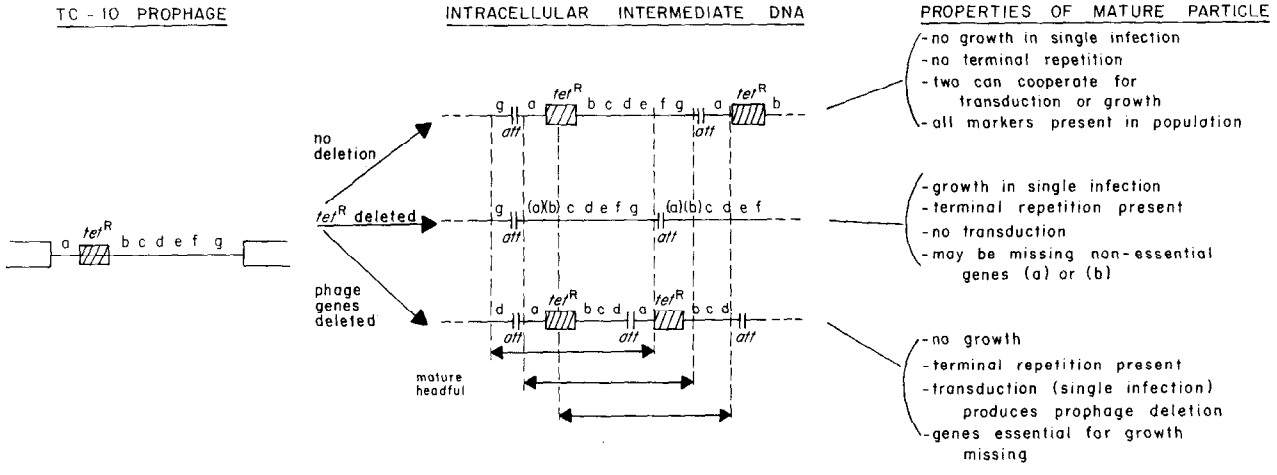


FIG. 6. The insertion model for P22 Tc-10. The consequences of an insertion of the *tet*^{R} genes into the P22 prophage genome and how the effects on function (i.e., growth or transduction) of such an insertion can be overcome by compensating deletions.

ticles in an HFT lysate can be made. The DNA in particles from the lysate should be lacking terminal repetition. This can be measured directly by the methods of Rhoades *et al.* (1968). If the DNA in defective particles is unable to circularize for lack of terminal repetition, one round or less of DNA synthesis should take place. The pattern of DNA synthesis should therefore resemble that of a P22 *erf*$^-$ infection in a *rec*$^-$ host. Electron microscopy of the heteroduplex DNA molecules formed by annealing wild-type P22 DNA with DNA of defective P22 Tc-10 particles should show the *tet*^{R} insertion.

Formation of tet^R *Prophage Deletions*

The inability to find *tet*^{R} prophage deletions after transduction at low m.o.i. with a heat-induced P22 Tc-10 lysate which behaves like a UV-induced lysate in every other respect suggests that the formation of prophage deletions is nonessential for the mechanism of high frequency transduction by a P22 Tc-10 HFT lysate. This result also suggests that the formation of prophage deletions is stimulated by UV.

A priori there is no reason why all the prophage deletions found so far should start next to the right-hand attachment site of the prophage. Perhaps UV stimulates the phage *int* system to make the deletions. In phage lambda, Davis and Parkinson (1971) found that the formation of deletions which removed the right-hand end of the prophage attachment site was dependent upon phage *int* function.

Comparison of P22 Tc-10 Particles with T4 "Light" Particles

The structure of mature DNA from phages P22 and T4 is similar: both DNAs are circularly permuted and terminally repetitious. It is therefore useful to compare P22 Tc-10 particles with T4 "light" particles (Mosig, 1968). Mosig (1963) showed that T4 "light" particles contain incomplete phage genomes and are therefore defective on single infection. However, two or more "light" particles can cooperate in a multiple infection to produce progeny phage. We find similar results with P22 Tc-10; however, the defective particles do not differ from wild type in density. The origin of T4 "light" particles is different from the origin of P22 Tc-10 particles: in the T4 case, normal phage DNA is packaged into an undersized phage head which cannot accommodate the entire genome; in the P22 case, phage DNA containing an oversized genome must be packaged into a normal phage head which cannot accommodate the oversized genome. However, the resulting defective particles are equivalent in the sense that both the T4 "light" particles and the P22 Tc-10 particles contain incomplete phage chromosomes whose nucleotide sequences are circularly permuted but not terminally repetitious.

A Comparison with Other Systems of Specialized Transduction

DB5000 appears to be identical to T10, a *tet*^{R} transductant, described by Dubnau and Stocker (1964) in their study of P22 transduction of drug resistance markers from an R factor of independent origin from 222. T10 was a "semidefective" *tet*^{R} transductant which was immune to P22 and which produced few viable phage. Plaque-forming phage could only be isolated if T10 were grown in a mixed culture with nonlysogenic cells. Cell-free filtrates of overnight cultures of T10 transduced *tet*^{R}; the addition of wild-type helper phage increased the number of transductants.

Another *tet*^{R} transductant, T1, which was immune to P22, but did not produce phage, was mapped close to *pro*A near the P22 prophage attachment site.

The high-frequency transduction of *tet*^{R} from an R-factor by phage ϵ_{15} described by Kameda *et al.* (1965) does not resemble our system. Their HFT lysate was derived by UV-inducing a *tet*^{R} transductant which had been lysogenized by wild-type ϵ_{15}. The resulting HFT lysate, unlike our DB5000 lysate, contained many viable plaque-forming phage. Transduction was independent of the m.o.i., and could not be improved by the addition of helper phage.

The λ-*gal* system has been a useful model system for the study of specialized transduction since so much is known about its

physical and genetic properties (Campbell, 1971). Therefore, in trying to understand specialized transduction by P22, we have compared the P22 *tet*^{R} HFT system with the λ *gal* HFT system (Table 5) to see whether the properties of a DB5000 HFT lysate could be interpreted in terms of the λ *gal* model.

Since a DB5000 lysate contains a mixture of defective transducing particles and viable phage, the most appropriate comparison seems to be between the DB5000 HFT lysate and a (λ) (λ *gal*) HFT lysate which also contains a mixture of defective transducing phage and viable phage. But whereas the (λ) (λ *gal*) HFT lysate contains only two kinds of particles, λ$^+$ and λ *gal*, in roughly equal proportion, a DB5000 HFT lysate consists mostly of defective particles which must be heterogeneous since they can complement each other to grow and to make *tet*^{R} transductants. At low frequency (5×10^{-5}) in a DB5000 lysate, we find viable "large-plaque-forming" phages, of which 5% also happen to be functionally and structurally integration deficient. If DB5000 were a double lysogen (P22) (P22*dtet*), a special mechanism would have to be postulated to account for the low yield of viable phage relative to defective transducing phage and for the high proportion of these viable large-plaque-forming phage which are also *int*$^-$. In addition, we have shown that the double lysogen (P22*c*$^+$) (*tet*^{R}P22 deletion) is very unstable. Thus a postulated (P22) (P22*dtet*) double lysogen would have to have a unique structure which renders it stable, since we know that DB5000 is very stable.

Each λ *gal* HFT lysate contains only one type of λ *gal* phage. To get λ *gal*s with different size substitutions, one must isolate independent *gal*$^+$ transductants from a LFT lysate. On the other hand, a single DB5000 HFT lysate can give rise to a whole range of *tet*^{R} prophage deletions. It is hard to imagine how so many different *tet*^{R} prophage deletions could arise from a single (P22) (P22*dtet*) double lysogen. Another argument against DB5000 being a double lysogen is that we have never seen a Tc-10 lysogen which was heterozygous for phage markers.

We conclude that our results with the P22 Tc-10 HFT lysate are more consistent with the insertion model proposed at the beginning of the Discussion than with a (λ)

TABLE 5

A COMPARISON OF SPECIALIZED TRANSDUCTION OF *tet*^{R} BY P22 WITH SPECIALIZED TRANSDUCTION OF *gal*$^+$ BY λ

	(λ$^+$) (λ*gal*) HFT lysate[a]	λ *gal* alone[a]	λ *pgal*[b]	DB5000 HFT lysate
Plaque formation	Yes[b]	No	Yes	Yes[c]
Linearity[d] of plaque formation	Yes	—	Yes	Large plaques—yes Small plaques—no
Transduction by plaque-forming phage	No	—	Yes	Large plaques—no Small plaques—yes
Ratio of defective particles to normal plaque-forming particles	1:1	All defective	All viable	20,000:1
Linearity[d] of transduction	No	Yes	Yes	No
Nature of transductants at:				
High m.o.i.	(λ)(λ*gal*)	(λ*gal*)	(λ *pgal*)	P22 Tc-10
Low m.o.i.	(λ*gal*)			Many different prophage deletions

[a] Based on Campbell (1957), Arber (1958), Campbell (1971).

[b] Only λ$^+$ makes plaques.

[c] Large and small plaques.

[d] Linearity indicates a single particle is sufficient.

(λ *gal*) double lysogen model. The main virtue of the insertion model is that it takes into account the DNA encapsulation mechanism which is presumably responsible for maintaining the circular permutation of P22 DNA in each cycle of growth. The P22 insertion model may be more relevant than the λ *gal* model for thinking about specialized transduction by other phages with circularly permuted DNA.

In particular, specialized transduction by phage P1 could be interpreted in terms of the insertion model proposed to explain specialized transduction of *tet*^{R} by P22. The DNA of phage P1, like that of phage P22, is circularly permuted and terminally repetitious (Ikeda and Tomizawa, 1968). Unlike P22, P1 is not inserted into the bacterial chromosome, but exists as a circular plasmid in the lysogenic cell. Any host gene which has recombined with the P1 genome is therefore an insertion. Stodolsky and Rae (Stodolsky and Engel, 1971; Rae, 1971) have independently proposed a similar model to explain the properties of P1*dl*.

P22 has been reported to transduce, by specialized transduction, the *pro*AB genes after lytic growth (Smith-Keary, 1966; Jessop, 1972), the *pro*AB genes upon induction (Wing, 1968), and *pro*AB, *pro*AB*lac*, *arg*F, and several other bacterial genes upon induction (Roth and Hoppe, 1972; J. R. Roth, personal communication). The appropriateness of our insertion model for explaining the properties of these other P22 specialized transducing phages remains to be tested.

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