VIROLOGY 45, 629–637 (1971)

Superinfection Exclusion by P22 Prophage in Lysogens of *Salmonella typhimurium*

I. Exclusion of Generalized Transducing Particles

JUDITH EBEL-TSIPIS AND DAVID BOTSTEIN

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Accepted April 26, 1971

P22-mediated generalized transduction in *S. typhimurium* occurs in both sensitive and lysogenic recipient bacteria, but the efficiency of transduction is significantly higher in sensitive cells than in wild-type P22 lysogens. This reduction in the efficiency of transduction is under the control of the prophage *sie*A gene; this gene is also responsible for the exclusion of superinfecting phage. Experiments with purified radioactive transducing particles demonstrate that exclusion of transducing particles in a *sie*$^+$ lysogen occurs at some step after the adsorption of transducing particles to the cell. This exclusion can be partially overcome by infection of the *sie*$^+$ lysogen with large numbers of nontransducing phage in addition to the purified transducing particles.

INTRODUCTION

The term "transduction" was first introduced by Zinder and Lederberg in 1952 to describe the ability of "filtrable agents" to transfer hereditary traits from one strain of bacteria to another. These early experiments were performed with P22-sensitive recipients and P22 wild-type transducing bacteriophage; under these conditions most transductants were found to be lysogenic for P22. In 1955 Zinder reported that transduction could also be carried out on cells which were lysogenic for P22 but that the efficiency of transduction (i.e., number of transductants obtained per phage added) was significantly lower in these lysogens than in the sensitive parents; this lower efficiency was observed when transducing lysates were prepared from either wild-type or "virulent" phages. The results presented in this paper demonstrate that this reduction in the efficiency of transduction is under the control of the prophage *sie*$^+$ gene.

Wild-type phage P22, when present as a prophage in *Salmonella typhimurium* LT2, exhibits a property called superinfection exclusion (*sie*$^+$) which has been described by Walsh and Meynell (1967) and Rao (1968). This exclusion property is unique to P22 and differs from the immunity repression common to temperate bacteriophages (Calendar, 1970). For example, in *E. coli* cells lysogenic for phage λ, superinfecting phage of the same immunity as the prophage are prevented from growing due to the presence of an immunity-specific repressor in the cell while superinfecting phage having an immunity different from that of the prophage can carry out a normal infection. Although P22 has an immunity repression system similar in some ways to that of λ, neither homoimmune nor heteroimmune bacteriophage can grow in cells lysogenic for wild-type P22. Wild-type P22 lysogens thus have an additional mechanism of excluding superinfecting phage which is not immunity specific; the role of the prophage in this exclusion became evident when mutants of P22, called P22 *sie*$^-$, were isolated. Lysogens of these mutant phages are still immune to P22, but they do not exclude virulent P22 or a number of heteroimmune phages (Rao, 1968).

In this paper we show that the exclusion

system defined by Rao's *sie*⁻ mutants is also responsible for the inhibition of transduction into P22 lysogens. Since the data indicate that generalized transducing particles contain primarily bacterial DNA, it would appear that the *sie*⁺ exclusion is quite nonspecific to the nature of injected DNA.

A more complete analysis of the genetic elements responsible for superinfection exclusion by P22 prophage is presented in the following paper (Susskind *et al.*, 1971).

MATERIALS AND METHODS

Bacteria. The following strains of *Salmonella typhimurium* were used: DB21, a prototrophic strain of LT2; DB25, a thymine-requiring derivative of DB21 (Botstein and Matz, 1970); PV78 *his*D23*met*C30 *gal*50*pur*C213 was kindly supplied by Dr. B. Magasanik; the lysogens PV78 (P22 *sie*⁺m_3) and PV78 (P22 *sie1*m_3) were made from PV78.

Bacteriophages. The following P22 phage strains were used (see also Susskind *et al.*, 1971): P22 *sie*⁺m_3, called hereafter wild-type P22; P22 *sie1*m_3, a derivative of a nonexcluding mutant isolated by Rao (1968); P22 $m_3c_2{}^5h_{21}$ a clear mutant carrying the morphological markers m_3 and h_{21} (Levine and Curtiss, 1961); P22 $c_1{}^7ts19.1$, a clear mutant phage which is temperature-sensitive for lysis and continues to make phage late in infection at the nonpermissive temperature of 39°.

Media. LB broth (1% Bacto-tryptone, 0.5% yeast extract, 0.5% NaCl, pH 7.0), lambda agar (1% Bacto-tryptone, 0.25% NaCl, 1.1% agar), and soft agar (0.8% nutrient broth, 0.5% NaCl, 0.65% agar) were the complex media used.

Ozeki-minimal agar (Ozeki, 1959) supplemented with 0.2% glucose or 0.2% galactose, 1 μg/ml thiamine HCl, and 20 μg/ml of adenine or amino acids where appropriate was used to plate for transductants. Minimal top agar (0.7% agar, 0.8% NaCl) was also used in some cases when plating was done by the overlay method.

Buffered saline (0.85% w/v NaCl, 0.066 *M*-phosphate buffer, pH 7.1) was used to resuspend phage which had been purified by centrifugation.

Dilution fluid (0.1% Bacto-tryptone, 0.7% NaCl) was used for all phage and bacterial dilutions.

Minimal medium supplemented with casein hydrolyzate (LCG20) is described by Botstein (1968).

Density-labeling minimal medium used to prepare purified transducing particles contains: 0.1 *M*-Tris (pH 7.4), 0.011 *M* $^{15}NH_4Cl$, 2.5×10^{-3} *M* $MgSO_4$, 0.012 *M* NaCl, 0.2% (w/v) glucose, 20 μg/ml phosphorus (as phosphate), 10 μg/ml thymine in D_2O

Chemicals. D_2O (99.1–99.8% pure) and $^{15}NH_4Cl$ (99% pure) were obtained from BioRad, Richmond, California. Thymine-methyl-^3H was obtained from Schwarz/Mann, Orangeburg, New York. Acid-hydrolyzed casein (vitamin and salt free; 10% solution) was obtained from Nutritional Biochemical Co., Cleveland, Ohio. Lysozyme (3× crystallized, Grade 1) was obtained from Sigma, St. Louis, Missouri.

Bacteriophage lysates. Phage stocks were generally grown on DB21. Cells were grown in LCG20 with shaking at 39° to a density of 2×10^8/ml. Phage were then added at m.o.i. = 5, and incubation continued either until the infected cells lysed or, in the case of cells infected with P22 $c_1ts19.1$, for 2 hr, when lysis was effected by treatment of the infected cells with lysozyme (1 mg/ml) and EDTA (0.01 *M*) for 15 min followed by $CHCl_3$. Phage lysates were purified and concentrated by differential centrifugation and resuspended in buffered saline.

The temperature of 39° was selected for phage growth because it was noted that lysates made at lower temperatures contained fewer transducing particles per plaque-forming unit; a comparison of two otherwise identical lysates, with one grown at 25° and the other at 39°, showed that the former had a 10-fold lower ratio of transducing activity to plaque-forming units than the high temperature lysate although the burst size in both cases was approximately the same.

Transducing particles. Density-labeled transducing particles were prepared relatively free of vegetative phage by the following procedure: DB25 was grown overnight at 37° in heavy minimal medium containing 10 μg/ml thymine and thymine-methyl-^3H

at a specific activity of 13 μCi/μg thymine. Under these conditions, a doubling time of 3 hr was normal. When the culture reached a density of 5 $\times$ 10^8 cells/ml, unlabeled thymine in D_2O was added to the culture (to a final concentration of 400 μg/ml) and growth was allowed to continue for 15 min. P22 $m_3c_2h_{21}$ phage were then added at a m.o.i. (multiplicity of infection) of 5 phage/cell and 2 min later NaCN (2×10^{-3} *M*) was added to the culture in order to inhibit further DNA replication. Adsorption was interrupted 10 min later and the infected cells were filtered on Millipore filters (GSWP04700), washed well with dilution fluid, and then resuspended in prewarmed LCG20 containing 200 μg/ml cold thymine. Cells were incubated with shaking at 39° for 150 min and then lysed with $CHCl_3$. Phage were purified by centrifugation as described by Botstein (1968). Transducing particles were purified away from plaque-forming units by repeated preparative CsCl equilibrium density gradient centrifugations. Figure 1 shows the density profile of the initial CsCl density gradient; Fig. 1a shows the distribution of the biological activity in the gradient, and Fig. 1b shows the distribution of ^3H counts. It can be seen that transducing particles prepared as above have an average density of 1.524 g/ml while plaque-forming activity has the normal density for P22 of 1.507 g/ml; these two types of particles can therefore be separated from one another. One can thus prepare density labeled radioactive transducing particles, containing only host DNA made prior to infection; these are essentially free of vegetative phage. These transducing particles will be referred to as purified transducing particles.

Transducing particles used in the experiments described in Tables 2 and 3 were prepared as above, but at a specific activity of 2 μCi/μg thymine.

Transduction procedure. PV78 or one of its lysogenic derivatives was grown at 38° in LB broth to a density of 2–4 $\times$ 10^8 cells/ml. One-milliliter aliquots of cells were added to 0.1 ml samples of phage and adsorption was allowed to occur for 15–20 min at this same temperature without shaking. Samples were then diluted appropriately and plated for

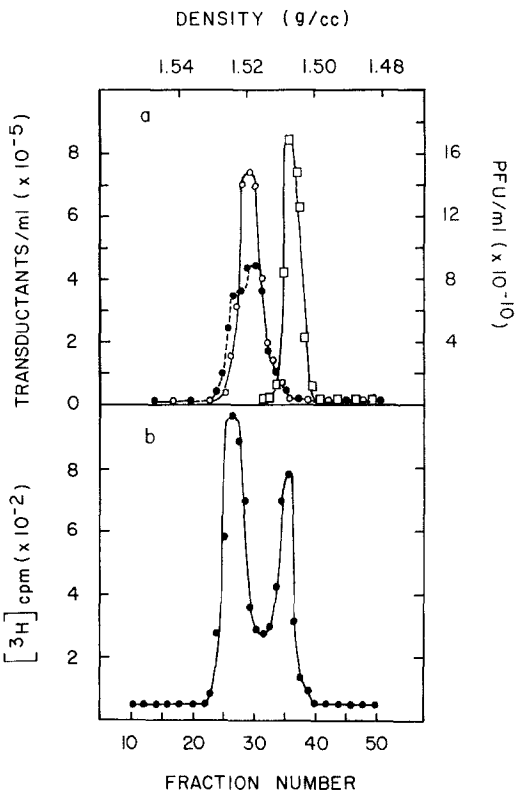

FIG. 1. CsCl equilibrium density gradient of density-labeled, radioactive transducing particles. Density-labeled transducing particles were prepared as described in Methods; the initial lysate was run on a preparative CsCl density gradient for 24 hr at 22,000 rpm in a Spinco SW 39 rotor (25°C). Three-drop fractions were collected into 0.4 ml of buffered saline from a puncture in the bottom of the tube and analyzed for biological activity (Fig. 1A) and radioactivity (Fig. 1B). Transducing activity was determined as described in Methods using PV78 (*sie1m*$_3$) as the recipient and plaque-forming activity was assayed as described by Adams (1959) using DB21 as indicator bacteria. (a) □—□, PFU/ml; ○—○, *his*$^+$ transductants/ml, ●—●, *gal*$^+$ transductants/ml. (b) Radioactivity was assayed by counting 50-μl aliquots from each fraction on a scintillation counter. ●—●, [^{3}H] cpm.

complete transductants to either *his*$^+$, *gal*$^+$, or *pur*$^+$. Transductants were scored after 48 hr incubation at 37°.

In order to be able to score transductants of nonlysogenic PV78, antiserum was added to the selective plates; since the transducing lysates were made from a clear phage, anti-

serum is needed to protect cells from phage growing on the plate. After adsorption of phage to PV78, the culture was diluted and an aliquot of cells was added to 2.5 ml of minimal top agar containing anti-P22 serum at $K = 20$ min^{-1}. This agar was then poured onto a selective minimal agar plate, and incubation and scoring were as described above.

Anti-P22 serum. Antiserum against P22 was made as follows: 0.5 ml of P22 c_1h_{21}, which had been purified on a CsCl density gradient and resuspended at 2.5×10^{11} phage/ml in buffered saline, was injected intramuscularly into a rabbit at 3-day intervals over a 3-week period. The rabbit was bled 5–7 days later. The blood was allowed to clot and the serum was adsorbed against DB21 in order to eliminate any antibodies against *S. typhimurium*. The cells were centrifuged out and the serum was sterilized by filtration through a Millipore filter. Serum is stored in 0.5 ml aliquots at −20°C. K values range from 200–300 min^{-1} (Adams, 1959).

RESULTS

Effect of Superinfection Exclusion on Transducing Efficiency

An investigation into the effects of superinfection exclusion on transduction was undertaken following the publication of Rao's (1968) paper on the isolation of mutants which did not exclude heteroimmune phage. Our observations clearly show that this prophage-controlled *sie*$^+$ function is also responsible for the reduction in the efficiency of transduction which Zinder (1955) had earlier described. Compared to either sensitive or P22 *sie*$^-$ lysogens, cells lysogenic for P22 *sie*$^+$ show a very much reduced efficiency of transduction.

A series of parallel transduction experi-

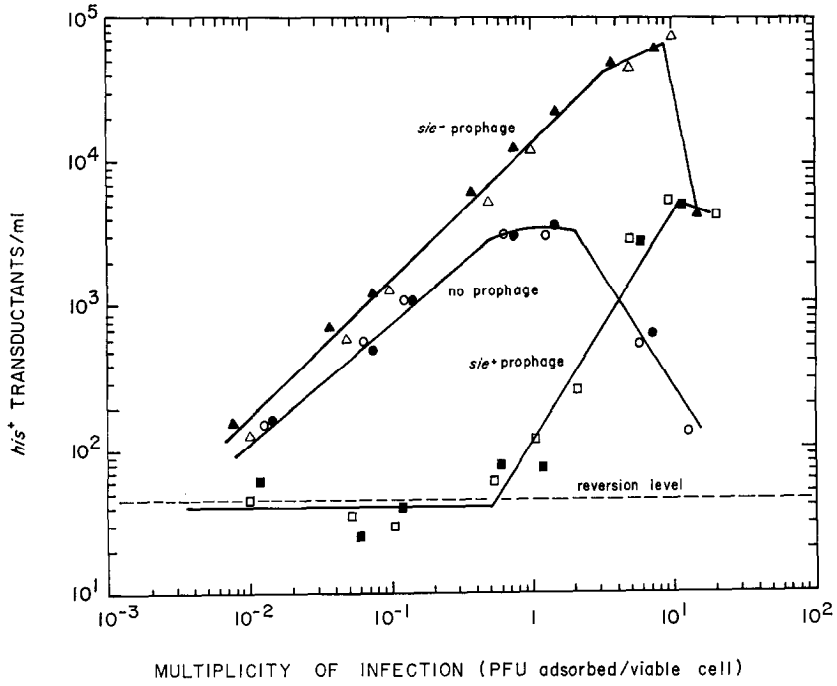

FIG. 2. Effect of phage multiplicity on the formation of *his*$^+$ transductants. Transducing lysates of P22 $c_1{}^7ts19.1$ were grown on DB21 and phage (0.1 ml) at the appropriate dilution were added to 0.9 ml cells. Following adsorption (15 min at 39°), infected cells were plated for *his*$^+$ transductants. Transductants of PV78 were plated in the presence of anti-P22 serum as described in Methods. Open and filled symbols represent the results of two experiments. △—△, ▲—▲, PV78 ($sie1m_3$) as recipient; ○—○, ●—●, PV78 as recipient; □—□, ■—■ PV78 (sie^+m_3) as recipient.

ments using a clear mutant of P22 were carried out on three kinds of recipient cells: PV78, PV78(*sie*$^+m_3$), and PV78(*sie*1m_3). Transductions were performed at adsorbed multiplicities of infection ranging from 0.01 phage/cell to 20 phage/cell. The results of two such experiments are shown in Fig. 2 and can be summarized as follows:

1. Transduction of PV78 for *his*$^+$ is roughly linear with phage multiplicity up to m.o.i. = 1; multiplicities of infection greater than 1 kill the sensitive cells even in the presence of antiphage serum on the plate. One cannot assay lysates of clear phage for transducing activity above this multiplicity in the sensitive host.

2. Transduction into a wild-type lysogen [PV78(*sie*$^+m_3$)] is very different and confirms Zinder's observation that the efficiency of transduction in a wild-type lysogen is lower than that found in a nonlysogen. At low multiplicities of infection, no *his*$^+$ transductants were observed above background while at higher multiplicities of infection (≥ 1) stable transductants were found but at lower levels than those found with the sensitive host or the *sie*$^-$ lysogen. In addition, the number of transductants formed in a (*sie*$^+$) lysogen is not linearly related (i.e., the log-log slope is greater than 1) to the phage input as is the case with transduction of a sensitive recipient.

3. Transduction of PV78 (*sie*1m_3) for *his*$^+$ is linear with number of phage up to m.o.i. = 10 and occurs with an efficiency which is slightly greater than that found in the nonlysogen. This demonstrates that one can transduce a *sie*$^-$ lysogen at efficiencies comparable to those obtained with sensitive recipients.

It is clear from these results that a prophage-controlled function (*sie*) is responsible for the exclusion of *his*$^+$ transducing particles. It should be noted that these *sie*$^-$ mutants were obtained by selecting for nonexclusion of phage (Rao, 1968).

In order to make certain that this *sie*$^+$-controlled exclusion of transducing particles was not limited only to particles carrying the *his*$^+$ genes, transductants for *pur*$^+$ and *gal*$^+$ were also scored in another experiment. The results of this experiment are shown in Table 1 and indicate that exclusion extends

TABLE 1

TRANSDUCTION FOR *his*$^+$, *gal*$^+$, AND *pur*$^+$ IN *sie*$^+$ AND *sie*$^-$ P22 LYSOGENS[a]

Multiplicity of transducing phage	Transductants/ml					
	his$^+$		*gal*$^+$		*pur*$^+$	
	sie$^-$	*sie*$^+$	*sie*$^-$	*sie*$^+$	*sie*$^-$	*sie*$^+$
None	25	25	<5	<5	<5	<5
0.1	810	50	35	<5	—	<5
1.0	9,500	140	780	15	500	<5
10.	60,000	7050	10,600	1100	5000	1000

[a] The donor phage $c_1ts19.1$ were grown on DB21 (prototroph) as described in Methods. Multiplicities of infection (m.o.i.) are not corrected for adsorption; in all cases 0.1 ml of phage was added to 0.9 ml of recipient cells at 2×10^8/ml in LB.

to these other markers as well. In all three cases, and at all multiplicities of infection examined, the level of transduction obtained with a *sie*$^+$ lysogen is depressed. In spite of this general depression of transduction, the relative frequencies of transductants for the three markers were approximately the same in all cases examined; that is, in both PV78(*sie*1m_3) and PV78(*sie*$^+m_3$) there were consistently more *his*$^+$ transductants than *gal*$^+$ or *pur*$^+$ transductants and slightly more *gal*$^+$ than *pur*$^+$ transductants. This pattern has repeated itself in other lysates, but its origin is not completely understood; this pattern could reflect either differences in the frequencies of different transducing particles in the lysates or differences in the probabilities of integration of various markers once inside the bacterial cell.

Effect of Superinfection Exclusion on Transduction with Purified Transducing Particles

In the foregoing experiments transduction has been examined using lysates which contain an excess of viable phage in addition to the transducing particles. In order to determine whether these viable phage affect the exclusion of transducing particles by *sie*$^+$ lysogens, transducing particles were purified and tested for their capacity to transduce *sie*$^+$ lysogens. Since the purified transducing particles were radioactive, it

was also possible to assay directly for their adsorption. Our results indicate that the *sie*$^+$ system is capable of excluding transducing particles at some step after adsorption and that this exclusion occurs in the absence of simultaneous phage infection.

Purified transducing particles were prepared as described in Methods. These transducing particles contain only bacterial DNA which was synthesized prior to phage infection and hence was radioactive and density labeled. An examination of the DNA extracted from these transducing particles reveals that this transducing DNA has an average density which is similar to that of the DNA of the host from which the particles were isolated; an upper limit on the amount of newly synthesized DNA encapsulated in transducing particles can be set at 6% of the total transducing DNA (data not shown).

In an unpurified lysate there is approximately one *his*$^+$ transducing particles per 2×10^4 plaque-forming units while in a purified lysate there are 20 *his*$^+$ particles per plaque-forming unit. It is generally difficult to assess the total number of transducing particles in a lysate due to the existence of abortive transductants, which constitute about 90% of all transductants (Stocker *et al.*, 1953), and to individual marker differences in transducing frequencies. However, with stocks of purified transducing particles one can make an estimate on the basis of specific activity calculations. This calculation requires that one know the specific activity of the bacterial DNA at the time of infection (2 μCi thymine-methyl-^{3}H/μg thymine), the size of the DNA in transducing particles (27×10^6 daltons) and that transducing particles contain unreplicated host DNA. The purified lysate used in this experiment had about 2×10^{-6} [^{3}H] cpm/particle, 10^5 [^{3}H] cpm/ml, and therefore about 5×10^{10} transducing particles per ml.

PV78(*sie*$^+m_3$) and PV78(*sie*1m_3) were infected with these purified transducing particles at several concentrations of particles, arranged so that most cells received transducing particles unaccompanied by viable phage. After 20 min at 39°, aliquots of each culture were assayed for *his*$^+$ transductants and for [^{3}H]-counts adsorbed to the cells. The results (Table 2) show that in all cases the ratio of *his*$^+$ transductants to cpm adsorbed was reduced more than 100-fold in *sie*$^+$ lysogens compared to the *sie*$^-$ control. The exclusion system therefore does not act by preventing adsorption of transducing particles to the cell, but rather at some later step in the transduction process. Since the effect of the *sie*$^+$ character on transduction is still observed with purified transducing particles, the expression of the exclusion system can in no way depend on simultaneous phage infection. On the con-

TABLE 2

EXCLUSION OF PURIFIED TRANSDUCING PARTICLES[a]

Volume added (μl/ml recipient cells)	Number of particles/cell[b]	*sie*$^-$				*sie*$^+$			
		Transduction		Absorption Ratio		Transduction		Absorption Ratio	
		his$^+$/ml	pur$^+$/ml	cpm/ml	*his*$^+$/cpm	*his*$^+$/ml	pur$^+$/ml	cpm/ml	*his*$^+$/cpm
5	0.7	43,500	4,720	150	290	0	0	240	—
20	2.8	211,000	24,600	920	230	160	10	738	0.22
50	7	543,000	50,700	2240	240	250	30	2020	0.12

[a] Exponential cultures of PV78(*sie*$^+m_3$) and PV78(*sie*1m_3) at 3×10^8/ml were infected with purified transducing particles for 20 min at 39°. One-milliliter samples were centrifuged, washed once with dilution fluid, and resuspended in 1 ml of dilution fluid. A fraction of the sample was used to assay transductants, and another fraction (200 μl) was counted in a scintillation counter. Samples were counted for 5 min 3 times; all values represent at least twice background (about 50% of the total added counts were recovered). Transduction values have been corrected for *his*$^+$ and *pur*$^+$ revertants (about 20 *his*$^+$/ml and 0 *pur*$^+$/ml).

[b] Calculated as described in the text. In no case was the number of viable phage/cell greater than 1×10^{-4}.

trary, as shown below, simultaneous phage infection tends to reduce the level of exclusion of the transducing particles.

Effect of Nontransducing Phage on Transduction Frequency in sie$^+$ *and* sie$^-$ *Lysogens*

The nonlinearity of response observed when a *sie*$^+$ lysogen was transduced with increasing numbers of phage particles (Fig. 2) suggested that the *sie*$^+$ lysogen was becoming increasingly susceptible to transduction as the total number of particles infecting the lysogen was increased. In order to test the possibility that the exclusion of transducing particles depends on the total particle multiplicity in an infected cell, an experiment was performed in which additional phage, free of assayable transducing activity (called "helper" phage), were added to the transducing lysate to raise the total particle multiplicity per cell. PV78(*sie*1m_3) and PV78(*sie*$^+m_3$) were infected with purified transducing particles (m.o.i. = 0.5 particle/cell) together with helper phage at several concentrations (m.o.i. 0, 5, 10, 20, 50). After adsorption, infected cells were assayed for survival, *his*$^+$ transductants, and unadsorbed phage; the results of this experiment are given in Table 3.

Addition of helper phage had no effect on transduction in the *sie*$^-$ lysogen while the addition of helper phage did markedly increase the transducing efficiency in the *sie*$^+$ lysogen. In no sample, however, did the number of *his*$^+$ transductants formed in the *sie*$^+$ lysogen reach the level found in the *sie*$^-$ lysogen; at most, one-fourth the expected number of *his*$^+$ transductants are formed in the *sie*$^+$ lysogen. It can be concluded that the system which is responsible for the exclusion of transducing particles is affected by the total number of particles infecting the cell. Nevertheless, the data suggest that exclusion cannot be completely overcome simply by the addition of extra infecting particles.

The magnitude of this effect is adequate to explain the nonlinear response observed when *sie*$^+$ lysogens were transduced by a mixture of phage and transducing particles (Fig. 2).

TABLE 3

EFFECT OF HELPER PHAGE ON TRANSDUCTION BY PURIFIED TRANSDUCING PARTICLES[a]

Multiplicity of helper phage	*sie*$^-$		*sie*$^+$		
	Survival	*his*$^+$ transductants/ml[b]	Survival	*his*$^+$ transductants/ml[b]	*sie*$^+$/*sie*$^-$
0	1	63,000	1	280	0.004
5	0.9	48,000	0.9	450	0.009
10	0.7	45,000	1	3000	0.07
20	0.7	37,000	0.9	9200	0.25
50	0.2	61,000	0.6	4300	0.07

[a] Particles were purified as described in the Methods. Helper phage (c_2h_{21}) were grown on PV 78, the recipient, and contributed fewer than 10 transductants/ml (*sie*$^-$). Recipient cells were grown in LB to 4×10^8/ml, centrifuged and resuspended in LB. Adsorption of mixtures of transducing particles plus helper was carried out at 39° for 20 min. The cells were plated immediately thereafter for survival and transduction.

[b] Corrected to 100% cell survival.

DISCUSSION

The experimental results may be summarized as follows:

1. Exclusion of transducing particles in wild-type P22 lysogens is under the control of the prophage *sie*$^+$ gene. Cells lysogenic for P22 *sie*$^+$ have lower transducing efficiencies than either sensitive cells or cells lysogenic for P22 *sie*$^-$. It should be noted that this exclusion is not absolute: complete transductants are formed in P22 *sie*$^+$ lysogens.

2. The exclusion of transducing particles can occur in the absence of superinfecting viable phage. Transducing particles which have been purified away from viable phage are excluded by *sie*$^+$ lysogens.

3. Exclusion interferes with the transduction process at some step after the adsorption of transducing particles to the cell. Experiments with purified, radioactive transducing particles demonstrate that the formation of transductants per [^{3}H] count adsorbed is 1000-fold lower in a *sie*$^+$ lysogen than in a *sie*$^-$ lysogen.

4. The *sie*$^+$ system responsible for the exclusion of transducing particles is affected by the total number of particles infecting the

cell although it cannot be completely overcome simply by the addition of up to 50 extra infecting particles (i.e., helper phage).

Transducing particles were prepared from cells whose DNA was density labeled. Since these particles were formed in unlabeled medium, but nonetheless contain fully labeled DNA, only a very small proportion (at most 6%) of the DNA in these particles could be phage DNA. Thus the exclusion by *sie*$^+$ lysogens is nonspecific in the sense that nonphage (transducing) DNA is excluded. Superinfection exclusion also affects the efficiency of abortive transduction (Ozeki, 1956). In abortive transduction, the transducing DNA does not recombine with the bacterial chromosome; it can function in the transduced cell but, since it does not replicate, it is transmitted linearly to progeny cells. Transducing particles responsible for the two types of transduction events are indistinguishable (Sheppard, 1962). Ozeki (1956) found that the ratio of abortive to complete transductants was the same for a given marker in sensitive cells and P22 *sie*$^+$ lysogens, thus demonstrating that exclusion operates equally well on abortive and complete transduction events.

In this paper, lysogens have been designated as either *sie*$^+$ (wild type) or *sie*$^-$ (as defined by Rao's *sie* 1 phage). In the following paper, Susskind *et al.* (1971) describe a more complete analysis of the genetic elements responsible for superinfection exclusion by phage P22 and have found that exclusion of phages is controlled by two unlinked phage genes, *sie*A and *sie*B. They have characterized P22 *sie* 1 as a double mutant, *sie*A$^-$*sie*B$^-$, and have found that the A exclusion system is the only system active against superinfecting P22 and P22-transducing particles. Our results on superinfection exclusion of transducing particles are therefore relevant only to the *sie*A system.

The A exclusion system is unusual in several ways. It is highly nonspecific and excludes to some extent all DNA which is injected into a P22 *sie*A$^+$ lysogen from a phage particle. It does not, however, exclude heteroimmune phage DNA which is transferred by conjugation into a P22 *sie*A$^+$ lysogen (Rao, 1968) or bacterial DNA entering by this route (Botstein, unpublished results). Exclusion is not accomplished by degradation of the superinfecting DNA to acid-soluble nucleotides (Rao, 1968) as is true of the T-even phages (Fielding and Lunt, 1970).

The question arises whether DNA is actually injected into the excluding lysogen from the phage head. Rao (1968) and Susskind *et al.* (1971) have evidence indicating that phage DNA is actually injected from phage particles into cells lysogenic for P22 *sie*A$^+$. The excluding step must therefore occur after the DNA is injected into the cell. At present, there is no reason to believe that excluded DNA can function in a *sie*$^+$ lysogen; Ozeki's finding (1956) that the formation of both abortive and complete transductants is inhibited in wild-type P22 lysogens provides evidence that transducing DNA cannot function in these lysogens. However, it should be noted that simple blocks at the transcriptional level for superinfecting DNA cannot in any case account for the inhibition of the formation of complete transductants, since it is not expected that transducing DNA need function before recombining with the host chromosome.

The observation that the degree of exclusion expressed by a *sie*$^+$ lysogen depends on the total particle multiplicity suggests that some component of the *sie*A excluding system can be saturated by input phage. This result, when considered together with the evidence mentioned above concerning the injection of superinfecting DNA into the cell, suggests that there exist within the lysogenic cell a number of sites (or protein molecules) which interact with superinfecting DNA. In the case of superinfecting transducing DNA, the product is incapable of forming either abortive or complete transductants. Similarly, the product made from P22 superinfecting phage DNA is incapable of participating in general recombination with the prophage and of forming an infective center. On the other hand, introduction of DNA by conjugation occurs normally into a *sie*$^+$ lysogen. It is therefore possible that the hypothetical sites (or proteins) responsible for exclusion act upon DNA as it is injected from a phage particle. This hypothesis suggests that the exclusion

system is located in or near the cell membrane.

ACKNOWLEDGMENTS

This work was supported by grants from the American Cancer Society (No. E-573) to D. B. and from the National Institutes of Health (No. AI 05388-09) to M. S. Fox. One of us (J. E. T.) was also a trainee under a microbiology training grant No. GM-00602 from the National Institute of General Medical Sciences to the Department of Biology, M.I.T.

We wish to thank Dr. Maurice Fox, in whose laboratory this work was done, for his interest in this work and for his perceptive criticism of the manuscript; Raymond White and Mimi Susskind for many valuable discussions, and N. Rao and M. Gough for providing us with phage strains.

REFERENCES

ADAMS, M. H. (1959). "Bacteriophages". Wiley (Interscience), New York.

BOTSTEIN, D. (1968). Synthesis and maturation of phage P22 DNA. I. Identification of intermediates. *J. Mol. Biol.* **34**, 621–641.

BOTSTEIN, D., and MATZ, M. (1970). A recombination function essential to the growth of bacteriophage P22. *J. Mol. Biol.* **54**, 417–440.

CALENDAR, R. (1970). The regulation of phage development. *Annu. Rev. Microbiol.* **24**, 241–296.

FIELDING, P. E., and LUNT, M. R. (1970). The relation between breakdown of superinfecting virus deoxyribonucleic acid and temporal exclusion induced by T4 and T5 bacteriophages. *J. Gen. Virol.* **6**, 333–342.

LEVINE, M., and CURTISS, R. (1961). Genetic fine structure of the C region and the linkage map of phage P22. *Genetics* **46**, 1573–1580.

OZEKI, H. (1956). Abortive transduction in purine-requiring mutants of *Salmonella typhimurium*. *Carnegie Inst. Wash. Publ.* **612**, 97–106.

OZEKI, H. (1959). Chromosome fragments participating in transduction in *Salmonella typhimurium*. *Genetics* **44**, 457–470.

RAO, N. (1968). Bacteriophage P22 controlled exclusion in *Salmonella typhimurium*. *J. Mol. Biol.* **35**, 607–622.

SHEPPARD, D. E. (1962). Density gradient centrifugation of bacteriophage P22. *Virology* **17**, 212–214.

STOCKER, B. A. D., ZINDER, N. D., and LEDERBERG, J. (1953). Transduction of flagellar character in *Salmonella*. *J. Gen. Microbiol.* **9**, 410–433.

SUSSKIND, M., WRIGHT, A., and BOTSTEIN, D. (1971). Superinfection exclusion by P22 prophage in lysogens of *Salmonella typhimurium*. II. Genetic evidence for two exclusion systems. *Virology* **45**, 638–652.

WALSH, J., and MEYNELL, G. G. (1967). The isolation of non-excluding mutants of phage P22. *J. Gen. Virol.* **1**, 581–582.

ZINDER, N. (1955). Bacterial transduction. *J. Cell. Comp. Physiol.* **45**, Suppl. 2, 23–49.

ZINDER, N., and LEDERBERG, J. (1952). Genetic exchange in *Salmonella*. *J. Bacteriol.* **64**, 679–699.