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

II. Genetic Evidence for Two Exclusion Systems

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Mutants of the *Salmonella* phage P22 which, as prophages, do not prevent the growth of superinfecting virulent P22 phage were isolated. These mutants, called *sie*A$^-$, retain some ability to exclude the heteroimmune phages L and MG178. Prophages carrying mutations at another locus, *sie*B, lose entirely the ability to exclude the heteroimmune phages. The *sie*$^-$ mutants of Rao (1968) were shown to be *sie*A$^-$-*sie*B$^-$ double mutants.

The *sie*A and *sie*B loci are located on the P22 genetic map near the *imm*I and *imm*C regions. The exclusion properties of *sie*A and *sie*B alleles are independent of the immunity specificity of the prophage. The A exclusion system (defined by *sie*A$^-$ mutants) appears to be nonspecific and excludes phages P22, MG178, or L. The A exclusion system is entirely responsible for the exclusion of generalized transducing particles by P22 lysogens. The B system, on the other hand, acts only on the heteroimmune phages. The two exclusion systems appear to act independently.

INTRODUCTION

Bacteriophage lysogens are immune to superinfection by homologous phage because of the synthesis of repressor(s) by the prophage. In P22 lysogens of *Salmonella typhimurium*, growth of superinfecting homologous phage is prevented not only by the immunity system, but also by a system of exclusion (Walsh and Meynell, 1967; Rao, 1968). Even if immunity is destroyed by induction, superinfecting P22 cannot grow in the lysogenic cell, and cannot complement or recombine with the induced prophage. The related phages L, MG178 and MG40 are also excluded, since they are unable to form plaques on P22 lysogens even though they are heteroimmune to P22.

Nonexcluding mutants of P22, called *sie*$^-$, or superinfection exclusion mutants, have been isolated by Walsh and Meynell (1967) and by Rao (1968). Lysogens of *sie*$^-$ mutants fail to exclude superinfecting phage, indicating that exclusion is a property of the prophage. The mutants isolated by Rao were found to be nonexcluding for both P22 and the heteroimmune phages, (L, MG178, and MG40), regardless of whether they had been selected for inability to exclude P22 or inability to exclude MG178. This suggested that a single mechanism might be responsible for the exclusion of the different superinfecting phages. However, this report will present evidence that there are two systems of exclusion by P22, and these systems differ in their specificity for the various phages.

MATERIALS AND METHODS

Bacterial Strains and Phages

Bacterial strains are derivatives of *Salmonella typhimurium* LT2. DB21, the wild-type nonlysogen, was described by Botstein and Matz (1970) PV78*his*D23*met*C30*gal*50*pur*·

C213 is described in the accompanying paper (Ebel-Tsipis and Botstein, 1971). The P22 prophage deletion strains DB147, DB136, DB5057, and DB5201 are described by Chan and Botstein (in preparation).

P22 strains are derived from the wild-type strain of Levine (1957). The morphological markers m , c_2^5, and h_{21} derive from the strains of Levine and Curtiss (1961); phages carrying combinations of these markers were constructed by recombination. P22*sie*1m_3 , a derivative of *ts*2·1 *ts*12·1 *sie*1 of Rao (1968), was made ts^+ and m_2 by M. Gough. The virulent mutant, *vir*-3, was described by Bronson and Levine (1970).

Wild-type L phage (Bezdek and Amati, 1967) and its clear-plaque mutants (Bezdek *et al.*, 1970) have been described. $Lc_{II}101$ was isolated and characterized by R. Chan. MG178c is a spontaneous clear-plaque mutant isolated by R. N. Rao. Recombinants between P22 and L (Bezdek and Amati, 1968) ($imm\mathrm{I_P}imm\mathrm{C_L}$ and $imm\mathrm{I_L}imm\mathrm{C_P}$ recombinants) are described in Results.

Media

M9CAA (Smith and Levine, 1964) and LB broth (Levine, 1957) have been described. Buffered saline is 0.85% (w/v) NaCl, 0.066 *M* phosphate buffer (pH 7). Green indicator plates (Bresch, 1953; Levine and Curtiss, 1961) were prepared with Alizarin Yellow G (Matheson, Coleman & Bell) and Aniline Blue (water soluble) (Fisher Scientific). With indicator plates or λ plates (Signer and Weil, 1968), soft nutrient agar (Levine, 1957) was used. Minimal plates for transduction experiments contained Ozeki's minimal medium (OM) (Ozeki, 1959), supplemented with 0.2% (w/v) glucose, 20 μg/ml adenine, 20 μg/ml methionine, and 1 μg/ml vitamin B_1. Minimal top agar contained 0.7% (w/v) agar and 0.8% (w/v) NaCl. Dilutions were made in growth medium, buffered saline, or DF (0.85% (w/v) NaCl, 0.1% (w/v) Difco nutrient broth).

Mutagenesis of Phage

Nitrosoguanidine. P22 wild-type phage was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine according to the procedure of Botstein and Matz (1970). The stock of mutagenized phage used to isolate *sie*⁻ mutants contained 2% clear-plaque mutants.

Hydroxylamine. P22 wild-type phage was mutagenized with hydroxylamine according to the procedure of Hall and Tessman (1966). One percent of the phage survived after 24 hr at 37°. Among the survivors, clear-plaque mutants appeared at a frequency of 1%. Phage diluted out of the mutagenesis mixture were used to prepare plate stocks (Adams, 1959) on DB21. Since each stock was prepared from a single plate, no two stocks contained mutants of the same origin.

Preparation of Lysogens of Mutagenized Phage

Nitrosoguanidine-mutagenized phage. The wild-type nonlysogen, DB21, was grown in M9CAA at 37° until the culture reached the late logarithmic phase of growth. The cells were pelleted by low-speed centrifugation, resuspended in buffered saline at a concentration of 5×10^8 – $1\ 10^9$ cells/ml, and kept in the cold. A small aliquot was inoculated, at a multiplicity of infection of about 20, with P22 phage previously mutagenized with nitrosoguanidine. After a 20-min incubation at 37° to allow for adsorption of phage, the suspension was diluted 10^4-fold in LB broth. The diluted cell suspension was divided into several 2-ml portions, which were shaken overnight at room temperature. Each overnight culture was diluted 60-fold in fresh LB broth and was incubated at room temperature until the cell density reached approximately 10^8 cells/ml. The cultures were infected with P22c_2 phage at a multiplicity of about 10 in order to kill surviving nonlysogens. Incubation at room temperature was continued for approximately 2.5 hr. These cultures were plated and screened for nonexcluding lysogens as described below.

Hydroxylamine-mutagenized phage. DB21 was grown in LB broth at 37° until the cell density reached 2×10^8 cells/ml. The culture was divided into several small portions, each of which was inoculated with an aliquot of a different stock of hydroxylamine-mutagenized P22. The multiplicity of infection was about 20. After shaking at room temperature overnight, the cultures were diluted

40-fold in LB broth, and incubation at room temperature was continued until the cell titers reached about 10^8 cells/ml. Each culture was infected with P22c_2 at a multiplicity of about 20 to kill surviving nonlysogens. After incubating 15 min at room temperature, the cultures were diluted 60-fold in LB broth and were grown overnight at room temperature.

Isolation of sie⁻ Mutants

For both lysogenization procedures, the final cultures were diluted and aliquots containing a few hundred cells were spread on the surface of indicator plates previously spread with 10^5 P22*vir*-3 phage per plate. The plates were incubated overnight at 37°.

Most of the colonies which resulted were round and greenish-white in color. Occasionally, colonies were seen which were partly or wholly dark blue-green in color, indicating lysis of cells had occurred. Such colonies were also frequently "nibbled"; that is, sectors were missing from their otherwise circular shapes. These colonies were picked and tested for immunity and exclusion properties by the streak test (see below). Strains which were immune to P22c_2 but sensitive to P22*vir*-3 were purified. Prophage was isolated from these strains by induction with ultraviolet light, and the resulting phage strains were purified. DB21 was lysogenized with these phages, and the resulting lysogens were purified and characterized.

The phage mutants isolated according to this procedure are given in Table 1, where they are classified according to group (see below) and according to mutagen treatment.

The six mutants derived from hydroxylamine-treated phage were isolated from separate plate stocks. Mutants 77 and 78 are derived from the same culture of DB21 infected with nitrosoguanidine-mutagenized phage. All other mutants came from different cultures of cells infected with nitrosoguanidine-mutagenized phage.

TABLE 1

ORIGIN AND CLASSIFICATION OF *sie*⁻ MUTANTS[a]

Group	Mutagen	
	Hydroxylamine	Nitrosoguanidine
I	27, 32, 33	44, 47, 65, 69, 70, 78, 79
II	23, 35, 37	46, 66, 74, 77, 80
III	—	71

[a] The phenotypes of the groups are described in the text and in Table 2.

Streak Tests

The immunity and exclusion properties of lysogens were easily and reliably determined by streak tests. Suspensions of different phages, each at a titer of 10^8 phage/ml, were streaked vertically on the surface of indicator plates and were air-dried. Bacteria from colonies on solid media were streaked across the plates, perpendicular to the phage streaks, and the plates were incubated overnight at 37°. The resulting greenish-white bacterial streaks were discolored dark blue-green in the area of intersection with phage able to cause lysis of cells. In the case of nonlysogenic, fully sensitive cells, the bacterial streaks were usually disrupted, as well as discolored, by phage growth. Streaks of nonexcluding lysogens were usually not disrupted by phage growth, but sensitivity of the cells to various phages could nevertheless be gauged by the degree of discoloration.

Determination of Efficiencies of Phage Growth

Efficiencies of plating were determined at 37° as described by Adams (1959). Platings were done without preadsorption, using λ plates, nutrient soft agar, and broth-grown exponential cells.

For transmission coefficient determinations, cells were grown in M9CAA at 37° to cell titers of 4×10^8 cells/ml. The cells were pelleted by centrifugation and were resuspended in one-half volume of buffered saline. P22*vir*-3 was added at a multiplicity of about 0.04 and the suspension was incubated for 10 min at room temperature. The suspension was treated with antiserum ($K = 5$) for 5 min at room temperature and was diluted in buffered saline and plated on DB21(P22*sie*1m_3), on which only the infecting virulent phage can form plaques. The titer of infective centers was corrected by subtracting the titer of phage which formed plaques if the suspension was treated with chloroform before dilution and plating.

Preparation and Testing of $imm I_P imm C_P$/$imm I_P imm C_L$ Double Lysogens

To prepare a double lysogen, DB21 was grown in LB broth at 37° until the cell titer reached 2×10^8 cells/ml. The culture was simultaneously infected with a P22 and an $imm I_P imm C_L$ phage (see Results), each at a multiplicity of 10. After incubation without shaking for 20 min at room temperature to allow for phage adsorption, the infected culture was diluted 30- to 60-fold in LB broth and was shaken at 37° until the cell concentration reached approximately 10^8 cells/ml (about 3 hr). The culture was diluted and aliquots were spread on indicator plates, which were incubated overnight at 37°.

In order to identify double lysogens, the resulting colonies were tested for ability of the clones to release phage of both immunity types. Cells to be tested were deposited onto the surface of λ plates overlaid with soft agar seeded with cells lysogenic for nonexcluding P22 (P22*sie*1m_3), and separately onto agar overlays seeded with cells lysogenic for nonexcluding $imm I_P imm C_L$ phage (derived from P22*sie*1m_3). The plates were incubated overnight at 37°. On both plates, doubly lysogenic strains formed colonies which were surrounded by a ring of lysis, indicating the release of phage able to grow on the surrounding lawn of lysogenic bacteria. The cells to be tested by this method were usually transferred with sterile toothpicks from colonies on solid media, but the test was more reliable if cells growing in LB broth were spotted onto the overlays with sterile capillary tubes. If the cells were to be taken from colonies on solid media, the reliability of the test was somewhat improved by irradiation of the cells with ultraviolet light before inoculation onto the overlays.

Strains found by this test to release phage of both immunity types, even after purification, were further tested by examination of the phage released after induction with ultraviolet light. Induced lysates were plated on DB21 at 37° on indicator plates. The morphology of plaques on these plates was noted, and several of these plaques were tested for immunity type by plating (see below) and for exclusion phenotype by streak tests.

Transduction

Transducing phage stocks were prepared by infection of DB21 grown in LB broth at 37° to a cell titer of 2×10^8 cells/ml. The multiplicity of infection was 1 for c^+ phage and 3–5 for c_2 phage. Partial lysis of infected cultures was observed within 1 hr of further incubation at 37°. Chloroform was added and the lysates were purified and concentrated by differential centrifugation as described by Botstein and Matz (1970).

The transduction procedure itself is described by Ebel-Tsipis and Botstein (1971).

Determination of Phage Immunity Types

In order to determine their immunity types, phages thought to be recombinants between P22 and L were tested for ability to grow on the following lysogens: DB21(P22*sie*1m_3), DB21 (L wild type), DB21 ($imm I_P imm C_L m_3$), and DB21($imm I_L \cdot imm C_P$). The prophages in these lysogens are all nonexcluding, including the hybrid phages, which are derived from P22*sie*1m_3. As shown by Bezdek and Amati (1968), each immunity class is characterized by a unique plating pattern on lysogens of the four immunity types. A plaque of the phage to be tested was stabbed with a toothpick onto soft agar overlays, each seeded with cells of one of the lysogenic strains. After overnight incubation of the plates at 37°, a ring of lysis around the site of inoculation of phage indicated ability of the phage to grow on the lysogenic lawn.

Determination of Phage Adsorption Rates

Cells grown in M9CAA at 37° to cell titers of $2–3 \times 10^8$ cells/ml were pelleted by centrifugation and resuspended in buffered saline. P22*vir*-3 was added at a multiplicity of about 0.1, and the suspension was incubated at 37°. Samples were periodically removed, diluted in DF containing chloroform, and plated on DB21(*sie*1m_3).

RESULTS

Isolation and Characterization of sie⁻ Mutants

We have isolated mutants of P22 which, as prophage, do not exclude superinfecting

P22, but which still exclude the heteroimmune phages L and MG178. These mutants were isolated by screening lysogens of mutagenized phage for cells which are sensitive to P22*vir*-3 (Bronson and Levine, 1970), a virulent mutant which is apparently not sensitive to repression by P22 prophage. Wild-type P22 lysogens are resistant to *vir*-3, while nonexcluding lysogens are sensitive.

Nineteen phage mutants were isolated and their lysogens characterized by their ability to support plaque formation by phages P22*vir*-3, Lc_{II} (a clear-plaque mutant of phage L), and MG178*c* (a clear-plaque mutant of phage MG178). As shown in Table 2, each of these three phages has a low efficiency of plating (e.o.p.; always measured relative to the parent nonlysogen, DB21) on the wild-type P22 lysogen, but a nearly normal e.o.p. on a lysogen of P22*sie*1m_3, a derivative of a nonexcluding mutant isolated by Rao. (It should be emphasized that the m_3 and h_{21} plaque morphology markers have no effect on the *sie* phenotype.) One of the mutants isolated by our procedure (Group III in Table 2) has an exclusion phenotype similar to that of *sie*1, since P22*vir*-3, Lc_{II}, and MG178*c* all plate efficiently on its lysogen. This phenotype is apparently the result of two mutations in different genes (*sie*A and *sie*B), as shown below.

Most of our nonexcluding mutants, comprising Groups I and II, show a different phenotype. Lysogens of Group I mutants plate P22*vir*-3 with high efficiencies, but they still exclude MG178*c* about as well as the wild-type lysogen. Lc_{II} phage plate on these lysogens with an intermediate e.o.p. of 10^{-3}, or 10^4-fold higher than on the wild-type lysogen. As shown below, this phenotype apparently results from mutation in a single gene, *sie*A.

Lysogens of Group II mutants resemble Group I lysogens in their overall plating behavior; however, Group II lysogens show high-frequency segregation of cells which are phenotypically much better able to exclude superinfecting phage. We have not investigated further the nature of this transition in Group II lysogens, and we have limited our subsequent studies to the Group I (*sie*A$^-$) and Group III (*sie*A$^-$*sie*B$^-$) mutants.

TABLE 2

NONEXCLUDING MUTANT PHENOTYPES

Prophage	Efficiency of plating[a]		
	P22 *vir*-3	Lc_{II}	MG178*c*
P22 wild	10^{-5}–10^{-4}[c]	10^{-7}	10^{-7}
Group I (10)[b]	0.6–1.0	10^{-3}	10^{-6}
Group II (8)[b]	0.05–0.2	10^{-3}	$<10^{-6}$
Group III (1)[b]	1.0	0.6	0.1
P22 *sie* 1 m_3	0.6	0.7	1.0

[a] Indicates titer on lysogen relative to titer on DB21 (the parent nonlysogen), in this and all subsequent tables.

[b] Number in parentheses indicates the number of mutants isolated in each group. For each group, the efficiency of plating of P22 *vir*-3 is given as the range of values observed for different mutants of the group.

[c] Number of plaques formed does not decrease linearly with dilution. These values are therefore approximate, in this and subsequent tables.

Group I and III mutants are apparently normal in their stability as prophages, since their lysogens have normal levels of spontaneously induced free phage in liquid cultures. In many cases, however, this is true only if the cultures are started by inoculation with colonies taken from solid media; if liquid cultures are serially subcultured, they rapidly accumulate high levels of free phage, many of which are clear-plaque, virulent mutants. These virulent mutants apparently arise during growth of the primary liquid culture, and after dilution in fresh medium are strongly selected, since they can grow on the nonexcluding cell culture to produce high titers of phage.

Components of the sie1 Phenotype

The ability of Group I mutants to exclude L and MG178, even though they cannot exclude P22, suggested that there might be two systems of exclusion by P22 prophage, only one of which is missing in the Group I mutants. This would imply that *sie*1 and our Group III mutant are double mutants which have lesions in both systems. This suggestion was verified, in the case of *sie*1, in the backcross P22*sie*1m_3h^+ × P22*sie*$^+m^+h_{21}$. Two

TABLE 3

COMPONENT GENETIC DETERMINANTS OF SUPERINFECTION EXCLUSION

Prophage	Efficiency of plating			Transmission coefficient[b]
	P22 *vir*-3	Lc II	MG178*c*	P22 *vir*-3
P22 wild	10^{-5} to 10^{-4}	10^{-7}	10^{-7}	$<10^{-3}$
sie 1 m_3h^+	0.6	0.7	1.0	0.8
sie A1 m_3h_{21}	0.7	10^{-4}	10^{-5}	0.7
sie B1 m^+h^+	10^{-5}	10^{-4}	10^{-3}	$<10^{-3}$

[a] P22 *sie*1m_3h^+ was crossed with P22 *sie*$^+m^+h_{21}$. m_3h_{21} and m^+h^+ recombinants were purified and tested for exclusion phenotype. Six of seven m_3h_{21} recombinants have the exclusion spectrum of *sie* A1; the other was the same as its *sie* 1 parent. Four of seven m^+h^+ recombinants are like *sie* B1; the others are *sie*$^+$.

[b] Number of chloroform-sensitive infective centers produced after adsorption, at a multiplicity of 0.04, relative to the nonlysogen, DB21.

types of recombinants were obtained with exclusion properties differing from those of either parent. One recombinant type, designated *sie*A1m_3h_{21} in Table 3, is similar in exclusion phenotype to the Group I mutants. We show below that *sie*A1 and the Group I mutants form a single complementation group. Mutants in this group are called *sie*A$^-$ mutants, and the system defined by them is called the A exclusion system. The other type of recombinant obtained in the *sie*1m_3h^+ × *sie*$^+m^+h_{21}$ cross is designated *sie*B1m^+h^+ in Table 3. The system defined by the *sie*B1 mutation is called the B exclusion system.

The results in Table 3 indicate that the B exclusion system is not active against superinfecting P22, since the *sie*B1 lysogen retains full capacity to exclude P22*vir*-3, and the *sie*A$^-$ lysogens, which presumably retain the B system intact, lose all ability to exclude *vir*-3. However, the B system is responsible for most of the ability of wild type lysogens to exclude MG178 and for part of their ability to exclude L phage.

The A exclusion system, as noted above, is the only system active against superinfecting P22. If it is assumed that *sie*B1m^+h^+ in Table 3 retains the A system intact, it is seen that the A system alone excludes L and MG178 to some extent.

Expression of Exclusion in the Absence of P22 Immunity

In order to determine whether the A and B exclusion systems are expressed in the absence of the P22 immunity system, phages were constructed which are heteroimmune to P22 but which carry a P22*sie*A$^+$ or a P22*sie*B$^+$ allele. These phages are recombinants between P22 phage and wild type L phage. The immunities of both P22 and L are determined by two immunity regions (Bezdek and Amati, 1968), *imm*I_P and *imm*C_P in P22 and *imm*I_L and *imm*C_L in phage L. Recombinants with *imm*I of one parent and *imm*C of the other parent (*imm*I_P*imm*C_L and *imm*I_L*imm*C_P recombinants) are temperate and heteroimmune to both P22 and L (Bezdek and Amati, 1968).

In Table 4, the exclusion properties of

TABLE 4

EXPRESSION OF EXCLUSION IN *imm*I_P*imm*C_L AND *imm*I_L*imm*C_P LYSOGENS[a]

Prophage	Efficiency of plating		
	P22 *vir*-3	Lc II	MG178*c*
L wild	1.2	—	0.1
P22 wild	10^{-5}–10^{-4}	10^{-7}	10^{-7}
immI_P*imm*C_L*sie*A$^+m_3$[c]	10^{-4}	—	10^{-2}
immI_L*imm*C_P*sie*B$^+$[c]	1.1	10^{-5}	10^{-6}
P22 *sie* 1 m_3	0.6	0.7	1.0
immI_P*imm*C_L*sie* A1 m_3	1.2	—	1.1
immI_L*imm*C_P*sie*B1	1.1	0.03[d]	0.2
P22 *sie*A$^-$ (9)[b]	0.6–1.0	10^{-3}	10^{-6}
immI_P*imm*C_L*sie*A$^-$ (9)	0.5–1.5		0.1–1.5

[a] The indicated P22 phages were crossed with wild-type phage L; *imm*I_P*imm*C_L and *imm*I_L-*imm*C_P recombinants were picked, and their lysogens in DB21 were tested for their exclusion phenotypes. Exclusion of L phage by *imm*I_P-*imm*C_L cannot be tested because *imm*I_P*imm*C_L lysogens are immune to L.

[b] These are Group I phages and their *imm*I_P-*imm*C_L derivatives.

[c] The P22 parent of these recombinants was P22 m_3, rather than P22 wild type.

[d] This value depends somewhat on the host strain used. In a similar set of experiments using DB53 (Botstein and Matz, 1970) lysogens in place of DB21 lysogens, this value is close to 1. Bezdek and Amati (1968), using yet another strain, also find efficiencies of plating close to 1 for this combination.

lysogens of *imm*I_P*imm*C_L and *imm*I_L*imm*C_P recombinants derived from P22*sie*$^+$, P22*sie*1, and P22*sie*A$^-$ phages are compared. The exclusion properties of these recombinants depend on those of their P22 parents; L prophage has little or no ability to exclude superinfecting phage, and the L parent does not contribute exclusion properties to the recombinants.

Lysogens of most *imm*I_L*imm*C_P recombinants derived from P22*sie*$^+$ plate P22*vir*-3 at an e.o.p. of about 1.0, Lc_{II} at an e.o.p. of 10^{-5}, and MG178*c* at an e.o.p. of 10^{-6} (roughly the phenotype of P22*sie*A$^-$*sie*B$^+$), indicating that the B system is present but the A system absent. These recombinants thus retain and express the *sie*B$^+$ allele of their P22 parent, even though the *imm*I_P allele has been replaced by *imm*I_L. As expected, lysogens of *imm*I_L*imm*C_P phage derived from P22*sie*1m_3 do not exclude P22*vir*-3, Lc_{II}, or MG178*c*, indicating that neither the A nor B system is present.

Lysogens of *imm*I_P*imm*C_L recombinants derived from P22*sie*$^+$ plate P22*vir*-3 at an e.o.p. of 10^{-4} and MG178*c* at an e.o.p. of 10^{-2} (i.e., the phenotype of P22*sie*A$^+$*sie*B$^-$), indicating that the A system is present but the B system is absent. The exclusion of L phage cannot be demonstrated by plating because L is repressed by *imm*I_P*imm*C_L prophage (Bezdek and Amati, 1968). These recombinants thus retain and express the *sie*A$^+$ allele of their P22 parent, even though *imm*C_P has been replaced by *imm*C_L. The P22*sie*B$^+$ allele is either not present or is not expressed in lysogens of these phages. When an *imm*I_P*imm*C_L*sie*A$^+$ phage derived from P22*sie*$^+$ was crossed with *imm*I_L*imm*C_P derived from P22*sie*1, no *sie*B$^+$ *imm*I_P*imm*C_P (P22) recombinants were found, indicating that the *sie*B$^+$ allele is not present, rather than simply not expressed, in the *imm*I_P*imm*C_L phage. As expected, lysogens of *imm*I_P*imm*C_L recombinants derived from P22*sie*1 or P22*sie*A$^-$ phage (Group I mutants) do not exclude P22*vir*-3 or MG178*c*, indicating that neither the A nor B system is present.

The Location of the sieA and sieB genes on the Phage P22 Genetic Map

The approximate location on the phage P22 genetic map of the *sie*A and *sie*B genes can be inferred from the experiments described in the preceding section. When wild-type P22 phage (*sie*A$^+$*sie*B$^+$) were crossed with wild-type L phage (phenotypically *sie*A$^-$*sie*B$^-$), recombinants carrying *imm*I_P and *imm*C_L were generally *sie*A$^+$*sie*B$^-$; the reciprocal recombinants (*imm*I_L*imm*C_P) were, on the other hand, usually *sie*A$^-$*sie*B$^+$. Thus *sie*A$^+$ appears to be linked to *imm*I_P and *sie*B$^+$ appears to be linked to *imm*C_P.

This result was confirmed using deletions of prophage P22 (Chan and Botstein, in

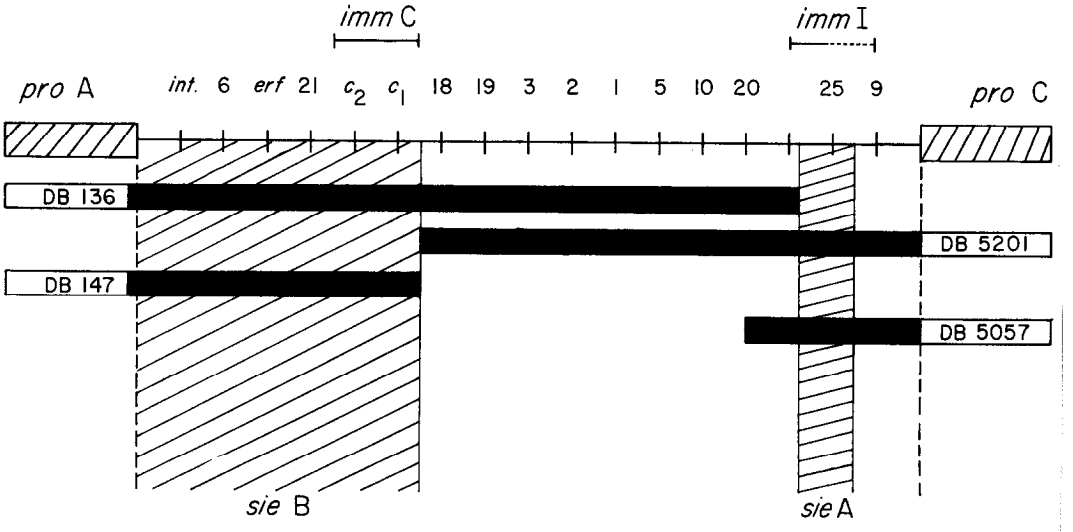

FIG. 1. Deletion mapping of the *sie*A and *sie*B genes.

preparation). As shown in Fig. 1, two classes of prophage deletions are available. Deletions of one class include all or part of the *pro*A gene of the host and a block of markers at the left end of the prophage; these deletions are variable in extent but always include *imm*C_P . Deletions of the other class include blocks of markers at the right end of the prophage; they are variable in extent but always include *imm*I_P .

The Location of sieB

The shortest deletion on the left, (in strain DB147), deletes *imm*C_P but not the next gene, gene 18. The prophage in this strain derives from a *sie*A⁻*sie*B⁺ parent. Nevertheless, all the left end prophage deletion strains, including DB147, are phenotypically *sie*B⁻, since wild-type L phage grows on them but not on their *sie*B⁺ parent. Thus we infer that a genetic element essential to the *sie*B⁺ phenotype is deleted even in the shortest left end deletion. On the other hand, the longest right-hand deletion (in strain DB5201), extends beyond gene 18; c_1^+ and c_2^+ markers can, however, be rescued from this strain. This deletion derives from a *sie*B⁺ parent also. Since this strain still restricts the growth of L phage, and is, by virtue of the deletion (see below) *sie*A⁻, it must still have an intact *sie*B⁺ system. This shows that all the genetic elements essential to the *sie*B⁺ phenotype must lie in the region between the left side (*pro*A) attachment site and gene 18.

The Location of sieA

All of the right-side deletions derive from a *sie*⁺ phage. All of them, even the shortest (in strain DB5057), delete *imm*I_P and their lysogens are fully sensitive, unlike lysogens of the parent phage, to vir_3 infection. Thus an essential genetic determinant of the A exclusion system must lie to the right of the end of the shortest deletion, i.e., to the right of gene 20. In order to confirm this, strain DB136, carrying the longest of the left-hand deletions, which derives from a *sie*A⁻ phage (*sie*A6 of Rao, 1968) and which deletes all or part of *imm*I_P as well as *imm*C_P , was infected with a *sie*A⁺ *am*N110 (gene 9) phage. Under these circumstances, many *am*⁺ progeny result by rescue from the prophage. These were tested for their *sie*A phenotype. About 20% of these were *sie*A⁻, showing that the *sie*A6 allele is still present in this extensive prophage deletion. Thus the *sie*A genes must lie between *imm*I_P and the right side (*pro*C) attachment site. Three factor crosses involving gene 9 and *sie*A indicate that *sie*A is to the left of gene 9.

It should be noted that in P22*sie*⁺ × L crosses, recombinants have been found between *sie*B and *imm*C_P . Thus although these markers are linked, they are separable. On the other hand, recombinants between *sie*A and *imm*I_P have never been observed; thus it is possible that *sie*A⁺ lies in the *imm*I region, even though mutations at the *sie*A locus do not detectably affect immunity.

Dominance and Complementation among sieA Alleles

In order to study dominance and complementation among *sie*A alleles, cells diploid for the *sie*A gene(s) were constructed and tested for their ability to exclude superinfecting phage. These diploid cells were double lysogens in which one *sie*A allele is carried on a P22 prophage and another on a heteroimmune prophage. These double lysogens were detected by their ability to release phage of both immunity types. The heteroimmune phages carrying P22*sie*A

TABLE 5

DOMINANCE OF *sie*A⁺ TO *sie*A⁻[a]

Prophages in double lysogen		No. of lysogens tested	Efficiency of plating P22 *vir*-3
*imm*I*pimm*C$_P$ / *imm*I*pimm*C$_L$			
*sie*A⁺	*sie*A⁺	2	$<10^{-9}$
*sie*A⁺	*sie*A78	2	10^{-3}; 10^{-2}[b]
*sie*A78	*sie*A⁺	2	$<10^{-9}$; 10^{-5}[b]
*sie*A78	*sie*A78	1	0.8
*sie*A⁺	—	—	10^{-5}–10^{-4}
*sie*A78	—	—	0.6
—	*sie*A⁺	—	10^{-4}
—	*sie*A78	—	0.9

[a] Double lysogens were prepared as described in Materials and Methods. Each of these was induced with UV light, and the genotypes of the prophages were checked.

[b] This variation is reproducible and not understood; it may be related to segregation of the double lysogens and thus reflect their exact genetic structure.

alleles are *imm*I_P*imm*C_L recombinants derived from P22 wild type, P22*sie*1, or P22 Group I and Group III mutants.

Table 5 shows the exclusion properties of double lysogens carrying various combinations of *sie*A$^+$ and *sie*A$^-$ phage. When both prophages have the same *sie*A$^-$ mutation, the double lysogen does not exclude P22*vir*-3. If one of the prophages is a *sie*A$^-$ mutant and the other is *sie*A$^+$, or if both prophages are *sie*A$^+$, the lysogen does exclude *vir*-3. The *sie*A$^+$ allele is thus dominant to *sie*A$^-$.

When the *imm*I_P*imm*C_L prophage is *sie*A$^+$, the double lysogen may exclude P22*vir*-3 more efficiently than a lysogen of either *imm*I_P*imm*C_L *sie*A$^+$ alone or P22 wild type alone. This effect is not understood. It is not simply a gene dosage effect, since it is observed regardless of whether the accompanying *imm*I_P*imm*C_P prophage is *sie*A$^-$ or *sie*A$^+$ (see also Table 6).

Complementation among *sie*A$^-$ alleles was tested with a set of double lysogens, each of which was constructed by coinfection of nonlysogenic cells with P22*sie*A44*m*44 (a Group I mutant) and an *imm*I_P*imm*C_L phage with one of 10 *sie*A$^-$ mutations. When double lysogens are prepared from phages with different *sie*A$^-$ mutations, it is not possible directly to determine whether the prophages have exchanged mutations or have become homozygous for one of the alleles. However, in the preparation of double lysogens listed in Table 5, the infecting P22*sie*A44*m*44 phage carried the morphological marker, *m*44, linked to *sie*A. Lysogens were used in complementation tests only if induced lysates contained both *m*44 and its m^+ allele, and most *imm*I_P*imm*C_P phage were also *m*44.

As shown in Table 5, all the *imm*I_P*imm*C_L·*sie*A$^-$ phages are unable to complement P22*sie*A44, whereas in the control double lysogen, the *imm*I_P*imm*C_L*sie*A$^+$ prophage does complement, causing exclusion of P22*vir*-3. Thus all the *sie*A$^-$ mutants tested belong to a single complementation group. In particular, *imm*I_P*imm*C_L*sie*A1 (derived from P22*sie*1), and *imm*I_P*imm*C_L*sie*A71 (derived from our Group III mutant), also fail to complement *sie*A44. This indicates that both *sie*1 and our Group III mutant have mutations in the same gene as the *sie*A Group I mutants. P22*sie*1 was shown above to have a second mutation, *sie*B1, which was separated from the *sie*A1 mutation by backcrossing with P22*sie*$^+$. Since our Group III mutant and P22*sie*1 have the same exclusion phenotype, we assume that the Group III mutant is also a double mutant with a mutation in the *sie*B gene.

It should be noted that our dominance and complementation tests are based on the assumption that an *imm*I_P*imm*C_L*sie*A$^-$ phage, derived from a P22*sie*A$^-$ mutant, actually carries the *sie*A$^-$ allele of its P22 parent; this is not necessarily so. It is possible that the P22*sie*A gene has been substituted by part of the genome of the L parent, which has no active *sie*A gene. Thus it is possible that some or all of our *imm*I_P*imm*C_L*sie*A$^-$ phage contain no P22*sie*A

TABLE 6

COMPLEMENTATION AMONG *sie*A$^-$ ALLELES IN *imm*I_P*imm*C_P/*imm*I_P*imm*C_L DOUBLE LYSOGENS[a]

Prophages in double lysogen		Efficiency of plating P22 *vir*-3
immI_P*imm*C_P /	*imm*I_P*imm*C_L	
*sie*A44*m*44	*sie*A$^+m_3$	$<10^{-4}$
	*sie*A44*m*44	1.1
	*sie*A47m^+	0.7
	*sie*A65m^+	0.8
	*sie*A71m^+	1.0
	*sie*A78m^+	1.1
	*sie*A79m^+	0.6
	*sie*A27m^+	0.9
	*sie*A33m^+	1.0
	*sie*A32m^+	0.3
	*sie*A1m_3	0.7

[a] Double lysogens were prepared, as described in Materials and Methods, by coinfection of DB21 with P22 *sie*A44*m*44 and various *imm*I_P*imm*C_L phages. Phages appearing in lysates after UV-induction of the lysogens were examined. Lysates of the *sie*A44*m*44/*sie*A44*m*44 lysogen contained phages of both immunity types, all carrying the *m*44 allele, and all *sie*A$^-$ in streak tests of lysogens. Candidates for other *sie*A44*m*44/*sie*A$^-m^+$ lysogens were used in the complementation test only if lysates contained both *m*44 and its m^+ allele, and most *imm*I_P*imm*C_P phage were also *m*44. All phages in these lysates were *sie*A$^-$ in streak tests of their lysogens. For the *sie*A44*m*44/*sie*A$^+m_3$ control lysogen, lysates contained both *sie*A$^-$*m*44 phages and *sie*A$^+m_3$ phages, and most *imm*I_P-*imm*C_P phages were *sie*A$^-$*m*44.

allele at all. This is very unlikely, however, because the *sie*A gene of P22 is so closely linked to $imm\mathrm{I_P}$ (see above) that in P22*sie*$^+$ × L crosses we have never observed an $imm\mathrm{I_P}imm\mathrm{C_L}$ recombinant which is phenotypically *sie*A$^-$. Thus the P22*sie*A gene may in fact be part of $imm\mathrm{I_P}$. We therefore assume that in P22*sie*A$^-$ × L crosses, the $imm\mathrm{I_P}imm\mathrm{C_L}$*sie*A$^-$ recombinants carry the *sie*A$^-$ allele of their P22 parents.

Exclusion of Transducing Particles

The frequency of generalized transduction of P22 lysogens by P22 transducing particles depends on the superinfection exclusion properties of the recipient lysogen (Ebel-Tsipis and Botstein, 1971). Table 7 gives the frequencies of stable *his*$^+$ transductants of various lysogens of *Salmonella typhimurium* strain PV78 (*met*$^-$*his*$^-$*gal*$^-$*pur*$^-$), using transducing stocks of P22$m^+c_2h_{21}$ or P22$m_3c_2h_{21}$ grown in the wild-type nonlysogen, DB21. The relative frequency of transduction of PV78(P22*sie*1), given as 1.0, corresponds to an absolute frequency of 5×10^{-6} or 2×10^{-5} transductants per infectious phage, depending on the stock of transducing phage used. The frequency of transduction of wild-type lysogens is reduced by a factor of 250, indicating that transducing particles are excluded by P22 prophage. The data show that the A exclusion system is essential to this exclusion, since *sie*A$^-$ mutants also fail to exclude transducing particles. A lysogen of *sie*B1, which lacks the B exclusion system, still excludes transducing particles about as effectively as the wild-type lysogen, indicating that the B system is not essential to exclusion of transducing particles.

Table 8 shows transduction frequencies for $imm\mathrm{I_P}imm\mathrm{C_L}$ lysogens, using $imm\mathrm{I_P}imm\mathrm{C_L}$ transducing phage. These lysogens, as shown above, have a *sie*B$^-$ phenotype; the B system is presumably absent in L phage. The transduction frequency for the $imm\mathrm{I_P}imm\mathrm{C_L}$*sie*A$^+$ lysogen is 300-fold lower than for the *sie*A$^-$ lysogen. From the results in both Table 7 and Table 8, we conclude that the A exclusion system works in excluding transducing particles independently of the B system.

Adsorption Properties of sieA$^-$ Lysogens

In order to exclude the possibility that superinfection exclusion is due simply to

TABLE 7

EXCLUSION OF P22 TRANSDUCING PHAGE BY P22 LYSOGENS[a]

	Prophage in recipient lysogen	Transducing phage	Frequencies of transduction	
			Absolute[d] (his$^+$ transductants per infecting phage)	Relative[e]
1	P22 wild	P22 $m^+c_2h_{21}$	2×10^{-8}	4×10^{-3}
	P22 *sie* 1 m_3		5×10^{-6}	1.0
	P22 *sie*A$^-$ (10)[b]		2×10^{-6}-4.5×10^{-6}	0.4-0.9
	P22 *sie*A71[c]		5×10^{-6}	1.0
2	P22 wild	P22 $m_3c_2h_{21}$	9×10^{-8}	4×10^{-3}
	P22 *sie* 1 m_3		2×10^{-5}	1.0
	P22 *sie*A1 m_3h_{21}		2×10^{-5}	1.0
	P22 *sie*B1		1×10^{-7}	5×10^{-3}

[a] Lysogens were prepared in strain PV78 (*met*$^-$*his*$^-$*pur*$^-$*gal*$^-$). Streak tests and efficiency of plating determinations verified that these lysogens did not differ from the corresponding lysogens of DB21 in ability to exclude P22 *vir*-3. Transduction experiments and preparation of transducing phage were carried out as described in Materials and Methods. Selection was for cells able to form colonies in minimal top agar overlays on OM minimal plates containing 0.2% glucose, 20 μg/ml adenine, 20 μg/ml methionine, and 1 μg/ml vitamin B_1.

[b] These are the 10 Group I mutants in Table 2. The range of values observed for different lysogens is given.

[c] This is the Group III mutant in Table 2.

[d] Corrected for reversion (approximately 1×10^{-8}).

[e] Frequency relative to the lysogen of P22 *sie* 1 m_3.

TABLE 8

EXCLUSION OF *imm*I$_P$*imm*C$_L$ TRANSDUCING PHAGE BY *imm*I$_P$*imm*C$_L$ LYSOGENS[a]

Prophage in recipient lysogen	Frequencies of transduction	
	Absolute[d]	Relative[e]
*imm*I$_P$*imm*C$_L$ *sie*A$^+$*m*$_3$[b]	3×10^{-7}	3×10^{-3}
*imm*I$_P$*imm*C$_L$ *sie*A33[c]	1.0×10^{-4}	1.0

[a] Procedures were the same as those in Table 6, except that the transducing phage was *imm*I$_P$-*imm*C$_L$ *sie*A$^+$*m*$_3$.

[b] This phage is derived from P22 *m*$_3$.

[c] This phage is derived from P22 *sie*A33, a Group I mutant.

[d] Corrected for reversion (approximately 1×10^{-8}).

[e] Frequency relative to the *imm*I$_P$*imm*C$_L$ *sie*A33 lysogen.

failure of phage to adsorb, lysogens of the Group I (*sie*A$^-$) mutants were tested for their ability to adsorb P22 phage. Cells lysogenic for wild type P22 adsorb P22 phage more slowly than nonlysogenic cells (Rao, 1968); this is because the prophage specifies a change in the structure of the O-antigen in the lipopolysaccharide layer of the cell surface, thereby altering the P22 phage receptor site (Stocker *et al.*, 1960).

Cells lysogenic for wild-type P22 adsorb P22*vir*-3 with a rate constant, or K value, of $0.7 \pm 0.1 \times 10^{-9}$ ml/min (Adams, 1959), whereas the K value for the nonlysogen is significantly higher (1.2×10^{-9} ml/min). Lysogens of seven of the Group I mutants (numbers 47, 65, 69, 70, 79, 27, and 32) have K values in the range $0.5–0.9 \times 10^{-9}$ ml/min and therefore are not significantly different from the wild-type lysogen in ability to adsorb P22. However, three Group I lysogens (mutants 44, 78, and 33), have rate constants of $0.2–0.3 \times 10^{-9}$ ml/min and therefore are significantly slower in adsorbing phage than the wild-type lysogen. These findings indicate that the sensitivity of the Group I mutants to superinfecting P22*vir*-3 is not due to an increased capacity to adsorb phage.

A possible explanation for the three mutants whose lysogens have very low adsorption rates involves the virulent phages which appear in liquid cultures of *sie*A$^-$ mutants. Since the isolation procedure involved the growth of lysogens, these virulent phage might have selected secondary prophage mutations affecting adsorption.

Phages Which Escape Exclusion

The plaques which appear at low frequency when phage are plated on excluding lysogenic strains were examined in order to determine whether they contain phages which can efficiently escape the A or B exclusion systems. If the exclusion systems operate as classical modification-restriction systems (Arber and Linn, 1969), it might be expected that these plaques would contain phages which are phenotypically modified, enabling them to escape exclusion when replated on the same lysogen. However, such phenotypically modified phage would be expected to lose their exclusion-insensitive phenotype after propagation in a nonlysogenic strain. Regardless of whether the exclusion systems are modification-restriction systems, the plaques which appear on excluding lysogens might also contain phages which are able to escape exclusion because of a stable genetic change.

A system. P22 phage are apparently sensitive only to the A exclusion system, since *sie*A$^-$ mutant lysogens plate P22*vir*-3 at an e.o.p. close to 1.0, whereas *sie*B$^-$ mutant lysogens fully exclude *vir*-3. Thus it is possible to screen for A system-insensitive phenotypes simply by plating *vir*-3 on a wild-type P22 lysogen.

Plaques of P22*vir*-3 which had appeared at low frequency on the wild type lysogen were suspended in broth, the surviving cells were killed with chloroform, and the phage were plated again on the same lysogen. The efficiencies of plating of these phage were about 100-fold higher than for the original *vir*-3 stock. However, these phage retained the ability to plate with higher e.o.p.'s after propagation and purification in the nonlysogenic strain, and thus are mutant, rather than phenotypically modified. The level of exclusion (about 100-fold) of these mutants is similar to that for exclusion by the A

system of L, MG178, and transducing particles.

B system. In order to examine sensitivity to the B system, the A system must be removed, since phages L and MG178 are sensitive to it. Plaques formed by Lc_{II} phage at a frequency of 10^{-3} on a lysogen of P22*sie*A44 (a Group I mutant) were examined. When picked and replated, these phage had an e.o.p. of 1.0 on the same *sie*A$^-$ lysogen, and they retained the ability to plate at this efficiency after purification and propagation in a non-lysogen. Although able to escape exclusion by the B system, these phage are still excluded by the A system as efficiently as the original Lc_{II}, since they plate with an efficiency of 10^{-3} on the *sie*B1 lysogen.

These B system-insensitive phages could either be mutants of Lc_{II} or recombinants between Lc_{II} and the P22*sie*A44 prophage. The latter is more likely, since we were able to obtain a B system-insensitive $imm I_P$-$imm C_L m_3$ recombinant from a vegetative cross between $P22 m_3 c_2 h_{21}$ and wild-type L phage.

Plaques formed when MG178*c* is plated on the P22*sie*A44 lysogen also contain phage which have an e.o.p. of 1.0 on the same lysogen when replated immediately or after propagation through the nonlysogen. These phage could either be mutants or recombinants between MG178*c* and the P22 prophage.

We have never observed a case in which the superinfection exclusion phenotype of the host in which phage were propagated influenced the capacity of the phage to grow on *sie*A$^+$ or *sie*B$^+$ lysogens. Thus we have no indication that any DNA modification system is involved.

DISCUSSION

We have shown that in wild-type P22 lysogens of *Salmonella typhimurium*, two systems of exclusion prevent growth of various superinfecting phages. Both exclusion systems are specified, at least in part, by the prophage. The A exclusion system, defined by the P22*sie*A$^-$ (Group I) mutants, is the only system which excludes superinfecting P22. The A system also contributes to exclusion of the heteroimmune phages L and MG178, and is entirely responsible for the exclusion of P22 transducing particles described in the preceding paper (Ebel-Tsipis and Botstein, 1971). The B exclusion system, defined by the P22*sie*B1 mutant, is not active against superinfecting P22 phage or P22 transducing particles, but is active against L and MG178. These relations are summarized in Table 9.

TABLE 9

PROPERTIES OF THE A AND B SUPERINFECTION EXCLUSION SYSTEMS

	Exclusion system	
	A	B
P22 phage	Excludes	No effect
Transducing particles	Excludes	No effect
L phage	Excludes	Excludes
MG178 phage	Excludes	Excludes

The A and B exclusion systems appear to act independently. Together they account for all of the exclusion exhibited by wild-type P22 lysogens. The A system excludes various superinfecting phages and transducing particles; where the B system is active as well (i.e., superinfection by phages L and MG178), the effect is superimposed on the effect of the A system alone. Thus the efficiency of plating of phages L and MG178 on the wild-type P22 lysogen is approximately the product of the efficiencies measured in strains exhibiting A and B exclusion separately.

The A Exclusion System

The A exclusion system interferes with superinfection by P22, L, and MG178 and with transduction by P22 transducing particles. The plating efficiencies for L and MG178 and the transduction frequency for P22 are each reduced about 10^2–10^3-fold by the A system. Exclusion of P22 is somewhat more efficient, since the efficiency of plating for P22*vir*-3 is reduced by a factor of 10^4–10^5. However, this greater stringency of the A system against P22 phage is not observed when the superinfecting P22*vir*-3 is a mutant selected for greater ability to grow on the

wild-type lysogen. The A exclusion system does not act on phage or bacterial DNA which enters the lysogenic cell by conjugation rather than injection from phage particles. Both L (Rao, 1968) and P22 (unpublished results) can grow in wild-type P22 lysogens after transfer as prophage from Hfr strains, and in conjugation experiments the yield of recombinants for bacterial markers is not grossly affected by the presence of the P22 A exclusion system in the recipient. The A exclusion system thus excludes both phage and bacterial DNA entering the lysogenic cell by injection from phage particles, but not phage or bacterial DNA entering by conjugation.

The A exclusion system appears to be absolutely nonspecific for the genetic nature of the DNA, so long as it is injected by any of a large number of smooth-specific *Salmonella* phages (Grabnar and Hartman, 1968; Grabnar, 1967; Kuo and Stocker, 1970). However, this general exclusion is not universal, since the *Salmonella* typing phage, Felix-O, plates with equal efficiency on a wild-type P22 lysogen and the parent nonlysogenic strain (unpublished results).

Rao (1968) has shown that superinfecting P22 cannot complement or recombine with an induced P22 prophage in the *sie*$^+$ lysogen. O-Antigen conversion (Walsh and Meynell, 1967) and synthesis of repressor (unpublished results) by superinfecting P22 are also prevented, although the resident prophage expresses both these functions.

The mechanism of action of the A exclusion system is obscure. It does not appear to act as a block to phage adsorption, since *sie*A$^-$ lysogens do not adsorb P22*vir*-3 faster than *sie*A$^+$ strains. The A system also does not appear to be a classical modification-restriction system (Arber and Linn, 1969) since the plaques which appear when P22*vir*-3 is plated on the wild-type lysogen do not contain phages which are phenotypically able to escape exclusion, and superinfecting P22 DNA is not degraded to acid-soluble fragments (Rao, 1968; Susskind and Botstein, unpublished results).

Preliminary experiments indicate that the excluded superinfecting P22 DNA does enter the cell, since it is available to a classical host DNA restriction system (c.f. Arber and Linn, 1969) which degrades DNA from phages grown in nonmodifying, nonrestricting mutant bacterial strains.

The sum of current information about the mechanism of the A exclusion system amounts to an implication of the route of entry and substantial evidence that the genetic information of injected superinfecting DNA is irrelevant.

Our P22 *sie*A$^-$ (Group I) mutants, selected for inability to exclude superinfecting P22*vir*-3, belong to a single complementation group. The *sie*A gene maps very near to *imm*I$_P$, and might be in the *imm*I region of non-homology with phage L, since the P22*sie*A$^+$ allele has not been separated from *imm*I$_P$ in crosses between P22 and L. The A exclusion system is expressed regardless of whether the *imm*C allele of the prophage is derived from P22 or L.

Cultures of P22*sie*A$^-$ lysogens tend to accumulate virulent phage on serial subculture in liquid media. These phage presumably arise by mutation from the P22 prophage. In the case of lysogens of P22*sie*1*m*$_3$, the virulent mutants which accumulate owe their virulence to single mutations in the *imm*I region (Bronson and Levine, 1971; C. Swanson and D. Botstein, in preparation). The high frequency with which P22 virulent mutants arise might have provided the selection pressure which led to the evolution of the A exclusion system.

The B Exclusion System

The B exclusion system reduces the efficiency of plating of L phage by a factor of about 10^3 and that of MG178 by a factor of about 10^5–10^6. Superinfecting P22 is not excluded by the B system. The plaques which appear when L or MG178 is plated on a *sie*A$^-$*sie*B$^+$ lysogen contain phages which have a stable, heritable ability to escape exclusion by the B system. These phages could be either mutants of the superinfecting phage or recombinants between the superinfecting phage and the resident P22 prophage; such recombinants would inherit from their P22 parent the allele(s) which allow superinfecting P22 to escape exclusion by the B system.

The frequency of plaque formation by Lc_{II} on P22*sie*A⁻*sie*B⁺ lysogens is higher than would be expected if the plaques were formed by mutants preexisting in the Lc_{II} stock. Furthermore, B system-insensitive recombinants can be obtained in vegetative crosses between P22 and L in a nonlysogenic host. Thus in the case of superinfecting Lc_{II}, the plaques which appear are probably formed by recombinants between the original superinfecting phage and the P22 prophage.

These findings suggest a possible explanation for the difference in efficiency of plating of L and MG178 on *sie*A⁻*sie*B⁺ lysogens. According to this hypothesis, the probability of plaque formation would be determined, at least in part, by the ability of the superinfecting phage to recombine with the P22 prophage during infection, thereby forming a recombinant which is still heteroimmune to P22 but which carries the P22 gene(s) responsible for the B system-insensitive phenotype of superinfecting P22. The greater ability of L to plate on *sie*A⁻*sie*B⁺ lysogens would then be explained by its ability to recombine with P22 at high frequency in vegetative crosses (Bezdek and Amati, 1968). Since MG178 appears to be more distantly related to P22, it might be expected to recombine much less frequently, possibly accounting for the 100–1000-fold lower efficiency of plating on *sie*A⁻*sie*B⁺ lysogens.

The supposition that superinfecting L phage escapes B exclusion by recombination with the P22 prophage requires that the excluded superinfecting phage DNA enter the cell. This implication is strongly supported by preliminary experiments which indicate that coinfection of a *sie*A⁻*sie*B⁺ lysogen with both B-sensitive and B-insensitive L phages results in efficient growth of both phage types; thus the B-insensitivity character appears to be *trans*-dominant.

It should be noted that the B exclusion system can be expressed by prophages having immunity specificity different from P22. Thus we have constructed *sie*B⁺ prophages with the immunity specificity $imm\mathrm{I_L}$-$imm\mathrm{C_P}$. Likewise, the ability to escape B exclusion can be expressed by appropriate recombinants between L and P22 ($imm\mathrm{I_P}$-$imm\mathrm{C_L}$ as well as P22 and L immunity types). Thus, neither B exclusion nor escape from it are completely immunity specific.

Several hypotheses can be proposed to explain the mechanism of action of the B system and to explain the B-insensitive phenotype. The B system may act to interfere with the synthesis or activity of phage function(s) necessary for growth. In the case of a superinfecting L phage, the B exclusion system prevents expression of these functions. A superinfecting phage (either L or P22) carrying the P22 alleles for these functions is, by hypothesis, not affected by the B exclusion system, and can thus act in *trans* to allow growth of all superinfecting phages.

Alternatively, the dominant B-insensitive phenotype may be due to the synthesis of a product which inactivates the B system, thus protecting both phages which are genotypically B-insensitive and those which are genotypically B-sensitive. This hypothesis makes no predictions about the mechanism of action of the B system, except that it would not prevent the superinfecting phage DNA from entering the cell and would not prevent the transcription and translation of the gene(s) responsible for the B-insensitive phenotype.

The P22 gene(s) which specify the ability to escape exclusion by the B system map, along with the gene(s) for the B system itself, between the left side (*pro*A) prophage attachment site and gene 18. Though the gene(s) for the B exclusion system are linked to $imm\mathrm{C_P}$, they occasionally segregate from $imm\mathrm{C_P}$ in crosses between P22 and L.

It is of some interest that the B exclusion system is formally analogous to the exclusion of $\mathrm{T4r_{II}}$ phage by *E. coli* cells lysogenic for phage λ. In both cases a mutable site mapping near the prophage repressor gene (*rex* in phage λ (Howard, 1967) and *sie*B in phage P22) governs exclusion of certain superinfecting phages. These superinfecting phages can escape exclusion if they carry appropriate alleles ($\mathrm{r_{II}^+}$ in the case of T4 and the P22 B-insensitive locus in the case of L). This escape is in both cases dominant in *trans*; mixed infection with an insensitive phage allows an excluded phage to grow.

Finally, both r_{II}^{+} and the P22 B-insensitive locus are dispensable in nonexcluding hosts.

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