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Superinfection Exclusion by Incomplete Genomes of Bacteriophage T4

J. D. CHILDS

Biology and Health Physics Division, Atomic Energy of Canada Limited, Chalk River, Ontario, Canada

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The genetic basis of superinfection exclusion by bacteriophage T4 was investigated by using incomplete genomes derived from the gene 66 mutant *E920g*. Incomplete genomes, which included a region of T4 between genes 42 and 44, were able to exclude superinfecting phage with an efficiency similar to that of complete genomes. Those genomes which did not include this region were unable to exclude superinfecting phage. A mutant with reduced ability to exclude superinfecting phage was isolated after mutagenesis with hydroxylamine. The mutation maps midway between *amN122* in gene 42 and *amB22* in gene 43. The efficiency of exclusion of superinfecting phage (as measured by the percentage of superinfected cells which failed to release any phage carrying selected markers of the superinfecting phage) by this mutant was 50 to 60%, whereas for wild type it was 85 to 95%. Uptake of ^{3}H-leucine by cells infected with the mutant was inhibited by superinfection with ghosts and it has therefore been designated *imm1*, for lack of immunity to superinfecting phage and ghosts. The formation of infective centers by cells infected with *imm1* or another *imm*$^-$ mutant (*imm2*) was not inhibited by superinfection with ghosts.

If *Escherichia coli* is infected with bacteriophage T4 and then superinfected with T4 a few minutes later, the superinfecting phage is unable to function or contribute markers to the progeny, and 40 to 60% of its deoxyribonucleic acid (DNA) is broken down to acid-soluble fragments a few minutes after superinfection. These processes are known as superinfection exclusion and superinfection breakdown, respectively (9, 13). Exclusion is not the result of DNA breakdown, i.e., in endonuclease I$^-$ mutants of *E. coli*, superinfecting phage is excluded as efficiently as in endonuclease$^+$ strains, but its DNA is not broken down (1, 11).

To investigate the genetic basis of superinfection exclusion, incomplete particles of T4 were used in this study. As incomplete genomes can induce some early enzymes and carry out limited DNA replication in single infection (16, 17; A. H. Doermann, *personal communication*), it seemed likely that at least some incomplete genomes could also exclude superinfecting phage. This prediction has been confirmed and has permitted the localization of the region responsible for superinfection exclusion in T4.

MATERIALS AND METHODS

The methods and media employed are those used by Chase and Doermann (3) and Doermann and Parma (7), except where otherwise noted.

Bacterial and bacteriophage strains. These were obtained from A. H. Doermann, except T4 *amE142*-(39) *imm2* and *imm2* from M. Vallée, and *E. coli* BC-251-(λh) from M. Russel. This *E. coli* strain restricts T4 amber and *rII* mutants. All experiments were performed at 30 C, and plates were incubated at 37 C. Incomplete particles of T4 were obtained from lysates of the gene 66 mutant *E920g*. Bacteriophage stocks were prepared by adding a single plaque, grown for 4 hr, to 10 ml of H broth containing log-phase *E. coli* CR63 (0.2 ml of overnight culture diluted into 10 ml of broth and aerated for 3 hr at 30 C). After 15 min of aeration, the culture was diluted 10-fold and aerated overnight. Bacterial debris was then removed by centrifugation at 3,500 × *g* for 30 min. Stocks of *E920g* or *E920g os2* (osmotic shock resistant), prepared in this way, normally contained three to five incomplete particles for every complete particle as measured by the method of Parma (18). The incomplete particles normally contained an average of about 70 to 72% of the T4 genome as measured by a modification of the genetic method of Mosig (5). Incomplete particles were separated from complete particles by cesium formate in heavy water density gradients. These gradients give improved separation of incomplete particles from complete particles compared to cesium chloride gradients. Also, osmotic shock-resistant T4 is inactivated by storage at 4 C in cesium chloride solution (15) but not in cesium formate solution.

Exclusion experiments. *E. coli* at 2 × 10^9 cells per

ml was mixed with an equal volume of the primary phage at a multiplicity of infection (MOI) of 0.001 to 0.05. After incubation at 30 C for 2 min, 0.1 ml of this mixture was added to 0.8 ml of prewarmed broth and aerated for 10 min. Then 0.1 ml of the secondary phage at 2×10^9 particles per ml (MOI = 2) was added, and after a further 10 min of aeration the culture was diluted into ice-cold broth and plated for infective centers. The bacterial strains used for both adsorption of the phage and for plating restricted both the primary and secondary phages. Thus, to form infective centers, the superinfecting genome had to complement the primary genome, and wild-type recombinants had to be produced. These bacterial strains varied in different experiments and are given in the text. As a control, all additions were made simultaneously in the presence of 1 mM NaCN and aerated for 20 min at 30 C. The efficiency of exclusion (E) was calculated from the following equation.

$$E = \left(1 - \frac{\text{titer of infective centers after delayed superinfection}}{\text{titer of infective centers after simultaneous infection}} \right) \times 100$$

Preparation of ghosts. These were prepared from JC110 (*rb50 imm1*) by the method of Duckworth (8), except that the ghosts were not collected by centrifugation after shock treatment. The preparation was contaminated with 4% viable phage.

Infective center formation in the presence of ghosts. *E. coli* CR63(λh) was used to restrict viable phage in the ghost preparation. Ghosts and phage were added at a MOI of 5 with a final bacterial concentration of 8×10^7 cells per ml at the times indicated in Table 6. Unabsorbed phage were inactivated by a 5-min treatment with antibody added 7 min after the final addition of ghosts or phage.

RESULTS

Exclusion by imcomplete genomes. A preliminary experiment was performed to determine whether incomplete genomes could exclude superinfecting phage. A lysate of *E920g* (gene 66) *amE355* (gene 24), consisting of approximately five *amE355* incomplete particles to one whole particle, or a lysate of only whole particles of *amE355* was used for the primary infection, and *amN52* (gene 37) was used for the secondary infection in *E. coli* B. Infected bacteria were plated with *E. coli* S/6. The efficiency of exclusion (as measured by the percentage of superinfected cells which failed to release any am^+ phage particles) was 70% for *E920g amE355* compared with 90% for *amE355*. The latter is typical for superinfection exclusion experiments with T4 (1, 10). This showed that at least some incomplete genomes could exclude superinfecting phage but that the average efficiency of exclusion was lower than that for whole genomes. The lower efficiency of exclusion could be the result of a decreased ability to exclude by all incomplete genomes or only by some of them. The latter would be the case if there was a gene responsible for exclusion and if only genomes which included this gene could exclude.

To distinguish between these two alternatives, the ability of incomplete genomes to exclude different superinfecting amber mutants in *E. coli* B was tested. If the only incomplete genomes able to exclude are those which carry an exclusion gene, then the efficiency of exclusion of any amber mutant will depend on the probability of an incomplete genome carrying both the am^+ allele and the exclusion gene. This probability decreases from nearly 100%, for an amber mutation adjacent to the exclusion gene, to a minimum depending on the maximum length of the incomplete genomes. For incomplete particles containing 70% of the T4 genome, which comprise the majority in lysates of *E920g* (5, 10, 18), it can be calculated from equation 5 of Mosig (14) that this probability decreases to 57% for a separation of more than 30% of the T4 genome. It follows that incomplete genomes should be able to exclude a superinfecting amber mutant with greatest efficiency where the amber mutation is adjacent to the exclusion gene. This should decrease to a minimum value for amber mutations at distances greater than 30% of the genome from the exclusion gene.

The efficiency of exclusion by *E920g amE355* is greatest for amber mutants defective in genes 41 and 42 and only slightly less in the adjacent genes 56, 44, and 46 (Table 1, Fig. 1). The lowest efficiency of exclusion is for amber mutants defective in genes 1, 7, 25, and 31. Each of these genes is more than 30% of the T4 genome from genes 41 and 42, except for gene 1 (28.4%) (5). This indicates that there is one region of T4 responsible for exclusion of superinfecting phage by incomplete genomes and it is located close to genes 41 and 42. The efficiency of exclusion of amber mutants defective in genes 41 and 42 (87 to 89%) indicates that incomplete genomes which carry this region can exclude as efficiently as whole genomes.

The efficiency of exclusion by genomes which do not include the gene 41 and 42 region can be calculated approximately from the data for amber mutants defective in genes 7, 25, and 31. The lysate used for the primary infection contained one complete genome for every five incomplete genomes which contained an am^+ allele for gene 7, 25, or 31, that is 16 and 84 genomes per 100, respectively. It can be calculated (*see above*) that, of these 84 incomplete genomes, 48 contained, in addition to am^+

alleles for genes 7, 25, or 31, the region responsible for superinfection exclusion near genes 41 and 42, and 36 incomplete genomes did not contain this region. Thus, 64 genomes per 100 (16 complete and 48 incomplete) contained the region responsible for superinfectiion exclusion and 36 genomes per 100 did not. In superinfection exclusion experiments using amber mutants defective in genes 7, 25, or 31 as the secondary infection, there were an average of 47 infective centers per 100 primary infections (Table 1). Of these infective centers, only 6.4 infective centers should have been derived from the 64 primary infections with genomes which contained the region responsible for superinfection exclusion, if the efficiency of exclusion by these genomes was 90%. The remaining 40.6 infective centers must have been derived from an almost equal number of primary infections, that is, from the 36 infections by the genomes which did not contain the region responsible for superinfection exclusion. This indicates that these genomes are unable to exclude superinfecting phage.

TABLE 1. *Exclusion of amber mutants by incomplete genomes*[a]

Secondary infection		Map position (% distance from *rIIB* clockwise)	% Exclusion
Mutant	Gene no.		
amE51	56	9	84.6
amN81	41	12	87.1
amN122	42	14	88.9
amN82	44	17	85.7
amN130	46	19	81.7
amlys882	*e*	38	61.9
amC42	1	43	58.2
amB23	7	48	48.2
amS52	25	67	54.8
amN54	31	76	56.1
amN52	37	94	68.5
Strain no. JC82: *amN81 amN82*	41, 44		91.6

[a] A stock of *E920g amE355* containing a ratio of five *amE355* incomplete particles for every complete particle was used for the primary infection of *E. coli* B. Infected bacteria were plated with S/6. The map position given for each amber mutation used for the secondary infection is the percentage of the T4 genome from *rIIB* from the map of Childs (5).

The ability of these genomes to exclude superinfecting phage was measured directly by using a purified preparation of incomplete particles from *E920g amH39* (gene 30) *os2* with less than 1% contamination with complete particles and helper phages JC86 and JC117. These carry amber mutations in genes 5, 25, and 34, and 1, 5, 25, and 34, respectively, which are located within 46% and 47% of the T4 genomes, respectively. More than 90% of incomplete genomes which carry am^+ alleles for all

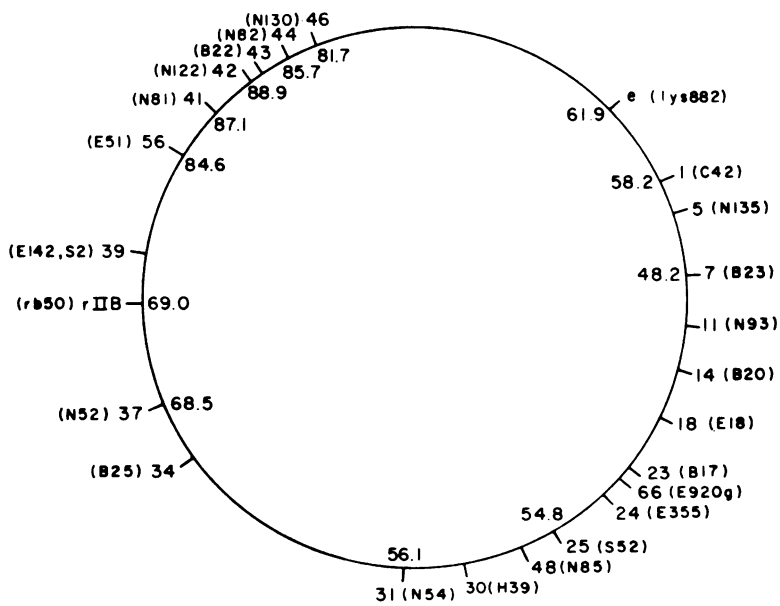

FIG. 1. *Map of T4 showing mutations used in this study. The mutation numbers are given in parentheses next to the number of the gene in which they are located. All are amber mutations except rb50 (gene rIIB) and E920g (gene 66). The numbers inside the circle are the efficiencies of exclusion, of those mutations tested, by incomplete genomes (see Table 1 for amber mutations and Table 3 for rb50).*

of these genes should lack the gene 41 and 42 region (5). As predicted, incomplete genomes failed to exclude JC86 and JC117, and, unexpectedly, rescue of incomplete genomes by superinfection appeared to be slightly more efficient than when infection was simultaneous (Table 2). The number of incomplete genomes rescued by simultaneous infection with these helper phages was also less than expected, being 14 to 16% of those rescued by *amN52* compared with an expected 32 to 35%. A possible reason for the latter may have been the requirement for four crossovers between the incomplete genome and helper phage, one on either side of gene 30 and one on each of the distal sides of genes 5 and 34 or 1 and 34. These would be required in the initial infection to form a wild-type recombinant able to grow on S/6. The efficiency of exclusion of *amN52* and JC82 by incomplete genomes in this experiment was similar although slightly less than that found in the previous experiment (Tables 1 and 2).

To map the exclusion region more precisely, the method used by Childs (5) to map T4 was modified to permit incomplete genomes to exclude the helper phage. Incomplete genomes were rescued by delayed superinfection (instead of simultaneous infection) with strain JC 25, which carried *rb50* and 20 amber mutations (see Table 3), in CR63(λh). Infective centers were tested for am^+ alleles derived from the incomplete genomes by the replica-plating technique of Doermann and Boehner (6). From this it was possible to locate the ends of each incomplete genome rescued between pairs of amber mutations of the helper phage. As all the incomplete genomes rescued were r^+, they each had one end in a clockwise and one in a counterclockwise direction from *rII* on the map of T4. Incomplete genomes with a clockwise end between *rII* and the exclusion region should be rescued with nearly 100% efficiency, whereas those with a clockwise end on the *rII* distal side of the exclusion region should be rescued with only 10% efficiency. The location of the exclusion region should therefore be revealed by an abrupt change in the efficiency of rescue of incomplete genomes with clockwise ends on either side of a single amber mutation, or on either side of the pair of amber mutations which span the exclusion gene.

The efficiency of rescue was calculated as the ratio of the number of ends between markers after delayed superinfection to the number found previously in the absence of exclusion (5). In this experiment, the average efficiency of rescue of incomplete genomes was 31% (401 incomplete genomes were rescued after delayed superinfection compared with 1,292 incomplete genomes after simultaneous infection). The expected distribution of the ends of 1,292 genomes was calculated from the data given by Childs (5). For example in that study, of 3,104 incomplete genomes tested, 791 had a clockwise end between *amN130*(46) and *amlys822*(*e*), which is equivalent to 329 out of 1,292. In this study only 33 genomes had a clockwise end in this region from the equivalent number of input incomplete genomes, the remaining 296 presumably having been lost by superinfection exclusion. The ratio of 33:329 (= 0.10) is therefore the efficiency of rescue of genomes with a clockwise end between *amN130* (46) and *amlys822*(*e*). Similar calculations were made for each interval.

An abrupt change in this efficiency of rescue was apparent on either side of gene 44 (Table 3). Between *rII* and gene 41 the ratio of recovery

TABLE 2. *Exclusion of amber mutants by incomplete genomes*[a]

Secondary infection			Simultaneous infection		Delayed superinfection
Strain no.	*am* mutations	Genes	% Recovery of incomplete genomes		% Exclusion
			Observed	Expected	
	N52	37	100	100	59.2
JC82	*N81 N82*	41, 44	94.7	93.0	81.3
JC86	*N135 S52 B25*	5, 25, 34	15.5	34.7	−4.1
JC117	*C42 N135 S52 B25*	1, 5, 25, 34	14.1	32.3	−25.7

[a] A purified preparation of incomplete particles of *E920g amH39*(30) *os2* contaminated with less than 1% whole particles was used as the primary infection in *E. coli* B. Infected bacteria were plated with S/6. The recovery of incomplete genomes by simultaneous infection with the multi-amber helper phages is given as a percentage of that obtained with *amN52*. The expected percentage of recovery (R) was calculated from the equation $R = [(L - D)/L] \times 100$, where L (length of incomplete genomes) = 70% and D = shortest distance separating the amber mutations of the helper phage. The results are the average of three experiments using JC117 and four experiments using *amN52*, JC82, and JC86.

TABLE 3. *Distribution of ends of incomplete genomes rescued by superinfecting phage*[a]

Secondary infection		Counterclockwise ends			Clockwise ends		
Mutation	Gene	Observed	Expected (no exclusion)	Efficiency of rescue	Observed	Expected (no exclusion)	Efficiency of rescue
rb50	*rIIB*	0	0		36	56.6	0.64
amS2	39	1	0.8		159	164.4	0.97
amN81	41	8	8.2	0.98	45	89.1	0.51
amN82	44	4	4.2		3	32.5	0.09
amN130	46	130	168.1	0.77	33	329.2	0.10
amlys882	*e*	63	85.7	0.74	6	83.2	0.07
amC42	1	14	29.6	0.47	7	34.5	0.20
amN135	5	5	62.4	0.08	12	83.3	0.14
amB23	7	10	55.4	0.18	18	58.3	0.31
amN93	11	5	43.7	0.11	16	54.9	0.29
amB20	14	9	62.0	0.15	16	62.0	0.26
amE18	18	2	27.1	0.07	24	71.2	0.34
amB17	23	3	42.5	0.07	1	31.6	0.11
amE355	24	9	91.6	0.10	11	75.3	
amS52	25	9	46.2	0.19	9	37.0	
amN85	48	23	131.9	0.17	3	18.7	0.22
amN54	31	70	250.6	0.28	2	8.5	
amB25	34	14	70.3	0.20	0	1.7	
amN52	37	22	111.5	0.20	0		
rb50	*rIIB*						
Total		401	1,292		401	1,292	

[a] Incomplete genomes from two lysates of *E920g os2* (245 from one lysate and 156 from another) were rescued by superinfecting phage JC25 in an infection in CR63(λh). JC25 carried *rb50* and 20 amber mutations, all of which are listed in the table, except *amA455*(34) and *amB252*(35). The ends of r^+ incomplete genomes were divided into clockwise and counterclockwise from *rII* on the genetic map of T4, and the total number between each pair of markers is given. The expected total number of incomplete genomes (in the absence of exclusion) was based on the ratio of plaques found after superinfection exclusion to the number found after simultaneous infection. The expected distribution of the ends of these genomes was then calculated from the data in Table 1 of Childs (5). The efficiency of rescue of genomes with ends in each region is the observed number divided by the expected number.

of incomplete genomes with clockwise ends between markers after delayed superinfection compared with simultaneous infection was 0.64 and 0.97 (average 0.88), on the *rII* distal side of gene 44 this ratio was 0.09 to 0.34 (average 0.16), and between genes 41 and 44 it was 0.51. This indicates that the exclusion region is probably between genes 41 and 44, or possibly between genes 44 and 46.

The experiment was repeated using a helper phage, JC53, with two additional markers between genes 41 and 44, *amN122* in gene 42 and *amB22* in gene 43. Infective centers were tested, first to determine whether they contained phage with gene 41^+ but not gene 44^+ alleles, to select those derived from incomplete genomes with a clockwise end between genes 41 and 44. These were then tested for the presence of the other am^+ alleles. It can be seen that the efficiency of rescue of genomes with ends in this region is not uniform (Table 4); between genes 41 and 42 it is similar to that between *rII* and gene 41 (0.82 and 0.88, respectively); between genes 42 and 43 it is slightly lower (0.55); and between genes 43 and 44 it is similar to the average on the *rII* distal side of gene 44 (0.13 and 0.16, respectively). This indicates that the exclusion region is

TABLE 4. *Distribution of clockwise ends of incomplete genomes, between genes 41 and 44, rescued by superinfecting phage*[a]

Secondary infection		Counter-clockwise ends		
Mutation	Gene	Observed	Expected (no exclusion)	Efficiency of rescue
amN81	41	55	66.9	0.82
amN122	42	18	32.5	0.55
amB22	43	8	61.0	0.13
amN82	44			
Total		81	160.4	

[a] Incomplete genomes, from a density gradient sample of *E920g os2* with 0.5% contamination with whole genomes, were rescued by superinfection with JC53, which carries *amN122* (gene 42) and *amB22* (gene 43) in addition to the markers carried by JC25 (see Table 3). Only incomplete genomes with a clockwise end between *amN81* and *amN82* were analyzed. The expected number of ends and efficiency of rescue were calculated as in Table 3.

probably between genes 42 and 43 or possibly between genes 43 and 44.

In these experiments there was some unexpected variability in the efficiency of recovery of genomes with clockwise ends in the intervals between *rII* and gene 42 and on the *rII* distal side of gene 43. This was probably because data from 38% of the gene sequences were not used owing to failure to recover all markers; loss of internal markers was apparent by interrupted sequences of am^+ markers (27% of the sequences) and loss of terminal markers by sequences corresponding to less than 60% of the physical map of T4 (11% of the sequences). The reason for not using these data was to permit comparison of the remaining data with that obtained in a previous study, in which the same criteria were applied (5). In that study, as in this one, loss of internal markers, particularly adjacent markers, made it difficult or impossible to assign ends to these incomplete genomes. The length of 60% of the T4 genome was chosen as an arbitrary figure, slightly shorter than the length of DNA contained by the smallest incomplete particles found in lysates of *E920g* (17). Data from sequences shorter than 60% of the T4 genome were not included, as the ends of these sequences would not correspond with the actual ends of incomplete genomes. In experiments where both parents were infected simultaneously, only 5 to 10% of the data were not used for the same reasons (4, 5). Loss of terminal markers in the remaining 62% of the genomes does not appear to have been serious, as most of the genomes with a clockwise end between *rII* and *amN82* (gene 44) had a counterclockwise end between *amC42* (gene 1) and *amN130* (gene 46) (Table 3), as found previously (5).

Isolation of exclusion mutant. To obtain an exclusion mutant, JC25, which carries *rb50* and 20 amber mutations (see Table 3), was treated with hydroxylamine for 49 hr, using the method of Tessman (19). The mutagen-treated stock was crossed to *rb50 amN122*(42) *amB22*(43), and am^+rb50 recombinants were selected. The parental amber mutations were chosen so the recombinants would inherit most of the gene 41–44 region, including all of the gene 42–43 region from the mutagen-treated parent and most of the remainder of the T4 genome from the untreated parent. The *rb50* mutation was present in both parents because it was required for subsequent exclusion experiments.

Recombinants were tested for their ability to exclude superinfecting *amN52* in *E. coli* BC-251-(λh), which restricts both parents. In these tests the efficiency of exclusion of superinfecting phage by *rb50*, the control, was 95%. Of 50 recombinants tested, five had efficiencies of exclusion of 82 to 90%. The recombinant with the least ability to exclude was chosen for mapping and numbered JC110.

Mapping the exclusion mutation. This was mapped by the method used to map another nonessential gene, exonuclease A (23). The exclusion mutant JC110, which carries *rb50*, was crossed with *amE51*(56) *amN81*(41) *amN122*-(42) *amB22*(43) *amN82*(44) *amN130*(46). The genotypes of recombinants were tested by the replica-plating method of Doermann and Boehner (6). Those resulting from a single crossover between any pair of amber mutations were tested for their ability to exclude superinfecting *amN52*(37) in *E. coli* B.

In each pair of reciprocal recombinant classes, except those resulting from a crossover between *amB22* and *amN122* (crossover interval 4) and two exceptions discussed below, the exclusion mutation segregated with only one class of recombinants (Table 5). In each case the class carried $amN122^+$ and $amB22^+$. This confirmed that the exclusion mutation mapped between *amN122*(42) and *amB22*(43). Of the 19 recombinants resulting from a crossover between *amN122* and *amB22*, 10 had the $exclusion^+$ and 9 had the $exclusion^-$ phenotype. Thus, the exclusion mutation is approximately halfway between *amN122* and *amB22*. Two exceptional recombinants were phenotypically $exclusion^-$ although five or six others within the same class were $exclusion^+$ (crossover intervals 3 and 5, Table 5). These probably resulted from double crossovers between *amN122* and *amB22*.

Superinfection with ghosts of T4. Duckworth (8) has shown that, if ghosts of T4 are added to *E. coli* prior to, or up to 2 min after T4 infection, then infective center formation is inhibited. After 2 min postinfection, a tolerance or immunity develops, and infective center formation is not affected by subsequent additions of ghosts. Vallée and Cornett (21) have isolated a mutant designated $immunity^-$ (imm^-), which fails to protect against the action of superinfecting ghosts as measured by uptake of ^{3}H-leucine; ghosts added to cells later than 2 to 3 min after T4 infection inhibit the uptake of ^{3}H-leucine in imm^--infected cells, but not in imm^+-infected cells. The imm^- mutation also fails to exclude superinfecting phage and is located between genes 42 and 43 (21, 22; Cornett, *personal communication*). The exclusion mutant JC110 has been tested by Vallée (*personal communication*), and she

TABLE 5. *Mapping the exclusion mutation*[a]

Recombinant genotypes	Crossover interval	No. screened	Exclusion phenotype		% Exclusion	
			Exc$^+$	Exc$^-$	Exc$^+$	Exc$^-$
−−−−−−−	1	5	5	0	93.0	
+++++++	1	NT[b]				
−+−−−−−	2	5	5	0	92.4	
+−+++++	2	5	0	5		48.8
−++−−−−	3	6	5	1	88.9	
+−−+++	3	5	0	5		51.0
−+++−−−	4	9	4	5	86.3	61.9
+−−−+++	4	10	6	4	88.0	58.9
−++++−−	5	5	0	5		49.0
+−−−−++	5	6	5	1	88.8	
−+++++−	6	6	0	6		73.1
+−−−−−+	6	5	5	0	89.6	

[a] The exclusion mutant JC110 (which carries *rb50*) was crossed with strain JC108 carrying the six amber mutations given below. The genotypes of recombinants and crossover intervals are given in the following order.

Mutation: *rb50* − *amE51*(56) − *amN81*(41) − *amN122*(42) − *amB22*(43) − *amN82*(44) − *amN130*(46)
Crossover interval 1 2 3 4 5 6

[b] NT, Not tested.

has shown that it also has the immunity$^-$ phenotype. The two mutations are therefore probably located in the same gene. The exclusion mutation carried by JC110 has been numbered *imm1* and that of Vallée and Cornett, *imm2*.

An experiment was performed to determine whether infective center formation by the two mutants could be inhibited by superinfecting ghosts. *E. coli* was infected with wild-type T4 or with either of the mutants and superinfected at 5 or 10 min with ghosts. It can be seen (Table 6) that ghosts added at these times do not inhibit the formation of infective centers by wild-type or either of the mutants by more than 20%. Ghosts added 10 min before infection, however, reduced the numbers of infective centers to 6%. This is similar to the number of surviving bacteria when ghosts alone were added.

TABLE 6. *Effect of ghosts on formation of infective centers*[a]

Time of addition (min)			Infective centers × 10^7 per ml	% Infective centers
0	5	10		
WT[b]	None	Broth	4.7	100
WT	Ghosts	None	3.9	84
WT	None	Ghosts	4.3	92
Ghosts	None	WT	0.3	6
amE142 imm2	None	Broth	8.5	100
amE142 imm2	Ghosts	None	7.8	92
amE142 imm2	None	Ghosts	10.6	125
imm2	None	Broth	4.7	100
imm2	None	Ghosts	4.1	87
amE51 imm1	None	Broth	4.0	100
amE51 imm1	None	Ghosts	4.7	86
E. coli CR63(λh)	None	None	8.4[c]	100[d]
E. coli CR63(λh) and ghosts	None	None	0.3[c]	4[d]

[a] Ghosts and phage were added to *E. coli* CR63(λh) at 8×10^7 cells per ml (MOI = 5). The percentage of infective centers was calculated from the number of infective centers formed with only phage added.

[b] WT, Wild type.

[c] Bacterial titer × 10^7 per ml

[d] Percentage bacterial titer.

DISCUSSION

The results show that incomplete genomes can exclude superinfecting phage with the same efficiency as whole genomes if they include the gene 42–43 region of T4. If they do not include this region, then they are unable to exclude superinfecting phage. The isolation and mapping of an exclusion mutation between genes 42 and 43 confirmed that the incomplete genomes which exclude, do so because they carry an exclusion gene. The efficiency of exclusion by the exclusion mutation in combination with *rb50* and various amber mutations was approximately 50 to 60%, except in combination with *rb50* and *amN130* when it was 73% (Table 5). As incomplete genomes without the exclusion gene have no measurable ability to exclude, it should be possible to isolate a deletion mutation also unable to exclude superinfecting phage.

The exclusion of superinfecting phage is probably a result of a change in the phage receptor sites and possibly the cell membrane. This is likely, because although adsorption of the superinfecting phage is normal, only 50% of the DNA of adsorbed superinfecting phage is injected into the host cell (1, 12, 19). Also, in endonuclease I^- cells, the DNA of the superinfecting phage which is injected is trapped between the cell wall and membrane (2). The degradation of superinfecting DNA in endonuclease I^+ cells probably occurs while the DNA is trapped between the cell wall and membrane, which would account for its rapid release into the growth medium.

A change in the receptor sites or membrane would also explain the finding that the uptake of ^{3}H-leucine by cells infected with the exclusion mutant JC110 is inhibited by superinfection with ghosts (Vallée, *personal communication*). A similar mutant, *imm2*, which also has reduced ability to exclude superinfecting phage, has been isolated (21, 22). It also maps between gene 42 and 43 (Cornett, *personal communication*), and therefore both mutations are probably in the same gene, *imm* (for immunity to superinfecting phage and ghosts). Unexpectedly, the formation of infective centers by *imm1*, the exclusion mutation carried by JC110, or *imm2* was not inhibited by superinfection with ghosts of T4. Either the cells can recover their ability to take up leucine or the reduction is not sufficient to prevent the release of some phage particles by most of the infected cells.

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